Original Research Article

Formulation of Adequate Complementary Food for Children 0–24 months Using Local Staple Foods

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ABSTRACT

Background: Malnutrition is still among the leading causes of death in children >5 years, contributing to about 33% of deaths in infants, and may sometimes result from poor complementary feeding practices. WHO recommends exclusive breastfeeding for the first six months of the baby's life, followed by gradual introduction of complementary foods, with continuous breastfeeding till baby is at least two years. Most babies are not exclusively breastfed for six months, and even more not breastfed till 24 months. Most complementary foods in developing countries are homemade, with plant-based ingredients, using improper processing techniques, making them low in nutrient content.

Aims: This work aimed at formulating Complementary foods from ten different proportions of yellow maize, rice, potatoes, egg whites, soybeans, pawpaw, watermelon, pineapple and oranges, using standard processing techniques.

Methodology: The blended (A to J) were formulated and were evaluated for colour, taste, flavor and consistency using nine scaled hedonic point, and the preferred five were evaluated for proximate composition, some vitamins and minerals, functional properties, and for microbes using standard AOAC methods.

Results: Results from sensory analysis showed that the preferred formulas were B, H, A, C, G, respectively. Moisture contents ranged from 4.6 - 10.5%, ash 2.65 - 3.70%, fibre 6.43 - 9.27%, protein ranged from 17.72 - 37.72%, fat ranged from 9.5 - 14%, carbohydrate 38.74 - 63.58%, and energy from 394.9Kcal - 433.2Kcal. Micronutrient analysis revealed that all the formulas had no vitamin C, while they had vitamin A contents ranging from 95.85 - 2340IU. Calcium from 378 - 632mg/100g, iron from 4.73 - 8.59mg/100g, phosphorous from 109.04 - 136.49mg/100g, and Zinc ranged from 1.47 - 2.35mg/100g. Magnesium had ranges of 53.32 - 85.19mg/100g, sodium ranged from 2.42 - 189.41mg/100g, while potassium ranged from 319.2 - 728.82mg/100g. For functional properties, water absorption and oil absorption capacity ranged from 1.5 - 4.4 and 1.5 - 1.95 respectively, Loose and packaged bulk density ranged from 0.51 - 0.54 and 0.78 - 0.92 respectively. The swelling index ranged from 1.03 - 1.11, foaming capacity and foam stability from 2.0 - 18.0 and 0.0 - 0.7 respectively, while the dispersibilities ranged from 52.5 - 92.5%. Microbial analysis revealed that none of the formulas contained yeast, C had no coliform while formula B had highest coliform (620 CFUs), while C had highest TBC (31000CFUs).

Conclusion: Formula C, with sweet potatoes as main starch source, looked most promising as a complementary food.

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Keywords: Keywords: Complementary food, Functional properties, Micronutrient, Malnutrition, Children, Breastfeeding

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1. INTRODUCTION

The first 24 months of a child's life are most important for optimal growth promotion, behavioral development and health [1]. At these early stages of life, appropriate nutrition is required to support the rapid growth and development of the child [2]. Undernutrition, in the forms of protein-energy malnutrition and micronutrient deficiency, is still a serious health problem in the world, especially in Africa. Infant malnutrition has been linked to morbidity and mortality [3]. WHO, in 2020, estimated that malnutrition contributed to about 45% of child deaths in children globally, with an estimated 149 million children stunted and 45 million wasted, with Africa having the highest number of each of these cases [4]. Arimond and Ruel (2004), were able to directly link malnutrition in 0 - 24months to poor breastfeeding and complementary feeding practices [5]. Even though exclusive breastfeeding is recommended for the first six months of the child's life, as breastmilk protects the baby from infections by virtue of its antibody and immunoprotective contents [6], less than 37% of infants are exclusively breastfed for the first six months of their life [7]. As from 6 months, however, breastmilk alone is not sufficient to meet the energy and nutrient needs of the child, and complementary foods must be introduced [8].

At early stage, the quantity and quality of complementary foods fed to the infant, the socio-economic state of the family, the general hygiene conditions of the complementary food and the environment, among other factors, play an important role on the health and growth of the child, and these factors often contribute to malnutrition, which may be difficult to reverse [9].

Sub-standard food processing techniques and poor hygiene conditions are some of the poor complementary practices that may lead to malnutrition [10]. The quality of the complementary food and its hygiene conditions can be affected by how it is processed [11].

WHO recommends that complementary foods be formulated at home [3]. However, formulating at home is usually done by inexperienced mothers and caregivers who do not take into account Recommended Dietary Allowances (RDAs),the need for hygiene and the essence of complementary food fortification. Africa is a developing continent, characterized by an overall poverty rate of about 43% [12]. This poverty rate is coupled with saturated households, poor living standards and pitiable hygiene conditions. A combination of all these factors plays a role in the quantity and quality of complementary foods fed to the infant, meal frequency and meal diversity.

This study therefore tackles the problem of malnutrition by formulating a low-cost complementary food out of readily available ingredients which will cater for the needs of Cameroonian infants, most of which are from households which are too poor to afford imported complementary foods. Also, there is little or no data available on the local consumed complementary foods in Cameroon such as pap, ekwang, soyaconya etc. These foods are produced by mothers and caregivers who have little on food processing, and about the importance of meeting the RDAs of infants. A complementary food, produced using processing techniques for nutrient optimization, whose nutrient content is known and documented is important.

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2. MATERIAL AND METHODS

2.1 Sample Collection, Preparation, Processing and Formulation

2.1.1 Sample Collection

Yellow maize, white rice, sweet potatoes, irish potatoes, soybeans, eggs, pawpaw, watermelon, pineapple, oranges, sugar, soybeans oil and artificial food flavouring were bought from the local markets in Muea and Mutengene, South West region, Cameroon.

