

# Physicochemical composition and antioxidant activity of Maize (*Zea mays*) based complementary food formulation using mixture design

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**Abstract**— Childhood malnutrition is a public health problem in developing countries. An attempt was made to produce maize based complementary flour from maize, soybean, germinated maize, carrot, egg shell flours, groundnut paste and table sugar using mixture design. Four independent variables were used to construct extreme vertices designs. Twelve formulations were generated using this design. Water, total digestible carbohydrates, crude proteins, lipids, sugars, ash, zinc, iron, fibers, total phenols, carotenoids, alpha tocopherol and antioxidant activity were the response variables. Mixture regression was used to fit the data in linear models. From the experimental models, the desirability test was carried out to define the best mixture in formulating balanced flour. Analyses of models indicate that they are adequate and useful predictors of the desired response. The best desirable formulation was 50.00 % of maize flour, 17.10% of soybean flour, 6% of groundnut paste and 11.90% of germinated maize flour. The maize based flour can satisfies the recommended energy and macronutrients requirement according to the codex Alimentarius commission standards. Maize based complementary flour can therefore be used in managing protein energy malnutrition. Moreover, the flour offers several healths beneficial due to the presence of many bioactive compounds. Taking micronutrients into consideration, the maize based formulation is most suitable for infants between ages of 12-23 months. In all cases, the weaning flours need to be fortified with more micronutrients or supplemented with synthetic nutrients to meet the micronutrients codex standard and this represent a challenge for future study.

**Index Terms**— Childhood malnutrition, complementary flour, formulation, balanced flour.

## 1 Introduction

Childhood malnutrition is a public health problem in developing countries. It usually occurs during the weaning period when breast milk alone is no longer sufficient to provide energy and nutrients for a growing child. Additional foods must then be added to the diet to supplement the breast milk for a satisfactory growth and development of the child [1],[2]. Traditionally, weaning foods are bulky porridge made from cereal flours. Such foods are high in carbohydrates and low in energy [1],[3]. In order to improve the nutritional and energy value of these foods, FAO / WHO recommends the addition of legumes as source of protein and/or lipids to cereals to make a balanced diet [4]. In avoiding micronutrient deficiencies, local fruits, vegetables and animal source foods can provide macro- and micronutrient-rich options for complementary feeding [4]. Addition of germinated cereal flours to cereal based flours is also recommended to reduce the viscosity of the porridge increasing by this the energy density. However, the major disadvantage of cereal based foods remains the presence of antinutrients such as phytates, tannins, and other secondary plant metabolites. These substances limit the bioavailability of nutrients, including iron, calcium, zinc, and in some cases proteins which are crucial to the development of infants [5]. Pretreatment like dehulling, roasting or toasting to reduce antinutrients has been recommend by the Codex alimentarius [4]. However, the efficiency of flour as weaning food depends on quality attributes such as taste, nutritional and hygienic value and the success in making a balanced diet requires taking into account parameters such as energy, carbohydrate, protein, lipid, vitamin and mineral content [6],[7]. Many legumes and oil seeds are high both in protein and lysine. Soybeans in particular have received much attention in recent years [8]. Wean-

ing flours formulations with high nutritional values are reported in the literature. To ensure the highest nutritional quality and good organoleptic properties, soybeans and cereal or tuber are often blend in various ratios and tested for the optimal nutritional quality [2],[3],[9]. This procedure is money and time consuming and many researchers have developed a computer tool in formulating balanced diet by taking into account availability, price etc [5],[6],[7],[10]. The unit operation usually consists of a mixture of ingredients in adequate percent in order to meet energy and nutrients requirement for the growth of the young child in respect of the WHO/FAO specifications for complementary flour. If it is clear that infant flours can be made from blending, the proportion of each ingredient in the mixture remains the key factor in meeting the nutritional goal. Mixture design method, a special type of Response Surface Methodology (RSM) has been proved to be effective in determining optimal proportion of ingredients to be blend in order to have a balanced diet [11]. Beside the nutritional value, the weaning flour can provide healths beneficial and these properties are often related to the presence of antioxidant compounds like phenols. A good correlation was observed between antioxidant activity of flour components and total phenol [12]. The objective of this study was to formulate complementary flour composed of maize (*Zea mays*), soybeans, groundnut and germinated maize with adequate nutrient density and high mineral bioavailability to meet the recommended macro and micronutrients specification of the Codex Alimentarius standard and for easy replication at the household level. Carrot flour and egg shell were added as source of vitamin and micronutrient respectively. Specifically, it aims at determining the optimal blend formulas and comparing nutri-

tional value of the maize based formulation regarding to their macro and micronutrients with the standard baby food proposed by Sanogo et al [7] and the codex alimentarius standard. Total phenol content and antioxidant activity of the formulated flours was also investigated.

## Material and methods

### Samples choice

A preliminary survey was carrying out in Douala city-Cameroon to identify the most common ingredients use in complementary food preparation. From this study, maize, soy, groundnut, carrot, egg shells were the common ingredients use in weaning food production.

### Sample collection

Maize (*Zea mays*), soy (*Glycine max*), groundnut (*Arachis hypogaea*), egg (*Gallus gallus domesticus*), carrot (*Daucus carota subsp*) were purchased from local market in Douala city. All the samples were kept at room temperature before processing.

### Processing technologies

Preliminary treatment of the raw materials

#### Cleaning and washing

Samples were manually sorted to remove husks, stone, damaged and colored foreign grains. Noxious seeds, insects and any foreign material were also removed. The eggs were released from their contents and the shells were cleaned with tap water. They were then boiled for 20 minutes in hot water before drying.

#### Dehulling

Maize and soy were completely dehulled to reduce the fiber and antinutrients content to acceptable levels. They were then roasted over low heat during 20 minutes. Sample were subsequently crushed and varnished to remove the fibrous film and other impurities. They were stored in plastic bags before grinding.

#### Toasting

This operation was carried out in a steel pot at 150 °C for 15 minutes. Maize and groundnuts were toasted to reduce antinutrient factors, microorganism, bulkiness of the formulated food and moreover to destroy insects and by this improving the quality.

#### Scraping

Carrots were scrap to remove the outer cortex, sized and cut into 0.5 cm thick slices before drying.

#### Germination of maize

Maize sample was divided in two groups. One group was use to produce germinated maize as sources of alpha amylase to reduce the bulk of the food when prepared for feeding and ultimately increase the nutrient density of the food. The other one was use to produce maize flour after washing several time with water before drying. Germination was carried out according to the technique described by Ariahu et al. [13]. Corn seeds were sorted and washed in 5% sodium chloride solution to suppress mould growth. The seeds are then soaked in tap water (in ratio of 1:3 (w/v)) for 12 hours at room temperature and then spread on jute bags. They were then covered with damp cotton and allow germinating at room temperature for 4 days. Water was spraying with an interval of 12 hours to facili-

tate the germination process [14]. At the end of germination, root hairs were removed from the germinated seeds.

