

Formulation of Infant Food Based on Local Cereals: Stability and Effects on Cognitive Development

ManéDiallo¹, Mady Cissé¹, Frédéric Dessor², Rachid Soulimani², Oumar Sock² and Stéphane Desobry^{2*}

¹Dakar Ecole Polytechnique (Higher Polytechnic School); Dakar BP 5085 Dakar-Fann; Senegal

²Université de Lorraine; Laboratoire d'Ingénierie des Biomolécules; 2 Avenue de la Forêt de Haye; 54500 Vandoeuvre; France

³Université de Lorraine; URAFPA; Neurotoxicology Laboratory; Campus Bridoux; Rue du General Delestraint 57070 Metz; France

Abstract

Our goal was to develop nutritious; accessible and stable infants flour which would improve cognitive and behavior abilities for African children.

Five formulations (F1 to F5) based on local cereals from Senegal were manufactured according to FAO/WHO nutritional requirements and composed to reach equal energetic value (400 Kcal/Kg). Flours formulation stability was studied by storage at 20°C and 5 relative humidities (0% to 95%) for 10 months. Flour dextrose equivalent; color and fat composition were followed and all formulated flours showed very good stability for a storage relative humidity below 75%.

During *in vivo* nutritional tests; a comparative weight study between mice fed with standard nutritive flour (control); and those fed with F1 to F5 formulations showed a similar slope of weight gain even if adaptation time to new feed initially retard mice growth. F2 and F3 showed the highest weight deficiency compared to the control.

Y and Morris Tests; used to characterize the behavior showed that F3; F4 and F5 formulations significantly increased psychomotor activity compared to the control.

Long term memory was not significantly different between all formulations; except for F5 which showed a clear improvement. F2 formulation composed of corn was the least efficient for working memory due to its low proteins quality. The low number of triplets can slightly affect the F2 alternation percent; even if this effect was not observed for the control. The other formulations gave results close to the control food. F4 and F5 formulations showed good nutritional qualities; F4; enriched with vitamins and minerals; as well as F5 enriched with fish oil gave very good results on all growth and compartment parameters. They might be effective for better cognitive and somatic development of children.

Keywords: Infant's food; Nutrition; Development; In Vivo study; Mice behavior

Abbreviations: AA: Arachidonic Acid; ALA: Alpha-Linolenic Acid; DE: Dextrose Equivalent; DHA: Docosahexaenoic acid; EFSA: European Food Safety Authority; EPA: Eicosapentaenoic Acid; EU: European Union; LA: Linoleic Acid; OA: Oleic Acid; WHO: World Health Organization

Introduction

In developing countries, infant mortality remains very high (50 to 110 per 1,000 live births in 2011) [1]. According to WHO, malnutrition contributes to 50% of these deaths. In Senegal, infant mortality rate is 56.4 per 1,000 births, and 35% are attributable to malnutrition. 27% of children suffer from chronic malnutrition and 11% severe malnutrition [2]. Children development is slowed by nutritional deficiencies (protein-energy, essential fatty acids, iron...). Delayed brain development, reduced psychomotor activity and cognitive performance are often observed. Malnutrition also causes immune capacity reduction that makes children vulnerable to infectious diseases. Malnutrition consequences are very high both individually and collectively, lead to considerable loss of human capital and low economic productivity, and perpetuate poverty.

Malnutrition is largely due to improper or insufficient diet during the first years of life and in particular, during the period of diversification that corresponds to the stage of increased foods variety (6-24 months). Until weaning, infants show no deficiency because breast milk offers nutritional and functional benefits. According to mothers practices

in Senegal [3], nearly 100% of mothers breastfeed while in European countries less than half of them gives breast [4]. It is at diversification age that the child becomes most vulnerable to malnutrition. In fact, most infant flours and dietary supplement developed for children in small family, artisanal or semi-industrial units show low nutritional value. Composition analysis and rheological behavior of gruels prepared from these products revealed that they contain insufficient levels of certain nutrients, especially minerals and vitamins [5-7]. All the necessary treatments to reduce anti-nutritional activities contained in the raw materials are not used. Moreover, gruels miss of both appropriate consistency and energy density. Thus, nutritional value improvement of infant formulas has been subject of numerous studies [5-9] and projects (Misolain Burkina Faso and Vitafortin Congo). These studies showed that few meals met the requirements for nutritional food supplementation. These meals included vitamin and mineral supplements and were obtained by technological processes that partially degraded starch

***Corresponding author:** Stéphane Desobry, Université de Lorraine, Laboratoire d'Ingénierie des Biomolécules, 2 Avenue de la Forêt de Haye, 54500 Vandoeuvre, France, Tel: +33 (0)383595880; Fax: +33 (0)383595804; E-mail: stephane.desobry@univ-lorraine.fr

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(incorporation of amylase sources, extrusion). Hydrolysis reduced the viscosity of flour added to increase the meal energetic value. The gruels remained liquid and easy to swallow. However, these flours presented a poor stability. Molds growth occurred when flours were stored above 12% w/w water and fat oxidation was significant. At present, in Africa, meals of high nutritional quality and milks enriched with essential fatty acids are often imported from Western countries and are not accessible to the entire population because of their high price.