2.1.2. Sample Preparation and Processing

2.1.2.1 Preparation of flours

Malted corn flour: 5kg of yellow maize (*Zea mays*) were sorted for stones, dirt, insects and bad grains, then soaked overnight using tap water, with the water level twice above the maize level. After one night, the maize was kept in a cool, dry place, and covered with plastic bags for germination to occur. After three days, when more than 80% of the maize had germinated, it was soaked again in water for 72 hours, the water changed every six hours. After the 72 hours, it was ground using an electric blender, into a smooth paste. 5 liters of water were added to the paste and stirred, and it was filtered using a clean, white muslin cloth, and the filtrate covered overnight in a clean bucket. The precipitate from the bucket was collected and dried at 50°C for 24 hours, till the moisture content was less than 5%. The resulting dry matter was ground using a dry electric blender, and sieved. The flour obtained was stored in zip-lock bags, inside an air-tight container at room temperature.

Rice flour: This was done using the method described by An-I [13], with slight modifications. 2kg of white, imported rice (*Oryza sativa*, subsp. indica) were sorted for stones, insects and dirt, and washed twice using running tap water. 10litres of water were placed on a gas stove, and left for 20 minutes till it started boiling. The washed rice was added to the boiling water on the stove, and left to cook for 10 minutes, after which it was removed and placed in a sieve to strain the excess water. The cooked rice was dried at 50°C for 24 hours, ground using a dry electric blender, and sieved using a 0.1mm sieve. The resulting flour was stored in zip-lock bags, inside an air-tight container at room temperature.

Sweet potato flour: This was produced using the method proposed by Maninder and Kawaljit [14], with slight modifications.70 healthy, medium-sized sweet potatoes (*Ipomoea batatas*) were washed using an iron sponge, and placed in a pot of water on a gas stove. They were boiled for 20 minutes, until tender, and peeled with a knife. The peeled potatoes were mashed in a clean bowl using a pestle and the resulting mashed potatoes were dried in an oven at 50°C for 24 hours, ground using a dry electric blender, and sieved using a 0.1mm sieve. The resulting flour was stored in zip-lock bags, inside an air-tight container at room temperature.

Irish potato flour: This was produced using the method described by Maninder and Kawaljit [14], with slight modifications. 200 healthy, medium-sized irish potatoes (*Solanum tuberosum*) were washed using an iron sponge, peeled and washed in running tap water. The potatoes were placed in a pot of water on a gas stove and boiled for 30 minutes until tender. The boiled potatoes were mashed in a clean bowl using a pestle and the resulting mashed potatoes were dried in an oven at 50°C for 24 hours, ground using a dry electric blender, and sieved. The resulting flour was stored in zip-lock bags, inside an air-tight container at room temperature.

Soy protein flour: 2kg of soybeans was sorted for stones, dirt, bad grains and insects, and roasted for 20 minutes at low heat using a gas stove, until they gave off a pleasant flavour. The roasted beans were soaked for 48 hours, with the water level twice above the soybeans level. The water was changed every 6 hours, and at the end of 48 hours, the soybeans were ground using an electric blender into a smooth paste. 5litres of water were added to the paste, stirred, and filtered using a clean, white muslin cloth. The filtrate was boiled on a gas stove for 30 minutes, and 3litres of acetic acid added, bit by bit, until a white precipitate was formed. The precipitate was separated from the supernatant by decanting the supernatant, and washed in running tap water to remove the acetic acid. The precipitate was dried in an oven at 50°C for 24 hours, ground using a dry electric blender, and sieved. The resulting flour was stored in zip-lock bags, inside an air-tight container at room temperature.

Egg white flour: 90 fresh eggs were cracked open, and the yolks removed. The whites were dried at 50°C for 24 hours, ground using a dry electric blender, and sieved. The resulting flour was stored in zip-lock bags, inside an air-tight container at room temperature.

2.1.2.2 Preparation of fruit juices

Fifteen medium sized oranges, 2 large pineapples, 2 large pawpaw fruits and one large watermelon were washed in running tap water and peeled. The peeled fruits were juiced individually using an electric juicer machine, sieved using a 0.1mm sieve, and stored in separate sterile containers at 4°C till when it was ready to be used.

2.1.2.3: Formulation

The flours and juices were mixed in ten different proportions as shown on table 1 below, to give ten different food blends. After mixing, the mixture was blended for 2 minutes using an electric blender, and the homogenized paste dried at 50°C, for 24 hours in a hot air oven. The resulting dry matter was ground using an electric blender, and sieved using a 1mm sieve. The blends were stored in air-tight bags, in a sealed container, at room temperature. After mixing of flours, juices and artificial flavours, the mixtures were homogenized using an electric blender, placed on sterile trays and dried at 50°C for 24 hours using a hot-air oven. The resulting dry matters were ground using a dry blender, sieved and the fine flours stored in sterile zip-lock bags at room temperature.

2.1.2.4 Sensory analysis

This was carried out by 20 trained panelists at the boardroom of the Faculty of Science, University of Buea. The panelists consisted of mothers who had babies between the ages of 0 – 24 months. It was done using the 9-point hedonic scale, as described by Dzung et al. [15]. The ten formulas were evaluated by each of the 20 panelists for colour, flavour, texture and taste using the 9-point hedonic scale, 1 representing 'dislike extremely', 5 representing 'neither like nor dislike', and 9 representing 'like extremely', and phosphatine® was used as a control complementary food. The best five formulas were picked after analysing the sensory analysis data, and these 5 were used for nutrient analysis, functional properties analysis and estimation of microbial load.

2.2 Nutrient analysis

2.2.1. Proximate Analysis

The moisture, ash, fibres and crude protein contents were determined using standard methods as described by AOAC [16]. The total carbohydrate content was estimated by method of difference, and energy values were calculated using the Atwater's conversion factors [17].

