

International Journal Of

Recent Scientific Research

ISSN: 0976-3031 Volume: 7(6) June -2016

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THE OFFICIAL PUBLICATION OF INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR) http://www.recentscientific.com/ recentscientific@gmail.com



Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research Vol. 7, Issue, 6, pp. 11763-11767, June, 2016

Research Article

EFFECT OF GERMINATING TIME ON PROXIMATE COMPOSITION AND ENZYMATIC ACTIVITY OF FOOD MIX

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ARTICLE INFO

ABSTRACT

Article History: Received 06th March, 2015 Received in revised form 14th April, 2016 Accepted 23rd May, 2016 Published online 28th June, 2016

Key Words:

Germination, Enzymatic Activity, Protease Activity, Anti- Nutritional factor, Minerals estimations. Despite praiseworthy advances in economic prosperity and in the field of medical therapeutics, malnutrition continues to be a significant public health problem in India. Given the grimness of the current situation, there is clearly a need to improve and improvise the management of Malnutrition. One of the suggested methods has been the use of nutritious premixes at home for children with uncomplicated malnutrition in order to improve their nutritional status . Amylase rich premixes can be used directly to address the twin problem of dietary bulk and poor energy density of most of the weaning gruels of the poor. These germinated cereal flour are extremely rich in enzyme alpha amylase. Keeping all the above discussed aspects in mind the present investigation was planned to develop amylase rich premixes. The purpose of this study was to develop standard and amylase rich premixes followed by estimation of their nutrient composition. For development of premixes germination process was used and the analysis of nutrient composition was done by standard techniques from the NIN, 2003.

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INTRODUCTION

Cereal based diets besides being deficient in micro nutrients lack in diversity. They contain high amounts of inhibitory substances which reduce bioavailability of several nutrients. Germination is one of the natural process that helps in mobilizing reserve materials and enhances the bioavailability of certain minerals. Germination cause important biochemical, nutritional and sensory changes in nutrient by enhancing their metabolism. It is a processing technology as it improves the digestibility of grains, enhance certain anti nutrients improves bioavailability of certain nutrients by reducing the effect of inhibitory components like oxalates, phytates and certain fibres. It also reduces certain oligosaccharides which causes flatulence thereby improving the overall nutritive value of the products. Germination also improves enzymatic activity (Amylase, Protease etc) and reduces the effect of certain undesirable components like trypsin inhibitor etc. Hence the present study was undertaken to develop germinated food mixes by using certified variety of seeds and to study the effect of germinating time on proximate composition and enzymatic activity.

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. WMO (Wheat Moth Based)

Food mix A by 24 hrs, Food Mix B by 36 hrs. And Food Mix C by 48 hrs. Samples were initially washed and cleaned with tap water before soaked for 6hr at room temperature (28 degrees), samples were placed under wet muslin cloth and left germinated for different durations. 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. The composition of food mix included wheat 50g, whole moth beans 25g, soya beans 10g, fat 5ml, milk powder 5g, sugar 5g. Samples were taken in triplicate for estimation of various components data was tabulated and ANOVA test was applied to analyse the difference.

RESULTS

Proximate Composition: Moisture content significantly increased after germination in all samples in comparison to control Sample was10.86 g however in food mix A it was 13.06/100 g, 15.3/100g, 15.43/100g for Food mix B, Food mix C germinated sample 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.

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The germination 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 control sample the ash content was 5.16/100 g however in food mix A it was 4.83/100 g, 4.53/100g, 4.2/100g for Food mix B, Food mix C germinated sample 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 were reported as there was increase in ash content with increase in germination time. This may be because of decrease in crude fat and carbohydrate content during germination because of enhancement in enzymatic activity which might have led to the apparent increase in ash content.

The protein content of standard and germinated samples were analyzed to be 13.63/100 g and 17.3 g/100g in the 24 h germinated sample (Food mix A) however, in Food mix Bthe 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 also reported 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 in 1992 estimated that during germination of beans, lentils, chickpea and pea's seeds there was increase in protein content protease activity get enhanced.

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 and germinated Food mixes were appraised to be 5.76% and 5.5% whereas 5.03% and 4.76% fiber was reported in Food Mix B and Food Mix C. 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 and Food Mix A were analyzed to be 7.53g/100g and 6.4g/100g however, in Food Mix B the values were 5.9 g/100g and inFood 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 significantly (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 content of Food mix prepared by non-germinated varietiesi.e. Sample NG was 57.03g/100g however as the germination time increased the carbohydrate content reduced from 52.9g/100g to 49.46/100gas estimated 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) quoted that during germination, carbohydrate was used as source of energy for embryonic growth which explained the changes of carbohydrate content after germination. Additionally, β -amylase activity hydrolyze the starch into simple carbohydrate, 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 α -amylase activities were found to parallel with the pattern of starch breakdown.

