

Development and Nutritional Evaluation of Instant Complementary Food Formulated from Vitamin A, Iron and Zinc Rich Crops

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ABSTRACT

The first 2 years of life are a critical period of rapid growth and brain development. During this period, nutrition and environmental factors play important roles in the growth and cognitive development of a child. In Ethiopia, poor feeding practices and shortfalls in food intake are the most important direct factors responsible for malnutrition and illness amongst children. The national prevalence for timely initiation of complementary feeding is 62.5%. The most common forms of malnutrition are Protein-Energy Malnutrition (PEM), vitamin A deficiency, iodine deficiency disorders, and iron deficiency anaemia. Therefore, in this study composite Complementary Flour (CF) was developed by incorporating carrot and pumpkin to enrich the CF with vitamin A. Other than Cereals, Iron and Zn rich beans were also used to address Fe and Zn deficiencies. The analysis of proximate, minerals, beta-carotene, phytate, bioavailability, and sensory were studied for formulated five complementary flour treatments. Ranking to select the optimum CF formulation was made based on their protein content, energy value, Zn, and beta-carotene amount. The ranking result showed $CF_4=30\%$ Wheat+20% Maize+25% Soybean+15% GLP-II+5% Pumpkin+5% Carrot was found to possess the most desirable nutritional value among the five formulated five complementary foods.

Keywords: Malnutrition; Micronutrients; Macronutrient; Bioavailability; Complementary food

INTRODUCTION

Ethiopia is one of the countries where infant and less than fivemortality rate is the highest, where 48 per 1000 live birth and 55 per 1000 live birth respectively [1]. About 50% of child death is related to malnutrition, which can be preventable through appropriate complementary feeding practice including Breast Feeding (BF). Breast Milk (BM) and complementary feeding could prevent 13% and 6% of under 5 child mortality respectively [2]. National prevalence for timely initiation of complementary feeding in Ethiopia is 62.5%.

The growth rate falters in developing countries children immediately after the introduction of weaning foods, which are usually of poor nutritional value [3-5]. The World Health Organisation recommends nutritionally adequate and safe complementary feeding starting from the age of 6 months with continued breastfeeding up to 24 months of age or beyond [6]. the prevalence of stunting, wasting, and underweight is 37%, 7%, and 21%, respectively [1]. The prevalence of anaemia adjusted for altitude among preschool children and school age was 34.4% and 25.6%. National prevalence of Iron deficiency among preschool age children and school age children, as measured by Soluble Transferrin Receptor (STFR), was estimated 29.6% and 19.5% respectively. The national prevalence of zinc deficiency was 35% in the preschool age children, 36% in school age children. The prevalence of subclinical vitamin A deficiency was 14%, and 10.9% in the preschool age children and school age children [7].

Traditional infant foods made of cereals and legumes may be low in several nutrients including protein, vitamin A, zinc, and iron. Despite children's high requirements for nutrients, their diets in developing countries are mostly comprised of cereals or starchy root crops which, when eaten exclusively, resulting in deficiencies of key nutrients such as iron, zinc, calcium, riboflavin, vitamin A, and vitamin C.

According to Mini Ethiopian Demographic and Health Survey,

Therefore, addressing micronutrient deficiency in children through

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complementary food is necessary. This study was conducted with the objective of developing protein rich complementary food with adequate amount of micronutrients such as iron, zinc, vitamin A and calcium using vitamin A rich vegetables and Bio-fortified Fe and Zn rich beans.

MATERIALS AND METHODS

Selection of ingredients

Wheat, GLP-II Bean, and soybean grains were obtained from the Ethiopian Institute of Agricultural Research centres. Maize, carrot and pumpkin were purchased from local market in Addis Ababa, Ethiopia. These ingredients were selected based on their nutritional benefits of protein, vitamin A, iron and Zn contents. Five complementary flours were formulated with the assumption that the formulations will provides nutritional requirements of complementary food for children under the age of two years old, who are breast feeding.

Preparation of the ingredients

Wheat flour preparation: Wheat grain was sorted, washed and soaked in potable water at room temperature for 24 hr. Then it was oven dried at 60°C until constant weight [8]. It was lightly roasted, milled, sieved, and packed in polystyrene bag.