Table 1. Formulation table for formulation of ten different complementary food blends

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Food samples/ Blends	A	B	C	D	E	F	G	H	I	J

Corn Flour (g)	58	0	0	0	29	29	29	0	0	0
Rice Flour (g)	0	58	0	0	29	0	0	29	29	0
SP Flour (g)	0	0	58	0	0	29	0	29	0	29
IP Flour (g)	0	0	0	58	0	0	29	0	29	29
Egg White Flour (g)	10	7	8	6	10	8	6	5	3	5
Soy Protein Flour (g)	10	7	8	6	10	8	6	5	3	5
Sugar (g)	8	10	0	7	10	5	7	5	7	5
Soybeans oil (ml)	5	5	5	5	5	5	5	5	5	5
Watermelon juice (ml)	50	50	50	50	50	50	50	50	50	50
Pineapple juice (ml)	0	10	0	10	0	0	0	10	0	0
Orange juice (ml)	10	0	10	0	10	0	0	0	0	0
Pawpaw juice (ml)	40	40	40	40	40	50	50	40	50	50
Milk flavour (ml)	5	5	5	5	5	5	5	5	5	5
Coconut flavour (ml)	5	5	5	5	5	5	5	5	5	5
Baking soda (g)	12	12	12	12	12	12	12	12	12	12

2.2.2. Micronutrient analysis

2.2.2.1 Mineral Analysis

Iron, zinc, phosphorous, sodium, potassium, magnesium and calcium contents were determined by Atomic Absorption Spectrophotometer, Hitachi Model 180-80, and Ion Chromatographic Analyzer ICA model IC 100 [18].

2.2.2.2 Vitamin Analysis

Vitamins A and C content of the food samples were estimated using methods described by AOAC [16]. For vitamin A, the optical density was read from a spectrophotometer at 436nm, and the β-carotene content of the samples calculated using the formula:

β-carotene (µg/100g) = (Absorbance at 436nm x volume x dilution factor x 100)/(weight x dry matter (%))

For vitamin C, titration was done using acetic acid as an indicator, and a vitamin C standard was used. The titration was done in duplicate by titrating 5 ml Diclorophenolindophenol (DCP) with each of the supernatants from the samples, and the volume of standard solution which changed the DCP to colourless recorded in each case. The vitamin C content in the samples was calculated using the formula:

Vitamin C (mg) = (Vol. of sample x vol.of vitamin C standard x conc. of vit C standard)/(volume of titrated sample)

2.2.3 Determination of Functional Properties

Water absorption capacity (WAC) and Oil absorption capacity (OAC) were determined using a modified method described by Lin et al. [19]; Bulk Density (BD) was determined by using the method described by Wang & Kinsella [20], with slight modifications. Foaming Capacity (FC) and foam stability (FS) were determined by the method described by Cherry & McWatter [21], while the method of Abbey & Ibeh [22] was used to determine swelling index. Dispersibility of each of the five blends was determined as described by Kulkarni et al. [23].

2.2.4 Microbial analysis

The total yeast, bacteria and coliform present in the samples was estimated as described by Olorunjuwon et al. [24]. Sabouraud Dextrose agar (SDA) was used as growth medium for yeast, Plate count agar (PCA) for bacteria and Violet Red Bile Agar (VRBA) for coliform. The microbial counts were read from the petri dishes as colony forming units

2.3 Subjects

The study was reviewed and approved by the Regional Delegation of Public Health for the South west, and informed consent was obtained from each subject/panelist prior to their participation in the study.

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2.4. Statistical Analysis

Raw data were computed using Microsoft EXCEL 2007. All data, were presented as mean ± SD and was analysed using one-way analyses of variance (ANOVA) using Graphpad software to test the level of significance at 5% probability ($p<0.05$). Bonferroni Test was used to separate the means where significant differences existed.

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3. RESULTS AND DISCUSSION

3.1 Sensory analysis

For food, the sensory qualities are important, as no one will consume any food which does not look, smell or taste nice, no matter how nutritious it may be. For complementary foods, their sensory properties are as important as their energy densities [25]. Sensory evaluation was difficult to carry out because of the low literacy level of most of the mothers involved. Based on the evaluation of colour, flavour, taste and consistency by the 20 panelists, the following observations were made and the following results generated as shown on table 2 below.

The results indicated that the most likeable formulas, based on their overall acceptability, were B, H, A, C, G, E, D, F, I and J, in order of merit. This implies B was most accepted, while J was the least accepted. An 88% acceptability rate was found for their complementary food formulated from fermented maize, rice, soybeans and fishmeal [26]. Phosphatine, which served as the control, had the best score in all four parameters, and therefore had the best overall acceptability as compared to all the ten formulas. Among the ten formulas, formula B had the best colour, followed by A, while I and J had the worst colours. B had the best taste, while formulas I and J

were tied for worst tastes. For the flavour, formula B had the best flavour, followed by formula A, while formula J had the worst. Formulas B, C and H were tied for best consistencies, while formula J had the worst. The least acceptable formulas (F, I and J) contained no pineapple juice and no orange juice. It could therefore be deduced that, the pineapple and orange juice used in this formulation played a positive role in the enhancement of the general acceptability of the formulas, either by improving their flavour, texture or taste.

Table 2: Mean and standard deviations of sensory scores of porridges made from the formulated diets and a commercial complementary food (Phosphatine)

Food Formulas	Mean Colour	Mean Taste	Mean Flavour	Mean Consistency	Overall Acceptability	Rank
F1 (A)	7.50±1.2	7.00±1.2	7.25±1.9	6.90±1.5	7.16±0.2	3rd
F2 (B)	7.70±1.1	7.25±1.3	7.60±1.2	7.25±0.9	7.46±0.2	1st
F3 (C)	7.00±1.3	7.00±1.2	6.70±2.5	7.25±0.6	6.99±0.2	4th
F4 (D)	6.00±1.3	6.40±1.2	6.70±2.0	6.65±1.1	6.44±0.3	7th
F5 (E)	6.85±2.1	7.15±1.1	6.60±1.6	6.50±1.6	6.78±0.3	6th
F6 (F)	6.60±1.0	6.40±0.9	6.50±1.7	6.65±1.3	6.54±0.1	8th
F7 (G)	7.05±1.1	7.00±0.6	6.70±2.0	7.10±1.0	6.96±0.2	5th
F8 (H)	7.50±1.4	7.15±2.3	7.25±1.2	7.25±1.3	7.29±0.1	2nd
F9 (I)	6.4±2.4	6.30±2.1	6.25±1.8	6.65±1.8	6.40±0.2	9th
F10 (J)	6.15±1.8	6.30±2.0	5.50±2.6	6.50±1.4	6.11±0.4	10th
11 (Phosphatine)	8.00±0.2	8.20±1.4	7.70±1.8	7.40±0.4	7.83±0.3	

3.2 Proximate analysis

Table 3 below gives a summary of the proximate composition of the formulas. The moisture contents of 4 out of 5 of the formulas were higher than the recommended 5% prescribed by the WHO as the maximum moisture content for complementary foods. Formula C was the exception, having moisture content of 4.6%. G had a moisture content of 6.4%, A had 7.6%, B having 8.35%, and H being highest with 10.5%. There was no statistical significance ($P > 0.05$) however, between the moisture contents of the formulas and the reference value.

