COMPARATIVE EVALUATION OF NUTRITIVE VALUES OF GERMINATED RICE, BARLEY, CHICKPEA AND JACKFRUIT SEED AND THE EFFECT OF PHYSICO-CHEMICAL AGENTS ON THE STABILITY OF ENZYMES OF CHICKPEA

A THESIS

BY

SHOMA DEVI
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Session: 2011-2012
Semester: January- June, 2012

MASTER OF SCIENCE (MS)
IN
FOOD ENGINEERING AND TECHNOLOGY

DEPARTMENT OF FOOD ENGINEERING AND TECHNOLOGY

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,DINAJPUR

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In partial fulfillment of the requirement for the degree of

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FOOD ENGINEERING AND TECHNOLOGY

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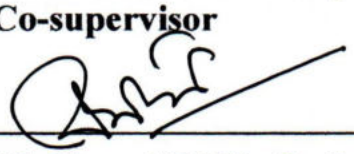

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JUNE, 2012

DEDICATED TO

MY

BELOVED PARENTS

ACKNOWLEDGEMENT

First of all, all praises are due to God, the supreme authority of the universe, who enabled the author to carry out the whole research and to build up this thesis.

The author cannot but expresses her heartfelt gratitude, indebtedness and sincere appreciation to her supervisor Professor, Dr. Mohammad Shiddiqur Rahman, Department of Agricultural and Industrial Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur for his continuous supervision, helpful advice, valuable suggestions, constructive criticism, affectionate and wholesome inspiration and scholastic guidance for the completion of this research work as well as reviewing the manuscript.

The author wishes to express her gratitude to her co-supervisor Dr. Md. Mahbubur Rahman, Senior Scientific Officer of Institute of Food Science and Technology of Bangladesh Council of Scientific and Industrial Research for his sincere guidance, valuable advice and encouragements throughout the period of this research work.

The author feesl proud to express her intense appreciation, gratitude and indebtedness to my respected Teacher N.H.M Rubel Mozmder, Department of Food Science and Nutrition, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for his invaluable suggestions and kind co-suggestions and co-operation during the research period.

The author wishes to render her thanks to Md. Moshiar Rahman and Umme Fatema Shahjadee, Senior Scientific Officer of Institute of Food Science and Technology of Bangladesh Council of Scientific and Industrial Research for their valuable co-operation and help during the Period of research work.

The author is extremely glad to take opportunity to express her heartfelt thanks and gratitude to her beloved teachers Professor Md. Ruhul Amin, Professor Dr. Md. Kamal Uddin Sarkar of Agricultural and Industrial Engineering Department, Associate Professor Dr. Maruf Ahmed, Assistant Professor S. M. Kamrul Hasan, Lecturer Shakti Chandra Mondal and Lecturer Md. Mojaffor Hossain of Food Processing and Preservation Deparment, Associate Professor Md. Sazzat Hossain Sarkar, Assistant

Professor Md. Sultan Mahomud, Lecturer Md. Sarwar Inam Auvie and Lecturer Md. Raihanul Haque of Food Engineering and Technology Department, Assistant Professor Md. Aslam Ali and Lecturer Habiba khatun of Food Science and Nutrition Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their good teaching and co-operation during the study period.

Grateful acknowledgement is extended to the author's friends who ensure her how important the friendship is.

Finally, the author is truly indebted to her parents and her brothers, who provide her real love, encouragement and inspiration which will never be forgotten.

The Author

ABSTRACT

The study was conducted with a view to determine the effect of germination on the changes of protein content, mineral content and enzymatic activity (α-amylase, protease) of rice, barley, chickpea and jackfruit seed as well as to determine the physico-chemical agents on the stability of the enzymes of chickpea. Protein content of rice and chickpea were increased 16.21% and 17.68% respectively at 24 hours then decreased drastically from 48-72 hours of germination. The protein content of barley and jackfruit seed were also increased 31.25% and 23.00% respectively at 48 hours of germination. Mineral content of rice and chickpea were increased at 24 hours of germination and then decreased gradually from 48-72 hours of germination. The mineral content of barley and jackfruit seed were increased at 48 hours of germination and then decreased gradually from 72 hours of germination. The α-amylase and protease activity of rice were increased significantly 51.27% and 155.55% respectively at 24 hours of germination and decreased gradually from 48-72 hours of germination. The α-amylase and protease activity of chickpea were increased tremendously 162.06% and 136.20% respectively at 24 hours of germination and decreased gradually from 48-72 hours of germination. The α-amylase and protease activity of barley were tremendously increased 72.93% and 57.14% respectively at 48 hours of germination and decreased gradually from 72 hours of germination. In jackfruit seed the α-amylase and protease activity remarkably increased 106.10% and 36.61% respectively at 48 hours of germination and decreased gradually from 72 hours of germination. The enzymatic activities of α-amylase and protease from chickpea were investigated after physical and chemical treatments. The enzymes α-amylase and protease have optimum pH 6.8 and 5.0; and optimum temperature 37°C and 38°C respectively. The activities of enzymes were increased in presence of metallic salts such as Ca^{2+}, $Mg^{2+}$and Mn^{2+} while Fe^{2+}, Zn^{2+} and Cu^{2+} inhibited the activities moderately. The activities of all the enzymes were completely inhibited in the presence of higher concentration of EDTA.

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CHAPTER 1

INTRODUCTION

CHAPTER I

INTRODUCTION

To make the food more palatable through germination is an ancient process. It is generally preceded by soaking the seeds in water. It improves the nutritive value of cereals and legumes and has been found to decreases the level of anti-nutrients present in cereal and maximizes the level of utilizable nutrients. Metabolic enzymes are activated and utilization or synthesis of wide range of chemical compounds occurs in seeds and results in the enhancement of nutritional quality during germination. The germinated seeds are rich in vitamins, minerals and are reported to contain important phytochemicals for disease prevention (Fernandez-Orozeo *et al.*, 2006). It is a simple biochemical enrichment tool to enhance the palatability result in increasing the digestibility and nutritive value. In cereal grains, germination increase oligosaccharides and amino acids concentration as observed in barley (Rimsten *et al.*, 2003), wheat (Yang *et al.*, 2001), oat (Mikola *et al.*, 2001) and rice (Manna *et al.*, 1995).

Rice is the seed of the monocot plants *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, it is the most important staple food for a large part of the world's human population, especially in Asia and the West Indies. It is the grain with the second-highest worldwide production, after maize (corn), according to data Awika, 2011. It is in the grass family of *Gramineae* and related to other grass plants such as wheat, oats and barley which produce grains for food (Padiberas Nasional Berhad, 2009). Rice also provides nutritionally significant amounts of thiamin, riboflavin, niacin and zinc with lesser amounts of other micronutrients (Kennedy *et al.*, 2002). There are about 40,000 different varieties of rice worldwide as report by (Brown 2008). White rice contains small amount of lipids (below 5% on dry weight basis) predominated with long chain fatty acids such as linoleic (18:2) and linolenic (18:3) acids. Rice is poor in nitrogenous substances with average composition of these substances being only 8% and fat content or lipids only negligible, i.e. 1% and due to this reason it is considered as a complete food for eating. It is an easily digestible food therefore it has been strongly recommended in preparing specific diets against stomach and intestinal disease processes as well as feeding the infants and old people.

Barley (*Hordium vulgare*) is a wonderful versatile cereal grain with a rich nutlike flavor and an appealing chewy, pasta-like consistency. Its appearance resembles wheat berries, although it is slightly lighter in color. Sprouted barley is naturally high in maltose, a sugar that serves as the basis for both malt syrup sweeteners. Historically, barley has been used for thousands of years. It was the primary staple of the Roman army's diet. They picked up that trick from the Greek gladiators who trained on it and were known as "barley eaters". In ancient Rome, a food made from spouted barley, honey, and colostrums was used to sustain infants whose mothers died in childbirth. Medical research conducted in Canada, the United States, and Australia has shown that barley can play a significant role in lowering blood cholesterol in hypercholesterolemic subjects. Other studies have shown that non-insulin dependent diabetics (Type II) had improved blood glucose levels as a result of including barley in their diet. Barley rates an astoundingly low 27 on the glycemic index. That's 22 percent less than skim milk. In addition, barley has high concentrations of tocotrienols, antioxidant compounds that work to suppress the activity of the first rate-limiting enzyme (HMG-CoA Reductase) in the liver, thus reducing cholesterol synthesis. Barley is one of the highest known sources of beta-glucans, carbohydrates that have remarkable immune boosting properties and have been shown to improve blood glucose and lipid levels among diabetics in clinical trials.

Chickpea (*Cicer arietinum* L.) is an ancient crop that has been grown in Bangladesh, India, the Middle East and parts of Africa and many other countries for many years. It is one of the most important pulses of Bangladesh. All the people of Bangladesh favor it. Chickpea is a plant in the family of *Fabaceae* (or *Leguminosae*), or a fruit of these specific plants, characterized by edible seeds, borne in pods that often open along two seams, by pea-shaped flowers, and by compound stipulate leaves (Mazur *et al.*, 1998). It is one of the important sources of protein, carbohydrate, dietary fibre and minerals (Tharanathan and Mahadevamma, 2003). This annual winter legume is a very good source of protein and carbohydrates, which together constitute about 80% of total dry food weight. The crude protein content of chickpea varies from 17% to 24% (FAO, FAOSTAT, 2003). Chickpea also provide a considerable fat to the human diet, which is in the range of 3.8% to 10%. In addition being an important source of protein and calories, chickpea is also rich in minerals and vitamins. Calcium and Iron are essential elements of the human diets but are usually deficient in diets of low-income people.

Chickpeas were probably introduced into the Indian subcontinent around 2000 B.C. (Ramanujam, 1970). Allchin (1969) feels that chickpeas are a more recent introduction into India. Presently, the most important chickpea producing countries are India (64%), Turkey (8%), Pakistan (7%), Iran (3%), Mexico (3%), Myanmar (3%), Ethiopia (2%), Australia (2%), and Canada (1%). In Bangladesh the crop is grown on well drained, sandy loam alluvial to clay loam soils. The young leaves are used as a vegetable. The green seeds from young pods are eaten raw or cooked, and contain a high amount of ascorbic acid (Vitamin C). The mature seeds are cooked as whole seed or after making "dal". The seeds can also be eaten after soaking in water overnight.

The jackfruit, *Artocarpus heterophyllus* Lam., of the family Moraceae, is also called jak-fruit, jak, jaca, and, in Malaysia and the Philippines, *nangka*; in Thailand, *khanun*; in Cambodia, *khnor*; in Laos, *makmi* or *may mi*; in Vietnam, *mit*. It is an excellent example of a food prized in some areas of the world and allowed to go to waste in others. Seeds are recalcitrant, i.e., they do not retain viability when dried or stored for extended periods. They should be planted immediately for best germination and seedling vigor. Seeds can be stored moist in a plastic container in the refrigerator for up to a few weeks. Stored seeds germinate more slowly than fresh seeds. No seed pretreatment is required. Hot water treatment has been used successfully to stimulate germination. The seeds, which appeal to all tastes, may be boiled or roasted and eaten, or boiled and preserved in sirup like chestnuts. They have also been successfully canned in brine, in curry, and, like baked beans, in tomato sauce. They are often included in curried dishes. Roasted, dried seeds are ground to make a flour which is blended with wheat flour for baking. In general, fresh seeds are considered to be high in starch, low in calcium and iron; good sources of vitamins B_1 and B_2.

Enzymes are organic substances produced by living cells that possess the ability to catalyze specific chemical reaction. Enzymes synthesize or break down chemical compounds, or transform them from one type to another type, according to the ability built in them individually. Most enzymes are highly specific they catalyze only one specific reaction or act upon only one isomer of a particular compound. Some enzymes are less specific and are able to catalyze several, usually related reactions. Also, the same reaction may be catalyzed by a large number of enzymes, different in their specific

characteristics and produced by different types of cells. All enzymes are proteins, metalloprotein, or conjugated proteins. The activity of pure proteinous enzymes is attributed to the reactive groups of the amino acids they contain. Enzymes have many uses in addition to their natural functions in the body. Manufacturers use enzymes in making a wide variety of products. For example, some detergents contain enzymes that break down protein matter, such as perspiration that causes stains. Enzymes are also used in the manufacture of antibiotics, beer, bread, cheese, coffee, meat tenderizers, vinegar, vitamins, and many other products.

Regarding the importance and points mentioned above, the objectives of present study are as follows:

1. To determine the proximate composition and mineral content of Rice, Barley, Chickpea and Jackfruit seed.

2. To determine the effect of germination in the protein content, mineral content and enzymatic activity of Rice, Barley, Chickpea and Jackfruit seed.

3. To study some of the physico-chemical properties such as optimum pH, optimum temperature, the stability of enzymes of chickpea towards denaturating agents such as Calcium, EDTA and various metals.

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

A conceptual framework for the study based on the ideas and concepts gathered from review work of existing literature of both theoretical and empirical nature will facilitate planning the study in a comprehensive manner. It also helps to know the previous research work carried out in the area and acts as a torch for new research.

The preparation of many indigenous or traditional germinated foods and beverages remains today as a house art. They are produced in homes, villages and small-scale industries. On the contrary, the preparation of others, such as soy sauce, has evolved to a biotechnological state and is carried out on a large commercial scale. In the distant past, there was no verified data on the economic, nutritional, technical and quality control implications of the indigenous germinated food. However, in the last 20 years, the numbers of books and articles dealing with indigenous germinated beverages and foods found around the whole world have rapidly increased. Hence, an attempt is made here to put together some of the closely related findings about the germination, cereal, legume, jackfruit seed and enzymes has been reviewed in this chapter.

2.1 Germination:

Germination is a natural process occurred during growth period of seeds in which they meet the minimum condition for growth and development. Literatures related to the germination of seed are presented in this section.

Germination is one of the oldest and most economical methods of producing and preserving food (Billings, 1998; Chavan and Kadam, 1989). In addition, germination provides a natural way to reduce the volume of the material to be transported, to destroy undesirable components, to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (Simango, 1997).

Germination has been suggested as an inexpensive and effective technology for improving the quality of legumes by enhancing their nutritional value, and germinated soybean constitutes an important proportion of the total consumption of food legumes in China, India, Burma and Indonesia (Circle and Smith, 1975).

Legumes are high-protein crops that have contributed to the human diet for centuries. They also provide a large amount of available carbohydrates, dietary fibre, watersoluble vitamins and minerals. However, pulses contain antinutritional factors such as trypsin inhibitors, α-galactosides and phytic acid, which can negatively affect their nutritional value, and that is the reason why legume seeds have to be processed prior to their consumption (Augustin and Klein, 1989).

Germination was reported to be associated with increase of vitamin concentrations and bioavailability of trace elements and minerals (El-Adawy *et al.*, 2004). Kaushik *et al.* (2010) found that germination improves calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content.

During the germination of seeds, a massive breakdown of the reserve substances begin with the help of amylolitic, proteolytic and lipolytic enzymes and the products are transported to the growing seedlings for their development. The remaining small amount of protein represent enzymes concerned in metabolic processes during seed development and germination (Millered and Thomson, 1975).

In Japan, six men and five women with impaired fasting glucose (pre-diabetes) or type 2 diabetes were randomly assigned to eat either white rice or sprouted brown rice three times a day. After a two-week washout, subjects switched groups. Researchers reported that "blood concentrations of fasting blood glucose, fructosamine, serum total cholesterol and traicylglycerol were favorably improved on the sprouted brown rice diet but not on the white rice diet" suggesting that diets including sprouted brown rice may help control blood sugar , (Yokoyama *et al.*, 2008).

