

# Biochemical and Enzymatic Changes Associated with Duration of Germination of Wheat Moth based Food Mixes

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**Abstract:** Germination is complex process during which the seed must quickly recover physically from maturation drying resume a sustained intensity of metabolism, complete essential cellular events to allow for the embryo to emerge, and prepare a subsequent seeding growth. Legumes consumed after processing and germination are most economical food. This process is also an appropriate low cost low technology option for household processing in lesser developed countries. It causes important changes in the biochemical, nutritional and sensory characteristics of products and it enhances the nutritional value and increases protein digestibility.

**Keywords:** Proximate composition, Anti Nutrient components, Biochemical Analysis, Amylase activity; protease activity.

## 1. Introduction

In germinated cereal grains hydrolytic enzyme are activated and they decompose starch non-starch polysaccharides and protein, which leads to increase of oligosaccharides and amino acid. The decomposition of the high molecular weight polymers during germination leads to the generation of bio-functional substance and the improvement of the organoleptic qualities due to softening of texture and increase of flavour. Germinated dry beans are receiving increasing attention due to enhanced flavour and nutritional qualities particularly throughout the breakdown of certain anti-nutrient, such as phytate, and flatulence. The process involves complex changes that propitiate of the breakdown of macromolecules, which increase the rate of starch and protein digestibility improving the content of amino acids and, consequently this results in more digestible foodstuffs and it is one of the reasons why legume sprouts have been used in the preparation of legume based, low cost weaning foods. Germination is also known to improve the vitamins and minerals contents. It has been reported that vitamin C and riboflavin are synthesized during germination. Legumes consumed after processing and germination, are most economical food. This process is also an appropriate low cost technology option for household processing in lesser developed countries. It cause important changes the biochemical nutritional and sensory characteristics in legumes by increasing in amino acid, protein digestibility, amino acid availability and certain vitamins, including thiamine, riboflavin niacin and ascorbic acid. A decrease in CHO content of legumes ranging from 35% to 40% on germination, has been also be reported by many workers. The decrease in total CHO and reducing sugar content was attributed to their consumption, as a source of energy during the germination process. An increase in the digestibility of starch/ CHO due to metabolic and structural changes, hydrolytic breakdown and increased amylolytic activity. Germinated legumes can be consumed as such or processed further for different products. These processing techniques, in turn, can alter the nutritional quality.

## 2. Methodology

Mixes were prepared by using certified varieties of seeds: Wheat (RAJ-3777), Moth Bean (RMO 257), soya bean (NRC 37 Ahilya 4), milk powder. Standard Food Mix S prepared by non-germinated seed. Food mix A by 24 hrs, Food Mix B by 36 hrs. And Food Mix C by 48 hrs. Germinated seed. Nutritional components like Protein, Fat, Fibre, Iron and Calcium; Anti nutrient components i.e. oxalic acid and phytic acid were estimated using standardized techniques. Statistical analyses for analysis of the results of nutritional estimation one-way ANOVA tests were used.

## 3. Preparation of Amylase Rich Premixes Germination

All the broken, cracked, or discolored seeds were removed. Tap water was used for washing and cleaning of seeds. The seeds were cleaned then soaked in distilled water for 12 hrs at 28°C (room temp) without direct contact with sun light. Samples were withdrawn at 24, 36 and 48 hr. of germination and dried in sun drying.

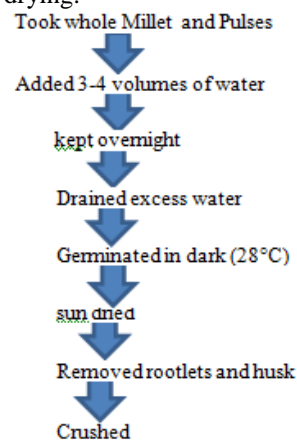


Figure 1: Flow chart for the preparation of Wheat based premixes

**Table 1:** Composition of premixes

<i>Food groups</i>	<i>Amount (gm)</i>
Wheat	50 g
Whole Moth Bean	25g
Oil seeds(soya bean)	10 g
Fat	5 ml
Milk powder	5 g
Sugar	5 g
Total	100 g

