# Development of Complementary Food 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 plays important role in the growth and cognitive development of a child. In Ethiopia, protein-energy malnutrition, vitamin A deficiency, iodine deficiency disorders, and iron deficiency anemia are the most common forms of malnutrition. 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. Proximate, minerals, beta-carotene, phytate, bioavailability and sensory analysis were conducted using standard procedures. Ranking to select the optimum CF formulation was made for all the treatments based on their protein content, energy value, Zn, and beta-carotene concentrations. According to the ranking result, complementary food consisting 30% wheat, 20% maize, 25% soybean, 15% GLP-II, 5% pumpkin and 5% carrot was found to possess the most desirable nutritional value among the five formulated complementary foods.*

**Keywords**: Complementary food, Macronutrients, Micronutrients and Bioavailability

## Introduction

Ethiopia is one of the countries where infant and under 5 mortality rates is the highest, 48 per 1000 live birth and 55 per 1000 live birth respectively (EDHS, 2019). About 50% of child death is related to malnutrition which can be preventable through appropriate complementary feeding practice. Breast milk and complementary feeding could prevent 13% and 6% of under 5 child mortality respectively (Kimani *et al.*, 2011). National prevalence for timely initiation of complementary feeding in Ethiopia is 62.5% (Habtewold *et al*., 2018).

The growth rate falters in children in developing countries immediately after the introduction of weaning foods which are usually of poor nutritional value (Black *et al*., 2008, Dewey *et al.*, 1992, Mosha *et al.*, 1998). Nutritionally adequate and safe complementary feeding starting from the age of 6 months with continued breastfeeding up to 24 months of age or beyond is recommended by the World Health Organisation (Dewey, 2003).

According to Mini Ethiopian Demographic and Health Survey (EDHS, 2019), the prevalence of stunting, wasting, and underweight is 37%, 7%, and 21%, respectively. The prevalence of anemia 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 and 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 (EPHI, 2016).

The magnitude of appropriate complementary feeding practice in Ethiopia is low which accounts 4.8% in 2011. There is evidence that shows appropriate complementary feeding practice reduces stunting and improves better health and growth (Black *et al*, 2013, UNICEF, 2011). A combination of nutritionally inferior diets and improper feeding practices are major contributing factors to the development of childhood malnutrition (Temesgen, 2013). 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 (Allen, 2006).

Therefore, addressing micronutrient deficiency in children through complementary food is a prior attention. This study is made with the objective of developing protein rich complementary food with adequate amount of micronutrients such as iron, zinc, vitamin A and calcium using vegetables and bio-fortified 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 Ethiopia. These ingredients were selected based on their known nutritional benefits of protein, Vitamin A, Iron and Zn contents.

## Preparation of the ingredients

Wheat flour: Wheat grain was sorted, washed and soaked in potable water at room temperature for 24 hr. Then it was oven dried at 60oC until constant weight (Ajiwe and Nwaigbo, 2014). It was lightly roasted, milled, sieved, and packed in polystyrene bag.

Maize flour: Maize grain was sorted, cleaned, thoroughly washed, and soaked overnight in potable water at room temperature. Then it was drained and germinated at 30oC in incubator for 48 hrs. The germinated seeds were oven-dried at 60oC for 24 hrs. To facilitate removal of the hulls, the grains were rubbed by hand (Marero *et al*., 1988) and roasted lightly to improve the taste, odour and to decrease cooking time. Then it was milled, sieved and packed in polystyrene bag.
GLP-II bean flour: The bean grain was sorted, washed, soaked overnight in potable water at room temperature, and then drained. The steamed 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 (Mitzner, 1984).

Soybean flour: Soybean grain without defects were cleaned, soaked in potable water 1:5 w/v for 15 hrs according to method described by Yimer (2008). 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 for 30 minutes and milled into flour, sieved and packed in polystyrene bag (Aduni *et al.*, 2016).

Pumpkin and Carrot flour: Carrot and pumpkin were peeled, sliced, freeze dried and milled to get powder separately (Aduni *et al*., 2016, Abebe *et al*., 2006). Then it was sieved and packed in polystyrene bag.

## Formulation of complementary foods

The complementary flours are formulated based on the FAO/WHO 1991 guidelines and recommendation of complementary food preparation for average breast milk consumption of children 6 to 24 months of age. The blends were prepared or mixed from the individual flour ingredients in the proportions as shown in Table1.

## Nutritional composition analysis

### Proximate composition

Proximate composition of triplicate food samples was determined using the method described by Association of Official Analytical Chemists (AOAC, 2005). Total ash was determined by incinerating 2 g of sample in furnace at 550$^{o}$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 a known weight of fat-free 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 (Pearson, 1976). 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 and expressed in calories (Guyot *et al*., 2007).

### Determination of minerals and phytate

Mineral contents were determined using graphite furnace atomic absorption instrument according to AOAC (2000). Phytate was determined using spectrophotometer at 500 nm according to Latta & Eskin (1980) method.

### Bioavailability

Mineral bioavailability was estimated using molar ratios of phytate to minerals. The mole of phytate and minerals was determined by dividing the weight of phytate and minerals with their atomic weight (phytate: 660g/mol; Fe: 56g/mol; Zn: 65g/ 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 (Norhaizan & Nor Faizadatul, 2009).