Maize flour preparation: Maize grain was sorted, cleaned, thoroughly washed, and soaked overnight in potable water at room temperature. Then it was germinated at 30°C in incubator for 48 hrs. The germinated seeds were oven-dried at 60°C for 24 hrs. To facilitate removal of the hulls, the grains were rubbed by hand and roasted lightly to improve the taste, odor and to decrease cooking time [9]. Then it was milled, sieved and packed in polystyrene bag.

GLP-II bean flour preparation: The bean grain was sorted, washed, soaked overnight in potable water at room temperature, and then drained. The drained beans were cooked for about 30 minutes in order to reduce anti-nutritional factors. Beans were cooled and the skin removed using hands. The de-skinned beans were then oven dried and roasted for about 5 min to improve its flavour and milled into flour, sieved and packed in polystyrene bag [10].

Soybean flour preparation: Soybean grains were cleaned, soaked in potable water 1:5 w/v for 15 hrs [11]. The beans were hulled by rubbing in-between palms, floating, and decanting the hulled seed coat. It was then oven-dried at 60°C for 24 hours and roasted under an open flame until golden brown for 30 minutes and milled into flour, sieved and packed in polystyrene bag [12].

Pumpkin and Carrot flour preparation: Carrot and pumpkin were peeled, sliced, freeze dried and milled to get pumpkin and carrot powder separately [12,13]. Then it was sieved and packed in polystyrene bag.

Formulation of complementary flour treatments

The complementary flours were formulated based on the FAO/ WHO 1991 guidelines and recommendation of complementary food preparation for average breast fed milk children 6 to 24 months of age [14]. The blends were prepared or mixed from the individual flour ingredients in the percentages or proportions as shown in Table 1.

Nutritional composition analysis

Proximate composition: Proximate composition of samples was determined using the method described by Association of Official

Analytical Chemists [15]. Total ash was determined by incinerating 2 g of sample in furnace at 550°C. Crude fat was determined by solvent extraction using Soxtec extraction system (Soxtec 8000, FOSS). Nitrogen content of samples was determined according to Kjeldahl using block digestion and steam distillation with Tecator Kjeltec Systems (Kjeltec 8400, FOSS). Crude protein, then estimated by multiplying N content with 6.25. Crude fiber was determined after digesting of defatted sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide (AOAC, 2005). Total carbohydrate was determined by subtracting the sum of protein, fat, ash and moisture contents from 100 [16]. The gross energy value, was estimated (in kcal/g) by multiplying the percentages of crude protein, crude fat and carbohydrate using the Atwater's conversion factors; 16.7 kJ/g (4 kcal/g) for protein, 37.4 kJ/g (9 kcal/g) for fat and 16.7 kJ/g (4 kcal/g) for carbohydrates [17].

Determination of Mineral and Phytate: Mineral content was determined using graphite furnace atomic absorption instrument according to AOAC [15]. Phytate was determined using spectrophotometer at 500 nm according to Latta and Eskin method [18].

Bioavailability: Mineral bioavailability was estimated using molar ration of phytate to mineral. The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with its atomic weight (phytate: 660 g/mol; Fe: 56 g/mol; Zn: 65 g/ mol; Ca: 40 g/mol). The molar ratio between phytate and mineral was obtained after dividing the mole of phytate with the mole of minerals [19].

Determination of Beta Carotene: Beta carotene content of the samples was determined using High Performance Liquid Chromatography (1220 infinity, Agilent). The carotenoids were separated on Agilent SB-C8 (4.6 × 150mm, 5 μ m) column operated at a flow rate of 0.5 ml/min with mixture of acetonitrile: methanol: chloroform (47:47:6) mobile phase and detected at 450 nm in UV detector. The beta carotene content was calculated using the formula:

Beta Carotene,
$$(mg / kg) = \frac{(C - B) \times V \times DF \times mcf}{m}$$

Where:

C=Instrument reading of the sample (mg/L)

B=Instrument reading of the blank (mg/L)

V=extract volume (mL)

DF=Dilution factor (if applicable)

mcf=moisture correction factor (to convert in to dry basis)

Sensory Evaluation: The formulated complementary flours were prepared into thick gruels and subjected to sensory evaluation to test their acceptability using a five point hedonic scale, where

 Table 1: Formulated complementary food treatments.

