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# Formulation of nutritious premixes based on natural ingredients and evaluating their efficacy for value addition

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**Abstract**

The objective was to formulate premixes using natural ingredients, namely, amaranth (*Amaranthus* sp, Premix-I) or cauliflower (*Brassica oleracea*, Premix-II) leaves, flax seeds *(Linum usitatissimum)*, cumin *(Cuminum cyminum)* and fenugreek seeds *(Trigonella foenum)* and evaluate their efficacy for value addition. Raw materials and premixes were analyzed for their chemical composition. Premixes were incorporated to unleavened flat bread at 6 and 12% levels and evaluated for nutritional and sensory quality. Results indicated that premixes were rich sources of protein, iron, calcium, total and β-carotene, dietary fiber and bioactive components. Products with premixes showed in vitro starch digestibility of 36%. In vitro digestible protein (80.3%), bioaccessible calcium (85.8%) and absolute iron (4.36 mg/100 g) were higher in premix incorporated products than control. Products with premix-I scored less for appearance and colour, though they were accepted for taste and texture. Products with premix-II were similar to control for sensory acceptability. In conclusion, premixes with natural ingredients can be used for value addition to obtain nutritionally superior products.



## Introduction

Amongst many approaches to tackle the problem of malnutrition, food based approach is considered the most sustainable one, despite being a long term strategy. It involves improving the dietaries of population to fight the existing nutrition insecurities through better selection of foods. While food security deals with availability and access to food, the nutrition security focuses on physiological needs for nutrients and its utilization (Shetty, 2009). The global challenge of micronutrient malnutrition can be addressed by sustainable food based approaches by integrating the framework of agriculture production as well as making available nutritionally optimal diets to population (Ejeta, 2009). The nutritional quality of traditional diets can be improved by value addition. It has been found that customer acceptance of traditional products is always better due to familiarity. Hence, exploring the possibility of value addition to traditional products is a better option to enhance the intake of micronutrients.

Our earlier work has shown that the nutritional quality of many traditional products can be improved by value addition to formulate products high in protein, energy, minerals, vitamins, fiber, etc or to produce low calorie or low carbohydrates products (Mamatha and Prakash, 1995; Shenoy and Prakash, 2002; Shanthala and Prakash, 2005; Dachana *et al.*, 2010; Gupta and Prakash, 2011a; 2011b; Dhinda *et al.*, 2012).

Green leafy vegetables are rich sources of micronutrients, though their use is limited in dietaries because of seasonality and poor keeping quality. Dehydration is a low cost technology which can be used to preserve greens for a long time. Flax seeds are rich in protein and fat (specifically omega -3 fatty acids) along with other essential nutrients. Spices are known to add special flavour to foods and additionally have physiological benefits due to their medicinal properties (Sreenivasan, 2008). Hence, the objective of the study was to formulate nutritious premixes utilizing all these natural ingredients and study their nutritional quality and sensory attributes after incorporating into a product.

## Materials and Methods

*Materials*

Raw ingredients used for the study namely amaranth leaves (*Amaranthus* sp), cauliflower leaves *(Brassica oleracea)*, flaxseeds *(Linum usitatissimum)*, cumin seeds *(Cuminum cyminum)*, fenugreek seeds *(Trigonella foenum)* and wheat *(Triticum aestivum)* were purchased from local market, and cleaned before use. All the chemicals used for analysis were of analytical grade and were procured from E-Merck, Mumbai, India and Qualigens Fine Chemicals,

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Mumbai, India. The dialysis tubing was procured from Sigma, USA, which had a molecular mass cut off of 8000 Kda. Glass double distilled water was used for all experiments.

*Methods*

The study involved preparation of dry premixes utilizing natural ingredients for possible incorporation to different recipes for value addition. All the raw ingredients and formulated premixes were analyzed for nutritional composition. The shelf stability of premixes was determined by moisture sorption isotherm. To study the efficacy for value addition, the premixes were evaluated for their sensory acceptability and nutritional quality by incorporating into a traditional product (unleavened flat bread) prepared with whole wheat flour and used as a staple in Indian dietaries.

*Preparation of premixes*

The greens were cleaned and washed in tap water. The edible portion was again rinsed in glass distilled water and oven dried at 60$^o$C for 8 hrs. The dried leaves were powered using a mixer and stored in clear PET (polyethylene terephthalate) containers. Flaxseeds and cumin seeds were cleaned and roasted at low flame for 5 minutes and then powdered using a mixer. Fenugreek seeds were soaked in water for 5 hours and water was drained from seeds. Seeds were placed over a wet cloth in a germinating chamber with humidity control at room temperature (28$^0$C) and allowed to germinate for 3 days in dark condition. The sprouted fenugreek seeds were dried in an oven at 60$^o$C for 10 hrs, powdered and stored. Wheat was cleaned and milled into flour, and stored for later use. Two premixes were formulated by mixing the ingredients in the following proportions, dehydrated greens powder- 200 g, roasted flaxseed powder - 200 g, roasted cumin seed powder - 100 g, sprouted and dried fenugreek seed powder- 100 g. The greens powder used were dehydrated amaranth for ‘Premix-I’ and cauliflower for ‘Premix-II’. These were mixed well, stored under refrigeration in airtight PET containers and used for further studies.

