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IMPACT FACTOR ~ 1.021
ABSTRACT

Malnutrition is a significant public health problem in India in spite of advance in economic prosperity which has lead to growth in field of medical therapeutics. Improvement in dietary quality should be major focus for reducing malnutrition. Nutritious germinated premixes can be used for malnourished children. Ragi based premixes can be one such alternative. In present research premixes were prepared using non germinated millet, pulse and oilseed as well as germinated (24, 36 and 48 hours) ingredients in order to investigate the effect of duration of germination on several nutritional and anti-nutritional factors. In present investigation germination helped in breaking down of complex macronutrients into simpler components with the help of enzymatic activity. The protein content of standard and germinated samples were analyzed to be 11.5g/100g and 15.46 g/100g in the 24 h germinated sample; however, in 36 h germinated samples the values were 17.1g/100g and 21.8g/100g after 48 hrs. The calcium content of standard premix was 189.36 mg/100g after 24 hrs of germination it was 206.3 mg/100g; 214.30 mg/100g (36 hrs) and 221.26mg/100g (48 hrs). The iron content of standard premix was 5.60mg/100g and 7.0 mg/100g (24 hrs germination). Thirty six and 48 hrs germinated premixes had iron content 7.6 mg/100g and 8.66 mg/100g. Phytic acid decreased from an initial average value of 1.88 mg/100gm in non-germinated sample to 0.33mg/100g in 48 hrs germinated sample. Oxalic acid reduced from 25.63 mg per 100 g in control sample to 6.73mg/100g in 48 hours germinated sample.

Keywords: Millets, Germination, proximate composition, anti-nutritional factors.

INTRODUCTION

Millet sustains one third of the world’s population and play a significant part of the diet in developing countries particularly India where they are eaten by a large section of the poor community. Millets particularly maize, sorghum, pearl millet, finger millet constitute a little less than 25 per cent of the total food grain production in India. However, they are generally regarded as coarse grains, their potential for augmenting the grain supplies and bridging the protein gap is increasingly realized. As nutritional well being is a sustainable force for health and development and maximization of human genetic potential. The nutritional status of a community has been recognized as an important indicator of national development. Approximately 8.1 million children under the age of five years (6.4%) suffer from different forms of malnutrition and it is one of the important co-morbidities leading to hospital admission in our country (NFHS 2005-06). The mortality associated with severe acute malnutrition is also high, ranging from 73 to 187 per 1000 (Pelletier, 1994).

One of the suggested methods for improving the quality of diet has been the use of nutritious premixes at home for children with uncomplicated SAM in order to improve their nutritional status (Lancet 2008). “Ragi” is considered as nutricereals as it is rich in macro, as well as micro nutrients along with phyto-chemicals. It is highly nutritious and is richer in protein, fat, minerals and dietary fibre than rice. It is non-acid forming, minimally allergic and an easy-to-digest grain. It also contains good amounts of crude fibre and phosphorus (Malleshi & Desikachar, 1985). Millets contain water soluble gum and β-glucan that is useful in improving glucose metabolism Ragi can be used in preparing amylase rich premixes by germinating technique at household level which can then be administered to children. During germination enzymatic activity gets enhanced due to which hydrolysis of complex molecules like starch, proteins increases which results in production of their simplified form like dextrin, peptones and peptides (Reddy et.al, 2003).

Through a decade of research and field trials, the affectivity of enzymes helps in lowering viscosity and reduction of bulk density of cereals and millet based...
weaning foods has been established. Hence these 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. Hence an effort should now be geared to popularise this useful yet inexpensive technology. Keeping all the above discussed aspects in mind the present investigation was planned to develop amylase rich Ragi based premixes.