#### Drying

Each sample (maize, germinated maize, carrot, egg shell) were placed in simple and aerated layers on pre-weighed drying trays, and dried at 45 ± 5°C in a cross flow cabinet dryer (Binder, FDL 115), with an air flow rate of 24 m<sup>3</sup>/h. Drying trays were periodically weighed all along the drying process. The seeds were dried to a moisture content of 10%. For each sample, drying experiment was conducted in triplicate and from the three values of tray weight, average of sample moisture was determined as a function of time.

#### Milling

All dry samples (maize, germinated maize, groundnut, soy, yam, carrot, egg shells) were ground into fine flour using a hammer mill (Cullati) (maize, germinated maize and soy) or a robot blender (Moulinex®) (groundnut, carrot, egg shell). Dried yam slices were grounded in a milling machine (Retsch ZM 200) equipped with a 1 mm sieve. Flour was then sieved through a sieve of 500µm, packaged in an air tight polyethylene bags and stored at -18 °C until analyzed

#### Processing of weaning flour using mixture design

##### Experimental design

##### Component mixture design

The composition of maize based complementary was as follow: maize, soybean, germinated maize, carrot, egg shell flours, groundnut paste and table sugar. Four independent variables were used to construct optimal mixture design sufficient to satisfy extreme vertices designs for maize based complementary food. The parameters ranges for the mixture design in preparing complementary food were obtained using STATGRAPHICS centurion version XV.II as shown in Table 1. The four independents variables were maize flour (A), soy flour (B), groundnut paste (C) and germinated maize flour (D). The choice of upper and lower levels takes into account the usual practices and the recommendations of the literature concerning complementary food. Thirteen variables were examined: water, total digestible carbohydrates, crude proteins, lipids, sugars, ash, zinc, iron, fibers, total phenols, carotenoids, alpha tocopherol and antioxidant activity.

Table 1: Summary of the levels of variation for each component for maize based complementary food

Code	Components	Low level	High level	Unity
A	Maize flour	36.0	50.0	%
B	Soy flour	12.0	22.0	%
C	Groundnut paste	6.0	15.0	%
D	Germinated maize flour	6.0	12.0	%

#### Formulation and production of flours

Twelve formulations for maize based complementary food

(Table 2) were generating by the software using data of Table 1 in creating mixture design. Ingredients were mixed according to proportions shown in Table 2.

#### **Modeling and validation of model**

Mixture regression was used to fit the data presented in Table 2 in linear models using STATGRAPHICS Centurion XV.II software. The form of equation was:  $y = b_1X_1 + b_2X_2 + b_3X_3$ . Where y represents the response variable and  $b_1, b_2, b_3$ , the regression model coefficients. This equation describes the variation in biochemical contents (water, total digestible carbohydrates, crude proteins, lipids, sugars, ash, zinc, iron, fibers, total phenol, carotenoids, alpha tocopherol and antioxidant activity) as a function of the percent of components variables (maize, soy, groundnuts paste, germinated maize flour). The statistical parameters used in evaluating and selecting the best fitted model were: coefficient of determination ( $R^2 > 0,9$ ), adjusted coefficient of determination (adjusted  $R^2$ ), predicted coefficient of determination (predicted  $R^2$ ), lack-of-fit, regression data (p value and F value) and the studentized residue which measures the difference between the observed response and the predicted response.

#### **Optimization**

This approach involves the use of mixture regression model to obtain the best mixture leading to the production of balanced flour according to the FAO/WHO specification. The multiple response optimization procedure of STATGRAPHICS Centurion XV.II software was used to generate a unique contour plot and a response surface graph for all the variables in function of the desirability and the optimal mixture was then displayed.

#### **Chemical analysis**

##### **Proximate composition**

Moisture was determined by drying at 103 °C until constant weight is achieved [15]. Ash was determined by heating dried sample at 550°C for 24 hours [15]. Crude protein content of flour has been analyzed according to Kjeldahl method: ASU L 17.00-15 - German certified method [16]. Total proteins were calculated by multiplying the evaluated nitrogen by 6.25. Crude fat content were quantify according to Weibull-Stoldt method: ASU L 17.00-4 German certified method [17]. Total dietary fibre was analyzed according to the AOAC 985.29 method after enzymatic digestion (alpha amylase, protease, amyloglucosidase) of sample and ethanolic precipitation of soluble fibre [18]. The levels of simple carbohydrates/sugars: maltose, sucrose, free glucose and fructose were determined in methanolic extraction by HPLC (Shimadzu Europa GmbH, Duisburg, Germany) equipped with an evaporative light-scattering detection (ELSD at 40°C, Gain=3). An X-bridge Amide (particle size 3.5 µm, 250 mm of length and 4.6 mm of internal diameter) column with 12 % carbon load was used. Acetonitrile (80%) and 0.1% ammonia in water (20%) was used as eluent and a gradient with changing flow rate under isocratic

conditions was applied: 0-30 min, 1-1.5 ml/min; 30-32 min, 1.5-1 ml/min; 32-35 min, 1 ml/min. External calibration was made using solutions of fructose, glucose, saccharose, and maltose at 0.2-2 mg/ml each (dissolved in 60% Methanol). Total available carbohydrate was calculated as 100% minus the sum of moisture, protein, fat, ash, and total dietary fiber obtained using proximate analysis. Levels of starch contents in the samples were estimated by calculation.

##### **Minerals analysis**

Determination of zinc and iron concentrations in the samples was performed using flame atomic absorption spectroscopy in acid digested ash according to the AOAC, 999.11 methods [19].

##### **Total phenols and antioxidant capacity**

Total phenols analysis was done according to a derived method of Folin-Ciocalteu colorimetric method after extraction in methanol solution (60% (v/v) [20]. The total radical trapping antioxidant potential assay of flour was determined according to the method described by [21] with some modifications. The antioxidant activity was evaluated as radical scavenging activity with ABTS (2, 2-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid).

##### **Determination of carotenoids profile and alpha tocopherol**

##### **Instrumentation**

The chromatography was carried out using a Shimadzu system (Columbia, MD) composed of CBM-20A System Controller, two LC-10ADvp pumps, SIL 10ADvp Auto sampler injector, CTO-10ASvp column oven, and SPD-20A photodiode array detection system set in a range of 100 - 500 nm (all from Waters, Milford, MA, USA). Alpha tocopherol and carotenoids were separated on a reversed C18 column (250 × 3 mm I.D.; particle size, 5 mm) from Merck KGaA (Darmstadt, Germany). The chromatography was carried out using a step gradient elution mode in which eluent A was a mixture of Methanol-Ammonium acetate (water solution) (90: 10 v/v) and eluant B: Methanol-Ammonium acetate-terButhyl methyl ether (8: 2: 90, v/v/v) at a flow-rate of 0.2 ml/min.