*Preparation of unleavened flat bread*

Unleavened flat bread is a staple dish consumed in a typical Indian meal everyday, hence they were chosen as a vehicle for evaluating the sensory quality of premixes. Flat breads were made by kneading wheat flour, salt (1%) and water into a dough, rolling the dough into a thin round shape and roasting on a hot pan on both sides till cooked. The formulated premixes were incorporated to flat bread dough at 6% and 12% levels. Flat bread without premix served as control. Freshly prepared flat bread was analyzed for moisture content, *in vitro* starch and protein digestibility, and bioaccessible iron and calcium. Freshly prepared flat bread were also subjected to sensory analysis. The nutritional value of serving size of products (utilizing 60 g of raw material mix) were computed using the analyzed values.

*Chemical analysis*

Standard methodologies (AOAC, 2005) were followed for estimating various chemical constituents of raw ingredients and premixes and are summarized below. The moisture content was estimated by oven drying and weighing method (method 926.12, 41.1.02). Total ash was estimated by incinerating the sample in a muffle furnace at 600$^o$C for 3-5 hrs and weighing (method 942.05, 4.1.10). This ash was further converted into solution and used for estimation of minerals. Fat was estimated using petroleum ether in a Soxhlet distillation apparatus by repeated extraction of ether extractives (method 948.22, 40.1.05). Protein was estimated following Kjeldahl distillation procedure for determination of nitrogen which was converted to protein using appropriate conversion factors (method 960.52, 12.1.07). For insoluble and soluble dietary fiber the method of Asp *et al*. (1983) was followed which is based on the separation of non-starch polysaccharides by enzymatic and gravimetric method. This method measures the dietary fiber equivalent to physiologically unavailable fiber. For estimation of total carotene and β-carotene, the ingredients were extracted in acetone and transferred to petroleum ether phase. Total carotene was read colorimetrically using petroleum ether for baseline correction. β-carotene was separated by column chromatography and read colorimetrically (Ranganna, 1986). Ash solution was used for estimation of iron and calcium. Iron was determined colorimetrically making use of the fact that ferric iron gives a red color with potassium thiocyanate (Raghuramulu, 2003). Calcium was precipitated as calcium oxalate, the precipitate dissolved in hot dilute sulphuric acid and titrated against standard potassium permanganate (Oser, 1965). Total polyphenols were estimated colorimetrically in methanolic extract of sample using Folin-Ciocalteau reagent with tannic acid as standard and the results were expressed as mg tannic acid equivalents /100 g sample (Matthaus, 2002). Tannins were estimated by colorimetric method based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution

(Ranganna, 1986). Oxalates were extracted with hydrochloric acid, precipitated as calcium oxalate from the deproteinised extract and were estimated by subsequent titration with potassium permanganate (Baker, 1952). For analysis of total starch, enzymic method was used which degrades starch to glucose, which can be read colorimetrically (Batey and Ryde, 1982; Raghuramulu *et al*., 2003).

*Analysis of in vitro digestible /bioaccessible nutrients*

*In vitro* starch digestibility was determined by modification of methods of Kon *et al*. (1971), Holm *et al*. (1985) and Som *et al.* (1992) wherein starch was treated with enzymes to effect hydrolysis and glucose was estimated in digested sample following gastrointestinal digestion. *In vitro* digestibility of protein was estimated by enzymatic method of Akeson and Stahman (1964). In vitro bioaccessibility of calcium and iron was determined by stimulated gastrointestinal digestion using pepsin for the gastric stage followed by pancreatin and bile salts for the intestinal stage. The proportion of mineral diffused through a semi permeable membrane was used to measure mineral dialysability (Luten *et al*., 1996). The dialysate was analyzed for iron by colorimetric method using α-α bipyridyl method (Raghuramulu *et al*., 2003). The calcium present in the dialysate was measured as indicated earlier for estimation of total calcium.

*Moisture sorption isotherm*

Moisture sorption isotherm of two premixes were determined at room temperature (25°C) using the principles based on Labuza (1976). A 5.0 g portion of sample was placed in weighed petri dishes and dehydrated in a vacuum oven at 70°C for 8 h. After drying, duplicate samples were placed in desiccators containing saturated salt slurries in the range of 0.115-0.848 water activity ($a_w$). The desiccators were saturated with the same solution 24 hours previously. The weights of the products were recorded every 24 hours till equilibrium was attained when the difference between two consecutive values was less than 1mg/g solids. Different salts used for the study and aw were potassium acetate -0.234; magnesium chloride -0.329; potassium carbonate -0.443; magnesium nitrate -0.536; sodium nitrite - 0.654; and potassium chloride -0.848. Moisture sorption curves were drawn with moisture uptake data of premixes.

*Sensory analysis*

The products prepared using two different premixes at 6% and 12% levels of incorporation were unleavened flat breads. The control product did not have any premix. The products were subjected to sensory analysis for the attributes of appearance, color, texture, aroma and taste with the help of a score card. The grading scale used was between 1-20 with the grades representing the quality attributes of 'Excellent', 16.1-20; 'Very good', 12.1-16.0; 'Good', 8.1-12.0, 'Satisfactory', 4.1-8.0, and 'Fair', 1-4.0. The panel members numbering 86 were students of the institution who were familiar with the sensory analysis techniques. The analysis of two types of the nutritious premixes incorporated to flat bread was done on two different days. The samples were presented under white light at room temperature and members were asked to sip water in between to minimize the possibility of carry over taste, if any. No accompaniment was given with samples.

*Statistical analysis*

The data of sensory analysis was subjected to statistical analysis making use of Minitab statistical software. Mean and standard deviation were calculated individually for scores obtained for different quality attributes for each set of products. Analysis of variance was done to find out significant difference between the quality attributes of nutritious premixes incorporated into flat breads.

