

**EFFECT OF SOAKING, SPROUTING AND COOKING ON
NUTRITIONAL AND BIOLOGICAL QUALITY OF COWPEA,
MOTH BEANS AND KIDNEY BEANS**

THESIS

By

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Submitted to



**CSK HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR-176 062 (H.P.) INDIA**

IN

Partial fulfilment of the requirements for the degree

OF

MASTER OF SCIENCE IN HOME SCIENCE

(FOOD SCIENCE AND NUTRITION)

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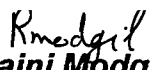
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CERTIFICATE I

This is to certify that the thesis entitled, **“Effect of soaking, sprouting and cooking on the nutritional and biological quality of cowpea, moth beans and kidney beans”** submitted in partial fulfilment for the requirements for the award of the degree of **Master of Science (Home Science)** in the subject of **Food Science and Nutrition** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ms. Kavita Mankotia (H-99-30-02)** daughter of **Lt. Col. O.P. Mankotia** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

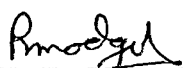
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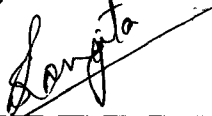
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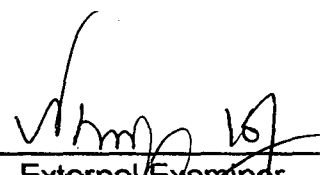
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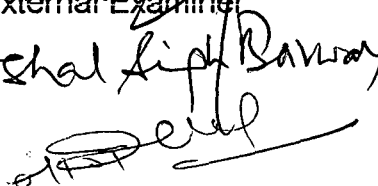


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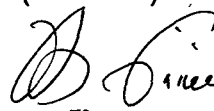


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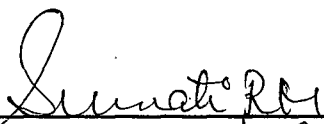


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(Kavita Mankotia)

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INTRODUCTION

CHAPTER-I

INTRODUCTION

Legumes are produced and widely consumed throughout the world particularly in tropical and sub-tropical areas of Africa, Asia and Latin America. For people who live in these areas, legumes constitute an important source of protein, vitamins (B group) and minerals (Barampama and Simard, 1995). Legumes also called as pulses belonging to the family leguminosae hold a great promise in meeting the protein requirement of vulnerable groups of vegetarian population, as legumes are rich and economical source of proteins (Anonymous, 1978). A significant part of the world population also relies on legumes as a staple food for subsistence, particularly in combination with cereals, as legumes increase the nutritional quality of predominantly cereal based diets (Burbano *et al.*, 1999 and Aparana *et al.*, 2000). Legumes provide proteins (20-24%), carbohydrates (50-60%), fats (2-3%) and minerals (2-4%) (Gopalan *et al.*, 1991).

India is a major legume producing country accounting for about 27-28 per cent of world legume production (Anonymous, 1989). Area under legumes in India is 228.47 lakh hectares yielding its annual aggregate production of 130.70 lakh tonnes (India, 2000).

The major legumes that find an important place in Indian dietaries are black gram, green gram, red gram, chickpea, kidney beans, cowpea, mothbean, lentil and soybean. Before consumption, legumes are processed in several ways so as to

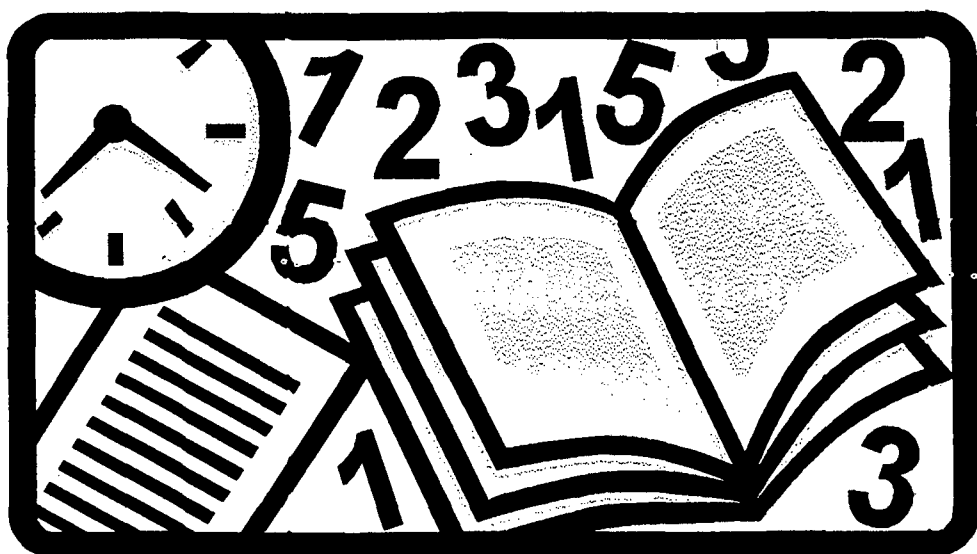
improve their nutritional and organoleptic qualities (Sangle *et al.*, 1993). The most common domestic methods used for processing of legumes are milling, dehulling, soaking, cooking and sprouting. These processing techniques may affect nutritional quality of legumes (Bishnoi, 1991; Kataria *et al.*, 1990).

The primary processing techniques such as dehulling of pulses might result in reduction of fibre and tannin contents but improve the appearance, cooking quality, palatability and digestibility of legumes (Singh, 1993). Secondary processing techniques such as soaking, cooking and sprouting improve the bio-availability of nutrients by partly or wholly removing some of the anti-nutritional factors and toxic constituents present in legumes (Singh and Jambunathan, 1984; Bishnoi and Khetarpaul, 1994; Singh 1995). These methods improve the protein and starch digestibility (El-Faki *et al.*, 1984).

Nutritional quality of the legumes is severely affected by certain antinutritional factors like protease inhibitors, saponins, phytic acid, tannins etc. These anti-nutrients interfere with the assimilation of nutrients present in legumes. The reduction or elimination of these antinutrients present in legumes might enhance the biological protein quality of the legumes (Modgil and Mehta, 1997). Domestic processing and cooking methods such as soaking, autoclaving, sprouting and roasting might help in reducing these anti-nutritional factors (Bansal *et al.*, 1998; Duhan *et al.*, 1989). Different processing techniques can either increase or decrease the nutritional quality of legumes.

No significant work has been done on the effect of different processing techniques on the nutritional and biological quality of kidney bean, moth bean and cowpea that are widely consumed in Northern India. In this back-drop, the present study was planned with the following specific objectives:

1. To study the effect of soaking, sprouting and cooking on the physico-chemical characteristics of the legumes.
2. To examine the effect of these different treatments on the micro and macro elements of the legumes.
3. To analyse the effect of these different treatments on the anti-nutritional factors in the legumes.
4. To see the effect of these different treatments on the biological utilization of proteins from the diets.



***REVIEW
OF
LITERATURE***

CHAPTER-II

REVIEW OF LITERATURE

The pre-requisite of any research to be conducted is to make clear all concepts related to the problems followed by a systematic and meticulous future planning. The knowledge of scientific literature in the related field of research problem, therefore, is of great importance in carrying out future research work. It not only provides impetus in understanding the problem in a precise, clear and comprehensive manner, but also help in gearing up and up-dating the research work. It becomes therefore, quite imperative for every research worker to critically review the related research work already carried out so as to get a broad spectrum and insight of the research problem. For this reason, the research work already done in India and abroad relating to the topic of present study has been reviewed under the following sub-heads:

- 2.1 Physical characteristics
- 2.2 Chemical composition
 - 2.2.1 Proximate composition
 - 2.2.2 Dietary fibre constituents
 - 2.2.3 Non-protein nitrogen and true protein
 - 2.2.4 Available carbohydrates
 - 2.2.5 Total calorific value
 - 2.2.6 Total minerals

- 2.2.7 Phytic acid
- 2.3 Effect of soaking, sprouting and cooking
 - 2.3.1 Proximate composition
 - 2.3.2 Dietary fibre constituents
 - 2.3.3 Non-protein nitrogen and true protein
 - 2.3.4 Carbohydrates
 - 2.3.5 Total calorific value
 - 2.3.6 Total minerals
 - 2.3.7 Phytic acid
- 2.4 Biological studies

2.1 Physical characteristics

2.1.1 Colour

Meshram *et al.* (1980) reported the colour of raw moth bean to be light brown. Shinde *et al.* (1991) reported the colour of cowpea varieties as white, red with white spots and red. Akinyele *et al.* (1986) reported the coat colour of cowpea cultivars was white-brown, maroon, brown, white and white grey and that of hilium was brown and black.

2.1.2 Weight

Attia *et al.* (1994) reported 100 seed weight in dry and cooked chickpea seeds ranged between 14.6 to 29.3 g and 30.1 to 68.1 g, respectively.

2.1.3 Density

Attia *et al.* (1994) reported density of dry and cooked chickpea seeds ranged between 1.284 to 1.305 g/ml and 1.098 to 1.15 g/ml, respectively. The average density of 18 cultivars of cowpea was reported to be 1.21 g/cm³ (Akinyele *et al.*, 1986).

2.2 Chemical composition

2.2.1 Proximate composition

2.2.1.1 Moisture

Meshram *et al.* (1980) reported that the moisture content of moth beans was 9.5 whereas a value of 10.50% was reported by Pawar and Ingle (1938). Emefu *et al.* (1992) recorded moisture content of cowpea flour was 11.5 per cent while Giami (1993) reported that the moisture in cowpea flour was 9.2 per cent. Yanez *et al.* (1995) reported moisture content in raw cultivars of *Phaseolus vulgaris* ranged from 14.0 to 15.4 per cent. Umoren *et al.* (1997) reported that moisture content of cowpea varieties ranged between 8.70 to 9.25 per cent. Samman *et al.* (1999) investigated 8 varieties of beans and found that moisture ranged from 12-14 per cent. Abu *et al.* (1999) reported that the moisture content of cowpea was 102.1 g/kg. Uwaegbute *et al.* (2000) reported moisture content in cowpea flour as 15.6 per cent. Aparana *et al.* (2000) reported the moisture content of blackgram, field bean, lentil and moth bean to be 10.20, 10.15, 10.05 and 8.40 per cent, respectively.

2.2.1.2 Proteins

Meshram (1980) reported that protein content of moth bean flour was 28.37 per cent whereas that reported by Pawar and Ingle (1988a) was 23.5 per cent. Akinlosotu and Akinyele (1991) reported that protein in cowpea flour was 25.80 per cent and Bakr and Gawish (1991) reported protein content in cowpea as 28.56 per cent. Giami (1993) reported that protein content of cowpea flour was 24.0 per cent. Barampama and Simard (1995) reported protein content of *Phaseolus vulgaris* cultivars to range from 21.8 to 26.9 per cent. Umoren *et al.* (1997) reported crude protein content of cowpea varieties to range between 22.31 to 25.70 per cent. Abu *et al.* (1999) found that the protein content of cowpea was 236.2 g/kg. Samman *et al.* (1999) investigated six varieties of beans and showed that protein varied between 18 to 22 per cent. Uwaegbute *et al.* (2000) reported that the protein content of cowpea flour was 24.2 per cent. Aparana *et al.* (2000) reported that the protein content in black gram, field bean lentil and moth bean was 21.25, 22.90, 22.50 and 25.42 per cent, respectively.

2.2.1.3 Crude fat

Crude fat content of moth bean was reported to be 1 per cent (Meshram, 1980) whereas Pawar and Ingle (1988a) reported that the crude fat in moth beans was 1.7 per cent. Giami (1993) reported that the ether extract of raw cowpea flour was 1.7 per cent. Barampama and Simard (1993) reported fat content in varieties of dry beans to range between 0.78 to 1.27 per cent. Siddhuraju *et al.* (1994) reported that the crude lipid in *V. aconitifolia* was 3.53 per cent. Singh *et al.* (1996) reported that the fat content in different varieties of rajmash ranged between 1.54 to 1.63 per

cent. Umoren *et al.* (1997) reported that the fat content of varieties of cowpea ranged between 1.80 to 2.14 per cent. Abu *et al.* (1999) reported that the ether extracts of cowpea was 14.5 g/kg. Samman *et al.* (1999) investigated six varieties of beans and showed that fat varied from 0.7-1.20 per cent. Uwaegbute *et al.* (2000) reported that the fat in cowpea flour was 3.5 per cent. Aparana *et al.* (2000) reported that the fat content in moth bean was 1.21 per cent.

2.2.1.4 Ash

Ash content of moth bean was reported to be 3.2 per cent (Meshram *et al.*, 1980) whereas Pawar and Ingle (1988a) reported that the ash content in moth bean flour was 3.7 per cent. Bakr and Gawish (1991) reported that the total ash in cowpea was 4.92 per cent whereas Giami (1993) reported that the ash content of cowpea flour was 3.1 per cent. Siddhuraju *et al.* (1994) reported that the ash content of *V. aconitifolia* was 2.83 per cent. Ash content of raw red kidney beans was reported to be 4.77 per cent (Williams *et al.*, 1994) whereas, Barampama and Simard (1995) reported that the ash content in common beans was 3.06 per cent. Umoren *et al.* (1997) reported that the ash content of varieties of cowpea was 3.30 to 3.95 per cent and Abu *et al.* (1999) reported that the ash content of cowpea was 32.0 g/kg. Samman *et al.* (1999) investigated six varieties of beans and showed that the ash varied between 3.46 to 5.00 per cent. Aparana *et al.* (2000) reported that the ash content of moth bean was 3.10 per cent. Uwaegbute *et al.* (2000) reported that the ash content was 1.3 per cent in cowpea flour.

2.2.1.5 Crude fibre

Meshram *et al.* (1980) reported that the crude fibre content of moth bean flour was 4.6 per cent. Rajaram and Janardhanan (1990) found that fibre content of *Vigna* species ranged between 4.08 to 6.90 per cent whereas, Tezoto and Sgarbieri (1990) reported that the crude fibre content of beans was 5.20 per cent. Siddhuraju *et al.* (1994) reported that the crude fibre content of *V. aconitifolia* was 4.47 per cent. Singh *et al.* (1996) found that the fibre content of common bean varied between 3.54 to 4.23 per cent. Umoren *et al.* (1997) found that fibre content of four varieties of cowpea varied between 2.88 to 3.75 per cent. Abu *et al.* (1999) reported that the crude fibre content of cowpea was 39.0 g/kg and Uwaegbute *et al.* (2000) reported that the fibre content in cowpea was 1.3 per cent.

2.2.2 Dietary fibre

Dietary fibre can be defined as those plant components that are resistant to hydrolysis by endogenous enzymes of human gastro intestinal tract. These components include cellulose, hemicellulose, pectins, gums and mucilages.

2.2.2.1 Neutral detergent fibre

NDF of cowpea varieties ranged between 47.2 to 58.3 per cent (Tamboliya *et al.*, 1975). Garcia, Lopez and Wyatt (1982) found NDF content was 5.8 per cent in raw beans. Vidal-Valverde and Frias (1991) found that the NDF content of raw chickpea, kidney bean and lentil was 12.2, 12.8 and 22.1 g per 100 g. Attia *et al.* (1994) reported that the NDF content in chickpea cultivars varied from 24.0 to 31.1 per cent. NDF content for raw kidney beans was to be 17.62 per cent (Williams

et al., 1994). Singh *et al.* (1996) reported NDF content of three varieties of rajmash to range between 3.54 to 4.23 per cent.

2.2.2.2 Acid Detergent fibre

The ADF content is a character that depends on seed size as well as on coat thickness since this constituent is found principally in the seed coat (Singh, 1984).

ADF content of cowpea varieties ranged between 35.9 to 49.4 per cent (Tamboliya *et al.*, 1975). Vidal Valverde and Frias (1991) found that the ADF content of raw chickpea, kidney bean and lentil was 4.96, 7.16 and 6.34 g per 100 g, respectively. ADF for raw red kidney beans was reported to as 7.18 per cent (Williams *et al.*, 1994) whereas, Singh *et al.* (1996) reported that the ADF content in three varieties of rajmash ranged between 26.60 to 32.86 per cent.

2.2.2.3 Hemicellulose

Vidal Valverde and Frias (1991) found the hemicellulose content of raw chickpea, kidney bean and lentil as 7.76, 5.63 and 15.74 g per 100 g on dry matter basis, respectively. Singh *et al.* (1996) reported hemicellulose in varieties of rajmash to be 8.01 to 10.31 per cent.

2.2.3 Non-protein nitrogen and true protein

Legumes contain a considerable proportion of NPN components, which means that crude protein value over estimate the protein content of the sample and would consequently affect the estimated protein intake in the diet. Kumar and Venkataraman (1975) reported that NPN and protein nitrogen on cowpea was 0.3 and 3.2 per cent, respectively.

Sgarbieri *et al.* (1979b) reported 3.4 to 4.9 per cent NPN in dry beans. Youssef *et al.* (1987) reported that the NPN and TP in faba beans was 0.5 and 28.6 per cent respectively. Kocchar *et al.* (1988) reported that the true protein of catalogued varieties of cowpea from International Institute for Tropical Agriculture ranged between 20.7 and 27.3 per cent. Souza *et al.* (1991) reported non-nitrogen extract in pigeon peas was 58.88 per cent. Siddhuraju *et al.* (1994) reported that the true protein in *V. aconitifolia* was 17.38 per cent.

2.2.4 Available carbohydrates

Food legumes are good sources of dietary carbohydrates. Most legumes contain 50 to 60 per cent carbohydrates (on a dry weight basis).

The carbohydrate content of moth bean flour was reported to be 53.33 per cent (Meshram *et al.*, 1980) whereas, Pawar and Ingle (1988a) reported that the carbohydrates in moth bean flour was 71.1 per cent. Chavan *et al.* (1989) reported reducing sugars and starch in cowpea as 0.2 and 40.4 per cent, respectively. Bakr and Gawish (1991) reported that the soluble carbohydrates in cowpea was 59.79 per cent. Barampama and Simard (1995) reported that the carbohydrates, soluble sugars and starch in common beans was 65.85, 7.82 and 43.80 per cent, respectively. Abu *et al.* (1999) reported that the carbohydrate content of cowpea was 56.41 per cent whereas, Uwaegbute *et al.* (2000) reported carbohydrates content in cowpea flour was 50.5 per cent.

2.2.5 Total calorific value

Akinlosotu and Akinyele (1991) reported that the energy content of cowpea was 428.5 Kcal/100 g whereas, Giami (1993) reported that the calorific value of raw

cowpea flour was 350.9 K cal/100 g. Siddhuraju *et al.* (1994) reported calorific value of *V. aconitifolia* as 388.45 K cal 100⁻¹ DM. Yanez *et al.* (1995) reported that the caloric value of raw cultivars of *Phaseolus vulgaris* ranged between 368 to 380 Kcal. Singh *et al.* (1996) reported the energy content of rajmash varieties to vary between 376.47 to 378.94 per 100 g dry weight.

2.2.6 Minerals

Minerals present in the human diet play a significant role as body building constituents. They are constituents of different enzymes, hormones and vitamins. Their presence in body fluid regulates the permeability of cell membranes, the osmotic pressure and water balance between intracellular and extra cellular compartments, the response of nerve to stimuli and maintenance of acid base equilibrium. Pulses are good sources of minerals constituting 3.0 to 4.5 per cent (Salunkhe *et al.*, 1982).

2.2.6.1 Calcium

Pawar and Ingle (1987) reported that the calcium content of moth beans was 2 mg/g. Akinlosotu and Akinyele (1991) reported that the calcium content of cowpea was 52.2 mg/100g. Barampama and Simard (1993) reported that the calcium content in dry beans was 55.2/100 g. Rincon *et al.* (1993) reported a value of 35 mg/100 g in mature dry cowpeas. Siddhuraju *et al.* (1994) reported a value of 356.15 mg/100g for calcium in *V. aconitifolia*. Singh *et al.* (1996) reported that the calcium in varieties of rajmash ranged between 265.0 to 273.0 mg/100 g.

2.2.6.2 Iron

Pawar and Ingle (1987) reported that the iron content of moth beans was 0.142 mg/g. Akinlosotu and Akinyele (1991) reported a value of 21.9 mg/100 g for iron in cowpea whereas Rincon *et al.* (1993) found iron value of 3.9 mg/100 g in mature dry cowpeas. A mean value of 7.73 mg/100 g for iron was reported by Barampama and Simard (1993) in dry beans. A value of 20.32/100 g for iron in *V. aconitifolia* was reported by Siddhuraju *et al.* (1994). Singh *et al.* (1996) reported that the iron content in varieties of rajmash ranged from 8.2 to 18.3 mg/100 g dry weight.

2.2.6.3 Zinc

Pawar and Ingle (1987) reported that the zinc content of moth bean was 52.4 mg/g. Akinlosotu and Akinyele (1991) reported that the zinc content of cowpea flour was 4.1 mg/100 g. A range of 2.7 to 2.9 mg/100 g of zinc was reported in cowpea (Farinu and Ingrao, 1991 and Rincon *et al.*, 1993). Siddhuraju *et al.* (1994) reported that the zinc content of *V. aconitifolia* was 7.49 mg/100 g. The zinc content of raw common beans was reported to be 2.85 mg/100 g (Barampama and Simard, 1995) whereas Singh *et al.* (1996) reported zinc content in varieties of dry beans to range between 3.7 to 5.5 mg/100 g dry weight.

2.2.6.4 Potassium

Potassium content of cowpea was reported as 1246 mg/100 g (Akinlosotu and Akinyele, 1991). Siddhuraju *et al.* (1994) reported that the potassium in *V. aconitifolia* was 1390.37 mg/100 g seed flour. Barampama and Simard (1995) reported that the potassium in the varieties of dry beans ranged from 442 to 631

mg/100 g whereas Singh *et al.* (1996) reported that the potassium content of three varieties of rajmash to vary between 1156.3 to 1187.5 mg/100 g ^{on} dry weight basis.

2.2.6.5 Copper

Siddhuraju *et al.* (1994) reported that the copper content of *V. aconitifolia* was 1.82 mg/100 g seed flour. Barampama and Simard (1995) reported that the copper content of common beans was 0.96 mg/100g whereas Singh *et al.* (1996) reported that the copper content of varieties of rajmash was between 1.3 to 1.7 mg/100 g ^{on} dry weight basis.

2.2.6.6 Sodium

Sodium content of *V. aconitifolia* was reported as 37.43 mg/100 g (Siddhuraju *et al.*, 1994). Barampama and Simard (1995) reported that the sodium content of common bean was 14.84 mg/100 g. Singh *et al.* (1996) reported that the sodium varied between 45.0 to 55.0 mg/100 g in varieties of rajmash.