These germinating activities have been utilized in the production of germinated foods and beverages, which are defined as those products that have been subordinated to the effect of microorganisms or enzymes to cause desirable biochemical changes. The microorganisms responsible for the fermentation may be the microflora indigenously present on the substrate, or they may be added as starter cultures (Harlander, 1992).

Rusydi *et al.* (2011) were evaluated the proximate content and fatty acid composition of germinated and non-germinated legumes (kidney, mung, soy bean and peanut) and rice varieties (red, black, Barrio, brown and milled). In germinated samples, moisture content increased significantly while carbohydrate, protein and fat were decreased significantly.

Total dietary fibre was increased in germinated samples except germinated kidney and mung bean. Germination also increased saturated fatty acids (SFA) in legumes, black, red and brown rice. Monounsaturated fatty acids (MUFA) decreased in all samples except germinated kidney, soy and Barrio rice. Polyunsaturated fatty acids (PUFA) increased in some germinated samples (mung bean, peanut, red, brown, Barrio and white rice) but decreased in other legume and rice samples. Generally, palmitic acid increased while stearic, oleic and linoleic acids decreased after germination. Overall, the proximate content and fatty acids of legume and rice varieties changed after germination and may be used as alternate resources for individuals with lifestyle diseases.

2.2 Cereal:

Cereal grains are major source of food for humans and for animals. In terms of total pounds produced, Maize is the leading cereal grain in the world. Cereal grains have been a primary source of nourishment for humans for thousands of years. From domestication of rice about 10,000 years ago in Yangtze Valley, China (Molina *et al.*,2011), to domestication of maize (corn) in Southern Mexico/Central America and wheat in the Fertile Crescent of the Near East around the same time (Gustafson *et al.*,2009) cereal grains have contributed immensely to transforming human civilization. Literatures related to the cereal grains are presented in this section.

Today, cereal grains are the single most important source of calories to a majority of the world population. Developing countries depend more on cereal grains for their nutritional needs than the developed world. Close to 60% of calories in developing countries are derived directly from cereals, with values exceeding 80% in the poorest countries. By comparison, approximately 30% of calories in the developed world are derived directly from cereals (Anon, 2003).

The three most important food crops in the world are rice, wheat, and maize (corn). The three cereal grains directly contribute more than half of all calories consumed by human beings. In addition, other minor grains like sorghum and millet are particularly major contributors of overall calorie intake in certain regions of the world, particularly semi-arid parts of Africa and India. For example, sorghum and millet contribute up to 85% of daily caloric intake in Burkina Faso and Niger (FAO, FAOSTAT.2011).

Rice is the single most important source of calories for humans. Among cereal, rice is grown mainly for direct human consumption with very little making it to other uses. Rice contributes approximately 21% of world per capita caloric intake, and 27% of per capita calories in the developing countries. In highest consumption countries, Vietnam, Cambodia and Myanmar, up to 80% of caloric intake is derived from rice. Of the 440 million metric tons (MMT) of polished rice produced in the world in 2010, 85% went into direct human food supply. By contrast, 70% of wheat and only 15% of maize production was directly consumed by humans. Rice production (and consumption) is highly localized; Asia produces the vast majority 92% of world rice, with China and India accounting for 50% of world rice production in 2010.

Rice has been considered the best staple food among all cereals and is the staple food for over 3 billion people, constituting over half of the world's population (Cantral and Reeves, 2002).

In cereal grains, germination increase oligosaccharides and amino acids concentration as observed in barley (Rimsten *et al.*, 2003), wheat (Yang *et al.*, 2001), oat (Mikola *et al.*, 2001) and rice (Manna *et al.*, 1995). In addition, MUFA was increased in germinated kidney, soy bean and Barrio rice, similar to the findings of Hahm *et al.* (2008).

Barley, the other major grain produced, is more tolerant of cold climates, and is thus mostly produced in northern Europe and northern parts of the U.S.A. and Canada. Sprouted barley flour is very digestible with lots of vitamins, minerals, and enzymes produced during the sprouting process. Archaeological evidence indicates that barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) were domesticated 10,000 years ago in the Fertile Crescent (Zohary and Hopf, 2001).

In an experiment at the University of Alberta, barley kernels were sprouted from 2 to 5 days, then oven-dried and milled. Researchers found decreases in dry matter, gross energy (calories) and triglycerides, and increases in fiber and diglyceride content. After the sprouted barley was fed to rats, scientists said that "digestibility data showed an enhancement of digestibility of nutrients in barley... implying that sprouting improved nutritional qualify of barley" (Chung, 1989).

An early maturation and a high level of adaptability to stressful conditions (including cold, drought, alkali, and saline soils) make it well suited for cultivation throughout the

world from boreal to equatorial regions. About two-thirds of the global barley crop is used for animal feed, while the remaining third underpins the malting, brewing, and distilling industries. Although the human diet is not a primary use, barley offers a wealth of potential health benefits (Baik and Ullrich, 2008) and is still the major calorie source in several parts of the world (Grando and Macpherson, 2005).

A number of methods have been employed with the aim of ameliorate the nutritional qualities of cereals. These include genetic improvement and amino acid supplementation with protein concentrates or other protein-rich sources such as grain legumes or defatted oil seed meals of cereals. Additionally, several processing technologies which include cooking, sprouting, milling and fermentation, have been put into practise to improve the nutritional properties of cereals, although probably the best one is germination (Mattila-Sandholm, 1998).

A germinated barley foodstuff (GBF) contained glutamine-rich protein and the hemicellulose-rich fiber. The fiber fraction modulates stool water content by its high water-holding capacity. The protein fraction which contains larger glutamine prevents experimental small bowel injury. Based on these observations, clinical studies were initiated in patients with mild to moderate active ulcerative colitis. The patients who had been unresponsive to or intolerant of standard treatment received 30 grams of GBF feeding daily in a nonrandomized, open-label fashion. At 4 weeks, this treatment resulted in a significant clinical and endoscopic improvement independent of disease extent. The improvement was associated with an increase in stool butyrate concentrations and in luminal Bifidobacterium and Eubacterium levels. After the end of GBF treatment the patients had an exacerbation of the disease. GBF was safe and well tolerated. These results indicate that GBF feeding is a potentially attractive treatment in patients with ulcerative colitis (Kanauchi, 2001)

Cereal grains are considered to be one of the most important sources of dietary proteins, carbohydrates, vitamins, minerals and fibre for people all over the world. However, the nutritional quality of cereals and the sensorial properties of their products are sometimes inferior or poor in comparison with milk and milk products. The reasons behind this are the lower protein content, the deficiency of certain essential amino acids (lysine), the low starch availability, the presence of determined antinutrients (phytic acid, tannins and polyphenols) and the coarse nature of the grains (Chavan & Kadam, 1989).

Germination also leads to a general improvement in the shelf life, texture, taste and aroma of the final product. During cereal germinations several volatile compounds are formed, which contribute to a complex blend of flavours in the products (Chavan & Kadam, 1989)

In general, natural germination of cereals leads to a decrease in the level of carbohydrates as well as some non-digestible poly and oligosaccharides. Certain amino acids may be synthesised and the availability of B group vitamins may be improved. Germination also provides optimum pH conditions for enzymatic degradation of phytate which is present in cereals in the form of complexes with polivalent cations such as iron, zinc, calcium, magnesium and proteins. Such a reduction in phytate may increase the amount of soluble iron, zinc and calcium several folds ([Chavan & Kadam, 1989], [Gillooly *et al.*, 1984], [Haard *et al.*, 1999], [Khetarpaul & Chauhan, 1990], [Nout & Motarjemi, 1997] and [Stewart & Getachew, 1962]).

The effect of germination on the protein and amino acids levels is a topic of controversy. For example, during the germination of corn meal the concentrations of available lysine, methionine, and tryptophan increase (Nanson & Field, 1984). In the same way, germination significantly improves the protein quality as well as the level of lysine in maize, millet, sorghum, and other cereals (Hamad & Fields, 1979). It appears that the effect of germination on the nutritive value of foods is variable, although the evidence for improvements is substantial.

2.3 Legume

Food legumes play an important and diverse role in the farming systems and in the diets of poor people around the world. They are ideal crops for simultaneously achieving three developmental goals in targeted population—reducing poverty, improving human health and nutrition, and enhancing ecosystem resilience. Food legumes also serve as a feed crop in many farming systems and fetch higher prices compared to cereals and are increasingly grown to supplement farmers' incomes. Literatures related to the legumes are presented in this section.

The Food and Agricultural Organization (FAO) yearbook (1991) has reported that for the world as a whole, nearly 9.6 million hectares are shown with chickpea. The world Chickpea production is 7.7 million metric tons.

Chickpeas were probably introduced into the Indian subcontinent around 2000 B.C. (Ramanujam. 1970),(Allchin, 1969).Chickpea seed has 38 – 59% carbohydrate, 3% fibre, 4.8% -5.5% oil, 3% ash, 0.2% calcium and 0.3% phosphorus. Digestibility of protein varies from 76- 78% and its carbohydrate 57-60% (Hulse, 1991).

Raw whole seeds contain per 100gm: 357 calories, 4.5-15.69% moisture , 14.9-24.6 gm protein , 0.8-6.4 gm fat , 2.1-11.7 gm fibre , 2.0-4.8 gm ash, 140-440 mg calcium, 190-382 mg phosphorus, 5.0-23.9 mg Fe, 0-225mg -carotene, 0.21 -1.1 mg thiamin, 0.12-0.33mg riboflavin and 1.3-2.9mg niacin (Duke, 1981).

The amino acid composition of seeds with 19.5% protein,5.5% oil is (per 100 gm): 7.2gm lysine, 1.4gm methionine, 8.8gm arginine, 4.0gm glycine, 2.3gm histidine, 4.4gm isoleucine, 7.6gm leucine, 6.6gm phenylalanine, 3.3gm alanine, 11.7 gm aspartic acid,16.0gm glutamic acid, 4.3gm proline and5.2 gm serine (Duke, 1981).

Pulses are an important source of protein also a good source of carbohydrate. Most of the pulses contain more than 50% carbohydrate (Copalan *et al*, 1981). There are considerable variations in the protein content among the different species of pulses (Swaminath *et al,* 1973).

Nutritional quality of protein and ascorbic acid level was increased in germinated chickpeas (Fernandez and Berry, 1988; Elemo *et al.*, 2011)

The fat content in pulses is not of much significance. Among the rest of the pulses, Chickpea seems to be relatively richer in fat content. Pulses are 3-4 times richer in minerals than rice. Most of the pulses are several times richer than rice in both calcium and iron content (Copalan *et al*, 1981).

2.4 Jackfruit Seed

Jackfruit is a tropical fruit appears in the market in spring and continues until summer. Fruits are consumed by all sector of community irrespective of age and gender but, the seeds are used in some local dishes. Though jack seeds are utilized at domestic level, scientific literature related to seed and its utilization is limited. Hence, an attempt is made here to put together some of the closely related findings about the jackfruit seed has been reviewed in this section.

The raw jackfruit seed had value that is significantly higher with the expectation of dry matter for all the parameters considered for approximate composition and energy content. For mineral content of processed seeds those subjected 60 minute duration of cooking had the highest value for micro and macro minerals. Also, seed subjected to 60 minutes duration of cooking had high percentage reduction in all anti nutritional factors with 49.72% reduction in phytin, 32.98% reduction in tannin, 50% rduction of oxalate, 44.25% reduction in saponin and 100% reduction of trypsin inhibitor, with acceptable value of crude protein (22.92%) energy content of 2.9 Kcal/gm, (Akinmutimi, 2006).

According to the Agricultural Journal, Food value per 100 gm edible portion of fresh jackfruit seeds contain, protein-6.6gm, fat-0.4gm, CHO-38.4gm, fibre-1.5gm, ash-1.25gm to 1.5gm and moisture- 51.6 to 57.77 gm. Presence of anti-nutritional factor such as fasin and trypsin inhibitor has been reported.

Neesha Maria, Consultant, Nutritionist mentioned that the Jackfruit seeds would give around 135 Kcal/100gms. It is a rich source of complex carbohydrate, dietary fibre, vitamins like Vitamin A, Vitamin C and certain B vitamins, minerals like calcium, zinc and phosphorus. They contain legans, isoflavones, saponins, that are called phyto nutrients and their health benefits are wide ranging from anti-cancer to antihypertensive, anti-ageing, antioxidant, anti-ulcer,etc. Jackfruit seed powder has the ability to relieve discomfort due to indigestion.

A study was conducted by Kumar *et al.* (1988) on the proximate composition of jackfruit seed flour. The seeds of *Kathari* and *Bharat Baramasi* varieties of jackfruit were found good sources of carbohydrates (28.01 and 26.83g %), protein (6.75 and 6.25g %), fat (0.78 and .89g %) and ash (1.27 and 1.16g %), respectively. The calorific value of both *Kathari* and *Bharat Baramasi* were found as 146.06 and 140.33 K.Cal/100g, respectively. *Kathari* variety was observed to be nutritionally excelling, compared to the *Bharat Baramasi* variety on the basis of most of the constituents analysed.

A systematic study was conducted by Odoemelam (2005) to evaluate the functional properties of jack seed flour. The flour had the 16 per cent (w/v) least gelation concentration. Water and oil absorption capacities were found as 2.30 mlg-1 and 2.8 mlg-1, respectively. Bulk density of the flour recorded as 0.61gml-1.The foam capacity of the flour was noted as 7.10 gml-1 and found gradually decreased to 2.00 gml-1 after 120min.

An emulsification capacity of flour was observed as 6.40 mlg-1 at pH 1 and was gradually decreased to 4.8 mlg-1 flour at pH 4, which is also the pH of minimum nitrogen solubility. Maximum nitrogen solubility of the flour noted 40.6 per cent at pH 10.

Rajarajeshwari and Jamuna (1999) assessed the use of jackfruit seed in product formulation. On incorporation of jack seed to two deep fat fried products viz., karasev and jamun revealed that, it was important to bring down fat absorption to a remarkable extent. In the savoury product none of the sensory attributes were affected, where as in sweet product the quality of texture and flavour was affected. For the presence of off flavour and after taste, no significant responses were obtained. The responses were significant with higher proportion of jack seed to the products.

Tananuwong *et al.* (2002) evaluated the possibility of substitution of jack seed flour in bread preparation. With increasing level of replacement, the water absorption capacity increased and bread dough peak time and dough stability time were reduced. The specific baking volume of the bread was reduced by 51 per cent at 5 per cent replacement with jack seed flour. Study revealed that, less than 5 per cent of wheat flour can be replaced with jack seed flour in the bread preparation.

2.5 Enzymes:

Enzymes are responsible for the breakdown of starch is widely distributed in nature. Among these are the amylases which act on starch, protease which act on protein etc. Amylases are used for the liquefication by the brewing industry. The area of enzymology is of special interest to both the biological and physical scientists. Enzymes are of universal occurrence in biological materials and life itself depends on a complex network of chemical reactions brought about by specific enzymes. Any alteration in the normal enzyme pattern of an organism may have far-reaching consequences. Enzymes, as catalysts, are of great interest to the physical chemist and investigation of the mechanisms of action of enzymes is a very important area of enzymology. Here some enzyme related literatures are listed below.

The area of enzymology has continued to grow rapidly for more than 60 years because of its importance to many field of sciences, especially biochemistry, physical chemistry, microbiology, genetics, botany zoology, food science, nutrition, pharmacology, toxicology, pathology, physiology, medicine, and chemical engineering. Enzymology has

important practical applications to activities as diverse as brewing and industrial fermentations, Such as pest control and chemical warfare, dry cleaning, sizing and detergents, analytical determinations, and recombinant DNA technology (Whitaken,1994).