## Results and Discussion

*Chemical composition of raw ingredients and premixes*

The chemical composition of raw ingredients and formulated premixes is presented in Table 1. The moisture content of amaranth and cauliflower leaves powder was 7.14 and 6.32% respectively. Green leafy vegetables are generally very low in fat and accordingly the fat content of amaranth leaves powder was 0.82% and that of cauliflower leaves powder was 1.28%. The values obtained were similar to that reported for food composition values of Indian foods (Gopalan *et al*., 1996). The protein content ranged from 5.64-5.90%. Greens are generally poor sources of proteins with fresh greens having less than 1% protein but since these were dry powders, an increase in protein content was seen. Similarly there was a remarkable increase in the ash content also. The greens are rich source of dietary fiber. Amaranth leaves powder had 6.6% of soluble fiber and 32.4% of insoluble fiber. In contrast, cauliflower leaves powder had a much higher content of soluble fiber which was 12.4%. The insoluble fiber however was in the same range with 32.6%. Similar values were reported by Kowsalya and Mohandas (1999) for fiber content of cauliflower greens.

Table 1. Chemical composition of raw ingredients and formulated premixes (per 100 g)

| Constituents | Amaranth leaves powder | Cauliflower leaves powder | Flaxseed | Cumin seeds | Fenugreek seed | Wheat flour | Premix-I (Amaranth leaves) | Premix-II (Cauliflower leaves) |
|---|---|---|---|---|---|---|---|---|
| **Moisture (g)** | 7.14 ± 0.02 | 6.32 ± 0.05 | 3.40 ± 0.01 | 5.14 ± 0.00 | 5.80 ± 0.00 | 7.92 ± 0.02 | 4.50 ±0.00 | 4.05 ±0.07 |
| **Fat (g)** | 0.82 ± 0.02 | 1.28 ± 0.05 | 40.25 ± 0.91 | 12.60 ± 0.50 | 6.74 ± 0.11 | 1.62 ± 0.01 | 20.0 ±0.05 | 21.66 ±0.05 |
| **Protein (g)** | 5.90 ± 0.17 | 5.64 ± 0.30 | 22.52 ± 0.18 | 16.24 ± 0.17 | 27.10 ± 0.06 | 12.02 ± 0.42 | 16.15 ±0.04 | 16.37 ±0.24 |
| **Ash (g)** | 20.03 ± 0.01 | 16.00 ± 0.01 | 3.55 ± 0.01 | 8.80 ± 0.00 | 3.25 ± 0.01 | 1.09 ± 0.01 | 9.81 ±0.07 | 8.33 ±0.07 |
| **Soluble fiber (g)** | 6.60 ± 0.04 | 12.40 ± 0.09 | 10.80 ± 0.26 | 8.20 ± 0.28 | 8.00 ± 1.13 | 1.60 ± 0.16 | 8.50 ± 0.23 | 10.43 ± 0.22 |
| **Insoluble fiber (g)** | 32.40 ± 1.13 | 32.60 ± 3.11 | 15.00 ± 0.28 | 32.20 ± 1.41 | 26.40 ± 0.56 | 4.60 ± 0.28 | 25.56 ± 1.28 | 25.63 ± 1.43 |
| **Calcium (mg)** | 619.5 ± 2.94 | 682.0 ± 1.04 | 205.5 ± 0.70 | 1029.5 ± 0.70 | 191.0 ±1.41 | 45.0 ± 1.41 | 524.5 ±1.53 | 583.5 ±2.02 |
| **Iron (mg)** | 21.65 ± 0.49 | 39.00 ± 0.70 | 2.66 ± 0.02 | 13.16 ±0.23 | 6.60 ± 0.03 | 6.50 ± 0.06 | 13.66 ±0.47 | 27.66 ±1.41 |
| **Total Carotene (mg)** | 23.808 ±2.10 | 49.526 ±1.44 | 0.256 ± 0.04 | 0.566 ±5.65 | 0.177 ±0.041 | 0.109 ± 0.027 | 19.270 ±0.59 | 44.670 ±2.41 |
| **β-carotene (mg)** | 15.116 ±0.34 | 17.278 ± 0.58 | ND | ND | ND | ND | 14.800 ±1.48 | 17.765 ±1.261 |
| **Polyphenols (mg)** | 137.5 ±11.67 | 425.0 ±15.35 | 1462.5 ±23.03 | 562.5 ±28.34 | 475.0 ±15.45 | 137.5 ±17.67 | 725.0 ±15.35 | 875.0 ±9.00 |
| **Tannins (mg)** | 6.05 ±0.17 | 1230.0 ±18.99 | 590.0 ±2.42 | 1300.0 ±5.07 | 1200.0 ±4.50 | 80.0 ±0.27 | 624.0 ±5.65 | 1070.0 ±10.14 |
| **Total oxalates (mg)** | ND | ND | Traces | Traces | 7.30 ±0.00 | 7.33 ±0.00 | 1.46 ±0.00 | ND |
| **Soluble oxalates (mg)** | ND | ND | ND | ND | 2.00 ±0.00 | 2.00 ±0.00 | 0.40 ±0.00 | ND |

ND:- Not detected. Values represent mean ± standard deviation of triplicate determinations