 Table 2 Nutrients estimations of Wheat Moth based Food

 Mixes

Nutrients	Food Mix Standard	Food Mix A 24 h	Food Mix B 36 h	Food Mix C 48 h
Moisture (%)	10.86 ± 1.34	13.06 ± 1.49	15.3 ± 1.8	15.43 ± 2.90
Ash content (g)	5.16±0.70	4.830.30	4.53±1.40	4.2±0.51
Protein (g)	13.63±1.05	17.3±1.34	19.97±0.85	24.1±1.70
Fat(g)	7.53±0.55	6.4±0.62	5.9±0.95	4.73±0.76
Fiber (g)	5.76±0.41	5.5±0.44	5.03±1.40	4.76±0.41
Carbohydrates (g)	57.03 ± 1.34	52.9±2.66	49.46±0.50	47.16±1.15
Mean±sd				

Anti-nutritional factors: Oxalic acid content of control samples was 25.63 mg per 100 g; in Food Mix Ait was estimated to be 22.83 mg per 100 g but as the duration of germination was increased from 24 to 36& 48 hours 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 from 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.

The control Food Mixes 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); 38.65 mg 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.

be contributed to the enzymes This can which makesolubilization 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;Khattak et al., 2007; Ghavidal and prakash, 2007; Liang et al., 2009).

 Table 3 Minerals estimations of Wheat Moth based Food

 Mixes

Nutrients	Food Mix Standard	Food Mix A 24 h	Food Mix B 36 h	Food Mix C 48 h
Calcium (Mg/100g)	17.66±0.55	21.43±0.90	25.44±0.73	27.21±1.45
Iron (Mg/100g)	9.2±0.01	10.58±0.27	11.76±0.49	12.92±0.06

Anti-Nutrients content of Wheat moth based (WMO) Food Mixes

 Table 4Anti-Nutrients content of Wheat Moth based

 Food Mixes

Nutrients	FOOD MIX Standard	FOOD MIX A 24 h	FOOD MIX B 36 h	FOOD MIX C 48 h
Oxalic	25.63±	22.83±	13.86±	6.73±
(Mg/100g)	13.55	2.10	1.95	1.46
Phytate	$102.33 \pm$	$82.83 \pm$	49.89±	$38.65 \pm$
(Mg/100g)	2.51	5.65	0.89	1.81

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 Mixes were gauging to be 17.66 mg/100g and 21.43 mg/100g in 24 h germinated Food Mixes. In premix prepared by using 36 hrs germinated grains the value were shown a higher shoot up of 25.44 mg/100g and 27.21mg/100g in 48 hrs germinated sample 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 fermentationThe iron content of standard premix and 24 hr germinated Food Mixeswas estimated to be 9.2mg/100g and 10.58 mg/100g respectively in our research. However, the iron content of 36 h and 48 hours germinated Food Mixes was estimated to be 11.76 mg/100g and 12.92 mg/100g.

This proves that during germination the net bioavailability of minerals improves this might be because of reduction in inhibitory components like oxalate and phytate.

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 α amylase there is production of protease too which is responsible of degradation of protein reserves thereby reducing dietary bulk and improving the digestibility and palatibilityGhavidel and Davoodi., 2011)

In the present study amylase activity in control samples was 12.1 maltose unit per 100 g; but as the duration of germination was increased from 24 to 48 hrs 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 & Davoodi (2011) analyzed α amylase activityas a function of germination time, the control samples being non germinated exhibited very low amylase activitywhich ranged from 7.0 to 18.1 maltose units/g dry matter. The α 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 valuesUriyo in 2001 also reported that increase in amylase activity with increase in duration of germination of cowpea a-Amylase levels increased from 85.6 to 720.9 µmole 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 α amylase activity had attained a maximum at 3 days germination and had begun to decline at 4 days. Sumathi in 1995also showed improvement in α amylase level of horse gram, moth bean and field bean during germination. These findings agree with other reports regarding α 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 control samples (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 nongerminated 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 respectivelySoaking 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, as the duration of germination was increased enzymaticactivity also increased.

 Table 5 Impact of duration of Germination on amylase

 activityWheat moth based (WMO) Food Mix

Amylase activity (maltose unit/g)				
Food mix Standard	Food Mix A	Food Mix B	Food mix C	
12.1±0.2	17.4±0.2	24.9±0.25	31.43±0.65	

Table 6 Impact of duration of Germination on amylase activity Wheat moth based (WMO) Food Mix

Protease activity(protease unit/mg)				
Food Mix Standard	Food Mix A	Food Mix B	Food Mix C	
3.86±0.25	2.63±0.25	3.93±0.15	4.2±0.17	

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 he researches revels 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 Food Mixes. These Food Mixes 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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How to cite this article:

Nidhi Chaudhary., Swati Vyas and Ila Joshi.2016, Effect of Germinating Time on Proximate Composition and Enzymatic Activity of food Mix. *Int J Recent Sci Res.* 7(6), pp. 11763-11767.

