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EFFECT OF SPROUTING ON PHYSICAL PROPERTIES AND FUNCTIONAL AND NUTRITIONAL COMPONENTS OF MULTI-NUTRIENT MIXES

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ABSTRACT

Multi-nutrient mixes with different combinations of cereals, millets, pulses, soy protein isolate and dairy whitener were formulated as per the nutritional requirements of school-going children. Effect of malted finger-millet or sprouted green gram present in the mixes on the nutritional and functional properties were studied and compared with their un-sprouted counterparts. On sprouting the Water absorption index and water solubility index increased significantly ($p \leq 0.05$) indicating the ability of flour to absorb water and an increase in the amount of soluble materials, which can be easily digested. The crude protein (CP) content of sprouted mixes ranged from 22.5 to 24.8 % and was significantly higher ($p \leq 0.05$) than un-sprouted mixes (15.5% to 18.7%). Fats were degraded significantly ($p \leq 0.05$) during sprouting/malting. A significant effect ($p \leq 0.05$) on oxalic acid, phenolic and antioxidants was found due to malting. Phenolics ranged from 103-115% mg eq of gallic acid/100g in un-sprouted samples and increased to 140% mg.eq of gallic acid/100g which was highly desirable. The oxalic acid content in un-sprouted combinations ranged from 93.2-101.2 mg% which decreased significantly ($p \leq 0.05$) to 21.6 mg% due to malting of finger-millet, making calcium in the mixes more available. Results indicate that sprouted mixes can be used to develop snacks for children with nutritional and functional benefits.

Key words: Multi-nutrient mixes, Sprouting/Malting, Oxalic acid, Phenolics, Water Absorption Index and Water Solubility Index.

INTRODUCTION

The critical period where children develop malnutrition coincides with the introduction of complementary foods, which are nutritionally inadequate in many developing countries (Khanam *et al.*, 2011). There is a need for nutritionally balanced, energy- dense, easily digestible foods with functional benefits to be formulated. A Cost- effective nutritious and functional multi-nutrient food mix prepared using locally available raw materials, which is easily assimilated by the body and promotes growth and healing is a good option. To achieve this objective, use of seasonal, local, low-cost and abundantly available raw food ingredients having high nutrition and functional properties like cereals, coarse cereals and millets, soybean, dairy ingredients and horticultural produce should be advocated.

Corn and wheat flour are commonly consumed source of cereals with functional properties and health benefits and are part of all meals in India. Sorghum and millet have considerable potential to be used as a human food and beverage source. In developing countries the commercial

processing of these locally grown grains into value-added food and beverage products is an important driver for economic development (Taylor, 2004). Finger millet (*Eleusine coracana*) also known, as 'ragi' is popular millet in India, consumed without de-hulling. It is the principal food grain of the rural population belonging to low-income groups in the Southern region. The tiny millet grain has a dark brown seed coat, richer in polyphenols compared to other continental cereals such as barley, rice, maize and wheat (Viswanathan *et al.*, 2009). Green gram is a widely consumed pulse and is an excellent source of protein (25%), high in dietary fibre, rich source of vitamins and minerals. Its low glycemic index and high folate content reduce blood glucose level and neural tube defects in newborn babies (Paul *et al.*, 2011). Soy Protein Isolate (SPI) is a commercial soy protein product having at least 90% protein (dry basis) which has been widely applied in the food industry as an important ingredient due to its nutritional value, desirable functional properties and low cost (Hao Hu *et al.*, 2013). Papaya (*Carica papaya L.*) is one of the important fruits of tropical and subtropical regions in the world.

The fruit is rich in β -carotene, vitamin-A and C, iron, calcium, protein, carbohydrates, phosphorous and good source of energy. It is generally converted into powders and added to food products to enhance its nutritional quality (Kandasamy *et al.*, 2012). Recent studies suggest that peanuts consumption might reduce the risk of heart diseases by lowering serum LDL-cholesterol level and reduce the risk of development of type II diabetes (Fraser *et al.*, 1992). The health benefits of peanuts have been attributed to the presence of minerals, vitamins, fatty acids, fiber, antioxidants, phenolics and bioactive compounds (Griel *et al.*, 2004). Roasting is known to increase their antioxidant activity (Mar win *et al.*, 2011). Thus, owing to the numerous nutritional and functional advantages of these ingredients, they were used to formulate four different types of multi-nutrient mixes.