2.2.6.7 Magnesium

Pawar and Ingle (1987) reported that the magnesium content of moth bean was 2.42 mg/g. Akinlosotu and Akinyele (1991) reported that the magnesium content of cowpea was 90.8 mg/100 g. Barampama and Simard (1993) reported magnesium content in varieties of dry beans ~~ranged~~ between 28.1 to 43.8 mg/100 g. Siddhuraju *et al.* (1994) reported that the magnesium content of *V. aconitifolia* was 259.89 mg/100g.

2.2.7 Phytic acid

In most of the legumes, phytin phosphorus accounts for about 80 per cent of the total phosphorus and is primarily present as a complex salt of minerals or

complexed with proteins. The phytates from complexes with multivalent cations such as Ca^{++} , Mg^{++} , Fe^{++} , Zn^{++} , Fe^{+++} which decreases their bioavailability. Moreover, phytates interact with proteins and form insoluble complexes many of which are unavailable to the human system under normal physiological conditions (Graf and Eaton, 1984).

Phytic acid content of dry bean cultivars varied between 0.54 to 2.93 per cent (Lolas and Markakis, 1975; Deshpande and Cheryna, 1983) whereas Tan *et al.* (1983) reported that winged beans contained 0.6 to 0.7 per cent phytate. Khokhar and Chauhan (1986) reported that the phytic acid content of four varieties of moth beans was 885, 866, 852 and 899 mg/100 g of beans whereas Pawar *et al.* (1986) have, however, reported a considerably lower value of 325 mg phytin phosphorus in raw moth beans. Bakr and Gawish (1991) have reported a phytic acid content of 320 mg/100 g in dry cowpea while Shinde *et al.* (1991) reported that the phytate phosphorus in three cultivars of cowpea was between 167 to 221 mg/100 g. Umoren *et al.* (1997) reported that the phytic acid content of cowpea varied from 2.55 to 5.26 mg/g.

2.3 Effect of pre-treatments on the nutrients content of legumes

The effect of pre-treatments before processing on nutritional quality of legumes is of great importance. Among the pre-treatments soaking, sprouting, cooking etc. are of great significance. Such processes not only improve the digestibility and palatability of legumes but also helps to remove deleterious effect of some of anti-nutritional constituents (Singh

2.3.1 Effect of soaking, sprouting and cooking on proximate composition

2.3.1.1 Moisture

In the year 1987 Padamshree *et al.* reported that the germinated, boiled and pressure cooked cowpea flour contained 8.0, 7.60 and 7.20 per cent moisture, respectively. Pawar and Ingle (1988b) found that soaked and cooked moth bean flour contained 11.40 and 13.9 per cent moisture, respectively. Giami (1993) reported that moisture content in germinated and heat treated cowpea flour was 11.00 and 10.30 per cent, respectively. Attia *et al.* (1994) reported that the moisture content in soaked and cooked chickpea cultivars to range between 9.50 to 11.41 per cent and 63.35 to 64.73 per cent, respectively. Yanez *et al.* (1995) reported that the moisture content of cooked beans cultivars ranged between 7.8 to 10.9 pr cent. In the year 1999 Bhama reported that the moisture content of sprouted and pressure cooked Bengal gram was 6.60 and 6.50 g/100 g. Uwaegbute *et al.* (2000) reported that the moisture content in 48 h germinated cowpea flour was 19.50 per cent.

2.3.1.2 Proteins

Pawar and Ingle (1987b) reported that protein content of moth bean soaked in distil water was 21.3 per cent as compared to 23.5 per cent of control. A decrease in protein by 11.9, 8.9, 6.9 and 6.8 per cent was reported by Srivastava and Bajpai (1981) in four varieties of pigeon pea on soaking whereas, an increase in protein was reported by King and Pushwastein (1987) on sprouting of legumes due to synthesis of new proteins. In 1987 Youssef *et al.* reported that protein content of germinated faba beans was 30.40 per cent. Padamashree *et al.* (1987) reported that the protein

content of germinated and pressure cooked cowpea flour was 26.60 and 20.67 per cent, respectively. A decrease in protein content after cooking was reported by Khalil *et al.* (1986a) in field pea, moth bean and pigeon pea. The decrease was 19.9, 10.2 and 8.2 per cent respectively. Pawar and Ingle (1988b) found an increase in protein content by 6 per cent during four days germination of moth bean and in 30 minutes cooked moth bean flour as 19.4 per cent. Akinyele and Akinlosotu (1991) found that protein content in soaked and germinated cowpea was 27.26 and 26.60 per cent respectively. Giami (1993) reported, that crude protein in germinated and cooked cowpea flour was 24.9 and 22.7 per cent, respectively. Attia *et al.* (1994) found that crude protein in cooked chickpea cultivars ranged between 20.3 to 23.0 per cent. Barampama and Simard (1995) reported protein content in soaked and cooked common bean was 20.80 and 20.18 per cent, respectively. Uwaegbute *et al.* (2000) reported protein content in 24 h germinated cowpea flour was 25.0 per cent.

2.2.1.3 Ash

Koehler and Burke (1981) reported that ash content of cooked dry bean cultivars ranged between 3.5 to 3.9 per cent. Padamashree *et al.* (1987) reported that ash content in cooked and germinated cowpea flour was 1.96 and 1.52 per cent, respectively. Youssef *et al.* (1987) reported that ash content of soaked and germinated faba bean was 3 and 3.7 per cent, respectively. Pawar and Ingle in (1988b) found that ash content in soaked and cooked moth bean was 3.28 and 3.1 per cent, respectively. Whereas, Abbey and Ibeh (1988) observed no significant difference in the ash content of heated cowpea when compared with that of raw cowpea flour. A value of 8.87 per cent for ash was reported in soaked, cooked

cowpea (Bakr and Gawish, 1991). Giami (1993) reported that the ash content in germinated and cooked cowpea flour was 3.2 per cent, respectively. Attia *et al.* (1994) reported ash content in cooked chickpea cultivars between 1.73 to 2.35 per cent. Barampama and Simard (1995) reported ash content of soaked and cooked common beans as 2.33 and 2.70 per cent, respectively. Bhama (1999) reported ash content of sprouted and pressure cooked Bengal gram as 5.50 and 5.56 g/100 g respectively. Uwaegbute *et al.* (2000) reported ash content of germinated cowpea flour was 8.0 per cent.

2.3.1.4 Fat

Khalil *et al.* (1986) reported the fat content after cooking to be decreased by 0.7, 0.5 and 0.5 per cent for field peas, moth bean and pigeon pea, respectively. Youssef *et al.* (1987) reported fat content of soaked and germinated faba beans was 1.35 and 1.26 per cent, respectively. Pawar and Ingle (1988) reported that the fat content of soaked and cooked moth bean was 1.60 and 1.10 per cent, respectively. Abbey and Ibeh (1988) reported the ether extract in heated cowpea flour as 2.3 per cent. Fat content of germinated and cooked cowpea flour was reported to be 1.52 and 0.50 per cent, respectively (Padamashree *et al.*, 1987). Giami (1993) reported fat in germinated and cooked cowpea flour was 1.4 and 1.7 per cent, respectively. Attia *et al.* (1994) reported that fat content of cooked chickpea cultivars ranged between 6.40 to 6.72 per cent. Total fat in cooked red kidney beans was reported to be 2.31 per cent as compared to 2.18 per cent of raw (Williams, *et al.*, 1994). Barmapama and Simard (1995) reported fat content of soaked and cooked common beans was

1.49 and 1.50 per cent respectively. Uwaegbute *et al.* (2000) reported fat content of germinated cowpea was 4.0 per cent.

2.3.1.5 Fibre

Youssef *et al.* (1987) reported crude fibre in soaked and sprouted faba beans was 1.00 and 1.00 per cent, respectively. Abbey and Ibeh (1988) found the fibre content in heated cowpea flour as 1.9 per cent. A value of 7.62 per cent for soaked and then cooked cowpea was reported by Bakr and Gawish (1991). Giami (1993) reported fibre content of cooked cowpea flour was 2.3 per cent and germinated cowpea flour as 2.2 per cent. Barampama and Simard (1995) reported fibre content for soaked and cooked faba beans was 3.87 and 3.91 per cent, respectively. Veena *et al.* (1995) studied effect of processing on the dietary fibre content and found it ranged from 23.2 to 26.6 g/100 g in raw and 16.0 to 31.5 g/100 g in processed legumes. Uwaegbute *et al.* (2000) reported fibre content in germinated cowpea flour as 1.5 per cent.

2.3.2 Dietary fibre constituents

All processing treatments significantly decrease the soluble dietary fibre content and increase the insoluble dietary fibre content. Chitra *et al.* (1996) reported that germination and fermentation decreased the total dietary fibre in all legumes.

2.3.2.1 Neutral Detergent Fibre (NDF)

Vidal Valverde and Frias (1991) found that the NDF content of open and pressure cooked kidney bean was 17.1 and 15.1 per 100 g as compared to 12.8 per 100 g in raw respectively.

Vidal Valverde and Frias (1992) reported that the NDF content decreased to 21.12 g/100 g on soaking followed by open pan cooking as compared to 21.3 g/100 g in raw lentil. Soaking alone did not significantly alter NDF while, soaking and cooking brought a considerable decrease in NDF content. Attia *et al.* (1994) reported NDF content in cooked chickpea ranged from 12.08-16.80 per cent.

Kaur (1997) found NDF content in raw, sprouted (24 h) and sprouted (48 h) rice bean as 14.5, 18.4 and 18.5 g/100 g, respectively. The NDF content of soaked pressure cooked and open pan cooked legumes were significantly higher than the soaked legumes. Increase on cooking was probably due to formation of new components such as maillard products formed from condensation of protein and tannin which may be isolated as fibre components (Vidal Valverde and Frias, 1991).

2.3.2.2 Acid Detergent Fibre (ADF)

The acid detergent fibres are known to increase on cooking (Johnston and Oliver, 1982; Herranz *et al.*, 1983).

Vidal Valverde and Frias (1991) found that the ADF content of open pan and pressure cooked kidney bean was 12.97 and 14.35 g/100 g as compared to 7.16 g/100g in raw. Vidal Valverde and Frias (1992) found that ADF content increased to 7.18 g/100 g on soaking and 10.37 g/100 g on soaking followed by cooking as compared to 6.7 g/100g in raw lentil.

Attia *et al.* (1994) reported ADF content in cooked chickpea ranged from 6.21 to 8.52 per cent.

2.3.2.3 Hemicellulose

The hemicellulose content of chickpeas after normal and pressure cooking was determined. It increased significantly with processing, only with normal cooking, in contrast the hemicellulose content of kidney beans decreased significantly with both types of cooking. The net effect of the cooking process was a significant decrease in hemicellulose (36-42% of the raw value). The hemicellulose content kidney bean was 4.08 and 0.61 g per 100 g as compared 5.65 g/100 g in raw (Vidal Valverde and Frias, 1991).

According to Vidal Valverde and Frias (1992) the hemicellulose content decreased to 14.05 g/100 g on soaking and 5.69 g per 100 g on soaking followed by cooking as compared to 14.49g/100 g in raw lentil.

2.3.3. Non-protein nitrogen

Sgarbieri *et al.* (1979a) reported 3.4 to 4.9 per cent non protein nitrogen in dry beans. Gupta (1982) reported wide variability in the content of non-protein nitrogen among different pulses. A decrease in the non-protein nitrogen after 15 hours were reported by Shrivastava and Kooner (1972). In contrast ^{to} others Chen and Thacker (1978) found increase in the non-protein nitrogen after five days of germination. Maturity of the seed is found to affect this constituent. True protein nitrogen was the major form at the mature stage of seed development (Gupta, 1982).

2.3.4 Available carbohydrates

Jood *et al.* (1986) reported that cooking decreased the concentration of sugars and starch of soaked seeds. Contrary to these observations, Kataria and

Chauhan (1988), Kataria *et al.* (1990), Youssef *et al.* (1987) reported that cooking brought about a significant increase in total soluble sugars, reducing and non-reducing sugars. These differences in the two cases could be explained mainly on the basis of the fact that in latter studies, the cooking water was not discarded whereas reported by Jood *et al.* (1986) the soaking and cooking water was rejected and seeds alone after drying were analyzed for various carbohydrate components. Pawar and Ingle (1988) reported carbohydrates in soaked and cooked cowpea as 58.51 per cent. Batra and Dhinsa (1989) reported the sucrose content of soaked and germinated lentil as 1.81 and 1.14 g/100g as compared to 1.71^{g/100g} of control.

Bakr and Gawish (1991) reported soluble carbohydrates in soaked and cooked cowpea as 58.51 per cent. Vidal Valverde *et al.* (1992) reported that cooking chickpea, kidney bean and lentils substantially decreased the total sugars content, but in the case of lentils, the decrease was less pronounced as the cooking water was not decanted before analysis. Pressure cooking of soaked seeds increased the total soluble sugars by 60 to 9.0 per cent, reducing sugars by 10.0 per cent and starch content decreased by 18.0 to 34.0 per cent.

2.3.5 Total calorific value

Akinyele and Akinlosotu (1991) reported that the energy content of soaked cowpea was 436 Kcal, whereas Giami in 1993 reported that the energy content of germinated and heat treated cowpea flour was 341.4 and 345.3 Kcal, respectively.

2.3.6 Minerals

Jood and Kapoor (1997) reported that increase in the extractability of minerals was the highest after cooking the sprouts, followed by sprouting,

autoclaving and ordinary cooking of soaked seeds. However the availability of minerals from soaked seeds, in the absence of further treatment was similar to the availability in unprocessed (raw) seed. According to El-Tinay *et al.* (1989) soaking and cooking treatments decreased mineral contents and reduced phytate content for most of the legume cultivars.

2.3.6.1 Calcium

Rahman *et al.* (1990) observed that during cooking of kidney bean, retention was almost 100 per cent for calcium, zinc and manganese. For sodium potassium, phosphorus, magnesium, iron and copper retention values were lower, but losses were not nutritionally important. The losses occurring during cooking may be because cooking involves a complex reaction system. Thereby Ca^{++} which is concentrated in the parenchymal cells migrate into the irons. Further, the permeability of cell wall is altered by the presence of Ca^{++} and Mg^{++} ion. Hence, more soluble calcium salt might be formed during these changes which get leached out (Kumar *et al.* 1978). Giri *et al.* (1981) reported the calcium content in green gram horse gram and red gram for different germination period and found the values ranging between 103.3 to 121.10 , 111.6 to 227.1 and 74.1 to 79.2 mg/100 g respectively. Augustin *et al.* (1981) reported calcium in cooked *P. vulgaris* varieties ranged from 0.10-0.20 mg. Pawar and Ingle (1987a) reported that the calcium content in soaked moth bean was 1.82 mg/g and that in cooked moth bean was 1.88 mg/g. Youssef *et al.* (1987) reported calcium content in soaked faba bean was 40.9/100 g and that in germinated faba bean was 63.4 mg/100 g. Barampama and Simard (1995) reported that the calcium content in soaked common beans was

101.25 mg/100 g and that in cooked common bean was 107.50 mg/100 g. Duhan *et al.* (2001) reported that the calcium content in 12 h soaked pigeon pea was 195.1 mg/100 gm.

2.3.6.2 Iron

Giri *et al.* (1981) reported, the iron content in green gram, horse gram and red gram for different germination periods and found that value ranged between 4.96 to 5.03, 8.96 to 9.00 and 4.37 to 4.38, respectively. Koehler and Burke (1981) reported that the iron content of cooked bean cultivars ranged from 6.8 to 7.5 mg/100 g. Pressure cooking reduced iron content which may have been due to leaching of minerals in cooking water. Augustin *et al.* (1981) reported that iron content in cooked *P. vulgaris* as 5.84 mg.

Rao and Deosthale (1983) reported that overnight soaking resulted in 20 to 40 per cent losses in iron while germination did not affect the mineral composition of pulses. Pawar and Ingle (1987a) reported that iron content of soaked and cooked moth bean was 0.137 and 0.139 mg/g, respectively. Youssef *et al.* (1987) reported that the iron content in soaked faba beans was 7.7 mg/100g. Akinyele and Akinlosotu (1991) reported that iron content in soaked cowpea as 15.0 mg/100 g. Rani and Hira reported (1993) that the iron content in pressure cooked beans reduced which may have been due to leaching of minerals in cooking water. The iron content in heat treated cowpea flour was reported as 6.2 mg/100 g (Giami, 1993) and that in germinated cowpea flour was 7.0 mg/100 g.

Barampama and Simard (1995) reported that the iron content in soaked common bean was 6.45 mg/100 g and that in cooked and soaked-cooked common

bean as 6.60, 5.80 mg/100g, respectively. Duhan *et al.* (2001) reported that the iron content in germinated pigeon pea was 10.35 mg/100g and in 12 h soaked pigeon pea was 10.47 mg/100 g and that of pressure cooked pigeon pea was 10.35 mg/100g.

2.3.6.3 Zinc

Augustin *et al.* (1981) reported that the mean value for zinc in cooked *Phaseolus vulgaris* was 2.0 mg per cent. Pawar and Ingle (1981) reported that the zinc content in soaked and cooked moth bean was 49.3 and 50.5 mg/1g respectively. Youssef *et al.* (1987) reported zinc content in soaked faba beans was 2.9 mg/g and that in germinated faba bean was 1.6 mg/100g.

Akinlosotu and Akinyele (1991) reported that the zinc content in cowpea germinated for different time ranged from 41-4.5 mg/100 g. Barampama and Simard (1995) reported that the zinc content in soaked common beans was 285 mg/100 g and that in cooked and soaked cooked common bean as 2.85 and 2.93 mg/100 g.

2.3.6.4 Potassium

Koehler and Burke (1981) reported that potassium content of cooked dry bean cultivars ranged from 1017-1077 mg/100 g. Augustin *et al.* (1981) reported the average content in varieties of cooked *P. vulgaris* was 1.29 mg per cent.

Youssef *et al.* (1987) reported that potassium in soaked faba beans was 1161 mg/100g and that in germinated beans was 1848 mg/100 g. Akinlosotu and Akinyele (1991) reported potassium in 48 h germinated cowpea flour was 1218 mg/100g.

Barampama and Simard (1995) reported that potassium content in soaked and soaked cooked common beans was 932.91 and 831.25 mg/100g and that in cooked common bean was 1035 mg/100g.

2.3.6.5 Copper

Augustin *et al.* (1981) reported that the average copper content in cooked varieties of *P. vulgaris* as 0.71 mg per cent. Youssef *et al.* (1987) reported that the copper content of soaked faba beans was 3.3 mg/100 g and that in germinated beans was 3.4 mg/100 g. Barampama and Simard (1995) reported that the copper content of soaked and soaked-cooked common beans was 106 and 1.01 mg/100 g, respectively and that in cooked common bean was 1.00/100g.

2.3.6.6 Sodium

Augustin *et al.* (1981) reported that the average sodium content in cooked varieties of *P. vulgaris* was 3.4 mg per cent. Youssef *et al.* (1987) reported that the sodium content in germinated faba beans was 243 mg/100 g and that in soaked faba beans was 226 mg/100 g.

2.3.6.6 Magnesium

Augustin *et al.* (1981) reported that the average magnesium content in cooked varieties of *P. vulgaris* was 0.17 mg per cent. Youssef *et al.* (1987) reported that the magnesium content in germinated faba bean was 172 mg/100g and that in soaked faba beans was 143 g/100g. Akinlosotu and Akinyele (1991) reported that the magnesium content in cowpea for different germination periods ranged between 90.5 to 94.0 mg/100 g.

Barampama and Simard (1995) reported that the magnesium content in soaked and soaked cooked common beans was 162.91 to 195.00 mg/100 g and that in cooked common beans was 165.00 mg/100 g respectively.

2.3.7 Phytic acid

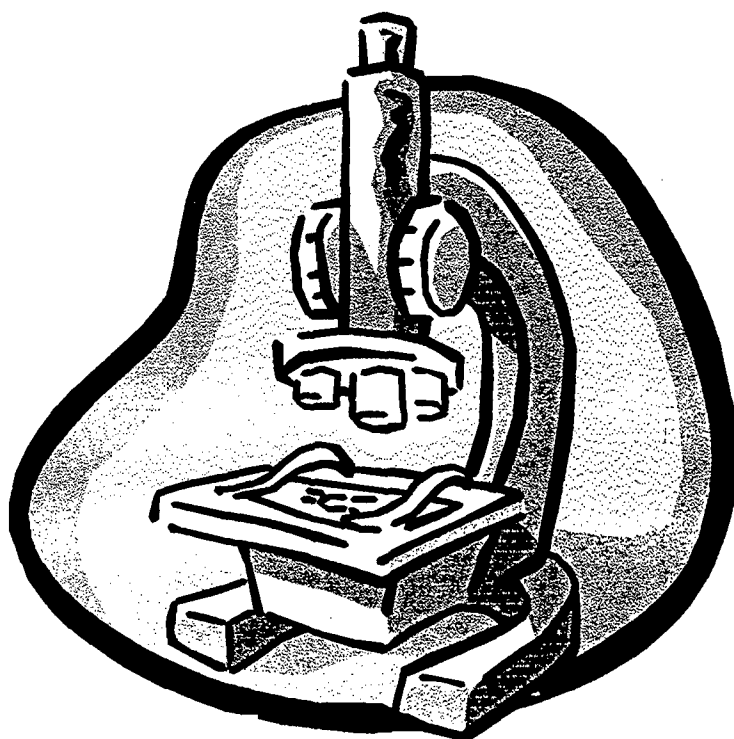
Tabekhia and Luh (1980) observed that soaking of dry beans in water for 12 h at 24°C resulted in decreased phytate content. Sprouting of moth bean varieties for 60 h at 25°C lowered phytic acid content varying between 48 to 53 per cent (Khokhar, 1984). Verma and Mehta (1988) found that on sprouting the phytin phosphorus decreased by 9.8 per cent as compared to whole ungerminated beans. Bakr and Gawish (1991) reported that the phytic acid in soaked and then cooked cowpea was 280 mg/100 g whereas Shinde *et al.* (1991) reported that the phytate phosphorus content of cooked cowpea cultivars ranged between 151 to 207 mg/100g. Attia *et al.* (1994) reported that phytic acid ranged from 0.38 to 0.76 per cent in cooked chickpea cultivars.

2.4 Biological studies

Chandershakher and Jayalakshami (1978) studied the protein quality of sprouted, roasted and autoclaved legumes on albino rats and found that processing of legumes especially heat processing has significantly beneficial effect in improving the protein quality. Roasted legumes promoted higher weight gains, gave higher PER values, resulted in better nitrogen retention and registered higher biological value. The next best method of processing legumes seems to be autoclaving or

pressure cooking a common method of legume cookery at home. They further observed that net protein utilization was improved from 44 per cent to 58 per cent in weanling rats for raw beans after soaking.

Previous studies have shown a decrease in some nutrients because they had discarded the water used for soaking, sprouting and cooking. Some studies have shown an increase in different nutrients. No research report is available on the affect of cooking, soaking and sprouting of cowpea, kidney beans and moth beans on the nutritional quality.