The low infant mortality rate (less than 0.5%) observed in industrialized countries is partly explained by strong support in food diversification and regulations. According to WHO recommendations and guidelines of the European Community (EC), all foods for infant feeding are carefully enriched with nutrients essential to infant development. It is therefore particularly important to provide nutritious flours to African children using local resources and taking the food diversification in industrialized countries as a reference to fight against child malnutrition and reduce infants' mortality rate.

Many authors [10-12] showed beneficial effects of supplementation with long-chain omega-3 (Poly Unsaturated Fatty Acids (PUFA) as EPA and DHA) on brain development, psychomotor performance and visual functions. In Europe, as children are often exposed to deficiencies in omega 3 fatty acids [13], since 1996, PUFA are included in all formulas according to the WHO and EU standards. Omega-3 rich fish oils are available in Africa, bordering ocean, and could easily be added to infant formulas.

Animal models have significantly contributed to improving health, well-being and cognitive performance [14]. The murine model is very similar genetically, immunologically, reproductively, physiologically and pathologically with man. This model was used to assess short- and long-term memory as well as learning abilities. These tests were widely used to demonstrate harmful or beneficial effects of a given substance on health, neurophysiology and memory [15-18].

As the temperature in Senegal is at 25 ±5°C all year long and the humidity can vary from 50% to 80%, it is essential that food formulation resist to these climatic conditions.

The aim of the present work was to formulate high quality flours for baby nutritional needs from local cereals in Senegal, test flour stability, and assess their impact on cognitive ability and behavior in mice.

Methods and Materials

Flour formulation

Raw materials selection: Selection criteria were based on bioavailability, aspect (color hardness) and nutritional value of available raw materials. The CNRA (National Centre for Agricultural Research) in Bambey, specialized in raw materials varieties in Senegal guided raw materials choice. Varieties of millet ('Sunna'), peanut ('Fourrier') and white cowpea (*Vigna unguiculata* [L.] Walp.) were selected. Yellow maize was specifically chosen for its protein content and sensory properties. Selected varieties were purchased at Dakar market. Physicochemical analyses were performed to characterize flours. Water content, protein and minerals were measured according to AOC method (1995), and fats according to Soxhlet method. The Cellulose content was obtained by difference (Table 1).

Formulas preparation: Formulas were made in accordance with FAO / WHO nutritional requirements standard (Table 2) [19] and taking into account the relative energy value / cost of each ingredient [20]. The recommended protein, fats, sugar and energy contents were parameters

that directed formula compositions. F1 to F5 formulation received the same energetic value (=400 Kcal/100g), close protein content (11.5±1.5 g/100g), and very close carbohydrates content (71.5±1.5 g/100g). The financial aspect was also considered and the final products (gruels) must have an energy value ≥ 300Kcal/100g and a cost below 2500F CFA (<4 euros) per Kg to be adapted to Senegalese population. F1 to F5 formulas are presented in Table 3 and were manufactured by Chocosen (Dakar, Senegal) following process flow diagram shown in Figure 1. F1 was enriched in millet, F2 in maize, F3 had a balanced composition, F4 was enriched with vitamins and minerals and F5 was enriched in PUFA from salmon oil extracted from by-products (salmon heads). Meals met children nutritional requirements. They all had an energy value ≥ 300 Kcal/100g which provides gruels energetic density between 100 and 120.

Stability Study

Storage of flours: Flour samples were placed in sealed enclosures containing saturated salt solutions for 10 months at 20°C. Salt solutions regulated the atmosphere relative humidity (MgCl₂ = 33%; NaBr = 59%; NaCl = 75%; KNO₃ = 95%).

Influence of water activity (a_w) on formulas physicochemical properties: From 1g samples taken from each formula in each a_w conditions, at given times, physicochemical analyses were carried out to evaluate changes in dextrose equivalent (once a month), color (every 15 days) and fatty acid profile (once a month).

Parameters	Unit	Millet flour (0.5 mm)	Cowpea flour (0.5 mm)	Yellow corn flour (0.5 mm)	Peanut flour (0.5 mm)
Moisture	%	8.0 ± 0.4	5.5 ± 0.2	8.1 ± 0.4	3.2 ± 0.2
Ashes	%	1.2 ± 0.1	3.1 ± 0.2	1.0 ± 0.1	2.0 ± 0.2
Proteins	%	7.4 ± 0.2	17.8 ± 0.6	9.1 ± 0.5	30.0 ± 1.0
Fats	%	4.8 ± 0.4	1.1 ± 0.2	2.7 ± 0.2	50.3 ± 0.7
Cellulose	%	2.3 ± 0.2	1.1 ± 0.2	1.4 ± 0.2	4.3 ± 0.2
Carbohydrates	%	76 ± 4	71 ± 4	77 ± 4	10 ± 3
Calcium	mg/100g	17.3 ± 0.7	8.2 ± 0.5	12.8 ± 0.5	4.9 ± 0.5
Phosphorus	mg/100g	205 ± 14	304 ± 20	219 ± 12	168 ± 20
Magnesium	mg/100g	39 ± 5	138 ± 12	49 ± 5	69 ± 8
Potassium	mg/100g	259 ± 22	1075 ± 126	238 ± 18	499 ± 43

Table 1: Physicochemical tests carried out on flours only.