Cereals, millets and legumes are generally pre-processed by fermentation, germination (malting), cooking, milling etc. in order to enhance their functionality and nutritional value. Germination/sprouting/malting is a biochemical process which involves transition of a seed from dormant state to vital active state. It is a simple technique that has been reported to improve the nutritive value of foods. Several studies on the effect of germination on legumes have found that germination can increase protein content and dietary fibre; reduce tannin and phytic acid content and increase mineral bioavailability (Rao and Prabhavathi, 1982; Hussein and Ghanem, 1999; Ghavidel and Prakash, 2007). Germination is reported to be associated with increase of vitamin concentrations and bioavailability of trace elements and minerals (El-Adawy *et al.*, 2004). Kaushik *et al.* (2010) found that germination improves calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content. In cereal grains, germination increase oligosaccharides and amino acids concentration as observed in barley (Rimsten *et al.*, 2003), wheat (Yang *et al.*, 2001), oat (Mikola *et al.*, 2001) and rice (Manna *et al.*, 1995) (Rusydi *et al.*, 2011). Since germination is cheap and more effective in improving nutritional value it was incorporated in mixes to contribute to the nutrition of people (Rusydi *et al.* 2011).

Several workers like Saha and Dunkwal, 2009; Tiwari and Awasthi, 2012, Premakumari *et al.*, 2012 etc. have prepared multi-grain mixes using cereals, pulses etc. However there is no study on the physical, nutritional and functional quality of a combination of different grains with legumes, dairy ingredients, protein isolates and fruits i.e. multi-nutrient mixes.

Therefore in the present study, four different types of multi-nutrient mixes using various proportions of cereals, legumes, millets, SPI, dairy ingredient, fruits and vegetables were formulated. The effect of malting of finger millet and sprouting of green gram present in the multi-nutrient mixes on the nutritional components, functional components and

physical properties were analysed and compared with their un-sprouted counterparts. These base mixes can be used further to develop nutritional rich functional ready to eat products for under-fed children.

MATERIALS AND METHODS

PREPARATION OF BASE MIX INGREDIENTS

Corn flour, wheat flour, Sorghum flour, Pearl millet flour, split green gram, finger millet, whole green gram, unsalted peanuts, dairy whitener, papaya, spinach, salt and sugar were procured from the commercial markets in Bhopal, India. Soy protein isolate containing 95% protein was procured from Sonic Biochem, Indore, India. Corn flour, wheat flour, sorghum flour and pearl millet flour were sieved through mesh of size 600 microns to obtain a uniform particle size. Finger millet, unsalted peanuts and split green gram were first cleaned thoroughly and made free from dust, dirt, stubbles and foreign matter. Peanuts (100g) were roasted at 130°C for 8 min followed by cooling at room temperature. All whole grains were then powdered using analytical mill (Cole Parmer, IL, USA) at high speed (20,000 rpm). Papaya was deseeded and de-skinned. The pulp was manually extracted and dried in trays at 60°C for 24h. The dried mass was then powdered using analytical mill (Cole Parmer, IL, USA) at high speed (20,000 rpm). Spinach leaves were thoroughly washed with water several times to remove any adhering dirt or dust and were dried at 60°C for 24hrs in a hot air oven (Meta-Lab scientific Industries, India) and powdered using analytical mill (Cole Parmer, IL, USA) at high speed (20,000 rpm). All powders were then sieved through mesh of size 600 microns.

SPROUTING/MALTING OF INGREDIENTS

Finger millet and whole green gram were first cleaned thoroughly and made free from dust, dirt, and foreign matter. Any seeds which were spoiled or with cracked hull were discarded and the remaining seeds were surface sterilized with 0.1% (w/v) potassium permanganate solution. For sprouting, seeds were soaked in distilled water for 4h at room temperature (RT). The excess water was drained, sample further rinsed with distilled water, seeds placed in a single layer on filter paper in sterile petridishes and placed in the Seed Germinator (Indosaw, India) at the 25°C, 90% RH for 24h for finger millet and 48h for green gram. After sprouting the seeds were dried in an oven overnight at 60°C. They were then cooled in a desiccator, powdered using analytical mill (Cole Parmer, IL, USA) at high speed (20,000 rpm) and sieved through mesh size of 600 microns.