The most preferred formula (B) had a moisture content of 8.35%, higher than the recommended 5%, making it unsuitable for long term storage. This implies formula C would have the longest shelf life, while formula H would easily favour the growth of microbes, hence a shorter shelf life. These findings are in line with the results obtained by Laryea et al. (2018) [27], who recorded a 4.8% moisture content for a complementary food made from sweet potatoes and soybeans. Mahmoud et al. (2014) [28] recorded a moisture content of 6.25% when they formulated a complementary food comprising of sweet potatoes, rice faba beans and peanut oil. This is close to the 10.5% moisture observed in formula H whose main starch sources were rice and sweet potatoes. The lowest moisture content observed in the formula made out of sweet potatoes and highest observed from that made out of sweet potatoes and rice could as well mean that most of the moisture from formula H could be coming from rice. This is justified by formula B, whose main starch source is rice, having the next to highest moisture content (8.35%) after formula H.

For the ash contents, three out of five formulas had higher ash contents than the recommended value of 2.9, even though the difference was not statistically significant (P>0.05) for all five formulas. Formulas A and H had the least ash contents (2.65%), and these were the only two formulas whose ash contents were within the reference range. G had an ash content of 2.95%, while C had 3.10%. the formula with the highest ash content turned out to be B, with 3.70%. In terms of ash content therefore, formulas A and H were the best. The presence of ash in the formulas is indicative of the presence of minerals in them, and hence the formulated complementary foods could be used in the fight against micronutrient deficiency in children. WHO recommends an ash content of 2.9g for every 100g of food sample for complementary foods, and the five formulas all have ash contents within this range, with some slightly higher, like formula B (the most preferred formula), whose ash content was up to 3.7%. Mahmoud et al. [28] found an ash content of 2.91% for their complementary food formulated from rice, sweet potatoes, faba beans and peanut oil. Tiencheu et al [29] recorded higher ash contents (4.32% - 4.85%) for their own formulations made out of egg whites, fermented maize, pawpaw and beans. Akinola et al. [30] recorded higher ash contents (5.21 – 7.52%) in their complementary foods formulated from yellow maize, soybeans, guinea corn, millet, groundnuts, carrots and crayfish.

The fiber content of all the formulas was very high; higher than the recommended value of 3.8% for complementary foods. However, there was no statistical significance (P>0.05) between the fibre content of any of the formulas and the standard value. Among the formulas, formula H had the highest fibre content (9.27%), followed by formula B with a fibre content of 7.32%, then formula C with 7.29%, and formulas A and G had the least fibre contents, with A having a value of 7.13%, and G, the least, with a fibre content of 6.43%.The fibre content of a sample is a measure of how much undigestible carbohydrates are present in that sample. The fibre content in the samples was extremely high, significantly higher than the recommended 3.8% by WHO. Complementary foods are supposed to be low in fibre, so that the baby gets a chance to eat as frequently as the need arises, without fear of him feeling too full as a result of slow digestion due to too much fibre present in the food. The findings in this study were contrary to findings reported by Shewangzaw et al. [31] from their complementary food formulas made from a mix of soybeans, teff, white maize and honey bee larvae, where they found much lower fibre contents in the range of 2.75 – 4.52. The high fibre content of all five formulas makes them not so ideal for a complementary food.
For protein contents, all samples had very high protein contents, higher than the standard value of 15% which is prescribed by WHO for complementary foods. This difference between the formulas and the standard value was however insignificant (P>0.05) for four out of five formulas.

Only formula B, with a protein content of 37.72%, showed a significant difference (P<0.05) between its protein content and the reference value. The lowest protein content was found in formula C (17.72%), followed by H with a protein content of 18.11%, then G with 20.13%, then A and B had the highest protein contents of 25.82% and 37.72% respectively. The high protein content of the five formulas could be considered a good thing, since protein energy malnutrition rates are still so high in Africa. However, in as much as a high protein diet is needed for infants, overnutrition is still a form of malnutrition, and very high amounts of any of these nutrients could result in toxicity. Mahmoud et al. [28] found a 7.48% and 4.94% protein content for rice and sweet potato flours respectively, implying that rice has more crude proteins than sweet potatoes. This is logical, considering the fact that the formula with rice had a higher protein content than the one with sweet potatoes. The germination and malting of the maize used in this study improved on its protein content, making the blend with maize as main starch source to have the second highest protein content, after the one with rice. Tadesse and Gutema [32] had lower protein contents in their complementary food formulated from beans (*Phaseolus vulgaris*), sorghum (*Sorghum bicolor*) and carrots (*Daucus carota*), and they had protein contents in the range of 8.34 – 12.56%, lower than WHO standards.

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The results obtained from the analysis of the fat content of the formulas showed them having fat contents in the range 9.5% - 14%, all of which are above the reference value of 8%. The only formula which showed a statistically significant difference (P<0.05) between its fat content and the standard value was formula G, with the highest value of 14%. B had a fat content of 11.5%, while A and C had 11%. The lowest fat content was recorded in H (9.5%), though this was still higher than the reference.

As in the case of the higher than recommended protein content, a higher fat content could be a good thing, as well as it could lead to toxicity. Aduni et al. [33] found fat contents in the range of 3.15 to 14.35% for their nine instant weaning foods made out of crayfish, carrot, irish potatoes, soybeans and Ndop rice. Jahan et al. [34] obtained similar results of fat content in their formulation using sweet potatoes, mung-beans, soybeans and wheat. Their formulas had fat contents in the range 9.29 – 11.40% for their three formulas.