Enzymes have many uses in addition to their natural functions in the body. Manufacturers use enzymes in making a wide variety of products. For example, some detergents contain enzymes that break down protein matter, such as perspiration that causes stains. Enzymes are also used in the manufacture of antibiotics, beer, bread, cheese, coffee, meat tenderizers, vinegar, vitamins, and many other products. Physicians use medicines containing enzyme to helps clean wounds, dissolve blood clots, relieve certain forms of leukemia and check allergic reactions to penicillin. Doctors also diagnose a number of diseases by measuring the amount of various enzymes in blood and others body fluids. Such diseases include anemia, cancer, leukemia and heart and liver ailments, (The world book encyclopedia, 1976).

Wang *et al.* (2003) were assayed the β-Amylase activities of barley cultivars collected from various areas of China, and as well as from Canada and Australia. Meanwhile a multi-location trial was conducted to determine variation of β-amylase activity in eight barley cultivars and the relationship between β-amylase activity and protein content. For 56 cultivars in study, β-amylase activity ranged from 458 to 1024 U/g, with a mean of 738 U/g. There was significant variation in both β-amylase activity and protein content for eight barley cultivars grown in four locations.

Amylase and invertase are the important hydrolytic enzymes which are found in fruits, pulses and plants. The activity of amylase, invertase and protease were studied on legume seeds (Rahman *et.al.*2006; Koshiba and Minamikawa, 1983; Morohashi, 1982).

CHAPTER 3

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present study on "Comparative evaluation of nutritive values of germinated rice, barley, chickpea and jackfruit seed and the effect of physico-chemical agents on the stability of enzymes of chickpea" was carried out during the period of May to November 2012. The material and methods adopted for the study are recorded in this section.

3.1. Experimental site

The experiment pertaining to the present investigation was carried out in the Food Enzymology Section of Institute of Food Science and Technology of Bangadesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

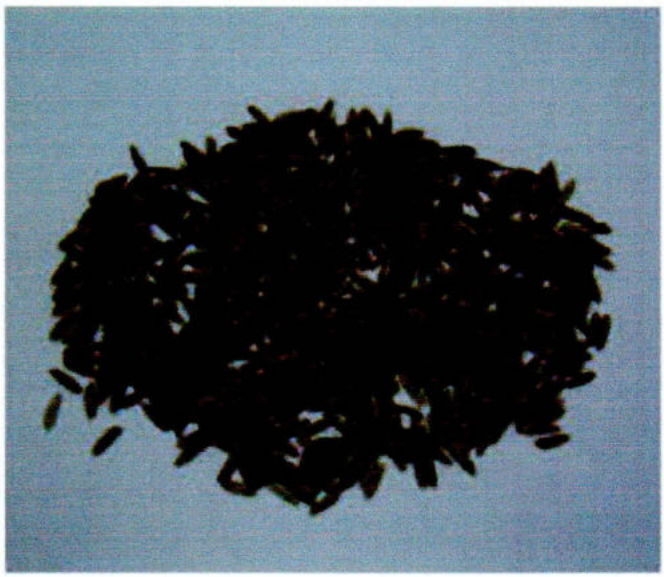
3.2. Sample Collection

The samples were collected from Bangladesh Rice Research Institute (BRRI) and Bangladesh Agricultural Research Institute (BARI). All the apparatus were provided by the Food Enzymology section of IFST of BCSIR. Chemicals and solvents used in the study were of analytical reagents grade.

3.3 Methods

3.3.1 Germination:

Seeds of rice, barley, chickpea and jackfruit seeds were washed and soaked in distilled water overnight. Seeds were placed on water soaked filter paper in sterilized petridishes. Distilled water was applied to the experimental seeds at an interval an of 24 hours, 48 hours, and 72 hours germination.



Photograph 1: Rice(BRRI-28)

Photograph 2: Barley (BARI-6)

Photograph 3: Chickpea (BARI-5)

Photograph 4: Jackfruit seed (Local variety)

3.3.2 Determination of moisture contents

Moisture content was determined by following the method of A.O.A.C. 2000.

Moisture content of seeds was determined by drying the seeds at 105°C in a drying oven till a constant weight was attained. A known quantity of seed sample was taken in crucible (the weight of the crucible was noted first) and it was kept for six hours at 105 °C in a drying oven. After drying for six hours, the sample was kept in a dessicator for an hour and the crucible with seeds was weighted. Drying and dessicating processes were continuing until a constant weight obtained.

Calculation: Moisture content was calculated by the following formula

$$\% \text{ moisture} = \frac{\text{Loss of weight}}{\text{Weight of samples}} \times 100$$

3.3.3 Determination of ash content:

Ash content was determined by following the method of A.O.A.C. 2000.

Procedure

The empty crucible was taken and dried in an oven at 105°C for an hour, removed and kept it in a dessicator and weighed up to constant weight. About 1 gm of particular seed sample was taken in the weighted empty crucible. 1 drop of Nitric acid was added and then the sample in the crucible was clarred on a low flame and the crucible was then kept in muffle furnace and temperature was allowed to rise to 650°C and kept it constant for five hours. Then it was removed, cooled and kept in a dessicator and weight of crucible was taken.

Calculation:

Ash content was calculated by the following formula:

$$\% \text{ ash} = \frac{\text{Weightof residue}}{\text{Weightof sample}} \times 100$$

3.3.4 Determination of protein content

Protein content of different seeds was determined by following the method of Micro-Kjeldahl (Wong, 1932).

Reagents and equipments:

a. Solid Sodium Sulphate
b. Concentrated sulphruic acid
c. Mercuric Oxide
d. 0.02 N Hydrochloric acid
e. Concentrated sodium hydroxide solution(5N Approximately)
f. 2% Boric acid
g. Few quartz chips
h. Nitrogen determination apparatus according to Paranas-Wagner, made of JENA glass-all connections with inter changeable ground joints.

Procedure

The protein was extracted by kjeldahl method. The principle of this procedure involves digestion of the sample with concentrated sulphuric acid (H_2SO_4) and digestion mixture, which causes oxidation and destruction of protein and conversion of the organic nitrogen to ammonia that remains in the acid mixture as ammonium bisulphate.

The amount of ammonia nitrogen is determined by making the digest alkaline followed by distillation of the liberate ammonia into standard acid solution and estimated titrimetrically.

Cleaned and dried 100 ml kjeldahl flasks were taken along with 0.5g sample in it with a piece of ash less filter paper. About 10 ml of concentrated H_2SO_4 and 10:1 gm digestion mixture (sodium sulphate and mercuric oxide) were added then the digestion chamber was heated (6 hours) until the content become clean. After completing the digestion, the flask was cooled and digested mixture was transferred in a 100 ml volumetric flask and diluted up to mark with distilled water. Ten(10 ml) of that solution was transferred in a microkjeldahl distillation apparatus after adding 5 ml of 50% NaOH and 2.5 ml of 15% $Na_2S_2O_3$. The solution was distilled with steam for 10 minutes. The distillate was

collected in excess of 2% boric acid solutions with indicator and was titrated by 0.02 N HCl. At the same time, a similar blank digestion was carried out without the sample.

Calculation:

The percentage of nitrogen in the sample was calculated by the following equation

$$\% \text{ Nitrogen} = \frac{(S - B) \times N \times 14 \times 10}{W} \times 100$$

Where,

S = Titration reading for sample

B = Titration reading for blank

N = Strength of HCl solution

14 = Factor

10 = Aliquot used

W = Weight of sample

The total nitrogen content was then converted into percentage of crude protein by multiplying with the factor 6.25% of crude protein = N × 6.25

3.3.5 Determination of fat content:

Fat content of seeds was determined following the methods by Mehlenbacher, (1960). The principle of this method lied in mixing the sample with a solvent n-Hexane which was then removed by distillation and the residue was dried and weighted. The extraction procedure was carried out in Sox let apparatus.

The fresh sample (5 gm) was weighted accurately and it was taken in extraction thimble. The thimble was then placed in a n-Hexane for an about eight hours.

Calculation:

$$\% \text{ of fat content} = \frac{W}{W_1} \times 100$$

Where, W = Weight of oil

W_1 = Weight of sample taken

3.3.6 Determination of Carbohydrate Content

The total percentage carbohydrate content was determined by the difference method as described by Edeogu *et al.* (2007). The content of the available carbohydrate is determined by difference, i.e. by subtracting from 100 the sum of the values (per 100 gms) for moisture, ash, protein and fat.

Calculation:

Carbohydrate = 100 – (Moisture + Ash + Protein + Fat)

3.3.7 Determination of Energy Content:

The energy content of the sample was determined by calculating the amount of protein, fat and carbohydrate.

Calculation:

Energy = (Protein × 4.1) + (Fat × 9) + (Carbohydrate × 4.1)

3.3.8 Determination of Mineral Content:

The minerals content were determined by the method of Anonymous, (A manual of Laboratory Techniques, 1976).

Preparation of Minerals Solution:

The ash (obtained in the previous experiments) is moistened with a small amount of glass-distilled water (0.5 to 1.0 ml) and 5 ml of distilled hydrochloric acid are added to it. The mixture is evaporated to dryness on a boiling water bath. Another 5 ml. of hydrochloric acid are added again and the solution evaporated to dryness as before. Four ml of hydrochloric acid and a few ml of water are then added and the solution warmed over a boiling water bath and filtered into a 100ml. volumetric flask using Whatman No.40 filter paper. After cooling the volume is made up to 100ml, and suitable aliquots were used for the estimation of phosphorus, iron and calcium.

3.3.8.1 Determination of calcium content:

Calcium content was determined by titrimetric method.

Reagents:

a. 6% Ammonium oxalate
b. Methyl-red indicator
c. Dilute sulphuric acid (2N)
d. Strong ammonia
e. 0.01N Potassium permanganate solution

Procedure:

An aliquot (25 ml) of mineral solution was diluted to about 150 ml with distilled water. A few drop of methyl red indicator were added and the mixture was neutralized with ammonia till the pink color change to yellow. The solution was heated to boiling and 10 ml of ammonium oxalate was added. The solution mixture was then allowed to boil for a few minutes and glacial acetic acid was then added to it till the color was distinctly pink. The mixture was kept aside in a warm place, and when the precipitate settled down the supernatant was tested with a drop of ammonium oxalate solution to ensure the completion of the precipitation. The precipitate was then filtered through whatman no.40 filter paper and washed with warm water till the precipitation become free of oxalate. The precipitate was transferred to a beaker by piercing a whole in a filter paper about 5 to 10 ml of dilute H_2SO_4 (2N) was poured over it. The solution was then heated to about 70°C and titrated against 0.01N $KMnO_4$ solution.

Calculation:

1 ml of 0.01N $KMnO_4$ solution = 0.2004 mg of Ca

$$\text{mg percent of calcium content} = \frac{\text{mg of calcium obtained}}{\text{Weight of sample}} \times 100$$

3.3.8.2: Determination of phosphorus content:

Phosphorus content was determined by the method of Boltz. (1958).

Reagents:

a. **Standard phosphorus solution:** 0.434 gm of potassium dihydrogen phosphate was dissolved in distilled water and diluted it to one litter. Now, each ml of solution contained 0.1 mg of phosphorus
b. **Molybdate reagents:** 100 gm of sodium molybdate was dissolved in distilled water and diluted it to 1 litter.
c. **Nitric Acid (2.5N)**

Procedure:

The sample stock (25ml), 5 ml of 2.5 N HNO_3 and about 15 ml of distilled water were taken in a 50 ml volumetric flask. Then 5 ml of molybdate reagent was added to the above mixture thoroughly whereby a yellow color was obtained. The absorbance of the solution was then measured at 380 nm. The blank cell was prepared by taking 25ml of distil water instead of stock sample solution.

A calibration curve was constructed in the usual manner by taking 0.5, 1, 1.5, 2, 2.5, 3.0 and 3.5 ml of the standard solution of phosphorus into seven different 50 ml volumetric flasks. To each flask, 5.0 ml of 2.5 ml of 2.5 N HNO_3 and 15 ml of distilled water and 5.0 ml of molybdate reagent were added and then diluted it up to the mark. The mixture was mixed thoroughly by shaking whereby a yellow color was obtained. The absorbance of these solutions were taken at 380 nm using a reagent blank that contained distilled water instead of phosphorous solution and 5 ml of 2.5 N $HNO_3$and 5 ml of molybdate reagent. The calibration curve was constructed by plotting absorbance against the respective concentration of the solution (Figure-3.1)

Calculation:

Percent of phosphorus content (mg per 100gms of sample)

$$= \frac{\text{Weight of total phosphorus obtained}}{\text{Weight of sample(g)}} \times 100$$

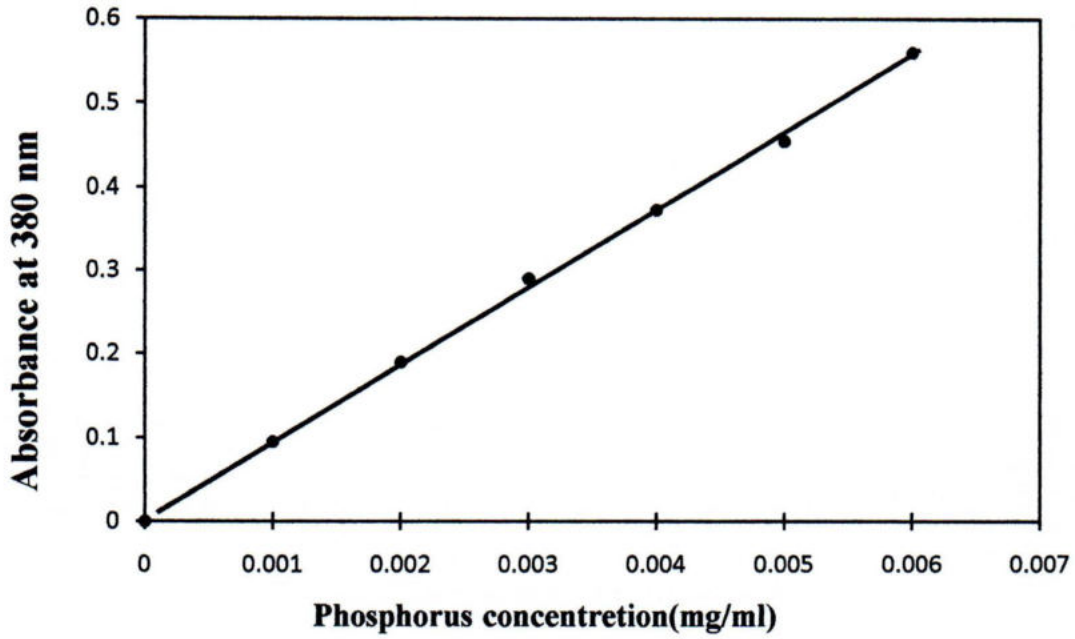


Figure 3.1: Standard curve of maltose for estimation of phosphorus

3.3.8.3 Determination of Iron Content:

Iron is determined calorimetrically making use the fact that ferric iron gives a blood-red color with potassium thiocyanate.

Reagents:

1. **30% Shulphuric acid**. (A.R) (30 ml. Conc. H_2SO_4 diluted to 100 ml)
2. **Saturated potassium persulphate solution:** 7gm potassium persulphate (A.R) were dissolved in glass-distilled water and the solution made up to 100 ml.
3. **Potassium thiocyanate 40% solution**: 40gm KCNS were dissolved in 90 ml. Glass distilled water, 4 ml. Acetone was added and the volume was made up to 100 ml.
4. **Standard iron solution:** 0.7022 gm (A.R) ferrous ammonium sulphate was dissolved in 100 ml glass distilled water, and the after addition of 5 ml. Of 1:1 HCL, the solution is made up to 1 liter and mixed thoroughly. (1ml=0.1mg. Fe). The standard solution is prepared fresh once in six months.

