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### Formulation and Evaluation of the Physicochemical Composition of a Rice Based Complementary Food Fortified with Spirulina

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#### Abstract

Childhood protein-energy malnutrition (PEM) is a status derived from an imbalance between nutritional and/or energy intake and needs and the weaning food remains the determining etiological factors in the onset of the disease. This work was undertaken to formulate a rice based complementary flour, enriched with spirulina capable of meeting the energy, macro and micronutrients needs of HIV infected children aged between 6 to 24 months suffering from MPE. Spirulina was used for it properties of boosting the immune system. A mixture design was develop using rice as source of carbohydrate, spirulina as source of protein and groundnut as source of lipids to make a balance diet according to standards. The results show that rice based complementary food formulated is balanced flour capable of meeting the energy and nutritional needs of weaning age children in accordance with the standards. The iron, calcium and magnesium contents also respect the standards. The weaning flour can be useful in managing protein and energy malnutrition of infected or non-infected child. However, zinc fortification is needed to fulfil the standard.

Keywords: Malnutrition, Complementary Food, Siprulina, Balance Diet, HIV Infected Children

#### Introduction

Childhood protein-energy malnutrition (PEM) is a status derived from an imbalance between nutritional and/or energy intake and needs. It is well known that weaning food is an etiological factor which contributes to the occurrence of PEM <sup>[1]</sup>. In fact, after the age of 6 months, breast milk is no longer sufficient to satisfy the energy requirement for a child. More energy is needed to be supply in form of complementary food. Therefore, an inadequacy of the complementary food will alter child nutritional status. Malnourished children have 11 times higher risk of death than those who are not <sup>[2]</sup>. This situation is further complicated when the child is HIV infected. The risk is increased in HIV positive child because the immune deficiency status is amplified <sup>[1]</sup>. PEM coupled with HIV is therefore a serious global public health problem and a greater attention should be paid to this vulnerable group. In Cameroon, protocol for managing malnutrition drawn up by the Ministry of Health is based on the new WHO guidelines. Solution recommended by the WHO is comprehensive care integrating nutritional therapy through a healthy and balanced diet<sup>[3]</sup>. International health authorities therefore encourage the production of complementary foods using local food items. For an effective treatment of PEM, the weaning food to be given to a HIV-infected child should also contain components capable of boosting his immune system since there is a cause-and-effect relationship between undernutrition and immune deficiency <sup>[4]</sup>. Spirulina (Spirulina platensis) is an alga known to boost the immune system. It has even been recommended by the WHO in the diet of young children <sup>[5]</sup>. It is rich in proteins of good biological value capable to promote growth and maintenance needs of young children <sup>[6]</sup>. It is also able to boost the immune system of HIV infected child <sup>[7]</sup>. However, a complementary food composed only of spirulina cannot meet the energy needs of the immune-deficient child. In a food formulation dynamic, a source of carbohydrates and lipids must be added to make a balanced diet. The use of local foods such as rice as a source of carbohydrates or groundnuts as a source of lipids is interesting because they are easily available and accessible at a lower cost. Combination of legumes and cereals is beneficial for filling the lysine deficiency of cereals. In addition, they are integrated among the local foods items used in traditional weaning practices in Cameroon. The major difficulty in mixing ingredients lies in determining the appropriate proportions to be mix in order to have flours meeting the macro and micronutrients needs of a child. The objective of this work is therefore to produce a balanced weaning food based on rice, spirulina, groundnuts, germinated maize capable of covering the nutritional and immune needs of malnourished children. More specifically, it is intended to produce, using mixture design a balanced diet meeting standards in terms of macro and micronutrients and to theoretically evaluate its nutritional quality.

#### Material and Methods Sample Collection

Maize (Zea mays), groundnut (Arachis hypogaea), egg (Gallus gallus domesticus) were purchased from local market in Douala city. Ndop rice (Oryza sativa) was bought from local distributor in Yaoundé city. Dried spirulina (S. plantesis) was got at the spirulina farm of Nomayos near Yaoundé. Samples were collected in plastic bag and transported to the laboratory. All samples were kept at room temperature before processing.