***MATERIAL
AND
METHODS***

CHAPTER-III

MATERIAL AND METHODS

An appropriate selection and application of scientific methodology is a pre-requisite for conducting research as it adds to clarity, precision, reliability and validity of the findings relating to a research problem. This chapter, therefore, elucidates in detail the procurement and preparation of samples, treatment of samples, physical parameters, proximate principles, nutritional analysis, animal experiment and statistical analysis that have been employed to achieve the specific objectives of the present study. For precision and brevity, this chapter has been discussed under the following sub-heads:

3.1 Procurement of materials

3.2 Preparation of samples

3.3 Physical parameters

3.4 Treatment of samples

3.5 Proximate principles

3.6 Nutritional analysis

3.7 Animal experiment

3.8 Statistical analysis

3.1 Procurement of material

Moth beans (*Vigna aconitifolia*), Cowpea (*Vigna unguiculata*) and Kidney beans (*Phaseolus vulgaris*) were procured from the local market of Palampur, Himachal Pradesh.

3.2 Preparation of samples

The legumes were cleaned manually to get rid of dust and other foreign materials.

3.3 Treatment of samples

Each legume was divided into four lots (Fig. 1). Following treatments were given to the individual lot :

3.3.1 Soaking

The legume grains were steeped in potable tap water for 12 h at $25\pm 2^{\circ}\text{C}$ in 10 litre capacity plastic containers (buckets). Grain to water ratio was 1 : 3. The containers were then covered with filter paper sheets (Whatman No.44). After 12 h, grains were dried in a cabinet tray drier at $50\pm 3^{\circ}\text{C}$ for 12 h.

3.3.2 Sprouting

The soaked legume grains as in 3.4.1 were kept in trays lined with wet sand beds of 2 cm thickness, covered over with wet filter paper sheets. The grains on the sand beds were also covered with filter paper sheet and allowed to germinate at ambient temperature ($25\pm 2^{\circ}\text{C}$ and 36% RH). Grains took 72 h to germinate, daily

regular sprinkling of the water at equal interval was carried out with the help of hand sprayer to keep the grains moist. When the sprouts were 1 to 2 cm long germinated grains were dried in cabinet tray drier at $50\pm 3^{\circ}\text{C}$ for 12 h.

3.3.3 Pressure cooking

The soaked legume samples as in 3.4.1 were pressure cooked with equal amount of water for 45 minutes for kidney beans, 35 minutes for cowpea and 25 minutes for moth beans in the pressure cooker. The pressure cooked samples were then dried in cabinet tray drier at $50^{\circ}\text{C}\pm 3^{\circ}\text{C}$ for 12h.

The dried treated and untreated samples of all three legumes were milled separately in Willey Mill, to a fine powder so as to pass through a 40 mesh sieve. Then these treated as well as untreated samples were kept in refrigerator in air tight plastic containers till further analysis.

3.4 Physical parameters

3.4.1 Weight

One thousand seeds in triplicate from each legume (both treated and untreated) were randomly selected and weighed on an electrical weighing balance.

3.4.2 Colour

Seeds of the individual legumes were observed visually for their colour and appearance.

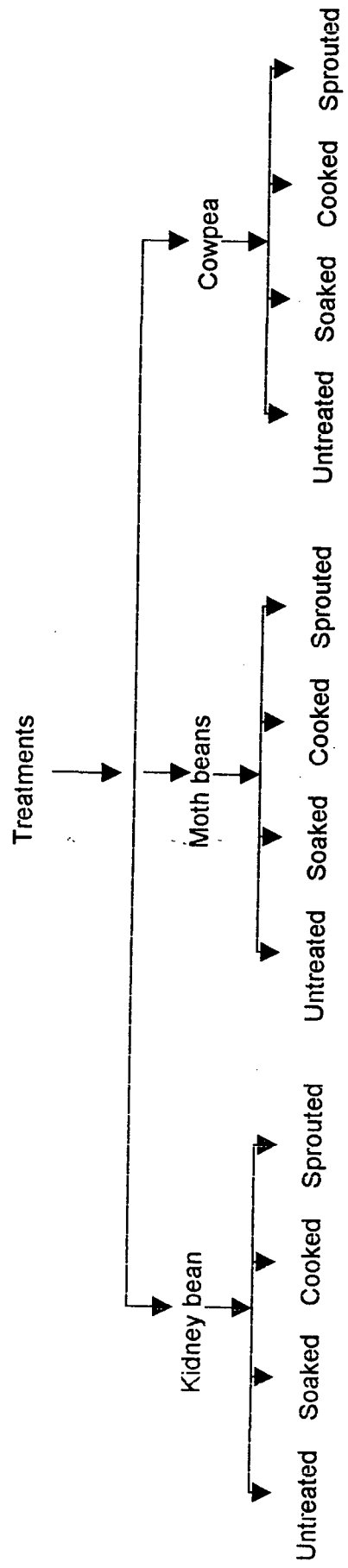


Fig. 1 Preparation of samples design

3.4.3 Seed size

Seed size included seed length and width. Fifty seeds in triplicate from untreated and treated samples were taken up randomly. Their length and width were measured using vernier calipers.

3.4.4 Density

One thousand seeds in triplicate from each legumes were weighed and put in a graduated cylinder containing known quantity of water and rise in water level was noted. Density was calculated by the following formula:

$$\text{Density} = \frac{W \text{ (g)}}{V \text{ (ml)}}$$

Where,

W = Weight of 1000 seeds (g)

V = Rise in water level (ml)

3.4.5 Shape

The seeds of the legumes were observed for their shape.

3.5 Proximate principles

Moisture, Ash, Crude fat and Crude fibre contents were analysed AOAC (1990) methods. Nitrogen was analysed by micro-kjeldhal method (1990) and multiplied by factor 6.25 for converting it into Crude proteins.

3.6 Nutritional Analysis

3.6.1 Non-Protein nitrogen

Non protein nitrogen was determined by the method of Becker *et al.* (1940).

Reagents

Trichloroacetic acid (10%) solution: Ten gram of trichloroacetic was dissolved in water and volume was made to 100 ml.

Procedure

Five hundred milligram sample in triplicate was extracted with 10 ml ice cold 10 per cent TCA and centrifuged at 10,000 rpm for 5 min. The precipitates were washed twice with 10% TCA. Supernatant was pooled and volume was made to 50 ml with 10 per cent TCA. 25 ml of aliquot was taken and digested for estimation of nitrogen. Nitrogen content was analysed by micro-kjeldhal method (AOAC, 1990).

3.6.2 True protein

True protein was calculated by the following formula :

$$\text{True protein} = (\text{Crude protein nitrogen} - \text{Non protein nitrogen}) \times 6.25$$

3.6.3 Energy

Energy in samples was determined by

Mayure (1962).

Reagents

1.5 N Potassium dichromate solution

Dissolved 73.54 g of potassium dichromate in water and volume was made to one litre.

0.15 N sodium thiosulphate

Dissolved 37.23 g of sodium thiosulphate in 100 ml of warm distilled water and volume was made to one litre.

Potassium iodide sodium bicarbonate solution

100 g potassium iodide (AR) and 32 g of sodium bicarbonate were dissolved in distilled water and diluted to 500 ml. These were prepared fresh.

Concentrated sulphuric acid

Procedure

One hundred twenty five mg sample was oxidized with 20 ml 1.5 N potassium dichromate solution and 40 ml of concentrated sulphuric acid for 90 minutes. The total volume was made to 250 ml with distilled water. To a 25 ml aliquot, 10 ml of potassium iodide sodium bicarbonate solution was added in dark and avoided the exposure to light for 25 minutes. The contents were diluted with 50 ml of water and iodine liberated was titrated against 0.15 N sodium thiosulphate using starch as indicator. End point was light green colour. The duplicate blanks were also run for each set. The amount of 1.5 N $K_2Cr_2O_7$ used for oxidizing the samples was calculated by subtracting the above reading from the blank. The energy value was calculated by following equation.

$$\text{Kcal/100g sample} = \frac{\text{ml of 1.5 N } K_2Cr_2O_7 \text{ used for oxidizing 1 g of sample}}{\text{oxidising coefficient}} \times 100$$

$$\text{Oxidising coefficient} = 23.39 - 0.069P + 0.00026 P^2$$

Where, P = true protein content of sample

3.6.4 Available Carbohydrates

Sugars (total, reducing and non-reducing) could not be extracted by method of Lane and Eynon^{n (1923)} as described by Ranganna (1995). Therefore other methods for total sugars and reducing sugars were adopted which are given below:

Total sugars other than starch were extracted according to procedure by Cerning and Guilbot (1973).

3.6.4.1 Reagents

Ethanol 80 per cent

Procedure

Extraction

Twenty five ml ethanol (80%) was added to 500 mg sample in a round bottom flask. The flask was connected to condenser and kept on a heating mantel for 30 minutes with occasional stirring. The extract was cooled centrifuged at 8000 rpm for 15 minutes and the supernatant was collected. The above procedure was repeated twice, each time extracting the residue in 25 ml ^{of} 80 per cent ethanol. The combined extract was evaporated to dryness in a beaker on a boiling water bath. The residue was dissolved in distilled water and made to 50 ml.

Reagents

Standard sugar solution

Dissolved 25 mg glucose in water and made to 100 ml. This solution contained 250 µg glucose per ml. For obtaining the standard curve, 0.1 ml to 1.0 ml of this solution was used.

Anthrone reagent (0.2% anthrone in 70 % sulphuric acid) :

This reagent was prepared fresh daily and allowed to stand for 30-40 min before use.

Procedure

Estimation

Freshly prepared 10 ml anthrone reagent was pipetted into a test tube (125 x 25 mm) chilled and kept in ice cold water. One ml of the sugar extract was taken and diluted to 10 ml with water. Out of the diluted extract one ml was taken and layered on the anthrone reagent. After cooling for 3-5 minutes the content were thoroughly mixed while still immersed in ice cold water. The contents in the tube were heated vigorously in a boiling water bath for 10 minutes and then immediately cooled in cold water. The absorbance was then read at 526 nm against a suitable blank.

3.6.4.3 Reducing sugars

Reducing sugars were estimated by Somogy's modified method (Nelson, 1944, Somogy, 1945).

Reagents

Copper reagent A

Dissolved 25 g anhydrous sodium carbonate, 25 g potassium sodium tartarate, 20 g sodium bicarbonate and 200 g anhydrous sodium sulphate in about 800 ml distilled water and diluted to one litre.

Copper reagent B

Disolved 15 g CuSO_4 in 100 ml distilled water containing two drops of HCl.

Arsenomolybdate reagent

Dissolved 25 g ammonium molybdate in 450 ml distilled water and 21 ml concentrated sulphuric acid with stirring, later 3 g sodium hydrogen arsenate in 25 ml distilled water was also mixed by stirring. The solution was kept in an incubation at 37°C for 24 hours before use. The reagent was stored in glass stoppered brown bottle in a refrigerator.

Mixed copper reagent

Copper reagents A and B were mixed in the ratio of 25 : 1 (v/v) before use.

Standard sugar solution

Dissolve 25 mg glucose and made to 100 ml with distilled water. This contained 250 µg glucose/ml.

Procedure

One ml of test extract obtained in 3.6.4.1 was taken in a blood sugar tube graduated at 25 ml. One ml mixed copper reagent was added in to it and then heated for 20 minutes in a boiling water bath. To this one ml arsenomolybdate reagent was added, thoroughly mixed and the contents were diluted to 25 ml. A

stable blue colour appeared quickly which was read at 520 nm against the suitable blank. The amount of reducing sugar was then determined by referring it to the glucose standard curve.

3.6.4.4 Non reducing sugars

The amount of non-reducing sugar was calculated by subtracting reducing sugars from the total soluble.

3.6.4.5 Starch

Starch from the sugar free pellet obtained after centrifugation in 3.5.4.1 was estimated by the method of Clegg (1956).

Procedure

Extraction

Added 5 ml of water to the aforesaid residue in test material and while stirring added 6.5 ml of 53 per cent perchloric acid. Stirred the content vigorously for 5 min and the occasionally for next 15 min. Added 20 ml water and centrifuged. Collected the supernatant in 100 ml volumetric flask. Added 5 ml water to the residue and repeated the extraction with 53 per cent perchloric acid, stirring occasionally for next 30 min. washed the contents of the tube into a volumetric flask. The test extract was made to 100 ml with water. It was then filtered, discarding first 5 ml of filtrate. A suitable aliquot of extract was used for glucose estimation using anthrone reagent by the method of Yemn and Willis (1954) as described under 3.5.4.2. Starch was calculated using the following formula :

$$\text{Starch} = \text{Glucose} \times 0.9$$

3.6.5 Neutral detergent Fibre, Acid Detergent Fibre and Hemicellulose

NDF and ADF were determined by the method of Van Soest and Wine (1967).

3.6.5.1 Neutral Detergent Fibre

Reagents

Neutral detergent solution

Distilled water	1 litre
Sodium lauryl sulphate	30.00 g
Disodium ethylene diamino tetra acetate (EDTA)	18.61 g
Sodium borate decahydrate	6.18 g
Di sodium hydrogen phosphate	5.00 g
2-Ethoxy ethanol	10 ml

EDTA and sodium borate decahydrate were weighed together in a large beaker. Some water was added and the solution was heated till the contents dissolved. Then it was added to the solution containing sodium lauryl sulphate. In a separate beaker hydrogen phosphate was weighed and dissolved by heating in the remaining water and was added to the beaker containing other ingredients. Adjusted pH between 6.9 to 7.1 per cent.

Decahydronaphthalen (AR)

Acetone (AR)

Sodium sulphate, anhydrous (AR)

Procedure

Five hundred mg dried sample (in triplicate) was weighed into a beaker of the refluxing apparatus. One hundred ml neutral detergent solution, 2ml decahydronaphthalen and 0.5 g, sodium sulphate were added to it. Then it was heated to boiling. Heat was reduced as boiling began to avoid foaming and allowed to reflux for 60 minutes. It was filtered through a weighed Gooch crucible with the minimum of hot water. Liquid was filtered and washing procedure repeated. Then the washing was done with acetone in the same manner and dried. The crucible was dried in hot air oven at 100°C for 8 hours and weighed after cooling. NDF was calculated using the following formula:

$$\text{NDF (\%)} = \frac{(\text{Weight of crucible + fibre content}) - \text{Weight of crucible}}{\text{Weight of sample}} \times 100$$

3.6.5.2 Acid detergent fibre

Reagents

Acid detergent solution

1 N sulphuric acid solution

Cety trimethyl ammonium bromide (CTAB)

Added twenty g of CTAB to 1 N sulphuric acid and made the volume to one litre

Decahydronaphthalen

Acetone

N-hexane

Procedure

Five hundred mg dried sample (in triplicate) was weighed in a beaker of the refluxing apparatus. 100 ml of acid detergent solution and 2 ml of decahydronaphthalen were added to it. The mixture was heated to boiling and refluxed for 60 minutes. Filtered it through a weighed Gooch crucible on filter manifold. Rinsed the sample into crucible with minimum of hot water (90 to 100°C). Finally the residue was washed twice with acetone in the same manner till colourless. All lumps were broken so that solvent came into contact with all particles of the fibre. The acid detergent fibre was dried at 100°C for 8 hours in hot air oven and weighed.

Calculations

$$\text{ADF (\%)} = \frac{(\text{Weight of crucible + fibre content}) - \text{Weight of crucible}}{\text{Weight of sample}} \times 100$$

3.6.5.3 Hemicellulose

Hemicellulose was determined as the difference between NDF and ADF.

$$\text{Hemicellulose} = \text{NDF} - \text{ADF}$$

3.6.6 Minerals

3.6.6.1 Digestion

One gram ground sample was taken in a 150 ml conical flask. To this 25-30 ml of di acid mixture (HNO_3 : HClO_4 in 5 : 1 v/v) was added and kept overnight. Digestion was done next day by heating till clear white precipitate settled down at the bottom. The crystal were dissolved by diluting in double distilled water. The contents were filtered through Whatman No.42 filter paper. The filtrate was made to

50 ml with double distilled water and used for determination of zinc, iron, copper and magnesium with help of atomic absorption spectrophotometer, model 3100, Perkin Elmer. Calcium, Sodium and potassium were determined with the help of flame photometer, Mediflame, 127.

3.6.6.2 Phosphorus

Phosphorus was determined colorimetrically by the method of Chen *et al.* (1956).

Reagents

Ascorbic acid solution (10%)

Ammonium molybdate solution (2.5%)

10 N H₂SO₄

Reagent C

Mixed 6 N H₂SO₄, water, 2.5% ammonium molybdate solution and 10 per cent ascorbic acid solution in the ratio of 1 : 2 : 1 : 1 v/v, respectively. This reagent was prepared fresh every day.

Standard phosphorus solution

Dissolved 0.351 g pure and dry anhydrous mono potassium dihydrogen orthophosphate in a few ml of water and added 10 ml of 10 N H₂SO₄ solution. The volume was made to one litre with water. This stock solution contained 80 µg P/ml. Diluted stock solution to one litre which served as working standard solution. It contained 2 µg P/ml. Two or three drops of chloroform were added for preserving this solution.

Procedure

One ml of the mineral extract from 3.6.6.1 was diluted to 10 ml. From this, 1 ml was pipetted in a test tube and made volume to 4 ml with water. Added 4 ml reagent C and mixed well. Incubated the contents at 37°C in a water bath for 90 min. Removed and allowed to cool to room temperature and read absorbance at 820 nm against a suitable blank. Standard curve was plotted using 1 to 8 ml of standard phosphate 0.2 O.D. correspond to 2 µg phosphorus.

3.6.7 Anti-nutritional factors

3.6.7.1 Phytic acid

Phytic acid was determined by the method of Davis and Reid (1979).

Reagents

0.5 N HNO₃

15.96 ml of 69.5 per cent HNO₃ (AR grade sp. Gravity 1.4) was diluted in water and volume made to 500 ml.

Ferric ammonium sulphate solution

Two hundred sixteen milligram ferric ammonium sulphate was dissolved in water and made to 500 ml.

Ammonium thiocyanate 10 per cent solution

Ten gram ammonium thiocyanate was dissolved in water and made to 100 ml.

Isoamyl alcohol

Sodium phytate standard

30.54 mg sodium phytate (5.5%, H₂O, 97% purity and containing 12 Na/mol) was dissolved in 100 ml 0.5 N nitric acid which gave a solution containing 200 µg phytic acid per ml.

Standard curve

Different concentrations i.e. 0.2 to 1.0 ml of standard sodium phytate containing 40-200 µg phytic acid was taken and made to 1.4 ml with water.

Procedure**Extraction**

One gram sample was extracted with 25 ml of 0.5 N nitric acid for 3 hours with continuous shaking in a shaker. After proper shaking, it was filtered through Whatman No.1 filter paper.

Estimation

Pipetted 0.5 to 1 ml filtrate in a test tube and diluted with distilled water to a final volume of 1.4 ml. One ml ferric ammonium sulphate solution was added to it. The tubes were after proper shaking placed in a boiling water bath for 20 minutes. After cooling down to room temperature under running water 5 ml isoamyl alcohol was added. The tubes were shaken well and then centrifuged at 3000 rpm for 10 minutes.

Finally, the colour intensity was read at 456 nm against isoamyl alcohol blank exactly after 15 minutes of addition of ammonium thiocyanate. The extraction at 465 nm in isoamyl alcohol layer was inversely proportional to phytate concentration.

Calculations

$$\text{Phytic acid (mg/100 g)} = \frac{\text{Reading of graph} \times \text{ml of volume made}}{\text{Weight of sample taken} \times \text{ml of aliquot taken}} \times 100$$

3.6.7.2 Phytate phosphorus

Phytate phosphorus was calculated as

$$\text{Phytate phosphorus, mg} = \frac{A \times 28.18}{100}$$

Where A = the phytate content, mg

3.6.7.3 Non-phytate phosphorus

Non-phytate phosphorus was calculated as a difference between the total phosphorus and phytate phosphorus.

3.7 Animal experiments

Eighty Wistar weanling rats aged 28 days weighing 32 ± 5 g were obtained from the Germ-free Small Animal House of the College of Veterinary Sciences, CCS Haryana Agricultural University, Hisar. These were divided into ten groups of eight rats each. The mean weight was adjusted to be same for all the groups. One group was fed casein and was used as control. Rest groups were assigned to the three pulses with three groups of each legume. One of the groups assigned to each legume was fed untreated and served as legume for control the respective legume, the remaining groups were fed treated legume viz., sprouted and cooked

(Experimental design is given in Fig.2). The diets were isoproteinous containing 10 per cent protein. The rats were aged individually in polypropylene cages. Weighed amount of feed and water was given *ad libitum*. The composition of diets is given in Table 1. The ingredients were mixed, homogenized and passed through 70 mesh sieve to ensure uniform distribution of vitamin and salt mixture. The composition of vitamin and mineral mixture has been given in Table 2 and 3 as recommended by BARR (1972).