For iron estimation all the reagents used should be free from iron. Used of glass-distilled water is preferred. Reagents used should be free from iron. Use of glass distilled water is preferred. If use of reagents containing traces of iron cannot be avoided, it should be seen

that the final solution of standard and the test contain identical quantities of these reagents containing iron as impurity.

Procedure:

To an aliquots (6.5 ml or less) of the mineral solution enough water is added (if necessary) to make up to volume of 6.5 ml followed by 1.0 ml of 30% H_2SO_4, 1.0 ml potassium persulphate solution and 1.5 ml 40% KCNS solution. The red color that develops was measured within 20 minutes at 540 nm. Iron content of these solutions were determined from a standard curve (figure: 3.2), constructed by using standard iron solution of different concentration in the same manner as before. From the results obtained, amount of iron present in chickpea were calculated.

Calculation:

Iron content (mg per 100 gm of sample)

$$= \frac{\text{mg of iron (Fe) obtained}}{\text{Weight of sample}} \times 100$$

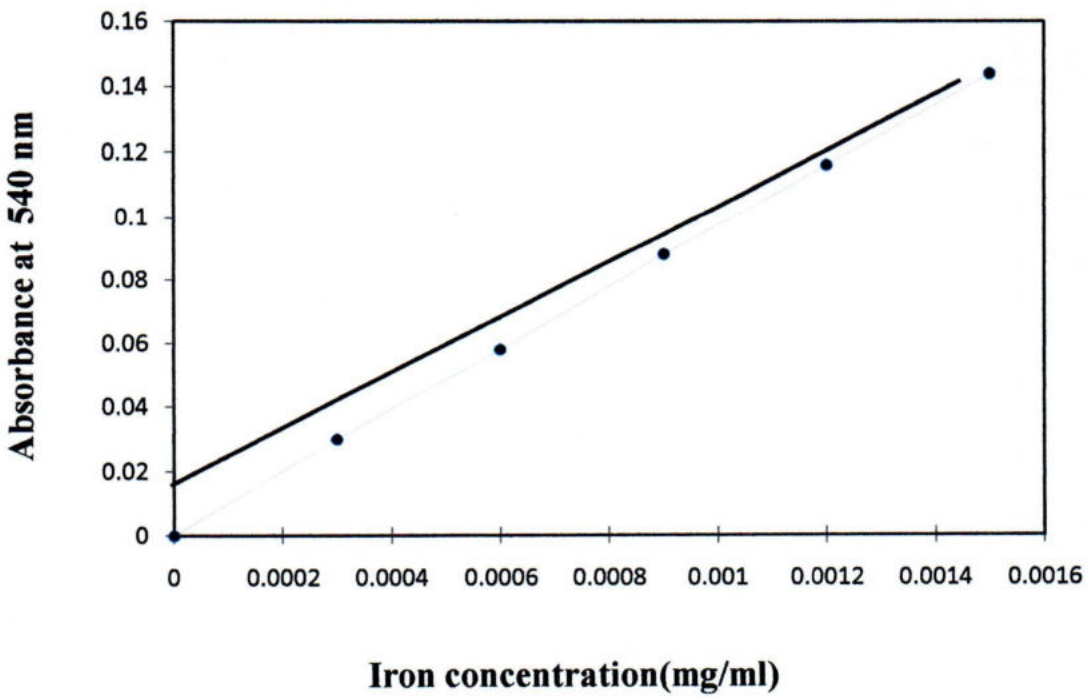


Figure 3.2: Standard curve of maltose for estimation of iron

3.3.9 Assay of Enzyme Activity:

Preparation of crude enzymes extract:

The seeds (2 gm) were grained in a mortar with cold 0.1M phosphate buffer of respective pH, for amylase 6.7, and for protease citrate buffer, 5.5 and finally crushed into paste using a homogenizer. The temperature was maintained at 4°C by putting ice in the outer chamber of the homogenizer. The suspension was then filtered through few layers of cheesecloth in the cold room.

The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 10,000 r.p.m. for 20 minutes at 4°C and used as crude enzyme extract.

3.3.9.1 Assay of protease activity:

Protease activity was assayed following the modified method as describe by Madhavan and Sridhar (1982). Hemoglobin was used as substrate. The protease activity was measured by estimating the amount of leucine released from hemoglobin. The amount of leucine released was calculated from the standard curve (Figure-3.3) prepared with leucine. One unit of protease activity was defined as the amount required for liberating 1 mg of leucine in 30 min at 37°C.

1.0 gm to 2.0 gm sample homogenize with 8 to 10 ml citrate buffer. The homogenized sample was collect in a centrifuge tube. The centrifuge was at 10,000 rpm for 20 minutes. Collect the supernatant and count the amount in ml. This is known as enzyme extract. Now 0.25 or 0.5 ml of enzyme extract and 3 ml substrate (0.1% hemoglobin) incubate at 37°C for 30 minute.

After 30 minutes of incubaton, stop reaction by adding 3 ml of 5% TCA. Then filter or centrifuge again and supernatant should be counted. Take 0.5 ml supernatant and add 0.5 ml H_2O (Distilled). Then add 1 ml ninhydrin solution. Now heat it for 20 minutes in boiling water bath. Cool and add 5 ml diluents. Now take OD at 570 nm.

Blank = 1 ml Citrate buffer + 1 ml Ninhydrin ⟶ Heat in boiling bath for 20 minute ⟶ Cool ⟶ Add 5 ml diluents ⟶ OD at 570 nm

Sample = 0.5 ml sample + 0.5 ml H_2O + 1 ml Ninhydrin solution ⟶ Heat for 20 minute ⟶ Cool ⟶ Add 5 ml diluents ⟶ OD at 570 nm

Color Reagent:

Preparation of Ninhydrin Solution:

5 gm of ninhydrine are dissolved in 188 ml of methyl cellusolve and a 62 ml filtered solution of $SnCl_2$ (100 mg) in citrate buffer is added. The solution is to be stored in a dark bottle in a refrigerator.

Ninhydrine → 1 gm in 37.6 ml methyl cellulose

$SnCl_2$ → 20 mg in 12.4 ml citrate buffer, pH= 5.5

Ninhydrin Solution = Ninhydrin + $SnCl_2$ → 37.6 ml + 12.4 ml = 50 ml

Diluent: H_2O: n-Propanol = 1:1

Substrate: 0.1% Hemoglobin in 50 ml Citrate buffer, pH = 5.5

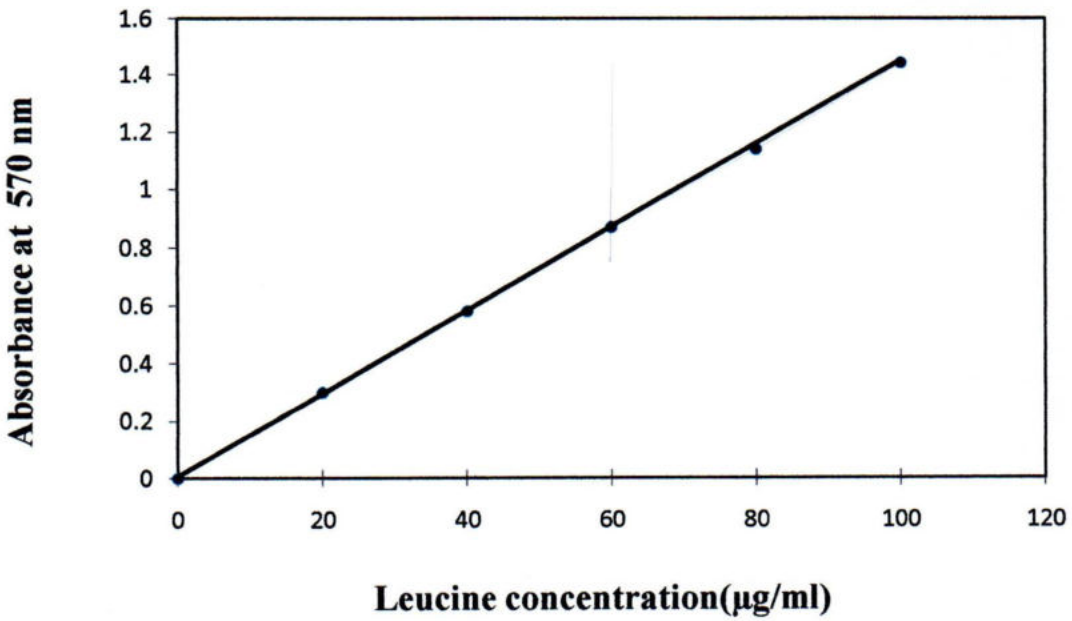


Figure 3.3: Standard curve of leucine for estimation of protease activity

3.3.9.2 Assay of α-amylase activity:

Amylase activity was assayed following the method as describe by Jayaraman (1981). One percent starch solution was used as substrate (1 gm in 100 ml of 0.1M phosphate buffer, pH 6.7). The amylase activity was measured by estimating the release of maltase. The amount of maltose released was calculated from the standard curve (Figure-3.4) prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 15 minutes at 37 ^{0} C.

Reagents:

i. 0.1M phosphate buffer, pH 6.7
ii. 1% starch solution in 0.1M phosphate buffer, pH 6.7
iii. 1% NaCl in distilled water
iv. 2N NaOH
v. Dinitrosalicylic acid (DNS)

Reagent: Simultaneously 1 gm of DNS, 200 mg, of crystalline phenol and 50 mg of sodium sulphite were placed in a beaker and then mixed with 100ml of 1% NaOH solution by stirring. If it is needed to store then sodium sulphite must be added just before use.

Procedure:

Three sets of experiments (Blank, control and sample) were performed for the measurement of amylase activity. The following different solutions were taken in different test tubes.

Substance	**Blank (ml)**	**Control (ml)**	**Sample(ml)**
0.1M phosphate buffer, pH 6.7	2.5	2.5	2.5
1% starch solution	2.5	2.5	2.5
1% *NaCl*	1.0	1.0	1.0

The contents in the test tube were mixed uniformly and test tubes were incubated in a water bath at 37^{0}C for 10 minutes. The 0.5 ml of crude enzyme extract and 0.5 ml distilled water were added to sample and control tubes respectively, whereas 1 ml distilled water was added to the blank test tube. Immediately after the addition of crude enzyme extract and distilled water. 0.5 ml of 2N NaOH was added to the control test tube.

The rest of the test tubes were incubated at 37^{0}C for 15 minutes and the reactions was then stopped by the addition of 0.5 ml of 2N NaOH. Then 0.5ml of DNS reagent was mixed to all the tubes. The tubes were heated in a boiling water bath for five minutes. After cooling at room temperature the absorbance was measured at 520 nm.

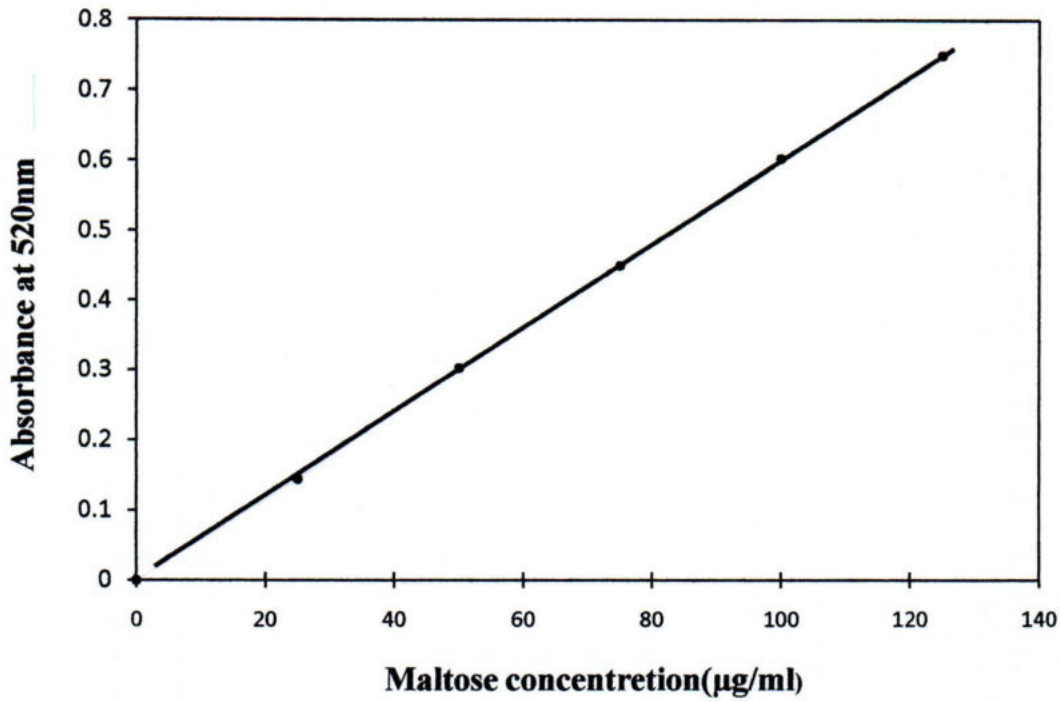


Figure-3.4: Standard curve of maltose for estimation of α-amylase activity

3.3.10 Effect of physico-chemical agents on the stability of α-amylase, and protease

3.3.10.1 Determination of optimum pH and optimum temperature

The activities of α-amylase and protease at different values (2-10) were measured at 37°C following the procedure as described in article 3.3.9.1 and 3.3.9.2. Starch and haemoglobin solution prepared in the above mentioned buffer of different pH values was used as substrate.

The activities of α-amylase and protease enzymes were measured at different temperatures (10-90°C) using buffer pH 6.7 and 5.5 for α-amylase, and protease respectively.

3.3.10.2 Effect of calcium:

Solid Calcium Chloride of different concentration was added to enzymes solution (5ml) for 10 minutes at 25°C. The mixtures were incubated with their respective substrates for 15 minutes at 37°C. The activities remaining were then assayed.

3.3.10.3 Treatment with EDTA:

EDTA of different concentrations were added to the enzymes solutions (5ml each) for 10 minutes at 25°C and incubated with the respective substrate for 15 minutes at 37°C by gently stirring. The remaining enzymes activities were then assayed.

3.3.10.4 Treatment with various metal ions and salts:

The effect of various metals on the enzymes activities were tested by pre- incubating enzymes solutions, 5 ml with specified concentrations of the reagent for ten minutes at 25°C. The mixture was incubated with their respective substrates for 15 minutes at 37°C and the remaining activities were assayed.

CHAPTER 4

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The results of the studies on rice, barley, chickpea, jackfruit seed in terms of nutritional composition, effect of germination in the protein content and mineral content, enzymatic activity at different geminating period and effect of physic-chemical agents on the stability of enzymes of chickpea are presented in this chapter.

4.1: The proximate composition of rice, barley, chickpea and jackfruit seed

Composition of raw rice, barley, chickpea and jackfruit seed were analyzed before the germination which were shown in the Table 4.1.