Flax seeds are known for their high protein and fat content and the estimated values were 22.52 and 40.25% respectively. The oil of flax seed is a rich source of ω-3 fatty acids (Cunnane *et al*., 1995; David *et al*., 1999). The ash content was 3.55%. Flax seeds are also good source of soluble fiber which was estimated to be 10.8%. The insoluble fiber was found to be 15.0%. The fat and protein contents of cumin (12.60 and 16.24%) and fenugreek seeds (6.74 and 27.10%) were slightly different than what was reported by other authors (Khanum *et al*., 2001), while the ash contents were 8.80 and 3.25% respectively. These two spices were also found to be a very good source of dietary fiber. These are associated with many health benefits which can be partly attributed to their fiber profile. Cumin is known to be a very good digestive adjunct, whereas fenugreek is recognized for its hypoglycemic and hypocholesterolemic effect (Neeraja and Rajyalakshmi, 1996; Sowmya and Rajyalakshmi, 1999; Sreenivasan, 2008). Soluble fiber content of cumin and fenugreek ranged from 8.0 – 8.2%. Fenugreek had slightly lower insoluble fiber content (26.40%) in comparison with cumin seeds (32.20%).

The nutritional composition of whole wheat flour used for the study was also determined and the analyzed values for fat, protein and ash were 1.62, 12.02 and 1.09% respectively. These are closer to values reported by other authors (Gopalan *et al*., 1996; Singh *et al*., 2005). The soluble fiber content was 1.60% and insoluble fiber content was 4.60%. The premixes had a moisture content of 4.05-4.50% as these were made of totally dry ingredients. The fat content of premixes (20-21.66%) was high due to addition of flaxseed. Similarly protein content was also high at 16.15% - 16.37% which would have been contributed from almost all ingredients, particularly flaxseeds and fenugreek seeds which have high protein content. Cumin seeds also have a considerable amount and even greens contribute to a certain extent for proteins.

The amaranth leaves and cauliflower leaves were rich source of calcium, the range of values being 619.5-682.0 mg/100 g. The calcium content of *Amaranthus tricolor*, another variety of amaranth greens, as reported by Gupta *et al*., (2005) was 239 mg/100 g which is much lower than the present study. This can be attributed to varietal differences. The iron content of cauliflower leaves was very high at 39.0 mg/100 g while amaranth leaves had lower iron content at 21.65%. The greens were very rich in both total and β-carotene, amaranth leaves had 23.808 mg of total carotene, of which 63.5% was estimated to be β-carotene. Cauliflower leaves had 49.526 mg of total carotene of which 35% was found to be β-carotene. It is to be noted that as these are very rich source of both total carotene and β-carotene, a small amount is sufficient to provide recommended dietary allowances (RDA) of vitamin A. Carotenoids are also known for their antioxidant function and hence they contribute to the general health. The RDA for β-carotene for an Indian adult is 4800 µg/day as per

Table 2. *In vitro* digestible / available nutrients in prepared products (per 100 g)

| Constituents | Control | Premix-I, 6% | Premix-I, 12% | Premix-II, 6% | Premix-II, 12% |
|---|---|---|---|---|---|
| Moisture (g) | 25.39 ± 0.32 | 27.39 ± 0.21 | 27.70 ± 0.28 | 26.6 ± 0.14 | 27.43 ± 0.14 |
| Total starch (g) | 53.18 ± 1.45 (71.3) | 46.75 c ± 1.68 (64.4) | 41.66 c ± 2.21 (57.2) | 47.32 c ± 1.22 (64.5) | 40.31 b ±0.63 (55.5) |
| Digestible starch (g) | 18.65 ± 1.34 (25) | 16.8 a ± 0.08 (23) | 15.21 b ± 0.12 (20.9) | 16.74 a ± 0.76 (22.8) | 14.79 a ± 0.79 (20.4) |
| Protein (g) | 9.10 ± 0.14 (12.1) | 10.71 c ± 0.43 (14.8) | 12.20 b ± 0.10 (16.8) | 10.78 c ± 0.33 (14.7) | 12.2 c ± 0.09 (16.8) |
| Digestible protein (g) | 5.82 ± 0.55 (7.28) | 8.55 b ± 0.01 (11.8) | 9.81 b ± 0.04 (13.5) | 8.15 b ± 0.55 (11.1) | 10.47 b ± 0.54 (14.4) |
| Ash (g) | 0.53 ± 0.70 | 1.92 c ± 0.02 | 2.54 c ± 0.03 | 2.07 c ± 0.04 | 2.76 c ± 0.14 |
| Calcium (mg) | 32.72 ± 0.82 (43.9) | 57.90 c ± 1.11 (79.80) | 83.85 c ± 0.91 (115.2) | 61.12 c ± 2.01 (83.3) | 83.3 c ± 0.56 (114.7) |
| Bioaccessible calcium (mg) | 26.0 c ± 0.57 (32.7) | 49.93 c ± 0.79 (68.8) | 70.74 c ± 0.50 (97.2) | 53.75 c ± 0.70 (73.2) | 70.61 c ± 1.78 (97.3) |
| Iron (mg) | 6.66 ± 0.14 (8.92) | 9.83 c ± 0.24 (13.54) | 11.84 c ± 0.25 (16.26) | 10.33 c ± 0.94 (14.07) | 12.44 c ± 0.30 (17.15) |
| Bioaccessible iron (mg) | 2.36 ± 0.14 (3.17) | 2.68 ns ± 0.41 (3.71) | 2.95 a ± 0.21 (4.07) | 1.64 b ± 0.41 (4.95) | 3.81 a ± 0.41 (5.25) |

Values represent mean ± standard deviation of triplicate determinations.
Figures in parenthèses indicate values on dry weight values.
Significant differences between control and other premixes on application of Students 'T' test; a: P<0.05, Marginally significant ; b: P<0.01, Significant; c: P<0.001, Highly significant, ns: not significant.

the revised RDA (ICMR, 2010).