COMPOSITION OF BASE MIXES

Four different types of multi-nutrient mixes were formulated with various proportions of ingredients mentioned in Table 1. The sprouted mixes were made by replacing the

finger millet and green gram powder with their respective sprouted/malted counterparts in the base mixes.

Table 1: Multi-nutrient combinations with proportions of various ingredients

Mixes	Ingredients (g)														
	Corn flour	Wheat flour	Green gram flour	SGGF	PMF	Sorghum flour	FMF	MFMF	DI	SPI	PP	SP	RP	Salt	Sugar
C1	48	20	7	-	-	5	-	-	5	7	-	5	-	3	-
SC1	48	20	-	7	-	5	-	-	5	7	-	5	-	3	-
C2	40	20	10	-	8	-	-	-	5	7	5	-	-	-	5
SC2	40	20	-	10	8	-	-	-	5	7	5	-	-	-	5
C3	45	20	5	-	-	8	-	-	5	7	5	-	-	-	5
SC3	45	20	-	5	-	8	-	-	5	7	5	-	-	-	5
C4	48	20	-	-	-	-	7	-	5	7	-	5	5	3	-
SC4	48	20	-	-	-	-	-	7	5	7	-	5	5	3	-

C- Combination, SC- Sprouted combination. SGGF- sprouted green gram flour, PMF- Pearl millet flour, FMF- finger millet flour, MFMF- malted finger millet flour, DI- Dairy ingredient, SPI- Soy protein isolate, PP- papaya powder, SP- Spinach powder, RP- roasted peanuts powder

PHYSICAL PROPERTIES

The main physical properties analysed for both un-sprouted and sprouted combinations are as follows:

BULK DENSITY

Packed Bulk density was evaluated by measuring the weight of known volume of sample. Samples were poured into a graduated cylinder, gently tapped ten times and filled to 10 ml. Results were expressed as g/ml. (Mandge *et al.*, 2011). The procedure was repeated 5 times and the average value was reported.

WATER ABSORPTION INDEX (WAI) AND WATER SOLUBILITY INDEX (WSI)

Water absorption index of the product was determined by method outlined by Anderson *et al.*, 1969. 2.5 g of ground sample was suspended in 30 ml of distilled water at 30 °C in a 50 ml tared centrifuge tube. The contents were stirred intermittently over 30 min period and centrifuged at 3,000 × g for 10 min. The supernatant liquid was poured carefully into tared evaporating dish. The remaining gel was weighed and WAI was calculated as the grams of gel obtained per unit gram of sample. WSI was determined from the amount of dried solids recovered by evaporating the supernatant from the water absorption index test described above (Anderson *et al.*, 1969). It was expressed as a percentage of solid in the sample extract (Mandge *et al.*, 2011).

LEAST GELATION CONCENTRATION

The Least Gelation Concentration (LGC) of the flour blends was determined using the modified method of Coffman & Garcia (1977). Sample suspensions of 2%, 4%, 6%, 8%, 12%, 14%, 16%, 18% and 20% (m/v) were

prepared in 10 ml distilled water in test tubes. The tubes containing the suspensions were then heated for 1 hour in a gentle boiling water bath, after which the tubes were cooled rapidly in water at 4°C for 2 hours. Each tube was then inverted one after the other. The LGC was taken as the concentration when the sample from the inverted test tube did not fall or slip.

PROXIMATE ANALYSIS

Combinations were weighed and dried at 60°C for nutritional analysis. After complete drying, the powder was sealed and stored in vacuum desiccators until used further for analysis. The moisture content, fat content and crude protein contents of the samples were estimated by the standard methods (AOAC, 1990). The ash obtained after combustion in the muffle furnace was used to prepare the ash solution, which was in turn used for the estimation of calcium. Calcium was precipitated in acidic medium as insoluble calcium oxalate by adding saturated ammonium oxalate solution. The precipitate was dissolved in dilute sulphuric acid (1:9), heated and the oxalic acid thus released was titrated against standard potassium permanganate solution in warm condition (60°C) to get the calcium content of the sample (Raghumulu *et al.*, 1993). Oxalic acid was analysed by extraction with hydrochloric acid followed by precipitation with calcium oxalate from de-proteinized extract and subsequent titration with potassium permanganate (Baker, 1952; Gupta *et al.*, 2005).