3.7.1 Feed efficiency ratio (FER) and protein efficiency ration (PER)

Feed intake, feed efficiency ratio (FER) and protein efficiency ratio (PER) were determined by the method of Chapman *et al.* (1959). Diet and water were provided *ad libitum* for 28 days. A weighed quantity of diet was given to rats and left over were collected and weighed after drying at 100°C daily. The body weight of rats was recorded. The amount of feed and protein consumed during this period

d. Feed efficiency ratio and protein efficiency ratio were worked out by
ula :

$$\frac{\text{Gain in weight (g)}}{\text{Feed consumed (g)}}$$
$$\frac{\text{Gain in weight (g)}}{\text{Protein consumed (g)}}$$

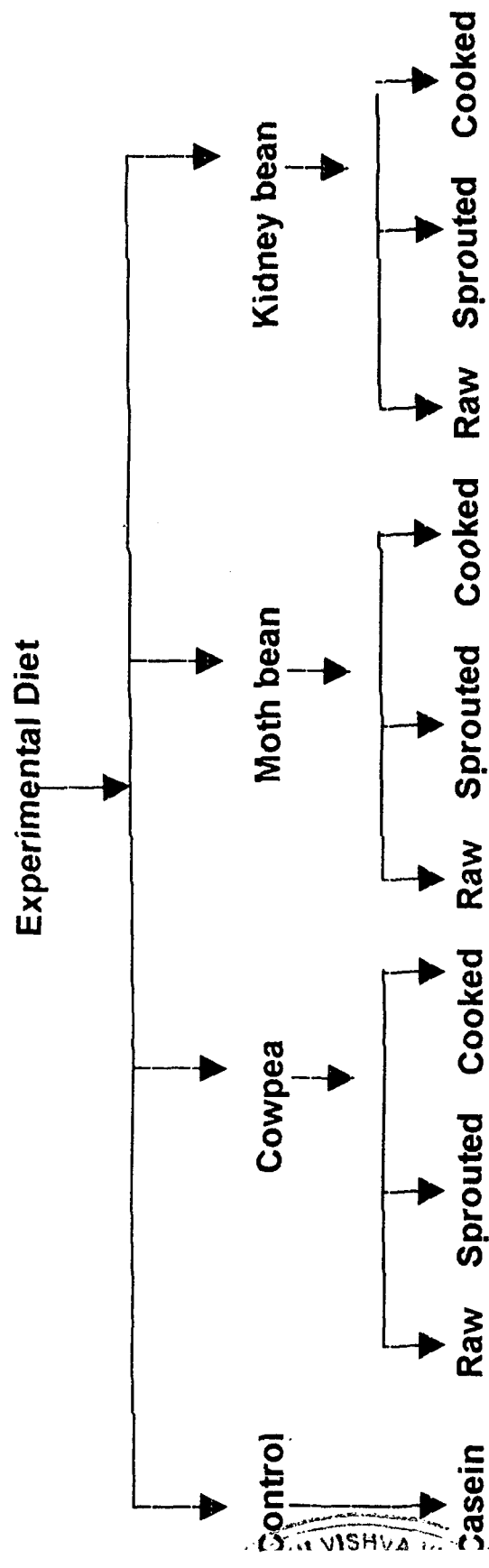


Fig. 2 Animal experiment design

Table 3.1 Composition of experimental diets (g/100 g)

Ingredients	Control diet	Legume diet									
		Kidney bean					Cowpea				
		Sprouted		Cooked		Raw	Sprouted		Cooked		Raw
		Raw	Sprouted	Sprouted	Cooked	Cooked	Sprouted	Sprouted	Cooked	Cooked	Raw
*Casein	13	-	-	-	-	-	-	-	-	-	-
**Groundnut oil	10	10	10	10	10	10	10	10	10	10	10
Sucrose	10	10	10	10	10	10	10	10	10	10	10
***Cellulose	5	5	5	5	5	5	5	5	5	5	5
Mineral mixture	4	4	4	4	4	4	4	4	4	4	4
Vitamin mixture	1	1	1	1	1	1	1	1	1	1	1
Choline chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Starch	56.8	23.75	24.28	23.38	23.38	25.75	26.22	25.50	23.51	24.28	24.28
Pulse	-	46.23	45.70	46.60	46.60	44.23	43.76	44.48	46.47	45.70	45.70

*All diets contained 10% protein including the crude protein from pulse source

**AI diets contained 10% fat including the crude fat from pulse source

*All diets contained 5% fibre including the crude fibre from pulse source

Table 3.2 Composition of mineral mixture (AIN 76A)*

Ingredients	G/kg mineral mixture
Calcium phosphate (dibasic)	500.00
Sodium chloride	74.00
Potassium citrate monohydrate	220.00
Potassium sulphate	52.00
Magnesium oxide	24.00
Ferric citrate	6.00
Maganese carbonate	3.50
Zinc carbonate	1.60
Cupric carbonate	0.30
Potassium iodate	0.01
Sodium selenite pentahydrate	0.01
Chromic potassium sulphate dodacahydrate	0.55
Starch	118.03

*Based on the National Academy of Science recommended levels for rats. (BARR Committee on Animal Nutrition, 1972)

Table 3.3 Composition of vitamin mixture (AIN 76A)*

Ingredients	g/kg vitamin mixture
Thiamine hydrochloride	0.6
Riboflavin	0.6
Pyridoxine hydrochloride	0.7
Nicotinic acid	3.0
Calcium pantothenate	1.6
Folic acid	0.2
Biotin	0.02
Retinol acetate	0.001
Cholecalciferol	0.0025
DL-Tocopherol	To provide 5000 IU of vit. E. activity
Menadione	0.005
Cyanocobalamine	0.001
Starch, finely powdered	To make 1000 g

*Based on the National Academy of Science recommended levels for rats. (BARR Committee on Animal Nutrition, 1972)

3.7.2 Determination of apparent protein digestibility (APD), True protein digestibility (TPD), Biological value (BV), Net protein utilization (NPU), Net protein ratio (NPR) and Protein retention efficiency (PRE)

These were determined by the method of Chick *et al.* (1954). The rats were taken over for these studies from the PER experiment. Out of 10 rats, 5 rats were continued in each dietary regime for PER. Another additional group of 5 rats was fed in protein free diet for endogenous and metabolic nitrogen excretion estimates. All the rats were transferred into metabolic cages for this part of study. After four days of adaptation period, the collection of faeces and urine was preserved by addition of few drops of one per cent H₂SO₄ to avoid the evaporation of nitrogen in the form of ammonia. Five days pooled urine and faeces were analysed for total nitrogen by micro-kjeldhal method. On the completion of experiment, final weight of rats were noted. APD, TPD, BV, NPU, NPR and PRE were calculated using following formulae:

3.7.2.1 Apparent protein digestibility was calculated as follows

$$\text{APD}\% = \frac{\text{Nitrogen intake} - \text{Faecal nitrogen} \times 100}{\text{Nitrogen intake}}$$

3.7.2.2 True protein digestibility

$$\text{APD}\% = \frac{\text{Nitrogen intake} - (\text{FN} - \text{MFN}) \times 100}{\text{Nitrogen intake}}$$

Where,

FN = Faecal nitrogen MFN = Metabolic faecal nitrogen

3.7.2.3 Biological value

$$\text{B.V.} = \frac{\text{N intake} - (\text{FN} - \text{MFN}) - (\text{UN} - \text{EUN}) \times 100}{\text{N intake} (\text{FN} - \text{MFN})}$$

Where,

N = Nitrogen

FN = Faecal nitrogen

MFN= Metabolic faecal nitrogen

UN = Urinary nitrogen

EUN= Endogenous urinary nitrogen

3.7.2.4 Net protein utilization

$$\text{NPU} = \frac{\text{T.D.} \times \text{B.V.}}{100}$$

3.7.2.5 Net protein ratio (NPR)

$$\text{NPR} = \frac{\text{Weight gain of test group} + \text{Loss in weight of protein free group}}{\text{Protein intake of test group}}$$

3.7.2.6 Protein retention efficiency

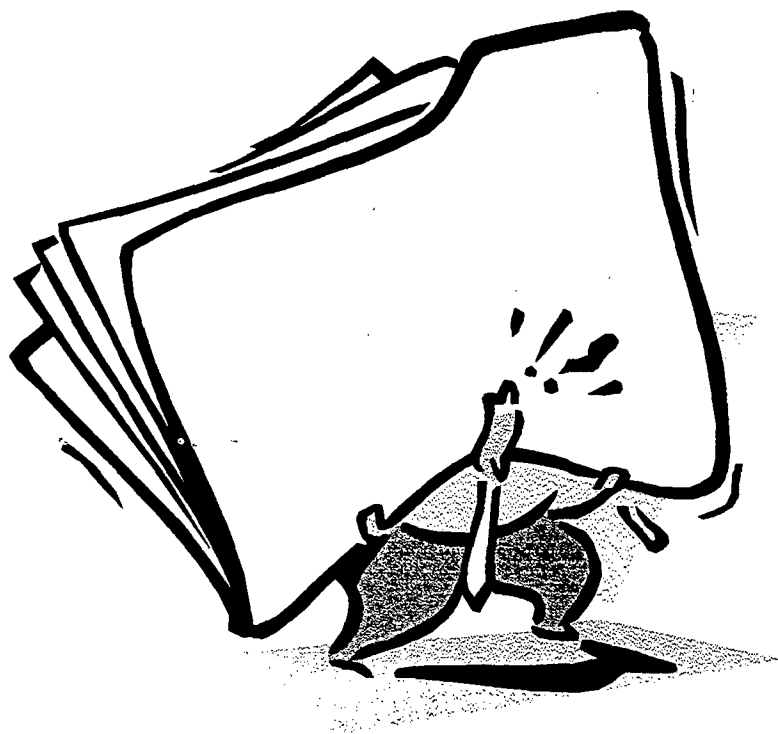
$$\text{PRE} = \text{NPR} \times 16$$

3.7.3 Internal organs

On completion of biological evaluation trial each rat was weighed and anaesthetized with diethyl ether. These were dissected and liver, kidney and spleen were taken out, cleaned of adhering matter and weighed.

3.8 Statistical analysis

The data from various laboratory experiments were subjected to statistical analysis for analysis of variance in a completely randomized design (Sendecor and Cochran, 1968).



RESULTS

CHAPTER-IV

RESULTS

The results of this study have been presented under following sub-heads:

- 4.1 Physical parameters
- 4.2 Proximate composition
- 4.3 Nutritional composition
- 4.4 Biological evaluation

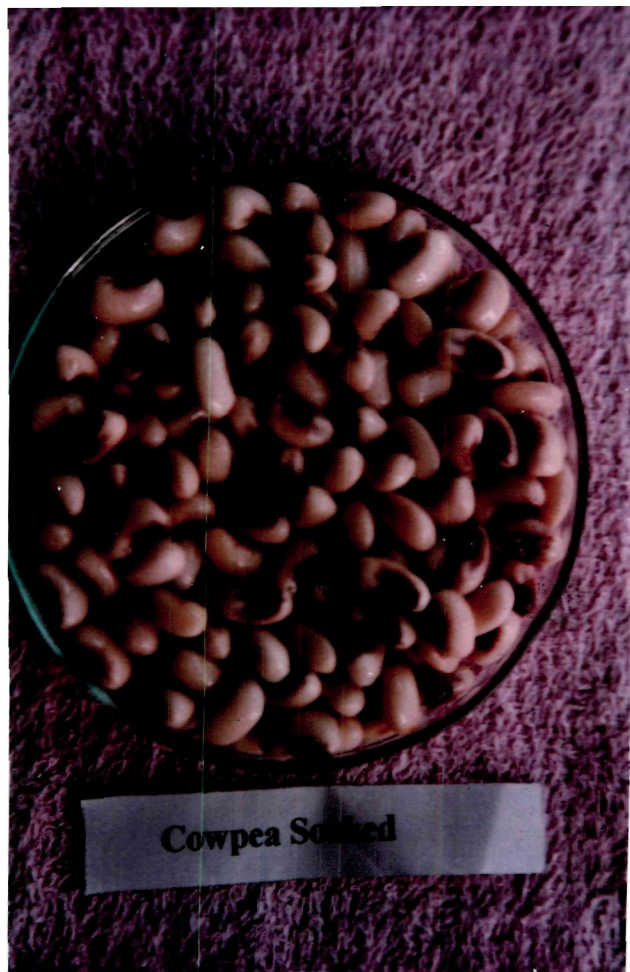
4.1 Physical parameters

A knowledge of the physical properties like colour, shape, length, density, 100 seed weight etc. of grain is important in their engineering, handling and storage characteristics. Among these, some of the characteristic as affected by various pre-treatments are presented.

Data on the various physical characteristics of untreated as well as treated seeds of cowpea, kidney beans and moth beans have been presented in Table 4, 5 and 6 and are discussed in the following sub-heads:

4.1.1 Colour

As is shown in Table 4, 5 and 6 colour of cowpea was cream while kidney bean was light brown in colour with red seeds and moth beans were light brown in colour.



Cowpea Soaked



Cowpea Sprouted

Cowpea Cooked

Plate 1 Soaked, sprouted and cooked cowpea

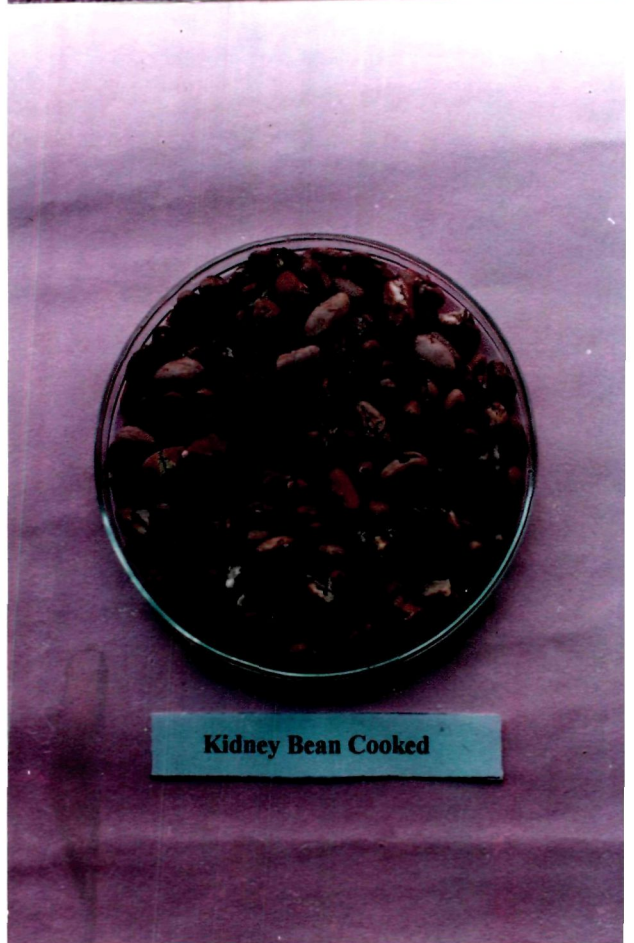
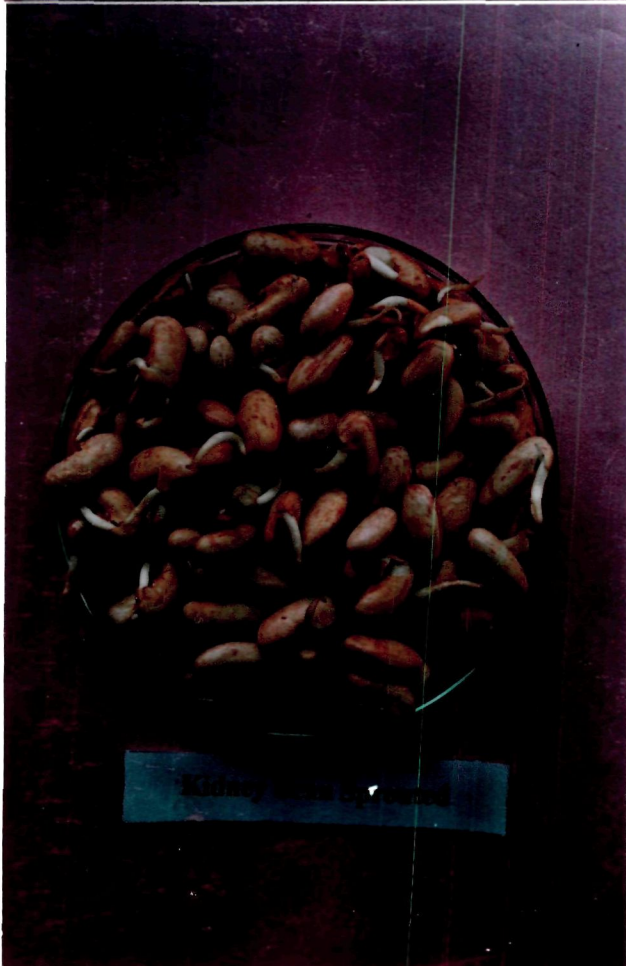
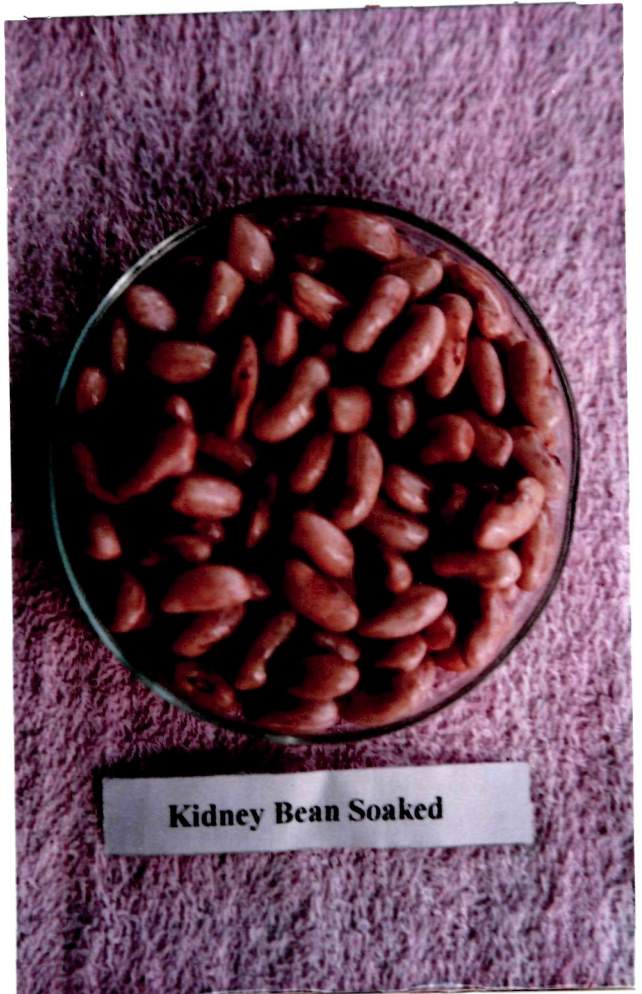
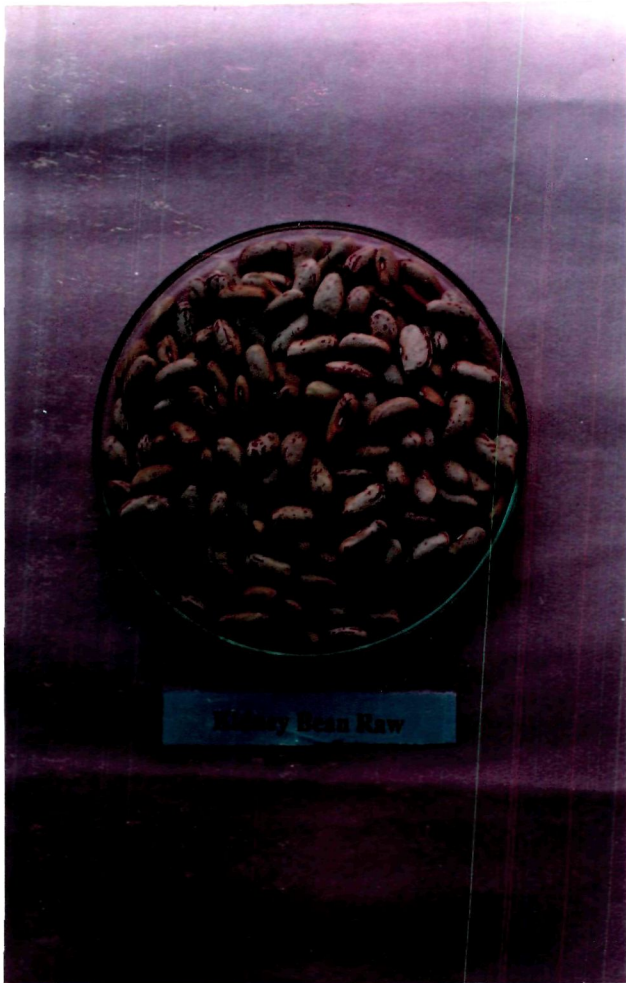


Plate 2 Raw, soaked, sprouted and cooked kidney beans



Moth Bean Raw

Moth Bean Soaked



Moth Bean Cooked

Plate 3 Raw, soaked and cooked moth beans

Table 4 Effect of soaking, sprouting and cooking on the physical characteristics of cowpea

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Colour	Cream				
Shape	Kidney shaped				
100-kernal weight (g)	17.80	35.33	31.66	40.33	1.66
Density (g/ml)	1.37	1.02	0.95	0.76	0.54
Length (mm)	10.33	13.40	14.07	13.03	1.06
Width (mm)	8.72	10.27	11.00	11.03	0.45

Table 5 Effect of soaking, sprouting and cooking on the physical characteristics of kidney beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Colour	Light brown with red spots				
Shape	Kidney shaped				
100-kernal weight (g)	28.64	56.33	51.33	62.67	2.69
Density (g/ml)	1.23	1.10	1.00	1.15	0.06
Length (mm)	15.03	18.60	19.13	17.37	1.11
Width (mm)	10.25	12.15	12.23	12.90	0.38

Table 6 Effect of soaking, sprouting and cooking on the physical characteristics of moth beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Colour	Light brown				
Shape	Cylindrical				
100-kernal weight (g)	3.27	6.38	6.00	7.77	0.39
Density (g/ml)	1.64	0.94	1.83	0.22	0.19
Length (mm)	5.13	7.17	8.30	8.37	0.40
Width (mm)	5.15	5.97	6.18	6.27	0.23

4.1.2 Shape

Plates 1, 2 and 3 show the shape of cowpea, kidney bean and moth bean respectively. Cowpea and kidney bean had kidney shaped structure, cowpea being smaller in size than kidney beans whereas moth bean was cylindrical in shape.

4.1.3 Weight

Table 4, 5 and 6 show the effect of soaking, sprouting and cooking on 100 kernel weight of cowpea, kidney beans and moth beans. The seed weight of the untreated as well as treated seeds of cowpea, kidney beans and moth beans varied significantly ($P < 0.05$). The highest seed weight (40.33, 62.67 and 7.77 g) was recorded in pressure cooked cowpea, kidney beans and moth beans respectively, and the lowest weight (17.80, 28.64 and 3.27 g) was observed in untreated control. Significant ($P < 0.05$) difference in the treated and untreated grains was observed. In case of moth beans the weight of soaked and sprouted seeds did not vary significantly.

4.1.4 Density

As depicted in Table 4, 5 and 6 density of untreated, soaked, sprouted and cooked cowpea was 1.37, 1.02, 0.95 and 0.76 g/ml respectively. Sprouting, cooking and soaking lowered the density. A significant ($P < 0.05$) difference was observed between the treatments when compared with each other. For kidney beans (Table 5) density ranged between 1.00 to 1.23 g/ml. Untreated kidney beans had significantly ($P < 0.05$) higher density when compared with soaked, sprouted and cooked counterparts. A significant ($P < 0.05$) difference was also observed among the treatments when compared with each other. The

density of the sprouted moth beans (Table 6) was the maximum (1.84 g/ml) and differed non-significantly when compared with the untreated counterpart (1.64 g/ml). The soaked and cooked grains of moth beans differed significantly ($P < 0.05$) when compared with each other and untreated grains.

4.1.5 Length

Data in Table 4, 5 and 6 show the average length of the untreated, soaked, sprouted and cooked samples of the cowpea, kidney beans and moth beans respectively. As is clear from the Table 4 length of the untreated cowpea was 10.33 mm and for the sprouted samples it was 14.07 mm. Significant ($P < 0.05$) difference was observed in the length of treated and untreated grains. For kidney beans untreated grains had significantly ($P < 0.05$) higher length (15.03 mm) as compared to sprouted counterpart 19.13 mm. Whereas in moth beans length ranged between 5.13 mm and 8.37 mm in untreated and cooked samples. Significant ($P < 0.05$) difference was observed between the untreated and cooked samples.

