Effect of Pre-cooking and Cooking methods on Phytate content in selected legumes

By

ASWATHY.S

(17PFD007)

A Thesis submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043

In partial Fulfillment of the Requirement for the

Degree of Master of Science in Food Service Management and Dietetics

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Saradha Ramadas 22.04.2019
Signature of the Head of the Department

22/4/19
Signature of the Supervisor

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CHAPTER I

INTRODUCTION

As defined by world health organization (WHO), health is a "state of complete physical, mental, and social well being, and not merely the absence of disease or infirmity." Health is a dynamic condition resulting from a body's constant adjustment and adaptation in response to stresses and changes in the environment for maintaining an inner equilibrium called homeostasis (WHO-2006).

Health and nutrition are important as ends in themselves and often are emphasized as critical components of basic needs in developing contries. Comparison of nation to nation standard data suggest that on the average health and nutrition in the developing world falls considerably short of that in the developed world(Behrman *et al.,1988).*

Over the last 20 years, nutrition of infants and young children has received a great deal of attention. The problems of under nutrition and the deficiency disease of children which followed the growing realization that these constitute one of the major medical and social problems faced by developing countries (Fomon 1967). In case of adults pre-existing micronutrient (vitamins and trace elements) deficiencies are often present in hospitalized patients. Deficiencies occur due to inadequate or inappropriate administration, increased or altered requirements, and increased losses, affecting various biochemical processes and resulting in organ dysfunction, poor wound healing, and altered immune status. Most of the enteral nutrition preparations also contain adequate amounts of vitamins and minerals, although bioavailability may be an issue. (Sriram *et al.,* 2009).

Minerals and micronutrient deficiencies are an important global health problems which affects around two billion people worldwide (WHO 2015). According to various studies some common minerals which cause deficiency disorders are iron, zinc, iodine and calcium. Deficiency of some minerals is measured directly by estimating mineral concentration in blood, urine or other body tissues. Food consumption patterns can be used to estimate the possible risks of mineral deficiency (Kumssa *et al.,* 2015).

Malnutrition and micro-nutrient deficiencies, continues to be a major health burden in developing countries. It is globally the most important risk factor for illness and death, with hundreds of millions of pregnant women and young children particularly affected (Muller 2015).

Mineral deficiency in developing countries is observed due to lack of nutritious food consumption or due to large consumption of plant based diet. These can lead to insufficient intake of minerals such as iron, calcium etc. Also the intake of anti-nutrients present in foods often leads to minerals and other micronutrient deficiencies. India's life expectancy has more than doubled and infant mortality, halved in the last fifty years. Ironically India has the highest number of malnourished people and our child malnutrition rate is dangerously high. With one sixth of the global population residing in India, one third of about two billion people suffering from vitamin and micronutrient deficit are in India (Kotecha 2008).

The wide spread prevalence of mineral deficiency (iron and zinc) among Indians is not due to the poor mineral content in their diets. It is the poor bioavailability of those minerals that makes the largely vegetarian population deficient in these nutrients. So the research field of India should be more focused on bio availability rather than bio fortification. The scientists say the intake of Fe and Zn through food sources is quite adequate but they are not available to the body since the phytic acid in vegetarian food binds with these minerals in the digestive tract, making their absorption difficult (ICAR 2017).

In 2012 the 'Times of India' reported that at least 20,000 children under the age of six in Tamil Nadu are so severely malnourished that their growth will be permanently stunted if immediate action is not taken. Though the numbers are dismal, Tamil Nadu fares better compared to other states. According to the report, which has figured up to march 2012,Tamil Nadu has the third highest number of children with normal weight among the big states, behind Madhya Pradesh and Maharashtra.

Anti-nutrients are plant compounds that reduce the body's ability to absorb essential nutrients. The most widely studied anti-nutrients include: phytate (phytic acid) mainly found in seeds, grains and legumes, it reduces the absorption of minerals from a food. Anti-Nutritional Factors (ANF) are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods or animal feeds and they play a vital role in determining the use of plants for humans and animals. There are reports from time to time of deaths after consumption of some type of beans despite cooking. (Soetan *et al., 2009)*

Anti-nutrients commonly found in plant foods have both adverse effects and health benefits. For example, phytic acid, lectins, phenolic compounds (tannins), saponins and enzyme (amylase and protease) inhibitors have been shown to reduce the availability of nutrients and growth inhibition. However certain compounds like lignins are also found to be helpful in reducing blood glucose (Thompson 1993). Anti-nutrients in foods are responsible for deleterious effects related to the absorption of nutrients and micronutrients. However, some anti-nutrients may exert beneficial health effects at low concentrations. The mechanism by which adverse and beneficial effects of food anti-nutrients operate are the same. Thus, manipulation of processing conditions and removal of certain unwanted components of foods may be required (Shahidi 1997).

Phytic acid is the primary source of inositol and storage phosphorous in plant seeds contributing 70% of the total phosphorous. The abundance of phytic acid in cereal grains is a concern in foods and animal feed industries because the phosphorous in this form is un available for absorption. Phytic acid is known as the food inhibitor which chelates micronutrient and prevents it to be bio available and prevent it to be bio available for monogastric animals, including humans, they lack enzyme phytase in their digestive tract (Higuchi 2014).

Phytic acid is the storage form of phosphorous is one of those anti-nutrients the paleo community telling you to avoid. It is often considered as an anti nutrient because it binds to minerals in the digestive tracts and making them less available to our bodies. Plant food such as nuts , beans, legumes and grains stores phosphorous as phytic acid. When phytic acid is bound to a mineral in the seed it is known as phytate.

The lack of dietary diversity can often leads to an insufficient intake of minerals. While the intake of phytic acid in higher quantities also leads to this disease conditions.Phytic acid is a potent inhibitor of native and fortification iron absorption and low absorption of iron from cereal and or legume based complimentary foods is a major factor in the etiology of iron deficiency in infants. Dephytinization of complimentary foods and legumes based infant formulas are technically possible but as phytic acid is strongly inhibitory at low concentrations (Hurrel 2004).

Several methods have been developed to reduce the phytic acid content in food and improve the nutritional value of food which becomes poor due to such anti nutrients. These include application of several pre-cooking and cooking methods.

Selection of foods with low phytic acid content or application of proper cooking and pre-cooking methods can reduce the interfering action of this anti-nutrient in absorption of minerals and micronutrients. The study was conducted to identifi the effect of leaching on phytic acid content in legumes and to find a better method to reduce the same antinutrient in legumes. Apart from these legumes containing good amount of phytic acid and effect of soaking and germination on this antinutrient was identified during the study period. For the study purpose some legumes where selected such as soybean, kidneybean, pintobean and peanut, which are having good amount of phytic acid content compared to other legumes. The phytic acid content was estimated before the application of any pre-cooking or cooking method. In selected quantities of selected legumes treatments like leaching, soaking, boiling, sprouting and steaming are applied to check their effect on phytic acid. Mineral, phytate are much higher in bran of certain cereals (Guttieri *et al.,* 2003)

The researchers say the phytic acid content among the predominantly vegetarian Indian population is relatively high. Methods for improving the phytic acid content of Indian foods should be prime place in the nutritional programmes and extention activities. Hence the study is put forward with the objectives,

- To find legumes which are having higher phytic acid content
- To find out the better method to reduce phytic acid
- Observe phytic acid content during different pre-cooking and cooking process

CHAPTER II
REVIEW OF LITERATURE

The topic entitled 'Effect of pre-cooking and cooking methods on phytate content in selected legumes' is dicussed under the following headings

2.1. Nutritional deficiency among humans

2.2. Anti-nutrients and nutritional bioavailability

2.3 Phytic acid as an antinutrient in plant foods

2.4 Effect of pre-cooking and cooking on phytic acid in plant foods.

2.1. NUTRITIONAL DEFICIENCY AMONG HUMANS

During last fifty years, the life expectancy in India has doubled and infant mortality halved. The range of progression in the fields of economy and politics is enormous. However, paradoxically, India has the highest number of malnourished people and the child malnutrition rate is unacceptably high. One third of the two billion people in India are suffering from vitamin and micronutrient deficiency (Kotecha, 2008).

Stein (2010) carried out a study on 'Global impacts of human mineral malnutrition'. It says that, malnutrition in the form of insufficient energy intake but the study mainly focuses on the "hidden" form of malnutrition, namely mineral malnutrition. It explains about the effect of disease that caused by mineral deficiency and the social and economic consequences to which they lead.

Studies done by National Nutrition Monitoring Bureau and National Institute of Nutrition, indicates that the diets of our rural population are inadequate and deficient in most of the nutrient. About 60% of the pre-school children are underweight. About 15% of children of 1-5 year of age suffer from short duration malnutrition (Rao, *et al.,* 1998)

According to Gupta *et al.,* (2015), more than half of the population is affected by micro-nutrient malnutrition and one third of world's population suffers from anaemia and zinc deficiency, particularly in developing countries. Iron and zinc deficiencies are the major health problems worldwide.

Hassan *et al.,* (2011) highlights on the report that, plants are the major sources of microelements to populace of the developing world. However, presence of anti-nutritional factors limits their optimal utilization. The results of the study states that the plant leaves are having more anti-nutrient contents like tannin.

Micronutrient malnutrition affects more than half of the world population, particularly in developing countries. Iron, zinc and vitamin A deficiencies are most serious health constraints worldwide (Jorge *et al.,* 2008).

Minerals are specific kinds of nutrients that our body needs in order to function properly. A mineral deficiency occurs when our body doesn't obtain or absorb the required amount of a mineral. Deficiencies can lead to a variety of health issues like weak bones, fatigue, or a decreased immune system (Butler 2016).

According to Stein *et al.,* (2007) the calculated annual burden of zinc deficiency in India is 2.8 million DALYs lost. Zinc bio-fortification of rice and wheat may reduce this burden by 20- 51%. Not only zinc bio-fortification save lives and prevent morbidity among millions of people, but it may also help accommodate the need to economise and to allocate resources more effectively.

Sommer *et al.,* (1983) states that, an average of 3481 pre-school-age rural Indonesian children were re- examined every 3 months for 18 months. The mortality rate among children with mild xerophthalmia was on average 4 times the rate, and in some groups 8 to 12 times the rate, among children without xerophthalmia.

WHO (2005) explains that the dietary requirement for a micronutrient is defined as an intake level which meets a specified criteria for adequacy, thereby minimizing risk of nutrient deficit or excess. These criteria cover a gradient of biological effects related to a range of nutrient intakes which, at the extremes, include the intake required to prevent death associated with nutrient deficit or excess.

Gupta *et al.,* (2014) opines that, mineral nutrients are fundamentally metals and other inorganic compounds. Animals and human obtain minerals from plant sources. Humans cannot utilize most foods without critical minerals and enzymes responsible for digestion and absorption. To protect humans from mineral nutrient deficiencies, the key is to consume a verity of foods in modest quantities.

Prentice *et al.,* (1994) gives the evidence on the relationship between dietary mineral supply and bone development in children. These data suggest that overt deficiencies of Ca, P and Zn are likely to produce rickets and growth retardation, while the effects of magnesium deficiency on human bone are unknown.

Brown *et al.,* (2001) summarises the recent research on the importance of zinc for human health. Many methods like FAO food balance sheets where used to estimate the zinc deficiency in people. The results of these analysis indicates that nearly half of the world's population is at risk for inadequate zinc intake, suggesting that public health programs are urgently needed to control zinc deficiency.

Nutritional anemia is one of India's major public health problems. The prevalence of anemia ranges from 33% to89% among pregnant women and is more than 60% among adolescent girl. Under the anemia prevention and control programme of the Government of India, iron and folic acid tables are distributed to pregnant women, but no such programme exists for adolescent girls (Toteja *et al.,* 2006).

Singh *et al.,* (2012) says that, the finger millet is the richest source of calcium and iron. Calcium deficiency leading to bone and teeth disorder, iron deficiency leading to anaemia can be overcome by introducing finger millet in daily diet. Maximum utilization of the millet is limited by the presence of phytates, phenols, tannins and enzyme inhibitors.

According to Flyman *et al.,* (2006) micro-nutrient deficiency is a universal problem, which presently affects over two billion people worldwide, resulting in poor health, low worker productivity, high rates of mortality and morbidity. Deficiency in micronutrients has led to increased rats of chronic diseases and permanent impairment of cognitive abilities in infants born to micronutrient deficient mothers.

