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Effect of Germination and Temperature on Phytic Acid Content of Cereals

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***Abstract* – Cereals are one of the staple food that is being consumed all over the world and are grown in large quantities than any other types of crops. Cereals have good nutritional value and provide most of vital nutrient that is required by the body. Phytic acid is one of the major anti-nutritional component that is found within the cereal. Phytic acid is also called inositol hexakisphosphate and is one of the major storage form of phosphorous in cereals. Phytase is the enzyme which is responsible for the reduction of phytic acid. There are some factors that affect the phytase activity and phytic acid content, germination and temperature are one of those factors. Many studies has shown that Germination and temperature have direct effect on phytase activity and phytic acid content of cereals.**

***Keywords* – Cereals, Phytic Acid, Phytase, Germination, Temperature.**

I. Introduction

Cereals are seeds or grains of Gramineae family. Cereals are the prevailing crops in the agricultural world, with a total harvesting of 2500 million tonnes globally in 2011, consisting of 723 million tonnes of rice, 704 million tonnes of wheat, 883 million tonnes of maize and other minor cereal in lesser amount [1]. There has been long history of cereals being used by humans for food [2]. Evolution of a numerous crops of cereals have showed that there is primarily the similar evolving pattern of all cereals crops from wild grasses to cultivated plants [3]. Cereals have advantage over many crops due to its ease of cultivation, growth, storage and transport. This ease of production and handling facility which is provided by cereals crop is the reason behind for being the staple for humans civilization for over 10,000 years [4]. Cereal as a whole grain is rich source of essential nutrients like carbohydrate, fats, oils, protein, vitamins and minerals [5]. Cereals like Rice, wheat, sorghum, barley and maize are grown in approximately 700 million hectares globally and together they constitute almost 40% of the energy and protein part of the human diet. Cereals contribute a vital role in the food security both at present and at future level [6]. One of the influential characteristic for the propagation of plant species is germination and development of new seedling. Germination is thought to be the most crucial phase in the life cycle of any plant because at the time of germination seeds are at high susceptibility to being getting damage, disease, and water or environmental problems [7]. The process of germination compromises of events beginning with the uptake of water by the dry mature seed and ending with the projection of the radicle through the seed envelopes [8]. During the period of germination the materials which are reserved gets degraded which are commonly utilized for respiration and synthesis of new cells preceding to developing embryo. Germination process affects the seed by increasing its nutritional contents [9]. There are several factors that affect the germination process, Temperature and moisture are one of those important factor which affect the germination process and the rate of germination of non-dormant seeds. For the germination of seed plant have base or minimum, optimum and ceiling temperatures. The lowest temperature at which germination occur is base or minimum temperature. The optimum temperature is that temperature in which germination rate are at highest and the maximum or ceiling temperature is that temperature above which no germination of seeds take place [10].