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The carbohydrate contents were all lower than the standard value of 64.68%, with the least carbohydrate content obtained from B (38.74%), and the highest from C (63.58%). H had a carbohydrate content of 59.24%, while G had 56.53%. B and A had the least values of 53.14% and 38.74% respectively. A comparison between these values and the standard value showed no statistically significant difference (P>0.05) between formulas A, C, G, H and the standard, meanwhile formula B, with its very low carbohydrate content, was statistically different from the reference value at $p < 0.05$. Anigo et al. [35] obtained dissimilar results for carbohydrate content from their formula which was a blend of soybeans, groundnuts, guinea corn, sorghum, corn and millet in different proportions. Their mixes had carbohydrate contents in the range of 88.75% - 90.89%. However, apart from formula B whose carbohydrate content was significantly low, the other four mixes had good enough, though substandard, carbohydrate levels, and this, with a mix of adequate protein content, makes the novel formulas suitable for complementary feeding. Even though the most liked formula was B, its extremely low carbohydrate content makes it not an ideal complementary food for a growing infant.

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Table 3: Proximate analysis of the formulae

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Samples	Moisture Content %)	Ash Content (%)	Fibre Content (%)	Protein Content (%)	Fat Content (%)	Carbohydrate. Content (%)	Energy Content (Kcal)
WHO standard	**5**	**2.9**	**3.8**	**15**	**8**	**64.68**	**400**
A	7.6±0.80^{a}	2.65±0.15^{a}	7.13±1.43^{a}	25.82±3.07^{a}	11.00±1.00^{a}	53.14±2.92^{a}	414.8±8.40^{a}
B	8.35±0.15^{b}	3.70±0.40^{a}	7.32±1.42^{a}	37.72±9.54^{b}	11.50±0.50^{a}	38.74±8.49^{b}	409.3±0.30^{a}
C	4.60±0.70^{a}	3.10±0.00^{a}	7.29±0.73^{a}	17.72±0.66^{a}	11.00±2.00^{a}	63.58±2.04^{a}	433.2±1.80^{b}
G	6.4±0.20^{a}	2.95±0.05^{a}	6.43±2.23^{a}	20.13±0.44^{a}	14.00±0.00^{b}	56.53±0.29^{a}	432.6±0.60^{b}
H	10.5±0.00^{b}	2.65±0.05^{a}	9.27±0.15^{a}	18.11±1.05^{a}	9.50±0.50^{a}	59.24±1.50^{a}	394.9±2.70^{a}

The superscripts a = statistical significance at $p < 0.05$ and b = significance at $p < 0.01$ compare to WHO reference pattern value

Comment [u34]: sure it included before 6 month child ?

The energy content of the five formulas, which is a function of the carbohydrate, fat and protein content of each one of them, was higher than the recommended value of 400Kcal for four out of the five formulas., it was noted that apart from formula H whose energy content was below (394.9Kcal) the reference value of 400Kcal, all other formulas had energy contents above this standard value. Formula C was highest, with an energy value of 433.2Kcal, followed by G (432.6Kcal). Formula A had a value of 414.8Kcal, while B had a value of 409.3Kcal. Comparing

these values with the standard value showed no statistically significant difference (P>0.05) between formulas A, B, H and the standard, while formulas C and G showed statistically significant differences from the standard (P<0.05). Araro et al. [36] got similar results in their complementary food mixes made with sweet potatoes, brown teff, and dark red kidney beans. Their mixes had energy levels in the range of 339.07 – 356.74%, values which were all slightly lower than the recommended value, but with no significant difference. The high energy levels of the five formulas, which are as a result of high protein, carbohydrate and fat levels, makes them a suitable complementary food.

3.3 Micronutrient analysis

Table 4 gives a summary of minerals and vitamins analyses of the five samples, pap and the WHO standard values for each of these micronutrients.
For the vitamin analysis, two vitamins were analyzed; A and C, with A representing the fat-soluble vitamins and C representing the water-soluble vitamins. The vitamin A content of the five formulas was estimated and recorded in terms of international units (IU). The analysis of vitamin A content in the five formulas showed a great vitamin A content in all five samples. The only formula whose vitamin A content was below the recommended value of 300IU was A, with a vitamin A content of 95.85IU. The other four formulas had values above 1000IU. The sample with highest vitamin A content was formula H, with a value of 2340.0IU, followed by formula C with 1403.35IU, then formula G with 1226.65IU, and then B with 1101.65IU. Comparing these values obtained for vitamin A with the standard value revealed no statistically significant difference (P>0.05) between sample A and the standard value, but all the other formulas had statistically significant difference (P<0.01) with the standard. The presence of vitamin A in sufficient amounts in the food is extremely important, as this could go a long way in contributing in the fight against micronutrient deficiency in Cameroonian children. The formula which was made up of rice and sweet potatoes (G) was highest in vitamin A content, followed by the formula which only had sweet potato as main starch source (C), and this is logical, as among all the starch sources used, sweet potatoes have been found to have the highest vitamin A content. Similar results were obtained for vitamin A content, for complementary food mixes which were sweet-potato based [27]. They had vitamin A content in the range 500 – 1766 IU, acceptable values of vitamin A for any complementary food. The range of values obtained for vitamin A content for the five novel formulas make them good choices for complementary foods, with the exception of formula A whose vitamin A content was significantly lower than the recommended value of vitamin A for complementary foods.

The analysis of vitamin C content for the novel formulas resulted in negative results for all five formulas. None of the formulas showed any positive results for the presence of vitamin C in them, as there was no amount of DCP within the range that could turn any of the formulas colourless. According to the results obtained, it was seen that there was very little or no vitamin C present in any of the five formulas. Either the vitamin was totally absent, or the amounts present were too minute to change DCP from pink to colourless. This is not good, especially for a growing child, especially as they need all the nutrients in the required amounts for proper formation and development. This implies the complementary food either needs to be fortified with vitamin C, or the child takes it alongside another complementary food which is rich in vitamin C, in order to balance up the RDA for vitamin C. Bassey et al. [37] found a vitamin C content of 1.54mg/100g in their complementary food mix formulated from cooking banana, cowpea and groundnuts. This value obtained is similar to the one obtained in this study, as 1.54mg/100g is closer to zero than it is to the standard value of 15mg/100g which is recommended by WHO.