4.1.6 Width

Data in Table 4, 5 and 6 show the average width of the untreated, soaked, sprouted and cooked grains of the cowpea, kidney beans and moth beans respectively. As is clear from the Table 4, width of untreated cowpea was 8.72 mm and for the cooked sample it was 11.03 mm. A significant ($P < 0.05$) difference was observed between the untreated and treated grains when compared with each other. For kidney beans untreated samples had width of 10.25 mm and for cooked 12.90 mm. A Significantly ($P < 0.05$) higher width was there in cooked grains of kidney beans when compared with sprouted and untreated grains. Whereas in moth beans

width ranged between 5.15 mm and 6.27 mm for untreated and cooked samples. Significant ($P < 0.05$) difference was observed between untreated and cooked grains of moth beans when compared with each other whereas cooked and sprouted samples differed non significantly.

4.2 Proximate composition

Proximate composition gives useful information about the nutritional and biochemical quality of foods. Nutritive value of any food and food product depends mainly on their proximate composition which includes determination of percentage of moisture, ash, crude fat, crude protein and crude fibre. The results of the proximate composition of cowpea, kidney beans and moth beans is given in Table 7, 8 and 9.

4.2.1 Moisture

Determination of moisture content is one of the most important and widely used analytical measurement. Moisture content is an index of stability and quality and also is a measure of yield and quality of food solids.

As is clear from the data in Table 7, 8 and 9 giving pre-treatments to legumes resulted in the loss of the moisture content which was determined on dry weight basis. As depicted in Table 7, untreated cowpea had significantly ($P < 0.05$) higher moisture content when compared with its soaked, sprouted and cooked counterparts. Cooked samples of cowpea had significantly ($P < 0.05$) higher values when compared to soaked and sprouted cowpea. Sprouted cowpea had the lowest moisture (4.86%). Similar trend was followed by kidney beans (Table 8) in which untreated and sprouted beans has moisture as 9.49 and 5.16 per cent, respectively.

Table 7 Effect of soaking, sprouting and cooking on the proximate composition, non-protein nitrogen and true protein of cowpea

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Moisture (%)	9.80	5.43	4.86	6.93	0.91
Ash (%)	3.02	3.08	3.12	3.13	0.17
Crude fat (%)	1.77	1.78	1.95	1.79	0.11
Crude fibre (%)	2.22	3.20	2.20	2.53	0.91
Crude protein (%)	22.18	21.54	22.61	21.14	1.27
Non-protein nitrogen (%)	1.05	0.97	0.76	0.34	0.06
True protein (%)	15.62	15.48	17.86	19.02	1.50

Table 8 Effect of soaking, sprouting and cooking on the proximate composition, non-protein nitrogen and true protein of kidney beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Moisture (%)	9.49	5.64	5.16	7.58	0.70
Ash (%)	3.92	3.63	3.95	4.18	0.15
Crude fat (%)	1.30	1.64	1.74	1.73	0.23
Crude fibre (%)	3.20	3.53	4.04	3.72	0.50
Crude protein (%)	21.63	20.43	21.85	19.96	1.59
Non-protein nitrogen (%)	0.70	0.43	0.51	0.35	0.09
True protein (%)	17.26	17.74	18.66	17.77	1.36

Table 9 Effect of soaking, sprouting and cooking on the proximate composition, non-protein nitrogen and true protein of moth beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Moisture (%)	7.35	6.19	6.53	5.80	0.28
Ash (%)	3.27	3.38	2.90	3.92	0.26
Crude fat (%)	1.29	1.13	1.53	1.49	0.19
Crude fibre (%)	3.83	3.70	3.52	3.51	0.53
Crude protein (%)	21.88	21.15	22.48	20.39	1.44
Non-protein nitrogen (%)	0.70	0.54	0.62	0.50	0.16
True protein (%)	17.51	17.78	18.61	17.27	0.60

Significant ($P < 0.05$) difference was there in untreated kidney beans when compared with treated counterpart but a non-significant difference was there in soaked and sprouted legume when compared with each other but differed significantly ($P < 0.05$) when compared with their cooked counterparts. Data in Table 9 show the moisture content for treated and untreated moth beans ranged from 7.35 to 5.80 per cent. Significantly ($P < 0.05$) higher moisture content was there in untreated legume and lowest for the cooked samples. Significantly ($P < 0.05$) difference was observed in the moisture content of treated and untreated grains.

4.2.2 Ash

Ash content of foods, in general, is defined as the inorganic residues left over after ashing. Foods also reveals the mineral constituents which plays an important role in human nutrition. The ash obtained is not necessarily of exactly the same composition as there may be losses due to volatilization of some interaction in between the constituents.

Data in Table 7, 8 and 9 show the ash content of cowpea, kidney beans and moth beans, respectively. Ash content for the cowpea ranged between 3.02 and 3.13 per cent for untreated and treated legume. There was no significant change in the ash content of soaked, sprouted and cooked legume compared with each other and untreated counterparts. Ash content in kidney beans was 3.92, 3.63, 3.95 and 4.18 per cent in the untreated, soaked, sprouted and cooked grains, respectively. Significantly higher ($P < 0.05$) ash content was there in cooked grains when compared with their untreated and treated counterparts. Significant difference was also observed in between sprouted and cooked grains. In the moth beans highest

ash content was there in cooked samples (3.92%) when compared with untreated, soaked and sprouted counterpart (3.27, 3.38 and 2.90% respectively). A non significant difference was observed in the ash content of the untreated and soaked samples whereas sprouted samples had significantly ($P<0.05$) lower ash content when compared with untreated soaked and cooked grains.

4.2.3 Crude fat

The ether extract or crude fat content of a food represents the true fat (tri-glycerides) and other materials such as phospholipids, sterols, essential oils, fat soluble pigments, waxes, carotenoids, chlorophyll and other pigments extractable with ether.

Data in Table 7, 8 and 9 show the crude fat content of the cowpea, kidney beans and moth beans respectively. As is clear from the Table 7 the untreated, soaked, sprouted and cooked cowpea had fat content of 1.77, 1.78, 1.95 and 1.79 per cent respectively. A non significant difference was there when cooked, soaked and untreated legumes were compared with each other. A significantly ($P<0.05$) higher fat content was there in sprouted legume when compared with its other counterparts. In the kidney beans a similar trend was followed in which untreated, soaked, sprouted and cooked samples had fat content as 1.30, 1.64, 1.74 and 1.73 per cent respectively. A significantly ($P<0.05$) lower value was there for untreated beans when compared with the treated beans which differed non significantly from each other. In moth beans crude fat was maximum for the sprouted sample and minimum for the soaked sample (Table 9), values being 1.29, 1.13, 1.53 and 1.49 per cent in untreated, soaked, sprouted and cooked grains, respectively. A significant

($P < 0.05$) difference was there in untreated and soaked grains when compared with sprouted and cooked counterpart.

4.2.4 Crude protein

Data in Tables 7, 8 and 9 depict the crude protein content of cowpea, kidney beans and moth beans respectively. In case of cowpea (Table 7) crude protein content was 22.18, 21.54, 22.61 and 21.14 per cent in untreated, soaked, sprouted and cooked grains respectively. As is clear from Table 7 a non significant difference was there in the treated and untreated grains when compared with each other, except between sprouted and cooked grains which differed significantly ($P < 0.05$) from each other. In case of kidney beans (Table 8) crude protein was 21.63, 20.43, 21.85 and 19.96 per cent in untreated, soaked, sprouted and cooked grains. Significantly ($P < 0.05$) higher crude protein was there in the untreated and sprouted grains when compared with their soaked and cooked counterparts. A non significant difference was observed in between untreated and soaked grains when compared with each other. In case of moth beans (Table 9) higher crude protein was in sprouted grains 22.48 per cent followed by 21.88, 21.15 and 20.39 per cent in untreated, soaked and cooked grain. Significantly ($P < 0.05$) higher crude protein was there in the sprouted grains when compared with their other untreated and treated

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viscous polysaccharides which gives plants, their structure and form but are not digested in the human system, are known as fibre content of food.

Data in Table 7, 8 and 9 show the crude fibre content of the untreated, soaked, sprouted and cooked cowpea, kidney beans and moth beans. Crude fibre in untreated soaked, sprouted and cooked cowpea was 2.22, 3.20, 2.20, and 2.53 per cent, respectively. A significantly ($P < 0.05$) higher value for the soaked legume was there when compared with untreated and sprouted counterparts. For kidney beans crude fibre was (Table 8) 3.20, 3.53, 4.04 and 3.17 per cent in untreated, soaked, sprouted and cooked grains respectively. A significant ($P < 0.05$) increase was found in sprouted kidney bean when compared with untreated and soaked counterpart whereas a non-significant difference was found in crude fibre content of sprouted and cooked beans when compared with each other. In the case of moth bean the crude fibre content of untreated, soaked, sprouted and cooked legume was 3.83, 3.70, 3.52 and 3.51 respectively. A non-significant difference was observed among the untreated, soaked, sprouted and cooked moth beans when compared with each other.

4.3 Nutritional composition

4.3.1 Non-protein nitrogen

As is clear from Table 7, non protein nitrogen in untreated, soaked, sprouted and cooked cowpea was 1.05, 0.97, 0.76 and 0.34 per cent respectively. A significant ($P<0.05$) difference was observed in non protein nitrogen untreated cowpea when compared with other treated counterparts. A significant ($P<0.05$) difference was also observed amongst all treatments when compared with each other. Similar was the trend for kidney beans where the non protein nitrogen of untreated grains was 0.70 and that for soaked, sprouted and cooked was 0.43, 0.51 and 0.35 per cent respectively. Untreated kidney beans had significantly ($P<0.05$) higher non protein nitrogen as compared to treated counter parts. A significant difference was observed between sprouted and cooked beans when compared with each other. Whereas, a non-significant difference was observed between soaked and sprouted and soaked and cooked kidney beans. In case of moth beans untreated, soaked, sprouted and cooked beans had non protein nitrogen of 0.70, 0.54, 0.62 and 0.50 per cent respectively. Untreated moth bean had significantly ($P<0.05$) higher non protein nitrogen when compared with its soaked and cooked counterpart. But differed non-significantly with sprouted. Non-significant difference was observed among soaked, sprouted and cooked beans when compared with each other.

4.3.2 True protein

True protein is determined by subtracting non-protein nitrogen from crude
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cent and 15.62 per cent respectively. The true protein was significantly less ($P < 0.05$) in untreated and soaked legume when compared with its sprouted and cooked counterparts but differed non-significantly when compared with each other. In the kidney beans (Table 8) the highest true protein was for sprouted beans (18.66%) and lowest was in the untreated beans (17.25%). Cooked and soaked kidney beans had true protein as 17.77 and 17.74 per cent respectively. A significant ($P < 0.05$) difference was there in untreated and sprouted samples when compared with each other. The true protein content of untreated, soaked, sprouted and cooked moth beans (Table 9) was 17.51, 17.78, 18.61 and 17.27 per cent respectively. The true protein content of sprouted moth beans was significantly ($P < 0.05$) higher than untreated, soaked and cooked counterparts. A non-significant difference was there among the untreated, soaked and cooked counterparts when compared with each other.

4.3.3 Sugars

Most important disaccharides present in plant food are sucrose and melibiose. The oligosaccharides are reducing and non-reducing.

4.3.3.1 Total sugars

Table 10, 11 and 12 shows the effect of soaking, sprouting and cooking on the total sugars of cowpea, kidney beans and moth beans respectively. Maximum total sugars were there in the cooked cowpea (11.76%) and lowest in the soaked cowpea (7.42%). As shown in Table 10 a significant ($P < 0.05$) difference was observed in total sugars content of untreated cowpea when compared with soaked, sprouted and cooked counterparts. A non-significant difference was there between soaked and sprouted cowpea when compared^{to} each other. A similar trend was followed in kidney

bean with highest sugars in cooked beans 9.92 per cent followed by 7.63 per cent in untreated and 6.75 per cent and 6.25 per cent in sprouted and soaked beans (Table 11). A significant ($P<0.05$) difference was observed among the treatments when compared with each other. In the moth beans (Table 12) highest value for total sugars was in cooked (12%) and lowest in soaked (8.5%). The sugars were significantly higher ($P<0.05$) in cooked moth beans when compared with the treated counterparts. Significantly ($P<0.05$) higher total sugars were there in cooked moth bean in comparison to sprouted and soaked counterparts. An insignificant difference was there between soaked and sprouted moth beans when compared with each other.

4.3.3.2 Reducing sugars

Data in Table 10, 11 and 12 show the reducing sugars content of untreated, soaked, sprouted and cooked cowpea, kidney beans and moth beans, respectively.

The reducing sugars content in untreated, soaked, sprouted and cooked cowpea was 0.48, 0.47, 0.44 and 0.53 per cent respectively (Table 10). A significant difference ($P<0.05$) was observed in between the untreated and treated cowpea when compared with each other. Cooked cowpea had significantly ($P<0.05$) higher reducing sugars as compared to untreated, soaked and sprouted counterparts. Similarly, in the kidney beans reducing sugars ranged between 0.51 to 0.38 per cent (Table 11). Significantly ($P<0.05$) higher reducing sugars were there in cooked kidney beans when compared with other counterparts. Reducing sugars for untreated, soaked, sprouted and cooked moth beans was 0.54, 0.49, 0.46 and 0.57 per cent respectively. As is clear from the data significantly ($P<0.05$) higher reducing

sugars were there in cooked legumes as compared to untreated, soaked and sprouted counterparts. Significant difference ($P < 0.05$) was also there in untreated, soaked and sprouted moth bean when compared with each other.

4.3.3.3 Non reducing sugars

Data in Table 10, 11 and 12 show the non-reducing sugars content of the cowpea, kidney beans and moth beans.

Table 10 shows the non-reducing sugars content of untreated, soaked, sprouted and cooked cowpea. In untreated, soaked, sprouted and cooked cowpea non-reducing sugar content was 8.44, 6.95, 7.06 and 11.14 per cent respectively. Significant ($P < 0.05$) difference was observed between untreated cowpea when compared to its soaked, sprouted and cooked counterparts. Cooked cowpea had significantly ($P < 0.05$) higher (11.14%) non reducing sugars as compared to untreated, soaked and sprouted cowpea. Non significant difference was observed in soaked and sprouted cowpea when analysed statistically. In the kidney beans (Table 11) value of non reducing sugars was 7.17, 5.84, 6.33 and 9.41 per cent respectively in untreated, soaked, sprouted and cooked grains. Significant difference ($P < 0.05$) was observed in the non-reducing sugar content of untreated kidney beans when compared with its soaked, sprouted and cooked counterparts. Significantly ($P < 0.05$) higher non-reducing sugars were there in cooked sample when compared with its soaked, sprouted and untreated counterpart. Though, sprouted kidney bean had higher value it was non significantly higher in comparison to soaked kidney beans. Table 12 shows that the cooked moth bean contained the highest non-reducing sugars (11.43%) followed by untreated (9.38%) and then sprouted (8.19%).

and least in soaked (8.01%). Significant difference ($P < 0.05$) was observed in non-reducing sugars of untreated moth bean when compared with soaked, sprouted and cooked counterparts. Cooked grains had significantly ($P < 0.05$) higher non-reducing sugars as compared to soaked and sprouted moth bean which differed non-significantly from each other.

4.3.4 Starch

Starch is one of the storage polysaccharides present in plants. All important food plants produce seeds containing starch as the carbohydrate reserve which serves as the principal food stored for use by the embryonic part in the initial growth stages. Starch is composed of two closely related polysaccharides amylose and amylopectin. Seed starches are classified either as cereals starch or tuber starch depending upon attaining power and physical properties.

Table 10, 11 and 12 show the starch content of the untreated, soaked, sprouted and cooked cowpea, kidney beans and moth beans. A significant ($P < 0.05$) difference was observed in the starch content of untreated and treated cowpea (Table 10) when compared with each other. Starch content was significantly ($P < 0.05$) higher in the untreated cowpea (46.13%) as compared to its soaked, sprouted and cooked (42.38, 32.25 and 31.13%) counterparts. A significant ($P < 0.05$) difference was also observed among all the treatments when compared with each other. In the kidney beans (Table 11) a similar trend was followed with highest starch content in untreated grains (41.63%) followed by soaked kidney beans (38.04%). Starch content was significantly ($P < 0.05$) higher in untreated kidney beans as compared to soaked, sprouted and cooked counterparts. A significant difference

was also observed in soaked, sprouted and cooked kidney beans when compared with each other. Similar trend was observed for starch content in moth beans (Table 12) with untreated, soaked, sprouted and cooked samples having starch as 50.25, 46.88, 41.25 and 40.13 per cent respectively. A significant ($P<0.05$) difference was observed in starch content of untreated and treated moth bean when compared with each other.

4.3.5 Energy

Table 10, 11 and 12 shows the effect of soaking, sprouting and cooking on the energy content of cowpea, kidney beans and moth beans.

The energy value for the untreated, soaked, sprouted and cooked cowpea was 422.32, 330.43, 336.77 and 323.92 Kcal respectively (Table 10). As is clear from the data, significantly ($P<0.05$) lower value for energy was observed in cooked cowpea as compared to untreated, soaked and sprouted counterparts of the cowpea. Significant ($P<0.05$) difference was also observed among untreated, soaked and sprouted cowpea when compared with each other. Similar trend in kidney beans with 365.76, 354.19, 350.32 and 347.84 Kcal energy in untreated, soaked, sprouted and cooked beans was observed (Table 11). In significant difference ($P<0.05$) was observed between untreated and treated kidney beans when compared with each other. In the moth beans (Table 12) untreated, soaked, sprouted and cooked moth beans contained energy as 406.59, 397.41, 346.10 and 326.75 Kcal, respectively. The untreated samples contained significantly ($P<0.05$) higher energy values as compared to sprouted and cooked counterparts. A non-significant difference was their between untreated and soaked moth bean when

compared with each other. A significantly ($P < 0.05$) higher energy value for their in soaked moth bean as compared to sprouted and cooked counterparts. No significant difference was their between sprouted and cooked moth beans when compared with each other.

4.3.6 Dietary fibre constituents

Dietary fibre occurs as structural material in the cell wall of plant. It includes NDF, ADF, lignin, hemicellulose, cellulose, pectin substances, gums and mucilages. Total dietary fibre is defined as those polysaccharides except starch, that are not available for digestion by gastrointestinal enzymes in human system. However, dietary fibre is subjected to some degradation by bacterial fermentation in the large intestine.

4.3.6.1 Neutral detergent fibre (NDF)

Data in Table 10, 11 and 12 show the NDF content of cowpea, kidney beans and moth beans.

The NDF content for untreated, soaked, sprouted and cooked cowpea was 3.20, 7.61, 7.39 and 8.00 per cent, respectively (Table 10). As is clear from the table cooked cowpea had significantly higher NDF as compared to untreated, soaked and sprouted counterparts. Untreated legume had significantly ($P < 0.05$) lower NDF as compared to the treated legume. In case of kidney beans NDF was 26.95, 22.84, 25.42 and 29.81 per cent respectively for untreated, soaked, sprouted and cooked kidney beans. As is clear from the data in Table 11, a significant ($P < 0.05$) difference was observed between untreated, soaked, sprouted and cooked kidney beans when compared with each other. Significantly ($P < 0.05$) higher NDF was in cooked kidney

beans as compared to soaked, sprouted and cooked counterparts. In case of moth beans (Table 12) cooked moth bean had highest NDF (24.46%) followed by untreated (22.83%), soaked (21.96%) and sprouted (17.88%) moth bean. A non-significant difference was observed among the untreated and soaked moth beans when compared with each other but a significant ($P < 0.05$) difference was there when untreated moth beans was compared with its soaked and sprouted counterpart. A significant ($P < 0.05$) difference was also there between soaked, sprouted and cooked counterparts when compared with each other.

4.3.6.2 Acid detergent fibre (ADF)

Data in Table 10, 11 and 12 show ADF content of untreated soaked, sprouted and cooked cowpea, kidney beans and moth beans.

As shown in the Table 10 ADF content for untreated, soaked, sprouted and cooked cowpea was 3.81, 3.80, 4.80 and 5.20 per cent, respectively. A significant ($P < 0.05$) difference was observed between the untreated cowpea when compared with its sprouted and cooked counterparts. But differed, non-significantly from its soaked counterpart which had almost similar ADF content. Cooked cowpea had significantly ($P < 0.05$) higher ADF as compared to its untreated and soaked counterparts. No significant difference was there between sprouted and cooked cowpea when compared with each other. As is clear from the Table 11 untreated kidney beans had, ADF content of 14.79 per cent, soaked 11.82 per cent, sprouted 11.75 per cent and cooked grains had 16.48 per cent. Significantly higher ($P < 0.05$) ADF was there in cooked kidney beans when compared with untreated, soaked and sprouted counterparts. A statistically non-significant difference was there in soaked

and sprouted kidney beans when compared with each other. Data in Table 12 shows the ADF content of untreated, soaked, sprouted and cooked moth beans was 10.83, 9.96, 7.55 and 14.46 per cent, respectively. Significantly ($P < 0.05$) higher ADF was the in untreated moth beans as compared to soaked and sprouted counterparts, but significantly ($P < 0.05$) less than cooked moth beans. A significant ($P < 0.05$) difference in ADF content was observed in all the untreated and treated moth beans when compared.

4.3.6.3 Hemicellulose

Hemicellulose are the polysaccharides containing pentoses, hexoses and uronic acid. They are hydrolysed by hot dilute acids, but are not acted upon by the digestive juices in the human intestinal tract.

Data in Table 10, 11 and 12 show the hemicellulose content of treated and untreated cowpea, kidney beans and moth beans.

As is clear from the data in Table 10 untreated cowpea had significantly ($P < 0.05$) lower hemicellulose (2.39%) as compared to the treated counterparts. The hemicellulose content of soaked cowpea was significantly ($P < 0.05$) greater than sprouted and cooked cowpea. The difference between sprouted (2.59%) and cooked (2.8%) was also significant ($P < 0.05$). In kidney beans Table 11 sprouted seeds had significantly ($P < 0.05$) higher hemicellulose (13.70%) in comparison to untreated, soaked and cooked, grains. The difference was also significant ($P < 0.05$) between untreated, soaked and cooked counterparts. There was no difference in the hemicellulose content of untreated and soaked moth beans (Table 12). The cooked

grains had significantly ($P<0.05$) lower value (10.00%) as compared to sprouted (10.33%), untreated (12.00%) and soaked (12.00%) moth beans.

4.3.7 Anti-nutritional factors

4.3.7.1 Phytic acid

Phytic acid is one of the anti-nutritional factor present in plant foods. It affects the digestion and absorption of minerals, so in the present study and attempt was made to analyse the phytic acid content of soaked, sprouted and cooked grains of three legumes.

