Functional and nutritional evaluation of supplementary food formulations

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Abstract Two type of ready to eat supplementary food formulations were developed by roller drying based on wheat, soy protein concentrate, whey protein concentrate, and green gram flour and were fortified with vitamins and minerals to meet the one third of the Recommended daily allowance (RDA). The supplementary food formulations contained 20–21% protein, 370–390 kcal of energy and 2,300 µg of β -carotene per 100 g serving. The physico-chemical, functional and nutritional characteristics were evaluated. The chemical score indicated that sulphur containing amino acids were the first limiting in both the formulations. The calculated nutritional indices, essential amino acid index, biological value, nutritional index and C-PER were higher for formula II. Rat bioassay showed higher PER (2.3) for formula II compared to formula I (2.1). The bioaccessibility of iron was 23%. Sensory studies indicated that the products were acceptable with a shelf life of 1 year under normal storage condition. However, the formulations were nutritionally better than only cereal based supplementary food formulations available commercially. The product could be served in the form of porridge with water/milk or in the form of small *laddu*.

Keywords Supplementary food · Fortification · Nutritional characteristics · Protein efficiency ratio

Introduction

Nutrition plays a vital role for normal growth and to maintain physical and mental fitness throughout the life (Awasthi and Kumar 1999). Nutrient deficiencies may contribute to growth retardation indirectly by reducing the intake of other growth limiting factors, such as energy and protein. Also, several micronutrients, including zinc, iron, and vitamin A, are associated with immune function and risk of morbidity, which in turn affect growth (Rivera and Martorella 1988). Growth retardation is highly prevalent in developing countries (Deonism 2000). Inadequate intakes of dietary energy and protein and frequent infections are well-known causes of growth retardation (Rivera and Martorella 1988; Mora et al. 1981; Habicht et al. 1995).

Childhood and adolescence diets influence, not only the immediate health of children, but may also have an important impact on adult health. The childhood diet must be adequate to support normal growth and development, and appropriate amounts of minerals are required since a deficient intake of certain minerals can produce diseases and lead to abnormal development (Camara et al. 2005). More than half of the preschool children are suffering from anemia. It is estimated that 75 million and 140 million preschool children have clinical and sub clinical vitamin A deficiencies (ACC/SCN 2000).

The critical period for developing childhood malnutrition coincides with the introduction of complementary foods, which are nutritionally inadequate in many developing countries (Gibson et al. 1998). Iron, zinc and vitamin-A are viewed as problem nutrients because of their low density in plant based complementary foods (Brown et al. 1998). In developing countries, retarded infant growth in the first year has been associated with decreased breast milk

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production and lack of hygienic complementary foods that have adequate energy and nutrient densities to promote normal growth (Dewey et al. 1998; Brown et al. 1998).

Protein energy malnutrition accounts for higher mortality rate in India (95/1000 live births) compared to developed countries (Bhandari et al. 1988; Dahiya and Kapoor 1994). School children constitute one of the important segments accounting 27% of the total population of India (Reddy et al. 1993). National Nutrition Monitoring Bureau (NNMB 1998) has indicated that pre-school children consume nearly 75% of the recommended energy. Energy consumption less than 80% of the requirement is reported to be a risk factor for malnutrition of pre-school children (Khabdiat et al. 1998). Micronutrient deficiencies are highly prevalent in low-income countries, and the most probable causes are low content in the diet and poor bioavailability (ACC/SCN 2000). Mineral deficiency is usually caused by low mineral content in the diet when rapid body growth is occurring when there is poor absorption of minerals from the diet (Favier 1993). Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of hemoglobin, myoglobin and cytochromes (Gibson 1994; Sandbeg 2002). The higher incidence of iron deficiency anemia is due to either insufficient intake of dietary iron or poor bioavailability or both or heavy worm load in the intestine. Bioavailability of iron from the dietary source depends on the iron content of the diet, actual composition of the diet and the absorption rate (Vijayalakshmi et al. 2008).