Complementary Food							
Treatment	Wheat (%)	Maize (%)	Soybean (%)	GLP-II bean (%)	Pumpkin (%)	Carrot (%)	
CFBT ₁	30	20	20	20	10	0	
CFBT ₂	30	20	20	20	0	10	
CFBT ₃	30	20	10	30	5	5	
CFBT ₄	30	20	25	15	5	5	
CFBT ₅	30	20	20	20	5	5	
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*CFBT: Complementary Flour Blend Treatment

1=dislike extremely, 2=dislike moderately, 3=neither like nor dislike, 4=like moderately and 5=like extremely. A total of 19 semitrained mothers panellists were used in this study from Ethiopian Institute of Agricultural Research staffs.

Thick gruels were made from the formulated composite flours, using 250 g of water for 100 g of sample. The boiled thick gruels were allowed to cool to approximately 45°C, before given to the panellists. During orientation, the panellists were familiarized with method, scorecard and the product being used in the study. Bottled water was provided for the panellists to rinse their mouth before and after evaluating each treatment sample.

Statistical Analysis: The data generated were statistically analysed for means and standard deviation and Analysis of Variance (ANOVA) was used to test the level of significance. Data were analysed by analysis of variance using general Linear Model procedure of SAS software. Duncan's New multiple Range Test was used to compare and separate means. Significance was accepted at p<0.05.

RESULTS AND DISCUSSIONS

Nutritional composition of complementary flour

Table 2 presents the nutritional compositions (g/100g dry weight) of formulated complementary flour treatments.

The moisture content of formulated CF is significantly different among samples at (p<0.05) and ranges from (6.7-7.3) g/100g. Moisture content of the composite flour was slightly higher than recommended values of <5% (FAO/WHO, 1991) but all the samples were in the acceptable limit of 10% moisture [14]. Flours with lower content of moisture (<10%) are suitable for extended shelf life. Higher moisture content can be due to different processing methods used such as soaking and germination. Low moisture contents of formulations are also convenient for the packaging and transport of products [20].

The protein content of all formulated complementary foods has significant differences at (p<0.05) and was found in the range of (17.69-20.36) g/100g. According to the guidelines of the Protein Advisory Group (PAG, 2007), weaning foods should have a protein content of at least 20% [21]. It is also recommended that the protein content should range from (14.52 to 37.70) g/100 g for maximum complementation of amino acids in foods and growth [22]. Higher content of protein may be the result of higher content of soybean, GLP-II bean and germinated maize. Dooshima *et al*, and Mohammed *et al*, reported a similar result of protein content in the development of complementary food [23, 24]. A study on quality evaluation of weaning foods formulated from some local

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cereals and legume blends also reported similar results of protein [8]. CF with high content of protein can be useful for children already suffering from protein energy malnutrition.

The fat content of all formulated complementary foods has significant differences at (p<0.05) and was found in the range of (4.75%-8.3) g/100g. The fat content of a food sample can affect its shelf stability; a low amount of fat is required for longer shelf life. Complementary food needs to ensure a minimum of 17% of energy as a lipid for infants who receive a medium amount of human milk [25]. In this study, the energy from fat ranges from (11.59-19.372)% which is in the recommended range. Higher fat content observed in treatment CFBT₄ and it may be due to a higher proportion of soybean than the other treatments. Fat is important in human diets as it provides essential fatty acids and facilitates the absorption of fat-soluble vitamins [26].

The fibre content of treatments shows no significant differences at (p<0.05). It ranges from (4.12-4.82) g/100g. FAO/WHO (1991) recommends the fibre content for complementary food not more than 5% [14]. Similar results of fibre (3.5-4.25) g/100g were reported by Desalegn *et al*, in formulating complementary food [27]. A higher amount of fibre can reduce the energy density of complementary foods which is not desirable. It also may affect the efficiency of the absorption of important nutrients from diets with marginal nutrient contents WHO [28].