Data in Table 13, 14 and 15 show the effect of soaking, sprouting and cooking on the phytic acid content of cowpea, kidney beans and moth beans. As shown in the Table 13, untreated, soaked, sprouted and cooked cowpea had phytic acid content as 306.33, 293.33, 285.67 and 269.33 mg/100 g respectively. A significant ($P<0.05$) difference was observed in the phytic acid content of untreated cowpea when compared with soaked, sprouted and cooked counterpart. Cooked cowpea had significantly ($P<0.05$) lower phytic acid as compared to soaked, sprouted and cooked counterpart. Sprouted legume had less phytic acid as compared to soaked legume but statistically difference was not significant. Phytic acid content of kidney beans as shown in Table 14 was 338.33, 283.33, 278.33 and 236.67 mg/100g respectively in untreated, soaked, sprouted and cooked legume. As is clear from the Table 14, untreated kidney beans had significantly ($P<0.05$) higher phytic acid content as compared to soaked, sprouted and cooked counterparts. Cooking reduced phytic acid significantly ($P<0.05$) in kidney beans. Sprouted kidney beans

Table 10 Effect of soaking, sprouting and cooking on total sugars, reducing sugars, non-reducing sugars, energy starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose of cowpea

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Total sugars (%)	8.92	7.42	7.50	11.67	1.43
Reducing sugars (%)	0.48	0.47	0.44	0.53	0.011
Non-reducing sugars (%)	8.44	6.95	7.06	11.14	1.65
Energy (K cal/100 g)	422.32	380.43	336.77	323.92	1.83
Starch (%)	46.13	42.38	32.25	31.13	2.80
NDF (%)	6.20	7.61	7.39	8.00	0.04
ADF (%)	3.81	3.80	4.80	5.20	0.72
Hemicellulose (%)	2.39	3.81	2.59	2.80	0.02

Table 11 Effect of soaking, sprouting and cooking on total sugars, reducing sugars, non-reducing sugars, energy, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose of kidney beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Total sugars (%)	7.63	6.25	6.75	9.92	0.38
Reducing sugars (%)	0.46	0.40	0.38	0.51	0.019
Non-reducing sugars (%)	7.17	5.84	6.33	9.41	0.64
Energy (K cal/100 g)	365.76	354.19	350.32	347.84	1.88
Starch (%)	41.63	38.04	30.75	33.38	3.30
NDF (%)	26.95	22.84	25.42	29.81	0.07
ADF (%)	14.79	11.82	11.75	16.48	1.11
Hemicellulose (%)	12.16	11.02	13.67	13.33	0.04

Table 12 Effect of soaking, sprouting and cooking on total sugars, reducing sugars, non-reducing sugars, energy, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and hemicellulose of moth beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Total sugars (%)	9.92	8.50	8.65	12.00	0.65
Reducing sugars (%)	0.54	0.49	0.46	0.57	0.019
Non-reducing sugars (%)	9.38	8.01	8.19	11.43	1.14
Energy (K cal/100 g)	406.59	397.41	346.10	326.75	1.87
Starch (%)	50.25	46.88	41.25	40.13	4.15
NDF (%)	22.83	21.96	17.88	24.46	1.11
ADF (%)	10.83	9.96	7.55	14.46	0.72
Hemicellulose (%)	12.00	12.00	10.33	10.00	0.03

had lower phytic acid than soaked, but statistically no significant difference was there between the two. In moth beans (Table 15) the untreated, soaked, sprouted and cooked legume had phytic acid as 311.33, 308.33, 302.67 and 287.67 mg/100 g. A non-significant difference was there in untreated, soaked and sprouted moth beans. Cooked moth bean had significantly lower ($P<0.05$) phytic acid content as compared to untreated, soaked and sprouted moth bean. Soaked moth bean had higher values for phytic acid as compared to sprouted but the difference was insignificant.

4.3.7.2 Phytate phosphorus

The Table 13, 14 and 15 show the phytate phosphorus content of cowpea, kidney beans and moth beans respectively. As clear from the Table 13, a significant ($P<0.05$) difference is there between untreated, soaked, sprouted and cooked cowpea when compared with each other. The phytate phosphorus content being 86.32, 77.02, 80.47 and 75.90 mg/100 g in untreated, soaked, sprouted and cooked grains. A similar trend was followed in kidney beans (Table 14). Cooked grains had significantly ($P<0.05$) lower phytate phosphorus in comparison to untreated, soaked and sprouted counterparts which too differed significantly ($P<0.05$) from each other. Phytate phosphorus being 95.34, 74.21, 78.43 and 66.60 in untreated, soaked, sprouted and cooked grains. The phytate phosphorus of untreated, soaked, sprouted and cooked moth beans (Table 15) was 87.73, 86.88, 85.29 and 81.07 mg/100g respectively. The difference was

significant ($P<0.05$) in untreated and treated counterparts when compared each other.

4.3.7.3 Non-phytate phosphorus

Non-phytate phosphorus is calculated as a difference between the total phosphorus and phytate phosphorus.

Data in Table 13, 14 and 15 show the non-phytate phosphorus content of cowpea, kidney beans and moth beans as affected by soaking, sprouting and cooking.

In case of cowpea Table 13 untreated, soaked, sprouted and cooked grain had phytate phosphorus as 327.68, 328.98, ~~322.86~~ and 327.43 mg/100 g respectively. A significant ($P<0.05$) difference was observed between the untreated and treated grains of cowpea. In kidney beans (Table 14) soaked seeds had significantly ($P<0.05$) higher non-phytate phosphorus (323.79%) in comparison to untreated and sprouted counterparts. An insignificant difference was there between soaked and cooked kidney bean. Non-phytate phosphorus was maximum (142.27) in case of untreated moth beans (Table 15) and minimum for sprouted seeds (121.71%). A significant difference ($P<0.05$) was there among untreated and treated counterparts of moth beans when compared with each other.

4.3.8 Minerals

The minerals are inorganic elements that serve as a constituent of skeletal structures, regulating acid base equilibrium and as a component of an activator of enzymes. The body contains about 24 macro and micro minerals, all of which must be provided by the diet. Bones and teeth are made up mainly of calcium,

magnesium and phosphorus. There are certain trace elements which are also of immense importance like copper, zinc and iron. Iron is an important constituent of blood. In the present study, an effort has been made to estimate calcium, phosphorus, potassium, magnesium, sodium, zinc, copper and iron in untreated, soaked, sprouted and cooked cowpea, kidney beans and moth beans

4.3.8.1 Calcium

Table 16, 17 and 18 show the calcium content of untreated and treated cowpea, kidney beans and moth beans.

As shown in Table 16, the calcium content of cooked cowpea (184.67 mg/100 g) was significantly ($P < 0.05$) higher than untreated, soaked and sprouted counterparts. Untreated and soaked seeds had similar calcium content (124.67 mg/100 g) and differed insignificantly from sprouted counterpart. Table 17 shows the calcium content of untreated and treated grains of kidney beans. The calcium content differed non significantly in untreated and treated counterparts of kidney beans when compared with each other. The highest calcium content being in cooked seeds (172 mg/100 g) followed by sprouted (164 mg/100 g) then soaked (159 mg/100 g) and least in untreated (152 mg/100 g). Data in Table 18 shows the calcium content of moth beans. The cooked beans had significantly ($P < 0.05$) higher calcium content (182.67 mg/100 g) as compared to untreated, soaked and sprouted counterpart. Sprouted moth bean had a higher value for calcium as compared to its untreated counterpart.

Table 13 Effect of soaking, sprouting and cooking on phytic acid, phytate phosphorus and non-phytate phosphorus of cow pea

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Phytic acid (mg/100 g)	306.33	293.33	285.67	269.33	11.39
Phytate phosphorus (mg/100 g)	86.32	77.02	80.47	75.90	1.39
Non-phytate phosphorus (mg/100 g)	327.68	328.98	322.86	328.43	1.03

Table 14 Effect of soaking, sprouting and cooking on phytic acid, phytate phosphorus and non-phytate phosphorus of kidney beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Phytic acid (mg/100 g)	338.33	283.33	278.33	236.67	15.88
Phytate phosphorus (mg/100 g)	95.34	74.21	78.43	66.60	1.59
Non-phytate phosphorus (mg/100g)	314.80	323.79	310.77	322.40	3.39

Table 15 Effect of soaking, sprouting and cooking on phytic acid, phytate phosphorus and non-phytate phosphorus of moth beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Phytic acid (mg/100 g)	311.33	308.33	302.67	287.67	11.35
Phytate phosphorus (mg/100 g)	87.73	86.88	85.29	81.07	1.35
Non-phytate phosphorus (mg/100g)	142.27	131.12	121.71	125.93	1.69

4.3.8.2 Sodium

Table 16, 17 and 18 shows the sodium content of untreated and treated Cowpea, kidney beans and moth beans.

Table 16 shows sodium content of cowpea. The sodium content was highest for cooked cowpea (41.50 mg/100 g) and it differed significantly ($P < 0.05$) from untreated, soaked and sprouted grains. An insignificant difference was there between untreated (37.50 mg/100 g), soaked (37 mg/100 g) and sprouted (38.50 mg/100 g) counter parts.

Table 17 shows the sodium content of kidney beans. The maximum was in cooked (475 mg/100 g) beans and it differed significantly ($P < 0.05$) from its untreated and soaked counterpart. Non-significant difference was there between cooked and sprouted grains when compared with each other. Table 18 shows the sodium content of untreated and treated moth bean as 51.67, 54.00, 55.00 and 58.33 mg/100 g respectively. The difference between the untreated and treated counterparts was statistically insignificant.

4.3.8.3 Potassium

Table 16, 17 and 18 show the potassium content of cowpea, kidney beans and moth beans. Data in Table 16 shows the potassium content of cowpea values as 126.17, 129.67, 133.17 and 135.00 mg/100 g, respectively for untreated, soaked, sprouted and cooked cowpea grains. The potassium content differed non significantly in untreated and treated counterpart when compared with each other. The maximum potassium content was in cooked grains (135.00 mg/100 g) and minimum (126.67 mg/100 g) in untreated cowpea.

The potassium content of kidney beans was 1013.42, 811.67, 1341.33 and 1013.42 mg/100 g for untreated, soaked, sprouted and cooked seeds respectively (Table 17). The difference was insignificant among the treatments when compared with each other. Table 18 shows the potassium content of moth bean as affected by different treatments. The potassium content was maximum in cooked seeds (130 mg/100 g) and minimum in soaked seeds (85 mg/100 g). The difference between untreated and cooked grains was insignificant. The soaked and sprouted grains differed significantly ($P < 0.05$) from each other and from untreated and cooked counterparts.

4.3.8.4 Magnesium

Data in Table 16, 17 and 18 show the magnesium content of untreated and treated seeds of cowpea, kidney beans and moth beans.

The magnesium content of cowpea for untreated, soaked, sprouted and cooked cowpea was 155.33, 155.33, 157.33 and 160.00 mg/100 g of magnesium respectively (Table 16). An insignificant difference was observed among the treated and untreated counterparts of cowpea when compared with each other. As clear from the Table 17 an insignificant difference was there between the untreated and treated grains of kidney beans. The magnesium content was 150, 144, 152.67 and 150.33 mg/100 g for untreated, soaked, sprouted and cooked beans. The untreated, soaked, sprouted and cooked moth beans had 162, 158.67, 169.33 and 174.67 mg/100 g magnesium respectively (Table 18). Cooked grains

had significantly ($P < 0.05$) higher magnesium as compared to soaked and sprouted counterparts.

4.3.8.5 Phosphorus

Data in Table 16, 17 and 18 show the phosphorus content of cowpea, kidney beans and cowpea.

Table 16 shows the phosphorus content of cowpea. The phosphorus content of untreated grains was significantly ($P < 0.05$) higher than soaked, sprouted and cooked counterparts values being 414.00, 406.00, 403.33 and 403.33 mg/100 g. There was no difference in the sprouted and cooked grains and non significant difference was there when compared with soaked grains of cowpea. Table 17 shows the phosphorus content of kidney beans. The untreated, soaked, sprouted and cooked kidney beans had 410, 398, 389 and 389 mg/100 g phosphorus respectively. The untreated seeds had significantly higher ($P < 0.05$) phosphorus as compared to soaked, sprouted and cooked counterparts. Soaked seeds had significantly ($P < 0.05$) higher phosphorus as compared to sprouted and cooked which had similar values. Data in Table 18 shows the phosphorus content of moth beans. The untreated seeds had significantly ($P < 0.05$) highest phosphorus than soaked, sprouted and cooked moth beans. Sprouted and cooked seeds had a similar value but were significantly ($P < 0.05$) lower when compared with soaked grains. Phosphorus content was 230, 218, 207 and 207 ^{mg/100g} in untreated, soaked, sprouted and cooked grains respectively.

4.3.8.6 Iron

Data in Table 16, 17 and 18 show the iron content of cowpea, kidney beans and moth beans affected by soaking, sprouting and cooking.

Data in Table 16 shows the iron content of cowpea, ^{which} was 11.77, 10.55, 6.97 and 7.48 mg/100 g respectively for untreated, soaked, sprouted and cooked seeds. There was a significant ($P < 0.05$) difference between untreated and treated counterparts. The maximum iron being in untreated and minimum in sprouted cowpea. The iron content was significantly ($P < 0.05$) higher in untreated kidney beans (Table 17) as compared to soaked, sprouted and cooked seeds. Sprouted and cooked seeds differed non significantly from each other but differed significantly ($P < 0.05$) from their soaked counterpart. Table 18 shows the iron content of moth beans. The iron content was significantly ($P < 0.05$) high in untreated seeds as compared to soaked, sprouted and cooked seeds. Soaked seeds had significantly ($P < 0.05$) higher iron than sprouted seeds but differed non significantly from cooked seeds. Iron content of sprouted and cooked seeds also differed non significantly from each other.

4.3.8.7 Zinc

Table 16, 17 and 18 show the effect of soaking, sprouting and cooking on the zinc content of cowpea, kidney beans and moth beans.

Table 16 shows the effect of different treatments on zinc content of cowpea. As clear from the Table cooked seeds had significantly ($P < 0.05$) higher zinc as compared to untreated, soaked and sprouted counterpart. A non significant difference was there between untreated (3.55 mg/100 g) and soaked (3.65 mg/100 g).

grains of cowpea. A significant ($P < 0.05$) difference was there between untreated and sprouted counterparts. Table 17 shows the zinc content of kidney beans. Cooked seeds had significantly ($P < 0.05$) higher zinc as compared to untreated soaked and sprouted counterparts. Soaked and sprouted kidney beans seeds had similar zinc content (3.45 mg/100 g). Table 18 shows the zinc content of moth bean. The cooked seeds had significantly ($P < 0.05$) higher zinc (2.87 mg/100 g) as compared to untreated and soaked counterparts but were non significant, from sprouted seeds. Untreated soaked and sprouted seeds also differed insignificantly from each other.

4.3.8.8 Copper

Table 16, 17 and 18 show the copper content of soaked, sprouted and cooked cowpea, kidney beans and moth beans.

Table 16 shows the copper content of cowpea. The copper content was significantly ($P < 0.05$) higher in cooked cowpea compared to untreated, soaked and sprouted seeds. The untreated seeds had higher copper (1.08 mg/100 g) than soaked (1.05 mg/100 g) and sprouted (1.07 mg/100 g) but statistically the difference was non significant.

Data in Table 17 depicts the copper content of kidney beans. The untreated, soaked, sprouted and cooked seeds had copper content as 1.79, 1.43, 1.67 and 2.33 mg/100 g respectively. The cooked grains had significantly ($P < 0.05$) higher copper content as compared to untreated, soaked and sprouted grains. Though, the untreated grains had higher copper than soaked and sprouted counterparts, the difference was statistically non significant. Data in Table 18 shows copper content of

Table 16 Effect of soaking, sprouting and cooking on macro and micro-nutrients of cowpeas

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Sodium (mg/100 g)	37.50	37.00	38.50	41.50	3.78
Potassium (mg/100 g)	126.17	129.67	133.17	135.00	18.15
Calcium (mg/100 g)	124.67	124.67	122.00	184.67	25.40
Magnesium (mg/100 g)	155.33	155.33	157.33	160.00	15.41
Phosphorus (mg/100 g)	414.00	406.00	403.33	403.33	7.58
Iron (mg/100 g)	11.77	10.55	6.97	7.48	1.00
Zinc (mg/100 g)	3.55	3.65	3.88	4.28	0.23
Copper (mg/100 g)	1.08	1.05	1.07	1.45	0.34

Table 17 Effect of soaking, sprouting and cooking on macro and micro-nutrients of kidney beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Sodium (mg/100 g)	416.67	406.67	425.00	475.00	50.84
Potassium (mg/100 g)	1013.42	811.67	1341.33	1013.42	81.50
Calcium (mg/100 g)	152.00	159.00	164.00	172.00	22.38
Magnesium (mg/100 g)	150.00	144.00	152.67	150.00	14.42
Phosphorus (mg/100 g)	410.00	398.00	389.00	389.00	1.88
Iron (mg/100 g)	16.22	12.52	7.35	7.92	4.73
Zinc (mg/100 g)	3.52	3.45	3.45	3.92	0.34
Copper (mg/100 g)	1.79	1.43	1.67	2.33	0.41

Table 18 Effect of soaking, sprouting and cooking on macro and micro-nutrients of moth beans

Parameter/Treatment	Untreated	Soaked	Sprouted	Cooked	CD (P<0.05)
Sodium (mg/100 g)	51.67	54.00	55.00	58.33	6.72
Potassium (mg/100 g)	124.67	85.00	103.33	130.00	13.09
Calcium (mg/100 g)	149.33	149.33	159.33	182.67	16.77
Magnesium (mg/100 g)	162.00	158.67	169.33	174.67	22.44
Phosphorus (mg/100 g)	230.00	218.00	207.00	207.00	1.63
Iron (mg/100 g)	8.50	7.67	6.92	7.42	0.52
Zinc (mg/100 g)	2.35	2.48	2.57	2.87	0.32
Copper (mg/100 g)	1.43	1.83	2.22	2.47	0.48

moth bean. The copper content was highest in cooked beans (2.47 mg/100 g) and it differed significantly from untreated and soaked counterparts. The sprouted seeds also had significantly higher copper (2.22 mg/100) than the untreated seeds. The difference was insignificant when soaked seeds were compared with untreated and cooked.

4.4 Biological evaluation

This part of the study deals with biological values of cowpea, kidney beans and moth beans. Biological evaluation has been recognized as a test criterion for assessing the quality of dietary proteins.

4.4.1 Feed consumed (Protein consumed)

Data in Table 19, 20 and 21 reveal the feed and the protein consumed by rats during 28 days period. Significant difference ($P < 0.05$) was observed between the feed and protein consumed in casein group and that of the respective legume groups. The feed consumed by the rats of the cooked legumes was significantly ($P < 0.05$) higher in comparison to untreated and sprouted counterparts. A significant ($P < 0.05$) difference was observed in feed consumption by rats fed on untreated and

Table 19 Effect of feeding untreated, sprouted and cooked cowpea on feed consumed, protein consumed, weight gain, feed efficiency ratio (FER) and protein efficiency ratio (PER)

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
Feed consumed (g)	270.53	122.64	124.03	148.98	24.84
Protein consumed (g)	27.05	12.26	12.40	14.90	2.50
Weight gain (g)	81.55	19.70	22.10	29.93	1.33
FER	0.30	0.16	0.19	0.20	0.02
PER	3.02	1.60	1.79	2.01	0.29

Table 20 Effect of feeding sprouted and cooked kidney beans on feed consumed, protein consumed, weight gain , feed efficiency ratio (FER) and protein efficiency ratio (PER)

Parameter/Group	Casein control	Sprouted	Cooked	CD (P<0.05)
Feed consumed (g)	270.53	137.23	162.20	16.28
Protein consumed (g)	27.05	13.72	16.20	1.62
Weight gain (g)	81.55	24.38	29.67	2.53
FER	0.30	0.17	0.18	0.03
PER	3.02	1.77	1.82	0.30

Table 21 Effect of feeding untreated, sprouted and cooked moth beans on feed consumed, protein consumed, weight gain, feed efficiency ratio (FER) and protein efficiency ratio (PER)

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
Feed consumed (g)	270.53	143.90	117.00	127.93	21.10
Protein consumed (g)	27.05	14.39	11.70	12.79	2.10
Weight gain (g)	81.55	24.74	26.30	29.78	1.73
FER	0.30	0.17	0.22	0.24	0.01
PER	3.02	1.72	2.25	2.33	0.14

untreated (19.70 g) cowpea. Weight gain was significantly ($P<0.05$) higher in groups fed on cooked cowpea in comparison to untreated and sprouted counterpart. In the kidney beans casein group had significantly ($P<0.05$) higher weight gain as compared to other groups (Table 19). Data for raw sample is not available for kidney beans as the rats died within second week of the trial due to flatulence. A significant ($P<0.05$) difference was there in sprouted and cooked legume for gain in weight. In moth bean casein, control group had significantly ($P<0.05$) higher weight gain (81.55g) followed by untreated 24.74 g. Sprouted 26.30 and cooked 29.78 g. A significantly ($P<0.05$) lower weight gain was there in untreated moth beans fed group when compared with sprouted and cooked legume which differed non-significantly from each other.

4.4.3 Feed efficiency ratio (FER) and Protein efficiency ratio (PER)

Data in Table 19, 20 and 21 show FER and PER of the rats fed on casein, untreated, sprouted and cooked. FER and PER was lowest for raw (0.16, 1.60) cowpea (Table 19) followed by sprouted (0.19, 1.79) and then cooked (0.20, 2.01) respectively. Significant ($P<0.05$) difference was observed in the FER and PER among the different groups of cowpea and casein control when compared with each other. Significant difference ($P<0.05$) was also there among untreated, sprouted and cooked grains. In case of kidney beans (Table 20) FER and PER was highest for casein control (0.30, 3.02) and it differed significantly from sprouted and cooked. In case of moth beans (Table 21) casein control group had significantly ($P<0.05$) higher than untreated, sprouted and cooked. Significant ($P<0.05$) difference was there between rats fed on untreated, sprouted and cooked

when compared. Cooked moth bean had significantly ($P<0.05$) higher FER as compared to untreated and sprouted grains fed groups.

4.4.4 Nitrogen balance

Data in Table 22, 23 and 24 show the effect of feeding untreated, sprouted and cooked cowpea, kidney beans and moth beans on nitrogen intake, excretion and retention. As is clear from the Table higher intake of nitrogen was in casein fed group for all the legumes and the difference was significant ($P<0.05$). The nitrogen intake for cooked legumes was significantly ($P<0.05$) higher as compared to respective untreated and sprouted counterparts of the three legumes. Nitrogen excretion was also higher in cooked cowpea, moth beans and kidney beans. It was significantly ($P<0.05$) higher as compared to sprouted and untreated counterparts. Similar trend was there for nitrogen retention. The casein control group had significantly ($P<0.05$) higher nitrogen retention as compared to untreated, sprouted and cooked legumes.