ESTIMATION OF FUNCTIONAL COMPONENTS

Phenolics were quantified by the Folin–Ciocalteu method of Singleton and Rossi (1965) as described previously by Siwela *et al.*, 2010. Samples were extracted with acidified methanol followed by centrifugation for 10

min. Sample extracts (0.5 ml) were mixed with 2.5 ml Folin–Ciocalteu phenol reagent in a 50-ml volumetric flask; 7.5 ml 20% (w/v) sodium carbonate was added. The contents were mixed and the flask made up to volume with distilled water, stoppered and thoroughly mixed. The flasks were left to stand at room temperature (approx. 25 °C) for 2 h, after which absorbance at 760 nm was measured. Gallic acid was used as a standard. The stable 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the mixes by method described by Lee *et al.*, 2003. 0.5 g of sample was extracted using aqueous methanol and centrifuged for 20 min at 2 °C. 100 µl filtrate was mixed with equal volume of methanol and 5 ml of DPPH reagent. After mixing the solution thoroughly, it was allowed to stand at room temperature for 20 min and absorbance was measured at 517 nm.

RESULTS AND DISCUSSION

The effect of sprouting on the physical properties, nutritional components and functional components of multi nutrient mixes were studied and the results have been presented below.

EFFECT OF SPROUTING ON PHYSICAL PROPERTIES

The physical properties of the mixes are given in table 2. The Bulk density of mixes ranged from 0.79g/ml to 0.85g/ml with a marginal non-significant ($p \leq 0.05$) increase in sprouted mixes. Bulk density is a measure of heaviness of flour (Nicole *et al.*, 2010) and is generally affected by the particle size and the density of the flour. It is very important in determining the packaging requirement, material handling and application in wet processing in the food industry (Adebowale *et al.*, 2005). For both sprouted and un-sprouted combinations, mixes that contained higher amount of corn flour showed higher WAI, which could be due to their higher amylose/amylopectin ratio (Nicole *et al.* 2010). Also, since all combinations contained Soy Protein Isolates, it may have contributed to high WAI, as also reported by Abioye, 2011. On sprouting the WAI increased significantly ($p \leq 0.05$) (Com 1: 2.26g/g and Com 2: 2.60g/g) indicating the ability of flour to absorb water. Among the sprouted combinations the highest WAI was obtained for samples containing sprouted green gram, which could be due to increased protein content of the green gram on sprouting. Protein sub-units have more water binding sites increasing water absorption capacity (Dev and Quensil, 1988) which in turn increases the gel forming ability of the mixes. From the results obtained, it was observed that sprouting had a statistically significant ($p \leq 0.05$) effect in increasing the WSI which may be due to increased activity of amylases and corresponding increase in the soluble polysaccharides content (Almeida-Dominguez *et al.*, 1996). The increase in WSI with sprouting is of significance since it gives an

indication that sprouting can be used to increase the amount of soluble materials, such as starch and amino acids, which can be easily digested (Pelembé *et al.*, 2002). Least Gelation concentration (LGC) for all the un-sprouted combinations was found to be 6% whereas it reduced to 4% on sprouting indicating good gelation ability and better gelation characteristics of the sprouted flours (Onyeka and Dibia, 2002, Kinsella, 1979).

Table 2: Physical properties of base mixes

Mix	Bulk density (g/ml)	WAI (g/unit g sample)	WSI (%)	LGC (%)
C1	0.81 ± 0.5	2.29 ± 0.0	14.64 ± 0.5	6 ± 0.0
SC1	0.84 ± 0.8	2.60 ± 0.1	21.36 ± 0.8	4 ± 0.0
C2	0.79 ± 0.6	2.11 ± 0.2	12.57 ± 0.3	6 ± 0.0
SC2	0.82 ± 0.9	2.26 ± 0.1	23.23 ± 0.3	4 ± 0.0
C3	0.80 ± 0.5	2.18 ± 0.4	19.29 ± 0.5	6 ± 0.0
SC3	0.82 ± 0.4	2.41 ± 0.5	21.54 ± 0.2	4 ± 0.0
C4	0.82 ± 0.5	2.22 ± 0.1	16.72 ± 0.7	6 ± 0.0
SC4	0.85 ± 0.6	2.26 ± 0.3	20.25 ± 0.5	6 ± 0.0