The energy values in all complementary flour treatments didn't show significant differences (p<0.05). It ranges from (369-385) kcal/100g of complementary food which is slightly lower than the recommended value of (400-425) kcal/100 g [14]. Lower energy values may be due to relatively lower fat contents of the formulation, as fat gives more than twice energy as compared with carbohydrate and protein.

The ash content of all formulated complementary foods has significant differences (p<0.05) and was found in the range of (2.38-2.66) g/100g. WHO recommends not more than 5% ash content for complementary foods and all the treatments are in the range of the recommendation. Mohammed *et al.*, reported similar results in the development of nutritionally enriched instant weaning food [24].

The carbohydrate content of all formulated complementary foods has significant differences at (p<0.05). The carbohydrate content was found in the range of (57.3-63.8) g/100g, the highest being treatment CFBT₃ and the lowest treatment CFBT₄. The carbohydrate levels of formulated CF were in the recommended range (41.13 to 73.79) g/100 g of the Codex Alimentarius Standards [14]. Bernard Tiencheu et al, also reported similar

Treatment	Moisture	Fat	Protein	Ash	Fiber	Carbohydrate	Energy (Kcal/100g)
CFBT ₁	7.04 ^{ba}	6.76 ^b	20.33ª	2.66ª	4.82ª	58.39 ^d	375.73 ^b
CFBT,	6.79 ^b	5.89 ^b	18.36 ^c	2.51 ^{bc}	4. 27 ^a	62.19 ^b	375.18 ^b
CFBT,	6.94 ^{ba}	4.75°	17.70°	2.61 ^{ba}	4. 12 ^a	63.89ª	369.113 ^b
CFBT₄	7.11 ^{ba}	8.30ª	20.37ª	2.38°	4.49ª	57.36°	385.61ª
CFBT ₅	7.32ª	6.32 ^b	19.52 ^b	2.51 ^{bc}	4.22ª	60.11°	375.41 ^b
Mean	7.04	6.4	19.25	2.53	4.38	60.39	376.21
CV	2.8	9.63	2.18	2.95	10.09	0.84	1.02

 Table 2: Proximate composition of formulated composite flour (g/100g dry weight).

CFBT1:30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Pumpkin; CFBT2:30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Carrot; CFBT3:30% Wheat+20% Maize+10% Soybeans+30% GLP-II+5% Pumpkin+5% Carrot; CFBT4:30% Wheat+20% Maize+25% Soybean+15% GLP-II+5% Pumpkin+5% Carrot; CFBT5:30% Wheat+20% Maize+20% Soybean+20% GLP-II+5% Pumpkin+5% Carrot; Means with the same letters (a-d) in a column are not significantly different (p > 0.05); CV Coefficient of Variance

results in formulated instant weaning food [29]. Lower results in carbohydrate content might be due to the higher content of protein and fat in the treatments.

Phytate and mineral content of complementary flours

Phytate, iron, zinc and calcium content of the complementary flour (mg/100g) were summarised in Table 3. There was significant differences (p<0.05) between all the mineral and phytate contents of formulated complementary flour.

The amount of iron in this study ranges from (4.36-4.92) mg/100g and they were significantly different at (p<0.05). According to the FAO/WHO recommendation, the suggested total quantity of minerals in formulated complementary food should be at least 50% of Reference Nutrient Intake (RNI) [14]. In this study, all the formulated complementary foods provide about 50% of iron RNI at medium bioavailability. Ijarotimi and Keshinro observed similar results of iron, which ranged from (3.79-4.25) mg/100g [30].

The amount of zinc in all treatments has significant differences (p<0.05) and were found in the range of (2.51-2.81) mg/100g. In this study, all the formulated complementary foods provide about 50% of zinc RNI at medium bioavailability. Desalegn *et al*, reported similar results in formulating quality protein maize based complementary food [27].

The calcium amount in all treatments has significant differences (p<0.05) and were found in the range of (77.92- 109.6) mg/100g. The recommended total quantity of calcium in a formulated complementary food should be at least 50% of RNI, which are 250. In this study, all the formulated complementary foods provide (15-21)% of calcium RNI.