Welch *et al.,* (2004) explains that, over three billion people are currently micro-nutrient malnourished, resulting in social costs including learning disabilities among children, increased morbidity and mortality rates, lower worker productivity, and high health care costs, all factors diminishing human potential, felicity, and national economic development.

2.2. ANTI-NUTRIENTS AND NUTRITIONAL BIOAVAILABILITY

According to Bandari *et al.,* (2003) the Nepalees wild yams may present a health-hazard potential, which intern demands proper processing before consumption to eliminate the effects of the anti-nutrients. The anti-nutrients which dominated in this yam species were, oxalates, phytate, tripsin inhibitor etc.

Ertop *et al.,* (1996) openes that, most of plant foods nuts and cereals contains anti-nutrient compounds. They reduce the mineral bio-availability and protein absorption of foods thanks to their chelating properties. They causes to micronutrient malnutrition and mineral deficiencies. Anti-nutrients reduce the maximum utilization of nutrients, and as consequence they obstruct an optimal bio availability of the nutrients present in a food and decrease its nutritive value.

The mineral content of legumes is generally high, but the bioavailability is poor due to the presence of phytate which is a main inhibitor of Fe and Zn absorption (Sandberg 2008). Some legumes also contain considerable amounts of Fe binding poly phenols inhibiting Fe absorption

Sharma *et al.,* (2013) experimented with high yielding cultivars of chick pea and lentils and analysed for their total ineral content, percent availability of minerals and level of anti-nutrients. Certain verities like, 'kabuli chick pea' was found superior. These cultivars contain highest percent availability of calsium and iron and lowest values of phytic acid and and poly-phenoles compared to other cultivan of chick pea and lentil.

The food constituents, such as dietary fiber, polysaccharides, oxalates, and polyphenolic compounds, may also play a major role in mineral bioavailability. Dietary fiber in whole wheat bread accounts for most of the poor availability of minerals. Davies *et.al* suggested that phytate rather than fibre largely determines the availability of zinc absorption Davies *et.al (*1977).

Gilani *et al.,* (2012) opines that the digestability of protein in traditional diets from developing countries such as India, Guatemala, and Brazil is considerably lower compared to the diet in developed countries. Food and feed products may contain a number of anti-nutrients which may be present in food naturally. Tripsin inhibitors, tannins in legumes, phytate in cereals and oil seed are naturally present anti-nutrients. Presence of such anti-nutrients in higher concentrations in diet may affect the protein digestion and bioavailability of nutrients.

According to Soetan *et al.,* (2009) *a*nti-nutritional factors (ANF) are compounds which reduce the nutrient utilization and/or food intake of plants or plant products used as human foods or animal feeds and they play a vital role in determining the use of plants for humans and animals. There are reports from time to time of deaths after consumption of some type of beans despite cooking.

Thompson (1993) found that anti-nutrients commonly found in plant foods have both adverse effects and health benefits. For example, phytic acid, lectins, phenolic compounds (tannins), saponins and enzyme (amylase and protease) inhibitors have been shown to reduce the availability of nutrients and leads to growth inhibition.

Ghavidel *et al.,* (2007) determined the content nutrients (protein, starch, ash, calcium, iron, phosphorous and thiamine) and anti-nutritional components (dietary fiber fractions, phytic acid and tannin), and in-vitro bio-availability of calcium and iron, and in-vitro digestibility of protein, and starch in control, germinated and de-hulled green gram, cow pea and chick pea. Germination caused significant increase in protein, thiamine, in-vitro iron and calcium bio-availability and in-vitro starch and protein digestibility contents of all the legume samples.

Holst *et al.,* (2008) explains that the effect of any dietary compound is influenced by the active bio-available dose rather than the dose ingested. Depending on the individual bio-available dose may cause different magnitude of effects in different people. The requirements of nutrients including phyto-nutrients, (e.g, flavonoids, Phenolic acid, and glucosinolates). These are not essential for growth and development but to maintain body functions and health throughout the adult and later phases of life.

According to Aletor *et al.,* (1995) several leafy vegetables species found in Nigeria were analysed for their proximate chemical composition, mineral constituents, energy values, phytin and oxalate materials in the fresh and air dried materials. The dry vegetables generally had higher phytate and oxalate values than the fresh ones, which makes the bio-availability of nutrients difficult.

Gemede *et al.,* (2014) explains that, anti-nutrients are compounds which reduce the nutrient utilization and or food intake of plants plant products used as human foods and they play a vital role in determining the use of plant for humans. The anti-nutrients might not always harmful even though lack of nutritive value. The balance between beneficial and hazardous effects of plant bio-active and anti-nutrients rely on their concentration, chemical structure, time of exposure and interaction with other dietary components.

2.3. PHYTIC ACID AS AN ANTINUTRIENT IN PLANT FOODS

Zhou &Erdman Jr. (1995) explains that phytic acid is a major phosphorous storage compound of most seeds and cereal grains, contributes about 1-7%of their dry weight. PA has a strong ability to chelate multivalent metal ions, especially Zinc Iron and calsium. The binding can result in poor bioavailability of minerals. Phytic acid is also considered as a natural anti-oxidant. Phytate rapidly accumulates in the seeds during the ripening period; sources of phytic acid in foods are cereals, legumes, oil seeds and nuts which are important for human nutrition. In legume seeds, phytate is located in protein bodies of endosperm.

Jasia *et al.,* 2017 identified the most concentrated sources of phytic acid tend to be oil seeds, whole grains and legumes. Roots, tubers and other vegetables may akso contain phytic acid, but usually in lower amounts. Phytic acid is isolated in the aleurone layer in most grains, making it most concentrated in the bran. In legumes it is found in the cotyledon layer.

Oberleas *et al.,* (1981) presented phytate content of several foods. Published zinc values were used to calculate phytate:zinc molar ratios. These ratios can be used to estimate the relative risk of having an inadequate intake of zinc. They may be used in planning menus to select the combination of foods that will supply the most available zinc to the daily diet. On the basis of animal experiments to date, a daily phytate:zinc molar ratio of 10 or less is thought to be acceptable in providing adequate dietary zinc, and daily ratios consistently above 20 may jeopardize zinc status. Many factors other than the daily dietary phytate:zinc molar ratio influence zinc nutriture, but the ratio concept is a tool which may contribute to a more accurate assessment of zinc status.

Sandberg in 2002 observed that the mineral content of legumes is generally high, but the bioavailability is poor due to the presence of phytate, which is a main inhibitor of Fe and Zn absorption. Some legumes also contain considerable amounts of Fe-binding polyphenols inhibiting Fe absorption. The mineral content of legumes is generally high, but the bioavailability is poor due to the presence of phytate, which is a main inhibitor of Fe and Zn absorption. Some legumes also contain considerable amounts of Fe-binding polyphenols inhibiting Fe absorption. Once, phytate and in certain legumes polyphenols, is degraded, legumes would become good sources of Fe and Zn as the content of these minerals is high.

The availability of phosphorus, when present in the form of phytate, depends on the species, the age of the experimental animal, and the level of phytase activity in the intestinal tracts of the specific species. Phytate is regarded generally as being less biologically available than most inorganic phosphorus (Fernandez *et al.,* 1997)

According to Gupta *et al.,* (2015) phytic acid is the major storage form of phosphorous in cereals, legumes, oil seeds and nuts. Phytic acid is known as a food inhibitor which chelates micronutrient and prevents it to be bio available for monogastric animals, including humans, because they lack enzyme phytase in their digestive tract.

'Phytic acid is a main reservoir of phosphorous in plants and contributes about 80% of the total phosphorous in cereal seeds'. However it is well known to possess anti-nutritional behaviour. Because it has strong affinity to chelate divalent ions,e.g. calcium, magnesium, and especially with iron and zinc (Vashisth *et al.,* 2017).

Hurrel *et al.,* in the year 2003 found that phytic acid in cereal-based and legume based complimentary foods inhibits iron absorption. Low iron absorption from cereal porridges contributes to the high prevalence of iron deficiency in infants from developing countries. They also found that phytate degradation improves iron absorption from cereal porridges prepared with water.

According to Gibson *et al.,* (2010) plant based complementary foods often contain high levels of phytate, a potent inhibitor of iron, zinc and calcium absorption. The study was don comparing the concentrations of phytate, iron, zinc, and calcium and the curresponding phytate: mineral molar ratios in many plant based complimentary foods. the study suggests that to prevent mineral deficiency in infants, dephytinization must be combined with enrichment with animal-source foods.

Ma G *et al.,* (2007) conducted studies in 60 food samples which are commonly consumed in china. They were analysed for its phytate content and mineral ratios. They found that, phytate in foods impair the bioavailability of calsium, iron and zinc, which to some extent depends upon food processing and cooking methods.

Weaning foods frequently contain phytate, an inhibitor of iron and zinc absorption, which act as an inhibitor of iron and zinc absorption, which may contribute to the high prevalence of iron and zinc deficiency seen in infancy. Extensive reduction in the phytate content of weaning cereals had long-term effect on the iron and zinc status of Swedish infants (Lind *et al.,* 2003).

In general, human do not produce enough phytase to safely consume large quantities of high-phytate foods on a regular basis. However, probiotic lactobacilli, and other species of the endogenous digestive microflora can produce phytase (Famularo, 2005). Thus humans who have good intestinal flora will have an easier time with food containing phyti acid.

Oberleas *et al.,* (1966) conducted studies on phytate content of foods and their effect on dietary zinc bio-availability. In this study they compared the phytate: zinc molar ratios which can be used to calculate the relative risk of having an inadequate intake of zinc. A daily phytate: zinc ratio of 10 or less is thought to be acceptable in providing adequate dietary zinc.

Woldegiorgis *et al.,* (2015) after their studies in anti nutrient composition in edible mushrooms stated that, phytic acid is a compound found only in plant foods is a phosphorous containing compound that binds with minerals and inhibits mineral absorption. Due to the structure of phytate which has a high density of negatively charged phosphate groups which forms very stable complexes with minerals and prevents their absorption.

Phytic acid is present in many plant systems, constituting about 1-5% by weight of many cereals and legumes. Concern about its presence in food arises from evidence that it decreases the bioavailability of many essential minerals by interacting with multivalent cations and or proteins to form complexes that may be insoluble or unavailable under certain physiologic conditions (Cheryan & Rackis.J 1980).

The formation of insoluble metal cation- phytate complex at physiological pH-values is regarded as the major reason for a poor mineral availability, because these complexes are essentially non-absorbable from the gastrointestinal tract. Most studies have shown an inverse relationship between phytate content and mineral availability (Lopes *et al.,* 2002).

The interaction between phytate and protein has been reported for several proteins including soyabeans, peanuts, blackgram .The reduced solubility of protein as a result of protein-phytate complex can adversely affect certain functional properties of proteins which are dependent on their hydration and solubility in addition; reduced bioavailability of phosphorous is a distinct possibility (Cheryan 1980).

Oatway *et al.,* (2001) report since its discovery, phytic acid has been catagorised as an anti nutritional component in cereals and legumes. Research has traditionally focused on its unique structure that gives it the ability to bind minerals, proteins, starch.

According to Campion *et al.,* (2013) in common bean, lectins, phytic acid, polyphenols and tannins exert major anti-nutritional effects when grains are consumed as a staple food. Reduced iron and zinc absorption, low protein digestibility and high toxicity at the intestinal level are the causes of their anti-nutritional effect.

Cakmak *et al.,* (1999) found that, Zinc (Zn) deficiency is a critical nutritional problem for plants and humans in Turkey. Zinc present in grains from Anatolia seems to be not bio-available. Phytate: Zn molar ratios in grains, a widely accepted predictor of Zn bio-availability. Zn nutritional status of school children in southern Anatolia, most children were found to be of shorter stature and had very low levels of Zn in hair.

Febles *et al.,* (2002) compared the phytic acid content of refined (hand-made and factory made) wheat flours, much consumed in the Canary Islands, have been determined in their study. A total of 200 samples of flours from different types (100 refined and 100 whole were analysed. The method proposed by Garcia- Villanova *et al.,* (1982) was used for determination of phytic acid. Refined flour had a lower content of phytic acid than whole flour.