Mean ± SD; C- Combination; SC- sprouted combination; WAI- Water absorption index, WSI- Water solubility index, LGC- Least gelation concentration

EFFECT OF SPROUTING ON NUTRITIONAL AND ANTI-NUTRITIONAL COMPONENTS

The results obtained for nutritional analysis of both sprouted and un-sprouted mixes are presented in Table 3. No statistically significant effect was found on the moisture and ash content of mixes on sprouting. The major effect of sprouting/malting was seen on protein and fat contents of the mixes. The crude protein (CP) content of sprouted mixes ranged from 22.5 to 24.8 % and was significantly higher ($p \leq 0.05$) than un-sprouted mixes where CP ranged from 15.5% to 18.7%. Similar findings were reported by Ghavidel and Prakash, 2007; Oshubó *et al.*, 2004 and Kaushik *et al.* 2010. The increase seen could be due to a compensatory increase in free amino acids and peptides (Adjei-Twum *et al.*, 1976) and increase in non-protein nitrogenous constituents during germination (Thapar *et al.*, 1974). Fats were degraded significantly ($p \leq 0.05$) during sprouting/malting process as also reported by several researchers (Mostafa *et al.*, 1987; Bau *et al.*, 1997). The decrease in oil contents on sprouting may be attributed to their utilization in the sprouting process as energy sources (Vineet Kumar *et al.*, 2006). The increase in respiration rate during germination brings about the release of energy from the breakdown of carbon compounds. Germination changes the stored insoluble nutrients in the cotyledons to soluble nutrients through the hydrolysis of macromolecules (Lorenz, 1980). The anti-nutritional factor analysed in our study was oxalic acid. The oxalic acid content ranged from 33.3 to 101.2 mg% in un-sprouted

mixes with maximum being in combination 4. This combination contained finger millet flour and spinach, which is known to have high amount of oxalic acid (Savage *et al.* 2000). On sprouting the oxalic acid content decreased significantly ($p \leq 0.05$) and ranged from 21.6-77.6 mg%. During germination, oxalate oxidase gets activated which breaks down oxalic acid into carbon di oxide and hydrogen peroxide and releases calcium (Illett, 1998). The effect of this change was seen correspondingly on calcium content of the mix which increased on sprouting as oxalic acid is known to interfere with calcium absorption (Proetti *et al.*, 2009). The maximum effect was seen in Combination 4 in which calcium content increased from 107.7 to 145.2 mg%. A 32.6% decrease in oxalic acid content of Combination 4 increased its calcium content by approximately 35% due to the sprouting of finger millet. The effect of sprouting on oxalic acid and calcium content of mixes is shown in Fig 1.

Table 3. Nutritional analysis of multi-nutrient mixes

Mix	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
C1	6.0 ± 0.3	17.8 ± 0.2	6.9 ± 0.4	5.8 ± 0.0
SC1	6.6 ± 0.0	23.6 ± 0.5	2.8 ± 0.3	6.8 ± 0.0
C2	6.0 ± 0.7	18.7 ± 0.5	4.6 ± 0.2	2.0 ± 0.0
SC2	6.9 ± 0.0	24.8 ± 0.2	1.8 ± 0.4	3.9 ± 0.0
C3	6.0 ± 0.6	15.5 ± 0.2	7.5 ± 0.1	1.8 ± 0.1
SC3	7.0 ± 0.1	22.7 ± 0.2	2.2 ± 0.5	2.9 ± 0.0
C4	5.8 ± 0.2	15.5 ± 0.5	6.9 ± 0.4	5.5 ± 0.0
SC4	6.6 ± 0.2	22.5 ± 0.5	2.6 ± 0.1	7.5 ± 0.1