##### **Sample preparation:**

Sample (0.5 g) was transferred in a polypropylene tube and water added. The mixture was shaken and allowed to stand for 30 min. Extraction of carotenoids and alpha tocopherol was carried out in a mixture of hexane-isopropanol 3: 2 (v/v) under stirring (15 min) using a programmable Rotator Mixer RM-Multi. The mixture was then subjected to centrifugation at 3800 × g for 5 min using a Thermo Scientific Heraeus Labofuge 200 centrifuge. This operation was repeated two times. After centrifugation, sodium chloride (0.1 M) (5 ml) was added to the supernatant. The solution was stirred and allowed to stand for 30 min. Hexane (7.5 ml) containing 0.005% BHT was then added. The mixture was shaken and the supernatant collected in a new tube. This last operation was repeated in 5 ml of hexane containing 0.005% BHT. The supernatants were collected and the volume adjusted to 20 ml with hexane. 200 µl of su

Table 2: Percentage composition of maize based complementary food (100%)

Formulation	Maize flour (%)	Soy flour (%)	Groundnut paste (%)	G M F (%)	Carrot flour (%)	Egg shell (%)	Sugar (%)
F1	50.0	22.0	7.0	6.0	2.5	2.5	10
F2	50.0	22.0	6.0	7.0	2.5	2.5	10
F3	50.0	14.0	15.0	6.0	2.5	2.5	10
F4	50.0	12.0	15.0	8.0	2.5	2.5	10
F5	42.0	22.0	15.0	6.0	2.5	2.5	10
F6	36.0	22.0	15.0	12.0	2.5	2.5	10
F7	50.0	17.0	6.0	12.0	2.5	2.5	10
F8	50.0	12.0	11.0	12.0	2.5	2.5	10
F9	45.0	22.0	6.0	12.0	2.5	2.5	10
F10	46.0	12.0	15.0	12.0	2.5	2.5	10
F11	50.0	22.0	7.0	6.0	2.5	2.5	10
F12	50.0	22.0	6.0	7.0	2.5	2.5	10

G M F: Germinated maize flour

pernatant was transferred in a clean polyethylene tube. The solution was then concentrated in a Techne sample concentrator, SBHCONC/1 under nitrogen (10-15 min). The residue was dissolved in 200 µl of isopropanol and ultrasonicated for 5 minutes. The liquid was then centrifuged at 5000 rpm for 5 minutes. The supernatant was placed in HPLC vial and ready to be injected into the column.

#### Quantification

Peaks were identified by their retention time and absorption spectra were compared to those of known standards (Sigma Chemicals). Carotenoids and alpha tocopherol were quantified using peak areas of the corresponding authentic standards.

### Results

#### Mixture design and biochemical composition

The mixture design used to calculate the model coefficient terms using mixture regression are presented in Table 3, 4 5 and 6.

#### Proximate composition of composite flours

The result of proximate composition is shown in Table 3. The protein content of the composite blends ranged from 13.64/100 g to 17.82/100 g while ash content varies from 3.85/100 g to 4.15/100 g. The highest protein and ash percentage is recorded for formulation 6 (16.4/100 g and 4.15/100 g respectively). The carbohydrate content of the composite flour blends is in the range value of 56.68 /100g to 67.07 /100g while moisture content ranged between 5.82 /100 g to 6.7 /100 g. The highest carbohydrate and moisture percentage is recorded for formulation 7 (67.07 /100 g and 6.7 /100 g respectively). Crude fibers content ranged from 4.08 /100 g to 5.75 /100 g while fat content varies from 10.17 /100 g to 15.67 /100 g. The highest crude fibers and fat percentage is recorded for formulation 5 (4.15 /100 g and 15.675 /100 g respectively). **Sugars composition of composite flours**

Fructose and maltose content (Table 4) ranged from 59.82mg /100 g to 74.26 mg/100 g and 221.85 mg/100 g to 536.97 mg/100 g respectively. The highest fructose and maltose content is recorded for formulation 6 (74.26 mg /100 g and 536.97 mg /100

g respectively). Glucose content varies from 190.26mg /100 g to 323.42mg /100 g. The highest glucose is recorded for formulation 8 (323.42 /100 g). Maize based formulation has highest content in sucrose as free sugars. Sucrose content is in the range values of 1289.13 /100 g to 1752.07 mg /100 g. The highest sucrose is recorded for formulation 9 (1752.07mg /100 g). This highest value of sucrose contributes to the increase in total sugars content. The highest total free sugars are recorded for formulation 9 (2167.57mg /100 g).

#### Carotenoid and alpha tocopherol composition of composite flours

Alpha tocopherol, lutein, zeaxanthin and β-cryptoxanthin content (Table 5) ranged from 944.83µg /100 g to 1190.66 µg/100 g, 79.57 /100 g to 102.17 µg /100 g, 264.83 µg /100 g to 364.27 µg and 34.69 /100 g to 47.85 µg /100 g respectively. The highest alpha tocopherol, lutein, zeaxanthin and β-cryptoxanthin content is recorded for formulation 8 (1190.66 µg /100 g, 102.17 µg /100 g 364.27 µg and 47.85 µg /100 g respectively). The values of alpha carotene vary between 236.65 µg to 224.83 µg/100 g. The highest alpha carotene content is recorded for formulation 6 (224.83 µg /100). The values of beta carotene ranged from 460.40 µg to 483.35 µg/100 g. The highest beta carotene content is recorded for sample 5 (483.35 µg/100 g). Lycopene content varies 0.86 µg to 1.09 µg/100 g. The highest lycopene content is recorded when soy flour is in highest ratio (22 %).

#### Total phenol composition and antioxidant activity of composite flours

The result of total phenol content and Trolox® Equivalent Antioxidant Capacity (TEAC) of the composite blends is shown in Table 6. The values of phenolic compounds and TEAC ranged from 6.62mg/100 g to 6.86mg/100 g and 0.12 to 0.14M of Trolox. The highest phenolic compounds percentage and TEAC activity is recorded for formulation 9 (6.86mg/100 g and 0.14M of Trolox).

Table 3: Proximate composition (g/100g) of maize based formulation

Formulation	Maize flour (%)	Soy flour (%)	Ground-nut paste (%)	Germinated maize flour (%)	Carrot (%)	Egg shell (%)	Moisture	Ash	Protein	Fat	Carbohydrate	Fibers
1	50	22	7	6	2.5	2.5	6.48	4.08	16.04	11.64	62.94	5.30
2	50	22	6	7	2.5	2.5	6.53	4.07	15.83	11.12	63.69	5.28
3	50	14	15	6	2.5	2.5	6.38	3.92	15.10	14.23	62.31	4.44
4	50	12	15	8	2.5	2.5	6.46	3.86	14.46	13.85	63.66	4.18
5	42	22	15	6	2.5	2.5	5.95	4.15	17.76	15.67	56.78	5.64
6	36	22	15	12	2.5	2.5	5.82	4.14	17.82	15.60	56.68	5.75
7	50	17	6	12	2.5	2.5	6.7	3.92	14.22	10.17	67.07	4.62
8	50	12	11	12	2.5	2.5	6.64	3.82	13.64	11.79	66.67	4.08
9	45	22	6	12	2.5	2.5	6.42	4.06	15.88	11.07	63.61	5.37
10	46	12	15	12	2.5	2.5	6.37	3.85	14.5	13.80	63.59	4.25
11	50	22	7	6	2.5	2.5	6.48	4.09	16.04	11.64	62.94	5.30
12	50	22	6	7	2.5	2.5	6.53	4.07	15.84	11.12	63.70	5.28