Phytic acid (myoinositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate) also known as Inositol-6-phosphate or phytate in its salt form is found at concentrations of 1–3% dry matter in most legumes and cereals. Phytic acid is also found in some vegetables and fruits [11], [12]. Phytic acid is a storage form of phosphate in plant seed and it often get accumulated in the vacuole of plant seeds after biosynthesis. Phytic acid provides phosphorus and myo-inositol upon the early growth of seedlings and during the germination of seed. In monogastric animals and by humans phytic acid is less digestible [13]. Due to high amount of phytic acid there is decrease in the absorption of nutrients and their bioavailability. Phytic acid is not digestible in the human gastrointestinal tract but it can be digested by dietary plant enzyme phytases and by phytases which are originating from enteric microorganisms [14]. In monogastric animal and in human phytate have anti-nutritional activities because of its strong chelation of Iron and Zinc which forms insoluble complexes that cannot be absorbed and this lead to Zinc and Iron deficiency [15]. Phytate problem is particularly with a cereal grains but mineral absorption from these foods can improve by pre-processing. There has been some concern that due to high phytate content in food, there could be high levels of toxic heavy metals due to natural accumulation in such foods [16]. Phytase is enzyme distributed expansively among various living forms. The greatest potential sources of phytase are microorganisms and then plant. Generally, there are four potential sources of phytase which are microbial phytase, plant phytase and phytase which is generated by the small intestinal mucosa, and the phytases which are associated with gut micro floral [17]. Phytase enzyme is responsible for the degradation of phytate or phytic acid. The phytase activity increases potentially during germination and consequently leads to degradation of phytates. It has been seen that there was decrease in the phytate content from 0.35% in native seeds to 0.11% during germination in oat seeds and this reduction in phytate content was believed due to increase in activity of enzyme phytase during germination [18]. Temperature also plays one of the major roles in germination, in phytase activity and in phytic acid content. Higher phytase activity and degradation of phytic acid in cereal occur at particular temperature. It has been reported that for hydrolysis of phytic acid in wheat meal dough, the optimum temperature was 55ºC, after 2 hour 80% of phytic acid was hydrolyzed and after 4 hour more than 95% was hydrolyzed. The hydrolysis was significantly lower at temperatures higher than 55ºC possibly because of inactivation of phytase enzyme [19]. We did comparative study on effect of germination and temperature on phytic acid content of cereals.

II. Germination

Germination is the process which begins with imbibition (uptake of water by seed) and end with embryonic axis (radicle) coming out of the seed. A mature and dry seed is called quiescent. The quiescent seed are the inactive organs with very low moisture content (5-15%) and with low metabolic activity to almost negligible level and at this stage seed can survive for several years. For the germination to initiate most of the quiescent seed needs to be only hydrated under such environment that support metabolic activity for the germination [20]. A set of stage must be completed before a seed can germinate which also include availability of food. Such stored food consist of protein, starch, lipid and other nutrients, and these stored food become accessible to the seed embryo via activity of particular pathways and enzymes [21]. There are many factors such as environmental and developmental which regulate the germination process. There are many plant hormones which are involved in the regulation of germination process and abscisic acid is one of them [22].

III. Germination Process

The seed of the most plant are dispersed in a dry and mature state and if these seed are non-dormant and they have favorable environment they will germinate to become a plant [23]. The process of germination is majorly done in three steps, (1) uptake of water by seed, (2) beginning of metabolic process and (3) growth of embryo or emerging of radical from the seed tissue [20]. So the germination is activated when the metabolic process of a seed overlap between the environmental conditions that is being experienced by the seed and those genetically requirements of the seed which is involved in breaking dormancy and initiating germination [24]. The thing which are associated with the completion of germination are biophysical, biochemical and molecular changes in the embryo and the surrounding tissues, and their interactions [8]. Imbibition or uptake of water exhibit three phase. In phase one swelling of seed and change in shape is caused by initial rapid water uptake by seed. In this phase recommencement of respiratory activities such as oxidative pentose phosphate and glycolytic respiratory pathways and synthesis of protein from existing mRNA, Repair of existing mitochondria and also repair of DNA. The second phase is initiated by slow down of water uptake by seed and during this phase repair of existing DNA and mitochondria is continued, and synthesis of proteins from newly synthesized gene transcripts, synthesis of new mitochondria, embryo expansion initiated and the seed covering layers starts getting weaken during this phase. The third phase is initiated when there is emergence of radicle through seed coat. During this phase movement of storage reserves which is deposited in storage organs like the endosperm in case of cereals, this also stimulate further increment in water uptake which leads to growth. Division of cell, synthesis of DNA and elongation of radicle cell also take place during the third phase [25].