MATERIALS AND METHODS

SAMPLE SELECTION AND PREPARATION

Ragi (GPU 67) samples were procured from the Agricultural research center of Tamil Nadu as it was not available in Rajasthan. Procurement of the certified Moth bean (RMO 257), Soya bean (NRC 37 Ahilya 4) seeds under experimentation were obtained from Durgapura Agricultural Research Centre, Jaipur. The test samples were initially cleaned manually by removing sticks and stones, other crop seed, weed seed and other foreign matter. The seeds were subjected to viability test by placing them in water. Those which floated on the top of water were discarded as unviable. Finally four different kind of premixes were prepared by using these seeds: Sample NG was prepared by using non-germinated seeds and it served as control sample. Sample A: in this grains were germinated for 24 hours, Sample B in which germination was done for 36 hrs and sample C for 48hrs.

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.

Took whole Millet and Pulses

Added 3-4 volumnes of water

Kept overnight

Drained excess water

Germinated in dark (28°C)

Sun dried

Removed rootlets and husk

Crushed

Table 1: Composition of premixes

<table>
<thead>
<tr>
<th>Food groups</th>
<th>Amount (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger millet (Ragi)</td>
<td>50 g</td>
</tr>
<tr>
<td>Moth bean</td>
<td>25 g</td>
</tr>
<tr>
<td>Oil seeds (soya bean)</td>
<td>10 g</td>
</tr>
<tr>
<td>Fat</td>
<td>5 ml</td>
</tr>
<tr>
<td>Milk powder</td>
<td>5 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>5 g</td>
</tr>
<tr>
<td>Total</td>
<td>100 g</td>
</tr>
</tbody>
</table>

ANALYSIS OF PROXIMATE AND ANTINUTRIENT COMPONENTS OF THE PREMIXES

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 wereused for estimations were first standardized. Moisture estimation was done by using oven drying method; Ash was determined by muffle furnace, protein by micro khejaldal method and crude Fiber by acid alkali (NIN, 2010). Anti nutrient components i.e. oxalic acid and phytic acid were even estimated titre metrically. The oxalic acid was extracted in HCL and precipitated as calcium oxalate by adding calcium chloride which is then washed and tittered with N/20 KMnO4 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 isomyo alcohol. Pink colour is dissolved in alcohol layer with ammonium thiocyanate, which is invariable proportional to phytic acid content. (Kawatra, 2000)

STATISTICAL ANALYSIS

Each sample was prepared thrice and readings of each parameter was taken five times finally their mean and sd were 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.

RESULTS AND DISCUSSIONS

PROXIMATE COMPOSITION

In the present study, we analyzed the influence of germination on the nutritive value of standardize control and germinated premixes. The results of the study are presented in Table 2 and 3. Moisture content significantly increased after germination in all samples in comparison to control non germinated Sample. Ruiz et al in 1990 reported in their study that the moisture content after 24 hr. at 37°C, raised from 30 to 40 % and the rise was approximately 75-85% after 48 hr. From this it is evident that during germination there is a marked increase in moisture content this may be contributed to the fact 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

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time increases more number of cells within the seeds are hydrated.

The germinated samples 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 4.1/100 g however in sample A was 4/100 g , 3.9/100g, 3.83/100g for sample B, sample C germinated sample respectively. Several researches also reported that as the soaking time increases there is loss of minerals as the seed utilizes then for emergence of rootlet and hence the ash content in reduced (Wang et al., 1997).

Few researches have reported contradictory results in the analysis of effect of germination on ash content of different variety of Mung bean, pea & lentil seeds. Where in the Ash content increased 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 control non germinated sample was 11.5g/100g and Sample A had 15.46 g/100g protein however, in Sample B the values were 17.1g/100g and Sample C 21.8g/100g protein respectively. Similarly, Mwika et al. (2000) reported that there was significant increase in protein content as the germination time increased, from 6.1% in non-germinated grains to 7.9% during the 96 h of germination. There was an overall increase of 29.5%. Opoku in 1981 reported that there was increase in millet protein which ranged from 14 to 40%. As the duration of germination increased the rate of respiration increase which results in loss of dry matter particularly carbohydrates which causes increment in other nutrient such as protein.