Table 4: Sugars composition (mg/100g) of maize based formulation

Formulation	Maize flour (%)	Soy flour (%)	Ground-nut paste (%)	Germinated maize flour (%)	Carrot (%)	Egg shell (%)	Fructose	Glucose	Sucrose	Maltose	Total sugars
1	50	22	7	6	2.5	2.5	66.06	190.26	1668.41	254.67	1937.47
2	50	22	6	7	2.5	2.5	67.03	212.17	1690.17	221.85	1983.87
3	50	14	15	6	2.5	2.5	59.82	191.62	1330.17	526.11	1589.23
4	50	12	15	8	2.5	2.5	60.20	235.78	1289.13	528.33	1594.97
5	42	22	15	6	2.5	2.5	67.42	189.38	1593.37	528.51	1860.19
6	36	22	15	12	2.5	2.5	74.26	320.18	1667.65	536.97	2080.63
7	50	17	6	12	2.5	2.5	67.98	322.57	1587.57	227.4	1998.22
8	50	12	11	12	2.5	2.5	64.08	323.42	1376.17	397.05	1780.57
9	45	22	6	12	2.5	2.5	72.73	321.17	1752.07	228.9	2167.57
10	46	12	15	12	2.5	2.5	64.76	190.26	1668.41	254.67	1937.47
11	50	22	7	6	2.5	2.5	66.07	212.17	1690.17	221.85	1983.87
12	50	22	6	7	2.5	2.5	67.04	191.62	1330.17	526.11	1589.23

Table 5: Alpha tocopherol et carotenoids composition (µg/100g) of maize based formulation

Formulation	Maize flour (%)	Soy flour (%)	Ground-nut paste (%)	Germinated maize flour (%)	Carrot (%)	Egg shell (%)	a-Tocopherol	Lutein	Zeaxanthin	β-Kryptoxanthin	a-Carotin	β-Carotin	Lycopin
1	50	22	7	6	2.5	2.5	1047.77	99.44	363.16	47.61	235.86	481.69	1.09
2	50	22	6	7	2.5	2.5	1068.00	99.85	363.26	47.61	235.76	480.92	1.09
3	50	14	15	6	2.5	2.5	1064.95	99.68	363.57	47.77	227.51	468.31	0.90
4	50	12	15	8	2.5	2.5	1109.72	100.55	363.87	47.83	225.23	463.44	0.86
5	42	22	15	6	2.5	2.5	944.83	86.69	306.63	40.21	236.65	483.35	1.09
6	36	22	15	12	2.5	2.5	989.03	79.57	264.83	34.69	236.65	480.04	1.09
7	50	17	6	12	2.5	2.5	1179.92	102.02	364.02	47.75	230.05	468.76	0.94
8	50	12	11	12	2.5	2.5	1190.66	102.17	364.27	47.85	224.83	460.40	0.86
9	45	22	6	12	2.5	2.5	1104.84	93.91	328.43	43.02	235.76	478.16	1.09
10	46	12	15	12	2.5	2.5	1139.19	95.8	336.00	44.15	225.23	461.23	0.86
11	50	22	7	6	2.5	2.5	1047.77	99.45	363.17	47.61	235.87	481.69	1.10
12	50	22	6	7	2.5	2.5	1068.00	99.85	363.27	47.62	235.77	480.93	1.10

Table 6: Total phenol, mineral composition (mg/100g) and Trolox equivalent activity (M of Trolox) of maize based formulation

Formulation	Maize flour (%)	Soy flour (%)	Ground-nut paste (%)	Germinated maize flour (%)	Carrot (%)	Egg shell (%)	Total phenol (mg/100g)	TEAC (M of Trolox)	Zn (mg/100g)	Fe (mg/KgMs)
1	50	22	7	6	2.5	2.5	6.74	0.13	3.59	3.12
2	50	22	6	7	2.5	2.5	6.76	0.13	3.58	3.07
3	50	14	15	6	2.5	2.5	6.62	0.13	3.49	2.91
4	50	12	15	8	2.5	2.5	6.64	0.13	3.45	2.75
5	42	22	15	6	2.5	2.5	6.66	0.14	3.46	3.56
6	36	22	15	12	2.5	2.5	6.78	0.14	3.30	3.58
7	50	17	6	12	2.5	2.5	6.83	0.12	3.49	2.68
8	50	12	11	12	2.5	2.5	6.76	0.12	3.42	2.55
9	45	22	6	12	2.5	2.5	6.86	0.13	3.46	3.08
10	46	12	15	12	2.5	2.5	6.72	0.13	3.35	2.77
11	50	22	7	6	2.5	2.5	6.7	0.13	3.59	3.12
12	50	22	6	7	2.5	2.5	6.8	0.13	3.58	3.07

### Mineral composition

The result of mineral composition of the composite blends is shown in Table 6. The values of zinc ranged from 3.30mg/100 g to 3.59 mg /100 g. The highest zinc percentage is recorded for formulation 1 (3.59 mg /100 g). Iron content ranged from 2.55/100 g to 3.58/100 g. The highest iron percentage is recorded for formulation 6 (3.58/100 g).

### Mixture design

#### Models

The 12 formulations and the responses (Table 3, 4 5 and 6) observed for maize based complementary flour were used to calculate the coefficients of linear models. Tables 7, and 8 present the values (specified in pseudo-components) of the regression coefficients terms of the linear model which rely the desired response (moisture, total digestible carbohydrates, crude proteins, fat, sugars, ash, zinc, iron, fibers, total phenol, carotenoids, alpha tocopherol and antioxidant activity) as a function of experimental components (maize flour ( $X_1$ ),soy flour ( $X_2$ ), groundnut paste ( $X_3$ ) and germinated maize flour ( $X_4$ )). The adjusted R-squared are higher (up than 90%) showing that the variability of the desired response has been explained by the model. The P-Value (Probability test F) for the different models is very small, indicating that they are a useful predictor of the desired response. The P-Value estimated in the lack-of-fit line is well above 0.05, so the selected model appears to be adequate. The studentized residual calculated are less than 3 indicating a good agreement between observed and predicted response.

#### Effect of variables on the desired response

##### Proximate analysis

Maize (A) (+7.10, +21.93) (Table 7) with highest coefficient values contributes mostly to the moisture and carbohydrate content of the flour respectively. Soy flour (B) with highest coefficient model terms has highest effect on ash, protein and fibers content of maize composite flour. Groundnut paste (C) with highest coefficient values (+21.93) contributes mostly to the fat content of the composite flour.

##### Sugar analysis

Soy flour (B) (Table 7) with highest coefficient model terms (+2376.67) had highly effect on sucrose content of the composite flours. Germinated maize flour (D) with highest coefficients terms contribute more to the reducing sugars (fructose (+84.88), glucose (+738.16), maltose (+47.31) and total sugars (+2733.39) content of the composite flours.