#### **Analysis of proximate and anti-nutrient components of the Food Mixes**

Nutritional Estimations were carried out with the basic aim to evaluate nutrient content of the prepared premixes by using standard biochemical techniques. All the techniques which were used for estimations were first standardized. Moisture estimation was done by using oven drying method; Ash was determined by muffle furnace, protein by micro kjeldal method and crude Fiber by acid alkali (NIN, 2010). Anti-nutrient components i.e. oxalic acid and phytic acid were even estimated titrimetrically. The oxalic acid was extracted in HCL and precipitated as calcium oxalate by adding calcium chloride which is then washed and titrated with N/20 KMnO<sub>4</sub> in the presence of dilute sulphuric acid at 70°C. The phytic acid is extracted in 0.5 M nitric acid and treated with ferric ammonium sulphate and isoamy alcohol. Pink color is dissolved in alcohol layer with ammonium thiocyanate, which is invariable proportional to phytic acid content. (Kawatra, 2000)

#### **4. Statistical Analysis**

Each sample was prepared thrice and readings of each parameter were taken five times finally their mean and SD was calculated in order to minimize human error. The premixes were prepared two times once in summer and once in winter to overcome seasonal variation. Data was analyzed by using Microsoft Excel software version 2007. For analysis of the results of nutritional estimation One-way ANOVA tests were used.

#### **5. Results**

##### **Proximate Composition**

Moisture content significantly increased after germination in all samples in comparison to control non germinated samples

Standard Food Mix Shad moisture content approximately 10.8g/100g however Food Mix A had moisture content of 13g/100g however, in Food Mix B the values were 15.3 g/100g and in Food Mix C it was 15.4g/100g respectively.

Ruiz et al 1990 reported in their study that the moisture content after 24 hr. Raised from 30 to 40% and the rise was approximately 75-85% after 48 hrs. From this it is evident that during germination the whole grains absorb moisture from the soaking medium for metabolism to initiate and this in turn influence the structure of the grain as the soaking time increase more numbers of cells within the seeds are hydrated.

The germinated sample showed a significant reduction in ash content ( $p < 0.05$ ), as the duration of germination increased reduction in ash content also increased in case of Standard Food Mix S it was 5.16/100 g however in Food Mix A it was 4.83/100 g, 4.53/100g, 4.2/100g was estimated in Food Mix B and C respectively.

Wang et.al 1997 reported that as the soaking time increase there is reduction in minerals as the seed utilizes then for emergence of rootlet. Contradictory results in the analysis of different variety of mungbean, pea & lentil seeds. Reported increase in ash content with increase in germination time. This may be because of decrease in crude fat and carbohydrate content during germination which might have led to the apparent increase in ash content

The protein content of Standard Food Mix S and germinated samples were analyzed to be 13.63/100g and 17.3 g/100g in the 24 h germinated sample (Food Mix A) however, in Food Mix B, the values were 19.97g/100g and Food Mix C had 24.1g/100g protein respectively

An increase in millet protein from 14 to 40 % was estimated by Opuku in 1981 as the duration of germination increased the rate of respiration increases which results in loss of dry matter particularly carbohydrate which causes increment in other nutrients such as protein. Camacho et al 1992 estimated that during germination of beans, lentils, chickpea and pea's seeds there is increase in protein content Ohtsubo et al 2005 found an increment in crude protein of germinated brown rice.

Fiber remains insoluble even on boiling with dilute acid or alkali. Fiber fraction of food products includes highly insoluble structural fibers viz., cellulose, lignin and hemicelluloses. The fiber content of Standard Food Mix S and germinated premixes were appraised to be 5.76% and 5.5% whereas 5.03% and 4.76% fiber was present in Food Mix A, Food Mix B and C germinated premixes respectively.