Table-4.1: Proximate Composition of rice, barley, chickpea and jackfruit seed

Parameters	Name of the Raw Materials			
	Rice (BRRI Dhan 28)	Barley (BARI Barley 6)	Chickpea (BARI Chola 5	Jackfruit Seed (Local variety)
Moisture (%)	14.70	14.06	10.20	63.40
Ash (%)	0.40	2.00	3.42	1.80
Protein (%)	6.86	11.20	17.80	7.00
Fat (%)	0.65	2.00	5.54	0.40
Carbohydrate (%)	77.39	70.74	63.04	27.40
Energy (kcal per 100 gm)	342.85	345.76	373.22	141.20

4.1.1: Moisture Content:

The moisture content of Rice, Barley, Chickpea and Jackfruit seed were found 14.70%, 14.06%, 10.20%, 63.40% respectively (Table-4.1). Cereal grains are usually harvested at 20-25% moisture content while 14% or less is considered safe for storing grains, 12% or less for storing seeds (Tames 2001). The moisture content of Rice and Barley are nearly similar to Tames. Moisture content of Jackfruit seed was 63.40% which is slightly higher than the value of Kumar *et al.* (1988), who reported the value of moisture content of Jackfruit seed was 56.34%. Moisture content of Chickpea was 10.20% which was similar to Duke (1981) who observed it as 4.5-15.69%.

4.1.2: Ash content:

The ash content of chickpea was 3.42 (table-4.1). Duke (1981) reported that the ash content of chickpea was 2.00%-4.80%. The ash content of chickpea is similar to Duke (1981). The ash content of rice, barley and jackfruit seed were 0.40%, 2.00% and 1.80% (table-4.1). Ali *et al.* (1992) reported that ash content of rice, barley and jackfruit seed are 0.20%, 3.90% and 1.20% respectively. The value of ash content of rice, barley and jackfruit seed are in accord with their findings.

4.1.3: Protein content:

The protein content of rice and barley were 6.86% and 11.20% respectively (table-4.1) which were slightly lower than the value 8.00% and 12.00% respectively mentioned by Potter and Hotchkiss (1996). The protein content of chickpea was 17.80%. This result has the similarity as Duke who mentioned the protein content of chickpea is 14.90%-24.60%. The protein content of jackfruit seed was 7.00%, which is slightly higher than Anonymous (1997) who mentioned the jackfruit seed contain 6.60% protein.

4.1.4: Fat content:

The fat content of rice and barley were 0.65% and 2.00% respectively (table 4.1). The value is slightly higher than the value of Ali *et al.* (1992) who mentioned that rice contain 0.50% fat and barley contain 1.30% fat. Jackfruit seed contained 0.40% fat mentioned by Anonymous (1997). Similar result was found in this study. The fat content of chickpea was 5.54%. Duke (1981) reported that chickpea contain 6.40% fat. The fat content of chickpea is slightly lower than finding of Duke.

4.1.5: Carbohydrate content:

Carbohydrate content of rice was 77.39% (table-4.1) which is slightly lower than the value of Ali *et al.* (1992) as reported the value of carbohydrate content of rice was 78.20%. Ali *et al.* (1992) observed that the barley contain 69.60% carbohydrate. The carbohydrate content of barley is slightly higher than the value of Ali *et al.* (1992). The carbohydrate content of chickpea was 63.04%. The carbohydrate content of jackfruit was 27.40% (table-4.1). This value is slightly higher than the value 25.80% which was reported by Anonymous (1997).

4.1.6: Energy content:

The energy content of rice was 342.85 kcal (Table 4.1). This has the similarity as Eggum, who mentioned that rice gives 349-373 Kcal energy per 100 gm (1983). Energy content of barley was 345.76 Kcal (Table 4.1) which was slightly higher than the value 336 Kcal observed by Fairbairn *et al.* (1999). The energy content of Chickpea was 373.22 Kcal. This value is as similar as Duke (1981), who observed it gives 575 Kcal energy. The energy content of jackfruit seed was 141.20 kcal (Table 4.1). This value is slightly lower than the value of 146 Kcal which was observed by Akinmutimi (2006).

4.2: Mineral content of rice, barley, chickpea and jackfruit seed

Minerall content of raw rice, barley, chickpea and jackfruit seed were analyzed before the germination which were shown in the Table 4.2.

Table-4.2: Mineral Content of Raw Materials

Parameters (mg/100gm)	Name of the Raw Materials			
	Rice (BRRI Dhan 28)	Barley (BARI Barley 6)	Chickpea (BARI Chola 5)	Jackfruit Seed (Local variety)
Calcium	13.23	28.23	260.80	96.19
Phosphorus	184.00	248.00	365.22	160.00
Iron	5.45	5.25	18.80	2.95

4.2.1: Calcium content:

Calcium content of rice was 13.23 mg/100gm (table-4.2), which is slightly higher than the value of 500 mg/lb mentioned by Potter and Hotchkiss (1996). The calcium content of barley was 28.23 mg/100gm (table-4.2), which higher than the value 26 mg/100gm reported by Ali *et al.* (1992). The calcium content of jackfruit seed was 96 mg/100gm. Anonymous (1997) mentioned that jackfruit seed contain 50mg calcium per 100 gm. These findings are slightly higher than Anonymous. Chickpea contained 260.80 mg/100gm calcium. Similar trend also observed by Duke (1981), who found that chickpea contains 140-440 mg/100gm calcium.

4.2.2: Phosphorus content:

Phosphorus content of rice, barley chickpea and jackfruit were 184 mg/100gm, 248 mg/gm, 365.22 mg/100gm and 160 mg/100gm respectively. The data were shown in the table 4.2. The values obtained for minerals are slightly higher than the values obtained by Ibukun (2008). This slight difference might be as a result of fertilizer application, rate of parboiling and the amounts of soil nutrients all of which affect the mineral contents.

4.2.3: Iron content:

The iron content of rice, barley, chickpea and jackfruit seed were 5.45 mg/100gm, 5.25 mg/100gm, 18.80 mg/100gm and 2.95 mg/100gm respectively which is slightly higher than the values reported by Ali *et al.* (1992).

4.3: Changes of protein content of rice, barley, chickpea and jackfruit seed during different germinating period

The results of changes of protein contents of rice under different germination conditions were summarized in the table-4.3.

Table-4.3:Changes of protein content of rice, barley, chickpea and jackfruit seed during different germinating period

	Variety	Duration of germination			
		0 hrs	24 hrs	48 hrs	72 hrs
Protein (%)	Rice (BRRI Dhan 28)	6.86	7.67	6.23	5.40
	Barley (BARI Barley 6)	11.20	12.21	14.70	10.62
	Chickpea (BARI Chola 5)	17.80	20.76	17.64	14.08
	Jackfruit Seed (Local variety)	7.00	8.05	8.61	7.34

It was observed that due to germination protein content of rice increased remarkably 11.81% at 24 hours of germination, whereas it was decreased drastically after 48 hours and 72 hours of germination. The variety of rice BRRI-28 showed the highest amount of protein 7.67% at 24 hours of germination. Maximum decrease was 21.28% at 72 hours of germination.

The changes of protein content of barley were shown in table 4.3. From the data it was observed that due to germination protein content increased significantly 31.25% at 48 hours of germination whereas it was decreased drastically from 72 hours of germination. The variety of barley BARI-6 showed the highest amount of protein 14.70% at 48 hours of germination and it decreased 5.18% at 72 hours of germination.

The highest amount of protein of chickpea (BARI-5 variety) was 20.76% at 24 hours of germination (table 4.3). Result revealed that it was decreased drastically from 48 hours and 72 hours of germination. Result shows that protein content increased at 24 hours of

germination and then decreased with the progress of germination which are in accord with the results of Rahman *et al.* 2006, Chrispeels *et al.* 1981and Lichtenfeld *et al.* 1981.

A graphical presentation of Changes of protein content of rice, barley, chickpea and jackfruit seed during different germinating period is shown below:

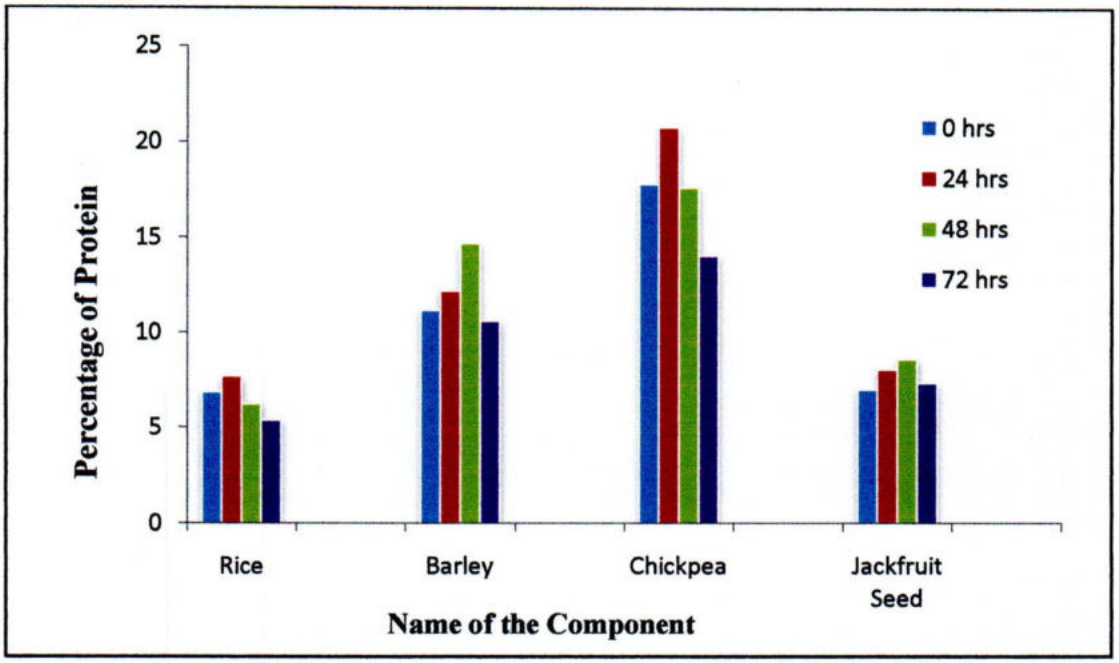


Figure 4.1: Changes of protein content during different period of germination

The changes of protein content of jackfruit seeds were summarized in the Table 4.3. It was observed that due to germination protein contents increased remarkably 23% at 48 hours of germination whereas it was decreased drastically from 72 hours of germination. The local variety of jackfruit seed showed the highest amount of protein 8.61% at 48 hours germination. It decreased 4.86% at 72 hours of germination.

Nutritional quality of protein an ascorbic acid level was increased in germinated chickpeas mentioned by Fernandez and Berry, (1988) and Elemo *et al.* (2011). Several studies on the effect of germination on cereals and legumes found that germination can increase protein content and dietary fiber, reduce tannin and phytic acid content and increase mineral bioavailability (Rao and Prabhavathi, 1982; Hussein and Ghanem, 1999; Ghavidel and Prakash, 2007). Hahm *et al.* (2008) also suggested that protein content of cereals and legumes is deceased at a certain period because amino acids are oxidized to carbon dioxide and water to generate energy for germination. Results are available that protein content increased at 24/48 hours of germination and then decreased with the

progress with germination which is in accord with the result of Chrispeels *et al.* (1981) and Lichtenfeld *et al.* (1981).

4.4: Changes of mineral content of rice, barley, chickpea and jackfruit seed during different germinating period:

Changes of Mineral content of rice barley, chickpea and jackfruit seed during different germinating period were shown in table 4.4.

Table-4.4: Calcium, Phosphorous and iron contents of Rice, Barley, Chickpea and Jackfruit seed during different periods of germination

Minerals	Variety	Duration of germination			
		0 hrs	24 hrs	48 hrs	72 hrs
Calcium (mg/100gm)	Rice (BRRI Dhan 28)	13.23	21.09	17.63	13.08
	Barley (BARI Barley 6)	28.86	32.06	46.01	29.98
	Chickpea (BARI Chola 5)	260.80	326.44	260.52	152.88
	Jackfruit Seed (Local variety)	96.19	176.34	208.35	186.41
Phosphorous (mg/100gm)	Rice (BRRI Dhan 28)	212.00	298.00	266.00	288.00
	Barley (BARI Barley 6)	248.00	288.00	376.26	224.20
	Chickpea (BARI Chola 5)	365.22	448.26	374.26	289.22
	Jackfruit Seed (Local variety)	130.00	160.00	186.00	112.00
Iron (mg/100gm)	Rice (BRRI Dhan 28)	5.45	7.22	6.85	5.66
	Barley (BARI Barley 6)	5.25	6.46	7.08	5.26
	Chickpea (BARI Chola 5)	18.80	30.65	22.82	14.26
	Jackfruit Seed (Local variety)	2.95	3.12	3.88	2.56

4.4.1: Changes of calcium content:

The changes of calcium contents of rice, barley, chickpea and jackfruit seed under different germination conditions were summarized in the Table 4.4. It is observed that the calcium content increased remarkably 59.41% and 41.27% at 24 hours of germination of rice and chickpea. It was decreased drastically from 48 hours and 72 hours of germination of rice and chickpea. The variety of rice BRRI-28 and chickpea BARI-5 showed the highest amount of calcium were 21.09 mg/100gm and 368.44 mg/100gm respectively at 24 hours of germination. The calcium contents of barley and jackfruit seed were increased significantly 62 .98% 116.60% respectively at 48 hours of germination. It was decreased drastically from 72 hours of germination. The variety of barley BARI-6 and local variety of jackfruit seed showed the highest amount of calcium were 46.01 mg/100gm and 208.35 mg/100gm respectively at 48 hours of germination.

Changes of calcium content of rice, barley, chickpea and jackfruit seed during different germinating period is graphically shown below:

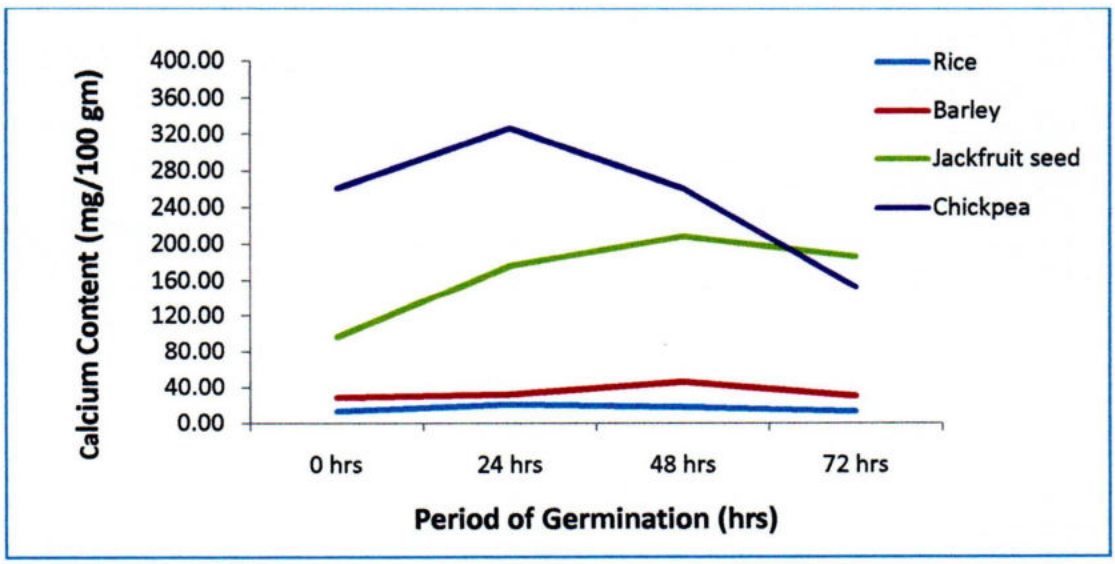


Figure 4.2: Calcium content of rice, barley, chickpea and jackfruit seed during different periods of germination

Kaushik *et al.* (2010) found that germination improves calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content. The decrease in calcium, iron and phosphorus content represents loss in minerals due to rootlet and washing of the seeds in water to reduce the sour smell during the period of germination (Tatsadjieu *et al.*, 2004). Findings of the present study are in agreement with those of previous workers.

