STORAGE EFFECT ON THE NUTRITIONAL QUALITY OF THREE MAJOR PULSES

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UNIVERSITY OF DHAKA, DHAKA, BANGLADESH

August 2003

STORAGE EFFECT ON THE NUTRITIONAL QUALITY OF THREE MAJOR PULSES

A THESIS SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
IN
BIOCHEMISTRY

OF THE
UNIVERSITY OF DHAKA

BY

MD. SHAHJAHAN

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DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
UNIVERSITY OF DHAKA, DHAKA, BANGLADESH

August 2003

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Dr. Md. Amiruzzaman, Ph.D.
Chief Scientific Officer (Retired)
Flat # W6, 12 lake Circus
Kalabaghan, Dhaka- 1205

Dr. H. K. M. Yusuf, Ph.D.
Professor
Department of Biochemistry
& Molecular Biology
University of Dhaka,
Dhaka, Bangladesh

Certificate

Certified that **Mr. Md. Shahjahan** has submitted the thesis entitled **"Storage Effect on The Nutritional Quality of Three Major Pulses"** in fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in Biochemistry of the University of Dhaka, Bangladesh. This is a piece of original research work carried out under our supervision. This thesis or any part thereof has not been submitted anywhere for any other degree or diploma.

Dr. Md. Amiruzzaman, Ph. D.
Supervisor

Prof. Dr. H. K. M. Yusuf, Ph. D.
Supervisor

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Dedicated to

the departed soul of

my beloved parents

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Md. Shahjahan

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ABSTRACT

The storage effects on the physico-chemical and nutritional qualities of three major pulses namely, lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.) and khesari (*Lathyrus sativus* L.) were studied. Six different types of storage containers viz. plain earthen *motka*, plain tin container, polythene lined jute bag, polythene lined *motka*, coal-tar coated *motka* and improved tin container were used. The storage period was up to 12 months. The effect of storage was studied at 3, 6, 9, and 12 months and compared with the parameters of fresh pulses. All experiments were repeated for three consecutive years to see any year-to-year variations. Physico-chemical parameters included moisture, germination, cooking time, water absorption and solids dispersed, and nutritional parameters included total protein, protein composition, protein digestibility, minerals and trace elements, crude fiber, free sugar and starch, starch digestibility (rapidly digestible starch and slowly digestible starch) and antinutritional factors e.g. trypsin inhibitor, chymotrypsin inhibitor, ODAP (of khesari) and phytic acid. All determinations were done by standard methods. The seeds stored in plain earthen *motka* absorbed significantly higher amount of moisture from the environment, which deteriorated the quality of seeds reflected in various physico-chemical as well as nutritional properties of all pulses. In this container, fungal infection, cooking time, and total sugar and reducing sugar of all the pulses increased significantly at a higher rate. On the other hand, water absorption, amount of solids dispersed, protein and starch content, digestibility of protein, phytic acid and germination percentage decreased significantly at a higher rate. The rapidly

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digestible starch decreased, but the slowly digestible starch increased significantly, giving an explanation of increased cooking time and decreased digestibility of stored pulses. The porousness of the earthen *motka* allowed moisture absorption from the environment. The seeds stored in different improved containers where moisture absorption was protected by improvement or modification, the changes in the physico-chemical properties were minimum and the nutritional parameters were retained close to fresh sample. Minor changes that occurred were presumably due to aging of seeds. SDS-electrophoretic analysis of protein profile of the pulses showed that the number and molecular weight of protein varied from pulse to pulse and between fresh and stored sample. For example, some lower molecular weight proteins showed up upon storage. Among the improved containers polythene lined jute bag, polythene lined *motka* and improved tin container were better for retention of various physico-chemical properties and nutritional qualities of the pulses. The farmers and traders may be suggested to use these improved types of containers for safe storage of their pulses.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical chemists
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of statistics
BRRI	Bangladesh Rice Research Institute
CDP	Crop Diversification Program
CIA	Chymotrypsin Inhibitor Activity
CRD	Completely Randomized Design
CU	Chymotrypsin Unit
DAP	Diamino Propionic Acid
DNS	Dinitrosalicyclic Acid
IVPD	*In vitro* Protein Digestibility
MS	Mean Square
MSC	Mean Square Container
MSD	Mean Square Duration
MSEa	Mean Square Error $_a$
MSE_b	Mean Square Error $_b$
MSY	Mean Square Year
MW	Molecular Weight
NRRL	Northern Regional Research Laboratory
ODAP	β-N Oxalyl-L-α, β-diamino propionic acid
OPT	*o*-Pthalaldehyde
RDS	Rapidly Digestible Starch
RS	Resistant Starch
SDS	Slowly Digestible Starch
SDS	Sodium Dodecyl Sulphate
TCA	Trichloroacetic acid
TEMED	Tetramethylethylendiamine
TIA	Trypsin Inhibitor Activity
TU	Trypsin Unit
TUI	Trypsin Unit Inhibited
HTC	Hard-to-cook
PCPM	Pectin, calcium, magnesium and phytin

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Chapter 1

INTRODUCTION

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The most crucial challenge confronting mankind today is to solve the worldwide problems of hunger and malnutrition. In developing countries like Bangladesh pulse has been selected as one of the potential crops for alleviating human malnutrition. The excellent nutritive value of pulse in terms of protein, calories, minerals and vitamins makes it highly complementary to a cereal-based diet in developing countries like ours. Judicious mixing of pulses with cereals to provide a good quality diet was in practice long before food scientists and nutritionists understood the nutritional importance of this practice. The protein quality of pulse for food is rather low because of its deficiency in certain essential amino acids such as methionine and cystine. But it is a rich source of lysine which is absent in most cereals (Evens and Bandemer, 1967; Jha *et al.,* 1984). Singh *et al.* (1997) reported that when pulses are mixed with cereals, the total dietary quality is elevated to 70 per cent of milk protein. Plants and plant products supply nearly 80 per cent of proteins consumed by humans in the developing countries. In this context, pulses are cheaper sources of proteins when compared to animal proteins in developing countries. In addition, pulses have been reported to reduce the levels of cholesterol and blood glucose (Soni *et al.*, 1982; Sukhminders *et al.*, 1992).

Inspite of good nutritional profile, pulses have several nutritional and processing problems such as presence of antinutritional factors, prolonged cooking time, poor digestibility and susceptibility to fungal and pest attack. Several antinutritional factors have been identified in pulses e.g. trypsin and chymotrypsin inhibitors, amylase inhibitors, polyphenols, phytic acid and flatulence factors (Williams and Singh, 1987). These antinutritional factors have profound effect on the nutritional quality of pulses (Singh, 1988). Investigation is therefore, necessary to see the levels of inhibition by different antinutritional factors present in pulses.

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Again pulses are subjected to losses during pre- and postharvest stages. A rough estimate of postharvest losses in pulses ranges from 9 per cent in the U. S. to 40 to 50 per cent in some developing countries (Pimentel, 1976). Huysmans (1970) found that out of the total 9.5 per cent losses of pulses, 8.5 per cent occurred during storage. Storage losses can be broadly categorized as a loss in weight, loss in quality and nutritive value. Bressani (1983) reported that during storage several chemical and bio-chemical changes occur in seed which lead to change in cooking behaviour and quality of seeds. This loss of pulses during storage period occurred under some adverse condition of storage.

In Bangladesh a number of pulse crops are cultivated covering an area of 0.475 million hectare with a current total production of 0.37 million metric tons (BBS, 2000). The present per capita availability of pulses is only 12 gm, but the suggested daily intake is about 45 gm (Yusuf, 1996). The acreage and production of pulses are decreasing because of strong thrust on rice and wheat production. But the demand of pulses from consumers' point of view has remained as high as ever and the prices have increased considerably. The increasing population growth has resulted in a sharp decline in the per capita availability and consumption of pulses in the country. To aggravate the situation a considerable portion of pulses is lost during storage.

In the country the harvested pulses are partly stored at farm level and partly at traders or millers level. Storage at millers level is done temporarily prior to milling. Bulk of the storage occurs at farm level. The Crop Diversification Programme (CDP) of Agricultural Marketing Department of Bangladesh conducted a survey on storage of pulses at farm level during the period 1991-92 and they reported that most of the farmers used gunny bag, earthen container, kerosine tin or polythene lined fertilizer bag for storing their pulses. All of these

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are traditional storage containers and cause huge storage losses of pulses. The CDP reported that lack of storage facilities especially storage containers was the major problem of farmers for storing of their pulses. Survey conducted by Bangladesh Agricultural Research Institute (BARI) on pulses storage revealed that farm level storage is beset with problems and shortcomings such as inadequate drying, lack of airtightness of the containers, use of porous containers, lack of protection against molds, insects and rodents etc. Since bulk of the harvested produce is stored at farm level, the market needs are fulfilled mainly by the supplies from farmers stock. It implies that if the farmers stock is not adequately protected against physical and nutritional losses, the product marketed is always a sub-standard one. In view of these deplorable situations occurring at farm level the Postharvest Technology Division of BARI has fabricated several improved storage structures viz. improved earthen motka, improved tin container, polythene lined jute bag etc. for safe storage of pulses at farm level. The initial findings of performance of these containers are very encouraging as regards appearance, colour, porosity, and general acceptability of stored pulses. The effect of these structures on physical and nutritional quality of stored pulses however, are yet to be fully studied. Therefore, the complete information on performance of these fabricated structures for pulses storage is lacking. In view of these information gaps on performance of devised containers, the present investigation has been planned to evaluate the quality of pulses, stored in these storage containers as well as in the traditional farm level storage structures. The information will help in evaluation of traditional and improved storage containers for farm and commercial level adoption so that this nutritious crop is stored without quantitative and qualitative deterioration.

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Objectives:

1. To investigate levels of various nutrients and antinutritional factors present in three major pulses – lentil, chickpea and khesari.

2. To study the effect of storage containers and duration of storage on physical, compositional and nutritional properties of stored pulses.

3. To study the digestibility of major nutrients viz. protein and starch in stored pulses.

4. To study correlation between various physical components and nutrients and various storage conditions of stored pulses.

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Chapter 2

LITERATURE REVIEW

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2.1. Moisture

Legume seeds contain moisture as bound water and absorbed water. The amount of free water present in seeds governs the rate of deterioration. Biological activity occurs when moisture is present. Insect attack grains at a moisture content of above 8 per cent if the storage temperature is above 19°C (Hall, 1970). Dobie (1982) reported that moisture content of seeds in equilibrium with a relative humidity of 70 per cent is about 14 per cent, thus legume seeds must be dried to below 14 per cent moisture content for protection against moulds.

Morris and Wood (1956) reported that beans with a moisture content above 13 per cent had deteriorated significantly in texture as well as flavours after six months of storage at 25°C. They also reported that beans stored at less than 10 per cent moisture content had maintained their cooking quality for two years as well as control samples stored at -23°C.

Chavan *et al.* (1989) reported that higher moisture content in seeds accelerates degradation of starch, proteins and lipids.

2.2. Cooking/cooking quality

Pulses are commonly cooked in boiling water for a period of 1 to 4 hours. Cooking can be achieved at atmospheric or high pressure. Other cooking methods include roasting, extrusion cooking and drum drying. Cooking is generally done to produce a tender, edible product, to develop the aroma and to inactivate antinutritional factors present in the seeds. The cooking water may or may not be discarded depending upon cultural and personal preferences (Sathe *et al.*, 1984).

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Cooking time means the time taken from the beginning of the test to that time when the seeds are ready to eat. This means that at least 80 per cent of them are soft enough to be masticated without the need of chewing, (Williams and Singh, 1987).

The term cooking quality or cookability refers to the condition in which the dry legume seeds achieve during cooking a degree of tenderness acceptable to the consumers. Several changes occur during storage of pulses, which influence the cooking behaviour of legumes.

Gulati *et al.* (1997) reported that cooking time of desi-chickpea varieties ranged between 50-62 minutes and that of kabuli types ranged between 53-70 minutes and cooking time increased significantly during storage and water absorption increased with increase in soaking period. They also reported that chemically hardened seeds required more cooking time.

Rosaiah *et al.* (1993) observed a wide range of variations in cooking time ranging from 32 minutes to 48 minutes in 9 different genotypes of mungbean. Similar results were also reported in case of pigeon pea where the cooking time of 8 different genotypes ranged between 18-27 minutes (Singh *et al.*, 1993).

Singh *et al* . (1984) also found a large variation in cooking time of different cultivars of pigeon pea and the mean cooking time was 30, 40 and 38 minutes for early, medium and late cultivars respectively.

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Shahjahan *et al.* (1995a) reported that the cooking time of existing cultivars and various advance lines of Chickpea commonly grown in Bangladesh ranged between 32 to 41 minutes. For commonly grown blackgram cultivars of Bangladesh the cooking time was found ranging between 21 to 25 minutes (Shahjahan *et al.,* 1995b).

Singh *et al.* (1988) reported that cooking time of whole seed of lentil was 35 minute and it was reduced to 8 minutes after dehulling, indicating a remarkable reduction in cooking time due to dehulling.

Khan *et al.* (1996) reported that the cooking time of four different improved lines of lentil grown in Pakistan ranged between 23 to 26.4 minute with an average of 23.0 minute for the dry seeds. On the other hand they observed that the cooking time came down significantly (6.3-8.0 minutes) when the seeds were soaked over night in water. The cooking quality of lentil was reported to depend on the seed coat, phytic acid and pectin contents of the seed coat, Ca + Mg/P ratio and amylase content (Bhatty, 1984).

Williams *et al.* (1983) opined that cooking time in chickpea might be affected by factors like starch contents, permeability of seed coat, internal structure and compactness of seed coat and endosperm material, while soaking the seeds in water. They also reported a positive and significant correlation between seed size and cooking time for chickpea. Similar observations were also reported for lentil (Erskine *et al.,* 1985).

Cabrejas *et al.* (1995) found that cooking time for the fresh and hard to cook beans varied when stored under tropical conditions and it also varied among cultivars. This was also

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accompanied in most instances by an overall decrease in the proportion of soluble-N fractions, particularly soluble proteins.

According to Sefa-Dedesh *et al.* (1979) the cooking time of stored pulses generally increased with storage time. Jacson and Variano (1981) reported that storage of pulses under invariable conditions of high temperature and high relative humidity decreased cookability of pulses. This is an irreversible phenomenon classified as "hard to cook" (HTC). (Stanley and Aguilerea 1985; Hentges *et al.,* 1991).

Hard to cook beans have depressed their economic value because of increased energy requirement for cooking, poor palatability and reduced quality of proteins (Stanley, 1990). It specifies acquiring resistance of seeds to softening during the cooking process resulting in poor cooking quality. (Gracia and Lajolo, 1994).

Several hypotheses have been proposed to explain the causes of bean hardening. Jones and Boulter (1983) and Hincks and Stanley, (1986) reported that the increases in moisture content due to high relative humidity (RH) during storage was one of the key factors in the initiation of hardening of seeds. Storage hardening and chemical hardening are well known indices to explain hard-to-cook tendency (Liu *et al.,* 1992; Reyes-Moreno *et al.,* 1994). The mechanisms responsible for the development of hard-to-cook phenomenon were stated to involve associations among phytic acid, minerals and pectin (Mulller, 1967).

Moscoso *et al.* (1984) indicated that the reduced cookability in matured bean seeds after storage under high temperature and high humidity might be probable result of a decrease in phytic acid phosphorus and an alteration in the ratio of monovalent to divalent cations in

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the tissue. Chitra and Singh (1998) reported that the PCMP number relating to contents of pectin, calcium, magnesium and phytin increased during storage with increasing cooking time, suggesting that hard-to-cook characteristics involved interaction between phytate, minerals and pectin.

Several physical and chemical factors were found to influence the cooking quality of pulses. Singh *et al,* (1984) reported that the amount of water absorbed, the solids dispersed during cooking and texture were highly correlated with cooking time of pigeonpea. They also reported a positive correlation between cooking time and seed size and negative correlation between 100 seed weight and solids dispersed. Similar observations were also reported by William *et al.* (1983) for chickpea and by Erskine *et al.* (1985) in lentil.

Akinyele *et al.* (1986) pointed out that there was positive correlation between crude protein content and cooking time of cowpea. Similar results were also reported by Cabrejas *et al,* (1995) for Kenyan beans.

Williams *et al.* (1983) and Badsha *et al.* (1987) reported strong and positive association of seed weight, seed volume, seed density and hydration capacity with cooking time.

Rosaiah *et al.* (1993) found that the mungbean containing higher percentage of proteins required shorter cooking time than that containing lower percentage.

Mattson (1985) reported that the difference in cooking quality among varieties of dry peas was related to their contents of phytic acid and calcium. According to him it was

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possible that when the phytic acid content was low, the pectin in the middle lamella formed insoluble calcium and magnesium pectates which contributed to poor cooking quality. Singh and Rao (1995) reported that cooking time of pigeon pea dhal significantly increased when the phytic acids levels were reduced by phytase enzyme.

Williams and Singh (1987) reported that the variations in cooking time might be due to different nutrient levels like water, nitrogen, phosphorus and type of water used in cooking as well as altitude of the location.

2.3. Antinutritional factors

Pulses are known to contain several antinutritional constituents. These are enzyme inhibitors, hemagglutinins (lectins), phytates, polyphenols, flatulence factors, cyanogenic compounds, lathyrogens, estorogens, goiterogens, saponins, antivitamins and allergens (Salunkhe *et al.,* 1985). These antinutritional factors are known to have deleterious or toxic effects for animals and man (Pusztai and Palmer, 1977; Grant *et al.,* 1983). Therefore, these constituents need to be inactivated by a suitable pre-treatment before they can be safely used as a food source (Grant *et al.,* 1986; Liener 1989)

2.3.1. Enzyme Inhibitors

The enzyme inhibitors i.e., protease and amylase inhibitors affect the nutritive value of proteins and carbohydrates. The oligosaccharides cause flatulence when consumed in large amounts of grains. The tannin combines with proteins to form a tannin-protein complex and thus interferes with protein digestibility (Salunkhe, 1985).

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Singh and Jambunathan (1981) isolated the protease inhibitor (trypsin inhibitor and chymotrypsin inhibitor) and they observed a considerable variation in the levels of these inhibitors in chickpea dhal. They observed that trypsin inhibition for deshi chickpea dhal ranged between 9.3 -14.6 with an average of 12.0 units/mg of meal and for kabuli type it ranged between 6.7-12.3 with an average of 9.4 units/mg of meal. On the other hand chymotrypsin inhibitor level for desi chickpea dhal ranged between 7.0-9.0 with an average of 7.7 units/mg of meal and that for the kubuli type ranged between 5.7-9.4 with an average, of 6.5 units/mg of meal.

Sustry and Murray (1987) reported the varietal differences in trypsin and chymotrypsin inhibitor in chickpea.

Cabrejas *et al.* (1995) reported that both fresh and hard-to-cook beans contained significant amounts of lectins, trypsin and amylase inhibitors. They further observed that HTC samples had significantly higher contents of lectins and amylase was significantly lower than those of fresh beans, while the amounts of trypsin and chymotrypsin inhibitors were the same.

Weder and Kahley (1998) isolated 23 proteinase inhibitors from Syrian small local lentils. They all inhibited human and bovine trypsin and chymotrypsin.

Barampama and Simard (1993) observed that the concentrations of antinutritional factors were highly influenced ($P<0.01$) by locality and combination of variety and locality. They also found significant differences in antinutritional factor contents of seeds among

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varieties for hemagglutinin (P<O.05), trypsin inhibitor and tanins (P<O.01), but not for phytic acid.

2.3.2. Phytic acid

Phytic acid (phytate, myo-inositol - 1,2,3,4,5,6 - hexakis phosphate) is widely distributed in legume seeds, stores 65 per cent to 85 per cent of total seed phosphorus (Raboy, 1997). Phytic acid is a strong chelator of various metal cations, especially potassium, magnesium and calcium forming phytate, although these salts can also contain other mineral cations such as zinc and iron (Raboy, 1997). Phytic acid could effect digestibility of pulses by chelating calcium or by binding with substrate or enzyme proteins. Phytic acid and associated minerals are deposited in protein bodies of the embryo and aleurone layer of seeds (Lott JNA *et al.,* 1995). It has been indicated that phytic acid lowers the bio-availability of minerals (Nolan and Duffmi, 1987) and inhibits poroteolytic enzyme (Serraino *et al.,* 1985) and amylolytic enzymes (Despande and Cheryan, 1984). Phytate also interacts with proteins, resulting in reduced protein solubility (Cheryan, 1980). Phytic acid is considered as the single most important antinutritional factor for the availability of iron, calcium and zinc (Ravindran, 1995). Sandberg and Svanberg (1991) stated that a 1.0 per cent level of phytic acid in the diet had significant effect on the availability of minerals.

A wide range of phytic acid content (100-810 mg/100 g) was reported in lentil (Davis, 1981; Davis and Warrington, 1986). They also reported that the phytate phosphorus amounted to about 50 per cent of total phosphorus of lentil seeds and splits. On the other hand in lathyrus seeds about 30 per cent of the total phosphorus was found in the form of phytic acid (Ferando, 1983). Srivastova and Khoker (1996) reported that the level of phytic acid in lathyrus seeds of Indian cultivars ranged between 1030 mg to 1090 mg per 100 g and

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for chickpea it ranged between 750 to 800 mg per 100 g seed (Duhan *et al.,* 1989). Chowdhury *et al.* (2002) reported the level of phytic acid content in Bangladeshi pulses like lentil (489 mg/100 g), chickpea (858 mg/100 g) and lathyrus (499 mg/100 g).

Duhan *et al.* (1989) reported that domestic processing and cooking including, soaking, cooking, autoclaving and sprouting of legume grains significantly lowered the phytic acid content. They found that soaking lowered phytic acid by 11-15 per cent in chickpea and 25-30 per cent in blackgram and cooking of soaked seeds lowered 20-26 per cent in chickpea and 35-40 per cent in blackgram grains where as the loss was 7-11 per cent and 6-9 per cent in these pulses respectively, when unsoaked seeds were cooked.

The germination of legume seeds results in a considerable decrease in phytic acid content. During germination process activity of the enzyme phytase increases, causing the phytic acid to break down. Srivastova and Khokar (1996) reported that the action of phytase during germination led to reduction in phytate content by 33 per cent in khesari seeds consistent with the findings in other legumes (32-48 per cent)

Long term storage of pulses reduces the phytic acid content. Moscoso et. al. (1984) reported that bean stored at 32^0C showed a consistent decrease in phytic acid phosphorous content during storage, with higher moisture samples showing the greatest decrease.

2.3.3. Neurotoxin (ODAP)

Lathyrus (*Lathyrus sativus* L.) commonly known as khesari dhal (India and Bangladesh) however, contains a low molecular weight neurotoxin named either β-N-oxalylamino-L- alanine (BOAA) or β-N- oxalyl-L-α, β-diamino propionic acid (β-ODAP)

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(Rao *et al.*, 1964; Spencer *et al.*, 1986). This non protein amino acid has been implicated in the development of the neuro-degeneratvie condition in human known as Lathyrism (Quereshi *et al.*, 1977).

Analysis of a large number of samples of L. sativus collected from the epidemic area of India indicated that ß-ODAP content in the dry seeds ranged from 1 to 25 g/kg (Roy and Rao, 1964). A nutritional survey of northwest Ethiopea showed that β-ODAP level of whole seed ranged from 2 to 8 g/kg (Tekle Haimanot *et al.*, 1993). In Bangladesh the ODAP content in the high-toxic Jamalpur variety ranged from 5 to 8 g/kg in the seeds (Hussain *et al.*, 1997; Dutta *et al.*, 1998).

Yu J-Z (1995) examined 50 varieties of grass pea grown in China and found that the ODAP content varied from 0.3 to 8.7 g/ka. The range of ODAP concentration in Pakistan genotypes was 1.5 – 4.9 g/kg (Khawaja, 1997).

Attempts have been made to reduce or eliminate the levels of β-ODAP in Lathyrus by selective breeding or by postharvest processing. Several low toxin lines have been developed in agricultural institutes worldwide (Campbell, 1989). However, so far a variety that is totally toxin-free has not yet been developed by traditional breeding methods.

Many simple processing methods such as roasting the seeds, soaking the seeds in water or in solutions (Lime water, sodium chloride, sodium bicarbonate and ascorbic acid), boiling and cooking, autoclaving, fermentation, germination and blending with other pulses have been reported for reducing or eliminating the neurotexin (Muslehuddin 1987; Pandey *et al.*, 1997; Geda *et al.*, 1997).

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Steeping grass pea seeds in a large volume of hot water for 1 hour leached out 90 per cent of the neurotoxin but under this condition a considerable proportion of its water soluble vitamins, particularly thiamin and riboflavin were lost (*Nagarajan* 1969). Tekle Haimanot *et al.* (1993) showed that steeping seeds in excess water leaches out 30 per cent of the β-ODAP, while roasted seeds showed elevated levels of β-ODAP.

Srivastova and Khokar (1996) reported that when the lathyrus seeds were soaked in boiled water highest losses (60-70 per cent) of β-ODAP were observed followed by trypsin inhibitors (42-48 per cent) and polyphenols (30-37 per cent). Ordinary cooking and pressure cooking of pre-soaked seeds were found to be most effective in reducing the levels of all the natural toxicants examined, whilst fermentation and germination were more effective in destroying amylase inhibitors by 69-71 per cent and trypsin inhibitors by 65-66 per cent. They further reported that dehusking of pre-soaked seeds significantly reduced β-ODAP levels. If the above processing method could be applied to low toxin varieties where the β-ODAP content is only one-tenth of that of the high toxin varieties, then with a similar 90 per cent reduction of this residual toxin, possibly the neurotoxin could be reduced to near zero or even completely removed. This might contribute to solving the problem of neurolathyrism and also to reducing food shortages in the semiarid areas of the world.

Kuo *et al.* (1995) worked with the high toxin content lathyrus seeds of variety Jamalpur (Bangladesh) and reported that solid-state fermentation of lathyrus seeds with Aspergillus oryzae NRRL 1988 for 48 hour followed by fermentation with *Rhizopus Oligosporus* sp. T-3 for another 48 hour eliminated more than 90 per cent neurotoxins. Other nutritional qualities were also improved in the fermented seed meal specially it increased the

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protein content and drastically reduced the flatulence factors. Kuo *et al.* (2000) also reported the similar results in low toxins varieties of grass pea.

2.4. Chemical composition

A wide variability exists in the chemical composition of different pulses. The chemical composition of pulses is influenced by the cultivar, geographic location and growth condition (Krober, 1968), In general, pulses contain 20-30 per cent protein, 1-7 per cent lipid and 2-5 per cent minerals (Saluakhe *et al.,* 1985).

The crude protein content in lentil seed of different Indian cultivars ranges from 21.7 to 31.4 per cent and fat content ranges from 0.6 to 3.8 percent Adsule *et al.* (1989). The starch content in lentil ranged from 35 to 53 per cent (Reddy *et al.,* 1984).

The crude protein in chickpea seed of different Indian cultivars ranged from 17 to 24 per cent extremely from 12.4 to 315 per cent and that for the dhal ranges from 20.5 to 29.6 per cent as reported by Agarawal and Bhattacharya (1980). Jambunathan and Singh (1978) reported that the starch content in chickpea seeds ranges between 41.0 and 50.8 per cent and for the dhal it ranges between 54.5-58.1 per cent and the fat ranges between 4.2-6.9 per cent with an average of 5.7 per cent.

The protein content in lathyrus seeds of Indian cultivars ranged from 20 to 33 per cent (Gupta, 1982). The lipid content of lathyrus sativus seeds is about 1.0 per cent, which is very low compared to other legumes (Pattee *et al.,* 1982).

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The total protein content of aging seeds decreases substantially during dry storage (Anderson, 1973; Roberts, 1979). This may be due to increased activities of proteolytic enzymes leading to degradation of proteins into amino acid.

2.5. Protein digestibility

One of the important factors determining the availability of nutrients in protein foods is their digestibility. This can be determined either in vitro using suitable hydrolytic enzyme system or *in vivo* conducting actual feeding of diet to experimental animals.

Legume proteins are considered to be difficult to digest due to antinutritional components and to flatus production that causes stomach and intestinal discomfort (Bressani and Elias, 1977).

Singh (1984) reported that the polyphenolic compounds present in the seed coat of grain legumes inhibited the activity of digestive enzyme.

Singh and Jambunathan (1981) reported that the *in vitro* protein digestibility showed larger differences between desi seed and dhal when compared with kabuli seed and dhal. They reported that the mean value of *in vitro* protein digestibility of seed and dhal of desi chickpea were 63.1 per cent and 71.0 per cent when for kabuli type the values were 72.7 per cent and 75.3 per cent respectively.

Bressani (1992) reported that the nutritive value and protein digestibility of grain legumes was improved by processing or cooking as these treatments destroyed the heat labile antinutritional factors. On the other hand Murthy and Vrs (1980), while studying the effect of roasting and puffing on the digestibility of Bengal gram observed that, the in vitro digestibility of raw sample decreased from initial value of 79 per cent to 57 per cent and 67

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per cent after roasting and puffing respectively. They reported that this decrease in digestibility might be attributed to the fat imbedded by the legumes during frying.

2.6. Starch digestibility

Starch is the main energy source in diets that containing cereals and grain legumes. There is good evidence that for monogastric animals, the digestibility of legume starch is lower than that of cereal starch. It was found that the ideal digestibility of legume starch is about 90 per cent, whereas the digestibility of starch derived from cereals is nearly 100 per cent. Borchars (1961) and Sandsted *et al.* (1962) reported that raw starches high in amylopectin were digested more quickly than those high in amylose. In general, legumes starch contains between 30 and 40 per cent amylose and 60-70 per cent amylopectin and cereal starch in general, have 20-25 per cent amylose and 75-80 per cent amylopectin (Schoch and Maywald, 1976). It has been found that high amylose/amylopectin ratio correlates with the lower digestibility of legume starch. Thorne *et al.* (1983) reported that the difference in the digestibility rate of high amylose and high amylopectin starch could be due to large surface area of amylopectin, which may make it more available for amylolytic attack.

Thorne *et al.* (1983) and Jenkins *et al.* (1987) reported that high levels of antinutrients (e.g., enzyme inhibitor, tannins, lectins, phytic acid) present in pulses often form complexes with nutrients such as starch which can reduce their digestibility. Another possible cause of the low digestibility of legume starch (below 80 per cent) may be due to insufficient crushing of the seeds which could reduce the accessibility of starch particle to digestive enzymes (Carre *et al.*, 1991).

Anderson *et al.* (1985) reported that higher percentage of proteins present in legumes affected the starch digestibility.

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Chapter 3

MATERIALS AND METHODS

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The experiment was conducted in the storehouse of Postharvest Technology Division, Bangladesh Agricultural Research Institute, Gazipur. The experiment was repeated for three consecutive years starting from 1998-1999 and continued up to 2000-2001. Three major pulses of Bangladesh Viz. lentil, khesari and chickpea were used for the study. The seeds of lentil (variety Faridpur local) were collected from farmers stock of the area Gazaria of Faridpur Sadar upzilla, khesari seeds (variety Manikgonj local) were collected from the Ghior Upzilla of Manikgonj district and chickpea seeds (variety- Nabin) were collected from Nachol (Barind region) of Chapai Nawbgonj district. The following 6 different types of storage containers were used for the study:

Traditional containers

i) Plain earthen *motka* (T_1)

ii) Plain tin container (T_2)

Improved containers

i) Polythene lined jute bag (T_3)

ii) Polythene lined *motka* (T_4)

iii) Coal-tar coated *motka* (T_5)

iv) Improved tin container (T_6)

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3.1. Description of the containers

i) Plain earthen *motka*: It was made of burnt clay and modified at the top having good sealing system. The lid was sealed with sealing materials made of clay, paddy husk and cowdung.

ii) Plain tin container: Container made of plain tin with wide lead, generally use by the farmers.

iii) Polythene lined jute bag: Gunny bag with a 0.05 mm polythene lined inside.

iv) Polythene lined *motka*: Same as plain earthen *motka,* only the inside was lined with 0.05 mm polythene to protect moisture abosorption.

v) Coal-tar coated *motka:* Same as plain earthen motka, the outer of side was coated with coal-tar to make it impervious to moisture.

vi) Improved tin container: Containers made of MS sheet/Tin with sealing system at the top.

The design of different containers are shown in photograph 1.

Each of the pulses was stored for 4 different periods viz. 3, 6, 9 and 12 months. There were 3 replications of each of the 6 different storage containers, each of the container was considered as a treatment. So there were 18 storage containers for each storage period making of a total of 72 storage treatments for each type of pulses.

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Plain earthen *motka*

Plain tin container

Polythene lined jute bag

Polythene lined *motka*

Coal-tar coated *motka*

Improved tin

Photograph 1 : Different Storage Containers

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3.2. Experimental design

Seed samples stored in different containers were from the same lot. Samples of seeds stored in containers were considered as experimental units and types of containers storing the samples as treatments. The seeds in the lot before storing were thoroughly mixed to make the lot as uniform as possible. The storage of seeds in each container was completely random. Thus the experimental design was considered as Completely Randomized Design (CRD) with six treatments, each replicated three times. Separate containers were used for different storing periods but seeds were from the same lot and stored at the same time. Thus, the measurements on seed quality were essentially the measurement over time and the analysis of variance of seed quality was performed by considering the storing period as a factor in the experiment and treating it as if it were a sub-plot or smallest experimental unit (Gomez and Gomez, 1983).

The storage containers were placed on wooden shelves. The climatic data on temperature and relative humidity for the experimental period were collected from the Meteorological Stations at Joydebpur and presented in Appendix figure 18, 19 and 20. The physico-chemical parameters of pre-stored (fresh) and stored samples after every storage period were determined.

3.3. Physico chemical properties

3.3.1. Moisture

The moisture of the pre-stored samples and stored samples after every storage period were determined by drying the sample at 100^0C for over night as described in AOAC, 1984.

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3.3.2. Germination

Placing 100 seeds in replicates of 25 seeds in sterile petridishes containing moist blotting paper as described by Draper (1994).

3.3.3. Cooking time

Cooking time was determined using the method of Williams *et al.* (1983). Twenty gram of dehulled and split dhal was put in 150 ml water after it started boiling. Boiled samples were removed at specified times for each of the pulses at an interval of 1.0 minute and examined their softness by thumb pressing method. Cooking time was the minimum time interval at which dhal was considered to be completely cooked by at least 2 persons out of a panel of 3 persons.

3.3.4. Water absorption

Water absorption during cooking was determined by heating 5.0 gm dhal in excess distilled water (35.0 ml) up to cooked state. Excess water was discarded and traces of water were removed by blotting paper. An increase in weight of seed sample after this treatment was the capacity of water absorption of seeds and expressed as g/g sample (Singh *et. al.*, 1991).

3.3.5. Solids dispersed

The percentage of solids dispersed by dhal in cooking water was determined by boiling 5.0 gm dhal up to cooking time. After sieving and washing the residue was dried at 110^0C for 3 hours and then percentage of solids dispersed was calculated from the difference between the original weight and weight of the residues (Singh *et al.*, 1984).

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3.3.6. Milling of dhal

The yield of dhal was determined after dehulling and splitting of seeds using the traditional grinding stone or chakki. The milled products were divided into four fractions viz. head dhal, broken, powder and husk using seive and bamboo kula and head dhal was used for estimation of various physico chemical properties.

3.4. Chemical analysis

3.4.1. Estimation of proteins

The standard microkjeldhal procedure of AOAC (1975) was used for the determination of nitrogen and crude protein was estimated by multiplying the nitrogen content by a factor of 6.25. True protein content in pulses was determined according to the method as a described by Lowry *et al.* (1951).

3.4.2. Minerals and trace elements

Minerals and trace elements were determined by wet digestion method as stated by Piper (1966). For digestion, triacid mixture containing nitric acid, perchloric acid and sulphuric acid in the ratio of 10:2:0.5 were used. Defatted samples (0.5g) were weighed and transferred to a 50 ml volumetric flask. After adding 6 ml of triacid mixture, the content was kept for cold digestion overnight. The flask was then placed in a sand bath and set to 230^0C and then digested for 3 hour (Till the white dense fumes of sulphuric acid appear). After digestion the mixture was cooled and dissolved in distilled water and the volume was increased to 50 ml. Suitable aliquots were analyzed for potassium, sodium, calcium, magnesium, iron, zinc, copper and manganese in an Atomic Absorption Spectrophotometer (Model 170-30, Hitachi). Phosphorus was estimated by colorimetric method as described by Jackson (1963). 5 ml of original extract was diluted to 25 ml. The yellow color produced by

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combination of phosphorus and vanadomolybdic acid was measured colorimetrically at 420 ηm and concentration of the phosphorus was determined using the blank and series of standards (0-20 ppm) with the help of a spectrophotometer (Spectronic® Geneys™8 UV/visible Spectrophotometer).

3.4.3. Estimation of crude fiber

Crude fiber is defined as the organic fraction (residue) of plant food left after sequential extraction with solution of 1.25 per cent sulphuric acid and 1.25 per cent sodium hydroxide and was determined according to the AOAC methods (1984). The crude fiber method essentially determines the cellulose and lignin content.

3.4.3.1. Reagents

(1) 0.255 N sulphuric acid

(2) 0.313 N sodium hydroxide

(3) Ethanol.

3.4.3.2. Procedure

Weighed out 2.0 g pulses flour and transferred to a special type beaker (Crude fiber beaker). After adding 200 ml of hot 0.255 N sulphuric acid the beaker was placed on a preheated plate of the digestion apparatus and digested the sample for 30 minutes rotating the beaker periodically to keep the solids or material from adhering to the sides. Filtered the sample through Whatman No. 42 filter paper placed in Buchner funnel using a vacuum pump. The residue was washed with hot water until the washings are free from acid (tested with Litmus paper). Transferred the residue (sample) back into the beaker containing 0.313N hot sodium hydroxide solution placed on the heater. The filter paper was then removed after

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washing the solution and the sample was then digested for 30 minutes. Filtered the sample through Whatman No. 42 filter paper placed on the Buchner funnel using the vacuum pump. The sample was washed until the washings were free from alkali (tested with Litmus paper). Finally the residue was washed with alcohol (about 25 ml). Transferred the residue with filter paper into a clean crucible and dry the produce at 100^0C overnight. The crucible was then transferred into a dessicator and cooled to room temperature and weighed (W_1). The residue was then ignited in a muffle furnace at 600^0 C for 30 minutes. Transferred the crucible into a dessicator and cooled to room temperature and weighed it (W_2). A blank was run simultaneously.

Weighed of the crude fibers = $(W_1 - W_2)$ – blank.

$$\% \text{ Crude fiber} = \frac{(W_1 - W_2) - \text{blank}}{\text{Wt. of the sample (g)}} \times 100$$

3.4.4. Estimation of sugar and starch

The total sugar and starch content in pulses were determined colorimetrically with anthrone reagent based on the method of McCready *et al.* (1950) and the reducing sugar content was determined by dinitrosalicyclic acid method as described by Borel *et al.* (1952).

3.4.4.1. Reagents for sugar and starch estimation:

1. Ethanol : 80 per cent

2. Anthrone reagents 0.2 per cent in 95 per cent H_2SO_4 : 100 ml of 95 per cent H_2SO_4 was cooled in an ice bath and then 0.2 g of anthrone (Fisher) was added and stirred until anthrone was completely dissolved. This solution was prepared fresh every time.

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3. Sodium potassium tartrate (50 g/100 ml).

4. 6N NaOH.

5. Dinitrosalicyclic acid (DNS) reagent : 10 g of crystalline phenol was dissolved in water, mixed with 22ml of 10 per cent NaOH and then volume to 100 ml with water. To 69 ml of this mixture, 6.9 g $NaHSO_4$ was dissolved (Solution A). Dissolved 255 g sodium potassium tartrate in 300 ml of 4.5 per cent NaOH and then 880 ml of 1.0 per cent 3, 5 dinitrisalicyclic acid was added to this mixture (solution-B). The solution A and B were mixed and then kept in dark place for 2 days, then filtered. The filtrate was the DNS reagent. Before use the regents 3, 4 and 5 were mixed in the proportion of 1:2:3.