Various long-term programs have effectively reduced infant mortality, malnutrition in several parts of the world (FAO/WHO 1993; FAO 1987). Development of supplementary foods based on locally available cereals and legumes has been suggested by the Integrated Child Development Scheme (ICDS) and Food and Agriculture Organization (FAO) to combat malnutrition among mothers and children of low socio-economic groups (Natrajan et al. 1979; Malleshi and Desikachar 1982; Anjum Khanam and Bhagya Swamylingappa 2009). Therefore, the objective of the present study was to develop two supplementary food formulations with quality protein and to provide recommended daily allowances of other nutrients.

Materials and methods

Wheat (*Triticum aestivum*), sesame seeds (*Sesamum indicum*), green gram dhal (*Phaseolus aureus* Roxb), and sugar were procured from a local market, of Karnataka, India. Whey protein concentrate was obtained from M/S Mohan Proteins Ltd (New Delhi, India). Red palm oil was obtained as gift from Agro processing and natural products Divisions, National Institute for Inter Disciplinary Science and

Technology, (Trivandrum, India) Vitamin premix was purchased from Nicholas and primal India, (Mumbai, India). Pepsin, pancreatin, standard iron, calcium and zinc were purchased from Sigma Chemicals Co., (St.Louis, USA). All other chemicals used were of analytical grade.

Preparation of soy protein concentrates Defatted soy flakes/grits were obtained from M/S Shakti soy, Coimbatore, Tamilnadu; it was processed according to the method of Obulesu and Bhagya (2006) and was powdered to pass through 60-mesh sieve.

Preparation of defatted sesame flour The sesame cake was made into grits and defatted by repeated extraction with hexane in a ratio of 1:5 (w/v). The defatted meal was powdered to pass through 60-mesh sieve.

Preparation of supplementary food formulations Supplementary food formula-I contained wheat flour, soy protein concentrate, whey protein concentrate, and sesame flour. In the formula - II, sesame flour was replaced with green gram dhal flour and fortified with red palm oil to meet the β -carotene requirement. All the ingredients except wheat were powdered to pass through 60 mesh sieve. The wheat was optimally roasted to golden brown at a temperature of 100–120 °C for 10 min and powdered. All the weighed ingredients were blended using a hobart mixer for 5 min (Table 1). Water was added to the mix in a ratio of 1:3 (w/v) to make a slurry and drum dried (Lab Plant M/S. Escher-Wyss, Ravensburg, Germany) at 1 kg/cm² with a drum speed of 3–4 rpm. The drum dried supplementary food was powdered to pass through 60-mesh sieve, powder sugar was added, and fortified with vitamin premix and minerals.

Chemical composition Supplementary food formulations were analysed for moisture, protein ($N \times 6.25$), fat, ash and crude fibre by AOAC method (2000). β -Carotene was estimated according to the method of Ranganna (1986). Phytic acid content was estimated according to the method of Thompson and Erdman (1982) by converting the ferric phytate; phosphorus content was analysed by Tausky and Shorr (1953). The Phytic acid content was derived from the phytate phosphorus content by multiplying by a factor of 3.55. Total iron, calcium and zinc were determined by Atomic Absorption Spectrometry (Shimadzu AAF-6701, Tokyo), using standard conditions as recommended by the supplier of equipment.

Amino acid analysis Supplementary foods containing 5 mg of protein were hydrolysed for 24 h under vacuum at 110 °C using 5.8 mol/L HCl. Amino acid analysis was carried out by pre-column derivatisation using phenylisothiocyanate. The phenylthiocarbonyl amino acids were analysed using a water

Table 1 Ingredients used in the supplementary food formulations

Ingredients g	Supplementary food-I	Supplementary food-II
Wheat flour (roasted)	53	43
Soy protein concentrate	10	10
Whey protein concentrate	5	10
Sesame flour	5	–
Green gram dhal flour	–	10
Red palm oil	–	5.5
Sugar	27	27
Ferrous sulphate	0.04	0.04
Calcium carbonate	0.9	0.9
Vitamin premix	0.09	0.09

Pico-Tag amino acid analysis system (Bidlingmeyer et al. 1984). Tryptophan was estimated by the acid ninhydrin method (Pinter and Molnar 1990).