4.4.5 Apparent protein digestibility (APD), Biological value (BV), True Protein Digestibility (TPD), net protein utilization (NPU), Net protein ratio (NPR) and Protein retention efficiency (PRE)

4.4.5.1 Apparent protein digestibility (APD)

Data in Tables 25, 26 and 27 show the effect of feeding casein control untreated, sprouted and cooked moth beans, kidney beans and cowpea to rats on APD. As clear from the Tables, the APD was significantly ($P<0.05$) higher for cooked as compared to untreated and sprouted counterparts of respective legumes. The APD for cooked cowpea, kidney beans and moth bean was 55.68, 62.96 and 57.62 per cent respectively. A significant difference ($P<0.05$) was also observed between

Table 22 Effect of feeding untreated, sprouted and cooked cowpea on nitrogen intake, nitrogen excretion and nitrogen retention

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
Nitrogen intake (mg)	694.35	317.38	440.75	487.00	2.30
Nitrogen excreted (mg)	311.30	110.53	198.44	214.66	1.53
Nitrogen retained (mg)	383.10	206.65	226.07	272.32	5.43

Table 23 Effect of feeding sprouted and cooked kidney beans on nitrogen intake, nitrogen excretion and nitrogen retention

Parameter/Group	Casein control	Sprouted	Cooked	CD (P<0.05)
Nitrogen intake (mg)	694.35	263.00	382.75	2.23
Nitrogen excreted (mg)	311.30	123.00	144.25	1.71
Nitrogen retained (mg)	383.10	140.00	239.00	4.09

Table 24 Effect of feeding untreated, sprouted and cooked moth beans on nitrogen intake, nitrogen excretion and nitrogen retention

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
Nitrogen intake (mg)	694.35	343.30	404.00	437.25	2.24
Nitrogen excreted (mg)	311.30	108.30	144.00	148.22	2.54
Nitrogen retained (mg)	383.10	235.00	260.00	289.00	5.23

the soaked and sprouted seeds of cowpea when compared with each other. Significant ($P<0.05$) difference was also observed in between the soaked and sprouted seeds of moth beans.

4.4.5.2 Biological value (BV)

Data in Table 25, 26 and 27 show the effect of feeding casein control untreated, sprouted and cooked cowpea, kidney beans and moth beans to rats on biological value. As clear from the Tables the BV was significantly ($P<0.05$) higher for cooked grains as compared to untreated and sprouted counterparts of respective legumes. The biological value for cooked cowpea, kidney beans and moth bean was 58.68, 66.37 and 75.67 per cent respectively. A significant difference ($P<0.05$) was also observed between the soaked and sprouted grains of cowpea and moth beans.

4.4.5.3 True protein digestibility (TPD)

Data in Table 25, 26 and 27 show the effect of feeding casein control untreated, sprouted and cooked moth beans, kidney beans and cowpea to rats on TPD. As clear from the tables the TPD was significantly ($P<0.05$) higher for cooked as compared to untreated and sprouted counterparts of respective legumes. The TPD for cooked cowpea, kidney beans and moth bean was 71.87, 69.33 and 84.06 per cent respectively. A significant difference ($P<0.05$) was also observed between the soaked and sprouted seeds of cowpea and moth beans.

4.4.5.4 Net protein utilization (NPU)

Data in Table 25, 26 and 27 show the effect of feeding casein control untreated, sprouted and cooked moth beans, kidney beans and cowpea to rats on NPU. As clear from the tables the NPU was significantly ($P<0.05$) higher for cooked

as compared to untreated and sprouted counterparts of respective legumes. The NPU for cooked cowpea, kidney beans and moth bean was 42.18, 46.01 and 63.68 per cent respectively. A significant difference ($P < 0.05$) was also observed between the soaked and sprouted seeds of cowpea and moth beans when compared with their respective counterparts.

4.4.5.5 Net protein ratio (NPR)

Data in Table 25, 26 and 27 show the effect of feeding casein control untreated, sprouted and cooked cowpea, kidney beans and moth beans to rats on NPR. As clear from the tables the NPR was significantly ($P < 0.05$) higher for cooked as compared to untreated and sprouted counterparts of respective legumes. The NPR for cooked cowpea, kidney beans and moth bean was 1.64, 2.4 and 3.20 per cent respectively. A significant difference ($P < 0.05$) was also observed between the soaked and sprouted seeds of cowpea and moth beans.

4.4.5.6 Protein retention efficiency

Data in Table 25, 26 and 27 show the effect of feeding casein control untreated, sprouted and cooked moth beans, kidney beans and cowpea to rats on PRE. As clear from the Tables the PRE was significantly ($P < 0.05$) higher for cooked as compared to untreated and sprouted counterparts of respective legumes. The PRE for cooked cowpea, kidney beans and moth bean was 26.24, 39.04 and 51.20 per cent respectively. A significant difference ($P < 0.05$) was also observed between the soaked and sprouted seeds of cowpea and moth beans.

Table 25 Effect of feeding casein control, untreated, sprouted and cooked cowpea on apparent protein digestibility (APD), Biological value (BV), True protein digestibility (TPD), Net protein utilization (NPU), Net protein ratio (NPR) and Protein retention efficiency (PRE)

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
APD (%)	85.19	51.37	53.47	55.68	0.06
BV (%)	86.73	54.39	55.37	58.68	0.48
TPD (%)	96.70	63.62	68.35	71.87	0.08
NPU (%)	83.87	34.60	37.85	42.18	0.06
NPR	3.44	3.11	1.91	1.64	0.18
PRE (%)	55.04	47.68	30.56	26.24	0.01

Table 26 Effect of feeding casein control, sprouted and cooked kidney beans on apparent protein digestibility (APD), Biological value (BV), True protein digestibility (TPD), Net protein utilization (NPU), Net protein ratio (NPR) and Protein retention efficiency (PRE)

Parameter/Group	Casein control	Sprouted	Cooked	CD (P<0.05)
APD (%)	85.19	57.88	62.96	0.06
BV (%)	86.73	66.83	66.37	0.33
TPD (%)	96.70	62.20	69.33	0.12
NPU (%)	83.87	37.84	46.01	0.04
NPR	3.44	2.40	2.40	0.05
PRE (%)	55.04	38.40	39.04	0.07

Table 27 Effect of feeding casein control, untreated, sprouted and cooked moth beans on apparent protein digestibility (APD), Biological value (BV), True protein digestibility (TPD), Net protein utilization (NPU), Net protein ratio (NPR) and Protein retention efficiency (PRE)

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
APD (%)	85.19	51.77	55.87	57.62	0.59
BV (%)	86.73	58.61	65.49	75.67	0.42
TPD (%)	96.70	60.06	62.20	84.06	1.58
NPU (%)	83.87	35.12	40.99	63.68	0.02
NPR	3.44	2.72	3.16	3.20	0.03
PRE (%)	55.04	43.52	50.56	51.20	0.07

1.5.6 weight of internal organs

1.5.6.1 Liver

Data in Table 28, 29 and 30 show the effect of feeding casein control, untreated, sprouted and cooked cowpea, kidney beans and moth beans to rats on liver. As clear from the tables, the liver weight was significantly higher ($P<0.05$) for casein control group in the three legumes as compared to the untreated, sprouted and cooked counterparts. Table 28 shows the effect of feeding cowpea on liver weight. The rats on untreated group had significantly higher ($P<0.05$) liver weight as compared to sprouted and cooked groups. The cooked cowpea fed group too had significantly ($P<0.05$) higher liver weight compared to sprouted group.

Data in Table 29 shows the effect of soaking, sprouted and cooked kidney beans on liver weight. The difference was insignificant.

Data of Table 30 shows the liver weight of rats fed untreated, sprouted and cooked moth beans. The weight gain was significantly higher ($P<0.05$) in untreated group as compared to sprouted and cooked group. The weight in sprouted and cooked groups differed insignificantly.

1.5.6.2 Kidney

Table 28, 29 and 30 show the effect of feeding casein, untreated, sprouted and cooked cowpea, kidney bean and moth beans on kidney weight of the rats.

As clear from the Table 28, in case of cowpea the kidney weight was maximum in cooked group (0.73 g) and it was significantly ($P<0.05$) higher than untreated but insignificant in comparison to casein control and sprouted. In case of kidney beans Table 29 the kidney weight was significantly ($P<0.05$) higher in casein

Table 28 Changes in weight of organs (g/100 g body weight) in rats fed untreated, sprouted and cooked cowpea

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
Liver (g)	6.19	4.23	3.72	4.02	0.21
Kidney (g)	0.53	0.49	0.55	0.73	0.21
Spleen (g)	0.21	0.17	0.13	0.27	0.05

Table 29 Changes in weight of organs (g/100 g body weight) in rats fed beans sprouted and cooked kidney

Parameter/Group	Casein control	Sprouted	Cooked	CD (P<0.05)
Liver (g)	6.19	3.78	3.88	0.29
Kidney (g)	0.53	0.20	0.27	0.07
Spleen (g)	0.21	0.24	0.39	0.08

Table 30 Changes in weight of organs (g/100 g body weight) in rats fed untreated, sprouted and cooked mott beans

Parameter/Group	Casein control	Untreated	Sprouted	Cooked	CD (P<0.05)
Liver (g)	6.19	4.50	4.04	4.19	0.22
Kidney (g)	0.53	0.44	0.50	0.53	0.06
Spleen (g)	0.21	0.17	0.17	0.21	0.03

fed group as compared to sprouted and cooked feed groups. Cooked and sprouted counterparts also differed significantly ($P < 0.05$).

In the moth beans the maximum weight gain in kidney was for casein and cooked feed groups which had similar values (0.53). The difference in weight gain of casein, untreated, sprouted and cooked groups was insignificant.

4.5.6.3 Spleen

Data in Table 28, 29 and 30 shows the spleen weight of rats fed on casein, untreated sprouted and cooked cowpea, kidney beans and moth beans.

As shown in the tables the gain in spleen weight was significantly ($P < 0.05$) higher in cooked group as compared to casein, sprouted and untreated groups. Casein group had significantly higher ($P < 0.05$) spleen weight when compared with sprouted counterpart. Untreated and sprouted group had insignificant difference.

The spleen weight was significantly ($P < 0.05$) high in cooked group (0.39 g) as compared to casein and sprouted counterpart.

In the moth beans (Table 30) the spleen weight was same for casein and cooked group (0.21 g) and was significantly ($P < 0.05$) higher than untreated and sprouted counterparts which too had similar values (0.17 g).



DISCUSSION

CHAPTER-V

DISCUSSION

The results of the present investigation have been discussed in the ensuing sections as under :

5.1 Physical parameters

5.1.1 Colour

As is shown in Table 4, 5 and 6 and plates 1, 2 and 3 colour of all the legumes differs from each other because all the legumes taken for studies are of different variety. Meshram *et al.* (1980) reported brown colour for moth beans, which is similar to the present case. Shinde *et al.* (1991) reported the colour of cowpea varieties as white, red with white spots and red. Akinyele *et al.* (1986) also reported the coat colour of cowpea cultivars as white brown, maroon, brown, white and white grey. In the present case creamish colour of cowpea might be due to varietal differences.

5.1.2 Shape

Shape of the legumes cowpea, kidney beans and moth beans has been depicted in Table 4, 5 and 6. Although, kidney beans and cowpea have kidney shaped structure, but kidney bean is larger in size than the cowpea which can be due to varietal differences. Moth bean is cylindrical in shape.

5.1.3 Weight

A glance at Table 4, 5 and 6 show the difference in 100 kernel seed weight with subsequent treatments of the three legumes viz., cowpea, kidney beans and moth beans. As is clear from the Table cooking resulted in an higher increase in weight of legumes as compared to soaking and sprouting. The trend is similar for all the legumes. The increase in weight of cooked seeds might be due to increase in the temperature which increases water absorption capacity of the grains as a result weight increased. Similar results have been reported by Attia *et al.* (1994) for chickpea who revealed that cooking increased the weight of chickpea seeds. According to them weight in dry and cooked chickpea seeds was between 14.6 to 29.3 g and 30.1 to 68.1 g.

5.1.4 Density

Table 4, 5 and 6 depict the result of soaking, sprouting and cooking on density of cowpea, kidney beans and moth beans. Attia *et al.* (1994) reported density of dry and cooked chickpea was between 1.284 to 1.305 g/ml and 1.098 to 1.159 g/ml respectively. The present study is at par with these results. Similar results have been reported by Gupta (1995) for faba beans according to her density decreased when faba bean was cooked. In uncooked seeds density was 1.22 g/ml whereas, for cooked faba beans it was 1.05 g/ml. The decrease in the density of cooked, soaked and sprouted seeds might be due to the leaching out of total solids.

5.1.5 Length

As is clear from the data in Table 4, 5 and 6 untreated samples of cowpea, kidney beans and moth beans have significantly ($P < 0.05$) lower length as compared

to their respective treated counterparts. Sprouting increased the length of cowpea and kidney beans seeds; this might be due to the reason that seeds are in the germination stage and as a result there are changes in physical appearance. During cooking, slight decrease in length may be due to shrinkage of seeds or rupturing of seeds. A non-significant difference was observed between sprouted and cooked samples of moth beans, but the difference was significantly ($P < 0.05$) higher when compared with soaked counterparts.

5.1.6 Width

As is clear from the data in the Table 4, 5 and 6, the width is maximum for the cooked seeds in all the legumes. This might be due to the reason that during cooking, due to the increase in temperature and shrinkage of seed, the mid section of the seed swells up as a result width increased.

5.2 Proximate composition

5.2.1 Moisture

Moisture content of cowpea, kidney beans and moth beans is depicted in Table 7, 8 and 9. As clear from the table, cooked samples of cowpea, kidney beans and moth beans have significantly higher moisture content as compared to their respective soaked and sprouted counterparts but lower than raw grain. Difference observed in the moisture content may be due to the reason that after treatment the treated samples were dried in the cabinet tray drier then ground and used for estimation whereas, untreated grains were directly ground and used for estimation. In the moth bean sprouted sample had more moisture as compared to soaked, this may

be due to the smaller seed size. Pawar and Ingle (1988) reported higher moisture content for cooked moth bean as compared to raw. Padamshree *et al.* (1987) reported moisture content of germinated and cooked cowpea as 8.0 and 7.20 per cent respectively.

5.2.2. Ash

Data in Table 7, 8 and 9 show that the cooking resulted in an increase in ash content of cowpea kidney beans and moth beans. Ash content was higher in sprouted kidney beans and cowpea as compared to soaked seeds, whereas, in moth bean sprouting resulted in decreased ash content. The difference between the ash content of soaked and sprouted seeds might be due to the reason that maximum ash is inorganic and present in bran portion of the seed and during soaking, sprouting and cooking they are hydrolyzed resulting in increased mineral content of whole seed. Pawar and Ingle (1988) reported that cooking reduced the ash content as compared to soaking whereas, Abbey and Ibeh (1988) observed a significant difference in the ash content of heated cowpea when compared with that of raw cowpea flour.

5.2.3 Crude fat

Data in Table 7, 8 and 9 depict the crude fat content of the untreated, soaked, sprouted and cooked grains of cowpea, kidney beans and moth beans, respectively. As is clear from the Tables, during cooking, there is a slight decrease in crude fat content but it was significantly higher in all the legumes. Sprouting resulted in increase in crude fat which might have been due to physico-chemical changes during germination and also cooking. The increase in fat during soaking, sprouting

and cooking might have also been due to breakdown of the germ portion and fat is mainly present in germ portion of the seed.

5.2.4 Crude protein

Table 7, 8 and 9 show the effect of soaking sprouting and cooking on the crude protein content of cowpea, kidney beans and moth beans. As is clear from the tables, soaking and cooking resulted in a decrease in crude protein in cowpea, kidney beans and moth beans. This may be due to the reason that during soaking some of the proteins leach out and during cooking denaturation of proteins occurs. In sprouted seeds an increase in the proteins might be due to synthesis of new proteins. Similar results have been reported by Pawar and Ingle (1988) who reported a decrease in protein content by 17.4 per cent on cooking and on soaking protein content decreased from 23.5 to 21.3 per cent in moth beans. Padmashree *et al.* (1987) reported protein content in germinated cowpea flour was 25.94 per cent whereas, in pressure cooked cowpea flour it was 20.67 per cent. These results are at par with present investigation.

5.2.5 Crude fibre

Data in Table 7, 8 and 9 depict the crude fibre content of untreated, soaked sprouted and cooked cowpea, kidney beans and moth beans, respectively.

As is clear from the Table, crude fibre increased in sprouted and cooked cowpea and kidney beans as compared to untreated grains which might be due to formation of protein fibre complexes (Bressani, 1993). A decrease in fibre was observed in the soaked samples in moth beans and cowpea which might be due to the chemical composition of dietary fibre because cowpea and moth bean have

slightly thin bran coat. Similar results have been reported by Barampama and Simard (1995). According to Gupta (1995) cooked and germinated faba bean had 3.37 and 3.43 per cent crude fibre in comparison to 2.34 per cent of untreated faba bean. The results of the present study are at par with these investigations. Youssef *et al.* (1987) reported no differenceⁱⁿ soaked and cooked faba beans.

5.3 Nutritional composition

5.3.1 Non-protein nitrogen and True protein

Data in Table 7, 8 and 9 show the NPN and TP of the cowpea, kidney beans and moth beans respectively. As is evident from the data NPN content was higher in all three untreated legumes and during soaking, sprouting and cooking NPN decreased. The decreased was maximum in cooked samples of cowpea and moth beans whereas less in sprouted kidney beans when compared with other treated counterparts. In case of TP soaking, sprouting and cooking resulted in an increase in TP when compared to its untreated counterparts incase of kidney beans and moth beans whereas in case of soaked cowpea a slight decrease in TP was there. Similar results have been reported by Gupta (1995). A decrease in NPN and increasing in TP during soaking, sprouting and cooking due to reason that non-protein substance might leach out. Some of the enzymes present in the seeds get activated during germination, leading to increased protein content of the seeds, thus reducing the non-protein content.

5.3.3 Sugars

5.3.3.1 Total sugars

Data in Table 10, 11 and 12 show the effect of soaking, sprouting and cooking on
incr
incr

and kidney beans but no significant difference was observed between soaked and sprouted counterparts. Increase in sugars might be due to the seed respiration and biochemical degradation of starch. Due to ^{the} process of glycolysis starch is converted to sugars. Kataria *et al.* (1990) reported decrease in total sugars as they had discarded the water used in soaking and cooking whereas, Kataria and Chauhan (1988) and Youssef *et al.* (1987) reported that cooking brought significant increase in sugars. These results are at par with the present study as the water was not discarded in this study too.

5.3.3.2 Reducing sugars

Data in Table 10, 11 and 12 show the effect of soaking, sprouting and cooking on the reducing sugars of cowpea, kidney beans and moth beans. As clear from the data, the reducing sugars were increased on cooking. This may be due to seed respiration and biochemical degradation of starch to glucose and maltose. Similar results have been reported by Kataria and Chauhan (1988) and Youssef *et al.* (1987).

5.3.3.3 Non-reducing sugars

Data in Table 10, 11 and 12 show the effect of soaking, sprouting and cooking on the non-reducing sugars of cowpea, kidney beans and moth beans. As clear from the data, non-reducing sugars increased on cooking. This might be due to seed respiration and biochemical changes within the seed. Similar results have been reported by Kataria and Chauhan (1988) and Youssef *et al.* (1987).

5.3.4 Starch

As is evident from Table 10, 11 and 12 soaking, sprouting and cooking resulted decreased starch content of cowpea, kidney beans and moth beans., Significantly higher starch content was noticed in untreated legume as compared to treated counterparts. This might be due to the reason that the process of seed respiration which increased and resulted in more hydrolyses of stored carbohydrates in seed grains. During cooking, heat further initiates hydrolysis of starch. Similar results were observed by Kataria *et al.* (1990) and Jood *et al.* (1986).

5.3.5 Energy

Soaking, sprouting and cooking resulted in decrease in the calorific value but the decrease was not very high (Table 10, 11 and 12). This decrease might be due to hydrolysis of starch due to seed respiration. During sprouting, seed was in germination stage as a result more chemical changes were there resulting in decreased starch. A decrease was observed in crude protein content during soaking and cooking which might have affected the calorific value. Increase in crude fibre might have also resulted in decreased energy. Jood *et al.* (1986) and Kataria *et al.* (1990) reported similar results.

5.3.6 Dietary fibres

5.3.6.1 Neutral detergent fibre (NDF)

Data in Tables 10, 11 and 12 show the NDF content of untreated, soaked, sprouted and cooked cowpea, kidney beans and moth beans. As clear from the tables cooked legumes had significantly ($P < 0.05$) higher NDF as compared to soaked, sprouted and untreated legumes. This increase in cooking is probably due

to formation of new components such as maillard products or those formed from condensation of proteins and tannins which may be isolated as fibre components (Vidal Valverde and Frias, 1991). In case of cowpea soaking, sprouting and cooking resulted in increased NDF whereas in case of kidney beans a decrease in soaked and sprouted and in moth beans a decrease in sprouted counterparts was there as compared to untreated and cooked counterparts. According to Vidal Valverde and Frias (1992) ADF content increased on soaking and cooking of lentils. The germination method and duration of germination also has a significant effect on the dietary fibre components. Bressani (1993) reported that an increase in fibre content in soaked and cooked legume might be due to formation of protein fibre complex due to chemical modifications induced by soaking and cooking.

5.3.6.2 Acid detergent fibre (ADF)

The data in Table 10, 11 and 12 show the ADF content of cowpea, kidney beans and moth beans respectively. As is clear from the Tables, the cooked grains had higher ADF as compared to untreated, soaked and sprouted counterparts. Soaked and sprouted seeds except in case of cowpea had lower ADF compared to untreated. The ADF is known to increase on cooking (Johnston and Oliver, 1982; Herranz *et al.*, 1981).

5.3.6.3 Hemicellulose

Data in Table 10, 11 and 12 show the hemicellulose content of cowpea, kidney beans and moth beans. In case of cowpea, untreated seeds had significantly ($P < 0.05$) lower hemicellulose and in case of moth beans cooked grains had significantly ($P < 0.05$) lower hemicellulose compared to untreated counterparts.

hemicellulose. According to Vidal Valverde and Frias (1992), the hemicellulose content decreased on soaking and cooking of lentils. Decrease in hemicellulose might have been due to the reason that during soaking, sprouting and cooking there was hydrolysis of starch, carbohydrates and other nutrient components. As a result, husk which is rich in bran was left behind and hemicellulose is the constituent of bran portion.