### Determination of Beta Carotene

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

$$Beta\ carotene, (\frac{mg}{kg}) = \frac{(C - B)\ x\ Vx\ DF\ x\ mcf}{m}$$

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 (1= dislike very much and 5 = like very much). A total of 19 semi trained mother panellists from Ethiopian Institute of Agricultural Research staffs were used. Thick gruels were made from the formulated composite flours, using 250g of water for 100g of sample. The boiled thick gruels were allowed to cool to approximately 45$^{o}$C before given to the panellists. During orientation, the panellists were familiarized with method, score card and the products to be evaluated. Bottled water was provided for the panellists to rinse their mouth before and after evaluating each sample.

### Statistical analysis

The data generated were statistically analysed for means, and analysis of variance (ANOVA) was used to test the level of significance. Data were analysed by analysis of variance (ANOVA) using general Linear Model procedure of SAS software. Duncan's new multiple range tests was used to compare and separate means. Significance was accepted at $p<0.05$.

# Results and Discussion

## Nutritional composition of composite flours

Table 2 presents the nutritional compositions of formulated complementary flours. The moisture content of formulated CF is significantly different among samples at $P<0.05$ and ranged from 6.7- 7.3 g/100g. Moisture content of the composite flour was slightly higher than recommended values of FAO/WHO (1991) which is < 5%, but all the composites were less than 10%. Flours with moisture contents of <10% are suitable for extended shelf life. Low moisture contents of formulations are also convenient for the packaging and transport of products (Oduro *et al*., 2007).

According to the guidelines of the Protein Advisory Group (PAG), weaning foods should have a protein content of at least 20% (PAG, 2007). It is also recommended that the protein content should range from 14.52 to 37.70 g/100g for maximum complementation of amino acids (FAO/WHO, 1994). The protein content of the formulated composite flours in this study has significant differences ($p<0.05$) and was found in the range of 17.69 - 20.36 g/100g. Higher content of protein might be due to soybean, GLP-II bean and germinated maize. Dooshima *et al*. (2015) and Mohammed *et al.* (2013) reported a similar result of protein content in the development of complementary food.

The fat content of all formulated complementary foods has significant differences ($p<0.05$) 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 in which 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 breast milk (Dewey and Brown, 2003). In this study the energy from fat ranges from 11.59-19.372% which is in the recommended range. Fat is important in human diets as it provides essential fatty acids and facilitates the absorption of fat soluble vitamins (Michaelsen *et al*., 2000).

The fiber content of treatments showed no significant differences at $p<0.05$. It ranged from 4.12-4.82 g/100g, which is within the recommended value of FAO/WHO (1991) for complementary food (not more than 5%). Similar results of fiber (3.5-4.25 g/100g) were reported by Desalegn *et al*. (2015) in formulated complementary food. A higher amount of fiber is not desirable as it reduces the energy density of complementary foods. It also may affect the efficiency of the absorption of important nutrients from diets with marginal nutrient contents (WHO, 1985).

The energy values in all complementary flour treatments didn't show significant differences ($p<0.05$). It was ranged from 369-385kcal/100g which was slightly lower than the recommended value of 400-425 kcal/100 g (FAO/WHO, 1991). 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. There was significant differences in carbohydrate contents of the composite flours ($p<0.05$). The carbohydrate contents were between 57.3 and 63.8g/100g, and within the recommended range (41.13 to 73.79 g/100 g) of the Codex Alimentarius Standards (FAO/WHO, 1994).

## Phytate and mineral contents

Phytate, iron, zinc and calcium contents of the composite flours is summarised in Table 3. There was significant differences ($p<0.05$) between all the mineral and phytate contents of formulated complementary flours. The amount of iron in this study was in the range of 4.36 to 4.92 mg/100g and they were significantly different ($p<0.05$). According to the FAO/WHO (1991) recommendation, complementary food should be at least 50% of reference nutrient intake (RNI). In this study, all the formulated complementary foods provide about 50% of iron RNI at medium bioavailability. Ijarotimi & Keshinro (2013) observed similar results of iron, which ranged from 3.79-4.25 mg/100g. The amount of Zinc in all treatments has significant differences ($p<0.05$) and were found in the range of 2.51-2.81mg/100g. In this study, all the formulated complementary foods provide about 50% of Zinc RNI at medium bioavailability. 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 is 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 result showed that the amount of Beta-carotene in all treatments has significant differences and was found in the range of 1.50 - 69.52µg/100g. It is shown that the β- carotene in the treatments increased as the proportion of carrot increased.

## Bioavailability of minerals

Bioavailability of micronutrients is the main concern 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 table 4, there is a slight differences in the bioavailability of all the treatments ($p<0.05$). The critical value for the phytate/Fe molar ratio more than 1 is being considered as poorly available. Zinc is available in all formulated complementary foods.

## Sensory evaluation

Table 5 presents the sensory acceptability of the formulated complementary flours. As it is shown in the table, there is no significant difference among all the treatments in all sensory parameters at five point hedonic scale. However, all the results are within acceptable limits. 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 method modified by Ijarotimi (2012). The formulation yielding the lowest score was considered to possess the most suitable nutritional characteristics. According to table 6, $CFB_4$ has the lowest rank score followed by $CFB_5$. Therefore, it was observed that $CFB_4$=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 and Recommendation

This research shows that complementary foods can be formulated from cereals, legumes, and vegetables for better nutritional benefits and organoleptic acceptability. In the present work, it was concluded that incorporating vegetables and beans increased both macronutrients and micronutrients. It also observed that the soaking, germination and mild roasting improved the taste and nutritional value of formulated foods by increasing bioavailability. Adding different types of fresh fruits and vegetables while preparing complementary food or after preparation can also increase the nutrition and bioavailability of micronutrients. The nutrition and bioavailability of the formulated complementary foods might be improved by adding enrichments like milk, egg, or fruits while preparing the diet.

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