4.4.2: Changes of phosphorus content:

The changes of phosphorus content of rice, barley, chickpea and jackfruit seed under different germination conditions were summarized in the Table 4.4.

From table 4.4, it is observed that the phosphorus content increased remarkably 50% and 49.02% at 24 hours of germination of rice and chickpea. It was decreased drastically from 48 hours and 72 hours of germination of rice and chickpea. The variety of rice BRRI-28 and chickpea BARI-5 showed the highest amount of phosphorus were 318 mg/100gm and 544.26 mg/100gm respectively at 24 hours of germination.

The phosphorus contents of barley and jackfruit seed were increased 51.72% and 43.08% respectively at 48 hours of germination. It was decreased drastically from 72 hours of germination. The variety of barley BARI-6 and local variety of jackfruit seed showed the highest amount of phosphorus were 376.26 mg/100gm and 186 mg/100gm respectively at 48 hours of germination.

A graphical presentation of Changes of phosphorus content of rice, barley, chickpea and jackfruit seed during different germinating period is shown below:

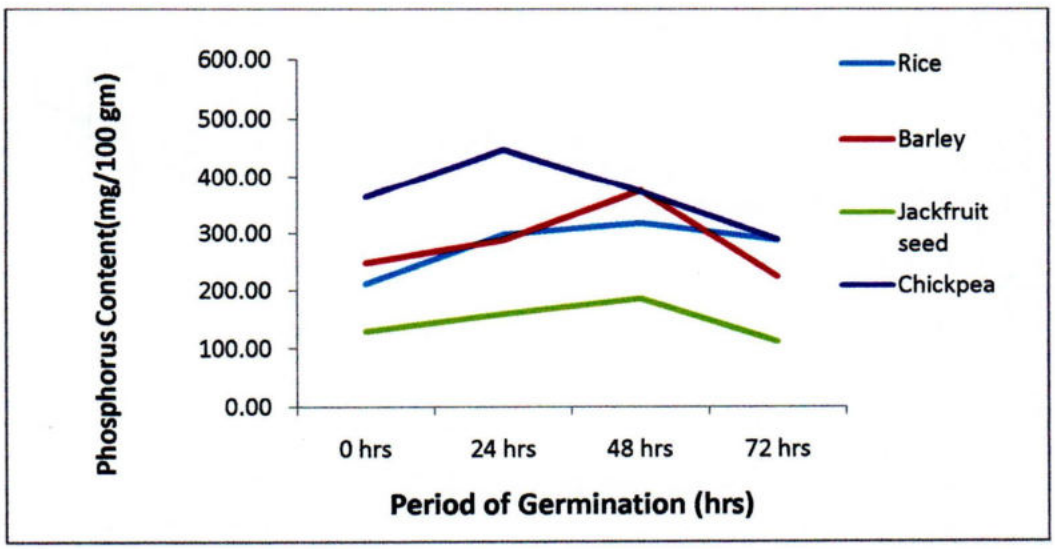


Figure 4.3: Phosphorous content of rice, barley, chickpea and jackfruit seed during different periods of germination

El-Mahdy *et al.* (1985) reported that germination of lentils led to a marked decrease in phytic acid and a considerable increase in inorganic phosphorus as well as non-phytate and organic phosphorus. The decrease in calcium, iron and phosphorus content represents

loss in minerals due to rootlet and washing of the rice in water to reduce the sour smell during the period of germination (Tatsadjieu *et al.*, 2004). Results of the present study are in conformity with those other workers.

4.4.3: Changes of iron content:

The changes of iron content of rice, barley, chickpea and jackfruit seed under different germination conditions were shown in the Table 4.4. From table it is summarized that the iron content increased 32.48% and 63.03% respectively at 24 hours of germination of rice and chickpea. It was decreased drastically from 48 hours and 72 hours of germination of rice and chickpea. The variety of rice BRRI-28 and chickpea BARI-5 showed the highest amount of iron were 7.22 mg/100gm and 30.65 mg/100gm respectively at 24 hours of germination. The iron contents of barley and jackfruit seed were increased 34.86% 31.53% respectively at 48 hours of germination. It was decreased drastically from 72 hours of germination. The variety of barley BARI-6 and local variety of jackfruit seed showed the highest amount of iron were 7.08 mg/100gm and 3.88 mg/100gm respectively at 48 hours of germination.

A graphical presentation of Changes of iron content of rice, barley, chickpea and jackfruit seed during different germinating period is shown below:

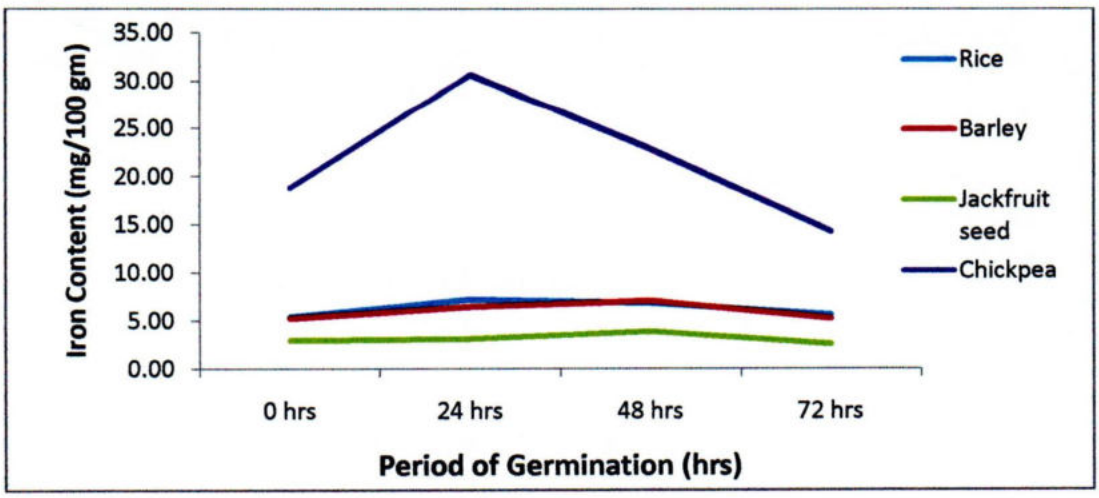


Figure 4.4: Iron content of rice, barley, chickpea and jackfruit seed during different periods of germination

An increase in the bioavailability of minerals and vitamins has been observed due to germination reported by Sulieman *et al.* (2006). Germinated seeds and grain showed an

increase in mineral content from 25 to 400 percent (Azuly, 1997) and (Pellel *et al.*, 1970) which are very much similar with the study. The decrease in calcium, iron and phosphorus content represents loss in minerals due to rootlet and washing of the rice in water to reduce the sour smell during the period of germination (Tatsadjieu *et al.*, 2004). Findings of the present study are in agreement with those of previous workers.

4.5: Enzyme activity of rice barley, chickpea and jackfruit seed during different germinating period:

Enzyme activity of rice barley, chickpea and jackfruit seed during different germinating period were summarized in table-4.5.

Table-4.5: α-amylase, and protease activities of rice, barley, chickpea and jackfruit seed during the period of germination

Relative activity	Variety	Duration of germination			
		0 hrs	24 hrs	48 hrs	72 hrs
α-amylase (unit/gm)	Rice (BRRI Dhan 28)	12.52	18.94	12.28	968
	Barley (BARI Barley 6)	13.26	18.67	22.93	13.27
	Chickpea (BARI Chola 5)	2.32	6.08	5.52	3.22
	Jackfruit Seed (Local variety)	4.26	6.01	8.78	4.81
Protease (unit/gm)	Rice (BRRI Dhan 28)	0.45	1.15	1.12	0.63
	Barley (BARI Barley 6)	0.56	0.62	0.88	0.58
	Chickpea (BARI Chola 5)	4.64	12.96	11.16	8.38
	Jackfruit Seed (Local variety)	1.42	1.59	1.94	1.15

4.5.1: Changes of α-amylase activity:

The α-amylase activity of the rice, barley, chickpea and jackfruit seed were presented in the Table-4.5.

During the different germination period the highest α-amylase activity was found at 24 hours in rice (18.94 unit/gm) while the amylase activity was decreased drastically from 48-72 hours due to germination. The present findings indicated that amylase activity increased 51.27% at 24 hours of germination and thereafter decreased. The maximum decrease was 22.68% at 72 hours of germination.

The highest α-amylase activity was found at 48 hours in barley (22.93 unit/gm) while the amylase activity was decreased drastically from 72 hours due to germination. The present findings indicated that amylase activity increased 72.92% at 24 hours of germination and thereafter decreased.

The highest α-amylase activity was found at 48 hours in jackfruit seed (8.78 unit/gm) while the amylase activity was decreased drastically from 72 hours due to germination. The present findings indicated that amylase activity increased 106.10% at 24 hours of germination and thereafter decreased. The maximum decrease was 12.91% at 72 hours of germination. The highest α-amylase activity was found at 48 hours in jackfruit seed (8.78 unit/gm) while the amylase activity was decreased drastically from 72 hours due to germination. The present findings indicated that amylase activity increased 106.10% at 24 hours of germination and thereafter decreased. The maximum decrease was 12.91% at 72 hours of germination.

In cereal grains, germination increase oligosaccharides and amino acids concentration as observed in barley (Rimsten *et al.*, 2003), wheat (Yang *et al.*, 2001), oat (Mikola *et al.*, 2001) and rice (Manna *et al.*, 1995). It was established that the amount of dry matters reduced in germinated wheat and the content of mineral substances, protein and activity of ferments α-and β-amylases, cellulase, proteases and maltase was greater than in non germinated wheat, observed by Kraujutiene *et al.* (2010). Results of the present study are in accord with their findings.

A graphical presentation of Changes of iron content of rice, barley, chickpea and jackfruit seed during different germinating period is shown below:

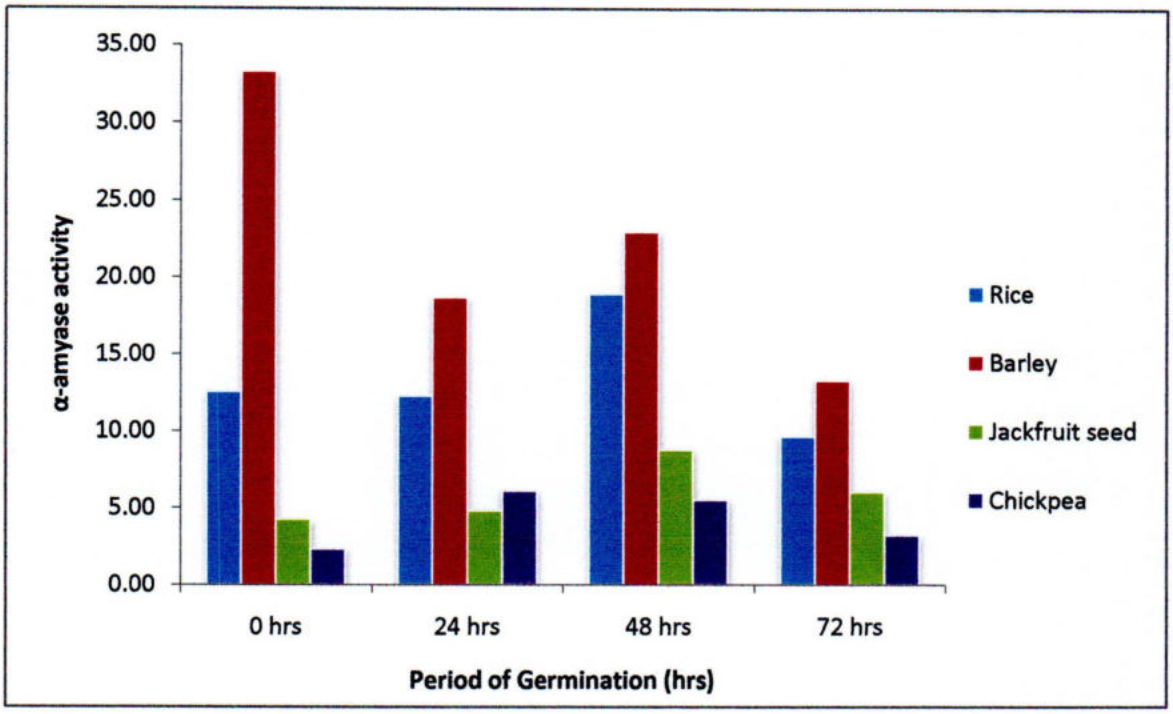


Figure 4.5: α-amylase activity of rice, barley, chickpea and jackfruit seed during the period of germination

Germination of seeds lead to breakdown of seeds reserves reported by Vanderstoep, (1981) and increased enzyme activity that leads to a loss of total dry matter and an increase in total protein observed by Lorenz, (1980). During the different germination period the highest α-amylase activity was found at 24 hours in BARI chola-5 (6.08 unit/gm) while the amylase activity was decreased drastically from 48-72 hours due to germination. The present findings indicated that amylase activity increased 162.06% at 24 hours of germination and thereafter decreased. The maximum decrease 38.65% at 96 hours of germination. This result is in agreement with Liza *et al.* (2010) who reported that the α-amylase activities increased at 24 hours of germination of chickpea and then decreased drastically from 48 hours.

4.5.2: Changes of protease activity:

Protease activity of the rice, barley, chickpea and jackfruit seed were presented in the Table-4.5.

During the different germination period the highest protease activity was found at 24 hours in rice (1.15 unit/gm) while the amylase activity was decreased drastically from 48-72 hours due to germination. The present findings indicated that amylase activity increased 155.55% at 24 hours of germination and thereafter decreased. The maximum decrease was 40% at 72 hours of germination.

The protease activity of barley BARI-6 was highest (0.88 unit/gm) at 48 hours of germination while the protease activity was decreased drastically from 72 hours due to germination. The present findings indicated that protease activity increased 57.14% at 48 hours of germination and the maximum decreased 3.57% at 72 hours of germination.

The highest protease activity was at in BARI chola-5 (10.96 unit/gm) at 24 hours of germination while the protease activity was decreased drastically from 48-72 hours due to germination. The present findings indicated that protease activity increased 136.20% at 24 hours of germination and thereafter decreased. This result is in agreement with Liza *et al.* (2010) who reported that the α-amylase activities increased at 24 hours of germination of chickpea and then decreased drastically from 48 hours.

Jackfruit seed showed the highest protease activity at 48 hours of germination (1.94 unit/gm) while the protease activity was decreased drastically from 72 hours of germination. The present findings indicate that protease activity of jackfruit seed maximum increased 36.61% at 48 hours of germination and at 72 hours it was decreased 19.01%.