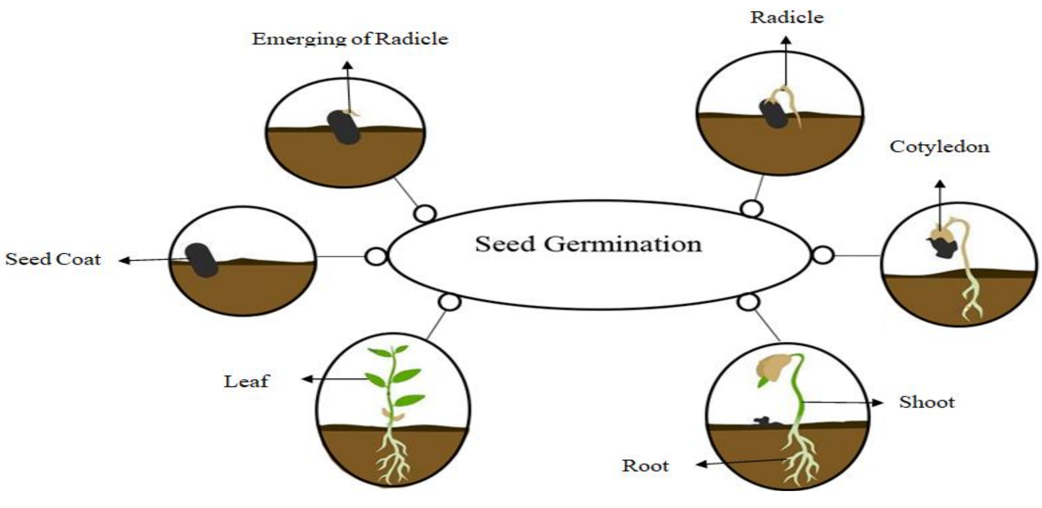

Fig. 1. Seed germination.

IV. Phytic Acid in Cereal Grains

Myoinositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate also known as Phytic acid ($C6H18O24P6$). One of the major storage form of phosphorous consisting of 1–5 % by weight in cereals, legumes, oil seeds and nuts is phytic acid. 50–85 % of total phosphorous in plants is represented by phytic acid [26]. Phytic acid has been widely utilized in antioxidant, anticancer agent, biosensor, cation exchange resin, nano-material and other fields due to its environmentally friendly, biocompatible, nontoxic and easily accessible organic acid and its special inositol hexaphosphate structure [27]. In monocotyledons seed like wheat, rice, millet, barley, e.t.c the accumulation site for the phytic acid is aleurone layer specially the aleurone grain. In terms of phytic acid deposition corn differ from other cereal because in germ more than 80% of phytic acid is found. The content of phytic acid of cereal varies from 0.5% to 2% [28]. The reason behind different products of milling contain different levels of phytates is because most of the phytic acid is found in the kernel's outer part. There is high amount of phytic acid which is found in the bran of cereals. White flours which have been low extracted is low in phytic acid content. The quantities of phytic acid in protein concentrates or isolates which are made from cereals or other raw materials depends on the raw material and method of processing, so method of processing also play a crucial role in the content of phytic acid in final product [29]. There is a strong ability of phytic acid to form chelate with multivalent metal ions, particularly zinc, calcium, iron and the residue of protein. The binding may end in very insoluble salts with poor bioavailability of minerals [30]. The relation of phytate with proteins begins in seeds during ripening, when phytate increase within the protein-rich aleurone layer of cereals. However, phytate-phosphorus may be a smaller amount nutritionally available since the phytate isn't quantitatively hydrolysable in human gut [28]. There are many methods which are available for the determination of phytic acid but however researcher has concluded that for the measurement of phytic acid for unprocessed product such as raw cereal or grains precipitation methods is useful and for processed foods HPLC, synchronous fluorescence, or other modern methods to determine the phytic acid content are useful [31]. Phytic acid is an anti-nutritional factor because it forms complexes which are insoluble with mineral such as Iron, Zinc, Magnesium and Calcium and making mineral unavailable for absorption by the body [28].