Increase in the amount of was also noted by Camacho et al. (1992) during germination of beans, lentils, chickpea and pea’s seeds. Ohtsubo et al., (2005) found an increment in crude protein of germinated brown rice. Our data, regarding the effect of germination on the proximate composition of mungbean seeds, agreed with Obizoba (1991) who reported increase in % moisture, % crude protein and % ash. According to him, total nitrogen, total non-protein nitrogen; protein nitrogen, true protein nitrogen also increased with germination. Parameswaran and Sadasivan (1994), Nazni et al., (2014), Khatoon and Prakash (2006), Urbano et al., (2005), Ghavidel and Prakash (2007), and Kaushik et al., (2010) also noted increase in the percent protein in germinated grains. Bau et al., 1997 Investigations have reported that during germination there was synthesis of enzyme protein which resulted in compositional changes as well as degradation of other components. Other researchers have explained that during germination moisture was imbibed in seed and hormones were activated resulting into synthesis of proteins and this helped in achieving germination. However several researches reported contradictory statements where in significant reduction in protein content post germination with consequent increment in amino acid content was observed, such changes were reported because of enhancement in the protease activity during germination (Vellupillai et al., (2009), Torres et al., (2007) and King and Puwastien (1987).

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 sample A germinated premixes were appraised to be 5.76% and 5.5% whereas 5.03% and 4.76% fiber was present in sample B and sample C germinated premixes respectively. Results shows that, during germination there was reduction in fiber content because during germination there in increase in temperature which results in cleavage of weak bonds between poly saccharides and breakdown of glycosidic linkage and hence solubilization of dietary fiber (Svanberg et al. 1987). According to the study done by Shah et al (2011) germination significantly increased the crude fiber contents, with mean values of 4.88 in the control and 6.4% at 96 h germination. However it can be noted that the only significant reduction was recorded in the 48 hrs Mung Bean (Ramzanand NM-98) germination, whereas further germination time increased the fiber content. During germination there is synthesis of cellulose and hemicellulose which act as structural carbohydrates and are important component of cell wall. Hence these results in increase in fiber content as reported in a study done by Chung et al (1998) where in fiber increased from 3.75 % to 5% during germination in barley.

In the present investigation the crude fat concentration decreased with germination time. The fat content of standard and Sample A germinated premixes were analyzed to be 2.03g/100g and 1.46g/100ghowever, in Sample B the values were 1.26g/100g and in Sample C it was 1.23g/100g respectively. Shah et al in 2011 studied the effect of germination time on different varieties of mungbeans (Ramzan and NM- 98) the ether extract values of the two varieties differed significantly (p <0.05). The crude fat concentration decreased with germination time from 1.79 to 1.4% and 1.71 to 1.39% as the germination time increased from 0, 24,48 and 96 hrs. Badshah et al. (1991) and Chung et al. (1998) also noted significant losses in lipid content during canola germination. Several researches have coded reason for reduction in fat content during germination which could be due to total solid loss during soaking prior to germination or use of fat as an energy source in germination process which was used as the major source of carbon for seed growth as fatty acids are oxidized to carbon dioxide and water to generate energy for germination.

In the present research the carbohydrate content of premix prepared by non germinated varieties i.e Sample NG was 58.36g/100ghowever as the germination time increased the carbohydrate content reduced from 58.36g/100g to 45.93g/100g in sample A it was 52.03g/100g; 50.03g/100g in sample B, 45.93g/100g in sample 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.

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Additionally, β-amylose 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 a-amylose activities were found to parallel with the pattern of starch breakdown.

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 premixes were gauging to be 189.36 mg/100g and 206.3 mg/100g in 24 h germinated premixes. In premix prepared by using 36 hrs germinated grains the value showed a higher shoot up of 214.30 mg/100g and221.26mg/100g in 48 hrs germinated sample .The iron content of standard premix and 24 hr germinated premixes was estimated to be 5.60mg/100g and 7.0 mg/100g respectively in our research. However, the iron content of 36 h and 48 hours germinated premixes was estimated to be 7.6 mg/100g and 8.66 mg/100g. Tizazu in 2010 suggested that germination was found to increase the level of minerals particularly calcium, iron and zinc. Sorghum flour germinated for 48 hours contained highest minerals content while lowest values of minerals were reported for un germinated sorghum flour. According to the research done by Mamiro et al., (2001) in vitro extractability of calcium and other minerals in finger millets and kidney beans increased significantly after germination in comparison to other processing techniques like soaking, autoclaving and fermentation. D’ouza (2013) highlighted in his research on field bean that germination or malting enhanced availability iron, calcium & other minerals. Soaking prior to cooking or germination is simple and more effective method that can be used both in the home and industries that produce food products.