3.4.4.2. Procedure

a) Extraction of sugars

Weighed 50 mg of pulse powder in a 1.5 cm x 100 cm centrifuge tube. A few drops of 80 per cent ethanol and 2.0 ml water was added, stirred thoroughly using a vortex mixture and then 8 ml of 80 per cent hot ethanol was added. Stirred the solution for 5 minutes and then centrifuge at 4000 rpm for 10 minutes. The supernatant was transferred into a 50 ml volumetric flask. To the residue added again 10 ml of hot 80 per cent ethanol, stirred for 5 minutes and it was then centrifuged. The residue was washed for two more times, each time combining the alcohol washing to the first extract and then volume to 100 ml with 80 per cent ethanol. The alcohol extract was used for sugar determination and the residue was used for starch determination.

b) Determination of total sugar

Pipetted out 0.1ml aliquot from the alcohol extract into screw capped test tubes and then 0.9 ml of distilled water was added. Prepared blank, 50 ml and 100 μg/ml glucose standard similarly. Cooled the tubes in an ice bath and then 2.0 ml of anthrone reagent was

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added. Mixed the solution using a vortex mixture. Loosely tighten the cap and heated the tubes in a boiling water bath for 7.5 minutes. Cooled to 25^0C and determined the concentration of samples at 630 nm using a spectrophotometer (Spectronic$^{(R)}$ Genesys^{Tm}8 UV/visible spectrophotometer).

c) Determination of reducing sugars

Mixed 1 ml of alcohol extract and 2 ml of DNS reagent in screw capped test tubes. Prepared a blank and 50 and 100 μg/ml glucose standard similarly. Loosely tighten the cap and boiled the solution in a boiling water bath for 10 minutes. After cooling, 10 ml of distilled water was added to make the volume up to 13 ml. Determine the concentration of unknown samples using glucose standard with the help of Spectronic$^{(R)}$ Genesys^{TM}8 UV/Visible Spectrophotometer at 500 nm.

d) Estimation of starch

After drying the residue in the tubes from the free sugar extraction at 70^0C, 5 ml of water was added to the tubes and stirred well to disperse the residue. Cooled in an ice bath while stirring; added 6.5 ml perchloric acid (52 per cent) and stirred for 15 minutes keeping cooled. After adding and mixing of 5 ml of distilled water the tubes were centrifuged at 4000 rpm for 10 minutes. The aqueous solution was poured into a 50 ml conical flask. The extraction was repeated with 6.5 ml perchloric acid on the residue, stirred for 30 minutes in the cold and then combined everything with first extract and made up to 50 ml and then filtered through Whatman No. 1. Pipetted out 0.1 ml of the percholoric acid extract into screw capped test tube and then 0.9 ml of water was added. Prepared 50 and 100 μg/ml glucose standard similarly. The blank consisted of $HClO_4$ diluted accordingly. Cooled and then 2.0 ml of anthone reagent was added. Mixed well, slightly tighten the cap and heated the tubes in a

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boiling water bath for 7.5 minutes. Cooled to 25^0C and then determine the conc. of unknown samples at 630 nm using the glucose standard with the help of spectrophotometer (Spectronic$^{(R)}$ Geneys^{TM}8 UV/Visible Spectrophotometer.

3.4.5. *In vitro* protein digestibility

The digestibility of pulses protein was determined by the method of Akeson and Stahmann (1964). In this method protein is digested by pepsin, followed by pancreatin.

3.4.5.1. Reagents

1. Pepsin solution (13.33 mg/100 ml of 0.1 HCl)
2. 0.2N NaOH
3. 0.1M Na-phosphate buffer solution containing 1M $Cacl_2$ and 0.01 per cent NaN_3
4. Pancreatin solution: 53.3 mg/100 ml of Na-phosphate buffer.
5. 20 per cent Tricholoroacetic acid solution.

3.4.5.2. Procedure

Pulses powder (200 mg) was accurately weighed and transferred into a 100 ml conical flask and then 15 ml of pepsin solution was added. The suspension was incubated for 3 hours at 37^0C in an incubator. After incubation 7.5 ml of 0.2N NaOH was added to shift the pH to about 7.5 to 8.0. After adding 7.5 ml pancreatin solution the flask was again incubated for 24 hours at 37^0C for digestion to occur. Adding 30 ml of 20 per cent TCA solution precipitated the undigested protein. The undigested protein was separated by Whatman No. 42 filter paper. The amount of undigested protein was measured by microkjeldhal method. The digestibility of seed protein was determined from the following formula.

$$\text{Digestibility } \% = \frac{\text{Total protein - undigested protein}}{\text{Total protein}} \times 100$$

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3.4.6. *In vitro* starch digestibility

In vitro digestibility of meal starch and isolated starch were determined using the enzyme amylase following the method described by Singh *et al.* (1982).

3.4.6.1. Reagents

i) 0.2M Phosphate buffer: (Prepared by mixing 45 ml of 0.2M using sodium dihydrogen phosphate and 55 ml of 0.2M disodium hydrogen phosphate, pH 6.9).

ii) Amylase solution: (20 mg α-amylase of Sigma Chemical Company EC 3.2.1.1, 2160 units/mg dissolved in 50 ml of phosphate buffer)

iii) 3.5 dinitrosalicyclic acid reagent

iv) Maltose solution: 1mg/ml of phosphate buffer.

3.4.6.2. Procedure

A suitable amount of each of the pulses flour (50 mg) or isolated starch (25 mg) was dispersed in 1.0 ml of 0.2M phosphate buffer in a screw capped test tube. Then 0.5 ml of amylase solution was added to the sample suspension and incubated at 37^0C for 2 hours. After incubation 2 ml of 3-5 dinitrosalicyclic acid reagent was quickly added and the mixture was heated for 5.0 minutes in a boiling water bath. After cooling, the solution was made up to 25 ml with distilled water and filtered prior to take measurement of absorbance at 550 nm. A blank was run simultaneously. Maltose solution of 0.2-1.0 mg/ml concentration was prepared similarly and the concentration of unknown samples were determined using the maltose standard at 550 nm. with the help of Spectronic® Genesys™8 UV/Visible spectrophotometer. The results were expressed as mg of maltose released per gram of meal powder or isolated starch meal.

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3.4.7. Determination of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistance starch (RS) of pulses

For nutritional purposes, starch in pulses may be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). All these three fractions were determined using controlled enzymatic hydrolysis of pulse starch with amylase and amyloglucosidase as described by Englyst *et al.* (1992). Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation with α-amylase and amyloglucosidase at 37^0C for 20 min and a further 100 min, respectively. Resistant starch (RS) was the starch not hydrolyzed after 120 min of incubation. Values for RDS and SDS obtained by the method reflect the rate of starch digestion in vivo.

Total starch present in pulses was determined colorimetrically with anthrone as described is by Borel *et al.* (1952).

3.4.7.1. Reagents

1. Sodium acetate buffer (0.1M, pH 5.2) : 13.6 g sodium acetate ($C_2H_3O_2Na.\ 3H_2O$) in 250 ml benzoic acid and then volumed to 1000 ml. Adjusted the pH by 0.1 M acetic acid.
2. α-Amylase (Sigma cat No. A6380, EC 3.2.1.1, 2160 units/mg solid) solution: 35 units/100µl sodium acetate buffer.
3. Amyloglucosidase (Sigma Cat No. A7255, EC. 3.2.1.3, 20300 units/mg solid) solution: 70 units/100 µl sodium acetate buffer
4. Standard glucose solution (0.3 mg/ml sodium acetate buffer).
5. Dinitrosalicylic acid (DNS) solution.

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3.4.7.2. Procedure

a) Measurement of free glucose (FG)

A portion (100 mg of pulse powder/isolated starch) was mixed with 2 ml of sodium acetate buffer in a screw capped test tube. The sample tubes were then placed in a shaking water bath at 100^0C for 30 minutes. Cooled the tubes to room temperature. Removed 1 ml of each of the sample solution, glucose standard and blank (Sodium acetate solution only) into each of the test tubes containing 2 ml ethanol and vortex mixed. The sample tubes were then centrifuged at 4000 rpm for 5 minutes. Pipetted out 1 ml of aliquot from the sample extract, glucose standard and blank in to each of the screw capped test tube, Then 2 ml of dinotrosalicyclic acid solution was added into each of the tube and heated for 10 minutes in a boiling water bath. Cooled the tubes and then 10 ml of water was added and mixed the solution in a vortex mixture. After 15 minutes, the absorbance of the sample solution, blank and standard were taken at 510 nm using a spectrophotometer (Spectronic® Genesys^{TM}8 UV/visible spectrophotometer). The percentage of the glucose was calculated using the formula

$$\% \text{ glucose} = \frac{At \times Vt \times C \times D}{As \times Wt} \times 100$$

Where,

At = Absorbance of test solution

Vt = Total volume of test solution

C = Concentration (mg glucose/ml) of standard

As = Absorbance of standard

Wt = Weight (in mg) of sample taken for analysis, which may be corrected for moisture

D = Dilution factor.

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b) Measurement of RDS and SDS

A portion (100 mg) of pulse flour or isolated starch was mixed with 2 ml of sodium acetate buffer in a screw-capped test tube. A blank containing sample solution was also run simultaneously. Equilibrated the samples and blank at 37^0C in a water bath. Taking the tube one at a time, immediately added 0.2 ml of each of the amylase and amyoglucosidase solution in each of the sample tube. Capped the tubes and placed in the shaking water bath at 37^0C securing firmly. After 20 minutes removed 0.5 ml of the hydrolysate (without stopping the shaking) into labelled tube containing 9.5 ml 66 per cent ethanol and mixed well. After a further 100 minutes removed a second 0.5 ml sample in the same way. The portion taken after 20 minutes was designated G_{20} and that taken after 120 minutes, G_{120}. After mixing, centrifuged the G_{20} and G_{120} tubes for 2-3 minutes at 3000 rpm to obtain a clear supernatant. Measured the glucose in these portions in the same way as measured for free glucose.

Calculation of RDS, SDS and RS

The values obtained for G_{20}, G_{120}, FG are combined to obtain values for RDS, SDS and RS and the results were expressed in percentage of total starch content.

$RDS = (G_{20} - FG) \times 0.9$

$SDS = (G_{120} - G_{20}) \times 0.9$

RS = Total starch – (RDS + SDS)

3.4.8. Estimation of Amylose

Amylose in starch is released by treatments with diluted alkali. By the addition of Tri-iodide ion, amylose produces blue color. The absorbance of blue color produced in aqueous solution was measured spectrophotometrically at 600 nm as described by Williams *et al.* (1958).

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3.4.8.1. Reagents

1. 0.1N NaOH

2. Iodine solution: Dissolved 2.0 g of potassium iodide in 75 ml water and then 0.2 g iodine was added. The final volume was made up to 100 ml

3. 0.1N HCl.

4. Amylose standard: Weighed 100 mg amylose in a 100 ml volumetric flask and 1 ml of 95 per cent ethanol and 9 ml of 1N NaOH were added. Allowed the contents to stand overnight at 40^0C and then made up to 100 ml with water.

3.4.8.2. Procedure

Weighed out 100 ml of pulses flour and transferred in to a 100 ml volumetric flask. Then 1 ml 95 per cent ethanol was added to disperse the flour. After adding 9 ml of 1N NaOH, the flask was incubated at 40^0C for overnight. The contents were then volume to 100 ml and mixed well in a rotary shaker for 30 minutes and then allowed the particles to settle. Pipetted out 5 ml of the supernatant of the sample solution and 1 ml of the standard solution into each of 100 ml beaker and then 30-40 ml water was added to each of the beaker. Adjusted the pH of the solution to 10.2 with 0.1N HCl. Then 1 ml iodine reagent was added and mixed well. Allowed them for 30 minutes and then volumed 100 ml with water and read the absorbance at 600 nm against the blank solution using Spectronic® Genesys™8 UV/Visible spectrophotometer.

The Amylose content was determined using the formula

$$\% \text{ Amylose} = \frac{\text{Absorbance (sample)}}{\text{Absorbance (standard)}} \times \frac{1}{1000} \times \frac{100}{5} \times \frac{100}{0.1}$$

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3.4.9. Antinutritional factors

3.4.9.1. Trypsin inhibitor activity

The trypsin inhibitor activity (TCA) was assayed according to Kakade *et al.* (1969). The method involved the use of 2 per cent casein and the spectrophotometric determination of the breakdown products by a given concentration of trypsin in the presence and absence of inhibitor.

a) Reagents

1. 0.1M phosphate buffer (pH 7.6)

2. Casein solution (2 per cent in phosphate buffer)

3. Stock trypsin solution: 5.0 mg trypsin (Worthington Bio-chemical Corporation, USA, 190 units/ mg protein) in 100 ml of 0.001M HCl.

4. 5 per cent trichloro acetic acid (TCA)

b) Procedure

Trypsin inhibitor was extracted by shaking 200 mg of pulses flour in a screw capped test tube with 10 ml of 0.1M phosphate buffer at room temperature for an hour and then filtered through Whatman No. 42.

i) Trypsin inhibitor activity

Pipetted 0.2 to 1.0 ml of sample extracts into a triplicate set of test tubes (one set for each level of extract), and the volume brought to 1 ml with phosphate buffer, 1 ml of stock trypsin solution was added to each tube and the tubes were placed in water bath at 37^0C. To one set of the triplicate tubes 6 ml of 5 per cent (W/V) trichloroacetic acid was added. These

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tubes served as blank for the other two sets. Then 2 ml of casein solution previously brought to 37^0C was added to each tube. The tubes were allowed to remain at 37^0C for exactly 20 minutes at which time the reaction was stopped by adding 6 ml of 5 per cent TCA to the experimental tubes. After standing for 1 hour at room temperature, the suspension was filtered and the absorbance of the filtrate was measured at 280 nm against the blank.

ii) Expression of activity

One trypsin unit (TU) is arbitarily defined as an increase of 0.01 absorbances units at 280 nm in 20 minutes per 10 ml of the reaction mixture under the conditions set forth herein. Trypsin inhibitor activity is defined as the number of trypsin units inhibited (TUI) per mg meal.

3.4.9.2. Chymotrypsin inhibitor activity

Chymotrypsin inhibitor activity (CIA) was assayed according to Kakade *et al.* (1970). The method involved the use of 1 per cent casein and the spectrophotometric determination of the breakdown products by a given concentration of chymotrypsin in the presence of the inhibitor.

a) Reagents

1. 01.M borate buffer, PH 7.6 : dissolved 6.183 g boric acid in water and volume to 1000 ml : dissolved 38.139 g disodium tetraborate in water and volume to 1000 ml. Mixed both the solution to get pH 7.6.
2. Casein solution: 1.0 per cent in 0.1M borate buffer.
3. 0.08M calcium chloride in 0.001M HCl
4. Chymotrypsin solution: 4 mg chymotrypsin (Worthington Biochemical Corporation USA, 61.4 units/mg protein) in 0.00M HCl containing 0.08M $Cacl_2$
5. Tricholoroacetic acid (TCA) reagent: (1.8 per cent TCA, 1.8 per cent anhydrous sodium acetate and 2.0 per cent glacial acetic acid.

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b) Procedure

Chymotrypsin inhibitor was extracted same as described for trypsin inhibitor except that 0.1M borate buffer (PH 7.6) was used in place of 0.1 m phosphate buffer.

i) Chymotrypsin inhibitor activity

Pipetted 0.2 to 1.0 ml of the sample extract into triplicate set of tubes (one set for each level of extracts), and the volume brought to 1 ml with borate buffer. Then 1 ml of chymotrypsin solution was added to each tube, and the tubes ware placed in water bath at 37^0C. To one set of the triplicate tubes 6.0 ml of TCA reagent was added, this set served as a blank for the other two sets. Then 2 ml of casein solution previously brought to 37^0C was added to each of the tube. The tubes were allowed to remain at 37^0C for exactly 10 minutes at which time the reaction was stopped by addition of 6.0 ml of TCA reagent to the experimental tubes. After standing at room temperature for at least 30 minutes the suspension was filtered and the absorbance of the filtrate was measured at 275 nm against appropriate blanks.

ii) Expression of activity

One chymotrypsin inhibitor unit (CU) is arbitrarily defined as an increase of 0.01 absorbance unit at 275 nm in 10 minutes per 10 ml of the reaction mixture under the conditions described herein.

3.4.9.3. Determination of lathyrus sativus neurotoxin: β-N-oxylyl-L-α, β diaminopropionic acid (ODAP)

The neurotoxin β-ODAP in *Lathayrus sativus* was determined by colorimetric method as described by Rao, (1978). In this method the toxin ODAP (BOAA) is first hydrolysed to DAP (∝, β-Diaminopropionic acid) by potassium hydroxide and the DAP is then reacted

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upon with OPT (*o*-phthalaldehyde) to produce an intense yellow color which obeys the Beers law and is therefore measurable spectrophotometrically at 420 nm.

a) Reagents

1. Ethanol 95 per cent
2. 3N KOH
3. Potassium tetraborate buffer (0.5M, pH-9.9)
4. OPT (*o*-phthalaldehyde): 100 mg in 1 ml 95 per cent ethanol and 99 ml borate buffer containing 200 µl mercaptoethanol)
5. ODAP standard (0.2 mg/ml water)

b) Procedure

i) Extraction of ODAP from dried powder of the lathyrus seed

A portion (50 mg) of lathyrus seed powder was mixed with 3 ml of distilled water in screw capped test tube and mixed thoroughly. The tubes were then heated in boiling water bath for 30 minutes. After cooling 7 ml of 95 per cent ethanol was added and placed in a shaker for over night shaking. The tubes were then centrifuges at 4000 rpm for 10 minutes. The supernatant was used for ODAP determination.

ii) Determination of ODAP

To 100 µl of extracted supernatant 200 µl of 3N KOH was added and then heated in a boiling water bath for 30 minutes. After cooling 700 µl water and 2 ml of OPT reagent was added. The concentration of the ODAP in the supernatant was determined at 420 nm using the measurement of concentration of standard ODAP by a spectrophotometer (Spectronic® Geneys™8 UV/Visible spectrophotometer).

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iii) ODAP standard

For preparation of ODAP standard 20,40, 60, 80 and 100 µl of standard ODAP were taken in each of the screw-capped test tube. Volumed this tubes to 100 µl with water and then 200 µl of 3N KOH were added to each of the tube and heated for 30 minutes in a boiling water bath. Cooled the tubes and 700 µl water and 2 ml of OPT reagent were added. After 30 minutes absorbance of the ODAP standard were measured at 420 nm from which the concentration of the unknown samples were determined.

3.4.9.4. Estimation of phytic acid

Phytic acid present in pulses was determined colorimetrically by the method described by Wheeler and Ferral (1971). Phytic acid was extracted with 5 per cent trichloroacetic acid. The extracted phytic acid and phytic acid chelators react with ferric chloride and forms ferric phytate. The available ferric ion after reaction is determined by developing blooded color with potassium thiocyanate.

a) Reagents

i) Trichloro acetic acid (5 per cent)

ii) Potassium thiocyanate (29 per cent)

iii) Ferric chloride solution (1 mg/ml water solution)

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b) Procedure

i) Extraction of phytic acid

Weighed 200 mg pulses flour and transferred in a 15 ml centrifuge tube and then 7.5 ml of 5 per cent TCA solution was added and vortexed the mixture. The mixture was incubated at 60^0C for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. The supernatant was transferred in a 25 ml volumetirc flask. The extraction was repeated for 2 more times and transferred the supernatant in to the volumetric flask and then volumed to 25 ml.

ii) Absorbance determination of extracted sample

Pipetted out 20 ml of extracted sample into a 75 ml Technicon tube and then 5 ml of ferric chloride solution was added. The tubes were then heated in a block heater at 95^0C for 45 minutes. Cooled and then volumed to 75 ml and filtered through Whatman No. 42 filter paper. Pipetted out 2.5 ml filtrate and then 2 ml potassium thiocyanate and 5 ml water was added in each of the tube. After mixing the absorbance was measured at 485 nm against a water blank.

iii) Preparation of standard graph

Pipetted out 5 ml of ferric chloride solution in a conical flask and then 20 ml water and 2 ml of 5 per cent TCA was added. The flask was heated in a block heater at 95^oC for 45 minutes. Filtered after cooling and from the filtrate pipetted out different volumes from 0.5 to 2.5 ml in different tubes. The volume was made up to 7.5 ml with water and then 2 ml of potassium thiocynate was added in each tube. After mixing the absorbance of the solution was measured at 485 nm. A standard graph of ferric ion was plotted. From the graph the slop was determined and then values for phytic acid were calculated on the assumption that four

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ferric ions combine with one molecule of phytic acid ($Fe_4P_6C_6H_6O_{24}$) (Anderson, 1963). Calculations were done on dry weight basis.

3.4.9.5. Determination of molecular of weight of protein by gel electrophoresis

Molecular weights of proteins present in pulses were estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) as described by laem-Li, (1970). Proteins soluble in SDS are separated on the basis of their charge and molecular weight. During elcetrophresis proteins of different mobilities travel as discrete zones which gradually separate from each other as electrophoresis proceeds. Indeed a linear relationship is obtained between the log MW of a protein and its relative mobility (Hedric *et al.*, 1968).

a) Reagents

Stock solution

1. **Acrylamide: BIS (30 per cent)**

   29.2 g acrylamide

   0.8 N'N' methylene bis-acrylamide

   Quantity stock (q.s.) 100 ml with distilled water.

   Filtered and stored at 4^0C (for 30 days)

2. **1.5M Tris-Cl, pH 8.8**

   18.15 g Tris base

   50 ml distilled water

   pH adjusted 8.8 with 1N HCl

   q.s. 100 ml with distilled water.

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3. 0.5M Tris-Cl, pH 6.8

3.0 g Tris base

50 ml distilled water

pH adjusted to 6.8 with 1N HCl

q.s. 50 ml with distilled water.

4. Ammonium persulphate

Dissolved 100 mg ammonium persulphate in 1ml water (prepared fresh every time)

5. Gel (15 per cent) Preparation

1.5M Tris-Cl.	6.25 ml
10 per cent SDS	0.25 ml
Acrylamide Bis solution	12.5 ml
Water	5.85 ml
TEMED (Tetramethylethylenediamin)	0.0075 ml
Ammonium persulphate	0.125 ml
	25.0 ml

6. Stacking gel (4 per cent) solution

05.M Tris-Cl	1.0 ml
10 per cent SDS	0.1 ml
Acrylamide Bis solution	1.3 ml
Water	7.55 ml
TEMED	0.01 ml
Ammonium persulphate solution	0.05 ml
	10.01 ml

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7. Sample buffer, pH 6.8 (3x)

0.15 per cent Bromophenol blue (in ethanol)	0.6 ml
0.5M tris-Cl	0.8 ml
Glycerol	2.6 ml
SDS (30 per cent)	3.2 ml
2-Mercaptoethanol	0.8 ml
	8.0 ml

8. Electrode buffer (pH 8-3)

Tris base	3.0 g
Glycine	14.4 g
SDS	1.0 g

Dissolved in water and volume to 1000 ml with water.

9. Staining solution

Coomassie brilliant blue	2.5 g
Methanol	500 ml
20 per cent glacial acetic acid	500 ml

10. Destaining solution

Methanol	500 ml
20 per cent glacial acetic acid	500 ml

b) Procedure

The plain glass plate was placed on the spacer containing plate and then a nylon thread was placed on the outer side of the spacer and tightened the plates with clips on either side.

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i) Casting the gels

Poured the resolving gel solutions leaving 1-1.5 cm at the top for the sample wells in stacking gel. Overlaid the resolving gel solution with small quantity of water to achieve a smooth and flat surface after polymerization. Removed excess water after polymerization and then stacking gel was added over the resolving gel surface. The comb was inserted into the stacking gel solution and allowed to polymerize (for about 1 hour) and removed the comb slowly.

ii) Sample preparation

A portion (5 mg) of isolated protein from each of the fresh and stored pulses was dissolved in 0.5 ml of 1.5 M tris-Cl. Then 20 µl of each of the sample solution diluted to 30 µl with sample buffer (3x-buffer) in eppendrop tubes. The tubes were then heated at 100°C for 3 minutes to the denatured the proteins.

iii) Preparation of protein calibration kit (Protein marker)

The proteins calibration kit (Amersham Pharmacia Biotech UK Ltd.) containing 6 highly purified and well characterized protein was used. The kit consists of phosphorylase b (94,000), Albumin (67,000), Ovalbumin (43,000), Carbonic anhydrase (30,000), Trypsin inhibitor (20,100) and α-Lactalbumin (14,400). The kit was mixed with 200 µl of 1x buffer solution to reconstitute proteins. Then 10 µl of this marker was transferred into eppendrop tube and heated for 3 minutes to denature the proteins.

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iv) Sample and marker loading

After setting the casting gel into buffer tank the comb was removed and 10 µl of sample solution and 5 µl of marker solution were poured in each of the well. The upper part of the buffer tank was filled with electrode buffer and overlaid the sample and protein marker. The lower tank was also filled with electrode buffer. Electric connections from power supply were set to a 40 mA for 30 minutes. The power supply was then increased to 70 mA. When the dye front (bromophenol blue) reached at the bottom (required about 3 hours) electric current was switched off and the gel was removed and transferred carefully and immersed in staining solution in a plastic tank. The gel was shaked for 2 hours in a shaking water bath at 37^0C. Destained the gel with constant shaking for overnight at 37^0C in a shaking water bath. Photographed the gel for calculating the molecular weight determination of different proteins.

v) Determination of molecular weight

Measured the migration distance of the proteins in the calobration kit and proteins of interest. Measured the migration distance of the dye marker. The corresponding Rf values were calculated by dividing migration distance of the protein by the migration distance of the dye marker. A calibration curve was constructed by graphing Rf values vs. log molecular weight for the proteins in the calibration kit. Then determined the molecular weight of the protein form the calibration curve.

3.4.9.6. Analysis of data

The format of the analysis of variance for measurement on seed quality over storing period based on a Completely Randomized Design is similar to that of a standard Split-Plot Design with type of container as main-plot and storing period as sub-plot treatments. An outline of the analysis of variance is given in Table 3.1.

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Table 3.1. Analysis of variance for Split-Plot design with container as main plot and storage duration as sub-plot based on CRD

Source of Variation	`Degrees of Freedom	Mean square	Computed F
Container (C)	c-l	MSC	MSC/MSE_a
Error(a)	r(c-l)	MSE_a	
Storing duration (D)	(d-l)	MSD	MSD/MSE_b
C X D	(c-l) (d-l)	MS(CXD)	$MS(CXD)/MSE_b$
Error (b)	cr(d-l)	MSE_b	

c = No. of types of container, d = No. of storing duration, t = No. of replications

The experiment was conducted in three years to capture if there is any, the effect of environmental variation during the growing period on the quality of stored seeds of pulses. Environmental variation in different year is unpredictable and hence year may be considered as a random factor. The appropriate analysis of data on seed quality is then the combined analysis of variance of split-plot over years considering year as random. An outline of the analysis of variance is given in Table 3.2.

Table 3.2. Analysis of variance for Split-Plot design with container as main-plot and storage duration as sub-plot combined over years based on CRD

Source of Variation	Degrees of Freedom	Mean square	Computed F
Year (Y)	y-l	MSY	MSY/MSF_a
Container (C)	c-l	MSC	MSC/MS (YXC)
YXC	(y-l) (c-l)	MS (YXC)	$MS (YXC)/MSE_a$
Pooled error (a)	yr(c-l)	MSE_a	
Storing duration (D)	(d-l)	MSD	MSD/MS (YXCXD)
Y X D	(y-l) (d-l)	MS (Y X D)	$MS(YXD)/MSE_b$
C X D	(c-l) (d-l)	MS (C X D)	MS(CXD)/MS(YXCXD)
Y X C X D	(y-l) (c-l) (d-l)	MS (YXCXD)	$MS(YXCXD)/MSE_b$
Pooled error(b)	ycr(d-l)	MSE_b	

y = No. of years, c = No. of types of containers, d = No. of storing duration, r = No. of replications

Al the data were analyzed with the help of a computer using the method of IRRISTATE Version 4.0

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Chapter 4

RESULTS AND DISCUSSION

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4.1. Interpretation of interaction

Analysis of variance (Appendix figure 1-6) of different factors showed interaction of some of the factors. They are described below.

4.1.1. Interaction between container and duration

It was observed that there was significant interaction between container (C) and the storage duration (D) of all the parameters of three pulses under study. Since the interaction between container and duration was significant the effect of container on quality of seeds were compared at each duration and vice versa.

4.1.2. Interaction between year and duration

The interaction between year (Y) and storing duration (D) of the protein, total sugar and reducing sugar of lentil and chickpea and protein, starch and reducing of khesari were significant. It was observed that during storage period proteins of different pulses was broken and decreased their quantity (Tables 4.24, 4.25 and 4.26). Similarly starches was also broken and decreased their quantity and increased the quantity of total sugar and reducing sugar (Tables 4.30, 4.31 and 4.32). The interaction indicated that the growing environment as well as the quality of pulses might have influenced the breakdown and hence interaction effect was observed. But the graph (Appendix figure 7-15) showed that the interaction effect was mainly due to a specific year especially the year 1999 and the interaction was small and may be neglected.

4.1.3. Interaction between year and container

The interaction between year (Y) and container (C) was observed only in cooking time and solids dispersed of chickpea. It was evident from the graph (Appendix figure 16-17)

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that the interaction of factors mentioned above were only due to the year 1999 out of three years. Again the interaction effect was quite small and may be neglected.

4.1.4. Interaction between year, container and duration

Significant interactions of year (Y), container (C) and duration (D) was observed in case of total sugar (TS) and reducing sugar (RS) of lentil and chickpea and starch and reducing sugar content in khesari. The graph (Appendix figure 1-6) showed that although the interaction of three factors was significant but it was not clear from the graph. The observed effect might be due to small experimental error and therefore the interactions may be neglected.

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4.2. Physico-chemical properties of pre-stored pulses

A wide variability exists in the physico-chemical properties of lentil, chickpea and khesari. The results of various physico-chemical properties are presented in table 4.1. It was observed that moisture content of lentil, chickpea and khesari were 8.16 per cent, 8.21 per cent and 8.15 per cent respectively. The cooking time for lentil was 8.08 $\pm$ 0.31 minutes while chickpea and khesari required much longer time for cooking (29.11 $\pm$ 0.55 and 20.21 $\pm$ 0.61 minutes respectively). Higher amount of water absorption (1.87 g/g dhal) was observed in lentil. Lentil also gave higher percentage of solids dispersed (77.55 per cent). Chickpea absorbed minimum amount of water (1.32 g/g dhal) and dispersed 55.77 per cent solids during cooking. Khesari absorbed 1.67 g/g water and dispersed 64.48 percent solids. The initial germination percentage of lentil was much lower (64.00 per cent) in comparison with chickpea (94.00 per cent) and khesari (93.78 percent). The results (Table 4.1) indicated that small seeded pulse lentil took less cooking time in comparison with large seeded pulses (chickpea and khesari). Similar results were also reported by Erskine *et al.* (1985) for lentil and by Singh *et al.* (1990) for chickpea.

Table 4.1. Physico-chemical properties of different pre-stored pulses

Parameters	Name of pulses		
	Lentil	Chickpea	Khesari
Moisture (%)	8.16 ± .09	8.21 ± .05	8.15 ±0.12
Cooking time (minutes)	8.08 ± 0.31	29.11 ± 0.55	20.21 ±0.61
Water absorption (g/g dhal)	1.87 ± .027	1.32 ± 0.02	1.67 ±.018
Solids dispersed (%)	77.55 ± 1.68	55.77 ± 0.64	64.48 ±0.96
Germination (%)	64.00 ± 2.83	94.00 ± 1.73	93.78 ±1.56

Each value is the average of 9 replications of 3 consecutive years

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4.3. Chemical composition of pre-stored pulses

The chemical composition of different pulses are presented in table 4.2. The results showed that khesari dhal contained the highest amount of crude and true protein (34.63 per cent and 30.55 per cent) followed by lentil (29.51 and 25.85 per cent respectively). Chickpea contained lowest amount of crude and true protein (23.46 and 20.82 per cent respectively). The starch content was higher in chickpea (56.25 per cent) and lower in khesari (51.80 per cent). But the amylose content was highest in lentil (40.00 per cent) and lowest in chickpea (31.49 per cent). The total sugar content was higher in chickpea (3.96 per cent), while the reducing sugar was higher in khesari (1.05 per cent). Crude fiber content of chickpea and khesari was higher than lentil. Chemical composition of lentil, chickpea and khesari are in agreement with the results reported by Saluakhe *et al.* (1985) and Reddy *et al.* (1984). The chemical composition of different pulse also reveal that a pulse rich in protein's generally poor in starch and vice versa.

4.3.1. Mineral content in pre-stored pulses

The mineral content in pre-stored lentil, chickpea and khesari are presented in table 4.3. It was observed that except sodium all the minerals studied were higher in chickpea followed by khesari. Khesari dhal contained higher amount of sodium. The results of mineral contents are in agreement with Salunkhe *et al.* (1985).

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Table 4.2. Chemical composition of different pre-stored pulses

Parameters	Name of pulses		
	Lentil	Chickpea	Khesari
Crude protein content (%)	29.51 ± 0.23	23.46 ± 0.26	34.63 ± 0.16
True protein content (%)*	25.84 ± 0.50	20.82 ± 0.35	30.55 ± 0.28
Starch content (%)	52.91 ± 0.2	56.25 ± 0.80	51.80 ± 0.72
Amylose (%)	40.00 ± 1.5	31.49 ± 0.68	34.87 ± 0.22
Total sugar content (%)	3.72 ± 0.11	3.96 ± 0.11	3.91 ± 0.10
Reducing sugar content (%)	0.95 ± 0.04	1.04 ± 0.04	1.05 ± 0.02
Crude fiber (%)	4.35 ± 0.17	8.7 ± 0.20	8.27 ± 0.22

Each value is the average of 9 replications of 3 consecutive years
* Total protein N − Free N

Table 4.3. Mineral contents of different pulses (mg/100g)

Parameters	Name of pulses		
	Lentil	Chickpea	Khesari
Calcium	80.22 ± 6.94	174.11 ± 9.93	129 ± 8.83
Phosphorus	283.67 ± 10.19	382.22 ± 10.87	326.33 ± 13.96
Magnesium	85.67 ± 4.69	156.67 ± 9.38	90.78 ± 5.61
Sodium	38.04 ± 1.25	31.47 ± 0.58	39.53 ± 0.63
Iron	5.4 ± 0.25	7.84 ± 0.16	6.76 ± 0.18
Copper	0.72 ± 0.40	2.38 ± 0.19	0.75 ± 0.07
Potassium	761.67 ± 21.79	845 ± 18.37	776.33 ± 18.65

Each value is the average of 9 replications of 3 consecutive years

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4.3.2. *In vitro* digestibility of protein and starch of pre-stored pulses

The *in vitro* digestibility of protein and starch of pre-stored lentil, chickpea and khesari are presented in table 4.4. The results showed that higher *in vitro* digestibility of protein (90.03 percent) was observed in case of lentil followed by khesari (88.46 percent. Similar results on protein digestibility of lentil and chickpea had been reported by Williams and Singh (1987) and for khesari by Salunkhe and Kadam (1989). The *in vitro* starch digestibility considering the meal was higher both in chickpea and khesari (48.44 mg maltose/g meal). On the other hand while considering the meal starch it was higher in lentil (91.43 mg maltose/g meal starch) followed by khesari 88.34 mg maltose/g meal starch. The digestibility of starch of different pulses is in agreement with Singh *et al.* (1982).

In lentil the amount of rapidly digestible starch and slowly digestible starch were slightly higher (58.10 per cent and 21.10 per cent respectively) and resistant starch slightly lower 20.80 per cent). Resistant starch was higher in chickpea (24.10 per cent) followed by khesari (22.62 per cent).

Table 4.4. *In vitro* digestibility of protein and starch

Parameters	Name of pulses		
	Lentil	Chickpea	Khesari
In vitro protein digestibility (%)	90.03 ± 1.02	85.35 ± 0.9	88.46 ± 0.90
In vitro starch digestibility (mg maltose/g meal)	48.44 ± 0.56	48.44 ± 0.44	45.83 ± 0.65
In vitro starch digestibility (mg maltose/g meal starch)	91.43 ± 0.92	85.33 ± 0.89	88.34 ± 0.89
Rapidly digestible starch (%)	58.10 ± 0.67	56.75 ± 0.69	56.37 ± 0.31
Slowly digestible starch (%)	21.10 ± 0.23	19.15 ± 0.39	20.96 ± 0.19
Resistant starch (%)	20.85 ± 0.25	24.10 ± 0.67	22.67 ± 27.0

Each value is the average of 9 replications of 3 consecutive years

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4.3.3. Antinutritional factors

4.3.3.1. Trypsin and chymotrypsin inhibitors

The utilization of pulses for human nutrition is constrained due to inherent antinutritional factors present in them. The amount of various antinutritional factors or their inhibition capacity in different pulses is presented in table 4.5. It was observed that the trypsin inhibition was higher in khesari (13.32 units/mg meal) followed by chickpea 12.5 units/mg meal. The trypsin inhibition was lower in lentil (9.53-units/mg meal). Chymotrypsin inhibition in khesari was 8.74 units/mg meal and was higher than lentil and chickpea where the inhibition were 7.14 units/mg meal and 7.03 units/mg meal respectively. The lower trypsin inhibition activity of lentil are in agreement with Hamza *et al.* (1986) where they had observed lowest trypsin inhibition activity and highest protein digestibility when compared with other legumes. The results on trypsin and chymotrypsin inhibition activity of chickpea and khesari are also in agreement with Singh and Jambunathan (1981) and Srivastova and khokher (1996) respectively.

4.3.3.2. Phytic acid

The phytic acid content in chickpea was higher (8.65 mg/g) followed by lentil (8.09 mg/g) (Table 4.5). Khesari dhal contained lower amount of phytic acid (7.42 mg/g). Davis (1981) reported that the phytic acid content in lentil was 7.63 mg/g. Duhan *et al.* (1989) reported that phytic acid content of chickpea varied significantly from 7.4 to 8.10 mg/g. Srivastova and Khokhar (1996) reported that phytic acid in khesari had ranged 10.3 – 10.9 mg/g.

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4.3.3.3. Neurotoxin ODAP

β-N-Oxalyl- ∝, β-diamino propionic acid (ODAP) is a low molecular weight neurotoxin present in lathyrus seed (khesari). It was observed that the amount of ODAP in khesari was 0.78 g/kg (Table 4.5). The ODAP content varied from variety to variety. In Bangladesh, the Jamalpur variety contained 5 to 8 g/kg (Dutta *et al.,* 1998; Hussain *et al.,* 1997; Roy *et al.*, 1990)). In India some high toxin varieties contained up to 28 g/kg ODAP in the seeds of lathyrus (Roy and Rao, 1968). In Ethiopia, an ODAP content of up to 5g/kg was reported (Kebede *et al.,* 1995).

Table 4.5. Antinutritional factors present in different pulses

Parameters	Name of pulses		
	Lentil	Chickpea	Khesari
Tripsin Inhibitor Activity (TIA)[a]	9.53 ± 0.42	12.51 ± 0.21	13.32 ± 4.53
Chytripsin Inhibitor Activity (CIA)[a]	7.14 ± 0.18	7.03 ± 0.25	8.74 ± 0.16
Phytic acid (mg/g)	8.09 ± 0.13	8.65 ± 0.13	7.42 ± 1.15
ODAP (%)	-	-	0.78 ± 0.02

[a] Inhibitor units/mg meal
Each value is the average of 9 replications of 3 consecutive years

4.4. Fungal infection

4.4.1. Lentil

As many as fungal species representing four genera were detected in pre-stored lentil. In stored lentil seven genera and one-unidentified bacteria were detected from the seeds stored in six different types of containers under three years of observations (1999, 2000 and 2001). The identified genera were *Aspergillus, Alternaria, Cladosporium, Curvularia, Fusarium, Panicillium* and *Rhizopus* (Table 4.6). In general, less incidence of seed associated mycoflora was detected in all the three years study irrespective of storing containers

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(treatments). Pooled data of three years indicated that among the fungal species, *Aspergillus flavus, Fusarium oxysporum* and *F. solani* prevailed more in seeds compared to other fungal species. It was found that microbial infection increased slightly with increasing storage period. Among the containers more microbial infection was recorded from the seeds stored in plain earthen *motka* (T_1) and least incidence was recorded in seeds stored in polythene lined jute bag (T_3). Although the fungal incidence was low in lentil but the incidence of storage fungi like *Aspergillus* increased with increasing storage period irrespective of containers. Prevalence of *Fusarim oxysporum* and *F. solani* were more or less static at both 6 months and 12 months of storage. The incidence of *F. oxysporum* and *F solani* were recorded 17 per cent and 15 per cent respectively on pre-stored seed samples but later it decreased in storage irrespective of containers (Table 4.6). Considering the total fungal infection all the storage containers showed more or less similar trend of fungal seed infection with the lowest in polythene lined jute bag (T_3) and coal-tar coated *motka* (T_5) and highest in plain earthen *motka* (T_1).