Rickard *et al.,* (1997) explains that, phytic acid is a compound found in cereal grains, legumes, nuts and oil seds. It is considered to be an anti-nutrient due to its ability to bind minerals, protein and starch at physiological pH

A study done by Sreerama *et al.,* (2011) shows the nutrient composition and anti-nutritional factors in cow pea and horse gram flours and is compared with chick pea flour. It is found that, fat content is higher in chick pea and lowest in horse gram. Resistant starch and phytic acid were significantly higher in cow pea flour.

Deficiency of zinc in humans due to nutritional factors and several disease states is observed. The high phytate content of cereal proteins is known to decrease the availability of zinc. Thus the pre-valance of zinc deficiency is likely to be high in a population consuming large quantities of proteins (Prasad 1984)

Phytic acid content of wheat, maize, barley, oats, soybean, cow pea, common bean, lupin and pea samples grown in Hungary were measured. In addition interaction of phytic acid with some isolated proteins has been studied by Hidvegi *et al.,* in 2002. Soy glycinin and sunflower seeds globulin interacted with the highest quantity of phytic acid and gluten proteins with the lowest one.

The availability of nutrients in plant foods may be affected by natural complexing agents. This is particularly true in the case of seeds containing phytic acid, such as legumes, cereals, and oil seeds. The phytic acid- protein interaction affects the protein availability of legumes negatively and that the, nature of the protein source play a prominent role (Carnovale *et al.,* 1988).

According to Lolas *et al.,* (1975) Beans as other seeds, are rich sources of phosphorous. The animal nutritional importance of phytic acid lies in its ability to chelate several mineral elements, especially calcium, magnesium, iron and zinc and thereby reduce their availability in the intestinal tract.

The relationship between nutrient phosphorous zinc levels and phytic acid in soybean were studied by Raboy *et al.,* in 1984. Electrophoresis and ion-exchange chromatography revealed that partially phosphorylated intermediates do not appear when phytic acid accumulation is greatly reduced by limiting the nutrient phosphorous or when accumulation is greatly accelerated by excess phosphorous.

Lott *et al.,* (2000) found that a very important mineral storage compound in seeds is phytate. This compound is important for several reasons as it is vital for seed development and successful seedling growth. It is often considered to be an anti-nutritional substance in human diets.

Phytic acid is found in cereals, legumes, nuts and oilseeds. It serves as the chief storage form of phosphorus. Phytic acid has long been considered as antinutrient, mainly due to its ability to bind with many divalent cations, proteins and starch and to consequently reduce their bioavailability. To combat phytic acid's anti-nutrient effects, particularly in nutritionally compromised populations, many ways of removing it from foods have been suggested.However, it has also suggested by many studies that consumption of phytic acid may convey some beneficial health effects (Jenab *et al.,* 2002)

The catalysis by iron of radical formation and subsequent oxidative damage has been well documented. Although many iron-chelating agents potentiate reactive oxygen formation and lipid peroxidation, phytic acid forms an iron chelate which greatly accelerates radical generation and suppresses lipid peroxidation. Phytic acid prevents browning and putrefaction of various fruits and vegetables by inhibiting poly-phenol oxidase (Graf *et al.,* 1987).

According to Feil (2001), phytic acid is the main storage form of phosphorous in many plant species. It is considered to be an anti-nutrient, because it binds nutritionally important cations and protein in the alimentary tract, thus lowering the bioavailability of minerals and protein in humans and other non-ruminants. Since phytic acid is a poor source of phosphorous for non-ruminants, the accumulation of excreted phosphorous in soil and water system can represent serious environmental problems in areas of intensive animal production and insufficient purification of municipal sewage. Thus, there are obevious reasons for reducing the level of phytic acid in the diet.

A 15 year old Indian boy is reported with nutritional rickets. Wills *et al.,* (1972) found that his diet contains higher amount of phytic acid. When the phytic acid was reduced the rickets healed. Reports of osteomalacia in adults and rickets in children among the immigrant population of the United Kingdom are essentially confined to Indians or Pakistanis. The high dietary phytic acid among the chapatti-eating Asian immigrants may be sufficient to impair calcium absorption and account for the nutritional rickets and osteomalacia in this population group.

Phytic acid in cereal grains and oil seeds is poorly digested by monogastric animals and negatively affects animal nutrition and environment. So breeding programs involving mutants with less phytic acid is done. These are aasociated with phytic acid reducing mutations. The mutation with certain genes will help in reducing phytic acid in hybid maize (Shi *et al.,* 2007)

2.4 EFFECT OF PRE-COOKING AND COOKING METHODS ON PHYTIC ACID IN PLANT FOODS

Germination is a highly effective method to reduce phytic acid observed by Ertop *et al.,* (2018) during the germination process they also found that the endogenous enzyme activity which has phytate degrading ability increases. This condition provide to degradation and decline anti-nutrients as phytic acid. They also concluded that, the processing methods of foods can significantly decrease anti-nutrients. Genetic improvement as well as pre-treatments such as fermentation, soaking, germination also improves nutritional quality.

Astely *et al.,*(1996) states that many traditional preparation methods (e.g.,Fermentation) reduce anti-nutrients, such as phytic acid, increase the nutritional quality of plant foods, and are widely used in socities where cereals and legumes are a significant part of the diet.

Peter Curcio (2002) in his article related to anti-nutrients comments that, along with saponins and lecithins, phytic acid is considered an anti-nutrient. Phytic acid is pre-dominantly found in the bran (80%) or outer most shell of whole grains. In legumes and seeds, phytic acid resides mostly in the endosperm. This is significant when you consider that most whole grains and high- fiber food products include all of the phytic acid , while processed or refined grains have it removed entirely.

According to Sehgal *et al.,* (2001) malting and blanching are effective in reducing poly-phenols ans phytic acid content inpearl millet grains. The results obtaind after the application of malting and blanching is that,there is a significant reduction in poly-phenoles (28%) and phytic acid(38%). The overall results suggested that malting with 72 hours of germination was most effective in reducing the anti-nutrient levels of pearl millet grains.

Khattab *et al.,* (2009) records that their studies on raw and treated cowpea, and kidney bean seeds which was investigated for their contents of anti-nutritional factors including tannin, phytic acid, tripsin inhibitors etc. Treatment applied included water soaking, boiling, roasting, autoclaving, fermentation and micronization. Kidney bean contained highest anti-nutrient content among all investigated seeds. Autoclaving and fermentation found to be most effective in reducing phytic acid.

Several processing methods germination, soaking, autoclaving, and fermentation are found to remove considerable amount of phytate in legumes. Germination reduces considerable amount of from the seeds or grains. Disappearance of phytate during germination depends on the phytase activity. Degradation of phytate can occur both during food processing and in the gastrointestinal tract. This degradation is of nutritional importance because it has been demonstrated that such controlled degradation improves the uptake of essential dietary minerals (Hurrel *et al.,* 1992).

Natural fermentation can obtain a large reduction in phytic acid in rice flour by the action of microbial as well as grain phytases. It can also be defined as a desirable process of biochemical changes. It destroys undesirable compounds to enhance the nutritive value and appearance of the food, to reduce the energy required for cooking and to make a safer product (FAO, 1995).

phytate degrading enzymes, phytases present naturally in the plant foods or present in yeasts or other micro-organisms used in food processing. In future their use in food processing could be feasible. In order to substantially increase iron absorption, phytate degradation has to be virtually complete. Recent findings suggests that the inositol penta-tetra and tri phosphates must also be degraded in order to improve iron absorption (Sandberg *et al.,* 1999)

Egounlety *et al.,* (2001)explaind the changes that are made by cooking and soaking on certain anti-nutrients including phytic acid in legumes such as soyabean, cow pea and ground bean. They also found that phytic acid decreased during fermentation of soyabean. The changes are beneficial especially in infant feeding based on cereal and legume- based foods.

Bishnoi *et al.,* (1994) expressed that all the pea verities differed significantly in their phytic acid content. All the domestic processing and cooking methods could reduce the contents of phytic acid and polyphenoles but germination for 48 hours seemed to have a marked lowering effect on the levels of these anti-nutrients in peas.

Huma *et al., (*2008) in their study reveals that dark colour legumes(red kidney bean) has a high level of phytic acid and tannin compared with light colour (white kidney bean and white grams). Soaking and cooking of legumes results in significant reduction in phytic acid and tannin contents. Maximum reduction was found for sodium bicarbonate soaking followed by cooking. These treatments also result in a slight reduction in nutrients such as protein, minerals and total sugars.

Manan *et al.,* (2003) in their study noted the effects of a traditional domestic cooking procedure on the phosphorous / phytate relationship and the nutritional quality of three verities of Pakistani peas and lentils were investigated. Cooking procedure which involved steeping, followed by boiling, of the seeds resulted in a reduction of considerable amounts of phytic acid from both peas and lentils.

Humaira *et al.,* in 2017 investigated the effect of processing on anti-nutritional factors of several traditional leafy vegetables collected in South Africa. The vegetables were boiled in a particular plant to distilled water ratio for certain time period. From the study it was found that boiling was effective in reducing the phytic acid, tannins, alkaloids, oxalic acid etc.it provides evidence that these leafy vegetables contributes to micronutrient malnutrition in developing contries and can be reduced using methods like boiling.

Helbig *et al.,* (2003) studied the effect of soaking in domestic processing on, the nutritive value of the common bean. The study concludes that soaking did not affect the tannin content of common bean and protein digestion. However soaking was capable of reducingthe phytate levels in the common bean.

Khalil *et al.,* (1995) carried out a study on faba beans. He compared the nutritive value of faba beans in raw condition and also after heat processing and germination. The study highlighted the fact that heat processing and germination resulted in significant reduction in tannins, phytic acid, vicine, trypsin inhibitor, and haemagglutinnin activity.

Duhan *et al.,* (1989) identified that phytic acid content of various cultivars shows slight variations. They surprisingly noted that phytic acid was lowered significantly by the common methods of domestic processing and cooking including soaking, cooking, autoclaving and soaking.

Ibrahim *et al.,* (2002) had done a research on efficacy of some treatments, namely soaking(in water and bicarbonate solution), pressure cooking, germination and fermentation in reducing or removal of anti-nutritional factors usually present in cow peas. The results showed that long time soaking in bicarbonate solution caused remarkable reduction in the anti-nutritional factors. Pressure cooking was more effective than normal cooking. Fermentation remarkably reduced phytic acid. However, tannins noticeably increased.

Kataria *et al.,* (2003) observed that domestic processing and cooking methods including soaking, ordinary and pressure cooking of soaked and un soaked seeds, and sprouting significantly lowered phytic acid, saponins and polyphenol contents of black gram seeds. They have also noted that soakingfor 18 hrs removed 28% of the phytic acid. Extent of removal was higher with longer period of soaking. Pressure cooking had a greater effect than ordinary cooking. Anti-nutrient concentrations declined during sprouting. Phytic acid was reduced to a greater extent compared to other anti-nutrients present in black gram seeds.

Brigide *et al.,* (2005) investigated effect of cooking in beans. Estimation of iron and anti-nutrients and the in vitro dialysis of iron were carried out. Cooking caused a decrease in all parameters except for phytate and iron availability.

Kaur *et al., (2002)* carried out a study on the effects of various processing methods viz. Pressure cooking with soaking, sprouting, sprouting and pressure cooking, dehulling and pressure cooking of the dehulled legume on the bioavailability of zinc were studied. The maximum retention of zinc was observed in dehulled soaked, and pressure cooked diets. Followed by sprouted and pressure cooked diets.

The effect of germination, cooking and canning on the changes in total phosphorous, inorganic phosphorous and phytate retention in black eyed, red kidney, mung, and pink beans were determined by Tabekhia *et al.,* (1980) in their study. Soaking the dry beans in water for 12 hours at 24° c resulted in a slight decrease in phytate. After germination for 96 hours or longer there was a significant breakdown in phytic acid.

Khokhar *et al.,* (1986) observed significant varietal differences in the contents of phytic acid , saponin and trypsin inhibitor activity of four verities of moth bean. Soaking, sprouting, cooking was applied on dry beans. It is found that soaking reduced phytic acid and sprouting reduced saponin. The other methos were less effective in reducing anti-nutrients.

Lestienne *et al*., (2005) explained the effect of soaking whole cereal and legume seeds on iron and phytate contents. In all the cereals except millets, the molar ratios of Phy/Fe were above than 14, and the ratios of Phy/Zn were above 20. While in legumes ratios were lower. Soaking of seeds led to a significant reduction in the phytate content of millets.