A graphical presentation of Changes of iron content of rice, barley, chickpea and jackfruit seed during different germinating period is shown below:

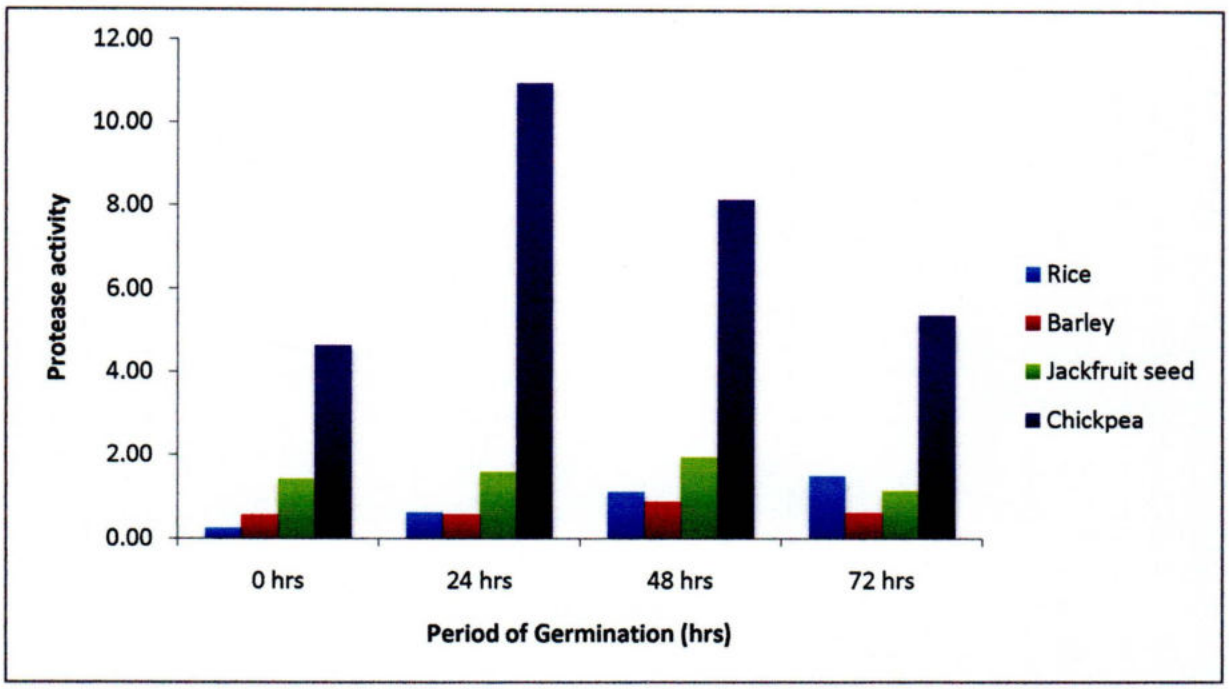
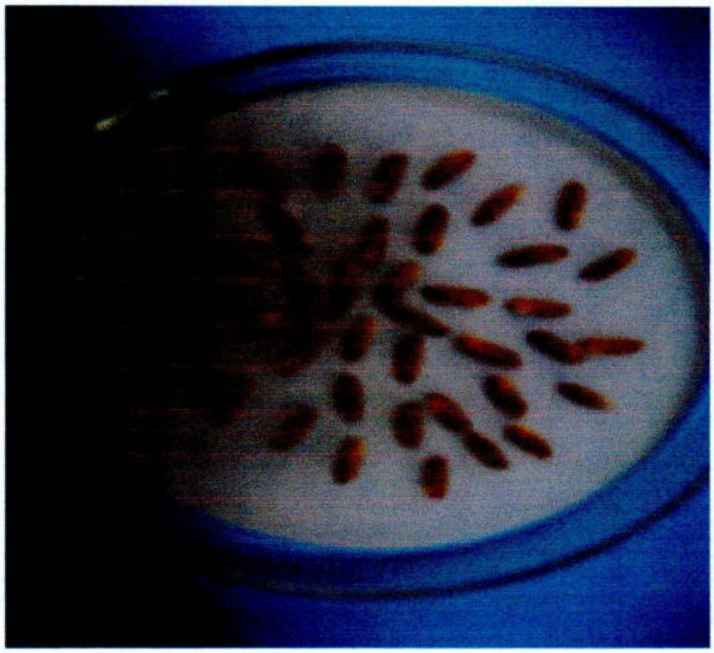
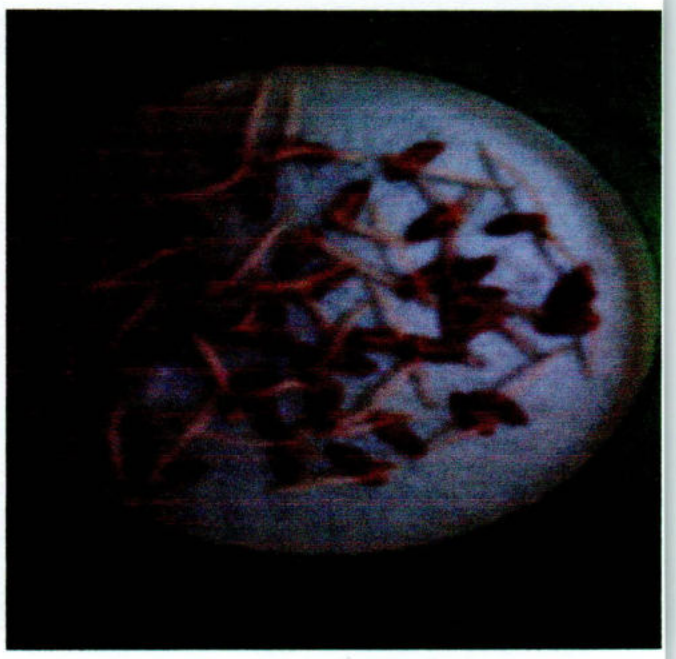


Figure 4.6: Protease activity of rice, barley, chickpea and jackfruit seed during the period of germination

Taraseviciene *et al.* (2009) mentioned that during germination metabolic enzymes are activated and utilization or synthesis of wide range of chemical compounds occur in seeds and results in the enhancement of nutritional quality. The protease activity of mugbean increased tremendously i.e from 131% to 161 % at 24 hours of germination and thereafter decreased Rahman *et. al.* (2007). α-amylase and protease activity of seeds increased at 24 or 48 hours of germination and then decline gradually (Kooshiba and Minamikawa, 1983). Here it is found that of four seeds were found to be increased tremendously at 24 or 48 hours of germination and then decline gradually which are in agreement with the results.



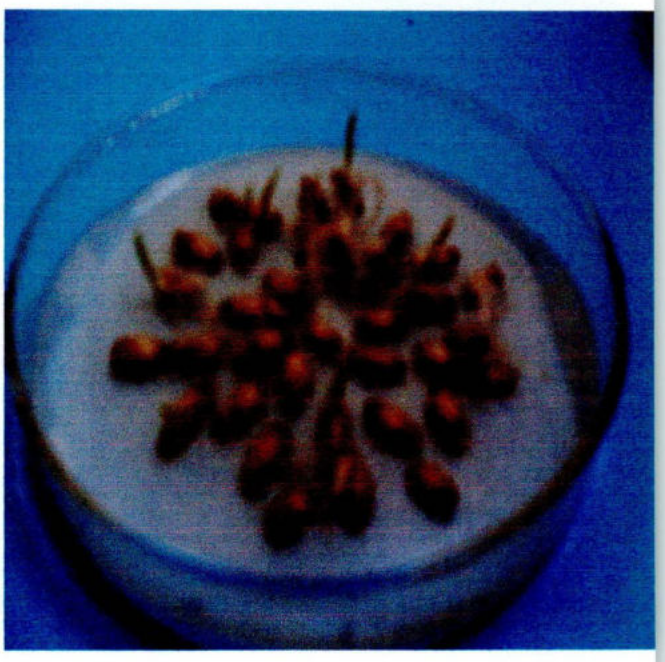
Photograph 5: 24 hours germinated rice



Photograph 6: 48 hours germinated rice

Photograph 7: 72 hours germinated rice

Photograph 5: 24 hours germinated barley



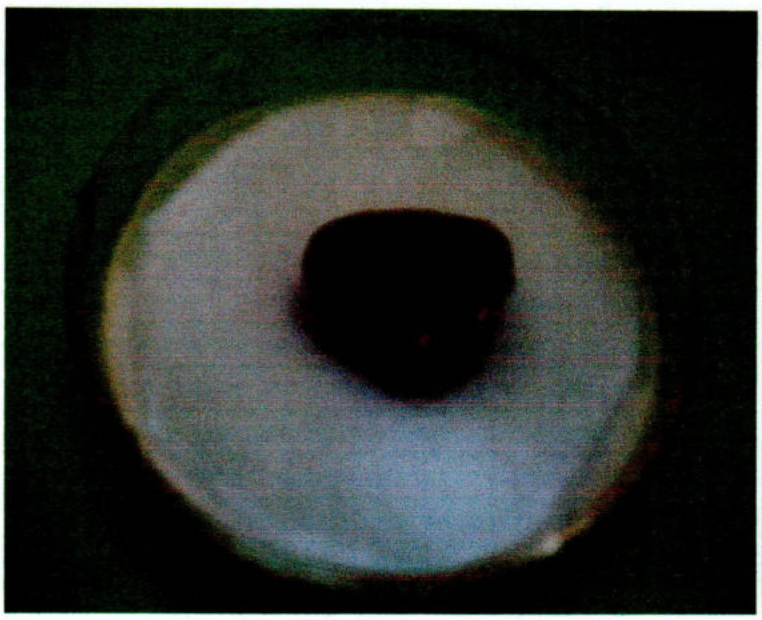
Photograph 6: 48 hours germinated barley

Photograph 7: 72 hours germinated barley

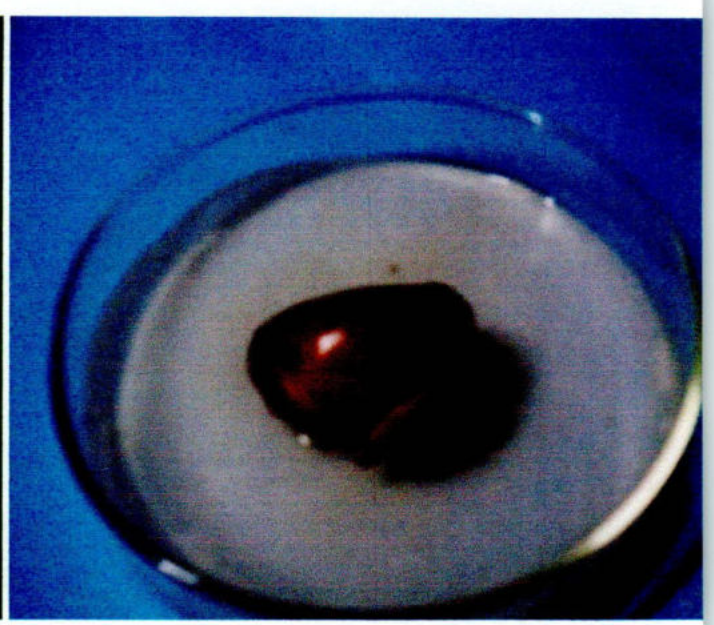
Photograph 8: 24 hours germinated chickpea

Photograph 9: 48 hours germinated chickpea

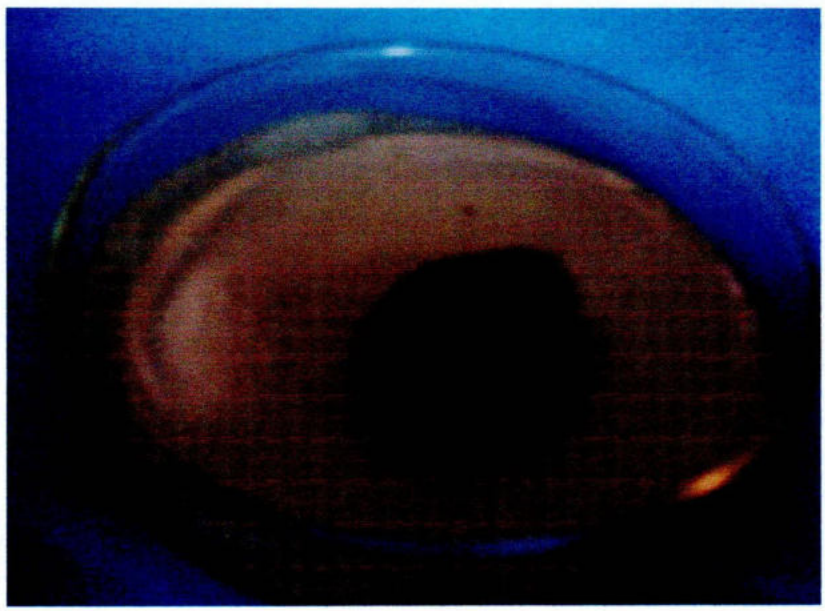
Photograph 10: 72 hours germinated chickpea



Photograph 11: 24 hours germinated Jackfruit seed



Photograph 12: 48 hours germinated Jackfruit seed



Photograph 13: 72 hours germinated jackfruit seed

4.6: Effect of Physico-Chemical Agents on the Stability of Enzymes of Chickpea:

4.6.1: Effect of Calcium

Table-4.6: Effect of calcium on the activities of α-amylase and protease

Concentration of $CaCl_2$(Molar)	Relative activities	
	α-amylase	Protease
0.000	100.00	100.00
0.001	105.00	105.97
0.005	109.30	107.33
0.010	111.75	110.93
0.050	116.25	115.21
0.100	121.62	118.03
0.300	128.50	122.00
0.500	133.00	127.05

The effect of calcium as metallic salt on the activities of α-amylase and protease is presented in the Table-4.6. The activities of enzymes were gradually increased with the increasing concentration of calcium and in the presence of 0.50M Ca^{2+}, the activities of α-amylase and protease became 133.00% and 127.05% respectively.

4.6.2: Effect of EDTA

Table-4.7: Effect of EDTA on the activities of α-amylase and protease

Concentration of EDTA(Molar)	Relative activities (%)	
	α-amylase	Protease
0.000	100.00	100.00
0.001	82.87	77.35
0.005	65.55	58.98
0.010	51.39	45.82
0.100	33.03	29.58
0.300	13.15	11.08
0.500	0.00	0.00

The relative activities of α-amylase and protease in presence of different concentrations of EDTA are presented in Table 4.7. The activities of α-amylase and protease are abolished gradually with the increase of EDTA concentrations and the enzymes lost their activities completely in the presence of 0.50M EDTA

4.6.3: Effect of various metallic salts:

Table-4.8: Effect of various metallic salts on the activities of α-amylase and protease

Test Salt		α-amylase	Protease
None		100.00	100.00
$MgCl_2$	0.001	104.10	104.55
	0.002	106.83	106.73
$ZnCl_2$	0.001	83.71	94.55
	0.002	75.29	90.70
$CuCl_2$	0.001	87.25	72.83
	0.002	77.10	55.15
$MnCl_2$	0.001	115.21	111.10
	0.002	124.65	119.20
NaCl	0.001	100.45	100.20
	0.002	99.91	99.17
KCl	0.001	100.00	100.00
	0.002	100.00	100.00
$FeCl_2$	0.001	68.81	63.41
	0.002	56.90	48.80

Table 4.8 represents the effect of various metallic salts on the activities of α-amylase and protease. The activities of enzymes were increased remarkably in the presence of Mn^{2+} salts while that was increased slightly in the presence of Mg^{2+} salts. Other metallic salts such as K^+ and Na^+ produce little or no inhibitory on the activities of the enzymes but the activities of all the enzymes reduce significantly in the presence of Zn^{2+},Cu^{2+} and Fe^{2+}. The activities of the enzymes increased significantly in the presence of divalent cation Ca^{2+} and Mn^{2+}suggesting the involvement of these divalent ions in maintaining the active conformation of the enzymes.