##### Carotenoids analysis

Germinated maize flour (D) with highest coefficient (+1394.99) contributes mostly (Table 8) to the alpha tocopherol content of the composite flours. Maize flour (A) with highest coefficient model terms (+117.27, +441.41, 58) has highest effect on lutein, zeaxanthin, beta criptoxanthin content of the composite flour respectively. According to the model terms, soy flour (B) contributes mostly to alpha carotene, beta carotene and lycopene (+252.91, +509.69, +1,447) content of the composite flours.

##### Total phenol and TEAC analysis

Germinated maize flour (D) (Table 8) with highest model term (+0.0072) has highest effect on total phenol content of composite flour. Soy flour (B) with highest coefficient model terms (+0.1535) contributes more to the antioxidant activity of the composite flours.

##### Zinc and iron analysis

Maize flour (Table 8) with highest coefficients model term (+3.66) contributes more to the zinc content of the composite flour. Soy flour (B) with highest coefficient model terms (+4.28) is the main contributors of iron content in composite flour. Soy flour (B) with highest coefficient (+4.28) is the main supplier of iron in composite flour.

##### Optimal formulation

From the experimental models, the desirability test was carried out to define the best mixture in formulating balanced flour having the same composition as the standard baby food proposed by Sanogo et al. [7]. The optimal mixture of ingredients to be blend in order to have balanced composite flour is presents in Table 9. The desirability test indicated that the best desirable formulation for maize based complementary flour is 50.00 % of maize flour, 17.10% of soy flour, 6% of groundnut paste and 11.90% of germinated maize flour.

##### Macronutrients content and gross energy of the formulated composite flour

The desirability test was carried out to produce baby flour having the same composition as the standard baby food proposed by Sanogo et al, [7]. Moisture content of the composite flour is 5.64 %. Protein, lipids and carbohydrates content are 14.26, 10.19 and 67% respect

Table 9: Optimal mixture of ingredients (%) in formulating balanced flour

Ingredients	Maize based complementary flour
Maize flour	50.00
Soy Flour	17.10
Groundnut paste	6
Germinated maize flour	11.90

tively. The value of ash content of the composite flour is 3.92% while fibers content is 4.64%. These values were calculated from the model equations. The gross energy of the formulated flour calculated is 416.70 Kcal/100g. Protein, lipids and carbohydrates contributes to about 13.69%, 22% and 64.31% of the total energy respectively.

Table 7: Linear coefficient terms, R<sup>2</sup> and R<sup>2</sup> adjusted regression analysis, p-values and lack of fit of proximate and sugar models

Parameters	Proximate Model terms							Sugars Model terms				
	Linear	Moisture	Ash	Proteins	Fat	Carbohydrates	Fibers	Fructose	Glucose	Sucrose	Maltose	Total sugars
A: Maize flour	b <sub>1</sub>	7.10	3.79	12.50	9.33	74.38	3.76	56.39	193.17	1554.17	12.06	1814.89
B:Soy Flour	b <sub>2</sub>	5.73	4.51	20.81	13.83	60.86	7.51	80.15	186.18	2376.67	19.56	2661.64
C:Groundnut paste	b <sub>3</sub>	5.43	4.01	17.88	21.93	56.18	4.81	60.64	190.42	2175.42	12.06	2433.64
D:Germinated yam flour	b <sub>4</sub>	6.57	3.74	12.75	9.06	74.45	4.23	84.88	738.16	1863.67	47.31	2733.39
	R <sup>2</sup> (%)	100	100	100	100	100	100	100	100	100	100	100
	R <sup>2</sup> adjusted	100	100	100	100	100	100	100	100	100	100	100
	Probability	0	0	0	0	0	0	0	0	0	0	0
	Lack of fit	0.95	0.92	0.997	0.997	0.99	0.99	0.997	0.997	0.997	0.995	0.995
	Error-type	0.0005	0.001	0.001	0.001	0.0016	0.001	0.0071	0.0071	0.0039	0.0018	0.0018

Table 8: Linear coefficient terms, R<sup>2</sup> and R<sup>2</sup> adjusted regression analysis, p-values, lack of fit of alpha tocopherol, carotenoid, total phenol, TEAC, sugar and mineral models

Parameters	Coefficient	Alpha tocopherol and carotenoid model term							Total phenol and TEAC model terms		Mineral models term	
		Alpha tocopherol	Lutein	Zeaxanthin	B-criptoxathin	α-caroten	β caroten	Lycopen	Total phenol	TEAC	Zinc	Iron
A: Maize flour	b <sub>1</sub>	1210.8	117.27	441.41	58	224.34	462.68	0,86	0.0067	0,109	3.66	2.25
B:Soy Flour	b <sub>2</sub>	835.4	76.70	263.47	34.34	252.91	509.69	1,447	0.0068	0.154	3.53	4.28
C:Groundnut paste	b <sub>3</sub>	889.102	77.42	264.74	34.86	226.81	467.88	0,85	0.0065	0.151	3.21	3.63
D:Germinated maize flour	b <sub>4</sub>	1394,99	87.57	267.23	35.01	224.33	448.85	0,85	0.0072	0.117	3.06	2.33
	R <sup>2</sup> (%)	100	100	100	100	100	100	99.91	95.79	99.86	100	100
	R <sup>2</sup> adjusted	100	100	100	100	100	100	99.87	94.22	99.81	100	100
	Probability	0	0	0	0	0	0	0.0012	0.0323	0.0018	0	0
	Lack of fit	0.92	0.987	0.973	0.996	0.997	0.996	0.997	0.878	0.995	0.9965	0.9835
	Error-type	0.0016	0.0049	0.0035	0.0004	0.0065	0.0029	0.0072	2.542x10 <sup>-5</sup>	0.00059	0.00072	0.0024



### Sugars composition of the formulated composite flour

The levels of sugars and starch in the infant formulas are presented in Table 10. Sugar values were calculated from the model equations. The closer values of total sugars calculated from the models and after summation of free sugars values indicated that the models are good predictors. The weaning formulation content 0.056% of fructose, 0.228% of glucose, 1.536% of sucrose and 1.836% of total sugars. From Table 10, the weaning flours have high content in sucrose (1.536g/100g) than other free sugars (fructose, glucose and maltose). The formulated flour content 66.03% of starch.

### Carotenoids and alpha tocopherol composition of the formulated composite flour

The levels of carotenoids and alpha tocopherol in the infant formula are presented in Table 10. The values were calculated from the model equations. Alpha tocopherol (the highest fraction of vitamin E) content of the formulated flour is 967.61 µg/100g. The weaning flour contains lutein (86.81 µg/100g), zeaxanthin (313.44 µg/100g) and lycopene (0.83 µg/100g) as non-provitamin A carotenoids. The complementary flour contains also alpha-carotene (195.72µg/100g), beta carotene (399.98µg/100g) and beta cryptoxanthin (41.13 µg/100g) as provitamin A carotenoids. These values were calculated from the model equations. The retinol activity of the flour is 112.52 RE.