In vitro digestibility of protein This was determined by pepsin followed by pancreatin digestion according to Akesson and stahman (1964). The digested protein relative to the total protein was expressed as% digestibility.

Chemical score The chemical score was calculated (FAO 1968) as

$$\frac{\text{g EAA in test protein}}{\text{total EAA in test protein}} \times \frac{\text{g total EAA in egg}}{\text{g EAA in egg}} \times 100$$

where,

EAA is essential amino acids.

Essential amino acid index (EAAI) and biological value (BV) EAAI was calculated according to the method of Oser (1951) and BV was calculated using the formula of Oser (1959).

$$BV = 1.09 \times EAAI - 11.7$$

Nutritional index (NI) NI was calculated using the formula of Crisan and Sands (1978).

$$NI = (EAAI \times \% \text{ protein})/100$$

Computed protein efficiency ratio (C-PER) This was calculated according to the method of Satterlee et al. (1979) using the formula

Table 2 Chemical compositions of supplementary food formulations

Constituents%	Supplementary food-I	Supplementary food-II
Moisture	3.0±0.20 ^e	3.0±0.10 ^d
Protein (NX6.25)	20.2±0.1 ^e	20.6±0.01 ^d
Fat	0.6±0.05 ^e	4.1±0.08 ^c
Ash	2.5±0.01 ^e	2.5±0.01 ^d
Acid insoluble ash	0.05±0.001 ^e	0.05±0.002 ^d
Crude fibre	2.0±0.01 ^e	2.0±0.02 ^d
Carbohydrates by diff.	71.7±0.1 ^e	67.7±0.3 ^b
Energy, kcal	373.0±1.5 ^e	390.0±1.8 ^a
β-Carotene, µg	200.0±0.05 ^e	2300.0 ±0.1 ^c
Iron	13.9±0.12 ^e	13.06±0.06 ^b
Calcium	478.5±1.5 ^e	447.5±2.5 ^c
Zinc	2.3±0.01 ^e	2.2±0.07 ^b
Thiamine,mg*	1.2	1.2
Riboflavin,mg*	1.0	1.0
Niacin,mg*	10.4	9.3
Vitamin B ₁₂ ,µg*	0.5	0.5
Folic acid,µg*	55.0	59.0
Phytic acid,g%	0.8±0.005 ^e	0.6±0.002 ^b

*Computed

Values are mean ± standard deviation of three determinations. Means followed by different superscripts in a row differ significantly at $p < 0.05$

Table 3 Essential amino acid composition (g%) of protein in the supplementary food formulations

Essential amino acid	Supplementary food-I	Supplementary food-II	FAO/WHO Reference pattern
Isoleucine	4.9±0.30 ^e	5.3±0.15 ^c	4.0
Leucine	9.4±0.13 ^e	9.7±0.18 ^d	7.0
Lysine	4.7±0.02 ^e	6.2±0.57 ^c	5.5
Methionine+Cystine	2.6±0.09 ^e	2.4±0.05 ^b	3.5
Phenylalanine			
+ Tyrosine	7.7±0.12 ^e	7.2±0.43 ^c	6.0
Threonine	4.6±0.07 ^e	5.2±0.08 ^b	4.0
Tryptophan*	1.6±0.13 ^e	1.8±0.14 ^a	1.0
Valine	5.7±0.10 ^e	5.9±0.09 ^a	5.0

*Determined by acid ninhydrin method

Values are mean ± standard deviation of three determinations. Means followed by different superscripts in a row differ significantly at $p<0.05$

$$C - \text{PER} = -2.107 + 7.1312 \times \text{SPC} - 2.5188 \times (\text{SPC})^2$$

where,

SPC is the EAA score ratio of sample to casein

Protein digestibility corrected amino acid score (PDCAAS)

This was calculated according to the method of Sarwar and McDonough (1990), using the essential amino acid composition of the test samples and the amino acid suggested by FAO/WHO/UNU (1990) for different age groups.