Hadi *et al.,* (2003) studied the effect of soaking (in water for 16 h) and extrusion conditions including barrel temperature (140°C and 180°C) and free moisture (18% and 22%) on anti-nutrients, total phytate phosphorous and protein digestability of whole meal of four kinds of legumes(peas, chick pea, faba and kidney beans). The results obtained indicated that the soaking and extrusion significantly decreased anti-nutrients such as phytic acid, tannins, phenols, alpha-amylase and trypsin inhibitors.

Chitra *et al.,* (1996) carried out a study with the objective to determine the effect of various types of processing on selected nutrition related parameters of commonly consumed Indian pulses and soybean. Germination reduced the phytic acid content of chick pea and pigeon pea seeds over by 60%, and that of mung bean, urd bean and soyabean by about 40%. Fermentation reduced phytic acid contents by 26-39%.in all the legumes with an exception of pigeon pea in which it was reduced by more than 50%. Autoclaving and roasting were more effective in reducing phytic acid in chick peas and pigeon peathan in urd bean, mung bean and soybean.

Rehman *et al.,* (2004) studied the thermal heat processing effects on anti-nutrients, protein and starch digestibility of black gram, chick peas, lentils, red and white kidney beans. Reduction in the levels of anti-nutrients along with an improvement in protein and starch digestibility was observed after cooking these food legumes. Anti-nutrients including tannin and phytic acid contents were reduced by different thermal heat treatments. Maximum improvement in protein and starch digestibilities was observed on cooking these food legumes at 121°C for 10 min.

The effect of boiling, roasting and autoclaving on the levels of some anti-nutrients factors present in the seeds of vegetable cow pea were studied by Udensi *et al.,* (2007). The reduction of trypsin inhibitor was found to be highest with autoclaving at 60 min. Boiling was more effective in reducing phytic acid and haemagglutinin respectively at 60 min than the other processing treatments at the same time.

Villlavicencio *et al.,* (2000) experimented with the Brazilian bean verities. They were irradiated and stored at ambient temperatures. The anti-nutrients phenolic compounds, tannins and phytate were determined. After soaking and cooking the samples a higher content of phenolic compounds and a lower phytate content was observed in the bean verities. Tannin content was not affected by soaking and cooking. Using radiation doses relevant for food did not affect the content of anti-nutrients in bean verities.

According to Montagnac *et al.,* (2009) Cassava is a valuable source of food for developing countries, but it contains highly toxic cyanogen compounds and anti-nutrients. Although phytate and poly-phenols have antioxidant properties, they interfere with digestion and uptake of nutrients. Fermentation and oven-drying are efficient processing methods to remove phytate and poly-phenols, respectively, from cassava roots. Sun drying the leaves, with or without prior steaming or shredding, removes about 60% phytate in cassava leaves.

Effects of different temperatures during soaking and germination on selected nutrients and anti-nutrients of mungean, were studied by Sattar *et al.,* in 1989. Soaking at 55°C resulted in larger decrease in the phytate and trypsin inhibitor activity than at 27°C whereas protein was little affected. A slight increasing trend in the protein content but decreasing trends in the phytate and trypsin inhibitor values were observed during germination regardless of the temperatures tested.

Comparative effects of extrusion cooking and conventional processing methods on protein content and reduction of anti-nutritional factor (phytic acid, condensed tannins, polyphenols, trypsin, haemagglutinating activity) levels in *Vicia faba* and *Phaseoulus vulgaris* seeds were studied. Thermal treatments was most effective in improving protein content and reducing anti-nutrients (Alonso *et al.,* 2000)

The effect of high temperature, high humidity storage on cooking quality and physicochemical properties of dry, mature red kidney beans was evaluated over a storage period of 9 months. The rate of softening of beans during cooking, and the dissolution of pectin during cooking is related to each other. The loss of cookability in mature bean seeds stored under high-temperature may probably results from a decrease in phytic acid phosphorous and alterations in the ratio of monovalent to divalent cations in the tissue (Moscosco *et al.,* 1984).

Effects of dehulling on phytic acid; trypsin, chymotrypsin, and alpha amylase inhibitory activities; and tannins of ten cultivars of dry beans were investigated by Deshpande *et al.,* (1982). Dehulling significantly increased the phytic acid content of beans.

The effects of germination, cooking and canning on the changes in total phosphorous, inorganic phosphorous and phytate retention in black eyed, red kidney, mung, and pink beans were determined in a study done by Tabekhia *et al.,* in 1980. Soaking the dry beans in water for 12 hr at 24°C resulted in a slight decrease in phytate. After germination for 96 hr or longer, there was a significant breakdown in phytic acid, and an increase in inorganic phosphorous.

Shimelis *et al.,* (2006) studied the effects of hydration, autoclaving, germination, cooking and their combinations, on the reduction/ elimination of anti-nutrients in *Phaseolus vulgaris*. Germination process reduced phytic acid and tannins which was due to metabolic activity.

The effects of different processing methods (soaking in water or solutions of sodium bicarbonate, citric acid, soaking plus cooking, germination) on anti-nutritional factors was observed by Yasmin *et al.,* in 2008. Simple soaking in water did not shown any reduction in tannin content. Phytic acid content was only reduced with germination technique.

Kidney beans (*Phaseolous vulgaris)* were being processed for inclusion into a weaning food formulation. They were sprouted for 96 h at 30°C and some nutrients and anti-nutrients were evaluated every 12 h. Tannin decreased to undetectable levels, and trypsin inhibitor substances and phytates decreased by 70.7% and 85.9% during the entire sprouting period (Mbithi *et al.,* 2001).

The possible role of phytic acid as a chelating agent in the softening of fresh beans during cooking was examined by Lugo *et al.,* in 1991. Its diffusion out of the grain of two bean verities was determined during the cooking process. The softening rate was five times higher in them. The obtained indicated that, phytic acid probably not a chelating agent during bean thermal softening.

The effects of germination on chemical composition, biochemical constituents and anti-nutritional factors of soybean were investigated. Both the total protein content and non-protein nitrogen increased after five days of germination. The phytic acid in the seeds was degraded by the phytase activated during germination, thus increasing the availability of the minerals present in the germinated seeds (Bau *et al.,* 1997).

The effects of domestic traditional processes such as dehulling, soaking, germination, boiling and autoclaving on the nutritional composition and anti-nutritional factors of mung bean seeds were studied by Mubarak in the year 2005. Germination and cooking process caused significant decrease in anti-nutritional factors and total ash contents. Germination was more effective in reducing phytic acid. Germination resulted in a greater retention of all minerals compared to other processes.

Kumar *et al.,* (2010) revealed that, phytate is the primary storage form of both phosphate and inositol in plant seeds. It forms complexes with dietary minerals, especially iron and zinc, and causes mineral-related deficiency in humans. Processing techniques such as soaking, germination, malting and fermentation, reduce phytate content by increasing the activity of naturally present phytase.

CHAPTER III

METHOTOLOGY

Research Design

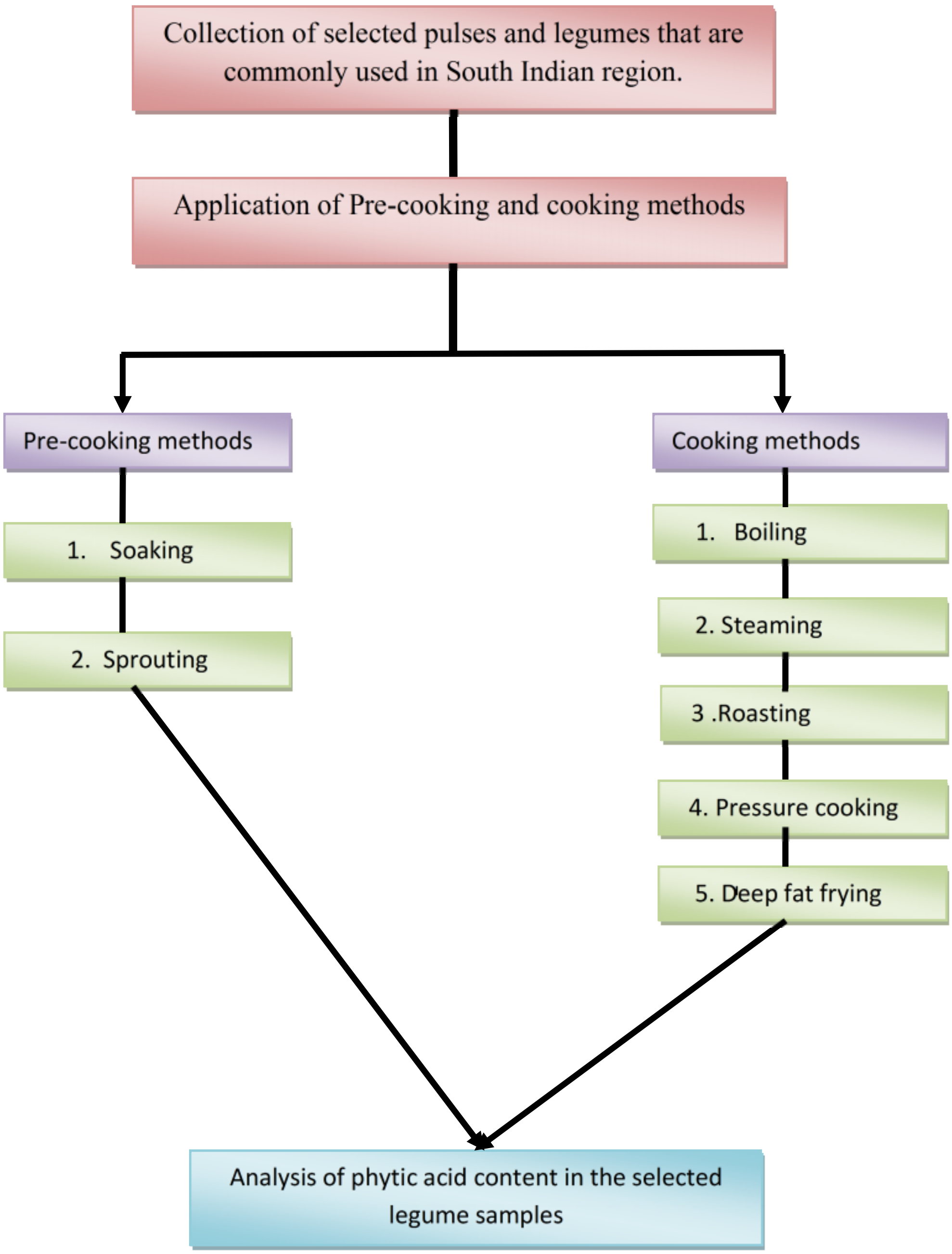


Figure 1

The topic entitled, "Effect of pre-cooking and cooking methods on phytate content in selected legumes" is discussed under the following headings,

3.1 Selection of commonly used pulses and legumes

3.2 Preparation of samples for experiment

- Precooking &cooking methods

3.3 Quantitative estimation of phytic acid in raw and treated legume samples

3.1 Selection of commonly used pulses and legumes

Pulses (beans, peas etc.) have been consumed for at least 10000 years and are among the most extensively used foods in the world. A wide verity of pulses can be grown globally, making them important economically as well as nutritionally. Pulses provide protein and fibre, as well as significant source of vitamins and minerals, such as iron, zinc, folate and magnesium, and consuming half a cup of beans or peas per day can enhance diet quality (Aukema *et al.,* 2014).

The commonly used pulses in our daily life like, ***soya bean***, ***cow pea***, ***kidney beans***, ***broad beans***, ***chick peas***, ***green peas, green gram***, ***black gram***, ***red gram***, ***horse gram*** were selected for conducting the experiments. These pulses are mainly used in south Indian cuisine as chutneys, sambar, and other main and side dishes.

Phytic acid is the phosphate ester of inositol. It contains six phosphate groups. Phytic acid is the major storage form of phosphorous comprising 1-5 % by weight in cereals, legumes, oil seeds and nuts (Vats and Banerjee, 2004).