Phytate

Phytates (inositol hexaphosphate) are compound which occurs naturally in many cereals and legumes. Phytates constitute the foremost storage form of phosphorous in cereals, legumes, nuts and seeds [32]. Phytate as phosphorus bound to inositol constitute of 60-80% of total phosphorus which is present in oilseeds, cereals, legumes and nuts. In fruits, root and berries lower concentration of phytate are found. In the germ of cereal phytate is concentrated in the aleurone layer and the scutellum while in endosperm phytates are not found. Phytates are accumulated within the globoids of the protein bodies in cereal and grains [33]. In human phytate act as antinutritional component in human diet because of its strong chelation of calcium, Iron and zinc which forms insoluble complexes that are not absorbed by the human digestive system and can lead to deficiency of minerals like iron and zinc [15]. This insoluble complex which is formed is the reason of poor bioavailability of the minerals. In most of the studies it has been shown that there is inverse relationship between the availability of mineral and phytate content. Many study has shown that iron absorption has strongly inhibited by the action of phytate. Overall phytate decreases the bioavailability of minerals. Both at acidic and alkaline pH, phytates are known to form complexes with proteins and this interaction may decrease protein solubility, proteolytic digestibility and enzymatic activity because of changes in protein structure. As the number of phosphate residues per myo-inositol molecule and the myoinositol phosphate concentration increases the inhibitory effect also increases [41]. There is reduction in the activity of different digestive enzymes such as pepsin, trypsin, acid phosphorylase and amylase due to interaction of phytates with these enzymes and this ultimately can reduce the nutrient utilization. Calcium salt of phytate can prevent the energy utilization from the lipid sources when it combine with lipid to form metallic soap in the digestive gut [17]. Germination progressively decreases the content of phytate. During germination there is potential for the increment of phytase activity and this subsequently is the reason for the degradation of phytates. During germination the content of phytate decreased from 0.35% to 0.11% in oat seed [18]. Milling, heat treatment, soaking, fermentation, and germination are the food processing and preparation methods that may remove or degrade phytates to a varying level [42]. The best possible way of reducing the phytate content is by hydroysis of phytate by the enzyme phytase and wet processing method such as soaking, fermentation and germination increases the phytase activity and thus eventually leads to degradation of phytate.

Table 1. Phytic acid content of different cereals.

Cereals	Phytic Acid Content (g/100 g)	Reference
Indian pearl millet	0.71–0.72	[34]
Wheat germ	1.1–3.9	[35]
Wheat bran	2.0–5.3	[35]
Brown rice	0.13- 0.27	[36]
barley	0.38–1.16	[35]
sorghum	0.87-2.2	[37]
Dry maize	0.71-0.76	[38]
Oat	0.42–1.16	[39]
Rye	0.54-1.46	[40]

V. Method to Reduce Phytic Acid in Cereal

There are some methods by which phytic acid content of a product can be reduced and these method should be utilized for the processing or development of any food product. Those methods or techniques that reduces the antinutritional factor of food product has always been a interest of study. Nutrient availability in foods are likely to improve by methods like Mechanical, thermal or biological processes. But here we will emphasize on wet processing method. There is reduction in phytic acid content in wet processing method which include soaking, germination and fermentation and this wet process increases the solubility of minerals in foods and this can improve the cereals or legumes in terms of bioavailability of minerals [43]. In wet processing method there is more nutrient retention when compared to any other method.

VI. Germination or Sprouting

Germination or sprouting is the process which is said to be done when the radicle come out of the seed. During the germination or sprouting many biochemical changes take place in the seed which ultimately enhances its nutritional properties (Lemmens et al. 2018). Germination play one of the important role in increasing phytase activity and reduction of phytic acid content of seed (Thompson et al. 1985). During germination phytase activity increases which is responsible for the reduction of phytic acid in plant seeds. For the initiation of germination or sprouting first step is soaking the dried seed so that it reaches to optimum moisture level which favors the respiratory, metabolic activities and movement of primary or secondary metabolites which ultimately triggers the germination [28]. During germination phytate is hydrolyzed in a sequential manner by enzyme phytase or jointly action of enzymes like phosphotase and phytase which do not allow the nutritional need of the plant by acceptance of phytate as substrate without an accretion of less phosphorylated myo-inositol intermediates [26].