The phytate content of all formulated complementary foods has significant differences at (p<0.05) and were found in the range of (112.17- 144.15) mg/100g. The highest phytate was observed in treatment CFBT₄ and the lowest in treatment CFBT₁.

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The result shows that the amount of Beta-carotene in all treatments has significant differences at (p<0.05) and was found in the range of (1.50-69.52) μ g/100g. The highest β -carotene was found in treatment CFBT₂ and the lowest in treatment CFBT₁. It was observed that the β -carotene in the treatments increased as the proportion of carrot increased in the formulations.

Bioavailability of minerals

Bioavailability of micronutrients is the main challenge in developing countries due to plant-based and monotonous eating habits. Micronutrients such as Ca, Fe and Zn are the main requirements for child growth.

As shown in the table, there was a slightly significant difference in the bioavailability of all the treatments at (p<0.05). Calcium is bioavailable in all formulated treatments while treatment CFBT₅ is being most available. There was a slight significance different at (p<0.05) for iron bioavailability. The critical value for the phytate/ Fe molar ratio is more than 1 is being considered as poorly available. Iron being the most important mineral; it is poorly available in all treatments. Zinc is available in all formulated complementary foods, which were being most available in treatment CFBT₁.

SENSORY EVALUATION

Table 5 presents the sensory acceptability of the formulated complementary flours. As shown in the table, there was no significant difference among all the treatments in all sensory parameters at five point hedonic scale. This shows that all the formulated complementary flours are acceptable to be fed to children from 6-24 months old.

Ranking and selection

A ranking system using four nutritional criteria, i.e, protein content, energy value, and Zn and Beta-carotene, were used to determine the optimal complementary food formulation according to the

Treatment	Phytate (mg/100g)	Zn (mg/100g)	Ca (mg/100g)	Fe (mg/100g)	B-Carotene Mcg µg/100g	
CFBT,	112.17 ^c	2.81ª	79.98°	4.92ª	1.50 ^d	
CFBT ₂	137.17 ^{ba}	2.72 ^b	86.23 ^b	4.67 ^b	69.52ª	
CFBT,	126.10 ^{bc}	2.51°	77.92°	4.72 ^b	29.61°	
CFBT ₄	144.15 ^a	2.81ª	109.60ª	4.36 ^d	39.48 ^b	
CFBT ₅	117.26 ^c	2.80ª	89.70 ^b	4.55°	39.53 ^b	
Mean	4.32	2.73	88.69	4.64	35.93	
CV	127.37	0.89	1.54	0.7	11.14	

 Table 3: Phytate, iron, zinc and calcium content of the composite flour (mg/100g).

CFBT₁: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Pumpkin; CFBT₂: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Carrot; CFBT₃: 30% Wheat+20% Maize+10% Soybeans+30% GLP-II+5% Pumpkin+5% Carrot; CFBT₄: 30% Wheat+20% Maize+25% Soybean+15% GLP-II+5% Pumpkin+5% Carrot; CFBT₅: 30% Wheat+20% Maize+20% Soybean+20% GLP-II+5% Pumpkin+5% Carrot; Means with the same letters (a-d) in a column are not significantly different (p>0.05); CV Coefficient of Variance

Table 4: The molar ratio between phytate and mineral of the composite flour.

Treatment	Phytate: Ca	Phytate: Fe	Phytate: Zn
CFBT	0.085 ^{bc}	1.937°	3.938 ^b
CFBT ₂	0.096 ^{ba}	2.492 ^b	4.977ª
CFBT ₃	0.098ª	2.270 ^b	4 .948 ^a
CFBT ₄	0.080°	2.806ª	5.062ª
CFBT ₅	0.079°	2.188 ^{cb}	4.126 ^b
Mean	0.088	2.338	4.61
CV	5.489	4.937	4.688
10			