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Table 4.6. Effect of storage containers and storage period on the prevalence of seed associated microbes in lentil

Fungi	Prevalence of fungi (%)												
	Pre-stored sample	T_1		T_2		T_3		T_4		T_5		T_6	
		6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months
Aspergillus flavus	1	3	7	2	4	2	3	4	4	2	4	2	2
A. niger	1	2	2	1	1	1	2	1	1	1	1	1	2
A. flaviceps	1	1	1	1	1	1	1	-	1	1	1	2	2
A. clavatonanika	-	-	-	-	-	-	-	1	-	1	1	-	-
Alternaria tenuis		1	3	2	2	1	3	1	2	1	1	1	1
Cladosporium sp.	1	-	-	-	1	-	-	1	1	-	1	-	1
Curvularia sp.	9	2	-	-	-	1	1	1	-	1	-	1	-
Fusarium osysporum	17	4	4	5	3	2	2	2	2	2	2	3	3
F. Solani	15	6	2	1	3	1	1	2	2	-	1	2	3
Penicilleum sp.	-	-	-	-	-	1	1	1	-	1	1	-	1
Rhizophus	-	-	1	-	-	-	-	-	-	-	-	-	-
Bacteria	-	-	-			-	-	-	-	-	1	-	-
Total fungal infection (%)	45	19	20	12	15	10	14	14	13	10	14	12	15

Each value is the mean of 3 consecutive years

T_1 = Plain earthen *motka*, T_2 = Plain tin container, T_3 = Polythene lined jute bag,
T_4 = Polythene lined *motka*, T_5 = Coal-tar coated *motka*,
T_6 = Improved tin container

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4.4.2. Chickpea

In pre-stored chickpea five fungal species represented three genera were detected. In stored chickpea a total of 9 microbes representing seven genera were detected from seeds stored in different containers for 12 months (Table 4.7). The identified fungal species were *Aspergillus flavus, A. niger, A flaviceps, Alternaria tenuis, cladosporium* sp., *Curvularia* sp., *Fusariums oxysporum, F. solani, Penicillum* sp. and *Rhizopus* sp. It was observed that irrespective of storage containers, *Aspergillus* sp. (*A. flavus* and *A. niger*) were more frequently associated with chickpea seeds during storage compared to other microbes. Between them *A. niger* was more prevalent compared to *A. flavus*. Association of *F. oxysporum* ranked next to *A. flavus* and *A. niger* to cause seed infection. In general, fugal infection on seeds in all storage containers increased with increasing storage time especially in case of *Aspergillus sp.* (storage fungi). Among the tested storage containers polythene lined jute bag (T_3) gave the consistent low fungal infection where cumulative total fugal infection were recorded 19 per cent at 6 months and 33 per cent at 12 months of storage. Seeds stored in polythene lined *motka* ranked next to polythene lined jute bag where 24 per cent and 42 per cent total seed infection were recorded respectively at 6 months and 12 months of storage followed by improved tin container (T_6) where 27 per cent and 24 per cent seed infection respectively were recorded at the same period of time. It was observed that the plain earthen *motka* (T_1) was proved ineffective to reduce seed infection where 64 per cent total seed infection was recorded after 6 months and 125 per cent after 12 months of storage against 29 per cent total seed infection of pre-stored chickpea seed sample.

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Table 4.7. Effect of storage containers and storage period on the prevalence of seed associated microbes in chickpea

Fungi	Prevalence of fungi (%)												
	Pre-stored sample	T_1		T_2		T_3		T_4		T_5		T_6	
		6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months
Aspergillus flavus	3	24	40	9	18	3	6	5	11	5	9	11	14
A. niger	1	21	69	43	48	4	18	10	25	17	36	9	22
A. flaviceps	1	-	3	-	-	-	1	-	-	1	-	-	-
Alternaria tenuis	-	-	-	-	1	-	-	-	-	-	1	-	-
Cladosporium sp.	3	2	3	1	4	1	2	-	1	-	-	-	-
Curvularia sp.	-	2	1	3	6	2	1	-	-	-	-	-	1
Fusarium osysporum	20	14	6	-	-	9	5	9	5	2	2	7	4
F. Solani	1	-	1	13	8	-	-	-	-	7	5	-	-
Penicilleum sp.	-	-	1	-	2	-	-	-	-	-	1	-	-
Rhizophus	-	1	1	-	1	-	-	-	-	-	-	-	-
Total fungal infection (%)	29	64	125	69	88	19	33	24	42	32	54	27	41

Each value is the mean of 3 consecutive years

T_1 = Plain earthen *motka*, T_2 = Plain tin container, T_3 = Polythene lined jute bag,

T_4 = Polythene lined *motka*, T_5 = Coal-tar coated *motka*,

T_6 = Improved tin container

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4.4.3. Khesari

Six fungal species representing four genera were detected in the seeds of pre-stored hesari. In stored khesari ten fungal spices were identified from the seeds stored for 12 months in six different storage containers. The identified fungal species were *Aspergillus flavus, A. niger, A. flaviceps, Alternaria tenuis, cladosporium* sp. *Curvularia* sp., *Fusarium oxysporum, F. Solani, Penicillium* sp. and *Rhizopus* sp. (Table 4.8). Cumulative data of three years revealed that the incidence of microbial infection on pre-stored seed was minimum but their incidence were increased with increasing storage period. Of the storage containers used, the minimum fungal infection was detected with seeds stored in polythene lined jute bag (T_3) where 14 per cent and 17 per cent infection were recorded respectively, at 6 months and 12 months storing (Table 4.8). Seeds stored in polythene lined *motka* (T_4) ranked next to seeds stored in polythene lined jute bag (T_3) to minimize total fungal infection (17 per cent and 23 per cent respectively at 6 months and 12 months storage) followed by seeds stored in improved tin container (T_6), and plain tin container (T_2), Coal-tar coated *motka* (T_5) and plain earthen *motka* (T_1). Among the recorded fungi, *Aspergillius flavus* was more prevailed in khesari seeds in all the treatments followed by *Fusarium oxysporum* and *A. niger.* Seeds stored in polythene lined jute bag (T_3) showed the minimal infection of *A. flavus* compared to other storage containers. About 4 per cent seed infection by *A. flavus* in seeds stored in polythene lined jute bag (T_3), 6 per cent in polythene lined *motka* (T_4), 8 per cent in coal-tar coated *motka* (T_5), 9 per cent in plain tin container (T_2) and improved tin container (T_6) and 10 per cent in plain earthen *motka* (T_1) were recorded against 2 per cent in pre stored seed sample after one year storage. It was observed that the minimal fungal infection was recorded in seeds stored in polythene lined jute bag (14 per cent at 6 months and 17 per cent at 12 months of storage) and highest infection was recorded in seeds stored in plain earthen *motka* (25 per cent at 6 months and 35 per cent at 12 months of storage).

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Table 4.8. Effect of storage containers and storage period on the prevalence of seed associated microbes in khesari

Fungi	Prevalence of fungi (%)												
		T_1		T_2		T_3		T_4		T_5		T_6	
	Pre-stored sample	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months
Aspergillus flavus	2	6	10	4	9	2	4	3	6	4	8	5	9
A. niger	-	2	9	4	5	3	4	4	8	4	7	4	6
A. flaviceps	1	1	2	2	4	2	2	2	2	2	3	2	2
Alternaria tenuis	2	1	3	-	1	-	-	-	-	-	1	-	-
Cladosporium sp.	-	3	3	2	3	1	3	2	2	-	4	-	-
Curvularia sp.	3	-	-	1	1	2	2	-	-	1	1	2	1
Fusarium osysporum	9	7	4	7	5	1	-	4	3	6	5	1	1
F. Solani	4	3	3	1	1	2	1	-	-	1	1	5	4
Penicilleum sp.	-	-	1	1	2	1	1	1	1	1	1	1	1
Rhizophus	-	2	3	-	-	-	-	1	1	2	1	2	1
Total fungal infection (%)	21	25	35	22	31	14	17	17	23	21	32	22	23

Each value is the mean of 3 consecutive years

T_1 = Plain earthen *motka*, T_2 = Plain tin container, T_3 = Polythene lined jute bag,
T_4 = Polythene lined *motka*, T_5 = Coal-tar coated *motka*,
T_6 = Improved tin container

It was evident from the results (Table 4.6- 4.8) that the polythene lined jute bag performed better for protecting seeds against fungal infection followed by polythene lined *motka*, improved tin container and coal-tar coated *motka*. The plain earthen *motka* proved to be ineffective for protecting seeds against fungal infection during storage.

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4.5. Physico-chemical properties of stored samples

4.5.1. Moisture content

Moisture is the most important factor for maintaining the quality of seeds. Infestation by insects and molds is influenced by the integration of grain moisture, relative humidity and storage temperature. In addition, at higher temperature several biochemical changes occur in the seeds which results in the loss of seed viability and deterioration in nutritional and cooking quality. All the pulse varieties of the experiment were stored at safe moisture level i.e. below 9.0 per cent.

4.5.1.1. Lentil

The initial moisture content of lentil was 8.16 per cent. After 3 months of storage it was observed that the moisture content of seeds stored in plain earthen *motka* increased significantly to 10.38 per cent and this increase was about 27 per cent higher over the initial moisture content (Table 4.9). The moisture content in seeds stored in plain tin container and coal-tar coated *motka* were 8.49 per cent and 8.92 per cent respectively and were significantly higher in comparison with seeds stored in polythene lined jute bag and coal-tar coated *motka*. The lowest moisture content (8.32 per cent) was observed in seeds stored in both polythene lined jute bag and polythene lined *motka* followed by seeds stored in improved tin container. The increase in moisture content in seeds stored in polythene lined jute bag and coal-tar coated *motka* was only 1.96 per cent higher over the initial moisture content. The results also showed that the moisture content of seeds stored in plain earthen *motka* increased significantly and continued to increase with increasing storage duration (Table 4.9). The moisture content in seeds stored in other containers also increased but considerably at a slower rate. After 6 months of storage, the moisture content in seeds stored in plain earthen *motka* was 11.21 per cent and was significantly higher than other treatments. The increase in

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moisture content at this stage was about 37.38 per cent higher over the initial moisture content and it was 40.32 per cent higher after 9 months of storage.

After 12 months storage the highest moisture content (11.98 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher than other treatments. The increase in moisture content in this treatment was 46.81 per cent higher over the initial moisture content. The lower moisture content (8.73 per cent) was observed in seeds stored in polythene lined *motka*, but it was at par with seeds stored in polythene lined jute bag. The increase in moisture content in this treatment was only 3.98 per cent over the initial moisture content.

Table 4.9. Moisture content of lentil during different stages of storage period

Treatments	Moisture content of pre-stored sample (%)	Moisture content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	8.16	10.38	11.21	11.45	11.98
Plain tin container (T_2)	8.16	8.49	8.65	8.99	9.23
Polythene lined jute bag(T_3)	8.16	8.32	8.44	8.66	8.76
Polythene lined *motka*(T_4)	8.16	8.32	8.46	8.57	8.73
Coal-tar coated *motka*(T_5)	8.16	8.52	8.69	8.80	8.93
Improved tin container(T_6)	8.16	8.33	8.54	8.73	8.95

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.18 and for rows = 0.18

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4.5.1.2. Chickpea

Chickpea was stored with an initial moisture content of 8.21 per cent. After 3 months of storage the moisture content of seeds stored in plain earthen *motka* increased to 11.10 per cent and was significantly higher than the other treatments (Table 4.10). The increase in moisture content in this treatment was 35.20 per cent higher over the initial moisture content. The second highest moisture content (8.83 per cent) was observed in seeds stored in plain tin container followed by 8.76 per cent observed in seeds stored in coal-tar coated *motka*. The lower moisture content (8.39 per cent) was found in seeds stored in polythene lined jute bag and it was only 2.2 per cent higher than initial moisture content. On the other hand the increase in moisture in seeds stored in improved tin container and polythene lined *motka* were 2.8 per cent and 3.5 per cent respectively. The moisture percentage in seeds stored in plain earthen *motka* also increased significantly after 6 months of storage and continued to increase up to 12 months of storage. After 6 months storage the increase in moisture content was 46 per cent higher over the initial moisture.

After 12 months of storage the highest moisture content (14.27 per cent) was observed in seeds stored in plain earthen *motka* and it was 73.8 per cent higher than the initial moisture content. The moisture content in other treatments also increased but considerably at lower rate. The lower moisture content (8.73 per cent) was observed in seeds stored in polythene lined jute bag. The increase in moisture content in this treatment was only 6.8 per cent followed by 7.4 per cent and 9.25 per cent by seeds stored in polythene lined *motka* and improved tin container respectively. These values except those in plain earthen *motka* are within the safe moisture level.

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Table 4.10. Moisture content of chickpea during different stages of storage period

Treatments	Moisture content of pre-stored sample (%)	Moisture content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	8.21	11.10	12.00	13.43	14.27
Plain tin container (T_2)	8.21	8.83	9.06	9.22	9.37
Polythene lined jute bag(T_3)	8.21	8.39	8.55	8.68	8.77
Polythene lined *motka*(T_4)	8.21	8.51	8.62	8.65	8.82
Coal-tar coated *motka*(T_5)	8.21	8.76	8.92	9.05	9.16
Improved tin container(T_6)	8.21	8.44	8.62	8.74	8.79

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.20 and for rows 0.28

4.5.1.3. Khesari

The initial moisture content of khesari seed was 8.15 per cent. After 3 months of storage the moisture content of seeds stored in plain earthen *motka* increased to 10.44 per cent and was significantly higher than the other treatments (Table 4.11). This increase in moisture content was 28 per cent higher over the initial moisture content. The lower amount of moisture content (8.26 per cent) was observed in seeds stored in polythene lined jute bag. The increase in moisture content in this treatment was only 1.35 per cent higher over the initial moisture content. It was observed that the moisture content in seeds stored in plain earthen *motka* were significantly increased with increasing storage time. After 6 months of storage the increase in moisture content in this treatment was 36.56 per cent higher over the initial moisture content and this increase was 43.07 per cent higher after 9 months of storage. The moisture percentage in other treatments was also increased but the rate of increase was quite low.

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After 12 months of storage the highest moisture content (12.24 per cent) was observed in seed stored in plain earthen *motka* and it was 49.81 per cent higher over the initial moisture content. On the other hand the lower moisture content (8.67 per cent) was observed in seeds stored in polythene lined jute bag and it was only 6.98 per cent higher over the initial moisture content.

Table 4.11. Moisture content of khesari during different stages of storage period

Treatments	Moisture content of pre-stored sample (%)	Moisture content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	8.15	10.44	11.13	11.66	12.24
Plain tin container (T_2)	8.15	8.40	8.62	8.75	9.02
Polythene lined jute bag(T_3)	8.15	8.26	8.42	8.58	8.67
Polythene lined *motka*(T_4)	8.15	8.28	8.41	8.57	8.70
Coal-tar coated *motka*(T_5)	8.15	8.41	8.52	8.66	8.99
Improved tin container(T_6)	8.15	8.32	8.46	8.62	8.77

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.13 and for rows = 0.16

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It was revealed from the analysis of variance (Appendix Tables 1, 2, 3 and 4) that there was no interaction of environment i.e., year with storing duration in different types of containers, but a strong interaction was noticed between storing duration and types of containers for all varieties of pulses. As evident from patterns of moisture uptake during storage (Figure 4.1, 4.2 and 4.3), the presence of interaction between storage period and types of containers was mainly due to plain earthen *motka* and besides this type of storage device the interaction between storing duration and types of containers was almost negligible. The results (Tables 4.9, 4.10 and 4.11) showed that moisture content of seeds stored in plain earthen *motka* for three months increased considerably and continued to increase with increase in storing duration at a slower rate. For other storing devices, the moisture content also showed an increasing trend with the increase of storing period but the rate of increase was quite low, almost negligible.

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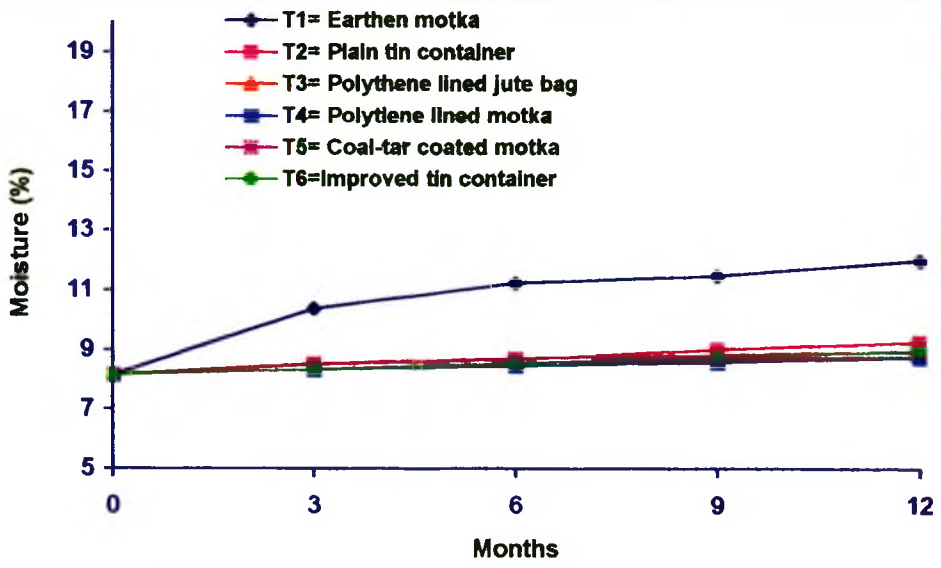

Fig. 4.1. Moisture content in lentil durirng different stages of storage period

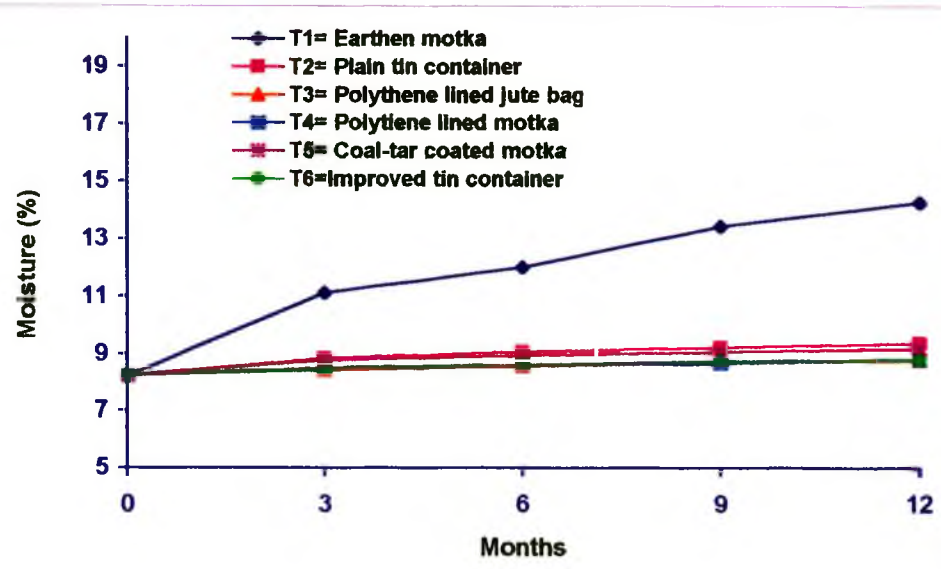

Fig. 4.2. Moisture content in chickpea during different stage of storage period

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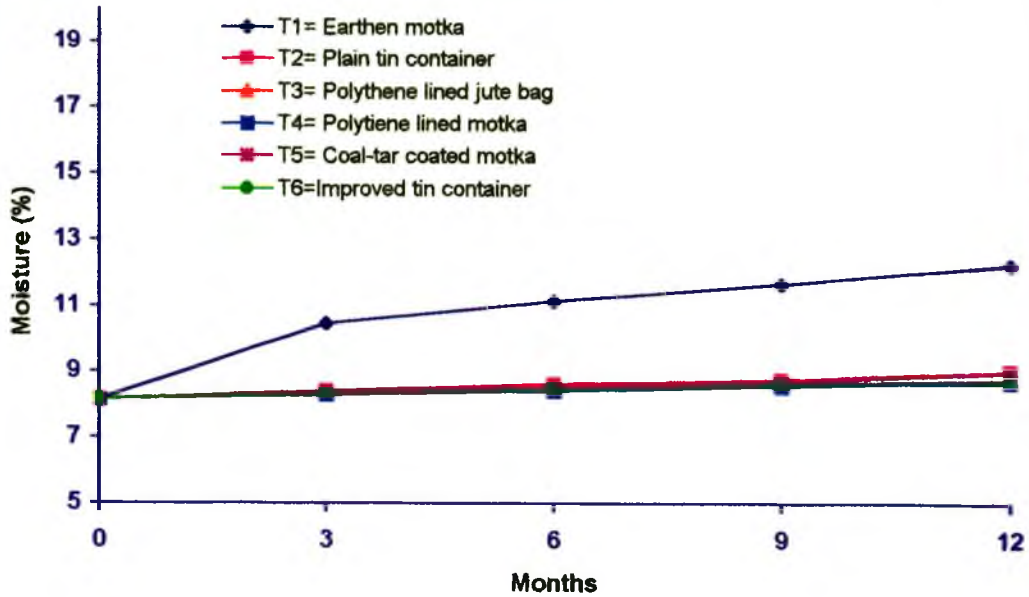

Fig. 4.3. Moisture content in khesari durirng different stages of storage period

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The increase in moisture content in plain earthen *motka* might be due to porousness of the container, which allowed the entrance of moisture from the environment. On the other hand, the polythene lining inside the *motka* or out side coating with coal-tar with good sealing system at the top prevents the moisture absorption into the seed. The improved tin container had a good sealing system at the top, which also prevents moisture absorption into the seed. The results indicated that for prevention of moisture, which is the key factor for deterioration of seed quality, polythene lined jute bag, polythene lined *motka* or coal-tar coated *motka* and improved tin containers may be used for safe storage of pulses.

4.5.2. Germination

Germination is one of the most important quality parameters of pulses.

4.5.2.1. Lentil

The germination percentage of pre-stored lentil was 64.00 per cent. Results showed that the germination percentage of lentil increased at the initial stages of storage (Table 4.12). It was observed that after 3 months of storage the higher percentage of germination (74.67 per cent) was observed in seeds stored in improved tin container and it was at par with all other treatments except seeds stored in plain earthen *motka*. The germination percentage increased in this treatment was 16.67 per cent higher over the pre-stored germination. It was revealed from the results that the germination percentage of lentil increased up to 6 months of storage and then started to decline (Table 4.12). After 6 months of storage higher amount of germination percentage (94.44 per cent) was observed in seeds stored in polythene lined *motka* and it was 42.56 per cent higher over the initial germination. This increase in germination was at par with seeds stored in plain tin container, polythene lined jute bag and improved tin container. The lower percentage of germination (88.89 per cent) was observed

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in seeds stored in both the plain earthen *motka* and coal-tar coated *motka* and the germination increased in these two treatments was 38.89 per cent over the initial germination percentage. The results indicated that after 6 months of storage the germination percentage of lentil declined and continued to decline up to the storage period (Table 4.12).

After 12 months of storage the higher germination percentage (92.22 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka* and improved tin container. The percentage of germination decreased by seeds stored in polythene lined jute bag was only 1.87 per cent lower over 6 months of storage but on an average the percentage increased over the initial germination was 44.09 per cent. The lowest percentage of germination (75.11 per cent) was observed in seeds stored in plain earthen *motka* and this was 15.50 per cent lower than 6 months storage seeds but it was 17.35 per cent higher over the initial germination percentage.

Table 4.12. Germination percentage of lentil during different stages of storage period

Treatments	Germination of pre-stored sample (%)	Germination (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	64.00	70.22	88.89	87.78	75.11
Plain tin container (T_2)	64.00	72.00	92.44	90.44	87.11
Polythene lined jute bag(T_3)	64.00	72.67	93.78	92.89	92.22
Polythene lined *motka*(T_4)	64.00	72.89	94.44	91.55	90.22
Coal-tar coated *motka*(T_5)	64.00	72.67	88.89	89.33	88.22
Improved tin container(T_6)	64.00	74.67	93.78	91.78	90.89

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 2.82 and for rows = 2.72

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4.5.2.2. Chickpea

The germination percentage of pre-stored chickpea was 94.00 per cent. It was observed that the germination percentage of chickpea decreased during storage period (Table 4.13). After 3 months of storage higher percentage of germination (93.33 per cent) was observed in seeds stored both in polythene lined jute bag and in improved tin container. The percentage of germination decreased by these two treatments was only 0.71 per cent. The lowest percentage of germination (20.44 per cent) was observed in seeds stored in plain earthen *motka* and it was 78.72 per cent lower than the initial germination of chickpea. It was revealed from the results that the germination percentage of chickpea decreased during storage period and continued to decrease up to the storage period.

After 12 months of storage higher percentage of germination (88.67 per cent) was observed in seeds stored in polythene lined *motka* and it was at par with seeds stored in polythene lined jute bag and improved tin container. The decreased in germination percentage observed by seeds stored in polythene lined *motka* was 5.67 per cent lower than the initial germination. The minimum germination percentage (2.22 per cent) was observed in seeds stored in plain earthen *motka* and was 97.63 per cent lower than the initial germination.

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Table 4.13. Germination percentage of chickpea during different stages of storage period

Treatments	Germination of pre-stored sample (%)	Germination (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	94.00	20.44	13.33	5.78	2.22
Plain tin container (T_2)	94.00	90.67	88.89	87.11	82.22
Polythene lined jute bag(T_3)	94.00	93.33	91.33	90.00	88.00
Polythene lined *motka*(T_4)	94.00	92.00	90.89	90.89	88.67
Coal-tar coated *motka*(T_5)	94.00	90.67	88.89	86.67	85.33
Improved tin container(T_6)	94.00	93.33	90.44	89.78	88.44

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 1.27 and for rows = 1.94

4.5.2.3. Khesari

The germination percentage of pre-stored khesari was 93.78 per cent. It was observed that during storage period germination percentage of khesari decreased (Table 4.14). After 3 months of storage the higher percentage of germination (93.11 per cent) was observed in seeds stored in polythene lined jute bag and it was only 0.71 per cent lower than the initial germination. This decrease of germination was at par with seeds stored in improved tin container. The lowest germination percentage (86.67 per cent) was observed in seeds stored in plain earthen *motka* and it was 7.58 per cent lower than the initial germination. It was revealed from the results that the germination percentage of khesari decreased with increasing storage period and continued to decrease up to the storage period (Table 4.14).

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After 12 months of storage higher percentage of germination (88.44 per cent) was observed in seeds stored both in polythene lined *motka* and improved tin container and it was 5.73 per cent lower than initial germination. This decrease was at par with seeds stored in polythene lined jute bag. The lowest germination percentage (74.22 per cent) was observed in seeds stored in plain earthen *motka* and it was 20.85 per cent lower than initial germination percentage.

Table 4.14. Germination percentage of khesari during different stages of storage period

Treatments	Germination of pre-stored sample (%)	Germination (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	93.78	86.67	84.00	80.44	74.22
Plain tin container (T_2)	93.78	90.22	88.30	86.22	82.22
Polythene lined jute bag(T_3)	93.78	93.11	91.55	89.33	88.00
Polythene lined *motka*(T_4)	93.78	91.11	90.22	89.33	88.44
Coal-tar coated *motka*(T_5)	93.78	89.56	88.44	87.55	86.22
Improved tin container(T_6)	93.78	92.67	90.67	89.78	88.44

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 1.20 and for rows = 1.24

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It was revealed from the above results that the initial germination percentage of lentil was low, but it gradually increased with increasing storage period and continued up to 6 months of storage and then declined (Figure 4.4). This might be due to higher initial seed hardness of lentil which has been decreased gradually with increasing storage period as reported elsewhere (Annonymous, 1987). The initial higher percentage of hardness of lentil seed with minimum germination and then gradual decrease in hardness during storage with increasing germination percentage had also been reported by Agrawal (1981) and by Singh (1985). The seed stored in plain earthen *motka* absorbed more water from the environment, which in presence of temperature deteriorated the quality of pulses leading to decrease in germination. Chickpea seeds, which were stored in plain earthen *motka*, decreased their quality severely which had been reflected in their germination (Figure 4.5). Chickpea seeds unlike lentil and khesari displayed severe reduction in germination when they were stored in plain earthen *motka*. On verification of moisture content and fungal infection incase of this pulse in the above mentioned container, it was revealed that chickpea absorbed comparatively higher amount of moisture than the other 2 pulses (Tables 4.9, 4.10 and 4.11). Similarly fungal infection was also higher in 12 months stored chickpea (Table 4.6, 4.7 and 4.8). These two parameters i.e. moisture and fungal might have effected drastically negatively on germination capacity of stored chickpea seeds. Similar results for soybean were also reported by Chavan *et al.* (1983). The moisture content in seeds stored in other containers was within the range of safe moisture level, maintaining their quality. Correlation co-efficient (Tables 4.46, 4.47 and 4.48) showed that the germination of all the pulses was negatively and significantly correlated with moisture content. These results are in agreement with Cahvan *et al.* (1983) where they had reported similar types of correlation with soybean seeds. Strong interaction observed (Figure 4.4, 4.5 & 4.6) was mainly due to plain earthen *motka* where as in other cases the interaction was very small almost negligible.

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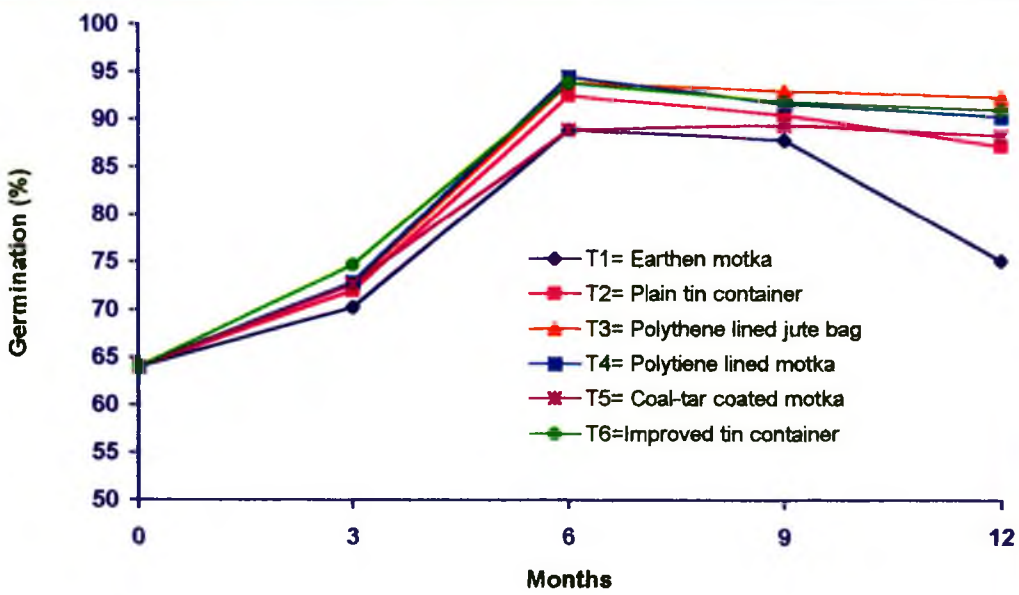

Fig. 4.4. Germination percentage of lentil during different stages of storage period

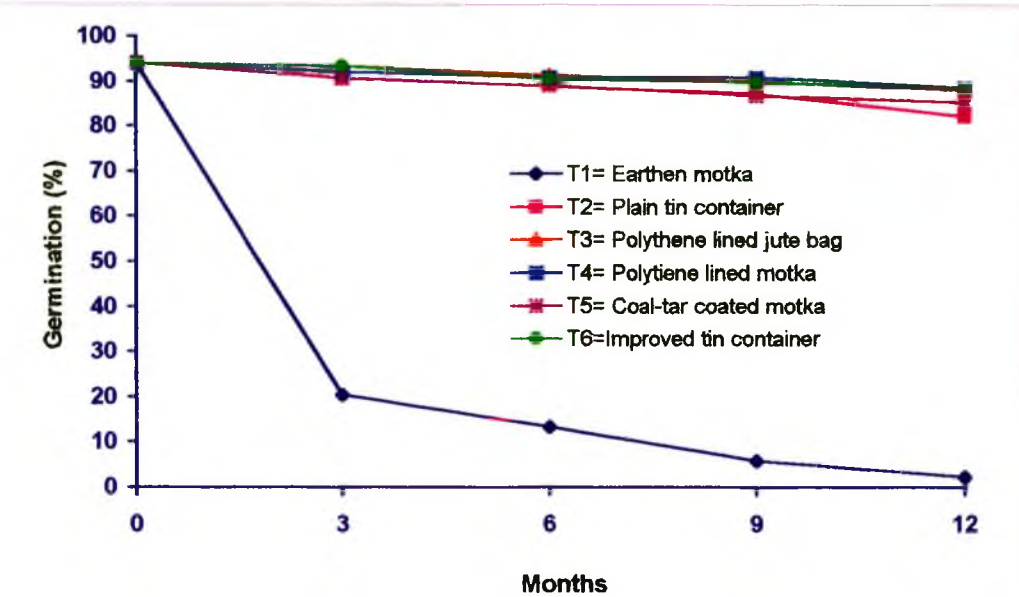

Fig. 4.5 Germination percentage of chickpea during different stages of storage period

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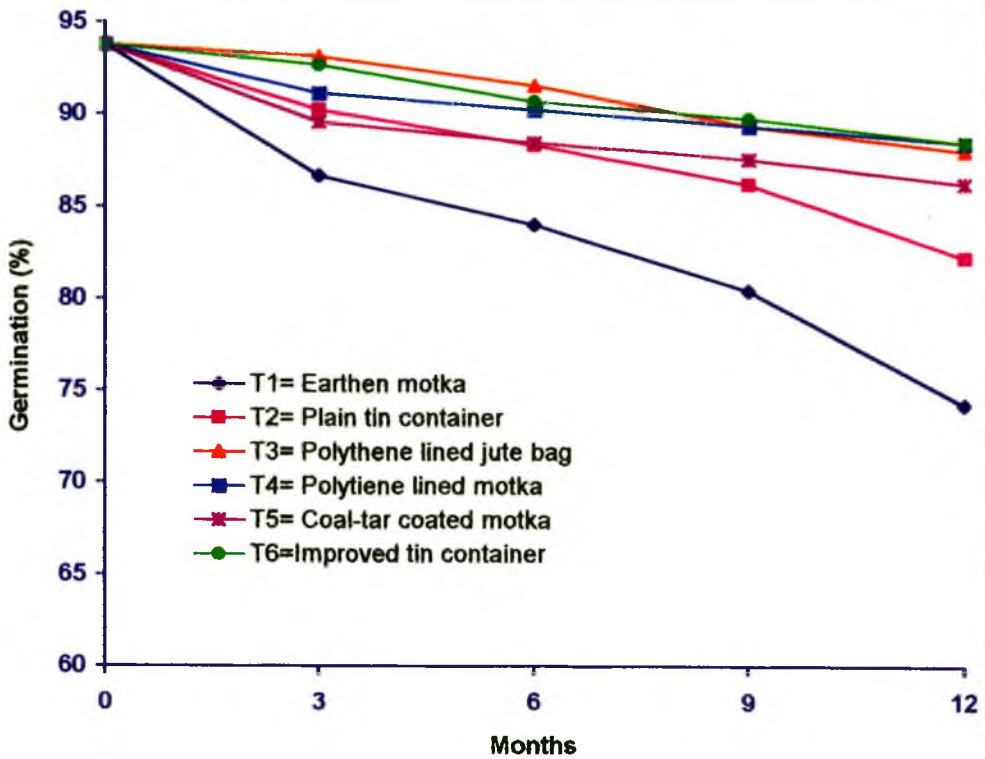

Fig. 4.6. Germination percentage of khesari during different stages of storage period

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4.5.3. Cooking time

Cooking time is one of the important parameters in evaluating the quality of pulse seeds. It is also an important factor influencing the consumer preference of pulses (dhal) because of time and fuel requirement. It is the time taken from beginning the test to that time when the seeds are ready to it.

4.5.3.1. Lentil

The cooking time of pre-stored lentil was 8.08 minutes. After 3 months storage the cooking time of lentil stored in plain earthen *motka* increased to 10.33 minute, which was significantly higher ($P \leq 0.05$) than other treatments (Table 4.15). The increase in cooking time in this treatment was about 27.87 per cent higher than the initial cooking time. The cooking time of the seeds stored in plain tin container and coal-tar coated *motka* have the similar cooking time (9.11 minutes) after 3 months of storage. On the other hand, the lower cooking time (8.67 minutes) was observed in seeds stored both in polythene lined jute bag and in improved tin container. The increase in cooking time by these treatments was only 7.3 per cent higher over the initial cooking time. With increasing storage period the cooking time of seeds stored in all the containers were increased and continued to increase up to storage period. The cooking time of seeds stored in plain earthen *motka* increased considerably and significantly.

After 12 months of storage, cooking time of seeds stored in plain earthen *motka* was 15.17 minutes, which was 87.7 per cent higher over the initial cooking time. The minimum cooking time of 9.83 minutes was observed in seeds stored in both the polythene lined jute bag and improved tin container after same storage period and was only 21.6 per cent higher than the initial cooking time.

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Table 4.15. Cooking time of lentil during different stages of storage period

Treatments	Cooking time of pre-stored sample (minutes)	Cooking time (minutes) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	8.08	10.33	11.50	13.50	15.17
Plain tin container (T_2)	8.08	9.11	9.39	9.89	11.33
Polythene lined jute bag(T_3)	8.08	8.67	9.05	9.55	9.83
Polythene lined *motka*(T_4)	8.08	9.11	9.44	9.78	10.22
Coal-tar coated *motka*(T_5)	8.08	8.89	9.61	10.00	10.77
Improved tin container(T_6)	8.08	8.67	9.11	9.72	9.83

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.34 and for rows = 0.38

4.5.3.2. Chickpea

The initial cooking time of chickpea was 29.11 minutes. After 3 months of storage the cooking time of seeds stored in plain earthen *motka* increased to 33.39 minutes and was significantly higher than other treatments (Table 4.16). This increase in cooking time was 14.70 per cent higher over the initial cooking time. The lowest cooking time (29.33 minutes) was observed in seeds stored in both the polythene lined jute bag and in improved tin container and it was only 0.75 per cent higher over the initial cooking time. The results (Table 4.16) revealed that the cooking time of chickpea increased with increasing storage period. The cooking time of seeds stored in plain earthen *motka* was increased considerably with increasing storage time and was significantly higher than other treatments in all the stages of storage period. After 6 months of storage the increase in cooking time given by seeds stored in plain earthen *motka* was 21.95 per cent higher over the initial cooking time and it has been increased to 41.43 per cent after 9 months of storage.

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After 12 month of storage the highest cooking time 44.22 minutes was observed in seeds stored in plain earthen *motka* and was significantly higher (P≤ 0.05) than other treatments. The increase in cooking time in this treatment was 52 per cent higher over the initial cooking time. On the other hand after the same storage period the lower cooking time of 31.83 minutes was observed in seeds stored in improved tin container followed by 31.89 minutes by seeds stored in polythene lined jute bag. The increase in cooking time in these treatments were 9.34 per cent and 9.54 per cent respectively after 12 months of storage.

Table 4.16. Cooking time of chickpea during different stages of storage period

Treatments	Cooking time of pre-stored sample (minutes)	Cooking time (minutes) during storage period (month)			
		3	6	9	12
Plain earthen *motka* (T_1)	29.11	33.39	35.50	41.17	44.22
Plain tin container (T_2)	29.11	29.72	31.11	32.00	32.94
Polythene lined jute bag(T_3)	29.11	29.33	30.44	31.17	31.89
Polythene lined *motka*(T_4)	29.11	29.55	30.61	31.33	32.11
Coal-tar coated *motka*(T_5)	29.11	29.61	31.11	31.89	32.61
Improved tin container(T_6)	29.11	29.33	30.33	31.55	31.83

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.48 and for rows rows = 0.65

4.5.3.3. Khesari

The cooking time of pre-stored khesari dhal was 20.21 minutes. After 3 months of storage the highest cooking time of 24.50 minutes was observed in seeds stored in plain earthen *motka* and was significantly higher (P ≤ 0.05) than other treatments (Table 4.17). The increase in cooking time in this treatment after 3 months of storage was 21.23 per cent higher over the initial cooking time. However, after 3 months of storage the lower and similar cooking time of 20.61 minutes was recorded with seeds stored in both the ploythene lined jute bag and coal-tar coated *motka* and it was only 1.98 per cent higher over the pre-stored cooking time. The cooking time of seeds stored in all the containers were increased with

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increase in storage period (Table 4.17). But the cooking time of seeds stored in plain earthen *motka* has been increased considerably and it was significantly higher in comparison with other treatments. After 6 months of storage the highest cooking time (26 minutes) was observed in seeds stored in plain earthen *motka* and it was 28.65 per cent higher over the initial cooking time.