#### **Processing Technologies**

#### **Preliminary Treatment of the Raw Materials**

Samples were manually sorted to remove stranger materials. Eggs shells, maize, soybeans and groundnuts were pretreated according to the method described by Leng *et al.*, <sup>[8]</sup>. Germinated maize as sources of alpha amylase (to reduce the bulkiness of the porridge) was produce according to the method of Ariahu *et al.* <sup>[9]</sup>. Rice was wash before drying. All dry samples (germinated maize, groundnut) were ground into fine flour using a hammer mill (Cullati) or a robot blender (Moulinex) (spirulina, egg shell). Dried rice 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.

#### **Formulation of Rice Based Complementary Foods**

The methodology of mixture design as a mathematical approach was used to calculate the proportions of ingredients needed in order to have balanced composite flour <sup>[10]</sup>. Optimal mixtures of ingredients in formulating balanced flour are shown in Table 1.

<b>Table 1:</b> Optimal mixture of ingredients (%) in formulating	
balanced flour	

Ingredients	<b>Rice based complementary flour (%)</b>
Riz	51.8
Spirulina	8.0
Groundnut paste	16.7
Germinated maize flour	8.5
Eggs shell	0.75
Table sugar	14.25
Total (%)	100

#### Chemical Analysis Proximate Composition

Moisture and ash were determined by AOAC method <sup>[11]</sup>. Crude protein has been analyzed according to Kjeldahl method <sup>[12]</sup>. Total fat content was quantified according to Weibull-Stoldt method <sup>[13]</sup>. Total dietary fiber was analyzed according to the AOAC 985.29 method <sup>[14]</sup>.

#### **Minerals Analysis**

Zinc and iron content in samples was performed using flame atomic absorption spectroscopy in acid digested ash according to the AOAC, 999.11 methods<sup>[15]</sup>.

#### **Results and Discussion**

# Macronutrients Content and Gross Energy of the Formulated Flour

Macronutrients contents of the optimal balanced formula are presented in Table 2. The weaning flour content 6.7 and 2.2g/100g of water and ash respectively. Proteins, lipids and

carbohydrates contents are 14.8, 10.0 and 75.1g/100g respectively. The gross energy value is 449.6 Kcal/100g. Carbohydrates, lipids and proteins contribute to about 66.8, 20 and 13.2% to the total energy respectively. This contribution is within the range of recommended values. A low-fat intake limits the absorption of liposolubles vitamins and the availability of essential fatty acids. High levels of proteins and lipids reduce the glycemic index <sup>[16]</sup>.

#### **Mineral content**

Iron, calcium, zinc and magnesium contents in the optimal formula are shown in Table 2. The balanced flour contents 18.5mg of iron, 380mg of calcium, 0.48mg of zinc and 138.1mg of magnesium per 100g respectively.

<b>Table 2:</b> Nutritional composition of the optimized rice-based
formulation

	Rice based complementary flour enrich with spirulina	Standard baby flour <sup>1</sup>	FAO/OMS <sup>2</sup> (INL98, 2004)			
Macronutrients						
Moisture (%) (%)	6.7	5	5			
Ash (%)	2.2	2	< 5.00			
Proteins (%)	14.8	13	13 à 15			
Lipids (%)	10.0	7	2.00			
Carbohydrates (%)	75.1	68.00	60 à 75			
Fibers (%)	4.8	5	< 5.00			
Gross energy (Kcal)	449.6	400	400			
	Contribution to total	energy				
Carbohydrates (%)	66.8					
Proteins (%)	13.2		6-15			
Lipids (%)	20.0		20-40			
	Micronutrients <sup>3</sup>	}				
Iron (mg/100g)	18.5		11.6; 5.8; 3.9			
Calcium (mg/100g)	380.0		400 - 500			
Zinc (mg/100g)	0.48		8.3; 4.1; 2.4			
Magnesium (mg/100g) Source: <sup>[16, 17, 18]</sup>	138.1		80 - 140			
Source: [16, 17, 18]						

#### Note:

Iron values are given for 5%, 10 % and 15% dietary iron bioavailability,

Zinc values are given for low, medium and high dietary zinc bioavailability

#### **Nutritional Quality of Weaning Foods**

The formulated rice based complementary flour is intended for HIV infected children growing normally or malnourished breastfed or not. According to the WHO, children who are HIV infected and growing normally should be managed with the same therapeutic feeding approaches as children who are not HIV infected. The daily food ration necessary to cover the energy needs of a weaning age child depends on the energy intake from the complementary food and its energy density <sup>[11]</sup>. Thus, the daily portion intake of energy, proteins and lipids as function of the quantity of complementary flour needed is presented in Table 3.The total energy requirements of a breastfed child age between 6 to 24 months is 615 kcal/day for 6–8 months, 686 kcal/day for 9–11 months and 894 kcal/day for 12–23 months. The gap of energy to be covered by the complementary food is evaluated at 200 kcal/day for 6–8 months, 300 kcal/day for

9–11 months and 500 for 12–23 months (Table 3); the remaining energy being provided by breast milk<sup>[10]</sup>.