Available lysine Available lysine in the supplementary food was determined by FDNB reactive lysine method of Carpenter (1960) as modified by Booth (1971).

Protein efficiency ratio (rat bio-assay method) PER was determined in the supplementary food, according to, ISI (1996). Casein was used as standard reference protein.

Bioaccessibility of iron Bioaccessibility of iron in the supplementary food samples was determined by an in vitro method

described by Luten et al. (1996). Iron present in the dialysates was analysed by α - α dipyridyl method (AOAC 1965).

Functional properties The food formulations were subjected to determination of various functional properties such as water holding capacity as described by Prasanna et al. (1972). Bulk density, was determined according to the method of Wang and Kinsella (1976), and Consistency (pat spread) was determined by the modified method of Bookwalter et al. (1968). Apparent viscosity of the supplementary food was measured by using Brookfield viscometer (RVmodel; Brookfield Engineering Laboratories, Inc, Stoughton, MA, USA) using spindle number 4 at 100 rpm. Colour measurement was determined using the Minolta CM 3500D (Osaka, Japan) instrument at visible wavelength. Colours of samples were measured by the C-Illuminating 2D view angle. The values of L (lightness), a (redness and greenness), and b (blueness and yellowness), were measured using a Hunter colour system.

Sensory Analysis A trained panel was employed for carrying out sensory evaluation following the method of Quantitative Descriptive Analysis (QDA) (Stone and Sidel 1998).

Table 4 Nutritional characteristics of supplementary food formulations

Parameters	Supplementary food-I	Supplementary food-II
In-vitro protein digestibility, %	92.0±1.5 ^e	92.7±2.25 ^d
EAA index, %	81.3	83.2
Predicted biological value, %	77.0	79.0
Nutritional index	14.2	15.4
C-PER	2.1±0.02	2.5±0.03
PER (Rat bio assay)	2.1±0.05 ^e	2.3±0.07 ^a
Chemical score	60	52
Limiting amino acid	SS Amino acids	SS Amino acids
PDCAAS		
2–5 years	0.7	0.9
10–12 years	1.0	1.0
Adults	1.0	1.0
Available lysine, g/100 g protein	2.8±0.11 ^e	2.6±0.04 ^d

Values are mean ± standard deviation of three determinations. Means followed by different superscripts in a row differ significantly at $p<0.05$

Table 5 Total and bioaccessible iron content in supplementary food formulations

Parameters	Supplementary food-I	Supplementary food-II
Total iron,mg%	13.9±0.12 ^e	13.0±0.06 ^b
Bioaccessible iron, mg%	3.3±0.13 ^e	3.1±0.04 ^d
Percent Bioaccessible	23.4±0.21 ^e	23.7±0.22 ^a

Values are mean ± standard deviation of three determinations. Means followed by different superscripts in a row differ significantly at $p<0.05$

Descriptors and panel were developed during initial session by the panelists. Each member was asked to describe the samples with as many spontaneous descriptive terms as they found applicable. The common descriptors such as colour, flavour and taste chosen by at least one third of the panel were compiled along with some impact descriptors for preparing scorecard. The panel consisted of 12 judges who regularly participated in sensory analysis studies and had experience in profiling of food products. The sample was served in petridishes coded with three digit numbers. Evaluation was carried out in booth rooms built in accordance with the ASTM standards (ASTM 1996).