### Polyphenol content and antioxidant activity of the formulated composite flours

The total phenol composition and TEAC activity of the optimal formulated maize based complementary food are presented in Table 10. These values were calculated from the model equations. The weaning flour content 6mg of total phenol and the antioxidant activity of the formulated flour is 0.104 M Trolox/100gMF.

### Zinc and iron composition of the formulated composite flours

Zinc and iron composition of the optimal formulated maize based complementary food are presented in table 10. The value of zinc and iron were calculated from the model equations. The weaning flour content 2.99 mg of zinc and 2.35mg of iron.

### Nutritional quality of weaning foods

The daily portion intake of energy, protein and lipids as a function of quantity of flour needed is presented in Table 11. They were calculated according to the recommendation of [22] for developing countries. According to these authors, complementary feeding interventions are usually targeted at the age range of 6-24 months, which is the time of peak incidence of growth faltering, micronutrients deficiencies and infectious illnesses in developing countries where the average expected energy intake (Table 11) from complementary foods is approximately 200 kcal at 6-8 months, 300 kcal at 9-11 months and 550 kcal /day at 12-23 months respectively. In this context, daily serving portions of 48.00g of complementary flour are needed to satisfy the 200 Kcal/day of energy for 6-8 months old child. A quantity of 71.99g will be sufficient to cover the 300 Kcal/day of energy for 9-11 months old child. The daily

serving portion of maize composite flour required to satisfy the 550 kcal/day requirement for 12-23 months old child is 131.99g. All these portions satisfied the recommended daily energy density value ( $\geq 0.8$  kcal/g). The estimated daily proteins and fat intake from complementary food were higher than the suggested intake at the age range of 6-24 months (Table 11). The daily suggested intake of vitamin A (200µg retinol activity) and  $\alpha$ -tocopherol (2.5mg) (Table 12) of all the serving portions of composite flour did not meet the requirement of FAO/WHO specification and the extend vary according to the daily serving portion related to the age of child. The average daily intake of vitamin A from complementary flours where about 27% for the smallest daily portion (48g intended 6-8 months old), 40.5% for the medium daily portions (72g intended for 9-11 month old child) and 74.26% for the highest daily proportion (132g intended 12-23 months old child). The average daily intake of  $\alpha$ -tocopherol from complementary flours where about 18.6% for the smallest daily portion (48-49g; 6-8 months of age), 27.86% for the medium daily portions (72-75g, 9-11 month old child) and 51% for the highest daily proportion (132-137g, 12-23 months old child). The average daily intake of zinc (Table 12) meets the suggested daily value in medium (2.05g) and high (1.2mg) dietary zinc bioavailability at all the daily serving portions (48.00, 71.99 and 131.99g). The formulated flour satisfied only the suggested daily iron value at higher daily serving portion of 132 to 137g intended for 12-23 months old child.

### Discussion

Twelve formulations were generated using extreme vertices designs. Proximate result from the twelve formulations is similar to the values obtained by Awolu et al. [23] for maize-based snack fortified with soybeans and tiger nut. The values of proteins and ash obtained from all the formulation are smaller than the value reported by Tiencheu et al. [2] for instant weaning foods processed from maize (*Zea mays*), paw-paw (*Carica papaya*), red Beans (*Phaseolus vulgaris*) and mackerel fish meal (*Scomber scombrus*). The values of moisture, carbohydrates, fat and sugar content of the formulations are higher than the values obtained by the same authors for maize based formulation. Total phenol, zinc and iron values are higher than the value recorded by Awolu et al. [23] for maize-based snack fortified with soybeans and tiger nut. From the model terms, it can be seen that experimental variables (maize, soy, germinated maize flours and groundnut paste) have an additive effect on response variables (water, total digestible carbohydrates, crude proteins, lipids, sugars, ash, zinc, iron, fibers, total phenols, carotenoids, alpha tocopherol and antioxidant activity) since all the values are positive but the extend vary from one variables to another. Maize flour with highest coefficient values contributes mostly to the moisture and carbohydrate content of the composite flour respectively.

Table 10: Nutritional composition of the optimized maize based formulation

	Maize	Standard baby flour*	FAO/WHO
<b>Proximate analysis</b>			
Moisture (%)	5.64	5	5
Ash (%)	3.92	2	< 5.00
Protein (%)	14.26	13	13 à 15%
Lipids (%)	10.19	7	2.00
Carbohydrates (%)	67.00	68.00	60 to 75
Fibers (%)	4.64	5	< 5.00
Gross energy (Kcal/100g)	416.70	400	400Kcal/100g
<b>Contribution to total energy</b>			
Protein (%)	13.69		6-15%
Lipids (%)	22.01		20-40%
Carbohydrates (%)	64.31		
<b>Sugars</b>			
Free fructose (g/100g)	0.056		
Free glucose (g/100g)	0.228		
Sucrose (g/100g)	1.536		
Maltose (g/100g)	0.016		
<sup>1</sup> Total sugars (g/100g)	1.836		
<sup>2</sup> Total sugars(g/100g)	1.834		
<sup>3</sup> Starch (%)	66.03		
Total available carbohydrate (%)s	67.00		
<b>Carotenoids</b>			
Alpha tocophero (µg/100g)	967.61		5
Lutein (µg/100g)	86.81		
Zeaxanthin (µg/100g)	313.44		
Beta kriptoxanthin (µg/100g)	41.13		
Alpha-carotene (µg/100g)	195.72		
Beta carotene (µg/100g)	399.98		
Lycopene (µg/100g)	0,83		
Pro vit A carotenoids (µg/100g)	950.26		
Retinol Activity equivalent (RE)	112.52		60-180/100kcal
None pro vit A Carotenoids (µg/100g)	87.64		
<b>Total phenol and antioxidant activity</b>			
Polyphenol (mg/100g)	6		
TEAC (MTrolox/100gMF)	0.104		
<b>Minerals</b>			
<sup>4</sup> Iron (mg/100g)	2.35		11.6, 5.8, 3.9
<sup>5</sup> Zinc (mg/100g)	2.99		8.3, 4.1, 2.4

<sup>1</sup> Total sugars = sum of free fructose, free glucose, sucrose and Maltose, <sup>2</sup> Total sugars calculated from the model, <sup>3</sup> Starch =total available carbohydrate minus sum of maltose, sucrose, free glucose, and free fructose, <sup>4</sup> Iron values are given for 5%, 10 % and 15% dietary iron bioavailability, <sup>5</sup> Zinc values are given for low, medium and high dietary zinc bioavailability

Table 11: Daily requirement values and daily portion intake of energy, protein and lipids as a function of quantity of flour needed

Age (months)	Daily requirement values				Maize-based formulation			
	Energy (Kcal)	Energy density	Proteins (g/day)	Lipids (g/day)	Quantity of flour needed (g)	Energy density (g/100Kcal)	Proteins (g/day)	Lipids (g/day)
6-8	200	≥ 0.8	2	0	48.00	4.17	7.13	5.09
9-11	300	≥ 0.8	5 - 6	3	71.99	4.17	10.26	7.33
12-23	550	≥ 0.8	5 - 6	9-13	131.99	4.17	18.82	13.44