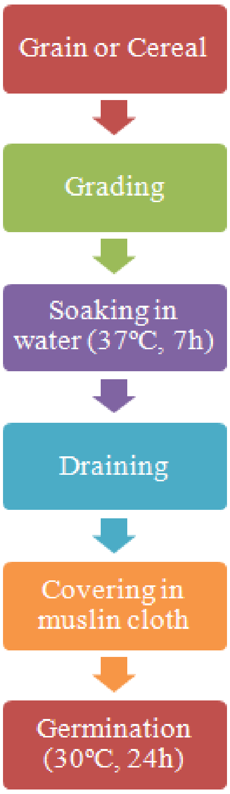

Fig. 2. Flow chart for production of germinated cereals or grains [28], [46].

Soaking

Soaking is one of the essential procedure in the process of germination or fermentation [28]. It has been reported that when sorghum flour is soaked in water for 24 hour at room temperature leads to reduction in the level of phytic acid by 16–21 %. Grains and beans also shows reduction in phytic acid when they are soaked and this result in bioavailability of minerals [26]. Soaking helps in the reduction of phytic acid because phytate is soluble in water and a there are major reduction in phytate which is seen by removal of the soaked water. It has been also reported that on enzymatic phytate hydrolysis during soaking temperature and pH play a significant role [47], [48]. Plant have intrinsic enzyme phytase which enzymatically hydrolyze the phytate. There is a significant amount of phytate (26–100 %) which was enzymatically hydrolysed if the soaking process is done at near to the optimal conditions (temperatures, 45-65°C & pH, 5.0-6.0) for phytate dephosphorylation by enzyme phytase [48], [49].

Fermentation

Due to health benefits of fermented food products it play a crucial role in a human diet. For food preservation, fermentation is one the economical and oldest technique that has been known to prevailed. As indicated by some of the earliest record that human has been taking soured milk as early as 2000 years ago [50]. Fermentation play an effective role in the reduction of phytic acid. In brown rice fermentation was most effective in the reduction of phytic acid by 53% to 95% [51]. Favorable pH condition is being provided by natural fermentation for enzymatic degradation of phytic acid [28]. When germinated pearl millet sprouts were fermented by using S. cerevisiae Saccharomyces diasticus, Lacto-bacillus brevis and L. fermentum at 30ºC for 72 hour then 88.3% was observed in the reduction of phytate [52]. These kind of phytic acid degradation can enhance the quantity of soluble minerals like zinc, calcium and iron. It has been accounted that when fermentation of millet is done for 12-24 hour could reduce the phytic acid content. Large reduction in phytic acid in rice flour can be achieved by natural fermentation by the enzymatic action of grain and microbial phytases. Hexa form of phytic acid (IP6) is reduced into lower form of phytic acid such as IP5, IP4, IP3, IP2, IP1 and myo-inositol by enzyme phytase and the lower forms of phytic acid have lower binding affinity with the minerals like zinc and iron [26].