ANTI NUTRITIONAL FACTORS
It can be concluded from the results in table3 that oxalic acid content of control samples (NG) was 25.63 mg per 100 g; in sample A it 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 and carbohydrates. Similarly statically significantly reduction was observed in phytic acid content. Phytic acid has been reported to from complexes with protein which then become more resistant to proteolytic degradation (Cheryan, 1980). Thus phytic acid being an anti nutrient lowers the bioavailability of both minerals & proteins.

Under our experimental conduction the control premixes had approximately102.33 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 (Sample A); 49.89 mg per 100 g(Sample B); 38.65mg per 100g (Sample C) these findings are similar to results reported by Tizazu et.al., (2011) they reported an improvement in minerals bioavailability of sorghum based complimentary foods, which reported statically significantly reduction in phytic acid levels (m/100g) (p <0.05) for different varieties of sorghum as germination time was increased from 36 to 48 hrs.

This can be contributed to the enzymes which cause 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, urd bean, and soyabean by about 40%. It has also been reported that germination or malting degraded the anti-nutrient presents in these food grains. Sudha Rani and Usha Anthony (2014) reported that there was significant effect on processing methods like germination and fermentation on the varieties of finger millet with different seed coat color has a high impact on the polyphenols content. 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 reduction in 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, Nazni et al., 2010).

Table 2: Nutrients estimations of Ragi Moth based premixes

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Moisture (%)</th>
<th>Ash content (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
<th>Fiber (g)</th>
<th>CHO (g)</th>
<th>Calcium</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>11.36±1.61</td>
<td>4.1±1.70</td>
<td>11.5±0.95</td>
<td>2.03±0.30</td>
<td>12.6±2.15</td>
<td>58.36±0.35</td>
<td>189.36±18.50</td>
<td>5.60±0.35</td>
</tr>
<tr>
<td>A</td>
<td>14.86±1.49</td>
<td>4±0.60</td>
<td>15.46±2.18</td>
<td>1.46±1.02</td>
<td>12.16±1.62</td>
<td>52.03±0.98</td>
<td>206.3±0.85</td>
<td>7.0±0.56</td>
</tr>
<tr>
<td>B</td>
<td>15.46±1.48</td>
<td>3.9±0.26</td>
<td>17.1±2.64</td>
<td>1.26±0.057</td>
<td>11.4±1.05</td>
<td>50.86±2.80</td>
<td>214.3±0.86</td>
<td>7.6±0.50</td>
</tr>
<tr>
<td>C</td>
<td>15.86±1.52</td>
<td>3.83±0.11</td>
<td>21.8±1.65</td>
<td>1.23±0.15</td>
<td>11.33±1.05</td>
<td>45.93±1.93</td>
<td>221.26±0.90</td>
<td>8.66±0.62</td>
</tr>
</tbody>
</table>

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ANTI NUTRIENTS ESTIMATIONS OF RAGI BASED (RMO) PREMIXES

Table 3: Anti-Nutrients estimations of Ragi Moth based premixes

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Oxalic (mg/100g)</th>
<th>Phytic (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG</td>
<td>25.63±13.55</td>
<td>102.33±2.51</td>
</tr>
<tr>
<td>A</td>
<td>22.83±2.10</td>
<td>82.83±5.65</td>
</tr>
<tr>
<td>B</td>
<td>13.86±1.95</td>
<td>49.89±0.89</td>
</tr>
<tr>
<td>C</td>
<td>6.73±1.46</td>
<td>38.65±1.81</td>
</tr>
</tbody>
</table>

CONCLUSION

The research reveals that germination improves the nutritional worth of the grains. This is an 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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