After 12 months storage the highest cooking time (29.44 minutes) was observed in seeds stored in plain earthen *motka* and was significantly higher ($P \leq 0.05$) than other treatments. The increase in cooking time in this treatment was 45.67 per cent higher over the initial cooking time. Seeds stored in polythene lined *motka* required the lower cooking time (22.22 minutes) after same storage period. The increase in cooking time in this treatment was only 9.94 per cent higher over the initial cooking time and its was at par with seeds stored in polythene lined jute bag and improved tin container.

Table 4.17. Cooking time of khesari during different stages of storage period

Treatments	Cooking time content of pre-stored sample (Minutes)	Cooking time (Min.) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	20.21	24.50	26.00	27.67	29.44
Plain tin container (T_2)	20.21	20.83	21.50	22.05	22.78
Polythene lined jute bag(T_3)	20.21	20.61	21.22	21.67	22.28
Polythene lined *motka*(T_4)	20.21	20.61	21.22	21.78	22.22
Coal-tar coated *motka*(T_5)	20.21	20.94	21.44	22.11	22.61
Improved tin container(T_6)	20.21	20.83	21.22	21.72	22.28

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.34 and for row = 0.37

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Cooking time is related with a number of physico-chemical parameters of seeds. Correlation tables (Tables 4.46, 4.47 and 4.48) showed that cooking time of all the three pulses were positively and significantly related with moisture content. These findings are in agreement with Narasimha and Desikachar (1978) where they had found the positive correlation of cooking time and moisture content of pigeonpea.

Cooking time is a heritable characteristic that differs widely among varieties. Several changes occur during storage period, which influenced the cooking behavior of pulses. The study showed that pulses stored in plain earthen *motka* have increased its cooking time significantly with increasing storage period (Figure 4.7, 4.8 and 4.9). The seeds stored in plain earthen *motka* absorbed higher amount moisture, which in presence of high temperature might have changed the chemical composition. In addition fungal infection of seeds of this treatment was much higher than other treatments which might have change the quality of seeds (Table 4.6-4.8). Gradually the seeds become hardened causing longer cooking time. Correlation co-efficient (Tables 4.46-4.48) showed that cooking time of pulses was positively correlated with the moisture of seeds. The present results are in agreement with the findings of Hincks and Stanley (1986) where they had reported that the increase in moisture contents due to high relative humidity during storage was one of the key factors in the initiation of hardening of seeds leading to longer cooking time. Paredes-Lopez *et al.* (1991) reported that storage of pulses under adverse conditions of high temperature and high humidity had rendered the seeds susceptible to hardening causing longer cooking time. Vimala and Pushpamma (1987) have also reported that during storage, the moisture, temperature and relative humidity fluctuations might lead to hardening of middle lamella leading to increase in cooking time. Black *et al.* (1998) had reported that grain hardness adversely affects the water absorption and hence delays the cooking time. The cooking time of seed stored in other containers had also increased significantly.

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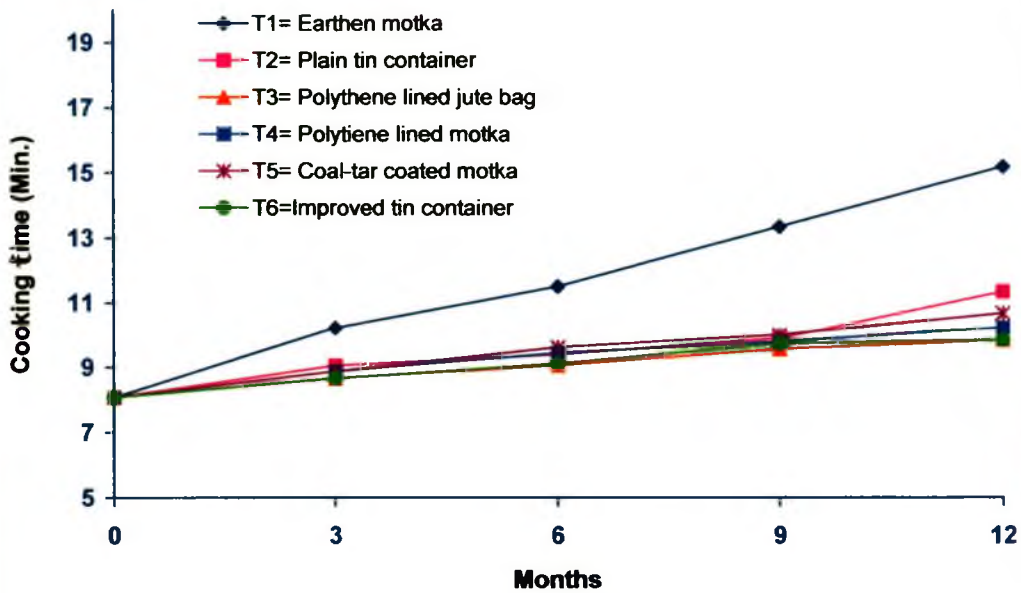

Fig. 4.7. Cooking time of lentil during different stages of storage period

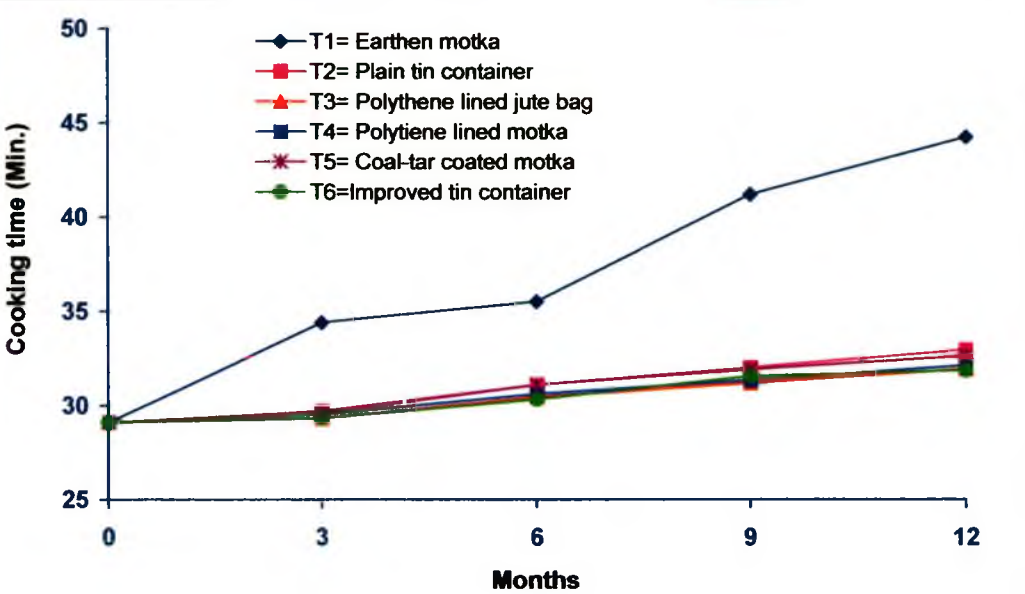

Fig. 4.8. Cooking time of chickpea durirng different stages of storage period

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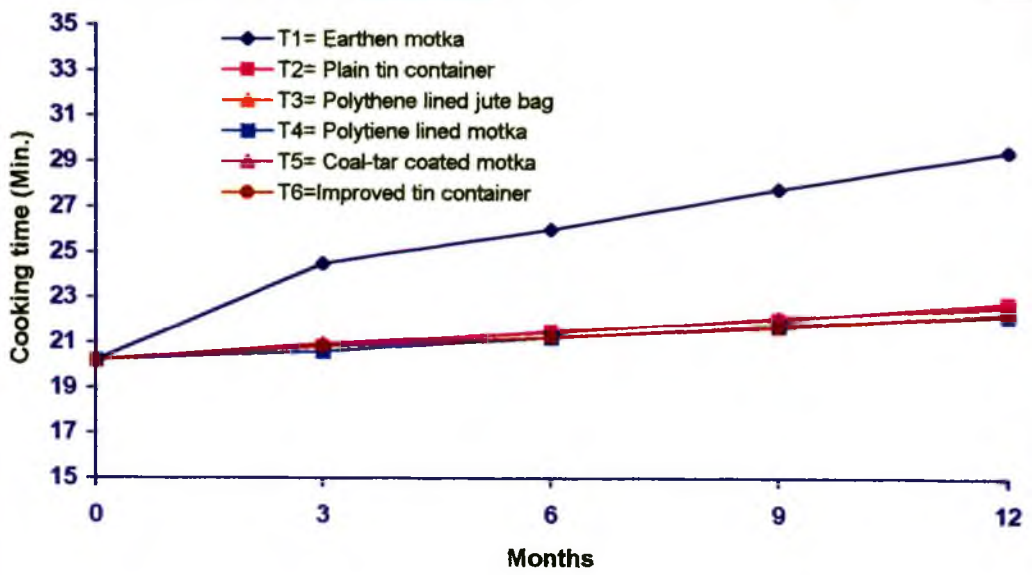

Fig. 4.9. Cooking time of Khesari during different stages of storage period

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4.5.4. Water Absorption

Water absorption capacity is one of the quality parameters of pulses. Grains, which absorb more water, will take less cooking time. It is the amount the water absorbed by seeds when it reaches the cooked state.

4.5.4.1. Lentil

The water absorption capacity of pre-stored lentil was 1.82 g/g dhal. It has been observed that during storage period the water absorption capacity of seeds decreased (Table 4.18). After 3 months of storage the highest water absorption of 1.80 g/g dhal was observed in seeds stored in polythene lined jute bag and was at par with seeds stored in coal-tar coated *motka* and improve tin container, but it was significantly higher than other treatments. The decrease in water absorption in this treatment was 3.74 per cent lower than the initial water absorption. The seeds stored in plain earthen *motka* absorbed lower water (1.72g/g dhal) and it was 8.02 per cent lower than the initial water absorption. The results showed that water absorption capacity of seeds of all the treatments decreased and continued to decrease significantly with increasing storage period.

After 12 months of storage, higher water absorption (1.68g/g dhal) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in coal-tar coated *motka* and improved tin container. The decrease in water absorption by seeds stored in polythene lined jute bag was 10.16 per cent than the initial water absorption. The lowest water absorption (1.48g/g dhal) was observed in seeds stored in plain earthen *motka* which was 20.85 per cent lower than the initial water absorption.

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Table 4.18. Water absorption of lentil during different stages of storage period

Treatments	Water absorption of pre-stored sample (g/g dhal)	Water absorption (g/g dhal) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	1.87	1.72	1.65	1.56	1.48
Plain tin container (T_2)	1.87	1.73	1.69	1.68	1.57
Polythene lined jute bag(T_3)	1.87	1.80	1.74	1.71	1.68
Polythene lined *motka*(T_4)	1.87	1.77	1.72	1.70	1.66
Coal-tar coated *motka*(T_5)	1.87	1.73	1.68	1.64	1.58
Improved tin container(T_6)	1.87	1.78	1.72	1.70	1.63

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.045 and for row = 0.046

4.5.4.2. Chickpea

The water absorption capacity of pre-stored chickpea was 1.32 g/g dhal. The water absorption capacity of chickpea decreased during storage period (Table 4.19). After 3 months of storage higher amount of water absorption (1.30g/g dhal) was observed in seeds stored in polythene lined jute bag and was at par with all other treatments except seeds stored in plain earthen *motka*. The decrease in water absorption capacity in this treatment was only 1.15 per cent lower than the initial water absorption. The lower water absorption (1.24 g/g dhal) was observed in seeds stored in plain tin container and it was 6.06 per cent lower than the initial water absorption. The results showed that water absorption capacity of seeds of all the treatments were decreased significantly with increasing storage period (Table 4.19).

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It was observed that after 12 month of storage, higher water absorption (1.16 g/g dhal) was observed in seeds stored in polythene lined jute bag and it was at par with other treatments except seeds stored in plain earthen *motka*. The decrease in water absorption in seeds stored in polythene lined jute bag was 12.12 per cent lower than the initial water absorption. The minimum water absorption of 1.04 g/g dhal was observed in seeds stored in plain earthen *motka* and it was 21.21 per cent lower than the initial water absorption.

Table 4.19. Water absorption of chickpea during different stages of storage period

Treatments	Water absorption of pre-stored sample (g/g dhal)	Water absorption (g/g dhal) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	1.32	1.24	1.21	1.14	1.04
Plain tin container (T_2)	1.32	1.26	1.24	1.19	1.13
Polythene lined jute bag(T_3)	1.32	1.30	1.26	1.22	1.16
Polythene lined *motka*(T_4)	1.32	1.29	1.25	1.22	1.15
Coal-tar coated *motka*(T_5)	1.32	1.28	1.23	1.19	1.14
Improved tin container(T_6)	1.32	1.28	1.26	1.21	1.15

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.048 and for row = 0.052

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4.5.4.3. Khesari

The initial water absorption capacity of khesari was 1.67 g/g dhal. The results showed that the water absorption of khesari decreased with increasing storage period (Table 4.20). After 3 months of storage the higher amount water absorption (1.65 g/g dhal) was observed in seeds stored in polythene lined jute bag and it was at par with water absorption observed in seeds stored in coal-tar coated *motka* and improved tin container. The decrease in water absorption in seeds stored in polythene lined jute bag was only 1.19 per cent lower than initial water absorption. The lower amount water absorption (1.51 g/g dhal) was observed in seeds stored in plain earthen *motka*, which was 9.58 per cent lower than initial water absorption. The results also showed that the water absorption capacity of khesari decreased significantly with increasing storage period.

After 12 month of storage significantly higher amount of water absorption (1.41 g/g dhal) was observed in seeds stored in polythene lined jute bag followed by seeds stored in coal-tar coated *motka* and improved tin container where in both the cases the water absorption observed was 1.38 g/g dhal. The seeds stored in polythene lined jute bag absorbed 15.56 per cent lower water than the initial water absorption. The lowest water absorption of 1.28 g/g dhal was observed in seeds stored in plain earthen *motka*, which was 23.35 per cent lower than the initial water absorption.

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Table 4.20. Water absorption of khesari during different stages of storage period

Treatments	Water absorption of pre-stored sample (g/g dhal)	Water absorption (g/g dhal) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	1.67	1.51	1.47	1.43	1.28
Plain tin container (T_2)	1.67	1.62	1.57	1.52	1.34
Polythene lined jute bag(T_3)	1.67	1.65	1.61	1.55	1.41
Polythene lined *motka*(T_4)	1.67	1.64	1.59	1.54	1.38
Coal-tar coated *motka*(T_5)	1.67	1.62	1.57	1.51	1.35
Improved tin container(T_6)	1.67	1.65	1.60	1.56	1.38

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.018 and for row = 0.020

Results on water absorption capacity of different pulses (Table 4.18-4.20) showed that as the storage period increased, the water absorption capacity decreased significantly in all the three pulses. Correlation co-efficient analysis showed that water absorption capacity of all the three pulses were negatively and significantly correlated with moisture content and cooking time of pulses (Tables 4.46, 4.47 and 4.48). These findings are in agreement with Vimala and Pushpamma (1985 and 1987) who reported that water absorption capacity of green gram, red gram, bengal gram and black gram had decreased significantly with increasing storage period. They also reported that minimum water absorption required maximum cooking time. Singh *et al.* (1984) had also reported negative and significant correlation of water absorption with the cooking time of pigeon pea.

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4.5.5. Solids Dispersed

Solids dispersion of pulses is one of the key factors for the quality of pulses. Higher percentage of solids dispersion is a good indicator of cooking quality. It is amount of solids dispersed by seeds in cooking water when it reaches the cooked state.

4.5.5.1. Lentil

The amount of solids dispersed by fresh lentil dhal during cooking was 77.55 per cent. Results showed that during storage the amount of solids dispersed decreased and it was continued to decrease with increasing storage period (Table 4.21). After 3 months of storage the higher solids dispersed (75.81 per cent) was observed in seeds stored in improved tin container and was at par with seeds stored in polythene lined jute bag and polythene lined *motka*. The decrease in solids dispersed by seeds stored in improved tin container were 2.24 per cent lower than the initial solids dispersed. The lower percentage of solids dispersed (70.49 per cent) was observed in seeds stored in plain earthen *motka* and it was 9.10 per cent lower than the initial solids dispersed of lentil. It was also observed that though the decrease of solids dispersed was significant in most of the cases, the rate of decrease was quite small up to 6 months of storage. After 6 months of storage the rate of decrease was significantly higher.

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After 12 months of storage the higher solids dispersed (67.25 per cent) was observed in seeds stored in improved tin container and it was 13.28 per cent lower than initial solids dispersed. This decrease was at par with seeds stored in ploythene lined jute bag, polythene lined *motka* and coal-tar coated *motka*. The lowest amount of solids dispersed (50.24 per cent) was observed in seeds stored is plain earthen *motka* and it was 35.21 per cent lower than the initial solids dispersed.

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Table 4.21. Solids dispersed of lentil during different stages of storage period

Treatments	Solids dispersed of pre-stored sample (%)	Solids dispersed (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	77.55	70.49	65.24	60.35	50.24
Plain tin container (T_2)	77.55	72.95	71.02	67.57	63.26
Polythene lined jute bag(T_3)	77.55	75.54	74.35	70.34	66.44
Polythene lined *motka*(T_4)	77.55	75.49	74.78	70.21	66.64
Coal-tar coated *motka*(T_5)	77.55	74.61	73.28	68.74	64.98
Improved tin container(T_6)	77.55	75.81	74.95	70.98	67.25

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.66 and for row = 0.81

4.5.5.2. Chickpea

The amount of solids dispersed by pre-stored chickpea into cooking water was 55.77 per cent. During storage the amount of solids dispersed by chickpea decreased (Table 4.22). After 3 months of storage the highest solids dispersed (54.29 per cent) was observed in seeds stored in polythene lined jute bag and was significantly higher than other treatments. The decrease in solids dispersed in this treatment was 2.65 per cent lower than the initial solids dispersed capacity. The lower solids dispersed (48.41 per cent) was observed in seeds stored in plain earthen *motka* and it was 13.79 per cent lower than the initial solids dispersed capacity. The results indicated that during storage period the amount of solids dispersed decreased significantly and continued to decrease with increasing storage period (Table 4.22).

After 12 months of storage the higher amount solids dispersed (44.80 per cent) was observed in seeds stored in polythene lined earthen *motka* and it was 19.54 per cent lower than initial solids dispersed. This decrease was at par with seeds stored in polythene lined jute

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bag, coal-tar coated *motka* and improved tine container. The minimum amount of solids dispersed (30.56 per cent) was observed in seeds stored in plain earthen *motka* and it was 45.20 per cent lower than the initial solids dispersed.

Table 4.22. Solids dispersed of chickpea during different stages of storage period

Treatments	Solids dispersed of pre-stored sample (%)	Solids dispersed (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	55.77	48.41	45.28	35.32	30.56
Plain tin container (T_2)	55.77	51.67	49.91	46.21	43.86
Polythene lined jute bag(T_3)	55.77	54.29	52.05	49.32	44.66
Polythene lined *motka*(T_4)	55.77	53.13	51.23	48.29	44.80
Coal-tar coated *motka*(T_5)	55.77	51.94	50.40	47.47	43.66
Improved tin container(T_6)	55.77	53.30	51.51	48.40	44.75

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.73 and for row = 1.18

4.5.5.3. Khesari

The amount of solids dispersed by pre-stored khesari dhal in cooking water was 64.48 per cent. During storage period the amount of solids dispersed by khesari decreased (Table 4.23). After 3 months of storage the highest amount of solids dispersed (62.10 per cent) was observed in seeds stored in improved tin container and was significantly higher than other treatments. The amount of solids dispersed decreased in this treatment was 3.69 per cent lower than the solids dispersed of pre stored khesari. The lowest amount of solids dispersed (51.53 per cent) was observed in seeds stored in plain earthen *motka*, which was 20.08 per cent lower than the initial solids dispersed. Results also showed that the amount of solids dispersed decreased significantly with increasing storage period and continued to decrease up to 12 month of storage.

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After 12 months of storage the higher amount of solids dispersed (52.09 per cent) was observed in seeds stored in polythene lined *motka* and it was 19.21 per cent lower than initial solids dispersed. This decrease was at par with seeds stored in polythene lined jute bag and improved tin container. The minimum amount of solids dispersed (37.95 per cent) was observed in seeds stored in plain earthen *motka* which was 41.14 per cent lower than the initial solids dispersed capacity.

Table 4.23. Solids dispersed of khesari during different stages of storage period

Treatments	Solids dispersed of pre-stored sample (%)	Solids dispersed (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	64.48	51.53	48.29	42.83	37.95
Plain tin container (T_2)	64.48	58.34	55.71	52.80	51.14
Polythene lined jute bag(T_3)	64.48	61.02	58.81	56.21	52.05
Polythene lined *motka*(T_4)	64.48	61.23	58.28	55.57	52.09
Coal-tar coated *motka*(T_5)	64.48	58.21	55.24	53.26	49.92
Improved tin container(T_6)	64.48	62.10	58.78	56.13	52.01

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.77 and for row = 1.21

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Analysis of variance (Appendix Table 1, 2 and 3) revealed that there was strong interaction between storing duration (D) and types of container (C) of different pulses. As evident from the graph (Figure 4.10, 4.11 and 4.12), the presence of interaction between storing duration and types of container was mainly due to earthen *motka*. The interaction between storing duration and other improved containers was small and almost negligible Results (Tables 4.21-4.23) showed that the amount of solids dispersion by all the three pulses stored in plain earthen *motka* decreased considerably and continued to decrease significantly at a larger ratio with increase in storage period. For other storage devices, the amount of solids dispersed was also decreased significantly but at slower rate. It was evident from the results that the seeds stored in plain earthen *motka* had changed their quality after absorbing higher percentage of moisture, which might lead to decreased in solids dispersion during cooking. Jones and Boulter (1983) reported that the seeds of *Pheseolus vulgaris* had become hardened when stored in high relative humidity under adverse condition leading to less water absorption and solids dispersion. Correlation coefficient (Tables 4.46, 4.47 and 4.48) showed that the amount of solids dispersed was negatively correlated with cooking time and positively correlated with water absorption. Similar relationship was also observed in case of pigeon pea as reported by Singh *et al.* (1984). Narasimha and Desikachar (1978) also reported negative correlation of solids dispersed with cooking time of pigeon pea.

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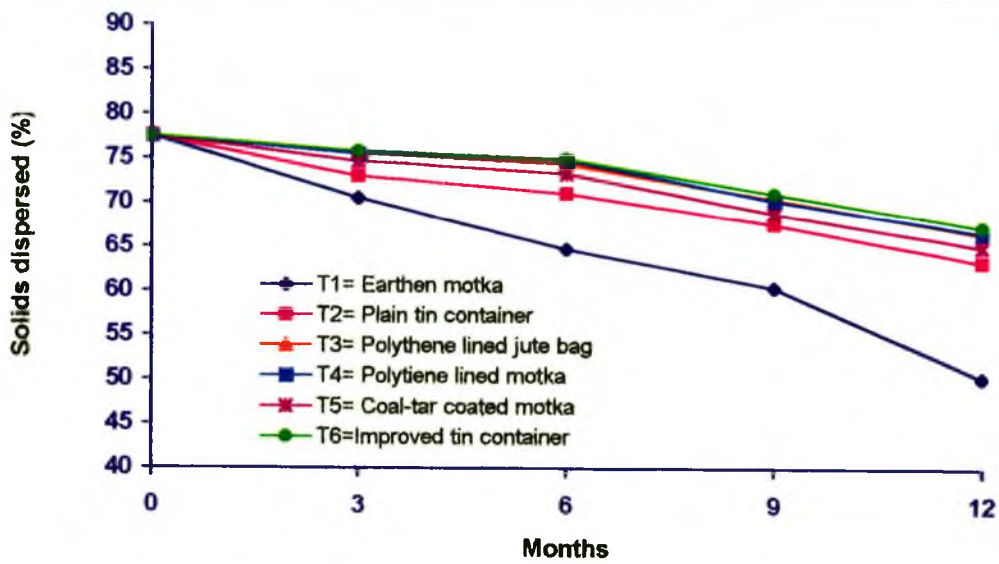

Fig. 4. 10. Solids dispersed of lentil during different stages storage period

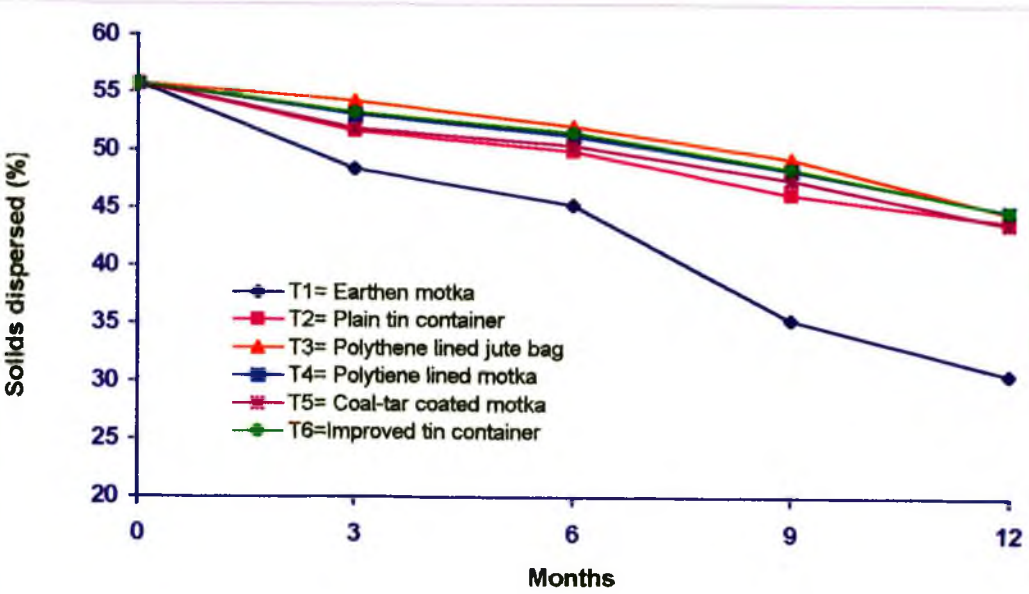

Fig. 4.11. Solids dispersed of chickpea during different stages of storage period

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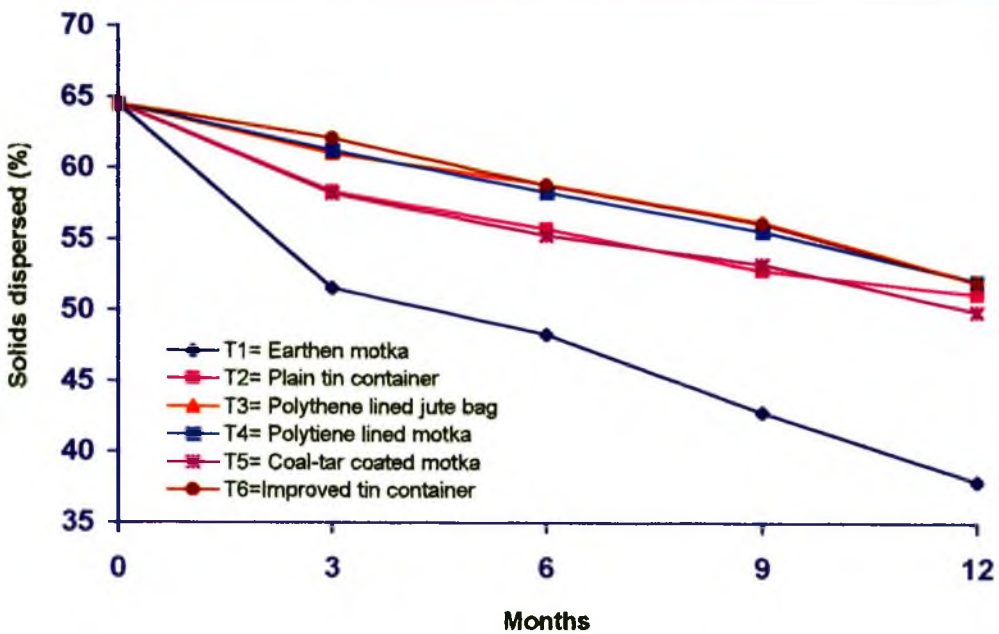

Fig. 4.12. Solids dispersed of khesari during different stages of storage period

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4.5.6. Protein Content

The protein content is one of the important quality parameters of pulses. The protein content of pulses are in the range of 20-30 per cent which make them consumable as protein rich food and hence these are often termed as 'poor mans meat'.

4.5.6.1. Lentil

The protein content of pre-stored lentil dhal was 25.84 per cent. After 3 months of storage the higher amount of protein (25.21 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with all other treatments except seeds stored in plain earthen *motka* (Table 4.24). The amount of protein decreased in polythene lined jute bag was only 2.43 per cent lower than the initial protein content. The lower amount of protein content (24.75 per cent) was observed in seeds stored in plain earthen *motka* and it was 4.22 per cent lower than the initial protein content. Results also showed that the protein content in lentil decreased significantly but at a slower rate during storage period.

After 12 months of storage the higher amount of protein (23.97 per cent) was observed in seeds stored in polythene lined jute bag and it was 7.23 per cent lower than the initial protein content. This decrease in protein content was at par with seeds stored in polythene lined *motka* and improved tin container. The lower amount of protein content (22.04 per cent) was observed in seeds stored in plain earthen *motka* and it was 14.70 per cent lower than the initial protein content.

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Table 4.24. Protein content of lentil during different stages of storage period

Treatments	Protein content of pre-stored sample (%)	Protein content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	25.84	24.75	23.95	23.12	22.04
Plain tin container (T_2)	25.84	25.06	24.34	23.59	23.13
Polythene lined jute bag(T_3)	25.84	25.21	24.88	24.46	23.97
Polythene lined *motka*(T_4)	25.84	25.15	24.78	24.37	23.87
Coal-tar coated *motka*(T_5)	25.84	25.08	24.58	24.16	23.64
Improved tin container(T_6)	25.84	25.20	24.90	24.42	23.90

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.17 and for row = 0.19

4.5.6.2. Chickpea

The protein content of pre-stored chickpea was 20.81 per cent. It was observed that during storage period the protein content decreased and continued to decrease significantly with increasing storage period (Table 4.25). After 3 months of storage the higher amount of protein content (20.34 per cent) was observed in seeds stored in polythene lined jute bag and it was 2.26 per cent lower than the initial protein content. This decrease in protein was at par with seeds stored in coal-tar coated *motka* and improved tin container. The lower amount of protein content (19.56 per cent) was observed in seeds stored in plain earthen *motka* and it was 6.00 per cent lower than the initial protein content.

At the end of 12 months of storage the higher amount of protein content (18.97 per cent) was observed in seeds stored in polythene lined jute bag and it was 8.84 per cent lower than the initial protein content. This decrease in protein was at par with the seeds stored in coal-tar coated *motka* and improved tin container. The lower amount of protein content (16.68 per cent) was observed in seeds stored in plain earthen *motka* and it was 19.84 per cent lower than the initial protein content.

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Table 4.25. Protein content of chickpea during different stages of storage period

Treatments	Protein content of pre-stored sample (%)	Protein content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	20.81	19.56	18.49	17.76	16.68
Plain tin container (T_2)	20.81	20.15	19.48	18.93	18.31
Polythene lined jute bag(T_3)	20.81	20.34	19.82	19.44	18.97
Polythene lined *motka*(T_4)	20.81	20.23	19.71	19.31	18.89
Coal-tar coated *motka*(T_5)	20.81	20.18	19.61	19.21	18.72
Improved tin container(T_6)	20.81	20.32	19.82	19.41	18.95

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.15 and for row = 0.14

4.5.6.3. Khesari

The protein content in pre stored khesari was 30.54 per cent. It was observed that during storage the protein content of khesari decreased (Table 4.26). After 3 months of storage the higher amount of protein (30.17 per cent) was observed in seeds stored in polythene lined jute bag and it was 1.21 per cent lower than initial protein content. This decrease in protein was at par with seeds stored in polythene lined *motka*, coal-tar coated *motka* and improved tin container. The lower amount of protein content (29.17 per cent) was observed in seeds stored in plain earthen *motka* and it was 4.48 per cent lower than the initial protein content. It was revealed from the results that the amount of protein content decreased significantly with increasing storage period.

After 12 months of storage the higher amount of protein content (28.55 per cent) was observed in seeds stored in polythene lined jute bag and it was 6.51 per cent lower than the initial protein content. This decrease in protein was at par with seeds stored in polythene lined *motka* and improved tin container. The lower amount of protein content (26.64 per cent) was observed in seeds stored in plain earthen *motka* and it was 13.55 lower than the initial protein content.

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Table 4.26. Protein content of khesari during different stages of storage period

Treatments	Protein content of pre-stored sample (%)	Protein content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	30.54	29.17	28.19	27.27	26.64
Plain tin container (T_2)	30.54	29.83	29.17	28.57	28.04
Polythene lined jute bag(T_3)	30.54	30.17	29.43	29.01	28.55
Polythene lined *motka*(T_4)	30.54	29.98	29.45	28.83	28.45
Coal-tar coated *motka*(T_5)	30.54	29.97	29.35	28.87	28.32
Improved tin container(T_6)	30.54	30.07	29.50	29.00	28.54

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.23 and for row = 0.22

The result (Tables 4.24-4.26) showed that the rate of protein decrease during storage period by the seeds stored in plain earthen *motka* was considerably higher than other treatments. This might be due to higher percentage of moisture content in seeds, which at higher temperature accelerated the degradation of protein by the proteolytic enzymes. Anderson (1973) and Roberts (1979) also reported similar results. It was evident from the correlation (Tables 4.46, 4.47 and 4.48) that protein content was negatively and significantly correlated with cooking time of pulses. Narasimaha and Desikachar (1978) also reported similar correlation in case of pigeon pea. But incase of chickpea they reported positive and significant correlation of protein content with cooking time.

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4.5.7. *In vitro* protein digestibility

The nutritional quality of pulses largely depends on its protein content and their digestibility. Higher digestibility indicates good quality of protein.

4.5.7.1. Lentil

The *in vitro* protein digestibility of pre-stored lentil was 90.02 per cent. It was observed that *in vitro* protein digestibility during storage period decreased (Table 4.27). After 3 months of storage the higher protein digestibility 86.39 per cent was observed in seeds stored in polythene lined jute bag and it was 4.03 per cent lower than the initial protein digestibility. This decrease in protein digestibility was at par with seeds stored in polythene lined *motka* and improved tin container. The lower amount of protein digestibility (82.87 per cent) was observed in seeds stored in plain earthen *motka* and it was 7.94 per cent lower than the initial protein digestibility. The results showed that during storage period the in vitro protein digestibility decreased significantly and continued to decrease with increasing storage period (Table 4.27).

After 12 months of storage the highest *in vitro* protein digestibility (80.74 per cent) was observed in seeds stored in polythene lined jute bag and was significantly higher ($p \leq 0.05$) than other treatments. The amount of protein digestibility decreased in this treatment was 10.31 per cent lower than the initial protein digestibility. The lowest amount of protein digestibility (68.12 per cent) was observed in seeds stored in plain earthen *motka* and the amount decreased by this treatment was 24.33 per cent lower than the initial protein digestibility.

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Table 4.27. *In Vitro* Protein Digestibility (IVPD) of lentil during different stages of storage period

Treatments	IVPD of pre-stored sample (%)	IVPD of lentil (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	90.02	82.87	79.21	74.96	68.12
Plain tin container (T_2)	90.02	84.25	83.28	82.02	76.44
Polythene lined jute bag(T_3)	90.02	86.39	85.44	83.21	80.74
Polythene lined *motka*(T_4)	90.02	86.07	85.27	83.02	79.94
Coal-tar coated *motka*(T_5)	90.02	84.86	83.81	82.30	77.43
Improved tin container(T_6)	90.02	86.03	85.25	83.08	79.84

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.79 and for row = 0.80

4.5.7.2. Chickpea

The *in vitro* protein digestibility of pre-stored chickpea was 85.35 per cent. Results showed that the protein digestibility decreased during storage period (Table 4.28). After 3 months of storage the higher protein digestibility (83.12 per cent) was observed in seeds stored in polythene lined *motka* and it was 2.61 per cent lower than initial protein digestibility. This decrease in protein digestibility was at par with seeds stored in polythene lined jute bag, coal-tar coated *motka* and improved tin container. The lower amount of protein digestibility (78.60 per cent) was observed in seeds stored in plain earthen *motka* and amount decreased by this treatment was 7.90 per cent lower than the initial protein digestibility. The results revealed that during storage period the protein digestibility decreased significantly and continued to decrease with increasing storage duration.

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After 12 months of storage the higher protein digestibility (67.06 per cent) was observed in seeds stored in improved tin container and it was 21.42 per cent lower than the initial protein digestibility. This decrease was followed by seeds stored in polythene lined *motka* where the protein digestibility observed was 66.61 per cent and it was 22.16 per cent lower than the initial protein digestibility. The lower amount of protein digestibility (64.09 per cent) was observed in seeds stored in plain earthen *motka* and it was 24.91 per cent lower than the initial protein digestibility.

Table 4.28. *In Vitro* Protein Digestibility (IVPD) of chickpea during different stages of storage period

Treatments	IVPD of pre-stored sample (%)	IVPD of chickpea (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	85.35	78.60	73.71	66.39	64.09
Plain tin container (T_2)	85.35	82.32	78.36	70.28	65.27
Polythene lined jute bag(T_3)	85.35	83.05	80.17	71.55	66.43
Polythene lined *motka*(T_4)	85.35	83.12	80.08	71.80	66.61
Coal-tar coated *motka*(T_5)	85.35	82.57	79.40	70.40	65.28
Improved tin container(T_6)	85.35	83.09	80.09	71.67	67.06

Each value is the mean of 9 replication over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.60 and for rows = 0.77

4.5.7.3. Khesari

The *in vitro* protein digestibility of pre-stored khesari was 88.46 per cent. Results showed that the in vitro protein digestibility decreased during storage period (Table 4.29). After 3 months of storage the highest amount of protein digestibility (86.49 per cent) was observed in seeds stored in improved tin containers and it was significantly higher ($p \leq 0.05$)

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than other treatments. The amount of protein digestibility decreased in this treatment was 2.23 per cent lower than the initial protein digestibility. The lower amount of protein digestibility (82.03 per cent) was observed in seeds stored in plain earthen *motka* and it was 7.26 per cent lower than the initial protein digestibility. The results showed that the amount of protein digestibility decreased significantly during storage period and continued to decrease considerably with increasing storage period (Table 4.29).

After 12 months of storage the higher amount of protein digestibility (76.83 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka* but significantly higher ($p \leq 0.05$) than other treatments. The amount of protein digestibility decreased by the seeds stored in polythene lined jute bag was 13.15 per cent lower than initial protein digestibility. The lowest protein digestibility (68.42 per cent) was observed in seeds stored in plain earthen *motka* and it was 22.65 per cent lower than the initial protein digestibility.