5.3.7 Anti-nutritional factors

5.3.7.1 Phytic acid

Data in Table 13, 14 and 15 show the phytic acid content of cowpea, kidney beans and moth beans. As clear from the Tables the phytic acid content was significantly ($P < 0.05$) higher in untreated legume as compared to treated counterparts. A significant decrease in phytate content was also observed by Kaur and Kapoor (1990). The decrease in phytin phosphorus content of legume seeds during soaking and sprouting can be attributed to leaching of phytate ions into soaking water under the influence of concentration gradient. A decrease in phytin phosphorus in moth beans was also observed by Pawar and Ingle (1987). It was suggested that enzymatic hydrolysis of phytate during germination resulted in decreased phytic acid. The decrease in phytic acid content due to cooking may be attributed to the formation of insoluble complexes between phytate and other components. Similar results were observed by Bakr and Gawish (1991).

5.3.7.2 Phytate phosphorus

As is clear from Table 13, 14 and 15 the phytate phosphorus was significantly ($P < 0.05$) high in untreated legume and decreased during soaked, sprouted and

cooked counterparts of the respective legumes. Decrease might have been due to decrease in the phytic acid content.

5.3.7.3 Non-phytate phosphorus

Data in Table 13, 14 and 15 show non-phytate phosphorus content of cowpea. The non-phytate phosphorus was highest in soaked seeds of cowpea and kidney beans whereas, untreated moth beans had highest non-phytate phosphorus.

5.3.8 Minerals

5.3.8.1 Calcium

Data in Table 16, 17 and 18 depicted the calcium content of cowpea, kidney beans and moth beans. The cooked grains of cowpea had significantly ($P < 0.05$) higher calcium content. Similar was the trend for kidney beans and moth beans. Kaur (1977) found 2.73 per cent increase in calcium on sprouting of rice beans. The increase in calcium content after cooking may be due to gradual decrease in phytate content.

5.3.8.2 Potassium

Data in Table 16, 17 and 18 show the potassium content of cowpea, kidney beans and moth beans. The potassium content was maximum for cooked seeds in cowpea and moth beans whereas, sprouted grains of kidney beans had maximum potassium and least was in the soaked counterparts. Youssef *et al.* (1987) has reported an increase in the mineral components.

5.3.8.3 Sodium

Data in Table 16, 17 and 18 show the sodium content of cowpea, kidney beans and moth beans. A significantly higher ($P < 0.05$) sodium content was there in cooked

seeds as compared to untreated. These high levels could be due to leaching out of minerals from seed coats in the soaking medium, which is thereby reabsorbed by the imbibed cotyledons and due to osmosis mineral from bran enter the endosperm part of the seed. This may be also due to leaching in of minerals as potable tap water was used. Youssef *et al.* (1987) have reported similar results.

5.3.8.4 Phosphorus

Data in Table 16, 17 and 18 show the phosphorus content of cowpea, kidney beans and moth beans. The phosphorus content was highest for untreated seeds and similar in sprouted and cooked counterparts of the respective legume. These high levels could be due to leaching out of minerals from seed coats in the soaking medium, which is thereby reabsorbed by the imbibed cotyledons and due to osmosis mineral from bran enter the endosperm part of the seed. This might be also due to leaching in of minerals as potable tap water was used. The results are similar to those of Duhan *et al.* (2004) for pigeon pea.

5.3.8.5 Iron

Data in Table 16, 17 and 18 show the iron content of cowpea, kidney beans and moth beans. As clear from the data, iron content was maximum in untreated seeds and decreased with treatments. A significant loss in iron content of cooked rice beans was reported by Kaur (1979). This might be due to leaching out of iron in the soaking water.

5.3.8.6 Zinc

Data in Table 16, 17 and 18 show the zinc content of cowpea, kidney beans and moth beans. The zinc content was highest for the cooked counterparts and

lowest for untreated seeds. This could be due to leaching out of minerals from seed coats in soaking medium, which is thereby reabsorbed by the imbibed cotyledons and due to osmosis mineral from bran enter the endosperm part of the seed. This might be due to leaching in of minerals as potable tap water was used. Youssef *et al.* (1987) has reported an increase in the mineral components. Rincon *et al.* (1993) reported a decrease of 12.2 per cent of zinc in pressure cooked faba bean.

5.3.8.7 Copper

Data in Table 16, 17 and 18 show the copper content of cowpea, kidney beans and moth beans. The copper content increased on cooking. These high levels could be due to leaching out of minerals from seed coats in the soaking medium, which is thereby reabsorbed by the imbibed cotyledons and due to osmosis mineral from bran enter the endosperm part of the seed. This might be due to leaching in of minerals as potable tap water was used. Similar results have been reported by Youssef *et al.* (1987).

5.4 Biological evaluation

5.4.1 Feed consumed, Protein consumed

Data in Table 19, 20 and 21 show the effect of feeding casein, untreated, sprouted and cooked legumes on the feed and protein consumed for cowpea, kidney beans and moth beans. The feed consumed by the casein control group was significantly higher ($P < 0.05$) as compared to treated counterparts. The feed consumed was maximum for the cooked counterparts of cowpea and kidney

beans. The data for the untreated seeds of kidney beans is not available as all the rats of this group died within second week of trial.

5.4.2 Weight

Data in Table 19, 20 and 21 show the weight gain by rats on casein control, untreated, sprouted and cooked legumes on the feed and protein consumed for cowpea, kidney beans and moth beans. In the cowpea casein control group had significantly ($P<0.05$) higher weight gain compared to untreated, sprouted and cooked counterparts. Significant ($P<0.05$) difference was observed between rats fed cooked cowpea when compared with untreated and sprouted counterparts. For kidney beans casein control group had significantly higher ($P<0.05$) weight gain (Table 20). A significant ($P<0.05$) difference in sprouted and cooked kidney beans was there. In the moth beans casein control group had significantly ($P<0.050$) higher weight gain (81.55 g) followed by cooked (27.87 g) sprouted (26.30 g) and untreated (24.74 g) moth beans. The increased weight gain in cooked group was due to the increased biological value of the feed.

5.4.3 Feed efficiency ratio (FER) and Protein efficiency ratio (PER)

Data in Table 19, 20 and 21 show the FER and PER of the rats fed on control, untreated, sprouted and cooked legumes on the feed and protein consumed for cowpea, kidney beans and moth beans. Significant ($P<0.05$) difference was observed in the FER and PER among the different groups of cowpea and casein control when compared. In the kidney beans FER and PER was highest for casein control which differed significantly ($P<0.05$) from sprouted and cooked counterpart. In the

moth beans, FER and PER was highest for casein control. Cooked moth bean had significantly ($P<0.05$) higher FER and PER in comparison to untreated and sprouted groups.

5.4.4 Nitrogen balance

Data in Table 22, 23 and 24 show the effect of feeding casein, untreated, sprouted and cooked legumes on the feed and protein consumed for cowpea, kidney beans and moth beans on nitrogen intake, nitrogen excretion and nitrogen retention. As is clear from the data, the highest nitrogen intake, retention and excretion was in casein control group in all the legumes. A significantly ($P<0.05$) higher nitrogen intake nitrogen retention and excretion was in cooked group when compared to sprouted and untreated counterparts. The low nutritional value and high fecal excretion of nitrogen in legumes have been attributed to the presence of residual anti-nutritional factors, efficiency of sulphur amino acids and reduced protein digestibility.

5.4.5 Apparent protein digestibility (APD), Biological value (BV), True protein digestibility (TPD), Net protein utilization (NPU), Net protein ratio (NPR) and Protein retention efficiency (PRE)

5.4.5.1 Apparent protein digestibility (APD)

Data in Table 25, 26 and 27 show the APD of groups of rats fed on casein control, untreated, sprouted and cooked to cowpea, kidney beans and moth beans. As depicted in the Tables, cooked legumes had higher APD as compared to untreated

to the improved digestibility. Similarly improvement in digestibility of sprouted grain in comparison to untreated, resulted in increased APD in sprouted legume.

Chandershekar and Jayalakshmi (1978) reported improved biological value after cooking and roasting which may be due to improved amino acid composition.

Wu et al. (1995) suggested that cooking resulted in the increased APD due to the reason that proteolytic enzyme inhibitors and toxic factors in beans were inactivated by heat processing.

5.4.5.2 Biological value (BV)

Data in Table 25, 26 and 27 show the BV of groups of rats fed on casein control, untreated, sprouted and cooked to cowpea, kidney beans and moth beans. As depicted in the Tables, cooked legumes had higher BV as compared to untreated and sprouted counterparts. The increase in BV observed in cooked legumes is due to the improved digestibility. Similarly improvement in digestibility of sprouted grain in comparison to untreated, resulted in increased BV in sprouted legume.

Chandershekar and Jayalakshmi (1978) reported improved biological value after cooking and roasting which may be due to improved amino acid composition.

Wu et al. (1995) suggested that cooking resulted in increased BV due to the reason that proteolytic enzyme inhibitors and toxic factors in beans are inactivated by heat processing.

5.4.5.3 True protein digestibility (TPD)

Data in Table 25, 26 and 27 show the TPD of groups of rats fed on casein control, untreated, sprouted and cooked to cowpea, kidney beans and moth beans. As depicted in the Tables, cooked legumes had higher TPD as compared to untreated

and sprouted counterparts. The increase in TPD observed in cooked legumes is due to the improved digestibility. Similarly improvement in digestibility of sprouted grain in comparison to untreated, resulted in increased TPD in sprouted legume.

Chandershekar and Jayalakshmi (1978) reported improved biological value after cooking and roasting which might be due to improved amino acid composition. Wu et al. (1995) suggested that cooking resulted in increased TPD due to the reason that proteolytic enzyme inhibitors and toxic factors in beans are inactivated by heat processing.

5.4.5.4 Net protein utilization (NPU)

Data in Table 25, 26 and 27 shows the NPU of groups of rats fed on casein control, untreated, sprouted and cooked cowpea, kidney beans and moth beans. As depicted in the Tables cooked legumes had higher NPU as compared to untreated and sprouted counterparts. The increase in NPU observed in cooked legumes is due to the improved digestibility. Similarly improvement in digestibility of sprouted grain in comparison to untreated, resulted in increased NPU in sprouted legume.

Chandershekar and Jayalakshmi (1978) reported improved biological value after cooking and roasting which may be due to improved amino acid composition. Wu et al. (1995) suggested that cooking resulted in increased NPU due to the reason that proteolytic enzyme inhibitors and toxic factors in beans are inactivated by heat

5.4.5.5 Net protein ratio (NPR)

Data in Table 25, 26 and 27 shows the NPR of groups of rats fed on casein control, untreated, sprouted and cooked to cowpea, kidney beans and moth beans. As depicted in the Tables cooked legumes had higher NPR as compared to untreated and sprouted counterparts. The increase in NPR observed in cooked legumes is due to the improved digestibility. Similarly improvement in digestibility of sprouted grain in comparison to untreated, resulted in increased NPR in sprouted legume.

Chandershekar and Jayalakshmi (1978) reported improved biological value after cooking and roasting which may be due to improved amino acid composition. Wu et al. (1995) suggested that cooking resulted in increased NPR due to the reason that proteolytic enzyme inhibitors and toxic factors in beans are inactivated by heat processing.

5.4.5.6 Protein retention efficiency (PRE)

Data in Table 25, 26 and 27 show the PRE of groups of rats fed on casein control, untreated, sprouted and cooked to cowpea, kidney beans and moth beans. As depicted in the tables cooked legumes had higher PRE as compared to untreated and sprouted counterparts. The increase in PRE observed in cooked legumes is due to the improved digestibility. Similarly improvement in digestibility of sprouted grain in comparison to untreated, resulted in increased PRE in sprouted legume.

Chandershekar and Jayalakshmi (1978) reported improved biological value after cooking and roasting which may be due to improved amino acid composition. Wu et al. (1995) suggested that cooking resulted in increased PRE due to

the reason that proteolytic enzyme inhibitors and toxic factors in beans are inactivated by heat processing.

5.5.6 Organ weights

5.5.6.1 Liver

Data in Table 28, 29 and 30 show the liver weight of rats as affected by different diets. As is evident from the tables the casein control group had significantly ($P < 0.05$) higher gain in weight for liver. The weight gain was significantly ($P < 0.05$) higher in untreated group of cowpea and moth beans. The weight gain in sprouted and cooked group differed insignificantly in kidney beans and moth beans.

5.5.6.2 Kidney

Data in Table 28, 29 and 30 show the kidney weight of rats as affected by different diets. In the cowpea the maximum kidney weight was in cooked group whereas in the kidney beans casein control group had higher kidney weight and in moth beans the weight gain was similar for casein and coked feed groups.

5.5.6.3 Spleen

Data in Table 28, 29 and 30 show the spleen weight of rats as affected by different diets. The spleen weight for significantly ($P < 0.05$) higher in cooked group in cowpea and kidney beans whereas in the moth beans it was similar to casein group. Casein group had higher spleen weight in comparison to untreated and sprouted counterparts of the legume.



SUMMARY

CHAPTER-VI

SUMMARY

Legumes are rich and economical source of protein in human diet. Before consumption, the legumes are processed in several ways to improve their palatability and organoleptic qualities. The processing techniques may affect the nutritional composition of the legumes. So the present study on cowpea, moth beans and kidney beans was conducted in the Department of Food Science and Nutrition, College of Home Science during the year 2000-2001, to study the effect of soaking, sprouting and cooking on the physico chemical characteristics, micro and macro elements, antinutritional factors of the legumes and on the biological utilization of proteins from the diets.

The colour of the cowpea was cream, moth bean was light brown and kidney beans was light brown with red spots. Shape of both cowpea and kidney beans was kidney shaped but cowpea was smaller in size than kidney bean whereas, moth bean was cylindrical. Weight was significantly ($P < 0.05$) higher in case of cooked samples of cowpea, kidney beans and moth beans (40.33, 62.67 and 7.77 g respectively). Soaking and sprouting of seeds also resulted in increased weight due to water absorption. The density was significantly ($P < 0.05$) higher in the untreated legumes and decreased on subsequent treatments. Soaking, sprouting and cooking increased the length and width of cowpea, kidney beans and moth beans. A

significant ($P < 0.05$) difference was observed in length and width of untreated counterpart.

Significant ($P < 0.05$) difference in the moisture content was observed in cooked grains of the cowpea, kidney beans and moth beans as compared to untreated, soaked and sprouted grains. The difference was insignificant in soaked and sprouted grains of the three legumes. Ash content was significantly ($P < 0.05$) higher in cooked samples of kidney beans and moth beans (4.18 and 3.92%) as compared to untreated, soaked and sprouted grains except in the cowpea where statistically no significant difference was ^{found} there in ash content. Soaking, sprouting and cooking resulted in an increase in crude fat content of all the three legumes. An insignificant difference was observed in crude fat content of soaked, sprouted and cooked grains but these differed significantly ($P < 0.05$) from untreated counterpart. Sprouting increased the protein content of legumes whereas, during soaking and cooking a decrease was observed. Significant difference ($P < 0.05$) was observed between untreated and treated seeds. There was increase in crude fibre content of cowpea and kidney beans after cooking whereas, in moth beans a decrease was observed on cooking. The NPN content of all the legumes decreased significantly ($P < 0.05$) in treated counterparts as compared to untreated. Soaking, sprouting and cooking resulted in decreased true protein content of moth beans whereas, true protein of cowpea and kidney beans increased on cooking. Significantly ($P < 0.05$) higher total sugars, reducing sugars and non-reducing sugars were observed in cooked cowpea, kidney beans and moth beans. The total sugars of ^{cooked} cowpea, kidney beans and moth beans was 11.67, 9.92 and 12.0 per cent respectively. A non-

significant difference was observed in soaked and sprouted grains for total sugars, reducing sugars and non-reducing sugars in the three legumes. The energy content of untreated cowpea, kidney beans and moth beans was 422.32, 365.76 and 406.59 Kcal respectively. Soaking, sprouting and cooking resulted in decreased energy content. The energy content of cooked grains was significantly ($P<0.05$) decreased in all the three legumes. Soaking, sprouting and cooking also resulted in decreased starch content of all three legumes. The untreated grains had significantly ($P<0.05$) higher starch content than soaked, sprouted and cooked counterparts of the legumes.

Significantly ($P<0.05$) higher NDF was in cooked grains of cowpea, kidney beans and moth beans. Soaking, sprouting and cooking resulted in increased NDF of cowpea whereas, soaked and sprouted counterparts of kidney beans and moth beans resulted in decreased NDF. An insignificant difference was there in ADF content of untreated and soaked grains of cowpea but cooked grains had significantly ($P<0.05$) higher ADF. In moth beans and kidney beans, soaking and sprouting resulted in decrease in ADF whereas, cooking resulted in increase in comparison to untreated. In the hemicellulose similar was the trend for cowpea whereas, in kidney beans soaked and sprouted had higher hemicellulose and in the moth beans sprouting and cooking reduced hemicellulose.

The phytic acid content of untreated cowpea, kidney beans and moth beans was 306.33, 338.33 and 311.33 mg/100 g. Phytic acid was significantly ($P<0.05$) reduced on soaking, sprouting and cooking. A similar trend was followed phytate phosphorus with least being in cooked cowpea, kidney bean and moth beans. The

non-phytate phosphorus was highest in soaked cowpea and kidney beans whereas in ~~the~~ moth beans in the untreated group it was higher.

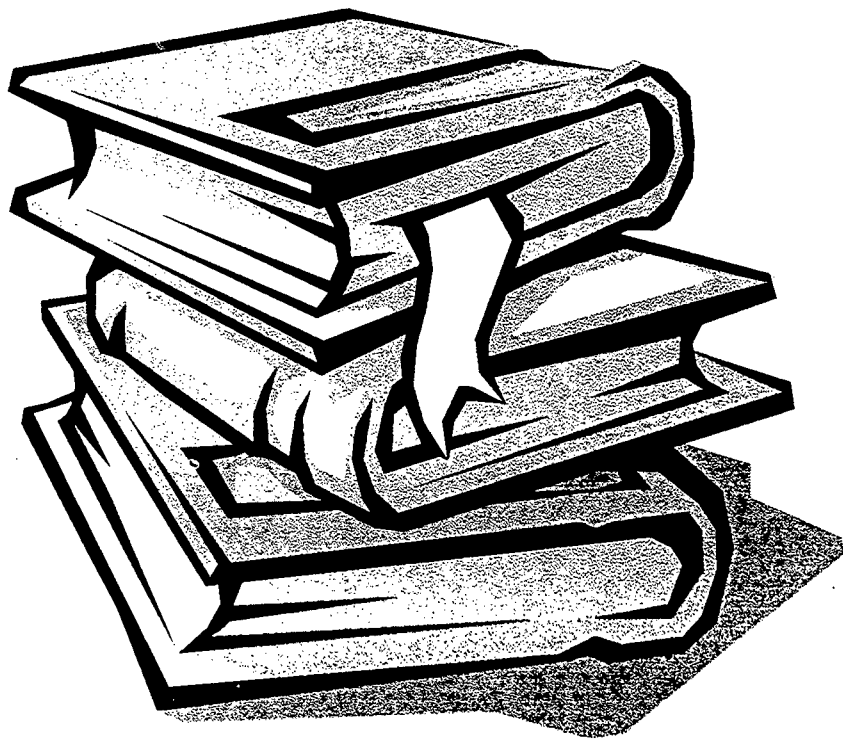
A significant ($P < 0.05$) increase in micro and macro minerals during cooking was observed in sodium, potassium calcium, magnesium, zinc and copper except for phosphorus and iron. An insignificant difference in minerals was there between soaked and sprouted counterparts.

Biological trial for evaluating the protein quality of cowpea, kidney beans and moth beans was conducted on rats, feeding them diets based on untreated, sprouted and cooked legumes. The data for the untreated kidney beans is not available because all the rats of this group died within second week of trial. Significantly higher ($P < 0.05$) feed consumption was in rats fed casein control diet. Cooked legume diet had significantly ($P < 0.05$) higher feed consumption and weight gain as compared to untreated and sprouted groups. FER and PER was significantly ($P < 0.05$) higher in cooked grains of cowpea and moth beans whereas, insignificant difference between sprouted and cooked diet of kidney beans.

Nitrogen intake, retention and excretion was significantly ($P < 0.05$) higher in casein group as compared to the legume groups, cooking resulted in higher feed intake by rats in comparison to sprouted and untreated groups.

APD, BV, TPD and NPU was significantly ($P < 0.05$) higher in casein group as compared to legume groups. The cooked group had significantly ($P < 0.05$) higher APD, BV, TDP and NPU for all the legumes except in ~~the~~ BV for kidney beans in which sprouted had higher values. The NPR and PRE was significantly higher in casein group. But in ~~the~~ legumes untreated cowpea had higher NPR and PRE.

as compared to soaked and cooked counterparts. In the kidney beans NPR was similar for sprouted and cooked grains and PRE was more in cooked grains. NPR and PRE was maximum in cooked in the moth beans. The casein group had significantly ($P < 0.05$) higher weight of the organs viz., liver, kidney and spleen. The weight gain in liver was significantly ($P < 0.05$) higher in untreated group of cowpea and moth bean. The casein control group of cowpea had higher kidney weight whereas, in the moth bean it was similar for casein and cooked feed group had higher kidney weight than casein group. The spleen weight was significantly higher ($P < 0.05$) for cooked group.



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LITERATURE CITED

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Appendix-I

Analysis of variance

Row	A	B	C	D	E	etc.	Sum	Mean
1								Sum
2								of
3								rows
4								
5								
Etc.								
Sum			Sum of columns					Grand sum

Analysis of variance

Source of variation	Sum of squares	Degree of freedom	Mean square	F (cal.)
Between treatments	C	No. of column1	$\frac{C}{\text{No. of column}} = X$	x/y
With in treatments	D	No. of rows-1 + No. of column-1x No. of rows-1	$\frac{D}{\text{No. of rows-1 +No. of column-1xNo. of rows-1}}$	

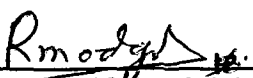
- A. $(\text{Grand Sum})^2/\text{toal number of observations} = C$
- B. $(\text{Square of all observations and add}) - C$
- C. $(\text{Sum of observations in one column squar columns/number of rows}) - C$
- D. $(\text{Sum of observations in one row squared anc columns})$
- E. $(B) - (C) + (D) = A$
If F calculated is greater than F tabulated
calculated, otherwise CD is not calculated.
CD = A x t at different levels of significance.

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|------------------------------------|--|
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ABSTRACT

Legumes are major sources of dietary proteins in the diets of vegetarian population of developing countries like India. Before consumption these are processed in several ways, which might affect their nutritional quality. In the present study an attempt was made to see the effect of soaking, sprouting and cooking on the physico-chemical, nutritional and biological quality of three legumes viz. kidney beans, moth beans and cowpea. Results of the investigations have shown that these pre-processing treatments resulted in an increase in physical parameters like length and width. Soaking, sprouting and cooking resulted in an increase in crude fat, crude fibre, true protein, carbohydrates, different dietary fiber and mineral constituents, but these treatments resulted in a decrease in calorific value and phytic acid biological quality of the treated legume was also higher. Cooking improved the quality of legumes as shown by the animal study.


(Signature of Major Advisor)


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