Flax seeds were also a fair source of calcium and iron and contained a small amount of total carotene. Cumin seeds were rich in both calcium and iron. Fenugreek comparatively had a lesser amount of calcium and iron. Wheat flour had a calcium content of 45.0 mg and iron content of 6.50 mg/100 g. Both the spices and wheat flour had small amount of total carotene, though β-carotene could not be detected.

The premixes were a good source of minerals. The calcium content was very high, with 524.5-583.5 mg/100 g. The iron content of amaranth leaves premix was 13.66 mg and this can also provide fair amount of iron. The cauliflower premix had a higher content of iron at 27.66 mg/100 g. This can provide much needed iron as Indian diets are particularly low in iron. The premixes were also found to be extremely rich source of total and β-carotene. Amaranth premix had 19.270 mg of total carotene and 14.80 mg of β-carotene. The cauliflower premix had still higher content of total carotene at 44.670 mg out of which 17.765 was β-carotene. A small amount of these premixes added to a dish can provide almost entire β-carotene needed for a day. Addition of dehydrated greens has been reported to increase the nutrient density of products remarkably (Gupta and Prakash, 2011a, 2011b).

The antinutrients are also present in foods along with mineral content, however, high concentration of antinutrients may strongly reduce the mineral availability, especially that of calcium as it forms insoluble complexes called calcium oxalates which is unabsorbable. These can also affect the calcium availability from other foods when present in high amounts by rendering the negative effect on the absorption rate (Weaver *et al*., 1987; Heaney *et al*., 1993). Another group of inhibitors of minerals absorption is polyphenols and tannins. The antinutrients estimated in the ingredients were polyphenols, tannins, total oxalates and soluble oxalates. Amaranth leaves had 137.5 mg of polyphenol and 6.05 mg of tannins/100 g and a small amount of total and soluble oxalates. In contrast, cauliflower powder had a higher content of polyphenol, (425.0 mg) and tannins (1230.0 mg/100 g). However, it did not have any oxalates. The values were closer to those reported by other authors (Kowsalya *et al*., 1999; Singh *et al*., 2005). Flaxseeds were a rich source of polyphenols (1462.5 mg) and also had a fair amount of tannins (590.0 mg/100 g). Cumin and fenugreek had fair amount of polyphenols ranging between 475-562.5 mg and a higher content of tannins ranging between 1200-1300 mg/100 g of sample. They had traces of total oxalates but soluble oxalates could not be detected in them. Wheat flour had small amount of polyphenols, tannins, total oxalates and soluble oxalates (137.5, 80, 7.33 and 2.0 mg/100 g respectively).

In premixes, the polyphenol content ranged from 725-875.0 mg/100 g. The tannin content was lower for amaranth premix (624.0 mg/100 g) and higher for cauliflower premix (1070.0 mg/100 g). The oxalate content was very low in amaranth premix and could not be detected in cauliflower premix.

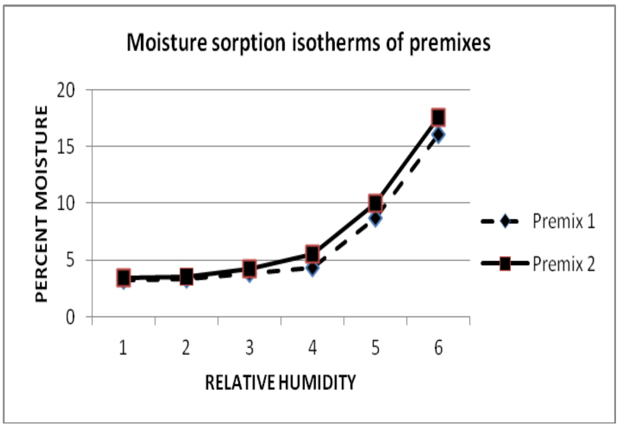


Figure 1. Moisture sorption isotherm curves of formulated premixes (RH indicators: 1- 0.234; 2 - 0.329; 3 - 0.443; 4 - 0.536; 5 - 0.654; 6 - 0.848)

*Moisture sorption of premixes*

The formulated premixes were subjected to moisture sorption studies to determine their storage stability. The premixes were stored at different relative humidity (RH) and moisture uptake was monitored by gravimetric technique every 24 hours till the samples attained maximum moisture level. The data of moisture uptake was used for drawing moisture sorption curves. The results are presented in Figure 1.

At RH of 22% the moisture uptake of the sample was low reaching up to 3.22% for premix-I and 3.48% for premix-II on 5th day. This indicates that at low RH the samples were quite stable. At RH of 33%, the moisture uptake was slightly higher than the previous value and at the end of storage period; it was 3.34 and 3.52%. On increasing the RH at 44%, again a consistent but small increase was seen which totaled up to 3.8 and 4.2%. At RH of 53%, the trend continued with a small increase in moisture uptake. However, at the next level of RH i.e. 65%, the moisture uptake was considerably high at 8.73% for premix-I and 9.99% for premix-II. At the RH of 93%, a very high moisture absorption was seen for both samples. For premix-I it was 16.1% and for premix-II it was 17.54%. At all levels of RH the moisture uptake by premix-II, i.e. the premix based on cauliflower leaves was slightly higher than the premix based on amaranth leaves.