VII. Effect of Germination on Phytic Acid Content of Cereal

Germination is the process which begins with the uptake of water by dry mature seed, various metabolic process set up and ends with emergence of radicle from the seed. It is a mechanism in which activation of the embryo take place by morphological and physiological changes in seed. Before the commencement of germination, seed absorbs water which results in the expansion and elongation of seed embryo and when the radicle emerges out of the covering of seed layers then the process of germination is completed [21]. During germination many biochemical changes take place. The chief storage form of phosphorus in plant tissues is Phytic acid or phytate when it is in the form of salt [53]. Phytic acid or phytate is formed when the maturation of the plant seed take place and it represents 60-90% of total phosphate in dormant seeds. Germination affects the content phytic acid in seed. Germination reduces the content of phytic acid up to 40% [54]. As reported by [9] when phytic acid content was compared for germinated soya bean and non-germinated soy bean the content of phytic acid was considerably decreased (21.74%) after germination and when phytic acid content was compared for germinated peanut and non-germinated peatnut, the content of phytic acid was also decreased for 38.11% after the completion of germination [55]. Also reported by Embaby et.al 2002 that there was reduction in phytic acid content of cowpeas after germination. They reported that there was reduction in phytic acid content from 17.4 to 22.9% when germination time increased from 24 hour to 48 hour [56]. Azeke [57], reported that all the cereal grain which was screened showed significant reduction in phytate content after the 10 days of germination [57]. During germination phytate is hydrolysed in a stepwise manner by the enzymes phytases or a joint action of phosphatases and phytases [58]. The reason for the reduction in phytic acid content during germination in cereal is that germination leads to increase in phytase activity and this lead to hydrolysis of phytate which ultimately degrade the phyctic acid and reduces its content in the germinated cereal [57]. As germination leads to increase in phytase (myo-inositol hexakisphosphate phosphohydrolase) activity and this eventually leads to degradation of phytic acid and liberates phosphate which results in lower inositol phosphates or inositol and this lower inositol phosphates have less binding ability and have low effect on iron and zinc bioavailability in humans [59]. In the normal or non-germinated cereal phytase activity is comparatively low when compared to the germinated cereals. The factor that affect the activity of phytase enzyme are type of cereal, the time, and conditions for germination. The phytate content in bran part is high. There is increase in the activity of enzyme phytases which degrades the phytate when time & temperature condition provides in range of 4-5 days at 15-25˚C in few cereals. This increase in the activity if enzyme is calculated to be three to ten folds in case of wheat, rice, barley, sorghum [57], [60]. Similar observation on effect of germination on phytic acid has been also reported by Ayet [61] which states that germination to the decrease in phytic acid which is due to hydrolysis of phytate phosphorus down to inositol monophosphate by enzyme phytase and the liberated phosphorous is more likely been transported to the embryo for the additional synthesis of organic phosphates. Hence in the rise in the activity of enzyme phytase during the germination of seed may be due to activation of the pre-existing enzyme or may be due to synthesis of phytase from the beginning of germination process [61].

VIII. Role of Temperature in Germination

The optimum condition such as oxygen, moisture and temperature is required for the germination of cereals and these conditions are specific for different types of cereal crops. When the temperature range reaches 30ºC to 35ºC there is increase in the rate of germination. The temperature at which germination of cereals grains takes place at short period of time that temperature is called optimum germination temperature [62]. So temperature plays an important role in seed germination. One of the most critical factors that affect the germination of the seed is temperature. The effect of temperature on germination of seed have been well depicted for many agricultural and vegetable species clarifying the critical lower temperature and upper temperatures for seed germination, and the temperature at the highest rate of germination and its germinative capacity [63]. Temperature is one of the major factor that affect verities of plant activities which also include seed dormancy and germination. Germination in some plant seeds take place at a wide range of temperatures while other germinate at a narrow range of temperature. Seed responses to temperature act as one of the key factor for timing of germination, and it is often considered most important environmental factor in the germination of plant seed [64]. Germination of seed occur between a (Tmin) and a ceiling (Tmax) threshold temperature, below and above this temperature range germination do not take place. The highest rate of germination occurs at optimum temperature (Topt). Different plant species have different base temperature (Tb) and this temperature is most likely near to the minimum temperature required for germination [65]. As different approach adopted by the species of different plant as a result of the heterogeneity of habitats, climatic seasons and the surrounding there is wide range of diversity found in the influences of temperature on the process of germination [66]. One of the main environmental factors which influences the initiation of seed dormancy during seed development and expression of seed dormancy during germination of seed is temperature. For the most species of crop as the temperature rises seed germination increases but some species of crop also shows higher percentage of germination at low temperature [67]. There is reduction in the rate of germination and also there is reduction in the dry mass of cereals due to prolong exposure to high temperature [68]. So temperature play one of the important role in the process of germination. Temperature has direct impact on the efficiency and rate of germination.