QDA method of intensity scaling was used. The scorecard consisted of 15 cm scale where in 1.25 cm was anchored as “low” and 13.75 cm as “high”. The panel was asked to mark the intensity of the attribute by drawing a vertical line on the scale and writing the code, but the overall quality (OQ) was evaluated on an intensity scale which was anchored at very poor, fair and very good to see

the liking or preference of supplementary food by panel members. The mean score of individual attributes were calculated and profilogram was drawn.

Storage studies About 250 g samples were packed in 300 gauge LDPE pouches and exposed to 90% RH and 38 ± 1 °C. The product samples were withdrawn periodically every month and subjected for sensory analysis and moisture estimation.

Statistical analysis All values are expressed as mean ± SD, *t*-test was performed using one-way ANOVA to obtain the significance between supplementary foods I and II.

Results and discussion

The chemical composition of supplementary food formulations I and II is presented in Table 2. The protein content of the two formulations ranged from 20.2 to 20.6 g%, and 373–390 kcal of energy. Baskaran et al. (1999) have reported that the protein content of 10.5–12.5% and 340 to 398 kcal energy for the supplementary food prepared from popped cereals. The fat content of the supplementary food I was low (0.6%) compared to formula II (4.1 g%), the increase in the fat content was due to the fortification of red palm oil. Red palm oil is rich source of provitamin A and antioxidant nutrient β -Carotene, tocotrienols, and tocopherols, which have the capacity to retard per-oxidation and scavenge free radicals (Rukmini 1994). The β -carotene content in the supplementary food II was higher (2,300 $\mu\text{g}\%$)

Table 6 Functional properties of supplementary food formulations

Properties	Supplementary food-I	Supplementary food-II
Bulk density, g/ml	0.8±0.01 ^e	0.7±0.02 ^d
Water holding capacity, ml/g		
At 25±1 °C	2.8±0.015 ^e	2.7±0.01 ^d
At 60±1 °C	3.3±0.035 ^e	3.1±0.02 ^c
Viscosity,cp		
At 25±1 °C	265.0±10.0 ^e	245.0±5.0 ^c
At 60±1 °C	530.0±20.0 ^e	440.0±20.0 ^b
Pat Spread		
At 25±1 °C	5.8±0.25 ^e	6.4±0.15 ^d
At 60±1 °C	5.3±0.1 ^e	6.0±0.05 ^b
Water required for the unit spread (g/g) of product		
At 25±1 °C	5	5
At 60±1 °C	5	5
Colour measurements		
<i>L</i> value (Lightness)	75.7±0.01 ^e	74.3±0.01 ^a
<i>a</i> value (Greenness)	1.1±0.01 ^e	0.5±0.01 ^c
<i>b</i> value (Redness)	15.9±0.01 ^e	24.1±0.01 ^a
ΔE value (Total colour)	21.6±0.005 ^e	28.7±0.01 ^a

Values are mean ± standard deviation of three determinations. Means followed by different superscripts in a row differ significantly at $p<0.05$

than in formula I (200 µg%); fortification of red palm oil increases the β -carotene content in the formulation which meets the total daily requirement. Singh et al. (2005) have reported that incorporation of dried and powdered cauliflower leaves into various formulations can help to meet the RDA of β -carotene. The Phytic acid contents of the two formulations was 0.8 g and 0.6 g%, respectively. The difference was due to the ingredients used in the formulation. The fibre content for both the formulations was 2% and these values are comparable with those reported for cereal based weaning and supplementary food formulations (Malleshi and Desikachar 1982; Gopalan et al. 2007). The nutrient composition of both the supplementary food formulations provided one-third of the RDA as recommended by ICMR (Indian Council of Medical Research). The mineral content of formula I was higher than formula II. The concentration of wheat was higher in formula I than in II, and the sesame flour was replaced by green gram dhal flour in formula I which contributed more iron, calcium and zinc to the formula. (Table 2) (Gopalan et al. 2007).