From the mineral analysis of the samples, the calcium content of the formulas was above the reference value (341.2mg/100g). This difference was however, not statistically significant (P>0.05), except for formula B whose difference with the reference value was statistically

significant at P<0.05. Among the five formulas, formula B, whose main starch source was rice, had the highest calcium content (632mg/100g), followed by C with 454mg/100g of calcium, then formulas G and H with calcium contents of 408.0mg/100g, and the formula with the least calcium content was formula A, with corn as main starch source, with 378.0mg/100g. It is of utmost importance that the novel formulas are up to standard with their calcium content, as calcium is extremely important for the brain and bone development of the infant. Plahar [38] found similar results for his sweet potato-based formulas which contained groundnuts, but lower calcium content in similar sweet potato-based formulas which did not contain groundnuts. He recorded a calcium content of 256.57mg/100g and 357.89mg/100g in the former formulas, while the latter formulas had calcium contents of 100.73 and 91.96mg/100g. Ajiwe and Nwaigbo [39] had dissimilar results in their formulas made from different proportions of yellow maize, millet, red sorghum, wheat, brown spotted African yam bean, bambara groundnut, pigeon pea and soybeans. From the 10 blends, they obtained calcium contents in the range of 42.19 – 140.76mg/100g, with the lowest value obtained from the mix of wheat, millet and pigeon pea, and the highest calcium content in the blend containing millet, pigeon pea and African yam bean.

The iron content of the formulas was generally lower than the reference value of 8.5mg/100g, except C, with sweet potatoes as main starch source, which had an iron content of 8.59mg/100g. The difference in iron content between all five formulas and the reference value was however not statistically significant (P>0.05). The iron contents ranged from 4.73mg/100g (formula H) to 8.59mg/100g (Formula C), with G coming below C with an iron content of 6.30mg/100g, followed by B with 6.27mg/100g, then A, with corn as main ingredient, with 6.03mg/100g. This could be explained by the fermentation process done on the corn, since fermentation has been shown to enhance the bioavailability of several micronutrients which are usually coupled to phytates in the unfermented grains. Satter et al. [40] found similar results from their complementary food formulated from wheat, soybeans, sugar, mango, skimmed milk and jackfruit. They had values for iron content in the range of 7.56 – 8.22mg/100g. Ikujenlola and Adurotoye [41] had much higher values of iron content in their complementary food formulated from high protein maize and steamed cowpea. Their values ranged from 260 – 390mg/100g, way above the recommended 8.5mg/100g standard value. The iron contents of the complementary foods, even though lower than normal, were not significantly different from the standard values, but the infants would also be recommended that these formulas be fortified with iron supplements in order that the infant's daily requirements for iron are met, as the role of iron in the body is very vital, and it is important that its RDA is always met.

The WHO standard for phosphorous in a complementary food is set at approximately 100mg/100g. All five formulas were found to be higher in phosphorous than the standard value. The range of phosphorous values were from 109.04 – 136.49mg/100g, with formula C, containing sweet potato as main starch source, having the highest phosphorous content. Except for formula C, the difference in phosphorous content between the standard values and the values obtained in the formulas was not statistically significant (P>0.05). There was a significant difference in phosphorous content between formula C and the standard at P<0.05. Among the formulas, C had the highest phosphorous content (136.49mg/100g), followed by A with a phosphorous content of 119.28mg/100g, then G with 114.63mg/100g, then B with 109.98mg/100g, and the least being H with 109.04mg/100g. Tiencheu et al. (2016) [29] had much higher values (286.37 – 365.08mg/100g) for phosphorous in their complementary food formulated from maize, pawpaw, red beans and mackerel fish meal, same as Anigo et al. [35] who had higher values in the range of 148.98 – 219.98mg/100g in their formulations made from guinea corn, sorghum, maize, millet, soybeans and groundnuts.

The analysis of zinc content revealed that all five samples were lower than the recommended value of 3.7mg/100g set by WHO. The range of zinc content of the five samples was 1.47mg/100g to 2.35mg/100g. This difference between the standard value and the values

obtained from the samples was statistically significant (P<0.05) for two out of five samples (C and G), but not statistically significant for the other three formulas (A, B and H). Among the five formulas, formula A was richest in zinc (2.35mg/100g), followed by B with 2.32mg/100g. Next was formula H with a zinc content of 1.87mg/100g, and the least were formulas C and G, whose zinc contents were 1.56mg/100g and 1.49mg/100g respectively. Gemede [42] had slightly higher values, in the range of 2.73 – 3.00 mg/100g for zinc content of his complementary food formulated from maize, pea and anchote flours, while Asouzu and Nkemjika [43] had similar results ranging from 1.52 – 2.61mg/100g in their complementary food formulated with maize and supplemented crayfish and carrot flour.

The analysis of magnesium content of the five samples showed that the two formulas with the highest magnesium contents were C (85.19mg/100g) and H (75.75mg/100g). Formula B had a magnesium content of 72.91mg/100g, while A had 70.6mg/100g. The least formula was G, with 58.32mg of magnesium per 100g of formula. The WHO standard for magnesium in complementary foods is 48.7mg/100g, and this standard was clearly met and surpassed by all five samples, though the difference was statistically insignificant (P>0.05) for all five samples. Bolarinwa et al. [44] had dissimilar values, ranging from 0.21 – 0.24mg/100g. Mohammed et al. [45] had similar results of magnesium content, a value of 54.44mg/100g for his complementary food mix made up of an improved variety of yellow maize, soybeans and African catfish meal.