*Critical values: Phytate: Ca>0.24; Phytate: Fe>1; Phytate: Zn>15

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Table 5: Sensory analysis of the complementary food (mg/100g). CFBT₁: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Pumpkin: CFBT₂: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+5% Pumpkin+5% Carrot; CFBT₄: 30% Wheat+20% Maize+20% Maize+20% Soybean+15% GLP-II+5% Pumpkin+5% Carrot; CFBT₅: 30% Wheat+20% Maize+20% Soybean+20% GLP-II+5% Pumpkin+5% Carrot; Means with the same letters (a-d) in a column are not significantly different (p>0.05); CV: Coefficient of Variance

Treatment	Appearance	Colour	Taste	Texture	Overall acceptability	
CFBT ₁	4.00 ± 0.095^{a}	4.00 ± 0.90^{a}	3.48 ± 0.99^{a}	3.87 ± 0.97^{a}	4.00 ± 1.17^{a}	
CFBT,	3.91 ± 0.95 ^a	3.61 ± 0.89^{a}	3.65 ± 0.88^{a}	$3.43 \pm 0.90^{\circ}$	3.91 ± 0.95 ^a	
CFBT ₃	3.70 ± 0.70^{a}	3.57 ±1.16 ^a	3.78 ± 0.85^{a}	3.47 ± 1.04^{a}	3.78 ± 0.90^{a}	
CFBT₄	3.81 ± 1.07 ^a	4.05 ± 0.69^{a}	3.85 ± 0.77^{a}	3.62 ± 0.91^{a}	4.24 ± 0.90^{a}	
CFBT ₅	3.78 ± 1.13 ^a	3.74 ± 1.01^{a}	3.83 ± 0.94^{a}	3.65 ± 1.15^{a}	4.09 ± 1.04^{a}	
mean	3.84	3.79	3.71	3.61	4	
CV	25.28	24.96	24.39	27.46	30.37	

CFBT₁: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Pumpkin: CFBT₂: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Carrot; CFBT₃: 30% Wheat+20% Maize+10% Soybeans+30% GLP-II+5% Pumpkin+5% Carrot; CFBT₄: 30% Wheat+20% Maize+25% Soybean+15% GLP-II+5% Pumpkin+5% Carrot; CFBT₅: 30% Wheat+20% Maize+20% Soybean+20% GLP-II+5% Pumpkin+5% Carrot; Means with the same letters (a-d) in a column are not significantly different (p>0.05); CV: Coefficient of Variance

Table 6: Ranking of formulated complementary foods.

Treatments	Protein	Energy	Zn	B -carotene	Total	
CFBT ₁	1	2	1	4	8	
CFBT,	3	2	2	1	8	
CFBT,	3	2	3	3	11	
CFBT ₄	1	1	1	2	4	
CFBT ₅	2	2	1	2	7	

CFBT₁: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Pumpkin; CFBT₂: 30% Wheat+20% Maize+20% Soybeans+20% GLP-II+10% Carrot; CFBT₃: 30% Wheat+20% Maize+10% Soybeans+30% GLP-II+5% Pumpkin+5% Carrot; CFBT₄: 30% Wheat+20% Maize+25% Soybean+15% GLP-II+5% Pumpkin+5% Carrot; CFBT₅: 30% Wheat+20% Maize+20% Maize+20% Soybean+20% GLP-II+5% Pumpkin+5% Carrot; Means with the same letter(s) in a column are not significantly different (p>0.05); CV: Coefficient of Variance.

method modified by Ijarotimi [31]. The formulation yielding the lowest score was considered to possess the most suitable nutritional characteristics. According to Table 6, CFBT₄ has the lowest rank score followed by CFBT₅. Therefore, it was observed that CBTF₄=30% Wheat+20% Maize+25% Soybean+15% GLP-II+5% Pumpkin+5% Carrot to possess the most desirable nutritional content among formulated food samples.

CONCLUSION

In conclusion, this research shows that complementary foods can be formulated form Vitamin A, iron and zinc rich crops for better bioavailability and organoleptic acceptability. Furthermore it was observed that the soaking, germination and mild roasting improved the taste and nutritional value of formulated flours and also improved the mineral bioavailability. It was concluded that a formulation of CFBT₄=30% Wheat+20% Maize+25% Soybean+15% GLP-II bean+5% Pumpkin+5% Carrot complementary food possess the most desirable nutritional value among the five formulated five complementary flour treatment.

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