Table 4.29. *In vitro* protein digestibility (IVPD) of khesari during different stages of storage period

Treatments	IVPD of pre-stored sample (%)	IVPD of khesari (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	88.46	82.03	78.85	73.62	68.42
Plain tin container (T_2)	88.46	83.78	80.97	77.12	73.23
Polythene lined jute bag(T_3)	88.46	85.54	84.07	79.15	76.83
Polythene lined *motka*(T_4)	88.46	85.60	83.77	79.05	76.80
Coal-tar coated *motka*(T_5)	88.46	84.05	81.21	77.76	74.01
Improved tin container(T_6)	88.46	86.49	84.39	78.07	75.94

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.69 and for rows = 0.85

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The presence of various antinutritional factors viz. protease inhibitor, polyphenol etc. inhibits protein digestibility. However, during cooking, most of the inhibitors degenerate and improve the digestibility (Kataria *et al.,* 1987). Pulses stored with high moisture and at high temperature deteriorate the quality of protein through Maillard reaction leading to decrease in their digestibility (El Fake *et al.,* 1984). In the present study pulses stored in plain earthen *motka* increased their moisture contents and crossed the safe moisture level (Table 4.9-4.11). As a result the protein quality deteriorated hence decreased their digestibility considerably at a higher rate (Figure 4.13-4.15). On the other hand the moisture content of seeds stored in other containers were within the rage of safe moisture level during the storage period. The changes that occurred during storage might have due to aging of seeds leading to slower rate of protein digestibility. Similar results were also reported by Kole and Gupta (1982) where they observed that during storage period some biochemical changes occurred in seed protein due to aging which lead to decrease in protein digestibility. The figure 4.13-4.15 revealed that the strong interaction between storage period and container was mainly due to plain earthen *motka*. The interaction between storing duration and other containers was small, almost negligible. Correlation coefficients (Tables 4.46, 4.47 and 4.48) showed that *in vitr*o protein digestibility were negatively and significantly correlated with moisture content in seeds. The results revealed that pulses stored in improved containers viz. polythene lined jute bag, polythene lined/coal-tar coated *motka* and improved tin container showed the better protein digestibility.

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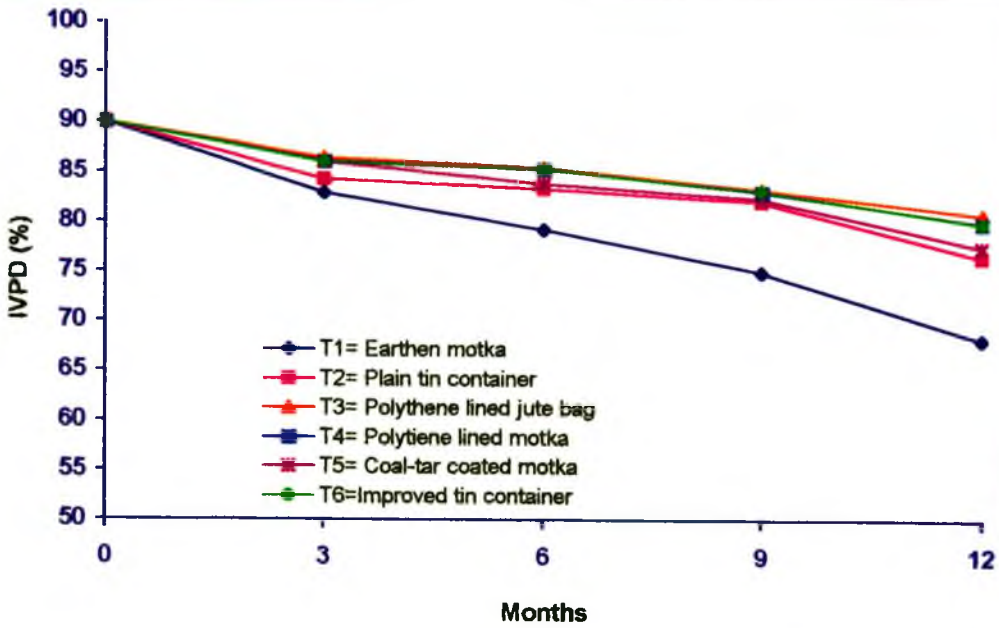

Fig. 4.13. In vitro protein digestibility on (IVPD) of lentil during different stages of storage period

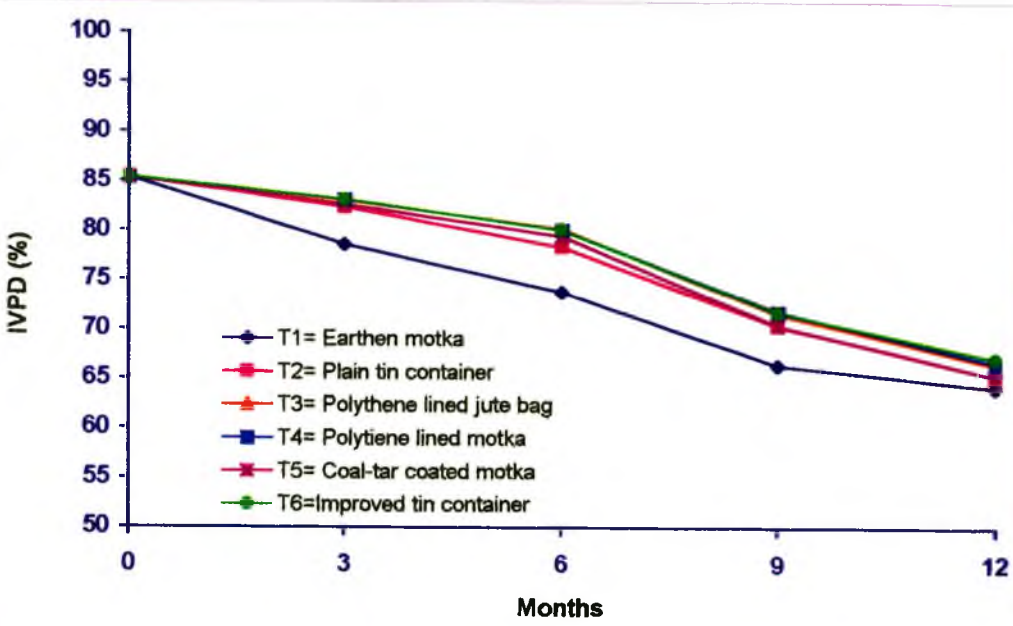

Fig. 4.14. In vitro protein digestibility (IVPD) of chickpea during different stages of storage period

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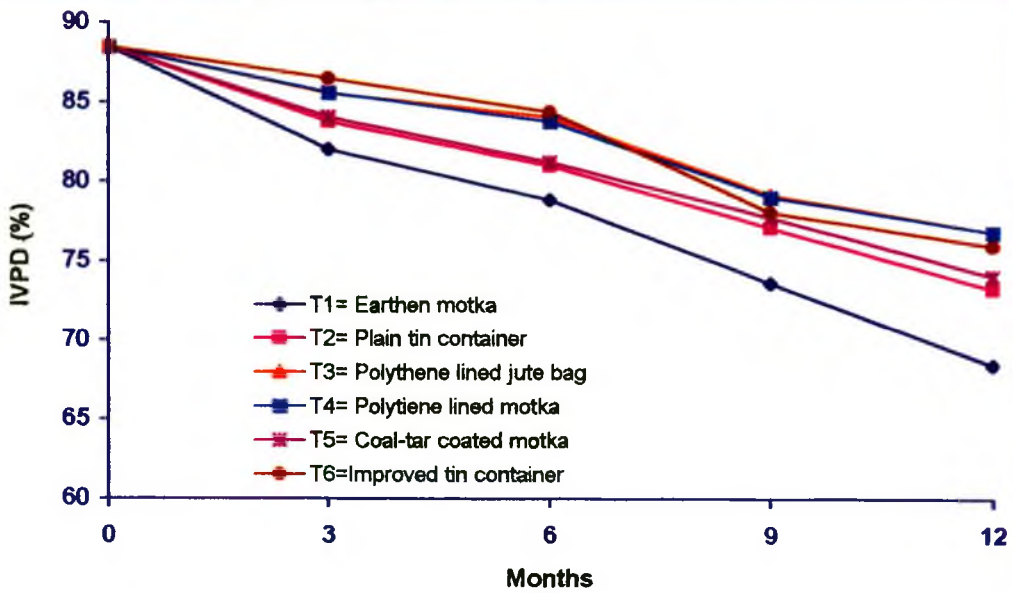

Fig. 4.15. In vitro protein digestibility (IVPD) of khesari during different stages of storage period

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4.5.8. Starch content

Starch is the major nutrients of seeds. It consists of amylose and amylopectin.

4.5.8.1. Lentil

The amount of starch content in pre-stored lentil was 52.91 per cent. It was observed that the amount of starch content decreased during storage period (Table 4.30). After 3 months of storage the higher amount of starch (52.57 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in improved tin container and was significantly higher ($p \leq 0.05$) than other treatments. The decrease in starch content by the seeds stored in polythene lined jute bag was only 0.64 per cent. The lower amount of starch content (50.69 per cent) was observed in seeds stored in plain earthen *motka* and it was 4.19 per cent lower than the initial starch content of lentil. It was observed that during storage period the amount of starch decreased significantly and continued to decrease up to storage period.

After 12 months of storage the highest amount of starch (46.89 per cent) was observed in seeds stored in polythene lined jute bag and was significantly higher ($P \leq 0.05$) than other treatments. The decrease in starch content in this treatment was 11.38 per cent lower than the initial starch content. The lowest amount of starch (43.98 per cent) was observed in seeds stored in plain earthen *motka* and it was 16.88 per cent lower than the initial starch content.

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Table 4.30. Starch content of lentil during different stages of storage period

Treatments	Starch content of pre-stored sample (%)	Starch content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	52.91	50.69	47.64	46.07	43.98
Plain tin container (T_2)	52.91	51.96	48.61	47.00	45.05
Polythene lined jute bag(T_3)	52.91	52.57	49.74	47.35	46.89
Polythene lined *motka*(T_4)	52.91	52.12	48.87	47.23	45.88
Coal-tar coated *motka*(T_5)	52.91	52.04	48.44	46.98	45.49
Improved tin container(T_6)	52.91	52.36	49.20	47.39	45.97

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.32 and for rows = 0.39

4.5.8.2. Chickpea

The amount of starch content in pre-stored chickpea was 56.23 per cent. It was observed that during storage period the starch content decreased (Table 4.31). After 3 months of storage the higher amount of starch (54.94 per cent) was observed in seeds stored in improved tin container and it was at par with the seeds stored in all the containers except the plain earthen *motka*. The decreased in starch content in this treatment was only 2.29 per cent. The lower amount of starch (52.18 per cent) was observed in seeds stored in plain earthen *motka* and it was 7.20 lower than the initial starch content. It has been observed that during storage period the starch content in chickpea decreased and continued to decrease up to the storage period (Table 4.31).

After 12 months of storage the higher amount of starch (46.74 per cent) was observed in seeds stored in polythene lined jute bag and it was 16.87 per cent lower than initial starch content. The decrease in starch content was at par with seeds stored in improved tin container. The lower starch content 42.98 per cent was observed in seeds stored in plain earthen *motka* and starch decreased by this treatment was 23.56 per cent lower than the initial starch content.

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Table 4.31. Starch content of Chickpea during different stages of storage period

Treatments	Starch content of pre-stored sample (%)	Starch content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	56.23	52.18	48.50	46.03	42.98
Plain tin container (T_2)	56.23	54.00	50.87	47.39	45.34
Polythene lined jute bag(T_3)	56.23	54.88	51.52	48.41	46.74
Polythene lined *motka*(T_4)	56.23	54.57	51.06	47.74	45.62
Coal-tar coated *motka*(T_5)	56.23	54.45	51.77	47.78	45.59
Improved tin container(T_6)	56.23	54.94	51.42	47.48	46.68

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.95 and for rows = 1.00

4.5.8.3. Khesari

The amount of starch content in pre-stored khesari was 51.80 per cent. It was observed that during storage period the starch content decreased (Table 4.32). After 3 months of storage the higher amount of starch (50.83 per cent) was observed in seeds stored in polythene lined jute bag and it was 1.87 per cent lower than the initial starch content. This decrease in starch content was at par with seeds stored in improved tin container, but significantly higher ($P \leq 0.05$) than other treatments. It was revealed that during storage period the starch content decreased significantly and continued to decrease up to the storage period (Table 4.32).

After 12 months of storage the higher amount of starch (42.08 per cent) was observed in seeds stored in polythene lined jute bag and it was 18.76 per cent lower than the initial starch content. This decrease was at par with seeds stored in polythene-lined *motka* and improved tin container but significantly higher than other treatments. The lowest amount of

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starch (38.23 per cent) was observed in seeds stored in plain earthen *motka* and it was 26.19 per cent lower than the initial starch content.

Table 4.32. Starch content of khesari during different stages of storage period

Treatments	Starch content of pre-stored sample (%)	Starch content (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	51.80	48.21	46.69	43.29	38.23
Plain tin container (T_2)	51.80	49.99	47.79	45.72	41.55
Polythene lined jute bag(T_3)	51.80	50.83	48.90	46.83	42.08
Polythene lined *motka*(T_4)	51.80	50.28	48.57	46.55	41.92
Coal-tar coated *motka*(T_5)	51.80	49.97	47.81	45.61	41.48
Improved tin container(T_6)	51.80	50.73	48.90	46.84	41.98

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.28 and for rows = 0.29

It was observed that the starch content of pulse seeds stored in different containers decreased with increasing storage period. It has been reported that during storage condition several biochemical changes occurred in seeds including breakdown of starch into sugar (Kole and Gupta, 1982). The activity of enzymes accelerated in presence of high moisture and high temperature and broke down the starch (Patte *et al.,* 1981). In the present study seeds stored in plain earthen *motka* had increased their moisture content through absorption of moisture from the environment (Table 4.9-4.11). As a result the activity of enzymes accelerated comparatively at a faster rate and increased the break down of starch into sugar leading to decrease of starch. The correlation co-efficient (Tables 4.46, 4.47 and 4.48) showed that starch content was negatively and significantly correlated with moisture content. It was evident from the Figure 4.16, 4.17 and 4.15 that the presence of interaction between storing duration and types of container was mainly due to earthen *motka* and aside, from this the interaction between storing duration and other types of containers was almost negligible.

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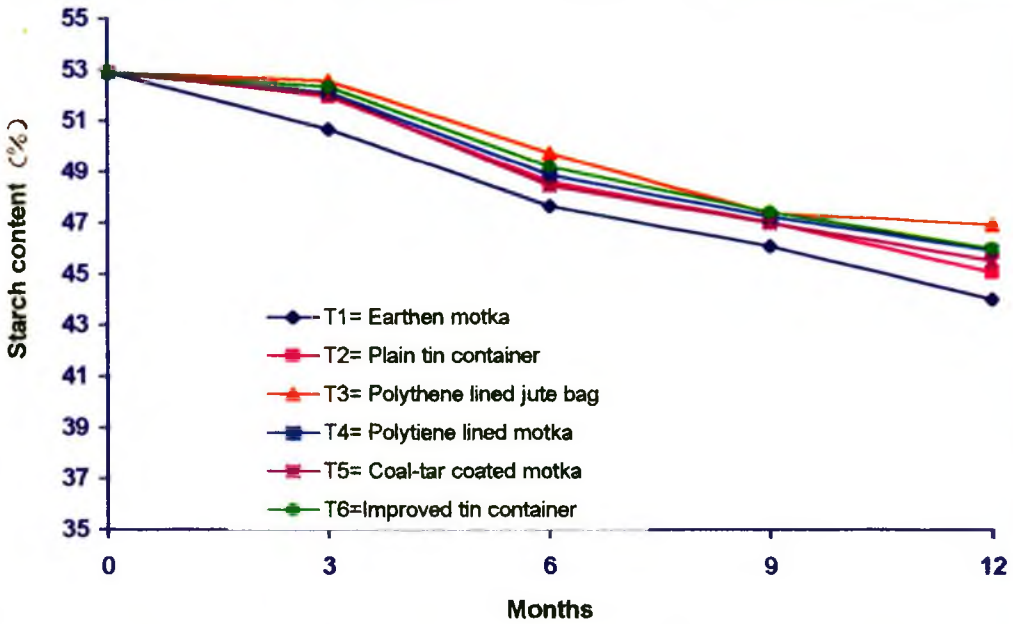

Fig. 4.16. Starch content in lentil durirng different stages of storage period

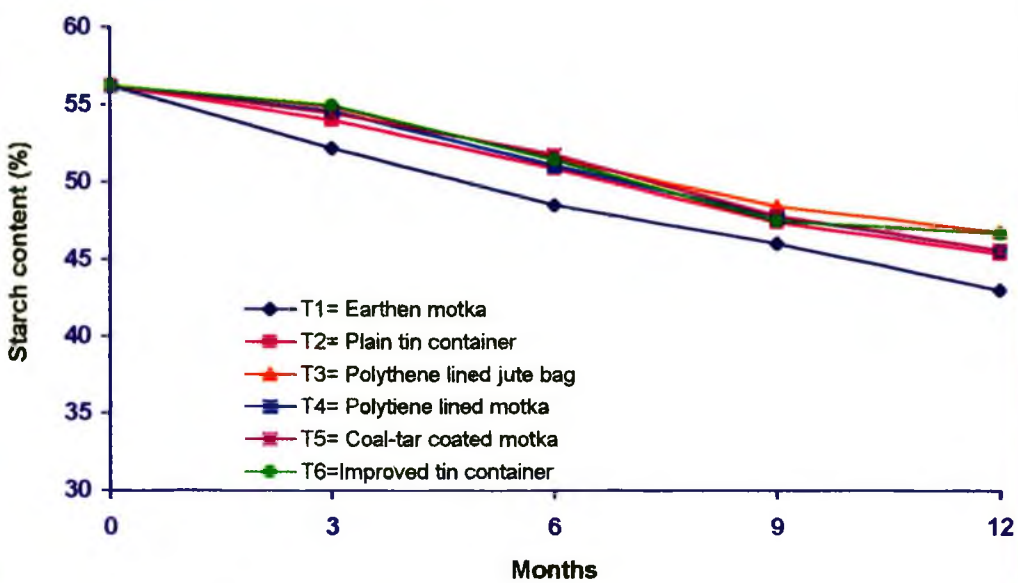

Fig. 4.17. Starch content in chickpea during different stages of storage period

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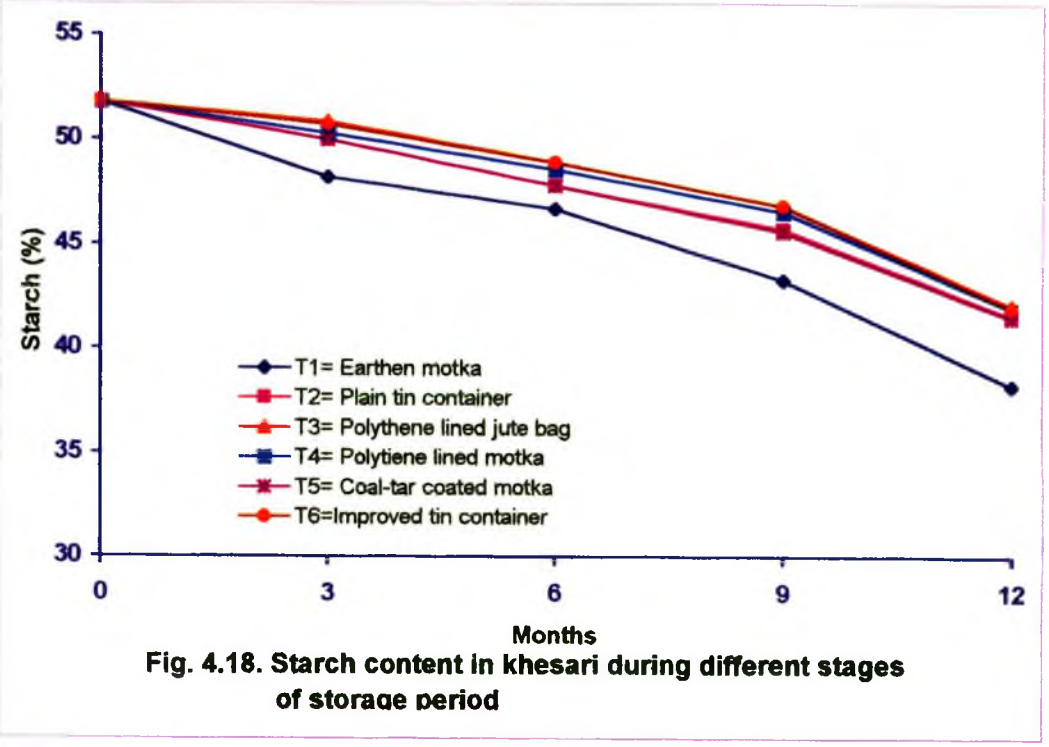

Fig. 4.18. Starch content in khesari during different stages of storage period

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4.5.9. Total sugar

4.5.9.1. Lentil

The amount of total sugar content in pre-stored lentil was 3.75 per cent. It was observed that the amount of total sugar increased during storage period (Table 4.33). After 3 months of storage the highest amount of total sugar (4.81 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher ($P \leq 0.05$) than all other treatments. The amount of total sugar increased by this treatment was 28.26 per cent higher over the initial total sugar content. The lower amount of total sugar (4.20 per cent) was observed in seeds stored in polythene lined jute bag and it was 12.00 per cent higher over the initial total sugar content. The results also showed that the amount total sugar increased significantly during storage and continued to increase up to storage period.

After 12 months of storage the significantly ($P \leq 0.05$) higher amount (8.02 per cent) of total sugar was observed in seeds stored in plain earthen *motka* followed by seeds stored in plain tin container (6.80 per cent). The increase in total sugar in seeds stored in plain earthen *motka* was 113.86 per cent higher over the initial total sugar content. The lower amount of total sugar (5.77 per cent) was observed in seeds stored in polythene lined jute bag and it was 53.86 per cent higher over the initial total sugar content.

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Table 4.33. Total sugar content of lentil during different stages of storage period

Treatments	Total sugar of pre-stored sample (%)	Total sugar (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	3.75	4.81	5.50	6.48	8.02
Plain tin container (T_2)	3.75	4.51	5.09	5.84	6.80
Polythene lined jute bag(T_3)	3.75	4.20	4.88	5.25	5.77
Polythene lined *motka*(T_4)	3.75	4.33	5.04	5.47	5.92
Coal-tar coated *motka*(T_5)	3.75	4.56	5.14	5.72	6.04
Improved tin container(T_6)	3.75	4.39	4.93	5.41	5.84

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.12 and for rows = 0.11

4.5.9.2. Chickpea

The total sugar content in pre-stored chickpea was 3.91 per cent. It was observed that the total sugar content increased during storage period (Tables 4.34). After 3 months of storage the significantly highest amount of total sugar (5.56 per cent) was observed in seeds stored in plain earthen *motka* and it was 40.40 per cent higher over the initial total sugar. The lower amount of total sugar (4.54 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka* and improved tin container. The total sugar increased in seeds stored in polythene lined jute bag was 14.64 per cent higher over the initial total sugar content. It was revealed that during storage period the amount of total sugar increased significantly with increasing storage period and continued to increase up to the storage period.

After 12 months of storage the significantly highest amount of total sugar (9.65 per cent) was observed in seeds stored in plain earthen *motka* and it was 143.68 per cent higher over the initial total sugar content. The lower amount of total sugar (6.14 per cent) was

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observed in seeds stored in improved tin container and it was 55.05 per cent higher than initial total sugar content. This increase was at par with the seeds stored in polythene lined jute bag, polythene lined *motka* and coal-tar coated *motka*.

Table 4.34. Total sugar content of chickpea during different stages of storage period

Treatments	Total sugar of pre-stored sample (%)	Total sugar (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	3.96	5.56	6.57	7.72	9.65
Plain tin container (T_2)	3.96	4.95	5.43	6.38	7.73
Polythene lined jute bag(T_3)	3.96	4.54	5.16	5.52	6.16
Polythene lined *motka*(T_4)	3.96	4.67	5.29	5.73	6.19
Coal-tar coated *motka*(T_5)	3.96	4.89	5.42	5.80	6.27
Improved tin container(T_6)	3.96	4.62	5.25	5.56	6.14

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.17 and for rows = 0.18

4.5.9.3. Khesari

The total sugar content in pre-stored khesari was 3.91 per cent. Results showed that during storage period the amount of total sugar increased (Table 4.35). After 3 months of storage the significantly highest amount of total sugar (4.77 per cent) was observed in seeds stored in plain earthen *motka* and it was 22.00 per cent higher over the initial total sugar content. The lower amount of total sugar (4.28 per cent) was observed in seeds stored in polythene lined jute bag and it was 9.46 per cent higher over the initial total sugar. This increase was at par with seeds stored in polythene-lined *motka* and improved tin container. The results also showed that during storage period the amount of total sugar increased significantly with increasing storage period (Table 4.35).

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After 12 months of storage the highest amount of total sugar (8.12 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher ($P \leq 0.05$) than all other treatments. The increase in total sugar was 108.95 per cent higher over the initial total sugar content. The lower amount of total sugar (6.04 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka* and improved tin container. The amount of total sugar increased by seeds stored in polythene lined jute bag was 54.47 per cent higher over the initial total sugar content.

Table 4.35. Total sugar content of khesari during different stages of storage period

Treatments	Total sugar of pre-stored sample (%)	Total sugar (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	3.91	4.77	5.63	6.57	8.17
Plain tin container (T_2)	3.91	4.48	5.16	5.81	6.77
Polythene lined jute bag(T_3)	3.91	4.28	4.90	5.32	6.04
Polythene lined *motka*(T_4)	3.91	4.41	5.03	5.55	6.17
Coal-tar coated *motka*(T_5)	3.91	4.46	5.14	5.71	6.59
Improved tin container(T_6)	3.91	4.40	4.94	5.36	6.06

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.17 and for rows = 0.18

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It was revealed from the results (Table 4.33-4.35) that the amount of total sugar increased in all the treatment with increasing storage time. Srivastova *et al.* (1988) reported that during storage condition pulses seeds increased their sugar content by slow breakdown of the starch present in the seeds. In the present study, the increased in total sugar in seeds stored in plain earthen *motka* was comparatively higher (Figure 4.19-4.21). It might be due to higher moisture content in seeds of the said treatment, which in presence of higher temperature accelerated the breakdown of starch in to sugar comparatively at a faster rate. Similar results were also reported by Pattee *et al.* (1981) while working with the peanut seeds. The results (Table 4.30-4.32) indicated that the breakdown of starch was comparatively minimum when the pulses stored in improved containers specially the polythene lined jute bag.

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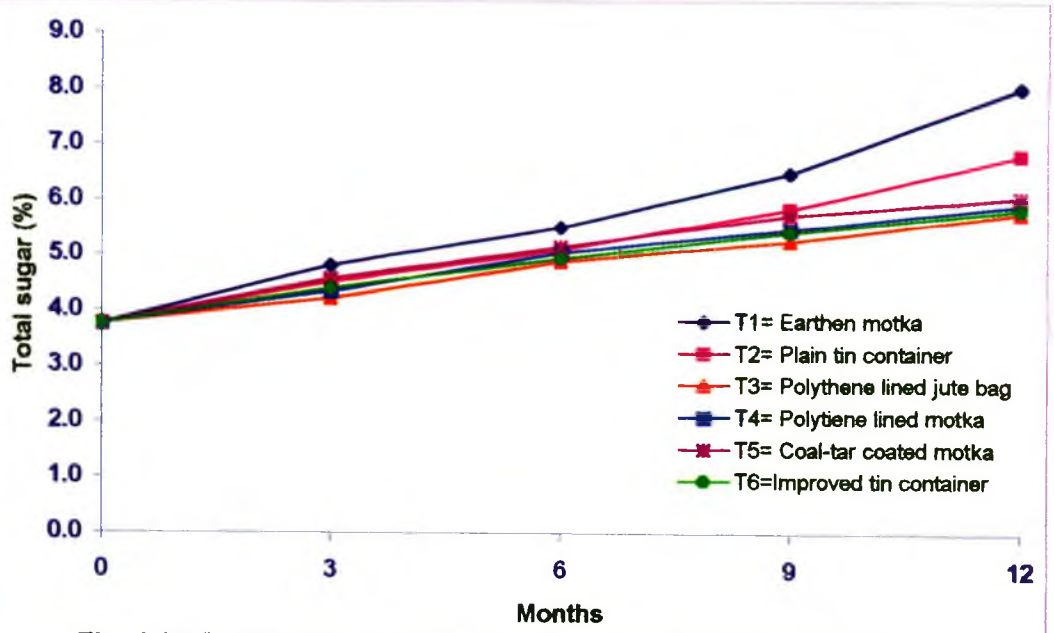

Fig. 4.19. Total sugar content of lentil during different stage of storage period

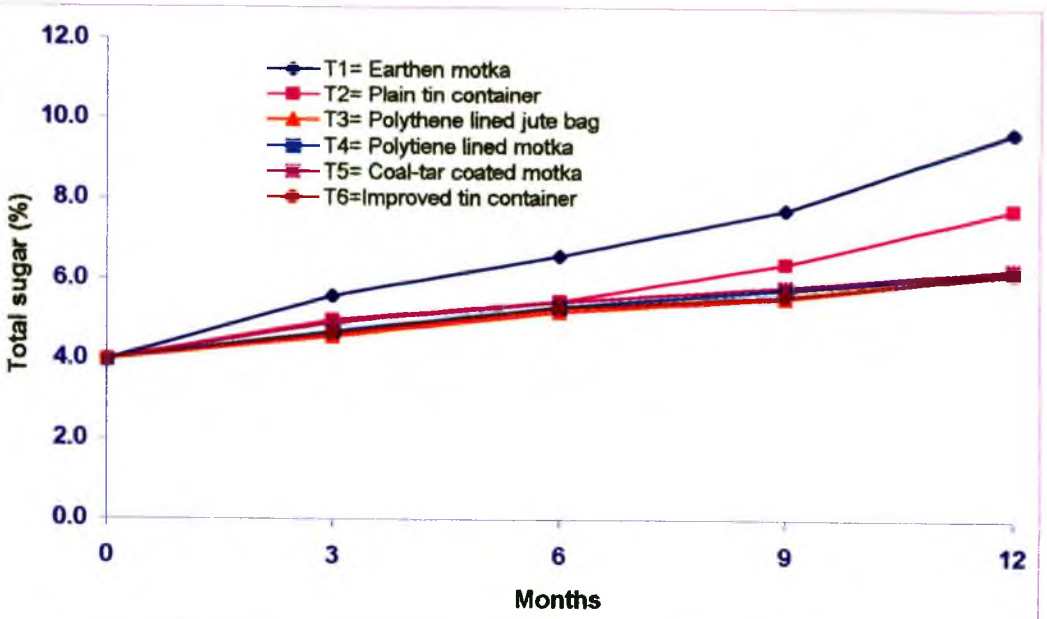

Fig. 4.20. Total sugar content of chickpea dulrng different stages of storage period

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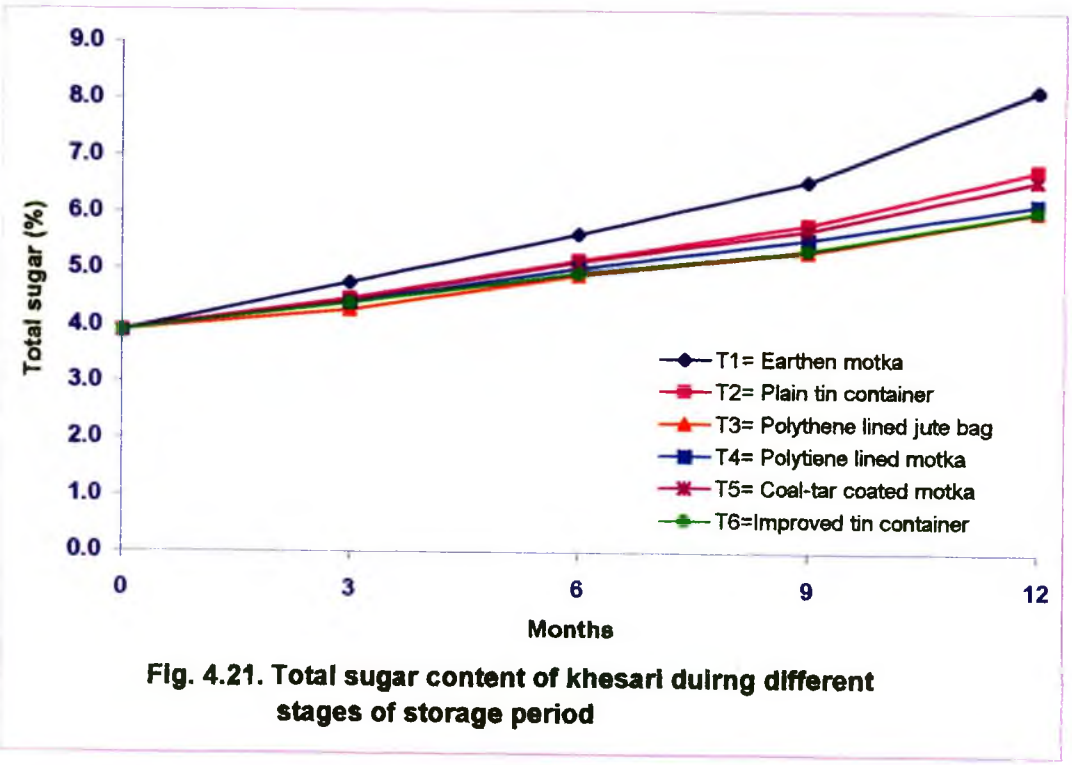

Fig. 4.21. Total sugar content of khesari duirng different stages of storage period

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4.5.10. Reducing sugar

4.5.10.1. Lentil

The reducing sugar content of pre-stored lentil was 0.94 per cent. The results showed that during storage period the reducing sugar content of lentil increased (Table 4.36). After 3 months of storage the highest amount of reducing sugar content (1.31 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher ($P \leq 0.05$) than all other treatments. The reducing sugar content increased by this treatment was 39.36 per cent over the initial reducing sugar content. The lower amount of reducing sugar (0.98 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka*, coal-tar coated *motka* and improved tin container. The results also showed that the amount of reducing sugar increased significantly with increasing storage period and continued to increase up to the storage period.

After 12 months of storage the highest amount of reducing sugar (3.62 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher than all other treatments. The amount of reducing sugar increased by this treatment was 285.10 per cent higher over the initial reducing sugar of lentil. The lower amount of reducing sugar (2.71 per cent) was observed in seeds stored in polythene lined jute bag and it was 188.29 per cent higher over the initial reducing sugar content. The increase in reducing sugar was at par with seeds stored in polythene-lined *motka* and improved tin container.

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Table 4.36. Reducing sugar content of lentil during different stages of storage period

Treatments	Reducing sugar of pre-stored sample (%)	Reducing sugar (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	0.94	1.31	2.80	3.29	3.62
Plain tin container (T_2)	0.94	1.13	2.18	2.69	3.13
Polythene lined jute bag(T_3)	0.94	0.98	1.89	2.16	2.71
Polythene lined *motka*(T_4)	0.94	1.02	1.91	2.32	2.78
Coal-tar coated *motka*(T_5)	0.94	1.09	1.98	2.38	2.88
Improved tin container(T_6)	0.94	1.02	1.91	2.32	2.75

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.089 and for rows = 0.10

4.5.10.2. Chickpea

The pre-stored chickpea contained 1.04 per cent reducing sugar. Results showed that the reducing sugar content increased during storage period (Table 4.37). After 3 months of storage the highest amount of reducing sugar (1.59 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher ($P \leq 0.05$) than all other treatments. The amount of reducing sugar increased by the seeds stored in plain earthen *motka* was 52.88 per cent higher over the initial reducing sugar content. The lower amount of reducing sugar (1.12 per cent) was observed in seeds both stored in polythene lined jute bag and improved tin container and was 7.69 per cent higher over the initial reducing sugar. The results also showed that during storage period the amount of reducing sugar increased significantly and continued to increase up to stored period.

After 12 months of storage the highest amount of reducing sugar (5.08 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher than all other

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treatments. The amount of reducing sugar increased in this treatment was 388.46 per cent higher over the initial reducing sugar content. The lower amount (3.12 per cent) of reducing sugar was observed in seeds stored in polythene lined jute bag and it was 200 per cent higher over the initial reducing sugar content.

Table 4.37. Reducing sugar content of chickpea during different stages of storage period

Treatments	Reducing sugar of pre-stored sample (%)	Reducing sugar (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	1.04	1.59	3.02	4.15	5.08
Plain tin container (T_2)	1.04	1.32	2.60	3.12	3.73
Polythene lined jute bag(T_3)	1.04	1.12	2.38	2.67	3.12
Polythene lined *motka*(T_4)	1.04	1.16	2.51	2.82	3.23
Coal-tar coated *motka*(T_5)	1.04	1.27	2.58	3.02	3.46
Improved tin container(T_6)	1.04	1.12	2.50	2.76	3.14

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.068 and for rows = 0.079

4.5.10.3. Khesari

The amount of reducing sugar present is pre-stored khesari was 1.05 per cent. Results showed that during storage period the amount of reducing sugar increased (Table 4.38). After 3 months of storage the highest amount of reducing sugar (1.34 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher than all other treatments. The amount of reducing sugar increased in this treatment was 23.80 per cent higher over the initial reducing sugar content. The lower amount of reducing sugar (1.09 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka*, coal-tar coated *motka* and improved tin container. It was observed that the amount of reducing sugar increased significantly during storage period and continued to increase with increasing storage period (Table 4.38).

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After 12 months of storage the highest amount of reducing sugar (4.71 per cent) was observed in seeds stored in plain earthen *motka* and it was significantly higher ($P \leq 0.05$) than other treatments. The amount of reducing sugar increased in this treatment was 348.57 per cent higher over the initial reducing sugar content. The lower amount of reducing sugar (3.05 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka* and improved tin container. The amount of reducing sugar increased by the seeds stored in polythene lined jute bag was 190.47 per cent higher over the initial reducing sugar content.

Table 4.38. Reducing sugar content of khesari during different stages of storage period

Treatments	Reducing sugar of pre-stored sample (%)	Reducing sugar (%) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	1.05	1.34	2.96	3.66	4.71
Plain tin container (T_2)	1.05	1.22	2.61	3.09	3.75
Polythene lined jute bag(T_3)	1.05	1.09	2.05	2.48	3.05
Polythene lined *motka*(T_4)	1.05	1.13	2.21	2.54	3.10
Coal-tar coated *motka*(T_5)	1.05	1.18	2.37	2.77	3.36
Improved tin container(T_6)	1.05	1.12	2.12	2.50	3.10

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.069 and for rows = 0.089

It was revealed from the results of three pulses that the amount of reducing sugar content increased with increasing storage period (Table 4.36-4.38). Similar results were also reported for pigeon pea (Srivastova, *et al.,* 1988). Kole and Gupta (1982) also reported that the amount of soluble sugar increased with increasing aging of seeds. The amount of reducing sugar content was considerably higher in case of seeds stored in plain earthen *motka* (Figure 4.22-4.24). It might be due to higher amount of moisture content in seeds, which in presence of high temperature accelerated the breakdown of starch in to sugar comparatively at a higher rate as reported by Pattee *et al.* (1981). It was revealed from the results (Tables 4.36-4.38) that the breakdown of starch into sugar was comparatively minimum when the pulses stored improved container especially the polythene lined jute bag.