The sodium content of the five formulas was analyzed and it was realized that there was a statistically significant difference (P<0.01) between four of the formulas (B, C, G and H) and the reference value of 60mg/100g. Only formula A had no statistically significant difference with the reference value. For the five formulas, the sodium content ranged from 102.42mg/100g (formula A), through 136.72mg/100g for formulas G and H, to 162.02mg/100g for formula C, and the most being formula B with 189.41mg/100g.

Also, the analysis of potassium content revealed that formulas B, G and H had similar potassium contents (611.49mg/100g), and this was the highest value observed among the five formulas. C had a potassium content of 728.82mg/100g, while A had least value (319.2mg/100g). The recommended value for potassium for a complementary food is 408.7mg/100g. Apart from formula A whose value for potassium content was below standard, all the other formulas had higher than the standard values for potassium content. Comparing these differences in value between the standard and the values obtained for the five formulas showed great statistical significance for all five formulas (P<0.01 for formula A, and P<0.001 for formulas B, C, G and H). Solomon [46] obtained values of 11.1 – 21.1mg/100g for sodium content, and 99.7 to 129.7mg/100g of potassium for a complementary food based on rice, maize, acha grains, soybeans, groundnuts, bambara nuts and crayfish, both of which were below the standard. Aduni et al. [33] on the other hand, had similar results for sodium and potassium contents for their complementary foods, with sodium ranging from 74.50 – 88.17, and potassium from 241.87 – 1322.27mg/100g. The most preferred formula (B) had the highest sodium content, and a satisfactory potassium content as well.

Table 4: Micronutrient analysis of the formulated complementary foods

SAMPLES	WHO standard	A	B	C	G	H

Vit. A (IU)	**300**	95.85±4.15[a]	1101.65±36.65[c]	1403.35±83.35[c]	1226.65±66.65[c]	2340.0±31.7[c]
Vit. C (mg/100g)	**15**	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]
Ca (mg/100g)	**341.2**	378.0±14.0[a]	632.0±12.0[b]	454.0±2.0[a]	408.0±16.0[a]	408.0±16.0[a]
Fe (mg/100g)	**8.5**	6.03±0.21[a]	6.27±1.3[a]	8.59±2.7[a]	6.30±0.8[a]	4.73±0.3[a]
P (mg/100g)	**100**	119.28±8.8[a]	109.98±2.3[a]	136.49±8.4[b]	114.63±1.4[a]	109.04±10.7[a]
Zn (mg/100g)	**3.7**	2.35±0.63[a]	2.32±0.37[a]	1.56±0.06[b]	1.47±0.18[b]	1.87±0.28 [a]
Mg(mg/100g)	**48.7**	70.6±21.74[a]	72.91±9.73[a]	85.19±12.01[a]	58.32±9.72[a]	75.75±6.31[a]
Na (mg/100g)	**60**	102.42±0.0[a]	189.41±14.2[c]	162.02±13.18[b]	136.72±12.13[b]	136.72±12.13[b]
K (mg/100g)	**408.7**	319.2±0.0[b]	611.49±22.5[c]	728.82±0.0[c]	611.49±22.5[c]	611.49±22.5[c]

The superscripts a = statistical significance at $p < 0.05$, b = significance at $p < 0.01$ and c = significance at $P < 0.001$, compared to WHO reference pattern value.

3.4 Functional properties

The results of the analysis of eight functional properties (water absorption capacity, oil absorption capacity, loose bulk density, packed bulk density, swelling index, foam capacity, foam stability and dispersibility) are presented in table 5 below.

Analysis of water absorption capacity (WAC) for the five formulas revealed formula A as the sample with the highest WAC, with a mean value of 4.4. C was highest after A, with a value of 3.1. Formula B, which had the best sensory attributes, had a WAC of 2.4, while G had a WAC of 2.5. The least WAC was recorded with formula H (1.5). The WAC of a food sample is an indication of the volume of water required to form gruels whose consistencies are suitable for infant feeding. According to Echendu et al. [47], the presence of carbohydrates in a food has a major influence on the WAC of the food. The WAC of a food is as a result of the ability of the proteins present in it to be able to bind water. This implies the variations of water absorption capacity observed in different foods may be as a result of the differences in protein present, differences in the concentration of each of these proteins and differences in their degree of interaction between these proteins and water [48]. According to them, a high WAC is as a result of more polar amino acids present in a flour. Also, Giami and Bekeham [49] reported that when the WAC of a flour is high, this promotes microbial activities, hence reducing its shelf life. Flours with high WACs also lead to the formation of thicker gruels, making them unsuitable as complementary foods. Based on this, formula H, with its low WAC, could be considered as the most desirable complementary food.

The analysis of oil absorption capacity (OAC) showed formula H, just like with its WAC, having the least value (1.5). This was closely followed, in ascending order, by formula C with an OAC of 1.6. Next was formula A with a value of 1.65 for OAC. Formula G had a value of 1.75, while formula B had the most OAC, with a value of 1.95. Apart from formula H whose WAC is as low as its OAC, the formulas which had low WACs were found to have higher OACs and vice versa. The OACs were generally lower than the WACs, implying that there were more hydrophilic interactions in the formulas with low OACs and more hydrophobic interactions in the formulas with high OACs.

For the loose bulk density (LBD), results showed H having the highest value of 0.54g/cm3, followed by G with a value of 0.53g/cm3. Next were C and A, with values of 0.52g/cm3, and the least value was obtained for B (0.51g/cm3). The packed bulk density (PBD) on the other hand revealed that the least value was obtained for A (0.78 g/cm3). In ascending order were A, G, C, B and H, with values of 0.78 g/cm3, 0.84 g/cm3, 0.87 g/cm3, 0.89 g/cm3 and 0.92 g/cm3 respectively.

A high bulk density is good functional property for a flour, as it determines the quality of mixing of that flour [50]. The bulk density of a particular sample reflects the amount of load the sample is able carry if it is allowed to rest directly on one another. Flours with higher bulk density are more advantageous as they ease the dispersibility of these flours. A major disadvantage of flours with high bulk density is their ability to limit the calory and nutrient density of a food, which can have a negative effect of the growth rate of the child [51]. This is because, diets which have a high bulk density would have lower amounts of flour particles which are willing to stick to each other, and this negative attribute reduces the energy content of these high bulk density diets [52]. High bulk density foods therefore need be prepared using a larger amount of water, making them lose their nutrient density, coupled with a pasty consistency, making them harder to be fed to the infant [53] Based on this major disadvantage of high bulk density foods, formula H would be the least preferable complementary food.