Results showed that, during germination there was reduction in fiber content because as there is increase in temperature there is cleavage of weak bonds between poly saccharides and breakdown of glycosides linkage and hence solubilization of dietary fiber (Svanberg et al 1987).

The fat content of Standard Food Mix S and Food Mix A germinated premixes were analyzed to be 7.53g/100g and 6.4g/100g however, in Food Mix B the values were 5.9 g/100g and in Food Mix C it was 4.73g/100g respectively. Shah et al in 2011 studied the effect of germination time on different varieties of mungbeans (Ramzen and NM-98) the ether extract values of two varieties differed significant ( $p < 0.05$ ) the crude fat concentration decreased from 1.79 to 1.4% and 1.71 to 1.39% as the germination time increased from 0, 24, 48 and 96 hrs.

In the present research the carbohydrate Standard Food Mix S prepared by non-germinated varieties i.e. Standard Food Mix S was 57.03g/100g however as the germination time increased the carbohydrate content reduced from 52.9g/100g

to 49.46/100g in Food Mix A it was 47.16g/100g; in Food Mix B, 45.93g/100g in Food Mix C.

Vidal-Valverde et al. (2002) explained that during germination, carbohydrate was used as source of energy for embryonic growth which explained the changes of carbohydrate content after germination. Additionally,  $\beta$ -amylase activity that hydrolyzes the starch into simple carbohydrate was increased (Suda et al., 1986). Starch in

cotyledon was broken down into smaller molecules such as glucose and fructose to provide energy for cell division while the seeds mature and grow (Vidal-Valverde et al., 2002; Nonogaki et al., 2010). Ohtsubo et al., (2005) explained that carbohydrate breakdown in which  $\alpha$ -amylase activities were found to parallel with the pattern of starch breakdown.

**Table 2:** Nutrients estimations of Wheat Moth based premixes

Nutrients	Standard Food mix S	Food mix A24 h	Food mix B36 h	Food mix C48 h
Moisture (%)	10.86 $\pm$ 1.34	13.06 $\pm$ 1.49	15.3 $\pm$ 1.8	15.43 $\pm$ 2.90
Ash content (g)	5.16 $\pm$ 0.70	4.830.30	4.53 $\pm$ 1.40	4.2 $\pm$ 0.51
Protein (g)	13.63 $\pm$ 1.05	17.3 $\pm$ 1.34	19.97 $\pm$ 0.85	24.1 $\pm$ 1.70
Fat(g)	7.53 $\pm$ 0.55	6.4 $\pm$ 0.62	5.9 $\pm$ 0.95	4.73 $\pm$ 0.76
Fiber (g)	5.76 $\pm$ 0.41	5.5 $\pm$ 0.44	5.03 $\pm$ 1.40	4.76 $\pm$ 0.41
Carbohydrates (g)	57.03 $\pm$ 1.34	52.9 $\pm$ 2.66	49.46 $\pm$ 0.50	47.16 $\pm$ 1.15

Mean $\pm$ sd

### Minerals contents

Calcium is the most abundant mineral in the body, body stores more than 99% of its calcium in the bones and teeth to help make and keep them strong. In the present study the calcium amounts in the Standard Food Mix S were gauging to be 17.66 mg/100g and 21.43 mg/100g in Food Mix A. In premix prepared by using Food Mix B the value were shown a higher shoot up of 25.44 mg/100g and 27.21mg/100g in Food Mix C

Mamiro et al 2001 reported that in vitro extractability of calcium and other finger millets and kidney bean increased significantly after germination in comparison to other processing techniques like soaking, autoclaving and fermentation. The iron content of Standard Food Mix S and Food Mix A premixes was estimated to be 9.2mg/100g and 10.58 mg/100g respectively in our research. However, the iron content of Food Mix B and Food Mix C was estimated to be 11.76 mg/100g and 12.92 mg/100g.