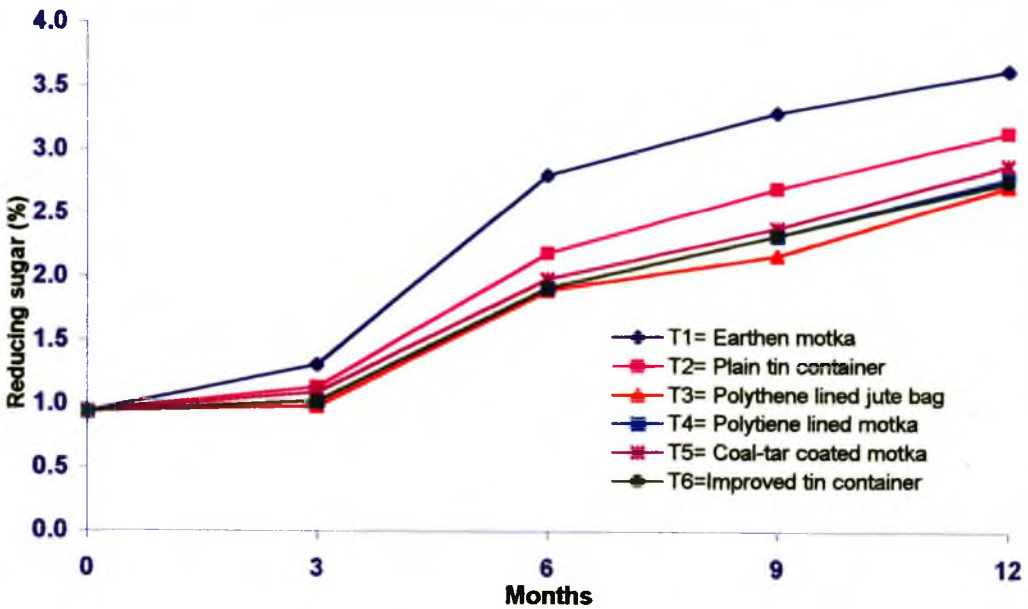

Fig. 4.22. Reducing sugar content of lentil duirng different stages of storage period

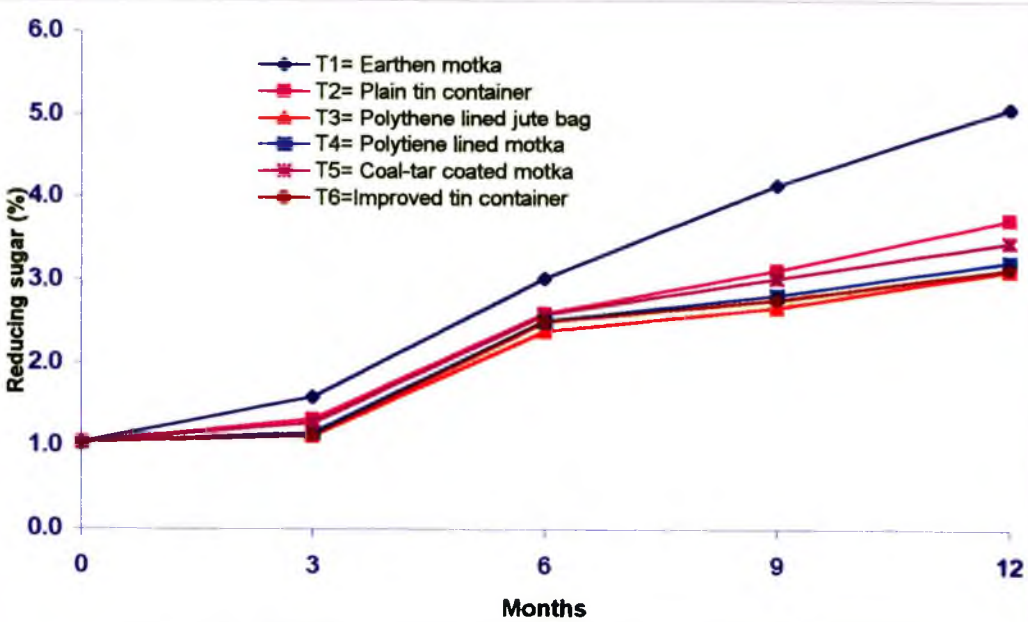

Fig. 4. 23. Reduicng sugar content of chickpea during different stages of storage period

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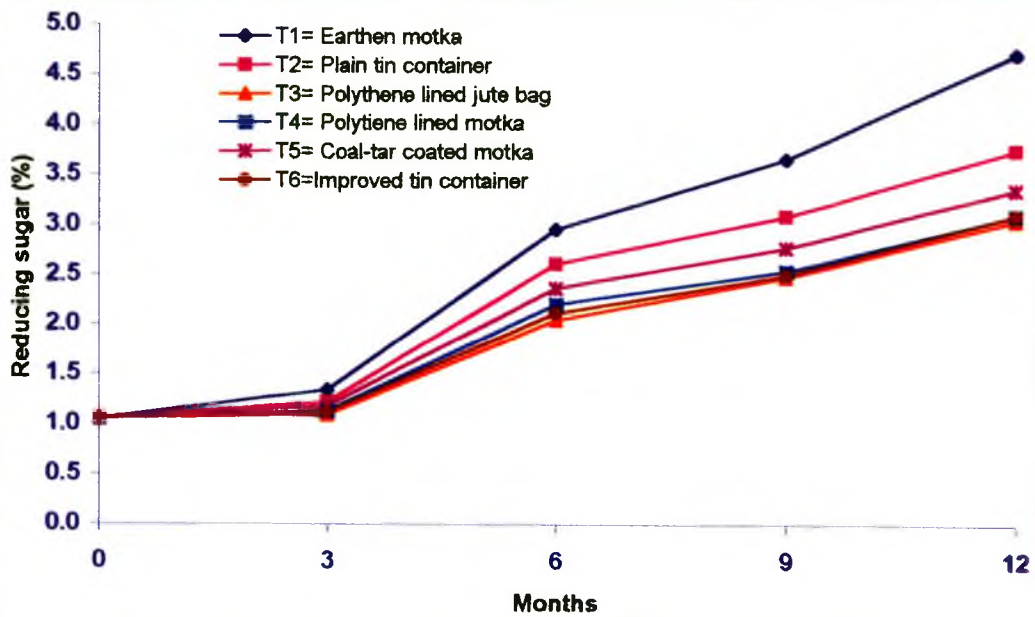

Fig. 4.24. Reducing sugar content of khesari duirng different stages of storage period

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4.5.11. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS)

For digestibility purpose pulses starch are classified into RDS, SDS and RS and all of these reflect the rate of starch digestion in vivo. RDS and SDS are measured after incubation at 37^0 C for 20 minutes and further 100 minutes respectively. RS is the starch not hydrolyzed after 120 minutes incubation.

4.5.11.1. Lentil

The RDS, SDS and RS of pre-stored lentil were 58.10 per cent, 21.10 percent and 20.80 percent respectively. It was observed that during storage period the RDS and RS of lentil gradually decreased and the SDS increased with increasing storage period (Table 4.39). After 6 months of storage the higher RDS (52.12 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in both the polythene lined *motka* and improved tin container. The decrease in RDS in seeds stored in polythene lined jute bag was 10.29 per cent lower than initial RDS of lentil. The lower RDS (48.69 per cent) was observed in seeds stored in plain earthen *motka* and it was 17.00 per cent lower than initial RDS of lentil. On the other hand the seeds of the same container gave the highest amount of SDS (31.78 per cent) and it was 50.62 per cent higher than initial SDS. The lower amount of SDS (28.78 per cent) was observed in seeds stored in coal-tar coated *motka* and was 36.39 per cent higher than initial SDS. The RS value was higher (19.53 per cent) with seeds stored in plain earthen *motka* and lower amount (18.88 per cent) was observed in seeds both stored in polythene lined *motka* and improved tin container.

After 12 months of storage the higher RDS (48.98 per cent) was observed in seeds stored in polythene lined jute bag and it was 15.70 per cent lower than initial RDS of lentil. This decrease in RDS was at par with seeds stored in polythene lined *motka* and improved tin

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container. The lowest amount of RDS (45.45 per cent) was observed in seeds stored in plain earthen *motka* and it was 21.77 lower than initial RDS. On the other hand the seeds stored in same container gave the highest percentage of SDS (36.24 per cent) and it was 70.75 higher than initial SDS. The lowest amount of SDS (33.60 per cent) was observed in seeds stored in improved tin container and it was (59.24 per cent) higher than initial SDS. The higher RS (18.31 per cent) has observed in seeds stored plain earthen *motka*. The lowest RS (17.28 per cent) was observed in seeds stored in polythene lined jute bag.

Table 4.39. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistance starch (RS) content of lentil during different stages storage period

Treatment	Fresh sample			After 6 months			After 12 months		
	RDS%	SDS%	RS%	RDS%	SDS%	RS%	RDS%	SDS%	RS%
Plain earthen *motka* (T_1)	58.10	21.10	20.80	48.69	31.78	19.53	45.45	36.24	18.31
Plain tin container (T_2)	58.10	21.10	20.80	49.95	30.74	19.31	47.44	34.61	17.95
Polythene lined jute bag(T_3)	58.10	21.10	20.80	52.12	28.95	18.93	48.98	33.74	17.28
Polythene lined *motka*(T_4)	58.10	21.10	20.80	52.09	29.03	18.88	48.74	33.77	17.49
Coal-tar coated *motka*(T_5)	58.10	21.10	20.80	51.84	28.78	19.38	47.92	33.98	18.10
Improved tin container(T_6)	58.10	21.10	20.80	52.09	29.03	18.88	48.83	33.60	17.57
LSD (5%)	-	-	-	0.55	0.57	0.25	0.55	0.57	0.25

LSD (0.05 %) for comparing means in rows for RDS = 0.68, SDS = 0.61 and RS = 0.24

4.5.11.2. Chickpea

The RDS, SDS and RS of pre-stored chickpea were 56.75 per cent, 19.15 per cent and 24.10 per cent respectively. It was observed that the RDS and RS were decreased gradually and SDS increased with increasing storage periods (Table 4.40). After 6 months of storage the higher amount of RDS (51.91 per cent) was observed in seeds stored in polythene lined jute bag and it was 8.52 per cent lower than initial RDS of chickpea. This decrease was at par with seeds stored in polythene lined *motka*. The lowest amount of RDS (46.87 per cent) was observed in seeds stored in plain earthen *motka* and was 17.41 per cent lower than initial

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RDS of chickpea. On the other hand the same treatment gave the highest amount of SDS (31.80 per cent) and it was 66.05 per cent higher than initial SDS of chickpea. The lowest amount of SDS (26.81 per cent) was observed in seeds stored in polythene lined jute bag which was 40.41 per cent higher than initial SDS of chickpea. This increase in SDS was at par with seeds stored in polythene lined *motka*. The higher amount of RS (21.64 per cent) after 6 months of storage was observed in seeds stored in plain tin container and was 10.21 per cent lower than initial RS of chickpea. The lowest amount of RS (21.18 per cent) was observed in seeds stored in improved tin container, which was 12.11 per cent lower than initial RS value.

After 12 months of storage higher RDS (47.14 per cent) was observed in seeds stored in polythene lined jute bag and amount decreased from the initial RDS was 18.69 per cent. This decrease in RDS was followed by seeds stored in improved tin container (Table 4.40). The lowest amount of RDS (42.56 per cent) was observed in seeds stored in plain earthen *motka* and it was 25.00 per cent lower than initial RDS of chickpea. On the other hand the same treatment gave the highest SDS of chickpea (36.31 per cent) after the same storage period and it was 189.60 per cent higher than initial SDS value. The lowest SDS (32.52 per cent) observed in seeds stored in polythene lined jute bag and it was 69.81 per cent higher than initial SDS value. After 12 months of storage the higher RS of chickpea (21.13 per cent) was observed in seeds stored in plain earthen *motka* and it was 11.49 lower than initial RS of chickpea. The lower RS (20.30 per cent) was observed in seeds stored in polythene lined *motka* and it was 12.32 per cent lower than initial RS value.

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Table 4.40. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of chickpea during different stages storage period.

Treatment	Fresh sample			After 6 months			After 12 months		
	RDS%	SDS%	RS%	RDS%	SDS%	RS%	RDS%	SDS%	RS%
Plain earthen *motka* (T_1)	56.75	19.15	24.10	46.87	31.80	21.33	42.56	36.11	21.33
Plain tin container (T_2)	56.75	19.15	24.10	48.83	29.53	21.64	45.90	33.13	20.97
Polythene lined jute bag(T_3)	56.75	19.15	24.10	51.91	26.89	21.20	47.14	32.52	20.34
Polythene lined *motka*(T_4)	56.75	19.15	24.10	51.74	26.91	21.35	46.42	33.28	20.30
Coal-tar coated *motka*(T_5)	56.75	19.15	24.10	49.35	29.02	21.63	45.90	33.76	20.51
Improved tin container(T_6)	56.75	19.15	24.10	51.44	27.38	21.18	47.00	32.62	20.38
LSD (5%)	-	-	-	0.27	0.50	0.53	0.27	0.50	0.53

LSD (0.05 %) for comparing means in rows for RDS = 0.28, SDS = 0.60 and RS = 0.64

4.5.11.3. Khesari

The RDS, SDS and RS of khesari were 56.37 per cent, 20.96 per cent and 22.67 per cent respectively. It was observed that like lentil and chickpea the RDS and RS of khesari gradually decreased, while the SDS increased with increasing storage period (Table 4.41). After 6 months of storage the higher amount of RDS (49.85 per cent) was observed in seeds stored in polythene lined jute bag and was 11.56 per cent lower than initial RDS of khesari. This decrease was at par with seeds stored in polythene lined *motka* and improved tin container. The lowest amount of RDS (46.38 per cent) was observed in seeds stored in plain earthen *motka* and it was 17.22 per cent lower than initial RDS of khesari. On the other hand the highest SDS (32.69 per cent) was observed in seeds of same container and it was 55.96 per cent higher over the initial SDS of khesari. The lower SDS (29.84 per cent) was observed in seeds stored in polythene lined jute bag and it was at par with seeds stored in polythene lined *motka* and improved tin container. The higher RS (20.93 per cent) was observed in seeds stored in plain earthen *motka* and it was par with all other treatments except seeds stored in improved tin container which showed lowest (20.21 per cent) of RS value.

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After 12 months of storage higher RDS (45.51 per cent) was observed in seeds stored in polythene lined *motka* and it was 19.26 per cent lower than initial RDS. This decrease in RDS was at par with seeds stored in polythene lined jute bag and improved tin container. The lowest RDS (41.74 per cent) was observed in seeds stored in plain earthen *motka*, which was 25.95 per cent lower than initial RDS. On the other hand the same treatment showed the highest SDS (38.38 per cent) after the same storage period and was 83.11 per cent higher than initial SDS. The lower SDS (33.25 per cent) was observed in seeds stored in polythene lined *motka* which was 68 per cent higher than initial SDS. This increase in SDS was at par with seeds stored in polythene lined jute bag and improved tin container. The higher RS (19.88 per cent) was observed in seeds stored in plain earthen *motka* and it was at par with all other treatments.

Table 4.41. Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) content of khesari during different stages of storage period

Treatment	Fresh sample			After 6 months			After 12 months		
	RDS%	SDS%	RS%	RDS%	SDS%	RS%	RDS%	SDS%	RS%
Plain earthen *motka* (T_1)	56.37	20.96	22.67	46.38	32.69	20.93	41.74	38.38	19.88
Plain tin container (T_2)	56.37	20.96	22.67	48.59	30.75	20.66	43.50	36.98	19.52
Polythene lined jute bag(T_3)	56.37	20.96	22.67	49.85	29.84	20.31	45.44	35.25	19.31
Polythene lined *motka*(T_4)	56.37	20.96	22.67	49.72	29.90	20.38	45.51	35.24	19.25
Coal-tar coated *motka*(T_5)	56.37	20.96	22.67	48.61	30.79	20.60	44.16	36.29	19.55
Improved tin container(T_6)	56.37	20.96	22.67	49.63	30.16	20.21	45.46	35.30	19.24
LSD (5%)		-	-	0.33	0.49	0.66	0.33	0.49	0.66

LSD (0.05 %) for comparing means in rows for RDS = 0.36, SDS = 0.48 and RS = 0.62

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The results thus show that the RDS of fresh samples of all the pulses was much higher in comparison with SDS (Table 4.39-4.41). Similar results were also reported in case of rice where RDS of fresh parboiled rice was 72 per cent and SDS was 24.65 per cent (Tetns *et al.*, 1997). The results also indicated that the RDS and RS of all the pulses gradually decreased and SDS increased with increasing storage period. The gradual hardness of seeds due to aging might have implication on decrease of RDS. This also accounts for longer cooking time and lower digestibility of stored pulses, as a described before.

4.5.12. Phytic acid

Phytic acid is an important antinutrients of pulses. The cooking quality of pulses largely depends on phytic acid content.

4.5.12.1. Lentil

The phytic acid content in pre-stored lentil was 8.09 mg/g. It was observed that during storage period the phytic acid content decreased (Table 4.42). After 3 months of storage higher amount of phytic acid (7.95 mg/g) was observed in seeds stored in polythene lined jute bag and it was 1.97 per cent lower than initial phytic acid content in lentil. This decrease was at par with seeds stored in polythene lined *motka*, coal-tar coated *motka* and improved tin container. The lower amount of phytic acid (7.16 mg/g) was observed in seeds stored in plain earthen *motka* and it was 11.50 per cent lower than the initial phytic acid content. The results also showed that the amount of phytic acid decreased significantly and continued to decrease up to the storage period.

After 12 months of storage, higher amount of phytic acid (6.92 mg/g) was observed in seeds stored in polythene lined jute bag and it was 14.46 per cent lower than initial phytic

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acid content. This decrease was at par with seeds stored in polythene lined *motka* and improved tin container. The minimum amount of phytic acid (5.14 mg/g) was observed in seeds stored is plain earthen *motka* and it was 36.46 per cent lower than the initial phytic acid content.

Table 4.42. Phytic acid content of lentil during different stages of storage period

Treatments	Phytic acid of pre-stored sample (mg/g)	Phytic acid (mg/g) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	8.09	7.16	6.45	5.80	5.14
Plain tin container (T_2)	8.09	7.54	7.16	6.76	6.33
Polythene lined jute bag(T_3)	8.09	7.95	7.55	7.23	6.92
Polythene lined *motka*(T_4)	8.09	7.89	7.51	7.17	6.82
Coal-tar coated *motka*(T_5)	8.09	7.74	7.38	6.94	6.49
Improved tin container(T_6)	8.09	7.94	7.53	7.17	6.83

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.24 and for rows = 0.23

4.5.12.2. Chickpea

The amount of phytic acid present in pre-stored chickpea was 8.65 mg/g. Results showed that the amount of phytic acid decreased during storage period (Table 4.43). After 3 months of storage the higher amount of phytic acid (8.49 mg/g) was observed in seeds stored in improved tin container and it was 1.84 per cent lower than initial phytic content. This decrease was at par with seeds stored in polythene lined jute bag and polythene lined *motka*. The lower amount of phytic acid (7.53 mg/g) was observed in seeds stored in plain earthen *motka* and it was 12.95 per cent lower than initial phytic acid content. The results indicated that the amount of phytic acid decreased significantly and continued to decrease up to storage period.

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After 12 months of storage the higher amount of phytic acid 7.47 mg/g was observed in seeds stored in polythene lined jute bag and it was 13.64 per cent lower than initial phytic content. This decrease was at par with seeds stored in polythene-lined *motka* and improved tin container. The minimum amount of phytic acid (4.68 mg/g) was observed in seeds stored in plain earthen *motka*. The amount of phytic acid decreased in this treatment was 45.90 per cent lower than the initial phytic acid content.

Table 4.43. Phytic acid content of chickpea during different stages of storage period

Treatments	Phytic acid of pre-stored sample (mg/g)	Phytic acid (mg/g) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	8.65	7.53	6.46	5.45	4.68
Plain tin container (T_2)	8.65	8.16	7.74	7.25	6.86
Polythene lined jute bag(T_3)	8.65	8.44	8.02	7.71	7.47
Polythene lined *motka*(T_4)	8.65	8.37	7.95	7.60	7.38
Coal-tar coated *motka*(T_5)	8.65	8.27	7.82	7.42	7.13
Improved tin container(T_6)	8.65	8.49	8.04	7.69	7.39

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.13 and for rows = 0.13

4.5.12.3. Khesari

The amount of phytic acid in pre-stored khesari was 7.42 mg/g. It was observed that during storage period phytic acid in khesari decreased (Table 4.44). After 3 months of storage the higher amount of phytic acid (7.23 mg/g) was observed in seeds stored in polythene lined *motka*. The amount of phytic acid decreased in this treatment was 2.56 per cent lower than initial phytic acid content. The lower quantity of phytic acid (6.29 mg/g) was observed in seeds stored in plain earthen *motka*. The amount of phytic acid decreased in this treatment was 15.23 per cent. The results showed that during storage period the amount of

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phytic acid decreased significantly and continued to decrease up to the storage period (Table 4.44).

After 12 months of storage the higher amount of phytic acid 6.38 mg/g was observed in seeds stored in polythene lined *motka* and it was 14.01 per cent lower than initial phytic acid content. This decrease was at par with seeds stored in polythene lined *motka* and improved tin container. The minimum amount of phytic acid (4.81 mg/g) was observed in seeds stored in plain earthen *motka* and it was 35.17 per cent lower than the initial phytic acid content in khesari.

Table 4.44. Phytic acid content of khesari during different stages of storage period

Treatments	Phytic acid of pre-stored sample (mg/g)	Phytic acid (mg/g) during storage period (months)			
		3	6	9	12
Plain earthen *motka* (T_1)	7.42	6.29	5.59	5.16	4.81
Plain tin container (T_2)	7.42	6.98	6.67	6.20	5.98
Polythene lined jute bag(T_3)	7.42	7.18	6.96	6.63	6.23
Polythene lined *motka*(T_4)	7.42	7.23	6.98	6.64	6.38
Coal-tar coated *motka*(T_5)	7.42	7.07	6.86	6.36	6.07
Improved tin container(T_6)	7.42	7.12	6.98	6.71	6.31

Each value is the mean of 9 replications over 3 consecutive years
LSD (0.05%) for comparing means in column = 0.21 and for rows = 0.23

The results show that as the storage period increased the levels of phytic acid decreased significantly in all the 3 pulses (Table 4.41-4.44). The reduction in phytic acid was considerably higher in seeds stored in plain earthen *motka*. This might be due higher moisture content in seeds of the said treatment (Tables 4.9-4.11) leading to high loss of phytic acid. These results are in agreement with the findings of Jones and Boulter (1983) where they

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reported that with the increase in moisture content, the level of phytic acid had decreased. Hentges *et al.* (1991) reported that during storage period, the high temperature in presence of high moisture in seeds accelerates the activity of phytase leading to decrease in phytic acid. Chitra and Singh (1998) had observed 56 per cent and 29 per cent loss of phytic acid in chickpea and soybean respectively after 12 months storage of seeds. They also reported that when phytic acid started to reduce during storage period the minerals specially Ca and Mg become free from phytic acid and associated with pectin substances or proteinacious materials, causing hard to cook phenomena. Singh and Rao (1995) had reported that the cooking time of pegeon pea dhal was significantly increased when the phytic acid levels were reduced by phytase. Correlation coefficient (Tables 4.46, 4.47 and 4.48) showed that phytic acid was negatively and significantly correlated with moisture and cooking time of pulses. Mattson (1985) also reported similar correlation. Analysis of variance of phytic acid (Appendix Tables 1, 2, and 3) revealed that there was strong interaction between storing duration and types of containers. As evident from the graph (Figure 4.25-4.27) that the presence of interaction was mainly due to plain earthen *motka*. The interaction between other containers were very small almost negligible. The moisture content of seeds stored in different improved containers were within the range of safe moisture level and hence reduction of phytic acid occurred comparatively at a slower rate. Though phytic acid chelates metals and makes them available, yet there is another implication of this factor as regards cooking quality. Cooking time is inversely proportional to phytic acid, so in earthen *motka*, the phytic acid has been reduced but its reduction has also caused deterioration in cooking and associated qualities. Hence, quality of pulses is maintained inspite of comparatively higher contents of phytic acid, when they are stored in polythene lined jute bag, polythene lined *motka*, coal-tar coated *motka* and improved tin container.

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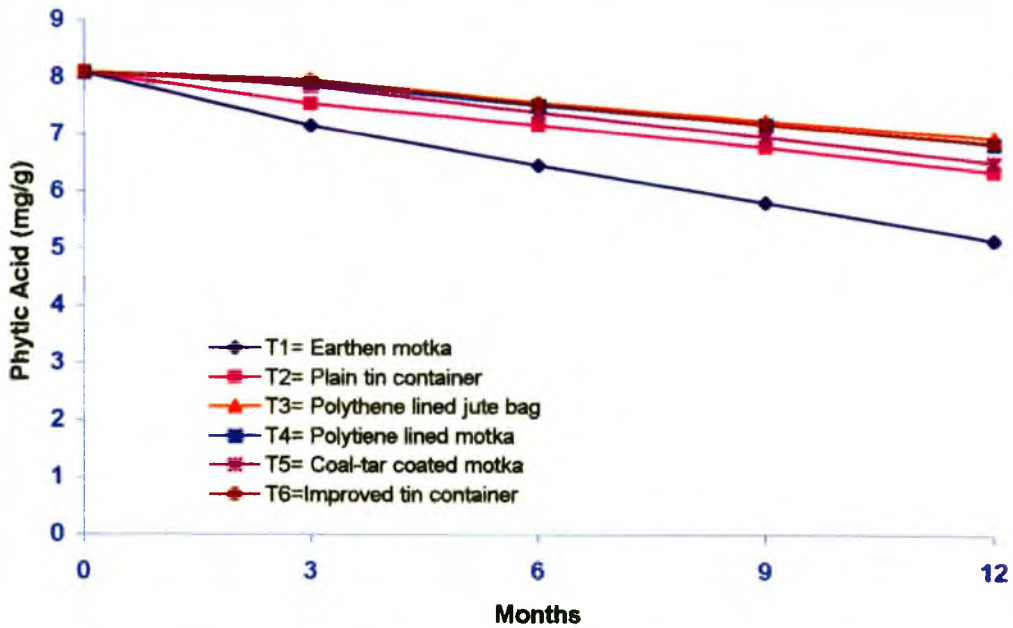

Fig. 4.25. Phytic acid content in lentil during different stages of storage period

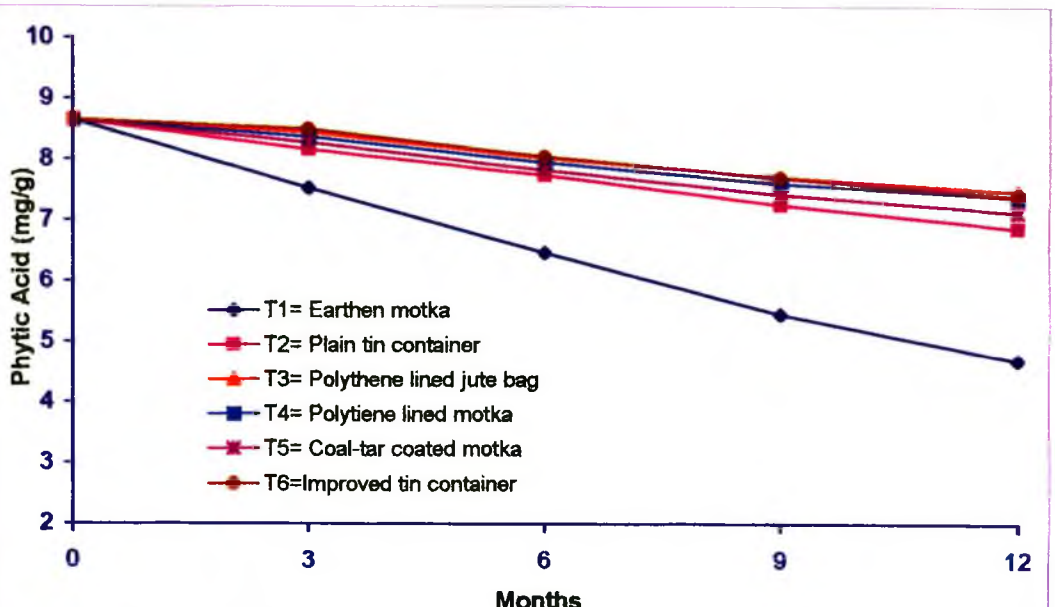

Fig. 4.26. Phytic acid content in chickpea during different stages of storage period

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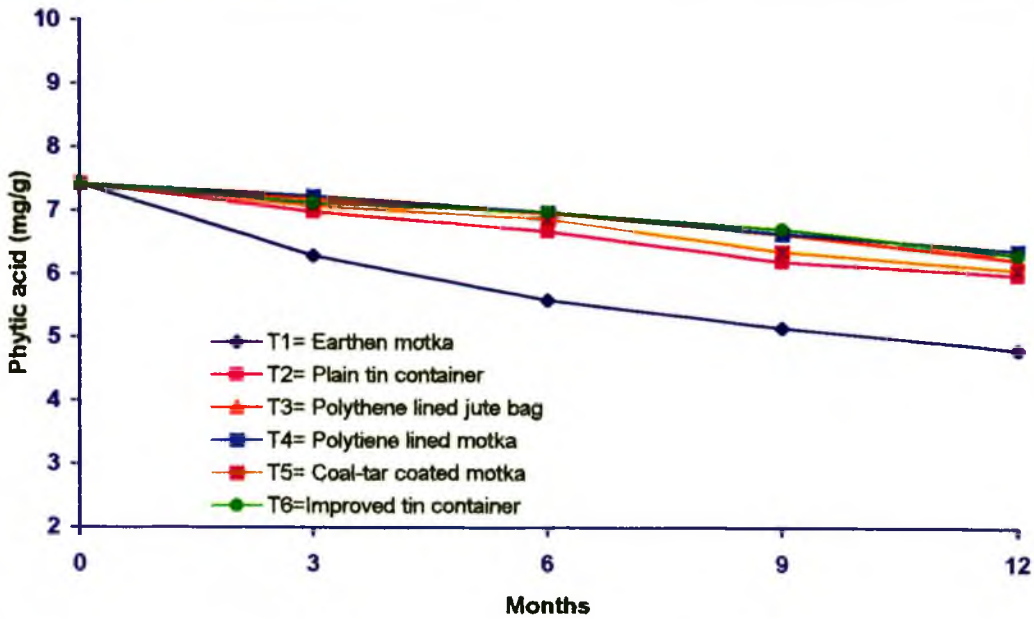

Fig. 4.27. Phytic acid content in Khesari during different stages of storage period

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4.5.14. Protein profile and molecular weight of proteins of different pulses

The proteins profile of different pulses is presented in Photograph 2. The molecular weight of different pulses was calculated from the formula $Y= 9465.5X^{-1.2289}$ obtained from the standard curve of marker proteins (Figure 4.28). It was observed that the number and molecular weight of proteins varied from pulse to pulse and also between fresh and stored samples (Table 4.45). The results indicated that there were 11 zones of protein in fresh sample of lentil with highest molecular weight of 89990 and the lowest one was 17000. On the other hand the stored sample gave 10 different zones. In chickpea the proteins of both the fresh and stored sample were distributed to 13 different zones but some of proteins of stored sample were of comparatively lower molecular weight. The protein of highest molecular weight 106000 was observed in fresh sample, whereas in case of stored sample the highest value was 97400. In khesari the different proteins of fresh sample were distributed to 13 different zones with highest molecular weight of 128000 and the lowest value of 18000. On the other hand the proteins of stored sample of khesari were distributed to 12 different zones with highest molecular weight of 128000 and the lowest of 18500. It was evident from the results (Table 4.45) that there was some decrease in molecular weight of some of the proteins due to increase in storage period. This might be due to aging of seeds, which caused some degradation of protein leading to decrease in protein content (Roberts, 1979).

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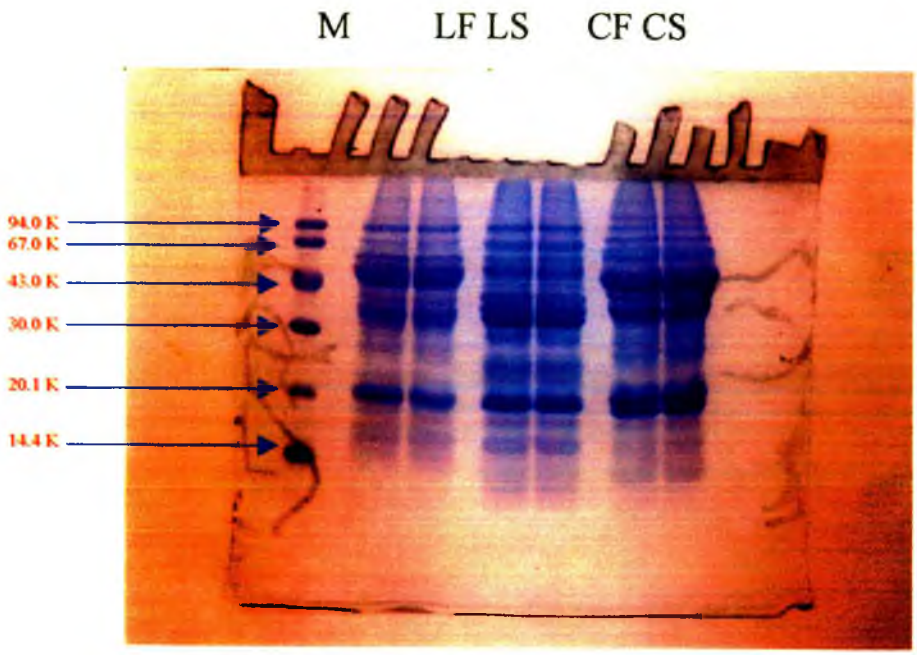

M=Marker, LF=Lentil fresh, LS=Lentil stored, CF=Chickpea fresh, CS=Chickpea stored, KF=Khesari fresh and KS=Khesari stored

Photograph 2. Protein pattern of fresh and stored pulses
Storage period in all cases was 12 months

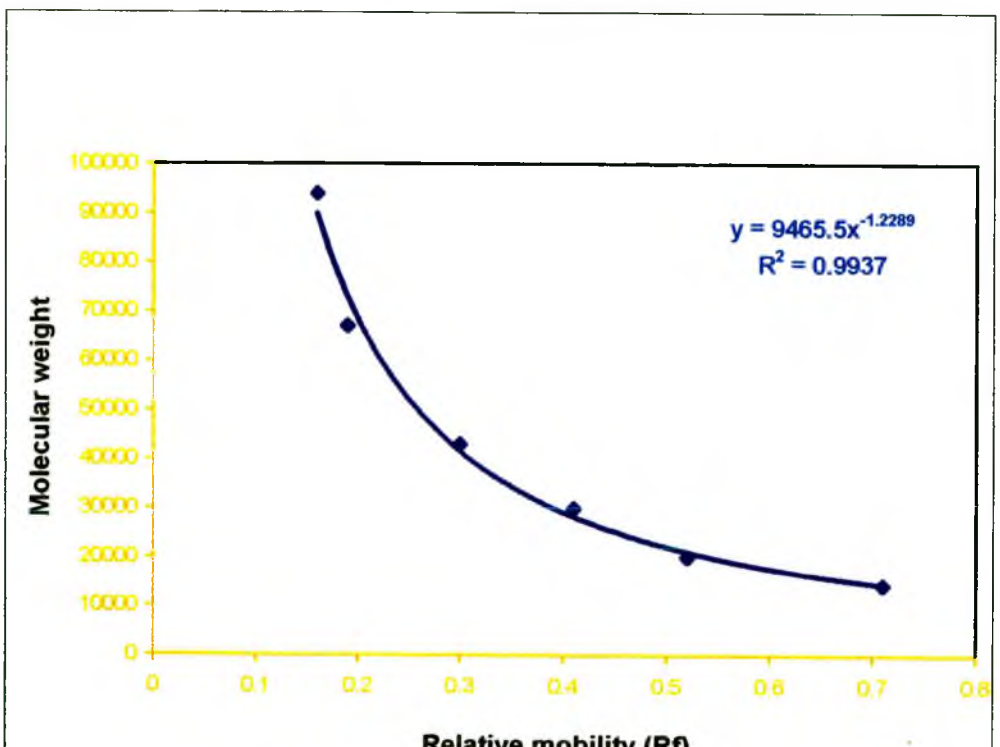

Fig. 4.28. Standard curve for the determination of apparent molecular weight of unknown proteins

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Table 4.45. Calculated apparent molecular weight of different proteins of fresh and stored sample of lentil, chickpea and khesari

Sl. No.	Molecular weight					
	Lentil		Chickpea		Khesari	
	Fresh	Stored	Fresh	Stored	Fresh	Stored
1	89990	89990	106000	97400	128200	128200
2	729000	72900	72900	72900	106000	10600
3	60800	60800	68400	60800	83500	83500
4	52000	43300	52000	52000	64400	64400
5	47300	38400	43300	41600	49600	49600
6	38400	33200	38400	37000	41600	41600
7	33200	27500	34400	34400	37000	37000
8	27500	20000	30100	29000	29000	29000
9	23300	18100	25300	24600	26700	28300
10	20100	17000	23300	23300	21700	21700
11	17000	-	21700	20000	20100	19700
12	-	-	18500	18500	193000	18500
13	-	-	17000	16400	18100	-

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Table 4.46. Correlation co-efficient of different parameters of lentil

	Moisture	Cooking time	Water absorp-tion	Solids dispersed	Protein content	IVPD	Starch content	Total Sugar	Reducing Sugar	Phytic acid	Germi-nation
Moisture	1										
Cooking time	0.909	1									
Water absorption	-0.697	-0.884	1								
Solids dispersed	-0.809	-0.951	0.925	1							
Protein content	-0.713	-0.902	0.957	0.967	1						
IVPD	-0.794	-0.952	0.957	0.985	0.967	1					
Starch content	-0.490	-0.721	0.886	0.826	0.904	0.831	1				
Total Sugar	0.495	0.720	-0.768	-0.808	-0.840	-0.811	-0.838	1			
Reducing Sugar	0.600	0.794	-0.904	-0.876	-0.938	-0.874	-0.975	0.830	1		
Phytic acid	-0.824	-0.953	0.949	0.982	0.974	0.977	0.867	-0.800	-0.920	1	
Germination	-0.042	0.115	-0.389	-0.188	-0.364	-0.219	-0.647	0.371	0.604	-0.299	1

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Table 4.47. Correlation co-efficient of different parameters of chickpea

	Moisture	Cooking time	Water absorp-tion	Solids dispersed	Protein content	IVPD	Starch content	Total Sugar	Reducing Sugar	Phytic acid	Germi-nation
Moisture	1										
Cooking time	0.964	1									
Water absorption	-0.617	-0.775	1								
Solids dispersed	-0.820	-0.927	0.942	1							
Protein content	-0.792	-0.898	0.952	0.977	1						
IVPD	-0.440	-0.617	0.940	0.844	0.862	1					
Starch content	-0.480	-0.656	0.949	0.856	0.897	0.977	1				
Total Sugar	0.793	0.895	-0.938	-0.963	-0.985	-0.817	-0.852	1			
Reducing Sugar	0.611	0.769	-0.942	-0.904	-0.947	-0.897	-0.951	0.915	1		
Phytic acid	-0.902	-0.968	0.878	0.972	0.974	0.758	0.796	-0.962	-0.883	1	
Germination	-0.969	-0.892	0.493	0.710	0.685	0.343	0.375	-0.676	-0.481	0.808	1

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Table 4.48. Correlation co-efficient of different parameters of khesari

	Moisture	Cooking time	Water absorp-tion	Solids dispersed	Protein content	IVPD	Starch content	Total Sugar	Reducing Sugar	Phytic acid	Germi-nation
Moisture	1										
Cooking time	0.987	1									
Water absorption	-0.593	-0.686	1								
Solids dispersed	-0.867	-0.923	0.870	1							
Protein content	-0.778	-0.859	0.901	0.966	1						
IVPD	-0.618	-0.720	0.935	0.909	0.956	1					
Starch content	-0.528	-0.642	0.979	0.851	0.908	0.958	1				
Total Sugar	0.640	0.746	-0.941	-0.907	-0.963	-0.974	-0.966	1			
Reducing Sugar	0.584	0.692	-0.892	-0.866	-0.949	-0.949	-0.930	0.969	1		
Phytic acid	-0.884	-0.935	0.854	0.983	0.967	0.894	0.828	-0.895	-0.865	1.000	
Germination	-0.868	-0.916	0.805	0.953	0.924	0.862	0.780	-0.881	-0.831	0.951	1

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Chapter 5

SUMMARY AND CONCLUSIONS

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Summary

Laboratory experiments on storage of three major pulses namely, lentil, chickpea and khesari, were conducted for three consecutive years starting from 1998-99 to 2000-2001. The objectives of the studies were to evaluate the various nutrients present in pulses and to find out the effect of storage in different containers for different periods of time on physical, compositional and nutritional qualities of the pulses. The pulses were stored separately in 6 different types of containers namely, plain earthen *motka*, coal-tar coated *motka*, plain tin container, improved tin container, polythene lined jute bag and polythene lined *motka*. Each of the containers was considered as a treatment. The pulse samples were collected from the major growing areas of each of the pulses. The experimental design was a Completely Randomized Design with 6 treatments each replicated 3 times. Pulses were stored for 4 different periods i.e. 3, 6, 9 and 12 months. The analysis of variance of seed quality was performed considering storing period as random factor and treating them in a sub plot and the treatments (containers) were in the main plot. Analysis were done on the basis of split plot over years considering years as factor. Findings are summarized and presented in Appendix Tables 7, 8 and 9. The findings are briefly presented as follows under major points:

Fungal infection

Pulse seeds stored in plain earthen *motka* had the highest fungal infection irrespective of types of pulses, hence this container proved ineffective in protecting against fungal infection. The seeds stored in polythene lined jute bag had the minimum fungal infection and performed better in obstructing fungal infection. The other improved containers like polythene lined *motka*,

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improved tin container and coal-tar coated *motka* also performed satisfactory in preventing fungal infection of pulses.