Using the moisture uptake value, sorption isotherm curves of both samples were drawn and the results are presented in Figure 1. As can be seen from the curve, up to RH of 53%, the moisture uptake by sample was considerably low. However, at higher RH of 65 and 93%, the samples absorbed 8.7-17.5% moisture which was extremely high. These results indicate that for longer shelf stability, these will require a proper packaging material which can inhibit water ingress. In contrast, it was reported that dehydrated greens powder were stable even at high RH with low moisture uptake when stored as such (Gupta and Prakash, 2011a), hence it is possible that ingredients other than green leaves powder added to premix were responsible for high moisture uptake during storage at high RH.

*Nutrient availability in prepared products*

The prepared products, namely flat breads were analyzed for *in vitro* starch (IVSD), and protein digestibility (IVPD), in vitro bioaccessible calcium and iron to determine their nutritional quality. All analysis were done in fresh products immediately after preparation. The results are presented in Table 2 and 3 and Figure 2.

In vitro *digestible starch and protein*

Table 2 gives the moisture, total starch content, IVSD and IVPD of prepared products. The moisture content of products ranged from 25.39% to 27.70%. Addition of premixes did not affect the moisture content of product though it was slightly higher in products prepared with premix. The total starch content for the control sample was 53.18% and decreased on addition of premixes. With 6% of premixes, it was 46.75% and 47.32% and with 12% premixes, it ranged from 40.31% to 41.66%.

The *in vitro* digestible starch in the control sample was 18.65%. Addition of premixes resulted in lower digestibility of starch and the values were in the range of 16.74-16.80% for 6% premix and 14.79-15.21% in the 12% premix added sample. When the values are computed on moisture free basis, we can observe a close correlation in total starch values as well as in starch digestibility with the following order, the control was highest followed by products with premix-I and then premix-II. This indicates that addition of premixes would also reduce the glycemic index of flat breads and it would be beneficial for diabetics. When starch digestibility is considered as % of total starch, it can be observed that for all products almost similar values were obtained (Figure 2A). Approximately 35.0-36.8% of starch was digestible in all products. On an average, 36% of total starch was digestible in flat bread and addition of premixes did not affect the percent starch digestibility.

The control sample had 9.10% protein, of which 5.82 g was digestible. The samples with premixes at 6% had a slightly higher content of protein (10.71-10.78 g/100 g ) due to the premixes being very rich in protein, of which 8.15-8.55 g was digestible. When the level of premixes was raised to 12%, the protein content increased to 12.20 g/100g with

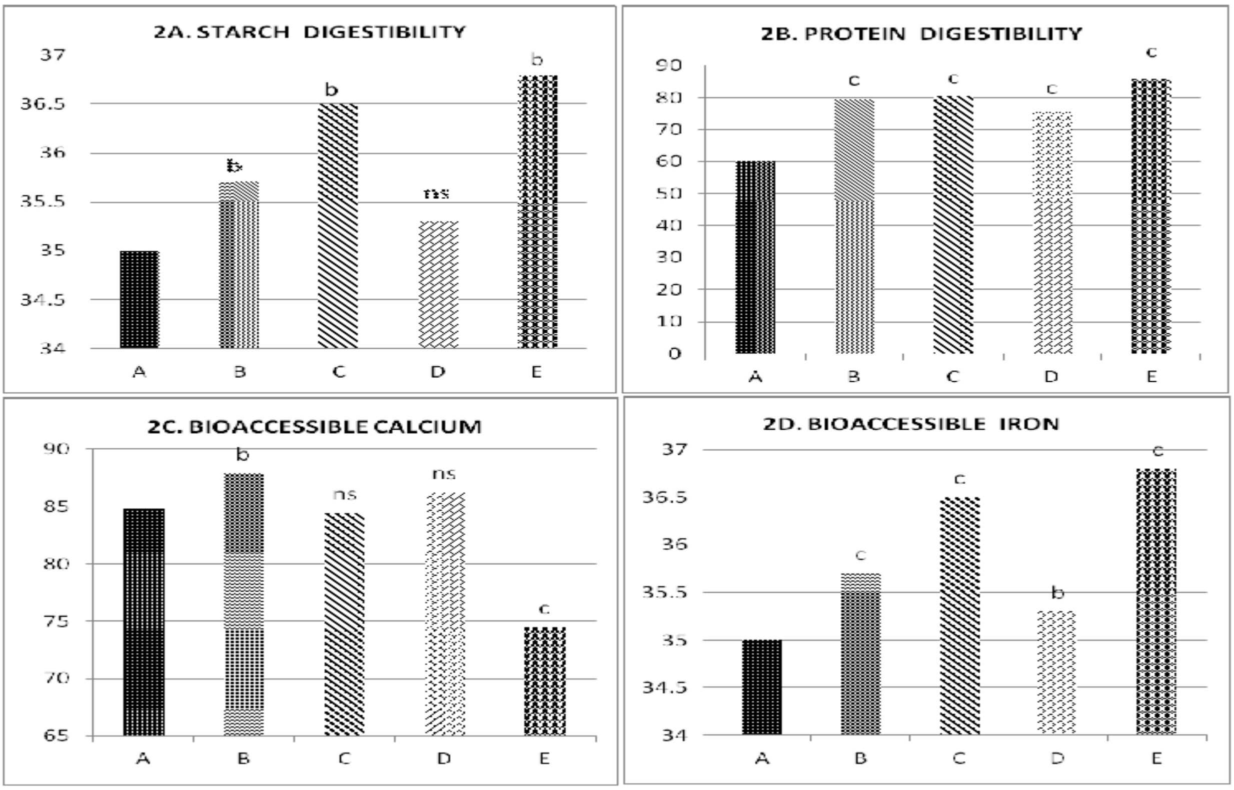


Figure 2. *In vitro* digestible/bioaccessible nutrients in products as percent of total [A: Control, B: Premix-I, 6%; C: Premix-I, 12%; B: Premix-II, 6%; Premix-II, 12%.]
Significant differences between control and other premixes on application of Students 'T' test:
b: P <0.01, Significant; c: P <0.001, Highly significant, ns: not significant