The amino acid composition of the supplementary food is presented in Table 3. The lysine content in the supplementary food II was higher than in supplementary food I, due to the higher concentration of whey protein concentrate in the formula. All other essential amino acids contained in food formulations meet the requirement of FAO/WHO reference pattern. The amino acid composition of the two formulations were comparable to that of FAO/WHO reference protein (FAO/WHO/UNU 1985).

The in vitro protein digestibility did not show any significant difference between two formulations. However, the digestibility of the supplementary food II was slightly higher. The chemical score based on essential amino acid composition was higher for formula I (60) than for formula II (52). The nutritional indices such as EAAI, which predicted biological value and nutritional index were slightly higher in formula II than in formula I. PER determined by rat bio-assay method was higher for formula II (2.3) than for formula I (2.1) clearly indicating better quality protein (Table 4).

The PDCAAS value of the supplementary food I was 0.7, 1.0 and 1.0 for the age group of 2–5, 10–12 years and adults, respectively. Similarly, the values were 0.9, 1.0, and 1.0 for supplementary food II (Table 4). The results clearly indicate that the quality of protein in formula II is better than formula I. The FAO/WHO (1994) have recommended that supplementary food for children should have PDCAAS value of $\geq 70\%$. This clearly shows that both the formulations adequately meet the nutritional needs of preschool children (2–5 yrs), school children (10–12 yrs), and adults (FAO/WHO 1991; FAO/WHO/UNU 1985).

Available lysine content of formula I was higher (2.8 g%) than formula II (2.6 g%). Bioaccessibility of iron determined in the formulations is given in Table 5. The accessibility of

iron was 23.4 and 23.7% in supplementary food I and II respectively, and the values are not significantly different ($p \geq 0.05$). Vijayalakshmi et al. (2008) have reported that supplementation of ascorbic acid and β -carotene from fruits into the mung bean preparations has increased the bioaccessibility by 12–13%. Pawar and Machewad (2006) have reported that reduction in antinutrients such as phytate and polyphenols improve the bioaccessibility of minerals. The bulk density of food formulations I and II were 0.8 and 0.7, respectively and they did not show significant difference ($p \geq 0.05$). The water holding capacity of the supplementary foods was determined at 25 ± 1 °C and 60 ± 1 °C (Table 6). There was no difference in water holding capacity between the two formulations. However, the water holding capacity was higher at 60 ± 1 °C than at room temperature because higher capacity of the starch molecules in this sample to absorb water and gelatinize better at higher temperature unlike at 25 ± 1 °C which had already undergone considerable cooking (Jowi et al. 2002). Pat spread was determined at 25 ± 1 °C and 60 ± 1 °C. The pat spread was higher at 25 ± 1 °C and the water required for the unit spread (g/g) of the product was 5 g/g at both the temperatures. The colour measurement of the supplementary food showed higher value for formula II. This could be due to the addition of palm oil as a source of β -carotene in the formulation, which contributed to the redness (Table 6).

Sensory analysis done on formula I and formula II is shown in the Fig. 1. The colour of both the formulations were slightly greenish and similar, as it can be seen from the graph. The pulsey aroma was more in formula I (6.9) than the formula II (6.5). Green gram aroma was predominant in formula I and green leafy aroma in formula II. The

Fig. 1 Sensory profile of supplementary food formulations

sweetness, texture and overall quality of the formulations did not differ significantly. However, the overall quality of the formula II was better than formula I.

Storage studies showed that the supplementary food formulations have about 4 months shelf-life under accelerated storage condition of 90% RH and 38 ± 1 °C. This implies a shelf-life of about 1 year under normal storage condition of 65% RH and 27 ± 1 °C in the same package.

Conclusion

The food formulations prepared are suitable to use as supplementary food for children above 6 months, as they provide all the required macro and micronutrients as recommended for the age group.

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