4.6.4: Optimum pH and temperature of α-amylase:

The activity of α-amylase at various pH values that varying from 2.0 to 10.0 are shown in the figure-4.7. The activity of the enzyme was greatly influenced by the pH. The α-amylase maximum activity at pH 6.8 and the activity were found to decrease more rapidly on the alkaline side than the acetic side. The buffers used were as follows:

pH 2.0 — 2.5 = KCl — HCl, pH 3.0 — 4.0 = AcONa — HCl

pH 4.5 — 5.5 = AcONa — CH_3COOH

pH 6.0 — 8.0 = NaH_2PO_4 — Na_2HPO_4

pH 8.5 — 9.0 = $Na_2B_4O_7$ — HCl, pH 9.5 — 10.0 = $Na_2B_4O_7$ — Na_2CO_3

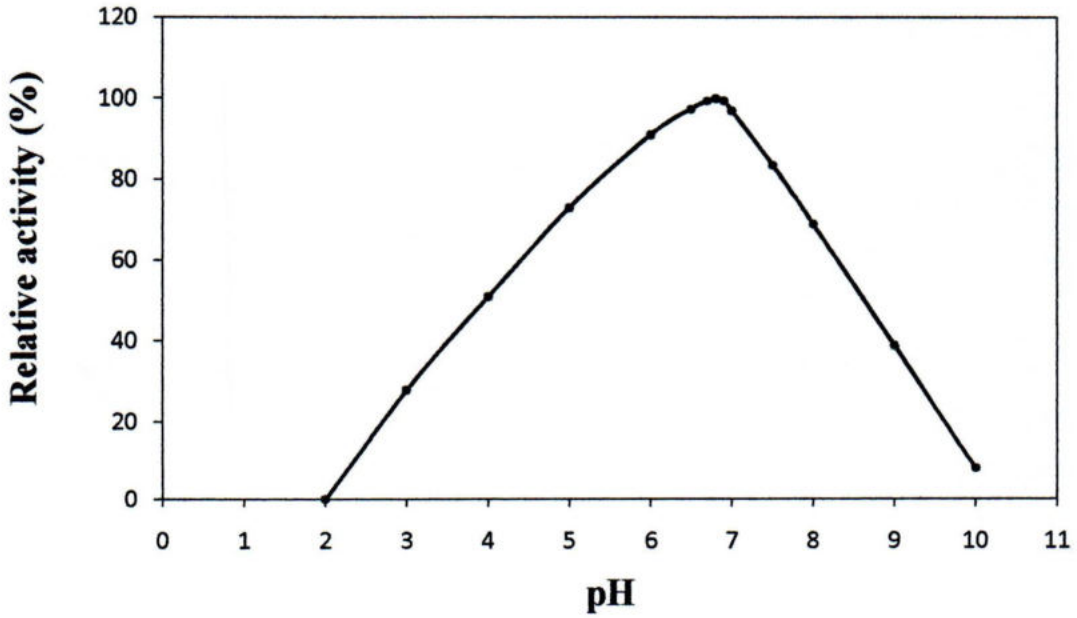


Figure 4.7: Effect of pH on the activity of α-amylase

The activity of α-amylase was found to be affected greatly with the changes of pH as well as temperature and the optimum pH and temperature reported in the study were very similar to that reported for α-amylase from poplar leaves, pH 6.5 (witt *et.al.*1996) and banana pulp (pH 6.9 and 38°C, Mao and Kinsella, 1981).

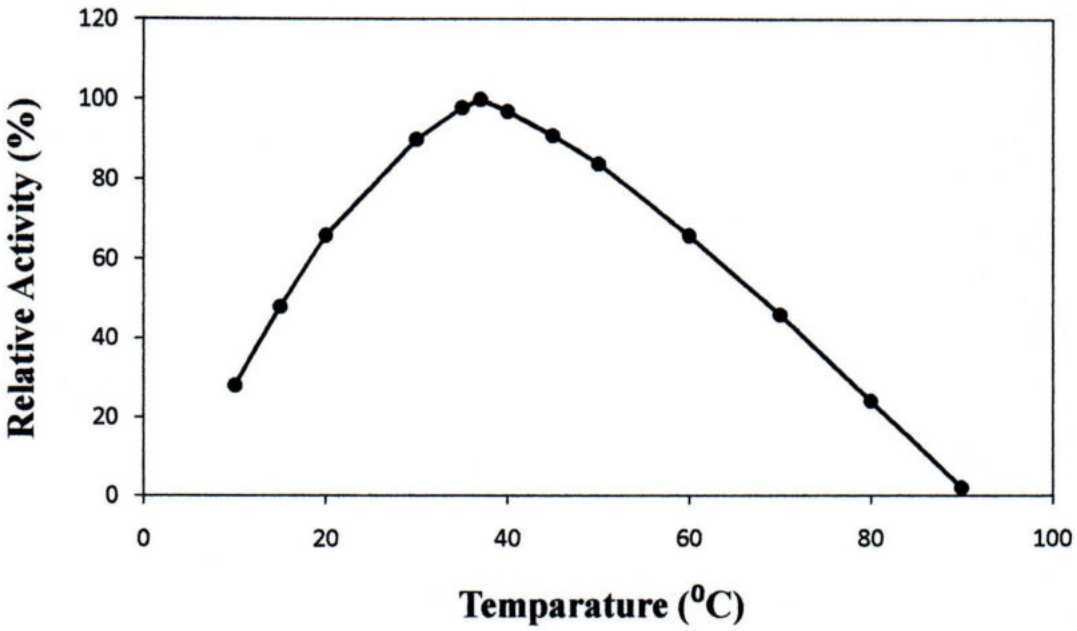


Figure 4.8: Effect of temperature on the activity of α-amylase

As shown in figure 4.8, the activity of the enzyme was also greatly influenced with the changes of temperature. The activity of α-amylase was increased rapidly with the rise of temperature and the maximum activity was observed at 37°C. With further rise of temperature, the activity of enzyme was decreased gradually and the enzyme lost about 76% of its activity at 80°C.

4.6.5: Optimum pH and temperature of protease

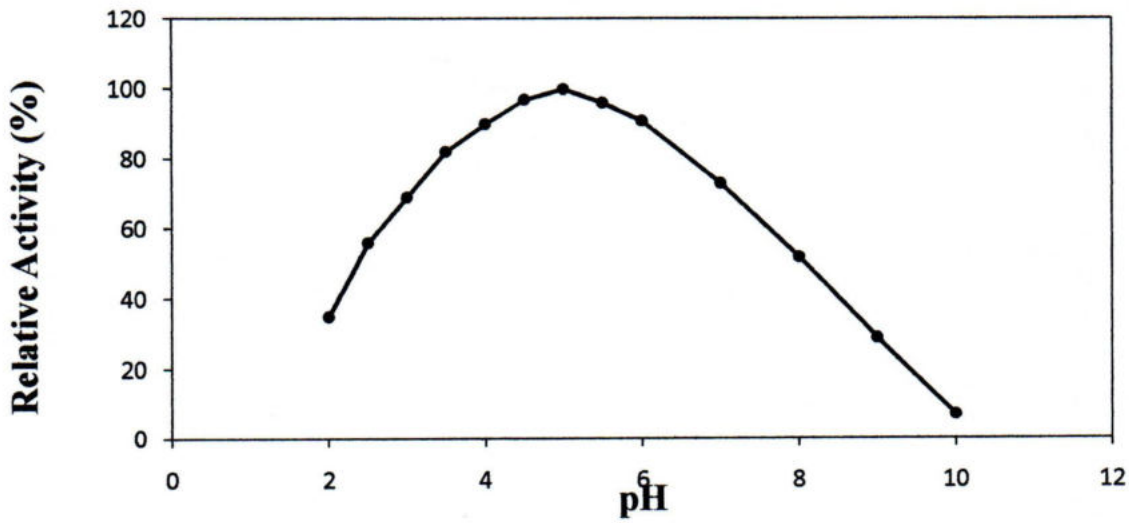


Figure 4.9: Effect of P^H on the activity of protease

The activity of protease at various pH values that varying from 2.0 to 10.0 are shown in the figure 4.9. The activity of the enzyme was found to be greatly affected by the pH changes. The protease gave maximum activity at pH 5.0. Further the activity was found to be decreased more rapidly above or below the optimum pH value.

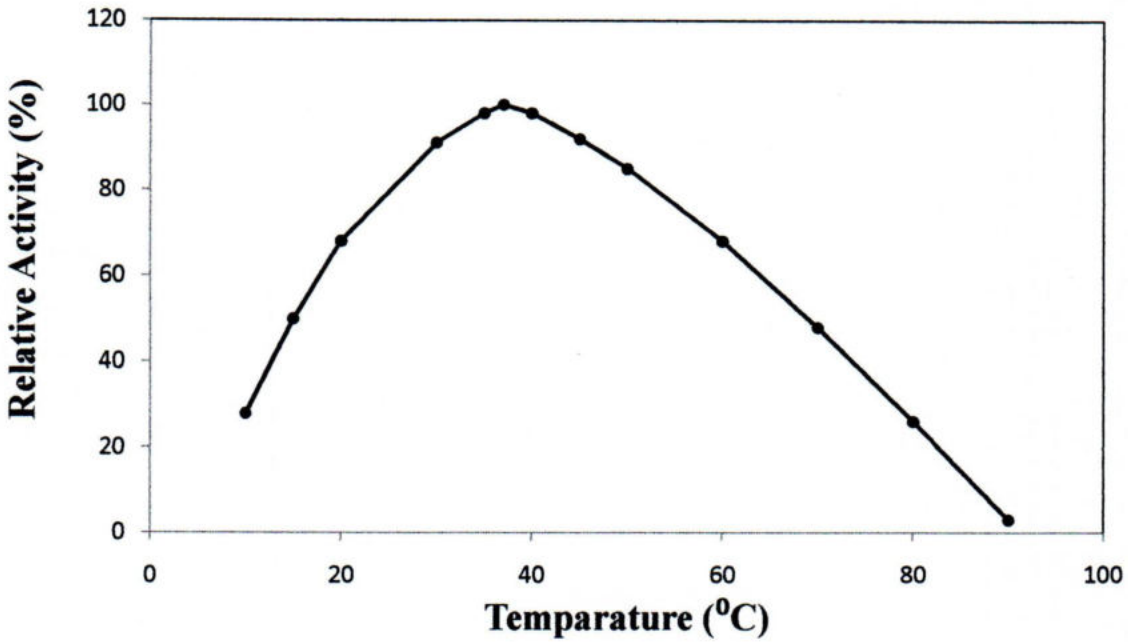


Figure 4.10: Effect of temperature on the activity of protease

As shown in figure 4.10, the activity of the enzyme was also greatly influenced with the changes of temperature. The activity of protease was increased rapidly with the rise of temperature and the maximum activity was observed at 38°C. With further rise of temperature, the activity of enzyme was decreased gradually and the enzyme lost about 74% of its activity at 80°C.

The activity of protease was greatly influenced with the changes of pH and temperature. The enzyme gave optimum pH value at the moderate acidic region, suggesting that the enzyme is acidic type. The changes in activity with the changes of pH might be associated with the ionization of groups located at the active site, which may be responsible for binding with the substrate. The decreased in activity of the enzyme at higher temperatures might be due to destruction of secondary or tertiary structure.

CHAPTER 5

SUMMARY AND CONCLUSION

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SUMMARY AND CONCLUSION

The study was conducted in the Laboratory of the Food enzymology of Institute of Food Science and Technology (IFST) at Bangladesh Council of Scientific and Industrial Reserch (BCSIR). The purpose of this investigation was to extensively study the effect of germination on the changes of the protein content and mineral content of rice, barley, chickpea and jackfruit seed. It also aimed at evaluating the enzymatic activity (α-amylase, protease) of rice, barley, chickpea and jackfruit seed at different germinating period. Moisture and proximate composition of raw rice, barley, chickpea and jackfruit seed were also observed. Another important objective of this study was to at evaluating the physico-chemical agents on the stability of enzymes of chickpea.

The proximate composition of rice, barley, chickpea and jackfruit seed were determined. The moisture content of rice, barley, chickpea and jackfruit seed were 14.70%, 14.06%, 10.20%, 63.40% respectively. The ash, protein, fat carbohydrate and energy content of rice were observed 0.40%, 6.86%, 0.65%, 77.39% and 342.85 kcal respectively. The barley contained 2.00% ash, 11.20% protein, 2.00% fat, 70.74% carbohydrate and 345.76 kcal energy per 100 gm. The chickpea contained 3.42% ash, 17.80% protein, 5.45% fat 63.04% carbohydrate and 373.22kcal energy per 100 gm. The ash, protein, fat carbohydrate and energy content of jackfruit seed were 1.80%, 7%, 0.40%, 27.40% and 141.20 kcal respectively. The calcium, phosphorus and iron content of rice were 13.23 (mg/1000gm), 184.00 (mg/100gm) and 5.45 (mg/100gm) respectively. Calcium, phosphorus and iron content of barley were 28.23 (mg/100gm), 248.00 (mg/100gm) and 18.80 (mg/100gm) respectively. The chickpea contained 260.80 (mg/100gm) calcium, 365.22 (mg/100gm) phosphorus and 18.80 (mg/100 gm) irons. The jackfruit seed contained 96.19 (mg/100gm) calcium, 160.00 (mg/100gm) phosphorus and 2.95 (mg/100 gm) irons.

The changes in the protein content, minerals content and enzyme activity of rice, barley, chickpea and jackfruit seed were analyzed at different hours of germination. Protein content of rice and chickpea were increased 16.21% and 17.68% respectively at 24 hours then decreased drastically from 48-96 hours of germination. The protein content of barley

and jackfruit seed were increased 31.25% and 23% respectively at 48 hours of germination.

Calcium, phosphorous and iron content of rice were increased 59.41%, 50% and 32.48% respectively at 24 hours of germination and then decreased gradually from 48-72 hours of germination. Calcium, phosphorous and iron content of barley were increased 62.98%, 51.78% and 34.86% respectively at 48 hours of germination and then decreased gradually from 72 hours of germination. Calcium, phosphorous and iron content of chickpea were increased 41.27%, 49.02% and 63.03% respectively at 24 hours of germination and then decreased gradually from 48-72 hours of germination. The calcium, phosphorous and iron content of jackfruit seed were increased 116.60%, 186.00% and 31.53% respectively at 48 hours of germination and then decreased gradually from 72 hours of germination.

The α-amylase and protease activity of rice were tremendously increased 51.27% and 155.55% respectively at 24 hours of germination and decreased gradually from 48-72 hours of germination. The α-amylase and protease activity of chickpea were tremendously increased 162.06% and 136.20% respectively at 24 hours of germination and decreased gradually from 48-72 hours of germination. The α-amylase and protease activity of barley were tremendously increased 72.93% and 57.14% respectively at 48 hours of germination and decreased gradually from 72 hours of germination. In jackfruit seed the α-amylase and protease activity tremendously increased 106.10% and 36.61% respectively at 48 hours of germination and decreased gradually from 72 hours of germination.

The enzymatic activities of α-amylase and protease from chickpea were investigated after physical and chemical treatments. The enzymes: α-amylase and protease have optimum P^H 6.8 and 5.0; and optimum temperature 37°C and 38°C respectively.

The activities of enzymes were increased in presence of metallic salts such as Ca^{2+}, Mg^{2+} and Mn^{2+} while Fe^{2+}, Zn^{2+} and Cu^{2+} inhibited the activities moderately. The activities of all the enzymes were completely inhibited in the presence of higher concentration of EDTA.

Since germination is cheap and more effective in improving nutritional value, it is hoped that this can contribute to nutrition of people. Cereals and legumes were used as these food groups provide significant amount of macro- and micronutrients to human. Although cereal grains and legumes constitute a major source of dietary nutrients all over the world, these are deficient in some basic components (e.g. essential amino acids) and germination may be the most simple and economical way of improving their nutritional value, sensory properties, and functional qualities. The effect of germination on the chemical and biochemical constituents of seeds vary greatly with plant species, seed varieties and the germination period. Usage and intake of germinated legumes and grains are not common locally. Overall, the protein and mineral content of rice, barley, chickpea and jackfruit seed changed after germination and may be used as alternate resources for individuals.

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