9.81-10.47 g digestible protein. When these were computed on dry weight basis, the increase in protein content of samples with premixes followed a similar pattern, which was also seen in digestible protein. When protein digestibility was considered as % of total proteins (Figure 2B), the control sample had a digestibility of 60.2% whereas in samples with premixes, this increased to 75.5-85.7%. It can be said that incorporation of premixes increased the protein content of flat bread and a higher extent of digestibility in comparison to control product.

In vitro *bioaccessibility of calcium*

The results given in Table 2 shows that the ash content of samples increased on addition of premixes indicating that premixes were good sources of minerals. The calcium content of control sample was 32.72 mg/100 g. All samples with premixes had a higher calcium content. Addition of higher quantity of premix increased the calcium content since the premixes were based on dried greens. The bioaccessible calcium was 26 mg/100 g in fresh control flat bread, it increased to 49.93-53.75 mg with 6% premix and 70.61-70.74 mg with 12% premix. The bioaccessible calcium when calculated as % of total calcium showed that for control product availability was 74.5% (Figure 2C). In samples with premixes, the bioaccessibility ranged from 84.4% to 87.9%. Hence, it can be seen that the premixes increased both the calcium content as well as bioaccessibility in products indicating the superior nutritional quality of premixes.

In vitro *bioaccessibility of iron*

The *in vitro* bioaccessibility of iron as determined by dialysis technique and the total iron content of all samples given in Table 2 shows that the control product had 6.7 mg iron/100 g and it was higher in all the products incorporated with premix as the premixes were rich in iron. Between the premixes, premix-II had a higher content than premix-I. The range of iron content in experimental products was 9.83-12.44 mg /100 g. The bioaccessible iron content was almost 1/3 or 1/4$^{th}$ of the total iron ranging from 2.36 -3.81 mg/100 g. In all the products with premixes, iron content as well as bioaccessible iron content was much higher than the control sample.

When available iron was calculated as % of total iron it can be seen that in control sample almost 1/3rd of total iron was available (Figure 2D). Samples with premix-I had lesser availability than control. However, in samples with premix-II availability was better. Though percent iron bioaccessibility was low in samples with premixes, the absolute amount of available iron was more indicating that a higher content of iron will be absorbed with value added

Table 3. Nutritive value of products per serving size and mean sensory scores of products prepared from premix-I and II at 6% and 12% level

| Products | Protein (g) | Fat (g) | Iron (mg) | Calcium (mg) | Soluble fiber (g) | Insoluble fiber (g) | Total carotene (µg) | β-carotene (µg) |
|---|---|---|---|---|---|---|---|---|
| Control | 7.21 | 0.98 | 3.90 | 27.0 | 0.95 | 2.76 | 0.006 | - |
| Premix-I, 6% | 7.36 | 1.63 | 4.46 | 44.3 | 1.18 | 3.51 | 760 | 530 |
| Premix-I, 12% | 7.51 | 2.30 | 4.42 | 61.6 | 1.43 | 3.64 | 1440 | 1070 |
| Premix-II, 6% | 7.37 | 1.69 | 4.66 | 46.4 | 1.25 | 4.86 | 1670 | 640 |
| Premix-II, 12% | 7.52 | 2.78 | 5.42 | 65.8 | 1.57 | 6.94 | 3270 | 1280 |

| Sensory attributes | Control | Incorporation levels 6% | Incorporation levels 12% | F- ratio (P- value) |
|---|---|---|---|---|
| **Flat bread with Premix I** | | | | |
| Appearance | 15.82 ± 2.58 | 14.28 ± 3.14 | 13.76 ± 3.42 | 10.693 (0.000***) |
| Color | 16.23 ± 2.44 | 14.19 3.51 | 13.38 ± 4.04 | 15.908 (0.000***) |
| Texture | 14.69 ± 3.14 | 14.75 ± 2.93 | 14.23 ± 3.65 | 1.1805 (0.308 $^{ns}$) |
| Aroma | 15.46 ± 2.52 | 15.57 ± 3.09 | 14.47 ± 3.48 | 3.1714 (0.0436*) |
| Taste | 15.55 ± 2.82 | 15.86 ± 2.72 | 15.12± 2.99 | 1.4798 (0.2296 $^{ns}$) |
| **Flat bread with Premix II** | | | | |
| Appearance | 15.57 ± 2.58 | 15.07 ± 3.14 | 15.25 ± 3.00 | 1.4891 (0.2278 $^{ns}$) |
| Color | 15.51 ± 2.71 | 14.88 ± 3.39 | 14.75 ± 3.38 | 2.57 (0.0790 $^{ns}$) |
| Texture | 15.32 ± 3.11 | 16.10 ± 2.46 | 15.6 ± 2.65 | 0.404 (0.6681 $^{ns}$) |
| Aroma | 15.09 ± 2.94 | 15.73 ± 2.91 | 15.36 ± 2.95 | 0.7791 (0.4600 $^{ns}$) |
| Taste | 15.44 ± 3.05 | 16.04 ± 3.35 | 15.53 ± 2.99 | 1.1677 (0.3129 $^{ns}$) |

products.