Sources of phytic acid in foods are cereals, legumes and nuts which are important to human nutrition. It represents 40-60% of total calorie intake for human in developed and developing countries respectively. In legume seeds, phytate is located in protein bodies of endosperm. Phytic acid content in whole seed ranged from 0.2 – 2.9% and is high in cotyledons (Ravindran *et al.,1995).*

Based on various studies, the legumes and pulses which are known to be higher in phytic acid content and are commonly consumed in south Indian region are selected for the experiments

The Legumes selected are:

1. ***Glycene max (Soya bean)*** **- Soy bean**
2. ***Vigna unguiculata (Cow pea)*** **- Thattapayaru**
3. ***Phaseolus vulgaris(Kidney beans)*** **- Rajma**
4. ***Vicia faba (Broad beans)*** **- Mochai paruppu**
5. ***Cicer ariectinum(Bengal gram)*** **- Konda kadalai**
6. ***Pisum sativum(Green peas)*** **- Pattani**
7. ***Vigna radiata (Green gram)*** **- Pachapayaru**
8. ***Vigna mungo(Black gram)*** **- Ulundhu**
9. ***Cajanus cajan(Red gram)*** **- Thuvaram parupu**
10. ***Macrotyloma uniflorum (Horse gram)***- **Kollu parupu**

All the pulses where brought from wholesale market in townhall, Coimbatore on 02.02.2019.

Human ethics committee report is enclosed in Appendix - I

3.2. Preparation of samples for experiment (Application of pre-cooking and cooking methods)

As s first step raw samples 10 grams of each raw sample (***soya bean***, ***cow pea***, ***kidney beans***, ***broad beans***, ***chick peas***, ***green peas, green gram***, ***black gram***, ***red gram***, ***horse gram***) was selected for the analysis of phytic acid. Then the same selected plants in selected quantities were subjected to various pre-cooking and cooking treatments in order to check the dephytinization activity of various pre-cooking and cooking methods in pulses and legumes. The phytic acid values obtained for raw samples were set as a standard or reference value for comparison of phytic acid content in various treated samples

The pre-cooking methods used are;

- **Soaking**
- **Sprouting**

Cooking methods used are;

- **Boiling**
- **Steaming**
- **Pressure cooking**
- **Roasting**
- **Deep fat frying**

RAW LEGUME SAMPLE

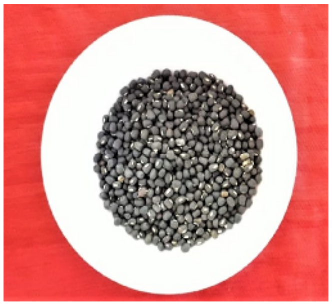
. *Glycene max (soya bean)*

. *Pisum sativum(peas)*

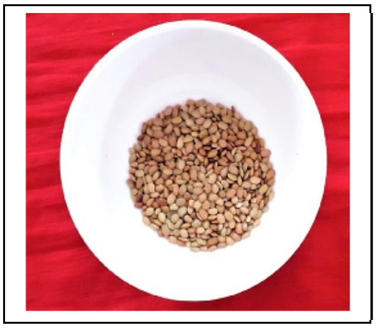
Vigna unguiculata (cow pea)

6. *Phaseolus vulgaris*
7. *(kidney beans)*

4. *Vigna radiata*
5. *(green gram)*



. *Vigna mungo(black gram)*



Macrotyloma uniflorum
(Horse gram)

Cajanus caja
(Red gram)

Cicer ariectinum
(Bengal gram)

Vicia faba
(Broad beans)

PLATE 1

Application of pre-cooking methods:

1. **Soaking**

10 grams of each selected samples (*soya bean*, *cow pea*, *kidney beans*, *broad beans*, *chick peas*, *green peas, green gram*, *black gram*, *red gram*, *horse gram)* were taken in separate bowl. They are added with 50 ml of water and coverd with a lid and kept undisturbed for up to 12 hours. After 12 hours the pulses were observed, it was noted that, they were bulged and increase in weight by absorbing water. Each of the samples were taken and pressed to remove excess water from it and taken for phytic acid estimation.

Note:

Many pulse particularly whole gram which have hard outer covering need soaking prior to cooking. During soaking water enters through the hilum or scar where the bean is attached to pod. It seeps around the periphery of the bean and causes the seed coat to wrinkle. Whole pulses are soaked in cold water overnight or in warm water (60-70°C) for 4-5 hours. It also reduces phytic acid and oligosaccharide. (Srilakshmi 2015).

2. **Sprouting**

Ten grams of all the selected raw samples were taken for sprouting. The samples were soaked in 50 ml of water and kept undisturbed for upto 12 hours. Then it is taken and water is drained out. The samples were tied with a cotton cloth and sprinkled water on them in between each 8-10 hours. The pulses got sprouted within 2 days. Sprouting depends on the room temperature. It is found that soyabean and kidney bean took more time (3 days) to get sprouted. May be it is because of their hard outer coverings. Green gram, cow pea, and horse gram were sprouted faster than other samples.

NOTE:

Whole grams are soaked overnight and water should be drained away and the seeds should be tied in a loosely woven cloth and hung. Water should be sprinkled twice or thrice day. In a day or two, germination will take place (Srilakshmi 2015).

SOAKED LEGUME SAMPLE

Glycene max (Soya bean)

Pisum sativum

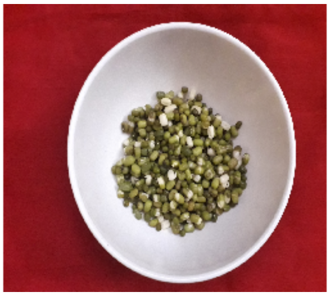
(Green peas)

Vigna unguiculata

(Cow pea)

Phaseolus vulgaris

(Kidney beans)



Vigna radiata

(green gram)

Vigna mungo

(black gram)

Macrotyloma uniflorum

(Horse gram)

Cajanus caja

(Red gram)

Cicer ariectinum

((Bengal gram)

Vicia faba

(Broad beans)

PLATE II

SPROUTED LEGUME SAMPLE

Glycene max
(Soya bean)

Pisum sativum
(Green peas)

Vigna unguiculata
(Cow pea)

Phaseolusvulgaris
(Kidney beans)

Vigna radiata
(green gram)

Vigna mungo
(black gram)

Macrotyloma uniflorum
(Horse gram)

Cajanus caja
(Red gram)

Cicer ariectinum
(Bengal gram)

Vicia faba
(Broad beans)

PLATE III

Application of cooking methods:

1. Boiling

The selected legumes were taken in 10 grams and each sample was boiled to find out the effect of cooking by boiling on phytic acid present in them. Each pulse were taken in 10 grams using a weighing machine and boiled at 100° C in separate vessels till it is cooked. It took more than 15 minutes to cook the samples by boiling.

After cooking the excess water was removed and the cooked samples were analyzed for its phytic acid content.

NOTE:

Boiling is cooking food by just immersing them in water at 100°C and maintaining the water at that temperature till the food is tender. Water is said to be boiling when large bubbles are seen rising constantly on the surface of the liquid and then breaking rapidly. Boiling point of water is 100°C (Srilakshmi 2015).

2. Steamed

Ten grams of each sample was taken and steaming process is applied to cook the samples. It took about 45 minutes to cook each sample by steaming.

The samples were taken and loaded in a steamer. At a time 3 samples were steam cooked and they were allowed to cool. After cooking the steam cooked samples were used for phytic acid analysis. Compared to other methods cooking by steaming was a time consuming process.

NOTE:

Steaming method requires the food to be cooked in steam. This is generated from vigorously boiling water or liquid in a pan so that the food is completely surrounded by steam and not in contact with the water or liquid. The water should be boiled before the food is placed in the steamer.

Wet steaming: here the food is in direct contact with the food
Dry steaming: here double boiler is used for cooking the food
(Srilakshmi 2015).

BOILED LEGUME SAMPLE

Glycene max
(Soya bean)

Pisum sativum
(Green peas)

Vigna unguiculata
(Cow pea)

Phaseolusvulgaris
(Kidney beans)

Vigna radiata
(green gram)

Vigna mungo
(black gram)

Macrotyloma uniflorum
(Horse gram)

Cajanus caja
(Red gram)

Cicer ariectinum
(Bengal gram)

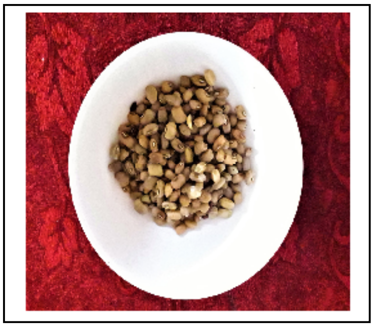
Vicia faba
(Broad beans)

PLATE IV

STEAMED LEGUME SAMPLE

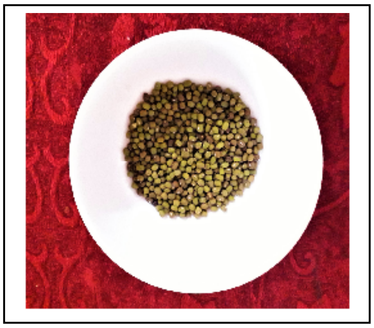
Glycene max
(Soya bean)

Pisum sativum
(Green peas)



Vigna unguiculata
(Cow pea)

Phaseolusvulgaris
(Kidney beans)



Vigna radiata
(Green gram)

Vigna mungo
(black gram)



Macrotyloma uniflorum
(Horse gram)

Cajanus caja
(Red gram)

Cicer ariectinum
(Bengal gram)

Vicia faba
(Broad beans)

PLATE V

3. Roasting

The selected samples are taken in 10 grams. They were roasted in a roasting pan.

The samples are roasted separately till their raw smell is gone and the outer covers of the legumes were opened and is cooked. Then the samples were taken to carry out the experimental part.

Roasting is when food is cooked uncovered on heated metal or a frying pan the method is also known as pan broiling (Srilakshmi 2015).

4. Pressure cooking

The samples are taken in 10 gram and are cooked in a pressure cooker. The samples like cow pea and green gram took less time to get cooked in a pressure cooker compared to other samples. The pressure cooked samples are taken to do the further experiments. Usually soaking is done prior to pressure cooking of pulses, but here it is not done. Pulses like green gram, green peas, and cow pea took 15-20 minutes to get cooked while other samples took 30-45 minutes to cook properly.

NOTE:

A relatively small increase in temperature can drastically reduce cooking time and this fact is utilised in pressure cookers. In pressure cooking, escaping steam is trapped and kept under pressure so that the temperature of the boiling water and steam can be raised above 100° C and reduce cooking time (Srilakshmi 2015).

5. Deep fat frying

Ten grams of all selected samples were taken seperately. They are deep fat fried using coconut oil in a deep bottom frying pan. When they are properly fried it is taken and wiped to remove excess fat from it. Then it is used for further analysis.

NOTE:

Food is totally immersed in hot oil and cooked by vigorous convection currents and cooking is uniform on all sides of the food. Cooking can be rapidly completed in deep fat frying because the temperature used is 180-220°C (Srilakshmi 2015)

ROASTED LEGUME SAMPLE

Glycene max
(Soya bean)

Pisum sativum
(Green peas)

Vigna unguiculata
(Cow pea)

Phaseolusvulgaris
(Kidney beans)

Vigna radiata
(Green gram)

Vigna mungo
(black gram)

Macrotyloma uniflorum

(Horse gram)

Cajanus caja

(Red gram)

Cicer ariectinum

(Bengal gram)

Vicia faba

(Broad beans)

PLATE VI

PRESSURE COOKED LEGUME SAMPLES

Glycene max (Soya bean)

Pisum sativum (Green peas)

Vigna unguiculata (Cow pea)

Phaseolusvulgaris (Kidney beans)

Vigna radiata (Green gram)

Vigna mungo (black gram)

Macrotyloma uniflorum
(Horse gram)

Cajanus caja
(Red gram)

Cicer ariectinum
(Bengal gram)

Vicia faba
(Broad beans)

PLATE VII

DEEP FAT FRIED LEGUME SAMPLE

Glycene max
(Soya bean)

Pisum sativum
(Green peas)

Vigna unguiculata
(Cow pea)

Phaseolusvulgaris
(Kidney beans)

Vigna radiata
(Green gram)

Vigna mungo
(black gram)

Macrotyloma uniflorum
(Horse gram)

Cajanus caja
(Red gram)

Cicer ariectinum
(Bengal gram)

Vicia faba
(Broad beans)

PLATE VIII

3.3 Quantitative estimation of phytic acid in raw and treated samples

Phytic acid in legumes and pulses were estimated using Haug and Lantzech (1983) method. The experiments were carried out using spectrophotometer. The standard procedure for estimation is as follows.