Table 12 Average daily intake of micronutrients from maize-based formulation as a function of quantity of flour needed

Age	Reference Nutrient Intake (INL <sub>98</sub> ) <sup>17</sup>	Daily ration of the formulated complementary Food (at least 50% of INL <sub>98</sub> )	Maize-based formulation		
			6-8 months	9-11 months	12-23 months
Energy requirement (Kcal)			200	300	550
Quantity of flour			48	71.99	131.99
Vitamin A µg retinol equivalent	400	200	54.01	81.00	148.52
Vitamin E mg (α-Tocopherol)	5	2.5	0.46	0.7	1.29
Zinc (mg) <sup>4</sup>	8.3; 4.1; 2.4	4.15; 2.05; 1.2	1.44	2.15	3.95
Iron (mg) <sup>5</sup>	11.6; 5.8; 3.9	5.5; 2.9; 1.95	1.13	1.69	3.10

Indeed, maize is a starchy seed with a high content in carbohydrates than soy bean or groundnut [24],[25],[26] and this justifies its use as good source of calories in many diets [27]. The highest moisture percentage recorded with the highest ratio of maize flour might be due to the fact that carbohydrates present in the seed can easily absorb water and this capacity is reduced with the incremental addition of soy flour because soy flour contain higher amount of solid matters with high emulsifying properties compared to corn flour [28]. Maize flour has also highest effect on lutein, zeaxanthin, beta cryptoxanthin content of the composite flour respectively. In fact, yellow maize is an effective source of lutein, zeaxanthin and beta cryptoxanthin [29]. This flour contributes more to the zinc content of the composite flour. If maize has a highest contribution in zinc content, it might be due mainly to the highest percentage of maize in the composite flour since zinc content in maize, soybeans and groundnuts seeds are in the same range of values depending of the cultivar and the time of harvest. Dakare et al. [30] reported an average value of 5.412mg ±2.23 for yellow maize. Asibuo et al. [31] reported ranged from 0 to 6.5 mg/100 g with a mean of 5.2 mg/100 g for groundnut (*Arachis hypogaea* (L)). Batal et al. [32] reported a range value of 3.8 to 5.8 with an average value of 4.8 mg and a range value of 1.4 to 2.8 with an average value of 2 mg of zinc in soybean and corn meal respectively. Germinated maize flour is generally uses as a source of alpha amylase to reduce the bulkiness of the gruel when preparing porridge. Beside this main role, it can be seen that it has a nutritional function. In fact, germinated maize flour contributes more to the reducing sugars,

tocopherol percentage recorded with the highest ratio of germinated maize flour might be due to germination. Indeed, during germination, storage carbohydrates decrease and reducing sugars increase due to the hydrolysis of starch and the requirement of energy by growing plant [33, 34]. It have also been reported that germination of seed increase also the level of bioactive compound like vitamin E [35, 36]. Germinated maize flour has highest effect on total phenol content of composite flour. Indeed, it has been reported that soybean and groundnut have high content in total phenol than maize and these compounds may increase during heat treatment [23]. Mujić et al. [37] reported a range value from 87.2 to 216.3 mg GAE/100g for five Croatian soybean seed cultivars. Chukwumah et al. [38] reported a range of values from 94.4 to 228.4 mg GAE/100g with an average content of 143.5 mg GAE/g of total polyphenol content in peanut cultivars. Total phenolic content ranged from 10.390 to 13.313 mg GAE/ kg was reported by Urias-Lugoa et al. [39] in maize (*Zea mays*). If germinated maize flour is the main contributor of total phenol content, this means that pretreatment like dehulling, roasting or toasting of maize, soybean or groundnut before grinding have contributed to reduce efficiently the total phenolic compounds of seeds and justify the role of germinated maize flour as the main supplier of phenols since it has not been subjected to these pretreatments. Soy flour stands as our main sources of protein and this is confirmed by it highest effect on protein content of the composite flour. Beside this primary role, it is also the main contributors of ash and fibers. Indeed, soybean is an excellent source of ash, fibers and proteins and a com-

plement to lysine-limited cereal protein [23],[28]. This flour had also highly effect on sucrose content of the composite flours. It has been reported that sucrose makes up 41.3-67.5% of the total soluble sugars in soybean seed [40] and it not obvious to see that it is the main sucrose supplier in the flour. According to the model terms, this flour contributes mostly to alpha carotene, beta carotene and lycopene content of the composite flour. This result is consistent since many studies have reported higher concentration of  $\beta$  carotene in soybean. Joung-Kuk et al. [41] reported a total average concentration of 6.6  $\mu\text{g/g}$  soybean seed. The contribution of this flour as the main supplier of iron is also noticeable. If soybean flour has a highest contribution in iron content of the composite flour, it might be due mainly to its highest content in iron than maize flour or groundnut paste. Indeed, Dakare et al. [30] reported a value of  $4.845 \pm 2.11$  for yellow maize. Asibuo et al. [31] reported ranged from 0.2 to 3.7 mg/100 g with a mean of 2.8 mg/100 g for groundnut (*Arachis hypogaea* (L)). Batal et al. [32] reported a range value of 1.9 to 44.3 mg with an average value of 17.2 mg and a range value of 1.8 to 3.6 with an average value of 2.5 mg of iron in soybean meal and yellow maize respectively. Soy flour contributes more to the antioxidant activity of the composite flours. Indeed, it has been observed in previous reports that there is a high correlation between the antioxidant activity assays and the content of in phenols compounds [37]. If soybeans flour is the main contributor of the antioxidant activity of composite flour, it means that others bioactive compounds present in soy flour had highest effect on antioxidant activity. One can think about alpha-carotene and beta carotene since soy flour with highest coefficient models terms had highly effect on alpha and beta carotene content of the composite flours but not total phenol. Groundnut paste is a leguminous and has been use as our main source of protein and lipids. According to the model terms, this paste contributes mostly to the fat content of the composite flour. This might be due to the higher amount of crude fat in groundnut paste than in soybean and corn flour [24],[25],[26]. In fact, groundnut paste is an edible oil source with about 33.6 to 55% of crude fat content [43]. Groundnut paste with highest coefficient values contributes mostly to the fat content of the composite flour. This is due to the higher amount of crude fat in groundnut paste than in soybean and corn flour [24],[25],[26]. In fact, groundnut paste is an edible oil source with about 33.6 to 55% of crude fat content [42]. Macronutrients content influence the nutritional quality of formulated complementary flour. The composite flours meet the requirement of energy and carbohydrates of the standard baby food [7]. The protein and lipids content was slightly higher than the standard value (13% and 7% respectively). This is because the level in composite flour was higher than theses values. However, the composite flour satisfied the requirement for energy (400Kcal/100g), protein (13 to 15%), carbohydrates (60 to 75 g/ 100 g), lipids (10