C- Combination; SC- sprouted combination; Mean ± SD

EFFECT OF SPROUTING ON FUNCTIONAL COMPONENTS

The content of functional components of mixes is given in Table 4. Phenolics are known to impart antioxidant properties and serve as radical scavengers thereby imparting several health benefits like reducing the risk of cancer, diabetes, cardiovascular diseases etc. (Cevallos-Casals and Cisneros-Zevallos, 2010). They also contribute to improving colour and sensory attributes of food (Troszynska *et al.*, 2006). Phenolics content of the mixes (mg/ 100g equivalent of Gallic acid) ranged from 103.5 to 115.0 which increased on sprouting to 121.7-139.7. The maximum increase of 18% in total phenolic content was seen in combination 3 due to sprouting of green gram. Similar effect of sprouting of green gram on phenolics was reported by Troszynska *et al.*, 2006. It has been reported that increase in phenolics during initial stage of germination is mainly to prevent seeds from oxidative damage (Cevallos-Casals and Cisneros-Zevallos, 2010). Antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species, which include reactive free radicals.

They scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi, Noguchi, & Niki, 2001). The antioxidant capacity measured as % radical scavenging activity ranged from 5.1 to 8.6 with maximum in Combination 2 and 3 as they contained papaya powder which is rich in carotene and is known to have good antioxidant activity and is a potent radical scavenger (Lim *et al.*, 2007). On sprouting the antioxidant capacity of all mixes increased significantly ($p \leq 0.05$) due to increase in activity of antioxidant enzymes like superoxide dismutase, glutathione peroxidase, catalase etc. The highest value of 9.8 % RSA was observed in sprouted combination 3 due to sprouting of green gram. Ramesh *et al.*, 2011 reported that on sprouting of green gram its antioxidant potential increases either due to increased amount of phenols or similar compounds or activation of enzymes. Therefore, sprouting increases the functional components in mixes improving quality of mixes.

Table 4: Values of functional components present in mixes

Mix	Phenolics (%mg eq of gallic acid/100g)	Antioxidants (% RSA)
C1	103.5 ± 4.9	5.1 ± 0.6
SC1	121.1 ± 4.1	7.7 ± 0.6
C2	113.2 ± 0.4	8.5 ± 0.5
SC2	130.7 ± 4.6	9.2 ± 0.1
C3	115.0 ± 1.3	8.6 ± 0.4
SC3	139.7 ± 5.8	9.8 ± 0.3
C4	112.7 ± 0.2	7.1 ± 0.1
SC4	124.7 ± 0.4	8.2 ± 0.1

C- Combination; SC- sprouted combination; Mean ± SD

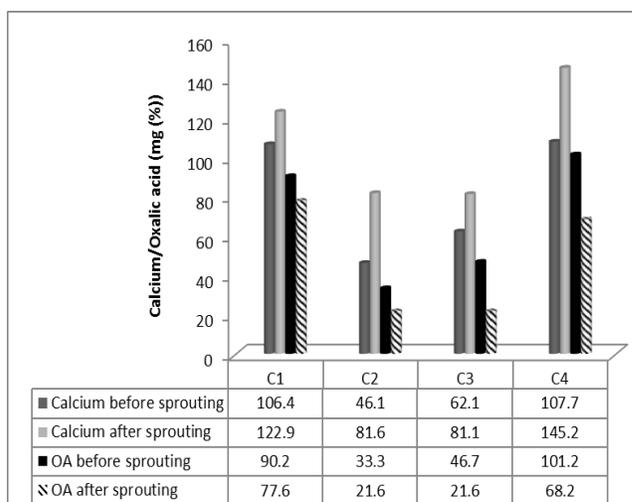


Figure 1: Comparison of calcium and oxalic acid content before and after sprouting in various combinations

CONCLUSION

The multi-nutrient mixes formulated from combination of various cereals, millets, pulses, SPI, dairy ingredient, roasted peanuts etc were rich in protein, minerals, vitamins, antioxidants, phenolic compounds, dietary fibres and other important nutrients. From the results obtained it can be seen that sprouting/malting helps in increasing the nutritional components and reducing the anti-nutritional components of multi-nutrient mixes which further enhanced their nutritional, physical and functional properties. Among all four samples, combination 3 was found to have maximum increase in functional components due to sprouting of green gram whereas combination 4 had maximum reduction in its anti-nutritional components. Thus, sprouting had varied effect on different combinations. Sprouting had marked effect on physical properties of the mixes too which increased their ability to be processed into various ready to eat products for children. Thus, sprouting was found to be a useful processing step in improving the quality and functionality of multi-nutrient mixes.

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