Reagents and equipments required for the experiment;

1. 0.2 N HCl
2. Acidic ammonium iron-III sulphate
3. 2,2-bipyridine solutions
4. Standard phytic acid concentrations
5. Vortex or other flask shaker
6. Water bath
7. Centrifuge
8. Spectrophotometer with cuvettes

Procedure for phytic acid estimation

Phytic acid was analyzed according to Haug and Lantzech (1983) method. According to this method an appropriate amount (0.8-1.0 gram) of sample was extracted with 0.2N HCl by taking 25 ml of 0.2N HCl in conical flask and shake for 1 hour on shaker at 30 C and 80 revolutions per minute. 0.5 ml of extract was taken into test tube fitted with a ground glass stopper. 1 ml of acidic ammonium iron-III sulphate solution of known iron content was added and the tubes were covered with a stopper and fixed with a clip. Tubes were heated in a boiling water bath for 30 min after cooling in ice water for 15 min; tubes were allowed to reach at room temperature. Contents of the tube were mixed and centrifuged for 30 min at 3000 revolutions per minute. 1 ml of the supernatant was transferred to another test tube and 1.5 ml of 2, 2-bipyridine solutions was added, light pinkish colour appeared. The absorbance was measured at 519 nm against distilled water. Decrease in iron, in the supernatant was measure for phytic acid contents.

Standards of known phytic acid concentration were prepared and absorbance was measured at 519 nm. Standard curve was made taking phytic acid concentration in micrograms on X-axis and Optical Density (O.D) on Y axis

ANALYSIS OF PHYTIC ACID

EXTRACTION OF SAMPLE

1 g of sample was extracted with 0.2N Hcl and shake it for 1h (at 30 C and 80 rpm)

0.5 ml of the extract was taken in a test tube fitted with a ground glass stopper

1 ml of acidic ammonium iron-III sulphate solution of known iron content was added and the tubes were covered with a stopper and fixed with a clip

The test tubes were heated in a boiling water bath for 30 min after cooling in ice water for 15 min; tubes were allowed to reach at room temperature

. Contents of the tube were mixed and centrifuged for 30 min at 3000 revolutions

1 ml of the supernatant was transferred to another test tube and 1.5 ml of 2, 2-bipyridine solutions was added to it then a light pinkish colour was appeared

The absorbance was measured at 519 nm against distilled water. Decrease in iron, in the supernatant was measure for phytic acid contents.

Decrease in iron, in the supernatant was measure for phytic acid contents.

Standards of known phytic acid concentration were prepared and absorbance was measured at 519 nm. Standard curve was made taking phytic acid concentration in micrograms on X-axis and Optical Density (O.D) on Y axis

CHAPTER - IV

RESULTS AND DESCUSSION

TABLE I

PHYTIC ACID CONTENT IN RAW FORM OF SELECTED LEGUMES

Table I highlights the data on phytic acid content of selected raw legume samples.

NAME OF THE SAMPLES	PHYTIC ACID IN 0. 1g SAMPLE (in mg)	PHYTIC ACID IN 100 g (g)	PERCENTAGE (%)
Soyabean	1.3	1.3	1.30
Green peas	0.7	0.7	0.70
Cow pea	0.78	0.78	0.78
Kidney beans	1.10	1.1	1.10
Green gram	0.84	0.84	0.84
Black gram	0.95	0.95	0.95
Horse gram	0.9	0.9	0.90
Red gram	1.6	1.6	1.60
Chick peas	1.3	1.3	1.30
Broad beans	0.97	0.97	0.97

From table I the normal phytic acid content of selected raw sample is clear. It shows that kidney bean is having higher amounts of phytic acid content compared to other samples. It contains 1.3g per 100g of sample. Red gram also contains relatively high amount of phytic acid and the quantity is 1.6g per 100g. Soyabean and chick peas contain 1.3 g phytic acid per 100g, that can be considered as higher amounts where other samples contain less amount of phytic acid, which is less than 1g per 100 grams of sample.

TABLE II

PHYTIC ACID CONTENT IN SOAKED FORM OF SELECTED LEGUMES

Table II shows the phytic acid content in soaked samples of legume.

NAME OF THE SAMPLES	PHYTIC ACID IN 1g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE%S
Soyabean	1.31	1.31	1.31
Green peas	0.61	0.61	0.61
Cow pea	0.21	0.21	0.21
Kidney beans	0.51	0.51	0.51
Green gram	0.79	0.79	0.79
Black gram	0.34	0.34	0.34
Horse gram	0.82	0.82	0.82
Red gram	0.60	0.60	0.60
Chick peas	0.56	0.56	0.56
Broad beans	0.40	0.40	0.40

The above table II shows the phytic acid content of various samples which are undergone soaking process prior to analysis of phytic acid content. While comparing the values of soyabean before soaking and after soaking, it is found that there is no much changes in the values. In green peas there is a reduction of around 0.1g of phytic acid in soaked sample. In cow peas the phytic acid reduced from 0.78 to 0.21g. Phytic acid content of kidney bean has undergone a remarkable reduction after soaking. It reduced to 0.51g from 1.10g. In green gram only a slight reduction in phytic acid is observed after soaking. In black gram the reduction is from 0.95g to 0.34g. In horse gram only .1g phytic acid is reduced after soaking. Red gram, which had a higher phytic acid content (1.6g) reduced by 1 g and the soaked sample contains 0.60g phytic acid in 100g of sample. After soaking the phytic acid present in raw chick peas were reduced to 0.56 g from 1.3 g. The difference is 0.74 g. 100 g raw sample of broad beans were containing 0.97 g of phytic acid, after soaking it was reduced to 0.40g.

From the values it is found that the sample, which has a higher phytic acid value in their raw samples, had shown a remarkable reduction of phytic acid content in their soaked sample. Here soyabean is an exception, where it has not shown any reduction in its phytic acid content after applying soaking process.

According to Egounlety *et.al* (2003) in cow peas, soy bean and ground bean was found to change their phytic acid content after soaking.

TABLE III

PHYTIC ACID CONTENT IN SPROUTED FORM OF SELECTED LEGUMES

The table highlights on the data of reduction of phytic acid in selected legumes after sprouting.

NAME OF THE SAMPLES	PHYTIC ACID IN 1g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE%S
Soyabean	0.21	0.21	0.21
Green peas	0.16	0.16	0.16
Cow pea	0.16	0.16	0.16
Kidney beans	0.23	0.29	0.29
Green gram	0.16	0.16	0.16
Black gram	0.22	0.22	0.22
Horse gram	0.18	0.18	0.18
Red gram	0.32	0.32	0.32
Chick peas	0.17	0.17	0.17
Broad beans	0.20	0.20	0.20

The given table III shows the results obtained after estimating the phytic acid content in sprouted samples. Phytic acid content is reduced from 1.3 to 0.21. Around 1.09 mg is reduced. Greenpeas is reduced to 0.16 from 0.7. The difference is 0.54 which is a small amount. Cow pea also undergone reduction of phytic acid(0.78-0.16) but the reduction is small.in case of kidney beans phytic acid in the raw samples were 1.10 which is comparatively high it undergone a remarkable reduction of 0.87 mg. Phytic acid in greengram was reduced from 0.84 to 0.16. 0.68 mg is reduced. Raw sample of black gram was containing 0.95 mg of phytic acid which was reduced to 0.22 mg. In case of horse gram phytic acid is reduced from 0.9 to 0.18 mg. Red gram is reduced from 1.6 to 0.32 mg. Chick peas is reduced from 1.3 to 0.17 mg of phytic acid. Broad bean was having 0.97 mg of phytic acid in its raw form, which is reduced after sprouting to 0.20 mg.

TABLE IV

PHYTIC ACID CONTENT IN BOILED FORM OF SELECTED LEGUMES

Table IV highlights the data on effect of cooking by boiling on phytic acid content of selected leumes.

NAME OF THE SAMPLES	PHYTIC ACID IN 0.1g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE %
Soyabean	0.32	0.32	0.32
Green peas	0.51	0.51	0.51
Cow pea	0.10	0.10	0.10
Kidney beans	0.21	0.21	0.21
Green gram	0.56	0.56	0.56
Black gram	0.22	0.22	0.22
Horse gram	0.6	0.6	0.6
Red gram	0.53	0.53	0.53
Chick peas	0.28	0.28	0.28
Broad beans	0.14	0.14	0.14

From the above table it is clear that boiling had reduced the phytic acid in soyabean remarkably. About 0.98g is reduced in soyabean by boiling. In green peas phytic acid was o.7 g in raw samples. Around 0.19g of phytic acid is reduced after boiling and become 0.51g. About 0.68 g of phytic acid is eliminated from cow peas after boiling. In kidney beans a notable change in phytic acid is observed. 0.89 g is reduced in kidney bean after boiling. In case of green gram the phytic acid is not reduced much. Only 0.28g is reduced in green gram. After boiling phytic acid content of black gram was reduced by 0.73g. In case of horse gram the reduction is only 0.3g, which is negligibly less. In red gram the reduction is 1.07g which is the highest reduction in the group. In chick peas also the reduction is 1.02g and is comparatively great reduction but not greater than red gram. Phytic acid content of broad bean is reduced by 0.83 g after cooking it by boiling.

Effect of cooking in water on the composition of beans and chick peas were studied by Wang *et.al* (2009). Significant variation is found in their tannin, phytic acid and other anti-nutrient content and cooking beans in water improved their protein content.

TABLE V

PHYTIC ACID CONTENT IN STEAMED FORM OF SELECTED LEGUMES

The table V gives a clear idea about the changes occurred in phytic acid content of selected legumes after cooking it by steaming.

NAME OF THE SAMPLES	PHYTIC ACID IN 0.11g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE %
Soyabean	0.33	0.33	0.33
Green peas	0.16	0.16	0.16
Cow pea	0.11	0.11	0.11
Kidney beans	0.31	0.31	0.31
Green gram	0.26	0.26	0.26
Black gram	0.22	0.22	0.22
Horse gram	0.20	0.20	0.20
Red gram	0.48	0.48	0.48
Chick peas	0.33	0.33	0.33
Broad beans	0.28	0.28	0.28

From the above table it is clear that phytic acid content of every sample has undergone some reduction after steaming. The reduction in soyabean is notable after steaming. A reduction of 0.97 g is occurred. Phytic acid in green peas reduced from 0.7 to 0.16. phytic acid in cow pea is cut down from 0.78 to 0.11. The fall is about 0.68g. The raw sample of kidney bean was containing 1.10 g of phytic acid which is diminished to 0.31 which has lessened 0.78g from actual value. Green gram drop down its phytic acid content from 0.84 to 0.26. In black gram the change is of 0.73 g. The phytic acid content of horse gram falls to 0.20g from 0.9 g. The highest reduction is noted in red gram after steaming which reduced to 0.48 from 1.6g. The reduction is of 1.12g. phytic acid in chick peas lessened after steam cooking. 0.97 gram is reduced from the normal sample. In broad beans the reduction is of 0.69g.

TABLE VI

PHYTIC ACID CONTENT IN ROASTED FORM OF SELECTED LEGUMES

Table VI indicates the level of phytic acid in legumes which are cooked by roasting.

NAME OF THE SAMPLES	PHYTIC ACID IN 1g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE %
Soyabean	0.4	0.4	0.4
Green peas	0.17	0.17	0.17
Cow pea	0.18	0.18	0.18
Kidney beans	0.48	0.48	0.48
Green gram	0.29	0.29	0.29
Black gram	0.27	0.27	0.27
Horse gram	0.21	0.21	0.21
Red gram	0.56	0.56	0.56
Chick peas	0.51	0.51	0.51
Broad beans	0.28	0.28	0.28

From the above table it is clear that phytic acid in soyabean is reduced considerably. The reduction is of 0.9 g. Phytic acid in in green peas reduced by o.53 g. Cow pea was containing 0.78g of phytic acid and is reduced after roasting to 0.18g. The lessening of phytic acid in kidney bean after roasting was found to be of 0.62g. The phyic acid present in green gram falls from 0.84g to 0.29g. Black gram at raw state, it was carrying 0.95g of phytic acid. After roasting it the phytic acid decreased to 0.27g. Horse gram also showed reduction in phytic acid after roasting. About 0.69g phytic acid reduction is observed in horse gram. The most remarkable effect of roasting on phytic acid is found in red gram. Reduction of phyic acid in red gram is about 1.1 g. In chick peas and broad beans there is reduction in phytic acid after roasting but not greater than in red gram. In both chick peas and broad beans the reduction is about 0.79g and 0.69g respectively.