to 25%) and fibers ( $\leq 5\%$ ) stipulated in the Codex alimentarius standards [43]. The ash values are above the stipulated recommendation (2%) in the Codex standards and in standard baby food. High ash content is indicative of more mineral elements in the flour blend which could be of immense benefit to the body [5]. Moisture content of the composite flours was higher than the values in standard baby flour but was within the acceptable limit of no more than 10% for long storage. Protein contributes to about 13.69% of the total energy intake while lipids contribute to 22% of the total energy. Those values meet the requirement percent in total energy of the codex alimentarius for protein (6-15%) and lipids (20-40%). Carbohydrates may contribute to about 64.31 to 67.89% of the total energy for weaning flour. This highest contribution of carbohydrates in total energy intake makes the composite flour suitable to be used in managing protein energy malnutrition since enough quantity of energy will be derived from carbohydrates sparing protein that can be used for it primary function of building the body and repairing worn out tissues rather than as a source of energy [44]. The formulated weaning flour contents free sugars. The values of fructose and glucose are in the range values reported by Tumwebaze et al. [45] for fructose (0-0.2) and glucose (0.1-0.2) in maize-based complementary food for Ugandan children 12 to 23 Months of Age. The value of sucrose and total sugars are lower than the value reported by the same author for sucrose (2.8-4.8) and total sugars (2.9-5.1). The weaning flours have high content in sucrose than other free sugars (fructose, glucose and maltose). This result is consistent with those reported by other researchers like Tumwebaze et al. [45]. Indeed, soybeans, maize were found to have higher content in sucrose than fructose, glucose or maltose. The formulated flour contents starch. Starch is an important part of our nutrition as energy supplier. The value of starch obtained in this study is in range values reported by many authors since maize is the main supplier of starch in the composite flour. Ndukwe et al. [27] reported a range value of  $59.72\% \pm 0.08$  for ten maize varieties grown in Eastern part of Nigeria. The value of alpha tocopherol (the highest fraction of vitamin E) of the formulated flour is higher than the range values (0.3 to 0.7 mg/100 g) of vitamin E reported by Nuss and Tanumihardjo [46] for total maize kernel of most varieties. This higher value might be due to the synergic contribution of others components to alpha tocopherol content of the composite flour. Carotenoids are natural pigments found in plants and some animals. Lutein and zeaxanthin, have gained interest due to their association with eye health [47]. Lycopene has retained attention as antioxidant in preventing chronic disease. The weaning flour contains lutein, zeaxanthin and lycopene as non-provitamin A carotenoids. The value of lutein and zeaxanthin are in the range value (5.5-330.3  $\mu\text{g}/100\text{g}$  (lutein) and 28.8- 209.0  $\mu\text{g}/100\text{g}$  (zeaxanthin)) reported by Scott and Eldridge [47] for varieties of maize. Maize, soybeans and

groundnut are a poor source of lycopene and the later was mainly provided by carrot flour added in lower percentage (2.5%) as source of carotene. The weaning food contains alpha-carotene, beta carotene and beta criptoxanthin as pro-vitamin A carotenoids. The values of alpha-carotene and beta carotene obtained in this study are higher than the values reported by Awoyale et al. [48] for alpha-carotene (16 $\mu$ g/100g) and beta carotene (41 $\mu$ g/100g) while the value of beta criptoxanthin is smaller than the value (138  $\mu$ g/100g) reported by the same authors in Ogi powder. The value of beta carotene was in the range value (291.63-565.09 $\mu$ g/100) reported by Beruk Berhanu Desalegn et al. [49] for maize based complementary food. The term pro-vitamin A carotenoids is a generic descriptor for all carotenoids exhibiting qualitatively the biological activity of vitamin A. The biological activity of carotenoids is referring as retinol equivalent [50]. 1RE equal to 1 $\mu$ g Retinol or 6 $\mu$ g of  $\beta$ -carotene or 12 $\mu$ g for others provitamin A carotenoids. The retinol activity of the flour is 112.52 RE. This value is lower than the value (127.3) obtained by Kunyanga et al. [51] and higher than the values reported by Beruk Berhanu Desalegn et al. [49] for maize based complementary food. The retinol activity of all the composite flour did not meet the requirement of FAO/WHO specification (60-180/100kcal). The maize based formulation satisfied only 46.9% of the codex alimentarius standard lower limit. A part from provitamin A activity, certain pro-vitamin A carotenoids have other functions.  $\beta$ -criptoxanthin is associated with a reduced risk of inflammatory disorders, such as rheumatoid arthritis and polyarthrits [52]. Beta carotene is associated with antioxidant activity. The weaning flour contents phenol and exhibit an antioxidant activity. The value of phenol obtained for maize composite flour is higher than the value (4.56-4.75) reported by Awolu et al. [23]. The antioxidant activity of the formulated flour is in the range value (0.059-0.104) reported by Chukwumah et al. [39] for groundnuts cultivars. The weaning flour contents zinc and iron. The value of zinc obtained for maize composite flour is higher than the value (2.7mg) reported by Kunyanga et al. [51] in maize based formulation while the value of iron is lower than the value (5.7mg) reported by the same authors. Beside nutritional contribution, the weaning flour offer health beneficial due to the presence of bioactive compounds. Lutein and zeaxanthin have gained interest due to their association with eye health [47]. Lycopene and  $\beta$  carotene has retained attention as an antioxidant in preventing chronic disease. The weaning flour content phenols and display antioxidant activity which offer several health beneficial due to their main roles as lipid stabilizers and as suppressors of excessive oxidation that causes cancer and ageing [12]. These findings suggest that maize based formulation have a great potentiality as weaning flour. It can offer several healths beneficial and it satisfies the recommended energy and macronutrients requirement according to the codex Alimentarius commission standards. It

can be used in managing protein energy malnutrition by preparing adequate weaning food with flour blended according to the percentage of ingredients stipulated in the optimal formula. Beside macronutrients, there are minerals and vitamins which contribute to the healthy development of a child. For micronutrient intakes and status, we focused on iron, zinc and vitamin A because as stated by Dewey and Adu-Afarwuah, [22], these are considered key 'problem' nutrients in many developing countries. The average daily intake of these micronutrients increases with the increase of the serving portion which is related to the age of the child. Taking into consideration their contribution in meeting the micronutrients daily intake suggested reference value, a highest daily serving portion of 132-137 intended for 12-23 months old child is the most suitable. Without any micronutrients fortification, the maize based flour formulated is most suitable for infants between ages of 12-23 months. In all the cases, the weaning flours need to be fortified with more micronutrients or supplemented with synthetic nutrients and this represent a challenge for future study.

#### Conclusion

The study has shown that maize composite flour produced from maize, soybean, germinated maize, carrot, egg shell flours, groundnut paste using optimal formula has a great potentiality as weaning flour as it can satisfies the recommended energy and macronutrients requirement according to the codex Alimentarius commission standards. Therefore, it can be used in managing protein energy malnutrition. Moreover, it offers several healths beneficial due to the presence of many bioactive compounds. Taking micronutrient into consideration, the maize based flour formulated is most suitable for infants between ages of 12-23 months. In all the cases, the weaning flours need to be fortified with more micronutrients or supplemented with synthetic nutrients and this represent a challenge for future study.

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#### Conflict of interest

No potential conflict of interest relevant to this article was reported.

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