Table 2. Germination temperature for different cereals.

	Temperature (ºC)		
Cereal	**Germination May Occur Between**	**Optimum Germination Range**	**Reference**
Wheat	4-37	12-25	FAO 2002
Maize	21-30	21-27	Greaves et al. 1996
Rice	10-42	25-35	Li et al. 2020
Rye	20-35	20-30	Lin et al. 2018
Barley	3-40	19-27	FAO 2002

IX. Effect of Temperature on the Phytic Acid Content of Cereal

There is reduction in the phytic acid content of cereal and legumes during processing. It has also been reported that during the process of baking of leavened and unleavened Iranian flat breads and during making of bread there is reduction in phytic acid content [69]. There is a report which states that thermal decomposition of phytic acid took place when it was heated to 150°C for one hour [70]. Change in temperature shows the difference in phytase activity. It was seen increase in the phytase activity of A. oryzae as the temperature increased upto 40ºC and then the activity became inactive at 50ºC. Similar observation was found in the phytase activity of A. niger, which showed increase in the phytase activity upto 45ºC and then became inactive at 60 ºC [71] So increase in phytase activity at specific temperature of different seed exhibit that there must increase in the reduction of phytic acid because phytase enzyme leads to degradation of phytic acid content in the seeds. As reported by vats & Banerjee that at temperature range between 45°C to 60°C there is usually high activity of phytase has been observed [72]. There is great reduction in phytic acid content in brown Bengal as compared to white Bengal gram when they are boiled or roasted [69]. In the study carried out by the Seye and his co-authors reported the result on effect of parboiling on the concentration of phytate of two millet verities. In their result they found that in both the verities of millet heat treatment like parboiling, hot soaking, steaming and drying before milling resulted in the content of phytate in the two verities of millets. It was also reported that for significant reduction in the concentration of phytate soaking at temperature 80 °C would be the best one and heat treatment like parboiling would be one of the effective method in the reduction of phyate content in millet grains [73]. Effect of temperature on phytic acid has also been reported by Dhanker & chauhan that there was significant decrease in the phytic acid content of Rabadi (a fermented pearl millet food) at temperature 35°C, 45°C and 50°C [74]. There was decrease in the phytic acid content of Sorghum cereal flour when it was evaluated after storage at 4°C, which also indicated that storage of cereal flour also contributed in the reduction of phytic acid [75]. In one of the study it has also been reported that upon heat treatment like boiling, baking, roasting in sand bath there is significant loss in phytic acid content of maize. There is also reduction in the phytic acid content during baking of maize chapati [38]. So it has been seen that temperature play essential role in the phytase activity and phytic acid content.

X. Conclusion

Cereals are one of the most important crop that is being cultivated at large scale than any other crops. Cereals have been staple food from long time ago. The reason for the successful incorporation of cereals in the field of agriculture is due to their easy cultivation, handling, storage and transportation. Cereal meets out daily nutritional intake in the diet. Cereals are good in nutritional value. Germination is one of the important processes where growth of embryo or seedling takes place. Proper environmental condition must be attained for efficient germination process and germination rate. Temperature plays one of the important roles in the germination process. There is high germination rate is observed when there is optimum temperature for the germination. Phytic acid is one of the anti-nutrients that is found in most of the cereal. The content of phytic acid in different cereal varies according to species and the environment where they have been grown and stored. Phytic acid is one of the major storage form of phosphorous in cereal. Phytic acid are considered anti-nutrient because it makes mineral unavailable for absorption by the body. There are many factor that affects the content of phytic acid in cereal. Germination, temperature and phytase activity are one of those factor that affect the content of p-

-hytic acid in cereals.

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