TABLE VII

PHYTIC ACID CONTENT IN PRESSURE COOKED FORM OF SELECTED LEGUMES

Table VII highlights the effect of pressure cooking on phytic acid in selected legumes.

NAME OF THE SAMPLES	PHYTIC ACID IN 1g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE %
Soyabean	0.29	0.29	0.29
Green peas	0.37	0.37	0.37
Cow pea	0.08	0.08	0.08
Kidney beans	0.18	0.18	0.18
Green gram	0.41	0.41	0.41
Black gram	0.16	0.16	0.16
Horse gram	0.53	0.53	0.53
Red gram	0.44	0.44	0.44
Chick peas	0.20	0.20	0.20
Broad beans	0.1	0.1	0.10

From the above table it is clear that phytic acid in soyaban is reduced by 1.01g after pressure cooking. In raw state green peas was containing0.7g of phytic acid. After pressure cooking decrease of 0.33g was observed. The amount of phytic acid in raw cow pea was 0.78 and after pressure cooking it does not shoed any change in its value. Initially kidney bean was found with comparatively high value of phytic acid (1.10g) which reduced by 0.92g as a result of pressure cooking. The PA value of green gram dropped from 0.84 to 0.41g. In the case of black gram phytic acid is reduced from 0.95g to 0.16g. Phytic acid in horse gram also reduced to 0.53g from 0.9g. Pressure cooking was found to be very effective in reducing phytic acid in red gram. The reduction is of 1.16g. Chick peas had also undergone a higher reduction of phytic acid. 1.1g is reduced in chick peas after pressure cooking. Broad bean had shown a reduction of 0.87 g f phytic acid.

TABLE VIII

PHYTIC ACID CONTENT IN DEEP FAT FRIED FORM OF SELECTED LEGUMES

Table VIII projects the data obtained after cooking selected legumes by deep fat frying.

NAME OF THE SAMPLES	PHYTIC ACID IN 1g SAMPLE	PHYTIC ACID IN 100 g	PERCENTAGE %
Soyabean	0.32	0.32	0.32
Green peas	0.13	0.13	0.13
Cow pea	0.15	0.15	0.15
Kidney beans	0.42	0.42	0.42
Green gram	0.27	0.27	0.27
Black gram	0.22	0.22	0.22
Horse gram	0.17	0.17	0.17
Red gram	0.53	0.53	0.53
Chick peas	0.49	0.49	0.49
Broad beans	0.24	0.24	0.24

The above table shows that, after deep fat frying phytic acid in soyabean reduced by 0.98g. Before cooking green peas was possessing 0.7g of phytic acid after cooking it has reached to 0.13g. In cow pea the reduction is of 0.63g. Kidney bean had a drop of 0.68g of phytic acid from the value of raw sample (1.10g). Green gram has undergone a reduction of 0.57g. At raw state black gram was containing 0.95g of phytic acid. After deep fat frying it was reduced to 0.22g. Horse gram, after cooking was observed to have 0.17g of phytic acid. Red gram has shown a notable reduction after deep fat frying (1.07g is reduced). Chick peas and broad beans also reduced in phytic acid. The reduction is of 0.33g and 0.73g respectively.

TABLE IX

EFFECT OF PRE-COOKING AND COOKING ON PHYTIC ACID CONTENT IN SELECTED LEGUMES

Table IX highlights on the, variation in phytic acid content between the selected legumes during different pre-cooking and cooking methods.

Process / Samples	Raw	Soaking	Sprouting	Boiling	Steaming	Roasting	Pressure cooking	Deep fat frying
Soyabean	1.3	1.31	0.21	0.32	0.33	0.4	0.29	0.32
Green peas	0.7	0.61	0.16	0.51	0.16	0.17	0.37	0.13
Cow pea	0.78	0.21	0.16	0.10	0.11	0.18	0.08	0.15
Kidney beans	1.1	0.51	0.29	0.21	0.31	0.48	0.18	0.42
Green gram	0.84	0.79	0.16	0.56	0.26	0.29	0.41	0.27
Black gram	0.95	0.34	0.22	0.22	0.22	0.27	0.16	0.22
Horse gram	0.9	0.82	0.18	0.6	0.20	0.21	0.53	0.17
Red gram	1.6	0.60	0.32	0.53	0.48	0.56	0.44	0.53
Chick peas	1.3	0.56	0.17	0.28	0.33	0.51	0.20	0.49
Broad beans	0.97	0.40	0.20	0.14	0.28	0.28	0.1	0.24

The above table (A) shows the sample wise and process wise comparison of reduction of phytic acid. From the table it is clear that, among raw samples kidney bean contain highest amount of phytic acid. Soaking doesn’t influence the phytic acid in soyabean. May be soaking with greater time (more than 12 h) or higher temperature can reduce it. In all the process cow had given a good response in reducing phytic acid. On comparing the cooking methods and reduction of phytic acid, sprouting and pressure cooking has found to be the most effective in reducing phytic acid.

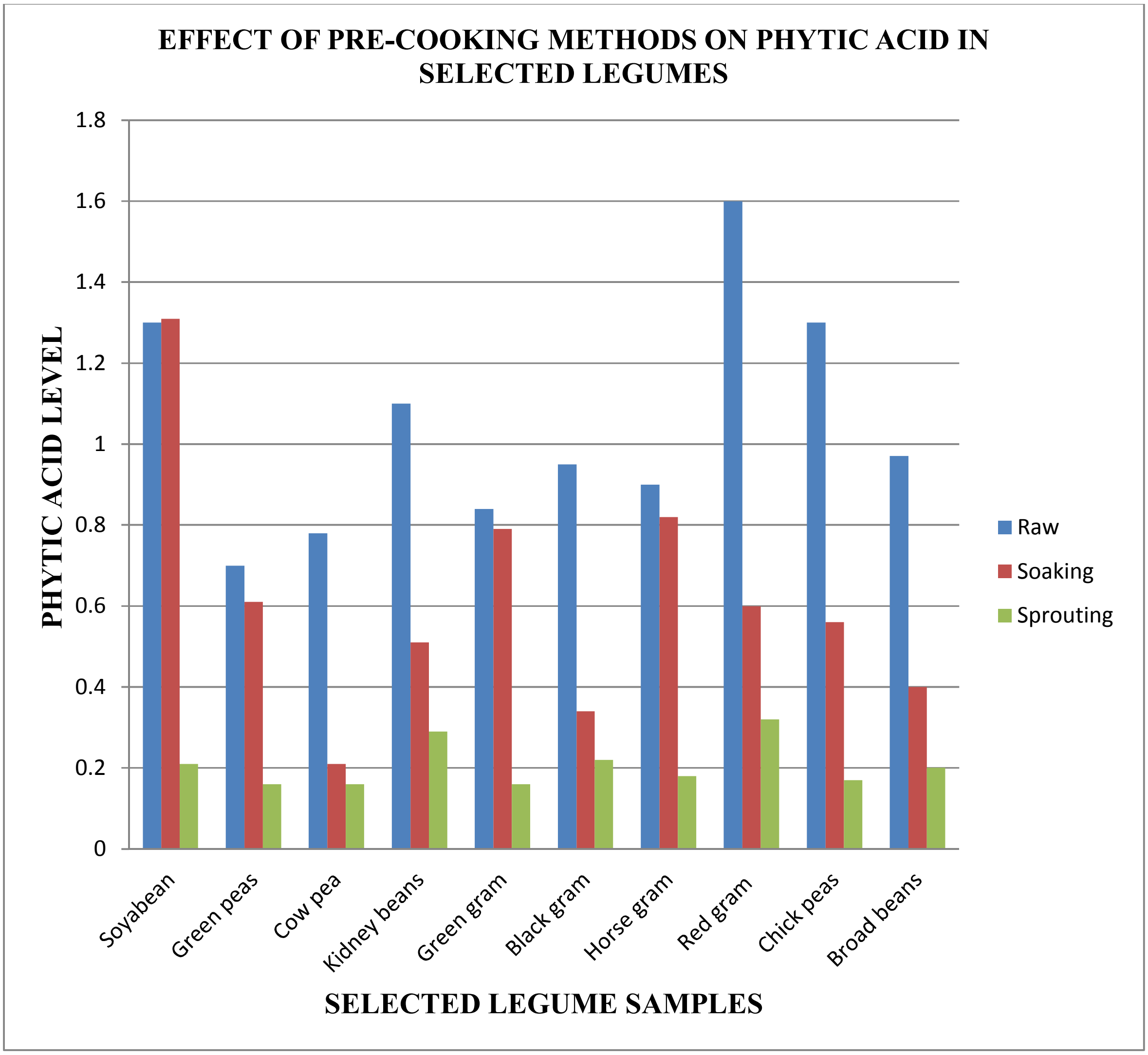


FIGURE II

The above figure shows that among all the pre-cooking method sprouting is found more effective in reducing phytic acid in selected legumes. Whereas soaking of selected legumes when compared to sprouting has shown very less effects on reducing phytic acid.

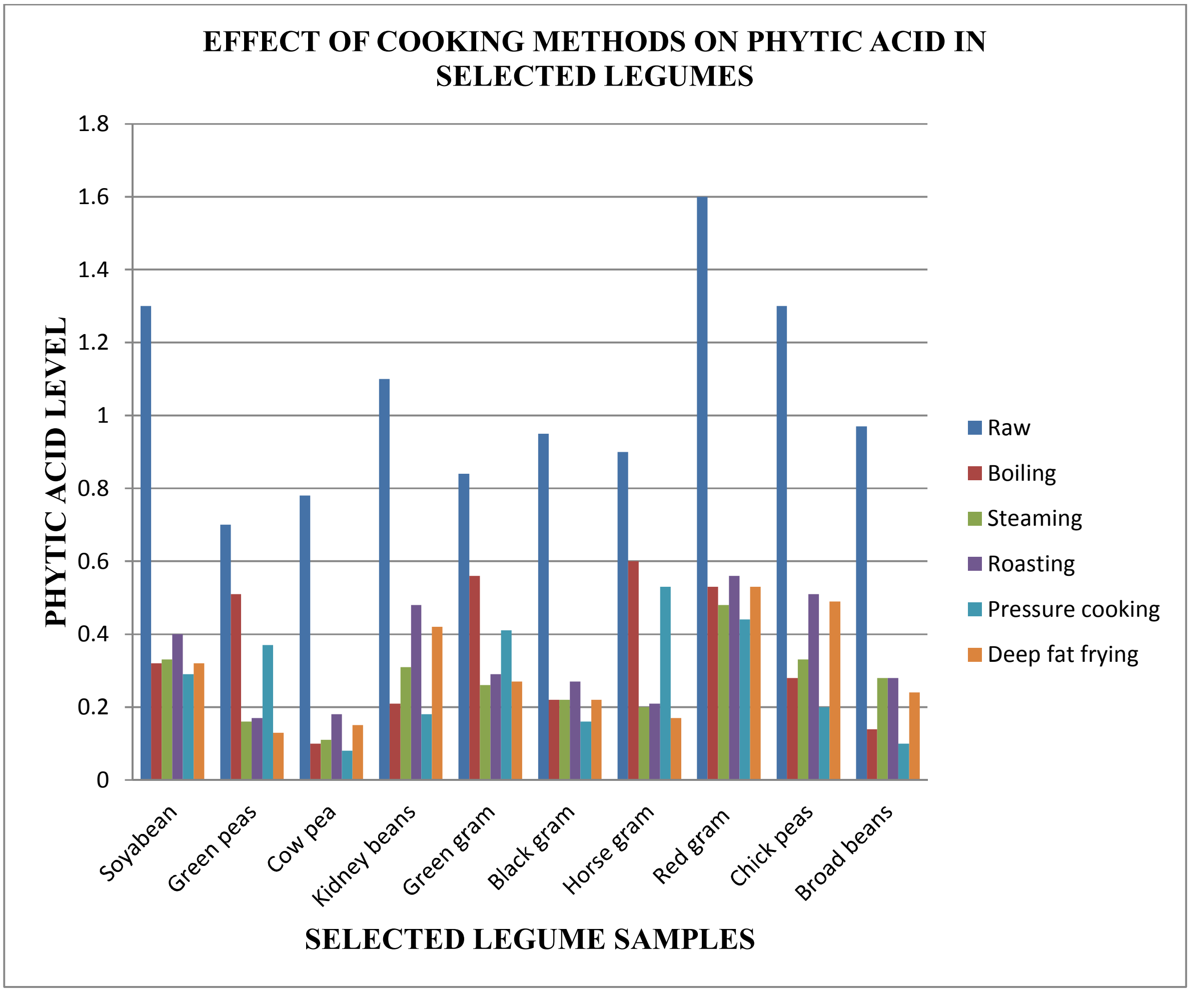


FIGURE III

The figure II shows the reducing effect of various cooking methods on phytic acid in seleced legumes. In most of the (7) legumes pressure cooking was found effective in reduction of phytic acid. Then is boiling and steaming. While comparing all the cooking methods including, boiling, steaming, roasting, pressure cooking and deep fat frying , roasting is found as a less effective method for reducing phytic acid in selected legumes. Almost all the cooking methods was found to be effective in cow pea to decrease phytic acid content.