**Table 3:** Minerals estimations of Wheat Moth based premixes

Nutrients	Standard Food mix S	Food mix A24 h	Food mix B36 h	Food mix C48 h
Calcium	17.66 $\pm$ 0.55	21.43 $\pm$ 0.90	25.44 $\pm$ 0.73	27.21 $\pm$ 1.45
Iron	9.2 $\pm$ 0.01	10.58 $\pm$ 0.27	11.76 $\pm$ 0.49	12.92 $\pm$ 0.06

### Anti-Nutritional Factors

Oxalic acid content of Standard Food Mix S was 25.63 mg per 100 g; in Food Mix A its was estimated to be 22.83 mg per 100 g but as the duration of germination was increased from Food Mix B and C the oxalic acid content gradually reduced.

Hence it can be concluded that long germination periods are sufficient to produce an appreciable reduction in the anti-nutrient contents and thus help in improving the utilization of available protein & carbohydrates. Similarly statically significant reduction was observed in phytic acid content. Phytic acid has been reported to form complexes with protein which then become more resistant to photolytic degradation (Cheryan, 1980). Thus phytic acid being an anti-

nutrient lowers the bioavailability of both minerals & proteins.

Under our experimental conduction the Standard Food Mix S had approximately 102.33 mg per 100 g phytic acid, however as duration of germination was increased the phytic acid content reduced to 82.83 mg per 100 g (Food Mix A); 49.89 mg per 100 g (Food Mix B); 38.65mg per 100g (Food Mix C) these findings are similar to results reported by Tizazu et al (2011); which stated that there was significant reduction in phytic acid levels (m/100g) ( $p < 0.05$ ) for different varieties of sorghum as germination time was increased from 36 & 48 hrs. Thus enhancing bioavailability of sorghum based complimentary foods.

This can be contributed to the enzymes which make solubilization of phytates which in turn releases soluble protein and minerals. According to Chitra et al. (1996) germination reduced the phytic acid contents of chickpea and pigeon pea seeds by over 60 % and that of mungbean, urad bean, and soya bean by about 40%. It has also been reported that germination or malting degraded the anti-nutrient presents in these food grains. Harmuth-Hoene et al. (1987) studied the influence of germination on biochemical properties of different cereals and legumes seeds. They observed that in wheat and mungbean, phytic acid was partially hydrolyzed. The decreased of phytic acid contents of germinated legumes has been frequently reported the reduction could be due to increase in endogenous phytase activity It could also be due to diffusion into the soaking medium also known as leeching out. Soaking of legumes in distilled water was an effective way of removing phytic acid from legumes as reported by several researchers (Ibrahim et al., 2002; Shimelis and Raksit, 2007; Khatkhat et al., 2007; Ghavidal and prakash, 2007; Liang et al., 2009). From this it can be concluded that germination enhanced the proximate composition of the food mixes the anti nutrient content was also lower in the germinated mixes because of synthesis of certain endogenous enzymes like phytase etc which thereby improved the net availability of nutrients.



**Table 4:** Anti-Nutrients estimations of Wheat Moth based premixes

Nutrients	Standard Food mix S	Food mix A24 h	Food mix B36 h	Food mix C48 h
Oxalic	25.63±13.55	22.83±2.10	13.86±1.95	6.73±1.46
Phytate	102.33±2.51	82.83±5.65	49.89±0.89	38.65±1.81

### Amylase and Protease Activity

Amylase is an enzyme produced during germination it can cleave amylopectin and amylose within the starch molecule thus resulting in production of maltose and lower molecular weight dextrins. Besides  $\alpha$  amylase there is production of protease too which is responsible of degradation of protein reserves thereby reducing dietary bulk and improving the digestibility and palatability

In the present study amylase activity in Standard Food Mix S (NG) was 12.1 maltose unit per 100 g; but as the duration of germination was increased from Food Mix C amylase activity also increased. In Food Mix A its was estimated to be 17.4 maltose unit per 100 g Food Mix B was 24.9 maltose unit per 100 g. and Food Mix C was 31.43 maltose unit per 100 g.