Moisture content

Moisture percentage of different pulses stored in plain earthen *motka* increased considerably and significantly at a high rate. After 12 months of storage the increase in moisture content were 46.8 per cent, 73.81 per cent and 50.18 per cent respectively for lentil, chickpea and khesari. The porousness of the earthen *motka* allowed absorption of moisture from the surrounding environment. The higher moisture content in presence of high temperature deteriorated the quality of seeds of this container, the changes being reflected in various physico-chemical properties of pulses. The improved containers viz. polythene lined jute bag, polythene lined *motka*, coal-tar coated *motka* and improved tin container protected stored pulses from moisture absorption and hence the increase in moisture content in these containers was minimum. The minimum increases in moisture content by lentil and khesari were 3.98 per cent and 6.98 per cent respectively stored in polythene lined *motka* and the minimum was 6.8 per cent for chickpea seeds stored in polythene lined jute bag.

Germination

Germination percentage of pulses decreased with increasing storage period. For lentil the initial germination percentage was lower and it increased during storage up to 6 months and then started to decline. The decrease in germination percentage was higher in pulses seeds stored in earthen *motka*. Storing lentil in plain earthen *motka* increased germination by 17.35 per cent over the initial germination but it was 15.50 per cent lower than that after 6 months storage seeds.

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Chickpea and khesari seeds of same treatments had their germination decreased by 97.63 per cent and 20.85 per cent respectively after 12 months of storage. The rate of decrease was much higher in case of chickpea. The improved storage containers performed better in retaining the germination quality of pulses. Among the improved containers polythene lined jute bag, improved tin container and polythene lined *motka* performed better for lentil, chickpea and khesari respectively.

Cooking time

The cooking time of pulses increased with increasing storage period. The increase in cooking time was higher in seeds stored in plain earthen *motka* irrespective of types of pulses. Lentil, chickpea and khesari stored in plain earthen *motka* had their cooking time increased by 87.7 per cent, 52.00 per cent and 45.67 per cent respectively after 12 months of storage. The higher moisture content in seeds stored in plain earthen *motka* deteriorated the quality of seeds and hence the cooking time has been increased. The seeds stored in improved containers viz. polythene lined jute bag, polythene lined *motka*, *coal-tar* coated *motka* and improved tin container also increased their cooking time but considerably at a slower rate. Lentil and chickpea showed their minimum increase in cooking time by 21.6 per cent and 9.34 per cent respectively after 12 months of storage and for khesari it was 9.94 per cent observed in seeds stored in both polythene lined jute bag and improved tin container. It was observed that large seeded pulses required higher cooking time than small seeded pulses.

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Water absorption during cooking

Water absorption is a common phenomenon associated with cooking of any food item and is measured by the amount of water absorbed by the food when it reaches the cooked state. The water absorption capacity of pulses decreased significantly with the length of storage period. The decrease was much higher in seeds stored in plain earthen *motka*. Lentil, chickpea and khesari of this treatment had their water absorption capacity decreased by 28.85 per cent, 21.21 per cent and 23.35 per cent respectively after 12 months of storage. The higher moisture content in seeds of this treatment deteriorated quality of seeds. As a result the seeds lost their water absorption capacity. The decrease in water absorption capacity was much lower in pulse seeds stored in improved containers viz. polythene lined jute bag, polythene *motka*, coal-tar coated *motka* and improved tin container. Among the improved containers polythene lined jute bag performed better where lentil, chickpea and khesari decreased their water absorption capacity by 10.16 per cent, 12.12 per cent and 15.56 per cent respectively.

Solids dispersed

Solids dispersion is another associated characteristic of the phenomenon of cooking and is measured by quantity of solids dispersed by a food when it reaches the cooked state. The amount of solids dispersed by pulses during cooking decreased with increasing storage period. The seeds stored in plain earthen *motka* dispersed minimum amount of solids and the decreases in lentil, chickpea and khesari were 35.21 per cent, 45.20 per cent and 41.14 per cent respectively after 12 months of storage. The decrease was minimum in seeds stored in different improved containers viz. polythene lined jute bag, polythene lined *motka* and improved tin container. Lentil seeds stored in improved tin container dispersed minimum quantity of solids

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and it was 13.28 per cent lower than initial solids dispersed. Chickpea and khesari stored in polythene lined *motka* dispersed minimum amount of solids and these were 19.54 and 19.21 per cent respectively lower than initial amount. The seeds stored in improved containers performed better in dispersing solids during cooking.

Protein content

The protein in pulses decreased during cooking and it was higher in seeds stored in plain earthen *motka*. Lentil, chickpea and khesari of this treatment had their protein decreased by 14.70 per cent, 19.84 per cent and 12.77 per cent respectively after 12 months of storage. All the improved containers performed better in preserving protein in seeds irrespective of types of pulses. Among them polythene lined jute bag showed better performance where the decreased in protein content were 7.23 per cent, 8.84 per cent and 6.51 per cent respectively by lentil, chickpea and khesari after 12 months of storage.

***In vitro* protein digestibility**

The *in vitro* protein digestibility of pulses decreased with increasing storage period. This decrease was much higher in seeds stored in plain earthen *motka* irrespective of types of pulses. In this treatment *in vitro* digestibility of lentil, chickpea and khesari decreased by 24.33 per cent, 21.91 per cent and 22.65 per cent respectively after 12 months of storage. The higher moisture content in the seeds of same treatment might have deteriorated the quality of protein and decreased the protein digestibility. Among the improved containers polythene lined jute bag performed better for lentil and khesari and for chickpea it was the improved tin container. The digestibility decreased by these treatments were 10.31 per cent, 21.42 per cent and 13.15 per cent

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respectively for lentil, chickpea and khesari after 12 months of storage. The results indicated that their might have some chemical changes in seeds protein due to aging of seeds which lead to decreased in protein digestibility but at a slower rate.

Starch content

The amount of starch content also decreased during storage. The starch content of lentil, chickpea and khesari stored in plain earthen *motka* decreased by 23.56 per cent, 26.19 per cent and 16.88 per cent respectively for lentil, chickpea and khesari after 12 months of storage. The improved containers viz. polythene lined jute bag, polythene lined *motka*, *coal-tar* coated *motka* and improved tin containers performed better in protecting the decrease in starch of pulses. Among the container polythene lined jute bag performed better where decreased in starch content was minimum. For lentil, chickpea and khesari this decrease were 18.38 per cent, 16.87 per cent and 18.76 per cent respectively after 12 months of storage.

Total sugar content

The total sugar content increased with increasing storage period. The increase in total sugar was much higher in seeds stored in plain earthen *motka* and it was 113.86 per cent, 143.68 per cent and 108.0 per cent respectively for lentil, chickpea and khesari after 12 months of storage. The moisture content in presence of high temperature might have influenced the break down of starch in to sugar. Lentil and khesari stored in polythene lined jute bag increased minimum amount of total sugar and was 53.86 per cent and 54.47 per cent respectively after 12 months of storage. For chickpea it was the improved tin container where the amount of total

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sugar increased was 55.05 per cent. The other improved containers viz. polythene lined *motka* and coal-tar coated *motka* also performed better in increasing minimum amount of total sugar.

Reducing sugar

The reducing sugar of pulses also increased with increasing storage period. The increase in reducing sugar was highest in seeds stored in plain earthen *motka* and lowest in case of seeds stored in polythene lined jute bag. The increases in reducing sugar in seeds stored in plain earthen *motka* were 285.10 per cent, 388.46 and 348.56 per cent respectively for lentil, chickpea and khesari after 12 months of storage. The seeds stored in polythene lined jute bag increased minimum amount of reducing sugar and this were 188.29 per cent, 200.00 per cent and 190.47 per cent respectively for lentil, chickpea and khesari after 12 months of storage. The increase in reducing sugar was also lower in case other improved containers.

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS)

The RDS and RS of pulses decreased and SDS increased with increasing storage period irrespective of types of pulses. The decrease in RDS and increase in SDS were higher in seeds stored in plain earthen *motka*. The decreases of RDS in lentil, chickpea and khesari for this treatment were 21.7 per cent, 25.00 per cent and 25.95 per cent respectively after 12 months of storage. The same treatment caused an increase of SDS by 36.24 per cent, 36.11 per cent and 38.38 per cent in lentil, chickpea and khesari respectively after 12 months of storage. The decrease in RDS and increase SDS were lower in case of pulses stored in improved containers. The seeds of lentil and chickpea stored in polythene lined jute bag showed their minimum decrease in RDS and it was 15.70 per cent and 18.69 per cent respectively. For khesari it was the

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seeds of polythene lined *motka* where the minimum decrease in RDS was 19.26 per cent. Lentil, chickpea and khesari increased their SDS at a minimum rate when stored in improved tin container, polythene lined jute bag and polythene lined *motka* respectively. The increase in SDS observed in these treatments were 59.24 per cent, 69.81 per cent and 68.00per cent respectively for lentil, chickpea and khesari.

Phytic acid

The phytic acid of pulses decreased with increasing storage period. The decrease was higher in seeds stored in plain earthen *motka* irrespective of pulses. The decrease in phytic acid in this treatment after 12 months of storage was 36.46 per cent, 45.90 per cent and 35.17 per cent respectively for lentil, chickpea and khesari. The decrease was much lower when the seeds were stored in improved container viz. polythene lined jute bag, polythene lined *motka*, improved tin container and *coal-tar* coated *motka*. Lentil and chickpea seeds stored in polythene lined jute bag decreased minimum quantity of phytic acid and it was 14.46 per cent and 13.64 per cent respectively after 12 months of storage. In case of khesari it was 14.01 decreased by seeds stored in polythene lined *motka*. It was observed that the seeds of plain earthen *motka* absorbed more water and deteriorated the quality of seeds, which caused higher decrease in phytic acid.

Protein profile and molecular weight of protein

The number and molecular weight of protein varied from pulse to pulse and also between fresh and stored sample. Molecular weight of some of the proteins decreased with increasing storage period, which might be due to degradation by internal or external protease.

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Conclusion

From above results, it is concluded that plain earthen *motka* is most unsuitable for storage of pulses, whereas this is used traditionally in rural Bangladesh. The study found that *motka* can be used if it is coal-tar coated or polythene lined inside. Under these conditions, the pulses keep quite well in all their physico-chemical and nutritional qualities. The study also found that other containers which could be used for safe storage of pulses seeds are polythene lined jute bag and improved tin container. The farmers and traders may be suggested to use these types of improved containers for safe storage of their pulses.

Suggestions for Future Research

- Processing of pulses for removal of various antinutritional factors.
- Implication of phytic acid contents on nutritional qualities of stored pulses
- Study on the bio-availability of various nutrients during storage
- Milling quality of pulses stored in different containers
- Processing pulses for various food products e.g., 'papad', 'bori', 'basan' etc.
- Occurrence of mycotoxin in store pulses.

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APPENDICES

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Appendix Table 1: F values of Anova for different parameters of lentil

Source of variance	Degrees of Freedom	MP	CT	WA	SD	PC	IVPD	SC	TSC	RSC	PAC	GP
Year (Y)	2	0.45	18.85	13.91	11.15	94.03	0.55	0.25	2.91	0.12	1.66	6.28
Container (C)	5	1487	261.15	37.08	476.67	123.54	491.35	66.04	781.02	209.51	1555	53.80
Y X C	10	0.56	0.71	1.15	1.26	0.16	0.58	0.27	0.20	0.77	0.24	1.00
Storing Duration (D)	3	130.11	343.91	106.79	2764	856.88	976.16	3608	2459	4022	435.70	501.62
Y X D	6	1.24	1.59	1.88	1.01	4.06	1.86	0.35	2.88	3.25	0.50	0.97
C X D	15	9.19	30.88	2.89	68.51	18.04	29.57	5.60	64.63	18.61	28.31	7.32
Y X C X D	30	1.40	0.83	0.47	0.33	0.52	1.28	0.95	1.71	1.57	0.56	1.09

MC = Moisture content, CT = Cooking time, WA = Water absorption, SD = Solids dispersed
PC = Protein content, IVPD = In vitro protein digestibility, SC = Starch content
TSC = Total sugar content, RSC = Reducing sugar content, PAC = Phytic acid content
GP= Germination percentage.

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Appendix Table 2: F values of Anova for different parameters of chickpea

Source of variance	Degrees of Freedom	MP	CT	WA	SD	PC	IVPD	SC	TSC	RSC	PAC	GP
Year (Y)	2	0.06	16.23	4.20	51.29	36.65	7.51	0.09	5.34	7.67	1.45	4.30
Container (C)	5	563.05	366.65	10.36	143.96	757.84	101.87	33.70	649.29	628.81	4226	4062
Y X C	10	3.01	0.71	0.78	4.41	0.19	0.98	0.90	0.67	0.58	0.33	0.94
Storing Duration (D)	3	169.10	719.83	69.14	1896	1234	7766	814.91	1417	10856	1490	320.03
Y X D	6	0.65	1.41	1.66	0.63	16.91	0.96	1.67	2.43	6.65	1.02	0.62
C X D	15	51.95	91.29	2.44	60.25	24.79	11.64	1.28	66.79	133.52	115.90	33.83
Y X C X D	30	1.47	0.88	0.97	0.59	0.92	0.68	1.52	2.50	2.03	0.71	0.30

MC = Moisture content, CT = Cooking time, WA = Water absorption, SD = Solids dispersed
PC = Protein content, IVPD = In vitro protein digestibility, SC = Starch content
TSC = Total sugar content, RSC = Reducing sugar content, PAC = Phytic acid content
GP= Germination percentage.

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Appendix Table 3: F values of Anova for different parameters of khesari

Source of variance	Degrees of Freedom	MP	CT	WA	SD	PC	IVPD	SC	TSC	RSC	PAC	GP
Year (Y)	2	0.11	7.86	5.50	8.56	14.48	1.45	1.56	0.69	23.66	3.43	0.52
Container (C)	5	3022	941.40	146.42	207.19	386.81	133.33	507.92	145.74	331.76	619.47	253.32
Y X C	10	0.90	0.52	0.29	1.34	0.17	0.84	0.06	0.29	1.74	0.77	0.24
Storing Duration (D)	3	256.89	392.93	1883	1412	558.10	2251	9122	1407	10125	387.16	227.05
Y X D	6	1.78	1.52	1.51	1.80	3.64	0.36	19.31	2.75	7.53	0.77	0.71
C X D	15	24.71	24.63	1.73	16.07	4.76	12.83	15.03	23.77	97.75	43.23	16.31
Y X C X D	30	0.86	0.44	0.57	0.42	0.69	0.55	3.59	1.04	2.46	0.74	0.22

MC = Moisture content, CT = Cooking time, WA = Water absorption, SD = Solids dispersed
PC = Protein content, IVPD = In vitro protein digestibility, SC = Starch content
TSC = Total sugar content, RSC = Reducing sugar content, PAC = Phytic acid content
GP= Germination percentage.

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Appendix Table 4. F values of Anova for RDS, SDS and RS of lentil

Source of variance	Degrees of Freedom	RDS	SDS	RS
Year (Y)	2	-	19.16	27.30
Container (C)	5	82.56	72.46	44.08
Y X C	10	1.86	1.73	1.07
Storing Duration (D)	1	1082.0	1547.0	829.0
Y X D	2	3.81	4.12	3.93
C X D	5	3.25	5.45	1.73
Y X C X D	10	2.02	1.98	2.00

Appendix Table 5. F values of Anova for RDS, SDS and RS of Chickpea

Source of variance	Degrees of Freedom	RDS	SDS	RS
Year (Y)	2	24.10	55.51	17.75
Container (C)	5	827.0	87.71	2.53
Y X C	10	1.94	2.16	2.11
Storing Duration (D)	1	1530.0	3083.0	57.73
Y X D	2	3.25	4.12	3.21
C X D	5	48.37	19.50	2.64
Y X C X D	10	1.99	1.98	1.88

Appendix Table 6. F values of Anova for RDS, SDS and RS of khesari

Source of variance	Degrees of Freedom	RDS	SDS	RS
Year (Y)	2	9.80	14.31	4.74
Container (C)	5	284.0	127.03	3.79
Y X C	10	1.78	2.08	0.68
Storing Duration (D)	1	1789.0	-	90.88
Y X D	2	4.86	3.81	2.85
C X D	5	4.68	5.26	4.76
Y X C X D	10	1.88	1.76	1.05

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Appendix Table 7. Summary of physico-chemical parameters of lentil

Parameter		Treatment					
		Plain earthen motka	Plain tin container	Polythene lined jute bag	Polythene lined motka	Coal-tar coated motka	Improved tin container
Moisture content (%)	Initial	8.16	8.16	8.16	8.16	8.16	8.16
	Final	11.98	9.23	8.76	8.73	8.93	8.95
Cooking time (Min.)	Initial	8.08	8.08	8.08	8.08	8.08	8.08
	Final	15.17	11.33	9.83	10.22	10.77	9.83
Water absorption (g/g dhal)	Initial	1.87	1.87	1.87	1.87	1.87	1.87
	Final	1.48	1.57	1.68	1.66	1.58	1.63
Solids dispersed (%)	Initial	77.55	77.55	77.55	77.55	77.55	77.55
	Final	50.24	63.26	66.44	66.44	64.98	67.25
Protein content (%)	Initial	25.84	25.84	25.84	25.84	25.84	25.84
	Final	22.04	23.13	23.97	23.87	23.64	23.90
In vitro protein digestibility (%)	Initial	90.03	90.03	90.03	90.03	90.03	90.03
	Final	68.12	76.44	80.74	79.94	77.43	79.84
Starch content (%)	Initial	52.91	52.91	52.91	52.91	52.91	52.91
	Final	43.98	45.05	46.89	45.88	45.49	45.97
Total sugar (%)	Initial	3.75	3.75	3.75	3.75	3.75	3.75
	Final	8.02	6.80	5.77	5.92	6.04	5.84
Reducing sugar (%)	Initial	0.94	0.94	0.94	0.94	0.94	0.94
	Final	3.62	6.13	2.71	2.78	2.88	2.75
Rapidly digestible starch (%)	Initial	58.10	58.10	58.10	58.10	58.10	58.10
	Final	45.45	47.44	48.98	48.74	47.92	48.83
Slowly digestible starch (%)	Initial	21.10	21.10	21.10	21.10	21.10	21.10
	Final	36.24	34.61	33.74	33.77	33.98	33.60
Phytic acid (mg/100g)	Initial	8.09	8.09	8.09	8.09	8.09	8.09
	Final	3.14	5.53	6.92	6.82	6.49	6.83
Germination percentage (%)	Initial	64.00	64.00	64.00	64.00	64.00	64.00
	Final	75.11	87.11	92.22	90.22	88.22	90.89

Initial : Pre-stored samples

Final : After 12 months storage

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Appendix Table 8. Summary of physico-chemical parameters of chickpea

Parameter		Treatment					
		Plain earthen motka	Plain tin container	Polythene jute bag	Polythene motka	coal-tar coated motka	Improved tin container
Moisture content (%)	Initial	8.21	8.21	8.21	8.21	8.21	8.21
	Final	14.27	9.37	8.77	8.82	9.16	8.79
Cooking time (Min.)	Initial	29.11	29.11	29.11	29.11	29.11	29.11
	Final	44.22	32.94	31.89	32.11	32.61	31.83
Water absorption (g/g dhal)	Initial	1.32	1.32	1.32	1.32	1.32	1.32
	Final	1.04	1.13	1.16	1.15	1.14	1.15
Solids dispersed (%)	Initial	55.77	55.77	55.77	55.77	55.77	55.77
	Final	30.56	43.86	44.66	44.80	43.66	44.75
Protein content (%)	Initial	20.82	20.82	20.82	20.82	20.82	20.82
	Final	16.68	18.31	18.97	18.89	18.72	18.95
In vitro protein digestibility (%)	Initial	85.35	85.35	85.35	85.35	85.35	85.35
	Final	64.09	65.27	66.43	66.61	65.28	67.06
Starch content (%)	Initial	56.25	56.25	56.25	56.25	56.25	56.25
	Final	42.98	45.34	46.74	45.62	45.59	46.68
Total sugar (%)	Initial	3.96	3.96	3.96	3.96	3.96	3.96
	Final	9.65	7.73	6.16	6.19	6.27	6.14
Reducing sugar (%)	Initial	1.04	1.04	1.04	1.04	1.04	1.04
	Final	5.08	3.73	3.12	3.23	3.46	3.14
Rapidly digestible starch (%)	Initial	56.75	56.75	56.75	56.75	56.75	56.75
	Final	42.56	45.90	47.14	46.42	45.90	47.00
Slowly digestible starch (%)	Initial	21.10	21.10	21.10	21.10	21.10	21.10
	Final	36.11	33.13	32.52	33.28	33.76	32.62
Phytic acid (mg/100g)	Initial	8.65	8.65	8.65	8.65	8.65	8.65
	Final	2.83	6.30	7.47	7.38	7.13	7.39
Germination percentage (%)	Initial	94.00	94.00	94.00	94.00	94.00	94.00
	Final	2.22	82.22	88.00	88.67	85.33	88.44

Initial : Pre-stored samples

Final : After 12 months storage

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Appendix Table 9. Summary of physico-chemical parameters of khesari

Parameter		Treatment					
		Plain earthen motka	Plain tin container	Polythene jute bag	Polythene motka	coal-tar coated motka	Improved tin container
Moisture content (%)	Initial	8.15	8.15	8.15	8.15	8.15	8.15
	Final	12.24	9.02	8.67	8.70	8.99	8.77
Cooking time (Min.)	Initial	20.21	20.21	20.21	20.21	20.21	20.21
	Final	29.44	22.78	22.28	22.22	22.61	22.28
Water absorption (g/g dhal)	Initial	1.67	1.67	1.67	1.67	1.67	1.67
	Final	1.28	1.34	1.41	1.38	1.35	1.38
Solids dispersed (%)	Initial	64.48	64.48	64.48	64.48	64.48	64.48
	Final	37.95	51.14	52.05	52.09	49.92	52.01
Protein content (%)	Initial	30.55	30.55	30.55	30.55	30.55	30.55
	Final	26.64	28.04	28.55	28.45	28.32	28.54
In vitro protein digestibility (%)	Initial	88.46	88.46	88.46	88.46	88.46	88.46
	Final	68.42	73.23	76.83	76.80	74.01	75.94
Starch content (%)	Initial	51.80	51.80	51.80	51.80	51.80	51.80
	Final	38.23	41.55	42.08	41.92	41.48	41.98
Total sugar (%)	Initial	3.91	3.91	3.91	3.91	3.91	3.91
	Final	8.17	6.77	6.04	6.17	6.59	6.06
Reducing sugar (%)	Initial	1.05	1.05	1.05	1.05	1.05	1.05
	Final	4.71	3.75	3.05	3.10	3.36	3.10
Rapidly digestible starch (%)	Initial	56.37	56.37	56.37	56.37	56.37	56.37
	Final	41.74	43.50	45.44	45.51	44.16	45.46
Slowly digestible starch (%)	Initial	20.96	20.96	20.96	20.96	20.96	20.96
	Final	38.38	36.98	35.25	35.24	36.29	35.30
Phytic acid (mg/100g)	Initial	7.42	7.42	7.42	7.42	7.42	7.42
	Final	2.90	5.76	6.23	6.38	6.07	6.31
Germination percentage (%)	Initial	93.78	93.78	93.78	93.78	93.78	93.78
	Final	74.22	82.22	88.00	88.44	86.22	88.44

Initial : Pre-stored samples

Final : After 12 months storage

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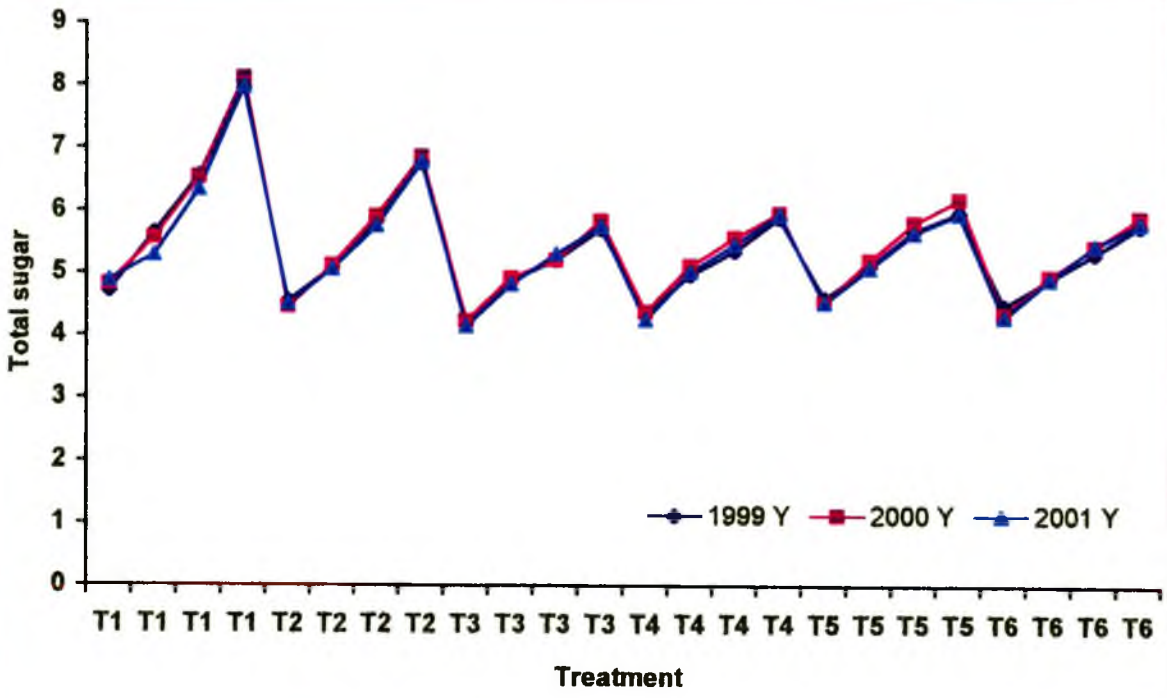
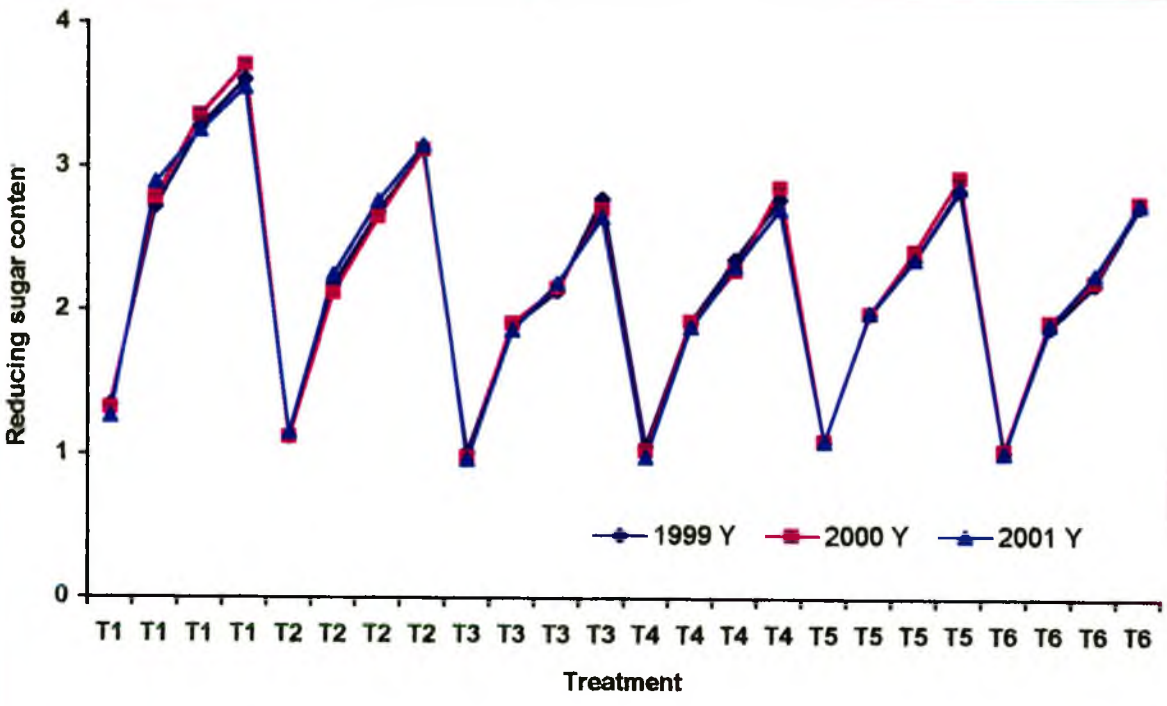


Appendix Fig. 1. Effect of Y X C X D for the total sugar in lentil



Appendix Fig. 2. Effect of Y X C X D for the reducing sugar of lenti

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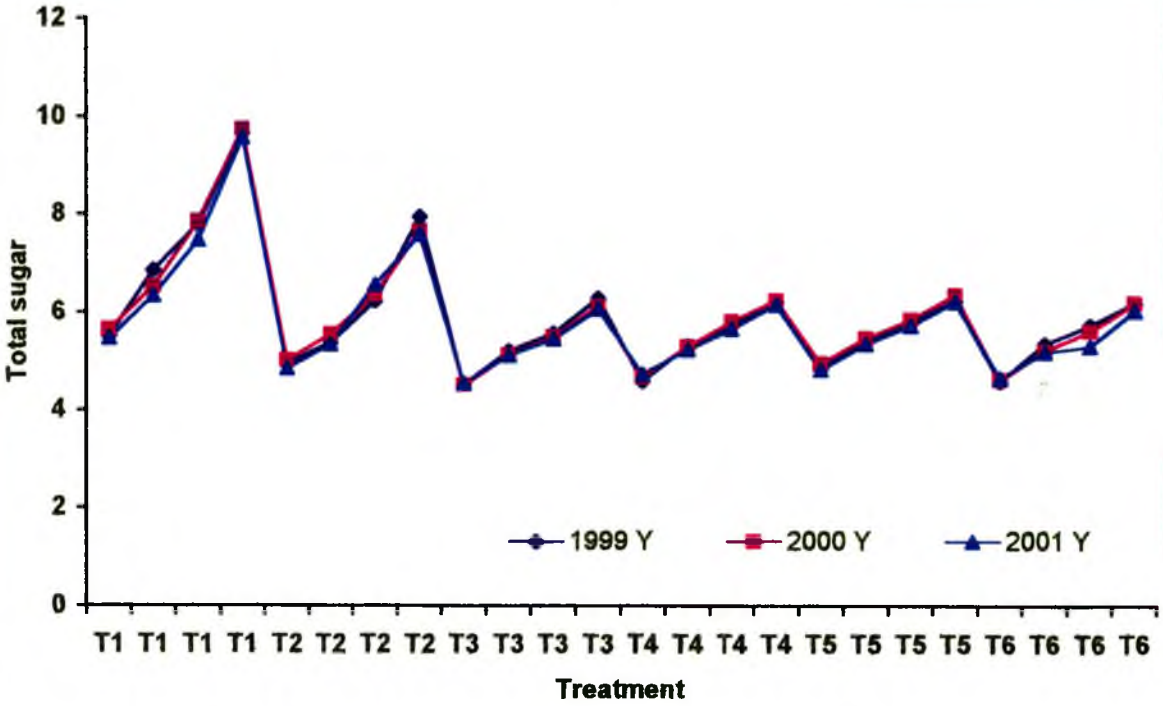
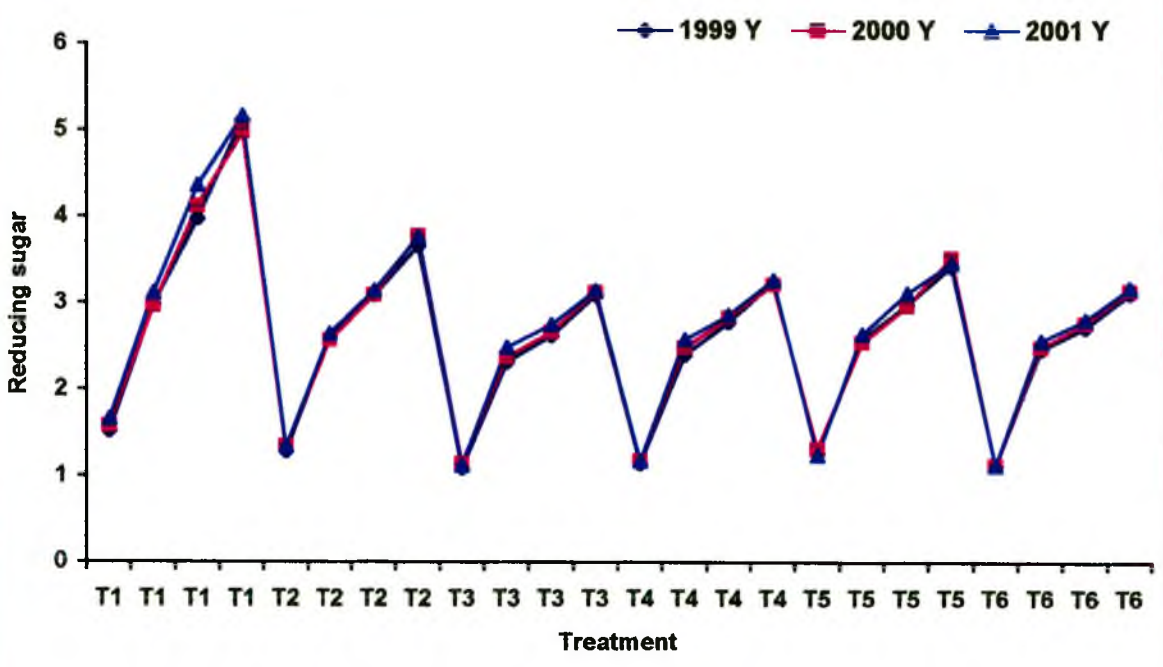


Appendix Fig. 3. Effect of Y X C X D for total sugar of chickpea



Appendix Fig. 4. Effect of Y X C X D for reducing sugar of chickpea

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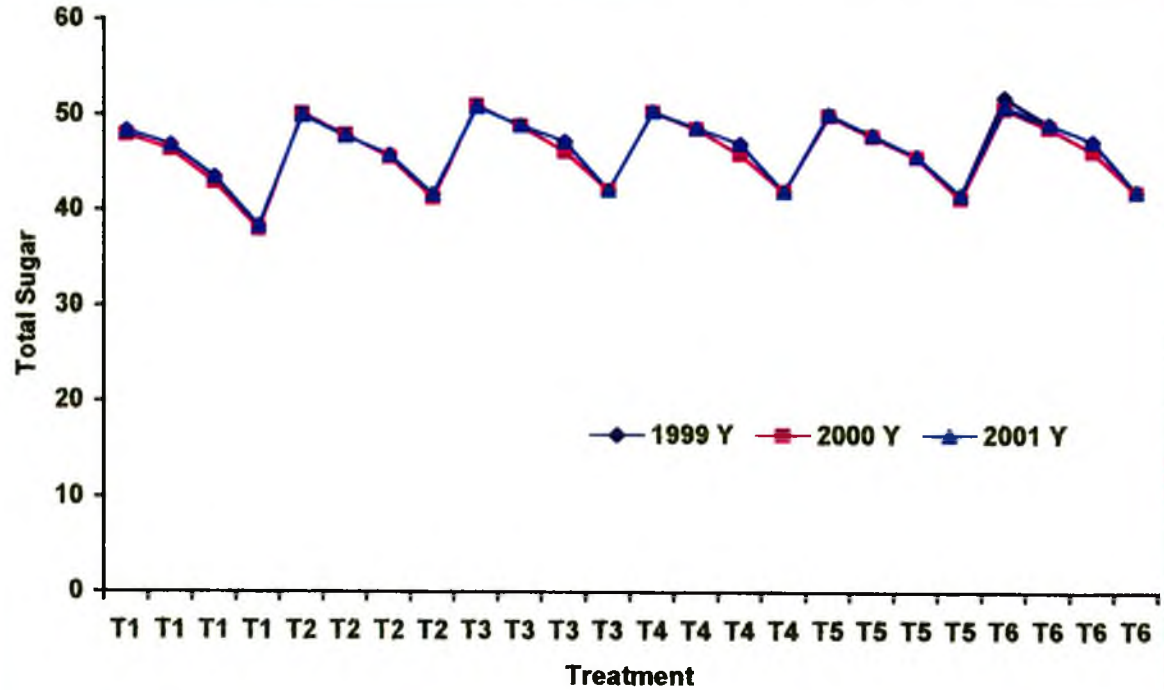
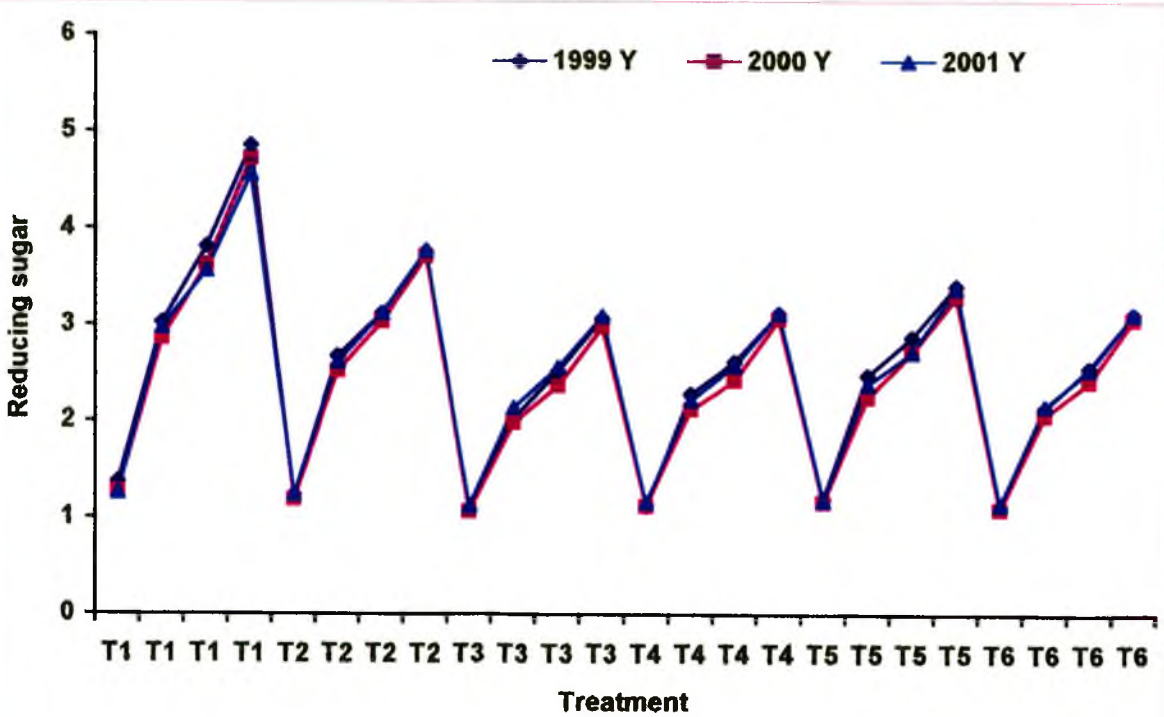