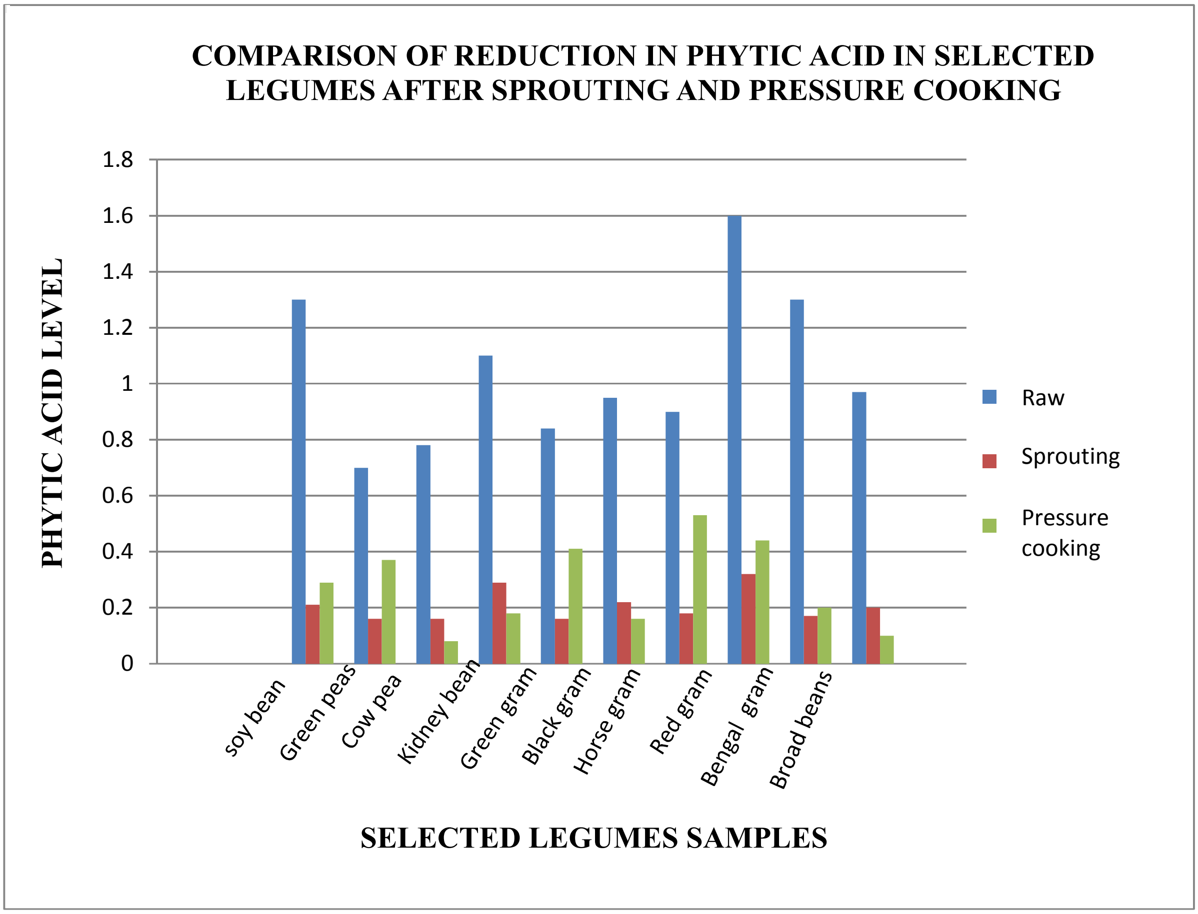


FIGURE IV

Figure III is the comparison of reduction of phytic acid after sprouting and pressure cooking. From the graph it is clear that in most of the selected legumes sprouting is effective in reducing the phytic acid.

A study done by Bisnoi *et al.* (*1994*) in selected pea verities has found that all the domestic processing and cooking methods could reduce the content of phytic acid and poly phenols but germination for 48 hours seemed to have a marked lowering effect on the levels of these anti-nutrients in peas.

Patterson et.al (2017) proved that processing of pulses (peas, beans, lentils, chick peas, and faba beans) is necessary to reduce or eliminate the anti-nutrient compounds. Conventional processing include soaking, dehulling, boiling and pressure cooking as well as germination and fermentation reduce the levels of phytate, protease inhibitors, condensed tannins, and saponins. Germination tends to be most effective at reducing phytate levels regardless of pulse type.

V. SUMMARY AND CONCLUSION

The study on "Effect of pre-cookig and cooking methods on phytate content in selected legumes" was carried out with a main objective to find out the better method to reduce phytic acid in legumes.

Various articles and reviews were reffered and noted the phytic acid activity in pulses and legumes. After considering various factors like, rate of consumption, anti-nutrients present, availability and price of legumes, 10 legumes were selected for the study. All the pulses selected were commonly consumed by South Indians.

The legumes selected for the study are, *Glycene max* (soya bean), *Vigna unguiculata* (cow pea),*Phaseolus vulgaris*(kidney beans),*Vicia faba* (broad beans), *Cicer ariectinum* (chick peas), *Pisum sativum*(peas), *Vigna radiata* (green gram), *Vigna mungo* (black gram), *Cajanus cajan*(red gram), *Macrotyloma uniflorum* (horse gram).

Selected legumes were under gone various pre-cooking and cooking treatments before the analysys of phytic acid. Before applying cooking treatments raw samples were tested for its phytic acid and used to comare with the treated samples to identify the chane or reduction in phytic acid.

The pre-cooking methods applied were soaking and sprouting. For soaking each sample were taken in 10 grams and soaked in separate vessals for 12 hours and after leeching out the water the samples were undergone phytic acid analysis. The same soking procedure was done as a step of sprouting. And the samples were tied in cotton cloth and kept for sprouting. After sprouting they were taken for phytic acid analysis.

The cooking methods applied were boiling, steaming, roasting, pressure cooking and deep fat fried. For each process 10 gram of all samples were taken and the procedures were done. Boiling was done in a vessel and the samples were boiled till the pulses are cooked. Steaming was done using a steamer. At a time 3 samples were steam cooked. Roasting was done using a pan. The samples were roasted separately till the outer covering of pulses breaks and raw smell changes. And they are allowed to cool. Pressure cooking of pulses is done with the help of a pressure cooker according to the pulses cooking time varies. Deep fat frying was done using coconut oil. In a deep bottom pan each sample were deep fat fried separately. Excess oil was wiped out. After preparing all the cooked samples they were taken for the analysis of their phytic acid content.

For the estimation of phytic acid, Haug and Lantzech (1983) method was used. According to this method an appropriate amount (0.8-1.0 gram) of sample was extracted with 0.2N HCl by taking 25 ml of 0.2N HCl in conical flask and shaken for 1 hour on shaker at 30 C and 80 revolutions per minute. 0.5 ml of extract was taken into test tube fitted with a ground glass stopper. Then1 ml of acidic ammonium iron-III sulphate solution of known iron content was added and the tubes were covered with a stopper and fixed with a clip. The tubes were heated in a boiling water bath for 30 min and kept for cooling in ice water for 15 minutes. The tubes were allowed to reach at room temperature. Then the contents of the tube were mixed and centrifuged for 30 min at 3000 revolutions per minute. From this 1 ml of the supernatant was transferred to another test tube and 1.5 ml of 2, 2-bipyridine solutions was added to it, a light pinkish colour was appeared. The absorbance was measured at 519 nm against distilled water. Decrease in iron, in the supernatant was the measure for phytic acid contents.

Standards of known phytic acid concentration were prepared and absorbance was measured at 519 nm. Standard curve was made taking phytic acid concentration in micrograms on X-axis and Optical Density (O.D) on Y axis

After finding out the phytic acid content in each of the raw and treated samples, the values of samples were compared to identify the change in phytic acid content in selected samples.

All the selected raw samples were containing some amount of phytic acid in it. After treatment, most of the samples were undergone reduction in phytic acid. In raw form red gram was containing higher amount of phytic acid then is chick peas and soya bean. After analysis of tables and graph it is found that sprouting is the best method to reduce phytic acid in legumes. Pressure cooking was also found effective in reducing phytic acid but not effective as sprouting.

CONCLUSION

Malnutrition and mineral deficiency disorders are common health problems around the world. According to studies conducted in developing countries like India are more exposed to mineral deficiency diseases and related problems. Mineral deficiency may occur due to many reasons.

The main causes of mineral deficiency are poor dietary intake and poor bioavailability. The bioavailability of minerals is affected by many chemical compounds namely anti-nutrients. They are naturally present in plants. The intake of anti-nutrients more than allowed level may affect our body system harmfully.

Even though they have bad effects such as their interference with mineral absorption, some of them also act as antioxidants and play a good role in keeping our body healthy. There are a number of anti-nutrients including, phytic acid, tannins, polyphenol, trypsin inhibitrs are some of them. Phytic acid is found in most of the legumes which we consume daily without knowing the activity of such anti-nutrients and their interactions with our body through diet. This study is trying to bring out the unseen phases of phytic acid as an anti-nutrient.

In this study the effect of phytic acid in legumes were analysed. Phytic acid is found to play a major role in reducing the bioavailability of minerals to human body. In most of the study reviewed says that phytic acid is an anti-nutrient mainly found in legumes and other plant seeds.

Consuming pulses and legumes in the raw form will lead to lack of mineral absorption by body as it contains more phytic acid and other anti-nutrients. Pre-cooking and cooking methods will help to reduce the amount of phytic acid in legumes. Sprouting is found to be a more effective method. So consumption of pre cooked and cooked legumes are found to be safe to eat as raw pulses contains greater amount of anti-nutrients.

Hence we cannot say that the mineral deficiency is completely due to the presence and interaction of anti-nutrients like phytic acid in legumes. But it plays a crusial role in bioavailability of minerals like iron, zinc and calcium. So before consumption of foods with higher amounts of such anti-nutrients they have to be treated with proper methods in which the reduction of anti-nutrients is high and improvement of essential nutrients is high.

According to the objectives of this study it is clear that all the pre-cooking and cooking method have some reducing effect on phytic acid content in selected legumes.The application of soaking in soyabean is not found effective. The best method for reduction of phytic acid in legumes is found to be sprouting. Red gram is found to have a higher amount of phytic acid in the raw form. Also chick peas and soyabean contains high amount of phytic acid but the quantity is not higher than in red gram. So intake of pre-cooked and cooked legumes is suggested for improved absorption of minerals by the body.

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INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women

Deemed to be University Under category 'A' By MHRD, (Estd. u/s 3 of UGC Act 1956)

Re Accredited with 'A' Grade By NAAC, Recognised by UGC Under Section 12 B

Coimbatore - 641043, Tamil Nadu, India

Seek and Ye Shall Find

24 January 2019

To
Ms.S.Aswathy
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear S.Aswathy,

Ref: Your proposal No.IHEC /18-19/FSMD /07entitled "Effect of Pre-Cooking and Cooking method on Phytate Content in Selected Legumes" submitted for approval to the IHEC on 30.09.18.

The study entitled "Effect of Pre-Cooking and Cooking method on Phytate Content in Selected Legumes" does not come under the purview of Institutional Human Ethics Committee as no human subjects are involved. If in case any human subjects are involved in the study hereafter you are requested to re-submit.

We wish you all the best in your research endeavours.

Regards,

S. Uma Mageshwari

Dr.S.Uma Mageshwari
Member Secretary

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore ★ Institutional Human Ethics Committee