Ghavidel and Davoodi (2011) analyzed  $\alpha$  amylase activity as a function of germination time, the non-germinated samples legumes exhibited very low amylase activity which ranged from 7.0 to 18.1 maltose units/g dry matter. The  $\alpha$  amylase activity improved by 10 to 150% over the initial value with the lowest in cowpea and highest in mung bean samples. It increase significantly ( $P < 0.05$ ) in all the legumes. however appreciable increase was observed in mungbean from 8.1 to 280.2 maltose unit/g dry matter at 0 to 72 h germination time followed by cowpea lentil and chickpea that had increases of exceeding 600, 500 and 200% respectively over the untreated initial values Uriyo in 2001 also reported that increase in amylase activity with increase in duration of germination of cowpea  $\alpha$ - Amylase levels increased from 85.6 to 720.9  $\mu$ mole maltose/ml of extract at 0 and 72 h germination time. Similar behavior was reported for cowpea by Malleshi et al (1989) whose investigation indicated that  $\alpha$  amylase activity had attained a maximum at 3 days germination and had begun to decline at 4 days. Sumathi in 1995 also showed improvement in  $\alpha$  amylase level of horse gram, moth bean and field bean during germination. These findings agree with other reports regarding  $\alpha$  amylase production during germination of plant seeds other than legumes such as maize (Helland et al, 2002); oats (Bodin, 1995); millet (Gimbi and Kitabatake, 2002) and sorghum (Lasekan, 1996 & Uvere et.al, 2000).

Protease activity in Standard Food Mix S (NG) was 3.86 protease unit per 100m g; but as the duration of germination was increased from 24 to 48 hrs an enhancement was also reported in protease activity in Food Mix A its was estimated to be 2.63 protease unit per 100 mg; Food Mix B was 3.93. protease unit per 100 mg; and Food Mix C was 4.2 protease unit per 100m g

According to research done by Ghavidel and Davoodi (2011). The results of soaking and germination studies on protease activity of legumes highlighted that the non-germinated sample had 0.71 to 1.53 protease unit/g dry

matter and the lowest and highest values were estimated to be of mung bean and chick pea respectively Soaking increased significantly ( $P < 0.05$ ) the protease activity of the legumes by 17-36% over the non-germinated samples. Chickpea had the highest protease activity at 72 h germination (6.21 protease unit/g dry matter) followed by lentil, cowpea and mung bean. Although, the maximum increase in enzyme activity at 72h germination over the initial values was in cowpea (310%), following by chickpea (306%), lentil (232%) and mungbean (216%). However, mung bean had 311% increases in enzyme activity in 48 h germination. Kikunage et al (1991) studied about the effect of germination on protease activity of chickpea and mung bean which support these results So as the duration of germination was increased enzymatic activity also increased.

**Table 5:** Impact of duration of Germination on amylase activity

Premixes	Amylase activity (maltose unit/g)			
	Standard Food mix S	Food mix A24 h	Food mix B36 h	Food mix C48 h
WMO	12.1±0.2	17.4±0.2	24.9±0.25	31.43±0.65

**Table 6:** Impact of duration of Germination on protease activity

Premixes	Protease activity (protease unit/mg)			
	Standard Food mix S	Food mix A24 h	Food mix B36 h	Food mix C48 h
WMO	3.86±0.25	2.63±0.25	3.93±0.15	4.2±0.17

## 6. Conclusion

Germination for a period of 36 to 48 hours was effective in improving nutritional value and reducing the levels of inhibitory substances. Duration of germination was positively correlated with enzymatic activity to the researches reveals that germination improves the nutritional worth of the grains. This is an in expensive technology by utilizing it we can reduce the bulk density and increase energy density of premixes. These premixes then can be used as weaning foods or supplementary foods for children, Germination helped in improving the nutritional value due to enzymatic degradation of carbohydrate, protein & fats. Thus the resulting products in easily digestible and can be used as weaning food. It was observed that during germination there was marked rise in protein content on the contrary the anti-nutrient components showed reduction resulting in improving the net availability of certain nutrients. As the duration of germination was increased the enzymatic activity was enhanced resulting in breakdown of complex nutrients into simpler one which are easily digestible by infants.

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