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 Table 3: Daily requirement values and daily portion intake of energy, proteins and lipids as a function of quantity of flour needed for a breastfed HIV infected child

	Daily requirement values [4]				Rice-based formulation			
Age (months)	Energy	Energy	Proteins	Lipids	Quantity of	Energy density	Proteins	Lipids
Age (montus)	(Kcal)	density	(g/day)	(g/day)	flour needed (g)	(g/100Kcal)	(g/day)	(g/day)
6 -8	200	$\geq 0.8$	2	0	44.48	4.5	6.58	4.45
9 -11	300	$\geq 0.8$	5-6	3	66.73	4.5	9.88	6.67
12 -23	550	$\geq 0.8$	5-6	9 -13	122.33	4.5	18.10	12.23

The energy density of the formulated complementary flour is 4.5 kcal/g (Table 3). This high energy density will allow the preparation of high energy density porridge <sup>[19]</sup>; the minimum recommended value being 0.8 kcal/g. For this purpose, a daily serving portion of 44.48g of complementary flour is needed to satisfy the 200 Kcal/day of energy for 6-8 months old healthy and breastfed child. 66.73g of the formulated food will be needed for a breastfed child in age ranging between 9 to 11 months (Table 3). The daily serving of rice composite flour required to satisfy the 550 kcal/day for 12-23 months old child is 122.33g. All these portions would satisfy the daily proteins and lipids intake requirements. Regarding the average child gastric capacity of 30 ml/kg of body weight <sup>[1]</sup>, the daily ration will be divided into 3 meals for a volume of 200 to 250 ml. For a non-breastfed child, the complementary flour should cover all the energy needed approximately 615 kcal/day at 6-8 months, 686 kcal at 9-11 months and 894 kcal /day at 12-23 months respectively. A daily serving portion of 136.79g, 152.58g and 198.84g respectively will be needed. Considering the energy intake of 449.6kcal per 100g of food and the weight of a tablespoon (20g), this quantity of food can be divided into 4 to 5 meals (2 tablespoons per meal) a

day. WHO/FAO (2013) has established levels of Reference Nutrient Intakes (RINL<sub>98</sub>) (Table 4) as a guide for amounts of vitamins and minerals that should be supplied when a formulated complementary food is eaten. This nutritional guideline suggests that a daily ration of a formulated complementary food should supply at least 50% and up to 100% of the WHO RINL98 daily total quantity of each of these vitamins and/or minerals. By considering the lower levels (50% of the WHO RINL98), the daily minimum suggested intake of iron (5.5; 2.9; 1.95) and magnesium (30) were satisfied at all the serving portions. The daily minimum suggested intake of calcium (250mg) was not satisfied only at 48.61 g serving portion (67.61%). The daily minimum suggested intake of zinc (1.2) was not satisfied at all the serving portions and the extent varying according to the age range. Feeding 6-8 months old child with 44.48g of rice based complementary food would satisfy only 21.35% of the 1.2 mg of zinc daily minimum requirement. A daily portion intake of 66.73g and 122.33 g of weaning food would satisfy only 32.00% and 58.71% of the 1.2 mg of zinc daily minimum requirement for 9-11 months and 12-23 months old infant respectively.