Appendix Fig. 5. Effect of Y X C X D for starch content in khesari



Appendix Fig. 6. Effect of Y X C X D for reducing sugar in khesari

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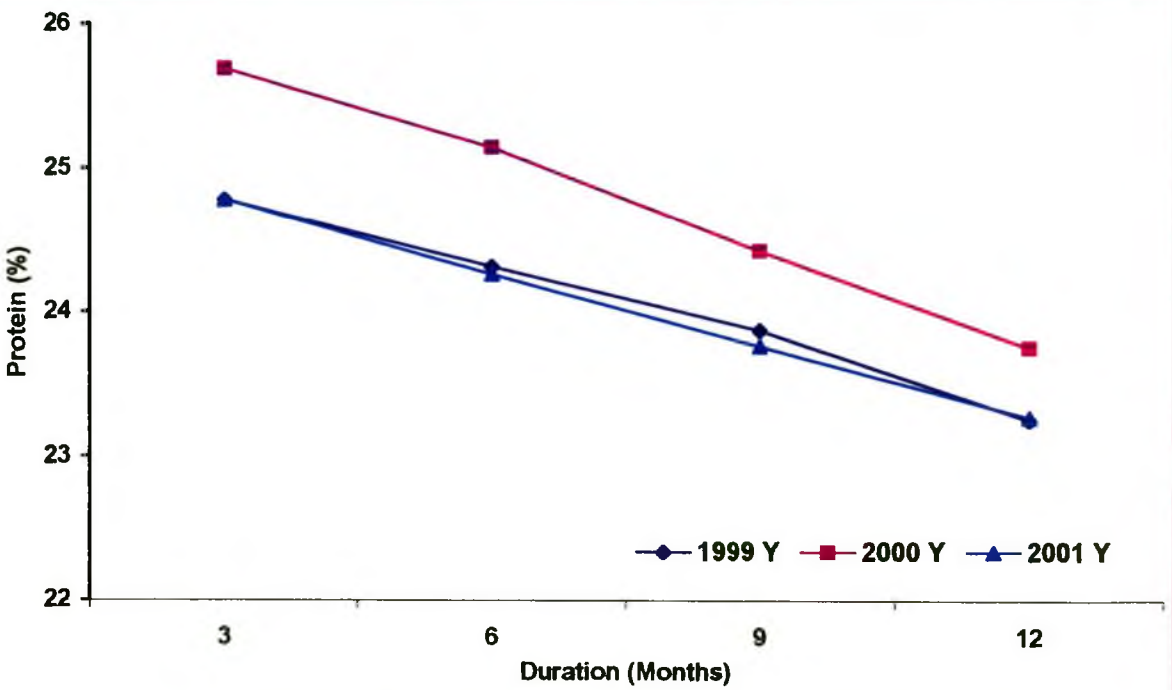
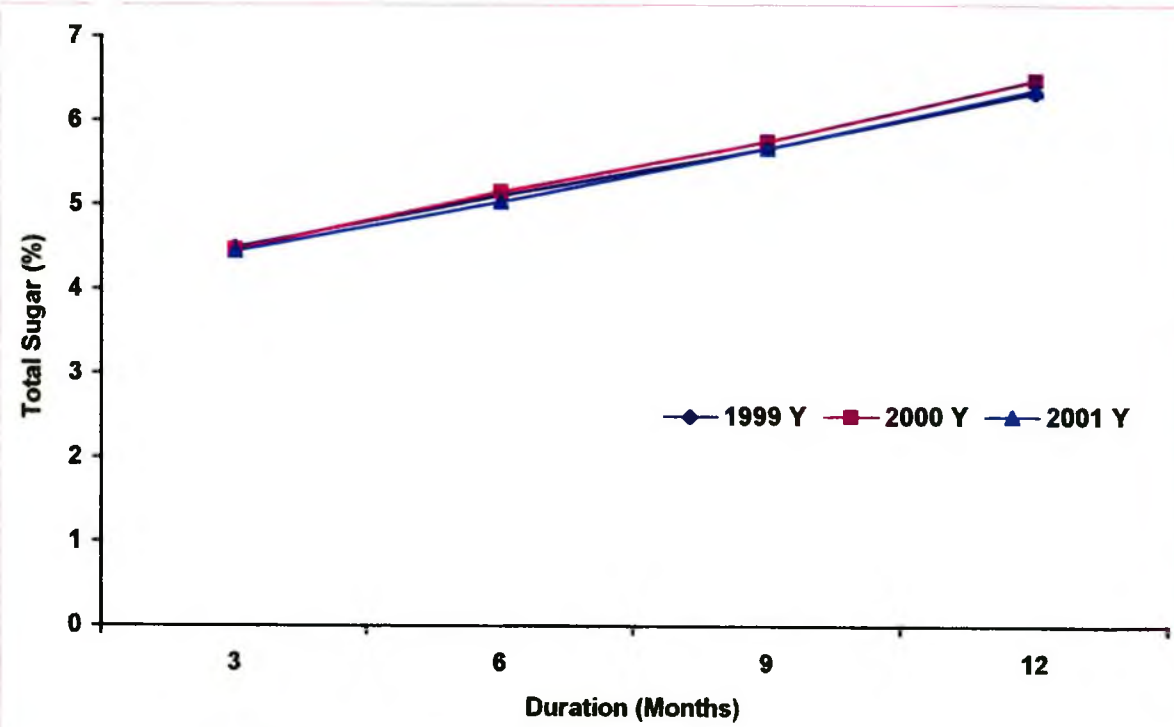


Appendix Fig. 7. Effect of Y X D for protein content of lentil



Appendix Fig. 8. Effect of Y X D for total sugar of lentil

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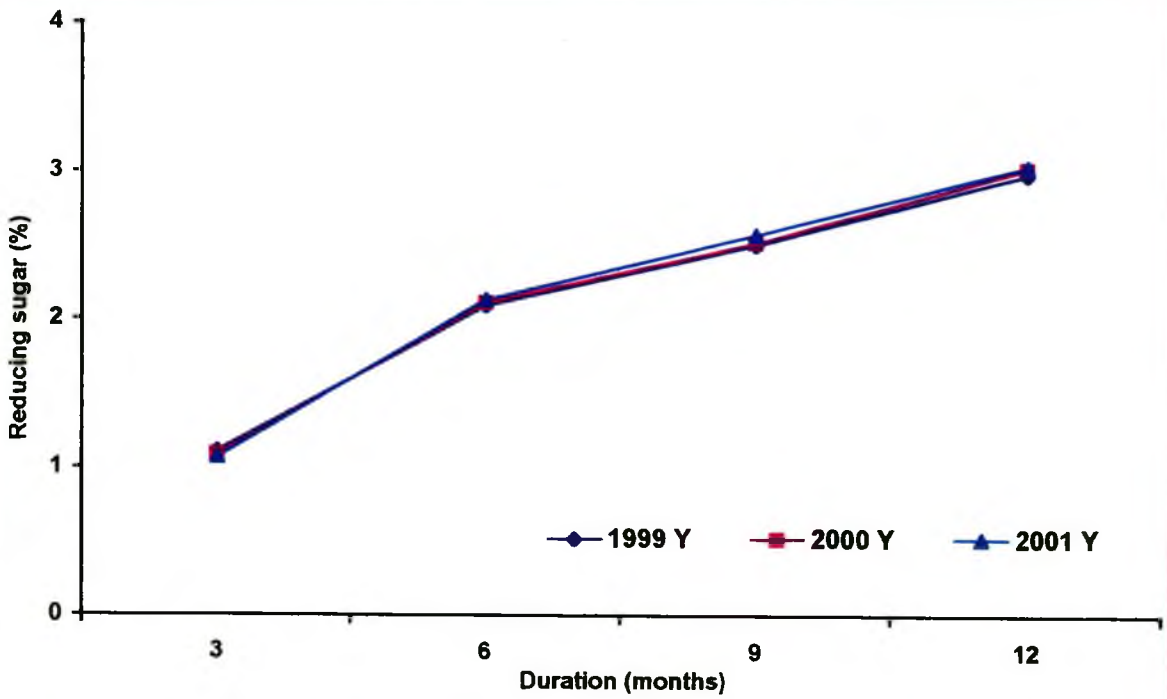
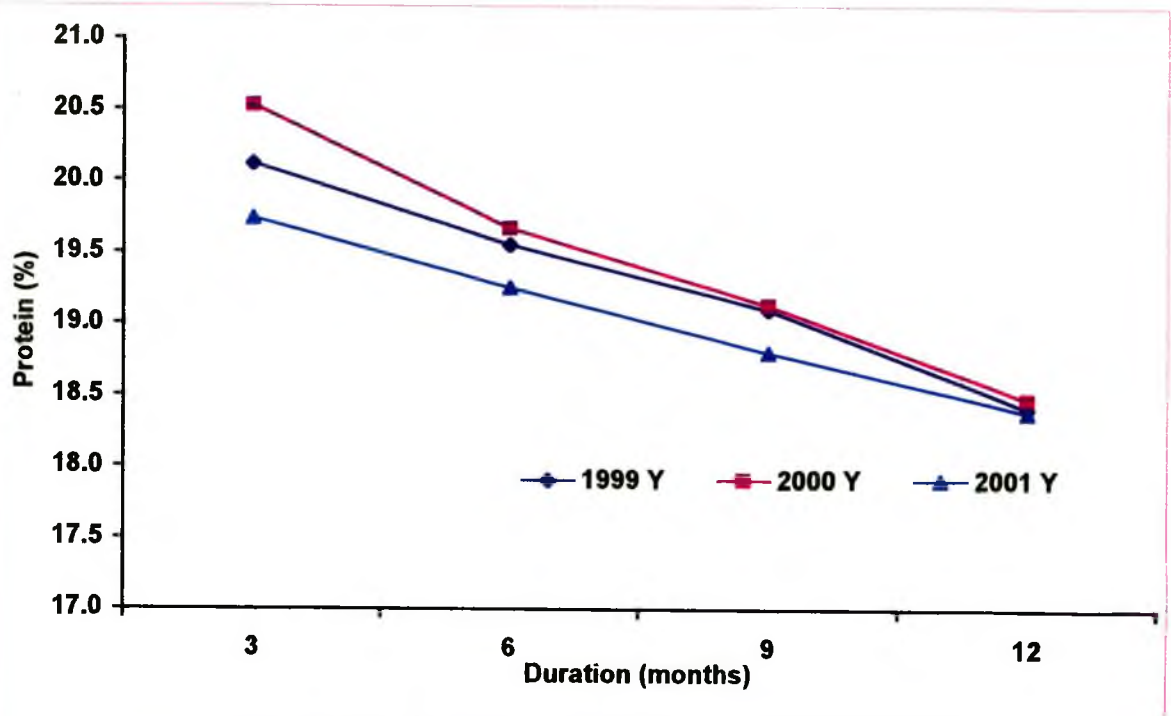


Appendix Fig. 9. Effect of Y X D for reducing sugar of lentil



Appendix Fig. 10. Effect of Y X D for protein of chickpea

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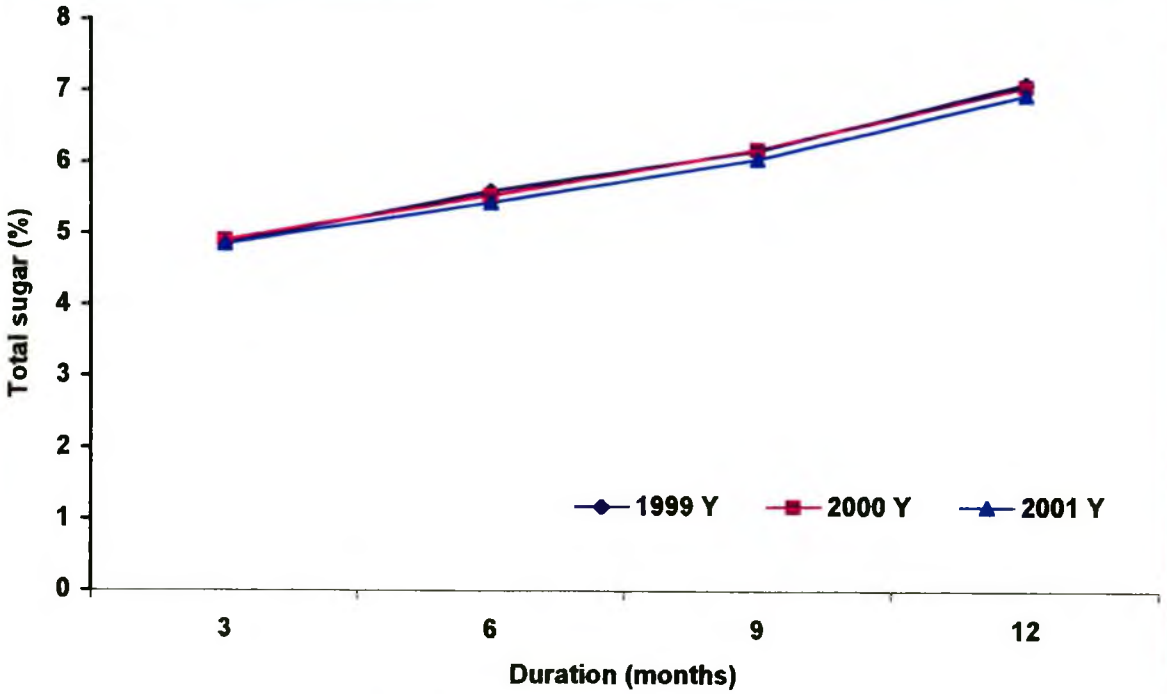
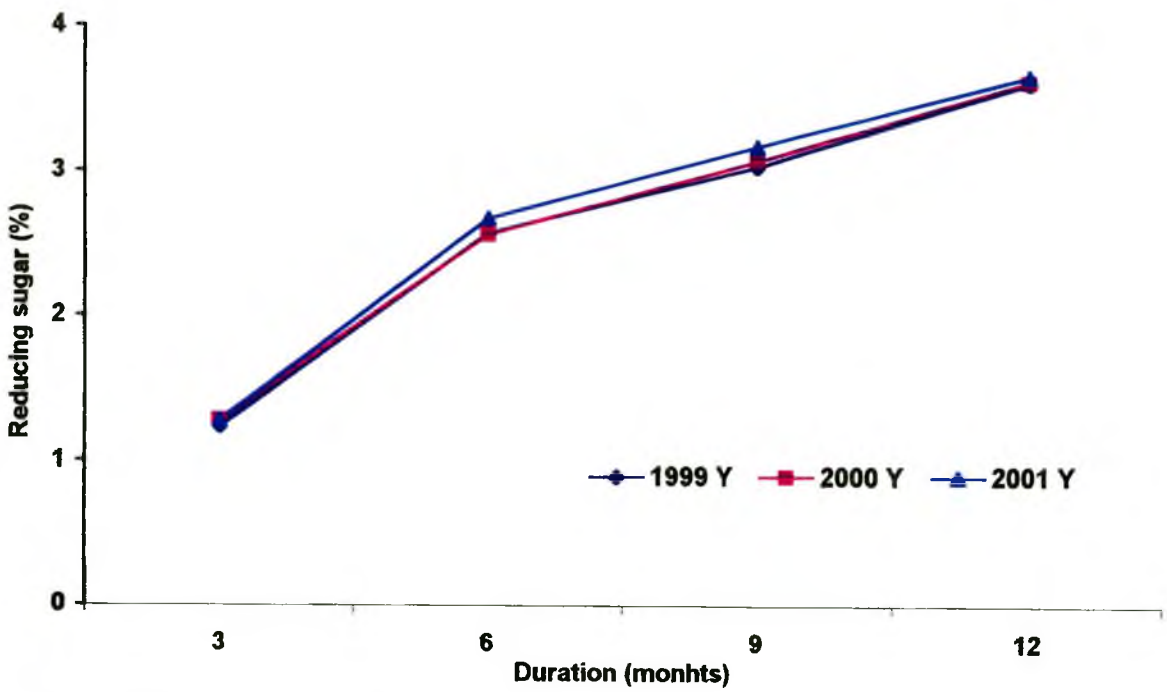


Appendix Fig. 11. Effect of Y X D for total sugar of chickpea



Appendix Fig. 12. Effect of Y X D for reducing sugar of chickpea

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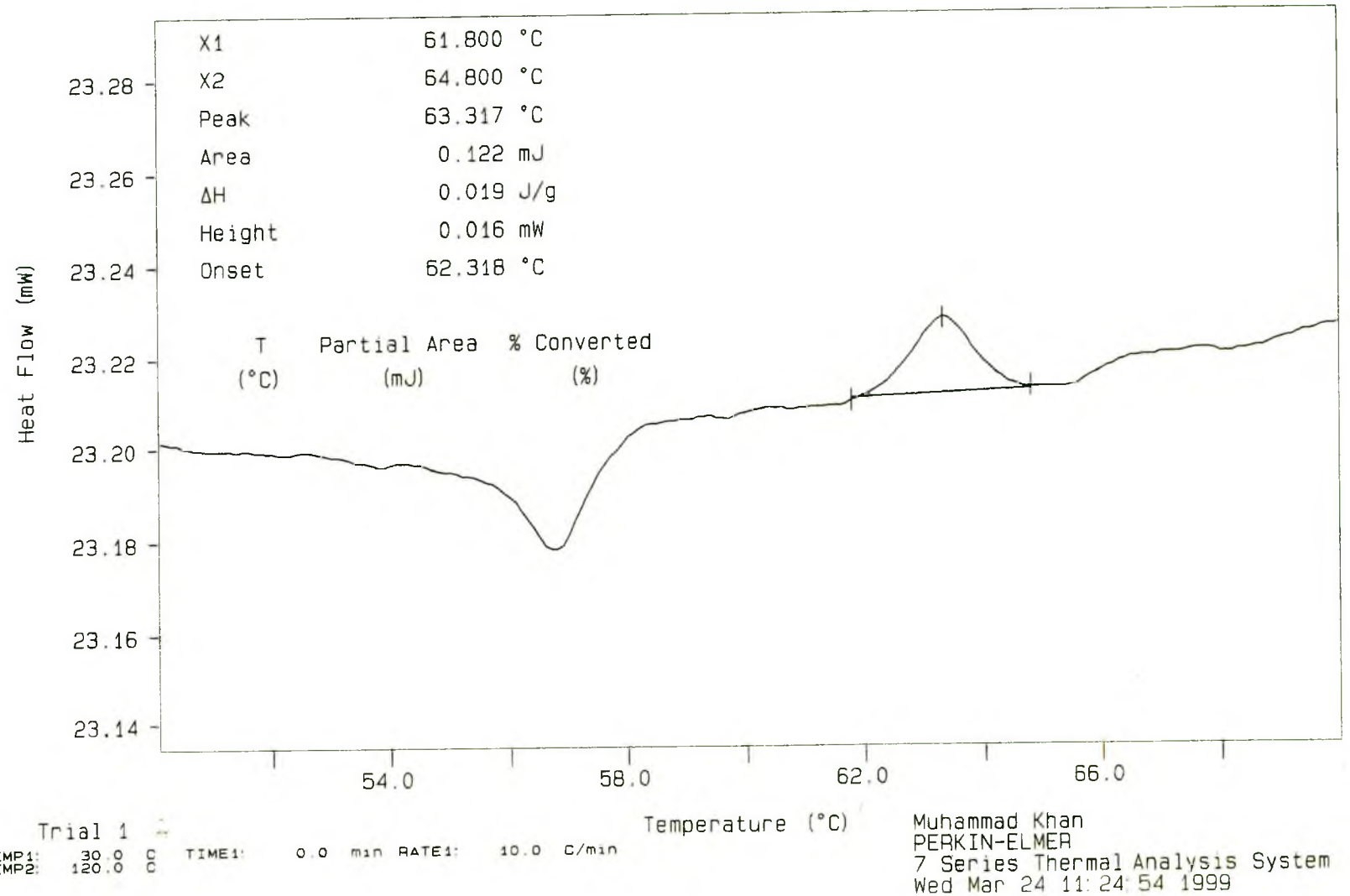
Sample Weight: 6.300 mg
v70-5f1



Trial 1
TEMP1: 30.0 C TIME1: 0.0 min RATE1: 10.0 C/min
TEMP2: 120.0 C

Muhammad Khan
PERKIN-ELMER
7 Series Thermal Analysis System
Wed Mar 24 11:24:54 1999

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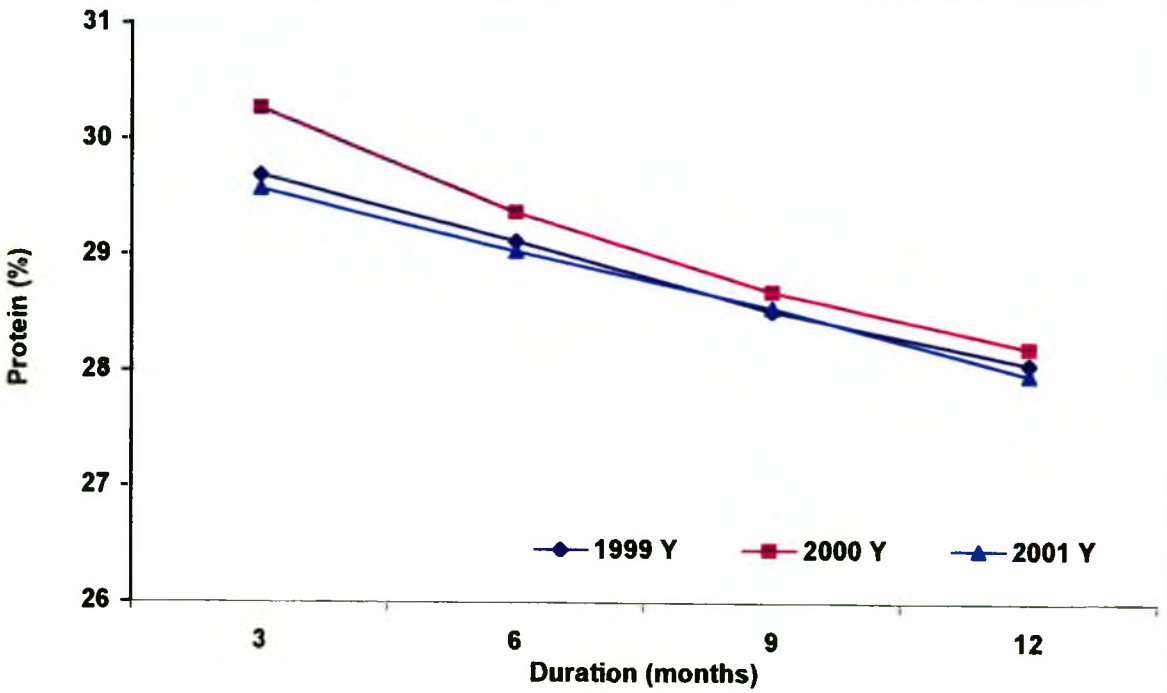
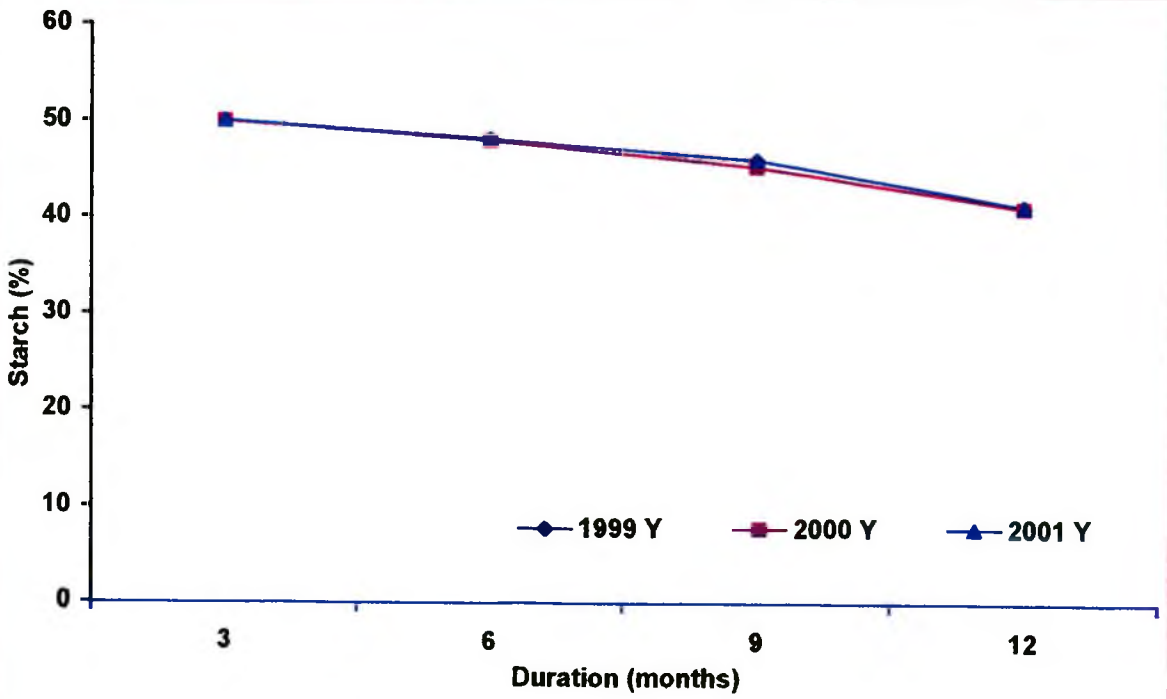


Appendix Fig. 13. Effect of Y X D for protein content in khesari



Appendix Fig. 14. Effect of Y X D for starch content in khesari

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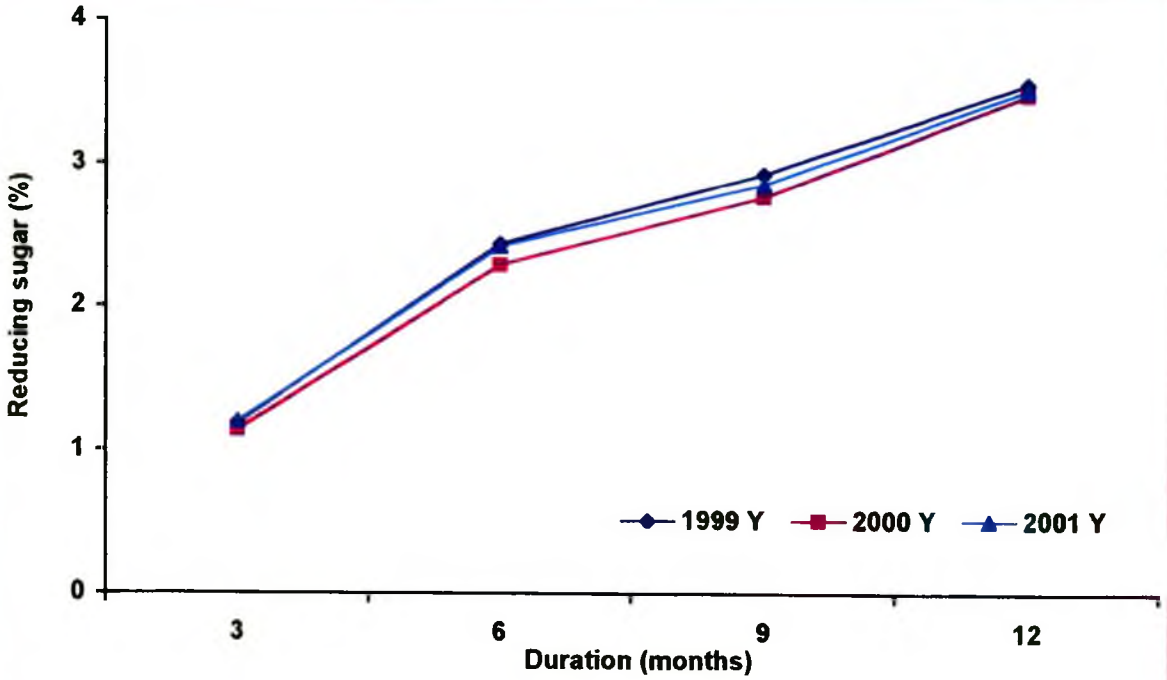
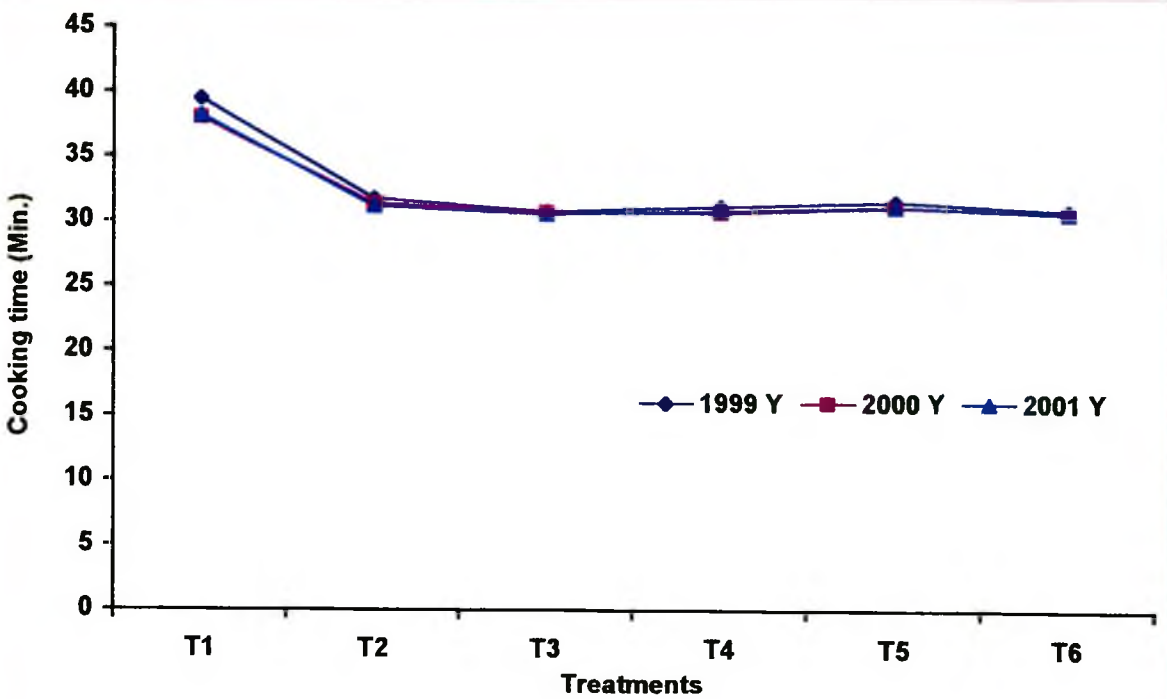


Appendix Fig. 15. Effect of Y X D for reducing sugar in khesari



Appendix Fig. 16. Effect of Y X C for cooking time of chickpea

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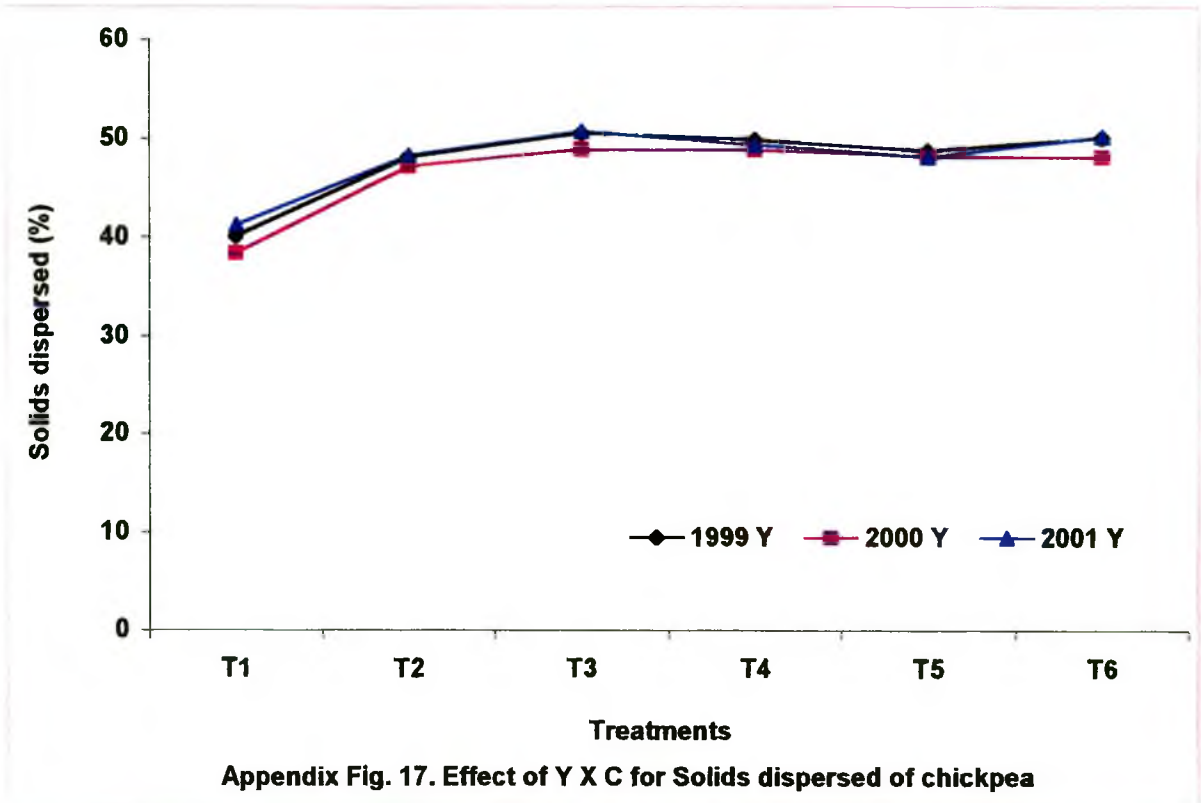

Appendix Fig. 17. Effect of Y X C for Solids dispersed of chickpea

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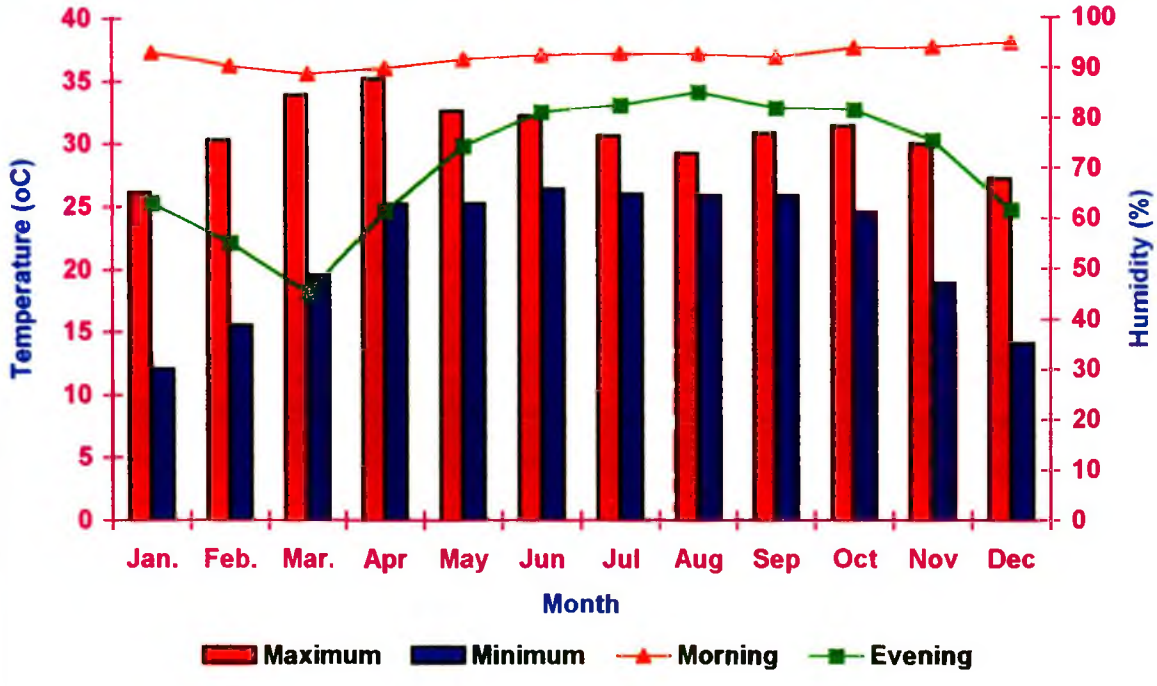
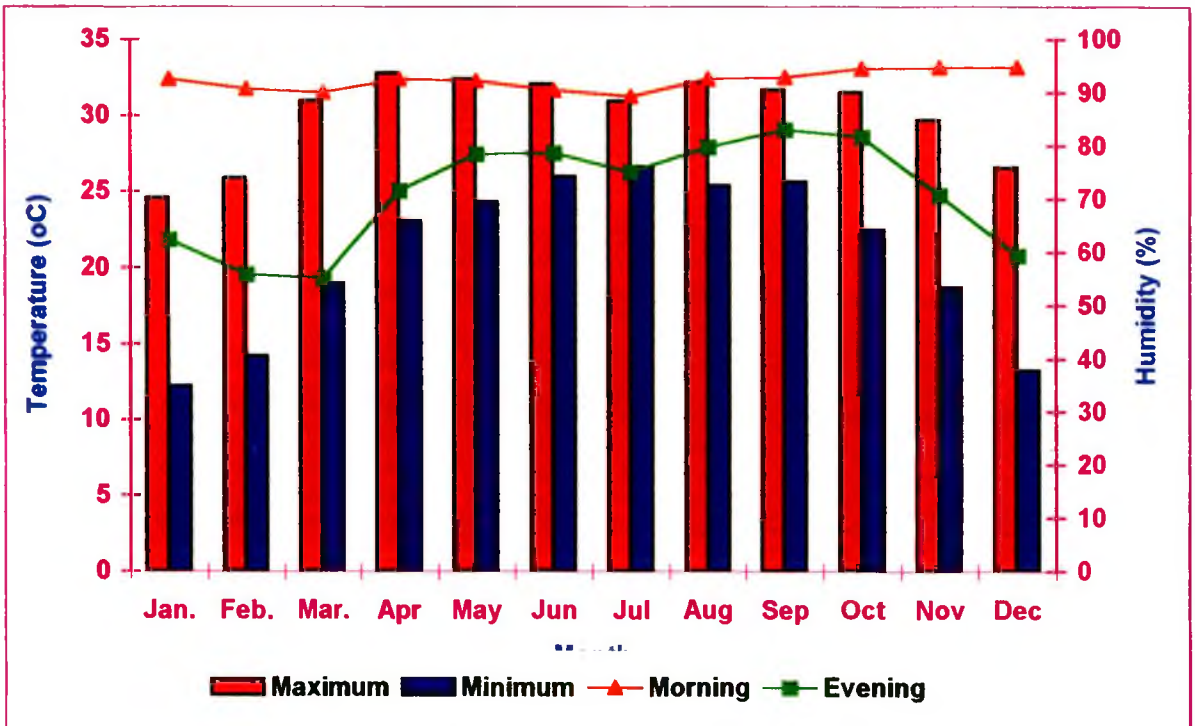


Appendix Fig. 18. Weather data for the year 1999



Appendix Fig.19. Weather data for the year 2000

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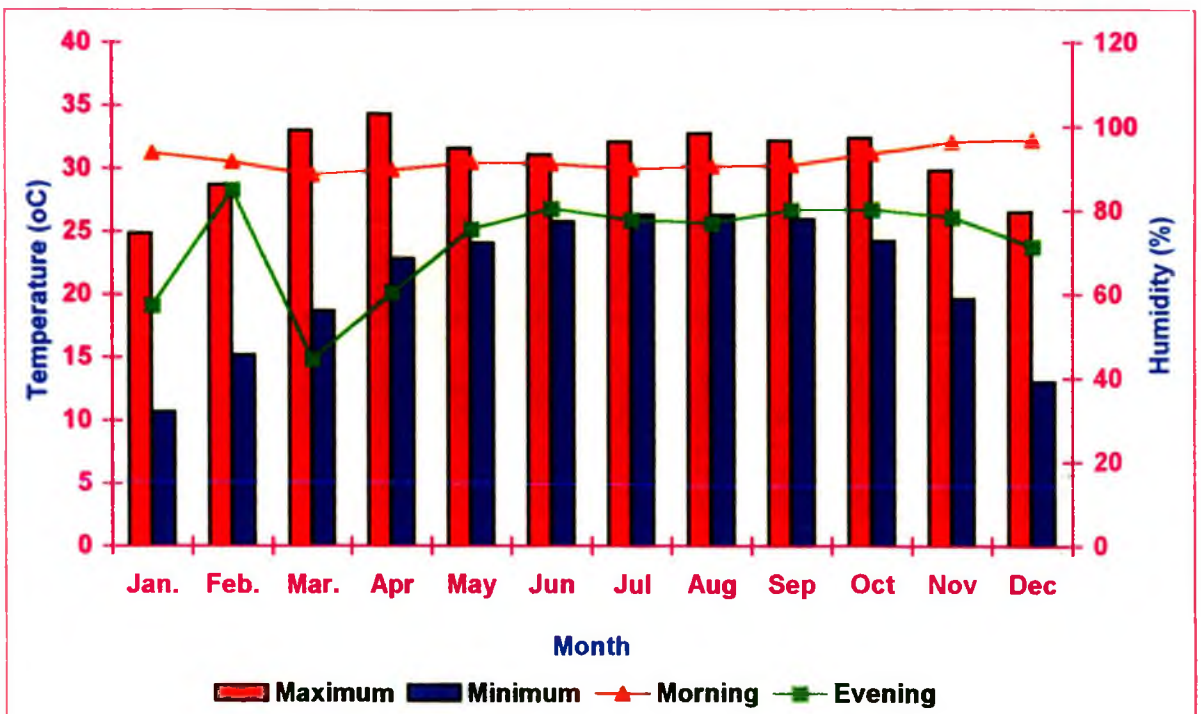

Appendix Fig. 20. Weather data for the year 2001