Though green leafy vegetables are a storehouse of nutrients, the process of dehydration is reported to bring down the content as well as bioaccessibility of nutrients (Gupta *et al.*, 2013). However, despite this, the formulated premixes were good sources of micronutrients, which can be attributed to combination of ingredients and a very low level of antinutrients.

*Nutritional value of prepared products*

The nutritional value of prepared products calculated from analyzed value of raw ingredients and premixes per serving size (two pieces flat bread) is given in Table 3. As can be seen the protein content of various products ranged from 7.25-7.41 g. These were almost similar to each other. In the experimental products there was an increase in the fat content which was due to addition of flaxseed in premixes which is very rich in fat. The iron content showed an increase in experimental products. The increase was in proportion to the amount of premix added. Products prepared with cauliflower premixes had higher iron content in comparison to products prepared with amaranth premix. An increase was also seen in the calcium content by 58-65% in products with 6% of premix and by 117-132% in products with 12% premix over the control value. This indicated that incorporation of premixes supplemented calcium intake. There was also an increase observed in soluble fiber content ranging between 24-65% over the control value. The insoluble fiber content also showed an increase of 25-50%. The addition of dried greens powder in premixes reflected in a tremendous increase in the content of total and β-carotene. The serving size of flat bread was sufficient to provide 11-13% (6% premix) or 22-27% (12% premix) of recommended allowance of β-carotene per day for an adult in a meal. This indicates that the experimental products had better nutritional composition than the control product and incorporation of premixes into different dishes can be recommended.

*Sensory analysis*

Organoleptic quality of foods is important from the consumer point of view, as taste is always a predominant determinant of acceptability of a new product. Sensory evaluation assists manufacturers in predicting the marketability of a product. The efficacy of formulated premixes was evaluated by incorporation into flat bread and subjecting it to sensory acceptability by a panel of 86 members. The results are summarized in Table 3.

*Flat breads with premix- I*

The sensory scores of flat breads indicated that for the quality attribute of appearance, the control product was given highest score followed by products with 6% and 12% of premix-I. On a grading scale these corresponded to quality of 'very good'. Similar trend was seen for the quality of color wherein the control scored the highest with 16.23 followed by premix-I, 6% product with 14.19 and premix-I, 12% with average score of 13.38. The differences between appearance and color scores of different products were statistically significant on application of ANOVA. This may have been due to the color of the flat bread which turned darker on addition of premix. The premix had a dark green and brown coloration since all the ingredients used had a dark color and this affected the appearance of product.

The scores given for the quality of texture ranged from 14.3-14.75 for all products with no significant differences. For the quality of aroma, highest scores of 15.57 was given to the product with 6% premix-I, this was followed by control at 15.46 and the product with 12% premix-I with 14.47. The latter was marginally lower than the former. For the quality of taste, product with 6% premix-I scored highest with 15.86 followed by control and product with premix-I, 12%. These values were similar to each other with no difference indicating that taste of all the three products was equally good. It can be said that amaranth premix influenced the appearance and color of flat bread adversely, however texture, aroma and taste were not influenced. Similar observations for sensory quality of flat bread were reported by Shanthala and Prakash (2005) on addition of curry leaf powder. When dehydrated greens were used for incorporation to other traditional products such as 'Dhokla' (a legume based fermented and steamed product) and 'Chutney powders' (spicy adjuncts eaten with meals), a higher level of addition was reported to bring down the acceptability scores, hence, there is an optimum level, which may be appreciated organoleptically, and a higher level may not be liked by subjects (Gupta and Prakash, 2011a).

*Flat breads with premix-II*

The results of sensory analysis prepared with cauliflower premix given in Table 3 show that for the quality of appearance the scores ranged from 15.17-15.57 and for the quality of color, they ranged from 14.75-15.51 signifying 'very good' on the grading scale. Unlike amaranth premix, flat breads made from cauliflower premix were easily accepted and were given grades similar to control. Statistically, there were no differences between any products. The attribute of texture was given highest score for the product with 6% premix at 16.10 followed by product with 12% premix at 15.60; the control was given a slightly lower score of 15.09 and 15.44 for aroma and taste responses. Similar trend was observed wherein the scores followed the order of highest being given to product with premix II, 6% followed by product with premix-II, 12% and then control. None of the product had any attribute which was significantly different. These results indicate that cauliflower premix was very well accepted with flat bread and was sometimes rated higher than control.

## Conclusion

Premixes were good source of minerals specially calcium and iron and very low in oxalate content. At higher RH, samples absorbed high moisture indicating that for longer shelf stability premixes proper packaging material is required. Addition of premixes did not affect the percent starch digestibility, and a higher protein digestibility was observed. Calcium and iron content as well as the bioaccessibility in products increased indicating better nutritional quality of both premixes and also the products prepared from both the premixes were organoleptically acceptable. It can be concluded that nutrient dense ingredients can be used in formulations such as premixes, which can be used for value addition to other products for increasing the nutritional quality. This can increase the consumption of micronutrients in diet and also provide dietary diversity in populations wherein a large part of meal comes from single staples.

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