	Reference Nutrient Intake (INL98)	Daily ration of the formulated complementary Food (at least 50% of INL <sub>98</sub> )	Rice-based formulation		
Age			6-8 months	9-11 months	12-23 months
Energy requirement (Kcal)			200	300	550
Quantity of flour (g)			44.48	66.73	122.33
Calcium (mg)	500	250	169.02	253.76	464.85
Iron (mg) <sup>5</sup>	11.6; 5.8; 3.9	5.5; 2.9; 1.95	8.23	12.35	22.63
Magnesium (mg)	60	30	92.09	168.81	61.38
Zinc (mg) <sup>4</sup>	8.3; 4.1; 2.4	4.15; 2.05; 1.2	0.21	0.32	0.59

Table 4: Average daily intake of micronutrients from maize-based formulation as a function of quantity of flour needed

The optimum formula has a moisture content of 6.7%. This value is higher than the value reported by Mohammed et al (3.18%) when formulating fermented yellow maize complementary flour enriched with soy and catfish flesh [20]. However, this value is closer to the range of values (6.7 and 7.3%) reported by Bekele *et al*<sup>[5]</sup>. The value of fibers (4.8%) recorded is higher than the ranges of values (0.9-1.32%) reported by Ponka et al [21]. Moreover, the work of Zanou et al<sup>[22]</sup> reported a value of 8g/100g higher than our values. The formulated food contains 10g/100g of lipids. This value is higher than the range of values (2.49 - 3.13g/100g)reported by Tadesse et al [23] in formulating sorghum based weaning food. This may be due to the addition of high fat groundnuts in our formulation. The formulated weaning flour content 18.5g/100g of iron. This value is higher than the range values (6.75-7.13g/100g) reported by Desalegn et

al <sup>[24]</sup>. Calcium content is 380.0mg/100g. This value is higher than the range values (77.92-109.6 mg/100g) reported by Bekele et al<sup>[5]</sup> and Amoin et al (300.5 mg/100g) <sup>[25]</sup> in formulating composite germinated flours. The incorporation of chicken egg shells into the formulation would be a supporting element. The magnesium content of the formulated flour is 138 mg/100g. Lower values have been recorded by Daou et al (55.55-97.88mg/100g) [26] and Sharoba (56.16-59.87mg/100g) <sup>[27]</sup> respectively. The composite flours meet the requirements of standards since the values of energy, moisture, ash, fibers, carbohydrates, proteins, lipids are closer to standards. The moisture content was less than 10% showing by this a long storage potential. Proteins and lipids contribute to about 14.8 and 20.0% of the total energy respectively. Those values meet the requirement percent in total energy contribution of the Codex

Alimentarius for proteins (6-15%) and lipids (20-40%). The highest contribution of carbohydrates (66.8%) 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 proteins which can be used for it primary function of building the body and repairing worn out tissues rather than a source of energy <sup>[28]</sup>. The impact of micronutrients cannot be separated from that of the additional energy or protein quantity or quality <sup>[29]</sup>. The weaning flour contents calcium, iron, magnesium and zinc. Those nutrients are among the nutrients believed to be "essential" because they are associated with linear growth, health, and cognitive development during childhood <sup>[26, 30]</sup>. The Values of calcium, magnesium, iron globally meet the percentage requirement of standards at all serving portions. The values of zinc partially satisfied standard requirement meaning that zinc is the limiting nutrient. The average daily intake of zinc is age and serving portion dependent. Regarding zinc, attention may be paid on bioavailability and phytates content rather than zinc content in food since high contain in zinc in food like cereal and legumes often result in poor absorption due to the high content of phytates. Spirulina is a microalga exempt of phytates. Meal should be provided 4-5 times per day, with additional nutritious snacks (such as pieces of fruit or bread or chapatti with nut paste) offered 1-2 times per day, as desired <sup>[31]</sup>. This combination if followed would probably increase the daily zinc requirement. The weaning flour formulation is also intended for malnourished children. Hence, in this context of MPE management, children with poor weight gain will need additional Kcal of 120-150Kcal for 6-11 months old and 160-190 kcal for 12-23 months old breastfed or not. The daily serving proportion will by this increase as well as the key nutrients to meet the standards. These findings suggest that rice-based formulation enrich which spirulina have a great potentiality as weaning flour since it can provide nutritional and health benefices. With these findings, sprirulina can be used to fortify rice based complementary flour to improve the micronutrients contents zinc remains the limiting micronutrients.

#### Conclusion

Rice based complementary food formulated is balanced flour capable of meeting the energy and nutritional needs of weaning age children in accordance with the standards. The iron, calcium and magnesium contents also respect the standards. The weaning flour can be useful in managing protein and energy malnutrition of infected or non-infected child. However, zinc fortification is needed to fulfil the standard.

#### **Declaration of Competing Interest**

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

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