The analysis of swelling index (SI) for the five formulas resulted in values in the range 1.03 – 1.11, with formula B having the least value for SI and formula C having the greatest. Formula H recorded a value of 1.08, while formulas A and G had the same value of 1.09 for SI. The SI and WAC of a food sample are used in determining its consistency, whether it is solid, liquid or semi-solid. Diets which have high swelling indices and high WACs absorb too much water during their preparation, making them voluminous, with low energy and low nutrient densities [54]. Formula B, with the least swelling index, would therefore be the most desirable complementary food.

Foaming capacity (FC) was also analysed, and the values ranged from 2.0 for formula B, to 18.0 for formula H. In ascending order, the foam capacities were A, B, C, G and H, with values 2.0, 9.85, 11.55, 15.0 and 18.0 respectively.

The foam stability was also analysed, and ranged from 0.0, through 0.1, 0.2, 0.5, to 0.7 for formulas G, B, C, A and H respectively. Therefore, H had the highest foam stability while G had the least.

According to Yadahally et al. [55], foam formation is as a result of the denaturation and aggregation of proteins in a sample when it undergoes heat treatment. Therefore, we would expect higher foam capacities from formulas with high protein contents, but controversially, this was not the case. Formula A, whose protein content was only second to B, was found to have the least value for foaming capacity. We could theorize that there wasn't much damage in proteins in formula A during processing. Formula H on the other hand, whose protein content was lowest, instead had the highest foaming capacity, indicating much more protein denaturation and aggregation during its processing. Generally, the foam formed by protein denaturation and aggregation is highly unstable. More stable foam is formed by native proteins than by denatured proteins [19].

The dispersibilities were also evaluated and results recorded (in %) showed that formula H had the greatest dispersibility of 92.5%, followed by C with a dispersibility of 77.0%, then A with 69.0%, and the least two were formulas G with 68.5% and B with 52.5%. For a flour, dispersibility is a measure of how much that flour can be reconstituted. Diets with higher dispersibility are better than those with lower dispersibilities. This implies that based on the dispersibility, formula H would be the most preferable complementary food.

Table 5: Results of functional properties analysis

Samples	A	B	C	G	H

WAC	4.40±0.2	2.40±0.1	3.10±0.7	2.50±0.6	1.50±0.1
OAC	1.65±0.15	1.95±0.15	1.6±0.1	1.75±0.05	1.50±0.1
LBD (g/cm^3)	0.52±0.02	0.51±0.01	0.52±0.02	0.53±0.03	0.54±0.01
PBD (g/cm^3)	0.78±0.02	0.89±0.02	0.87±0.04	0.84±0.07	0.92±0.09
Swelling Index	1.09±0.01	1.03±0.01	1.11±0.01	1.09±0.03	1.08±0.01
Foam Capacity	2.00±2.0	9.85±2.15	11.55±0.45	15.0±1.0	18.0±2.0
Foam Stability	0.50±0.1	0.10±0.1	0.20±0.2	0.00±0.0	0.70±0.1
Dispersibility (%)	69.0±1.0	52.5±2.5	77.0±2.0	68.5±3.5	92.5±0.5

3.5 Microbial analysis

The coliform count, total bacteria count and yeast counts were evaluated, and for total bacteria count, results were read in the petri dishes with dilution factors 10-3. For the yeast count, the dilution factor was 10-1 were read, and for coliform count, the petri dishes with dilution factor 10-1 were read. Table 6 below gives a summary of these results of microbial analysis that was done on the five formulas.

For the coliform count, formula C had the least, with no coliform present in the sample. Formula A had 370CFUs, formula B was the highest, with 620CFUs, while formula G had 220CFUs and formula H had 120. Formula B with the best sensory attributes turned out to have the highest coliform count, making it highly unsuitable for complementary feeding. Only formula C, with zero coliform count, can be considered as a good complementary food.

None of the samples were positive for yeast, as there was no growth in any of the petri dishes containing growth medium for yeast.

Table 6: Results of microbial analysis

SAMPLE	**DF**	**A**	**B**	**C**	**G**	**H**
TCC (CFUs)	**10^{-1}**	370	620	0	220	120
TYC (CFUs)	**10^{-1}**	0	0	0	0	0
TBC (CFUs)	**10^{-3}**	1.1 x 10^4	1.9 x 10^4	3.1 x 10^4	2.8 x 10^4	1.0 x 10^4

CFU : Colony Forming Units; TBC : Total Bacteria count ; TYC: Total Yeast count.

The total bacteria count was extremely high in all five formulas, with formulas A and H having the least number of CFUs; 11000 and 10000CFUs respectively. Formulas B and G had 19000CFUs and 28000CFUs respectively, and formula C was most loaded with bacteria, with a total of approximately 31000 CFUs. The total bacterial count of the five formulas was extremely high, ranging from 1.0 x 104 in formula H to a value as high as 3.1 x 104 in formula C. Conversely, formula C which was void of coliform turned out to have the highest count in other bacteria. This makes formula H the “safest” among all five complementary foods.

4. CONCLUSION

Formula B, though most preferred, is not as desirable for a complementary food. It may have a more desirable taste, flavour and texture than the other five, but it is too high in microbial load, and not as nutrient-rich, especially when compared to formula C. C on the other hand, with sweet potatoes as main starch source, happens to be the most suitable complementary food, considering how it met most of the standard values, more than any of the other five formulas. Sweet potatoes are a rich source of macro and micro nutrients, and need to be exploited more as a source of food for complementary feeding of infants.

Comment [u35]: how do you asured this prepared formula is preferd for new born ?

CONSENT AND ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

Comment [u36]: helsinki principle should declared in method part first in Ethical aproval

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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