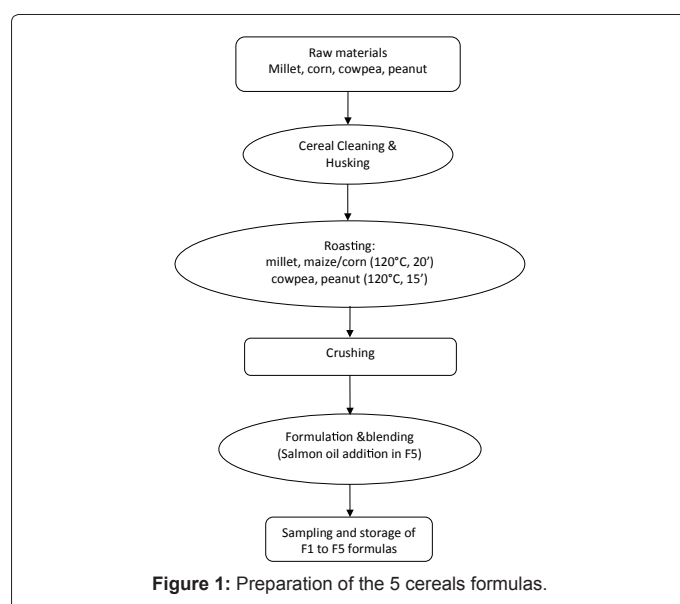


Figure 1: Preparation of the 5 cereals formulas.

Dextrose Equivalent value: Measuring dextrose equivalent of flour stored at different relative humidity was made to observe moisture influence on starch hydrolysis. The method used was based on linear relationship between reducing sugars and cryoscopic lowering [21-23]. The apparatus used was the advanced cryoscope 4250 (Grosseron, France).

Color: Colorimetric measurements on flour were performed using a colorimeter CR300 (Minolta, France). The L values (luminance), a and b (chromaticity coordinates) have been evaluated during storage.

Profile of fatty acids: Fatty acid profiles of F4 and F5 formulas, rich in fats, were obtained by lipids extraction from flour according to the Folch method [24], based on the conversion of fatty acids to methyl esters by BF₃ (Boron trifluoro methyl). These esters were identified and quantified by gas chromatography [25]. The apparatus (Shimatzu, France) was supplied of carrier gas (nitrogen) and composed of an injector at 250°C, a capillary column (50 m length, 0.25 mm diameter, 0.5µm thickness filled with polyethylene glycol terephthalic acid) (Perichrom, France), a controlled and programmable oven, a flame ionization detector at 220°C and a recorder/integrator. A temperature program was used to ensure the best possible separation: 120°C for 2 minutes, temperature increase from 120°C to 180°C at 40°C/min, 2 min bearing of 180°C, increased temperature of 180°C to 220°C at 3°C/min, 25 min bearing at 220°C. The peak integration was performed using Winilab 3 software (Perichrom, Saulx-le-Chartreux, France).

The peaks obtained were identified using pure standards of fatty acids provided by Supelco (Belle melting, USA). Pure AGPIs from marine source were used to identify the F5 formula peaks.

Nutrition and behavior study

Animals and housing conditions: Sixty male albino mice of Swiss strain were used (Janvier Laboratories, France). This choice was based on the fact that male animals have a constant hormonal cycle in contrast to female mice and avoid possible influences of this cycle on behavior outcomes. The mice were 3-weeks old at the beginning of the experiments. They were housed in an animal house responding to directives of the European Union Council of 24 November 1986 (86/809/EEC). Animals were placed in individual standard cages at a constant temperature of 22±2°C and humidity of 55±10%. The light cycle is 12h/day and 12h/night was reversed because mice have a circadian rhythm night and that tests should be made during the day.

Weight monitoring : Mice were fed ad libitum every 4 days for 60 days. Weights were checked every 4 days for 60 days.

Y-maze: A black wooden Y-form, composed of three separate parts was used. Each leg measured 40 cm long, 13 cm high, 10 cm wide at the end and 3 cm wide at the center point where they converge to form equal angles. The arms are marked by the letters A, B and C to identify the different arms explored by the mouse placed in the maze.

The mouse explored the maze for 3 minutes and at the end of test, a sequence of letters reflecting alternation behavior was obtained. Test result was obtained by determining alternation ratio, corresponding to the consecutive visit of 3 different arms from the total number of triples (number of visited arms minus 2). This report was expressed as a success percentage for evaluating immediate memory work. The test was performed after 1 month of formula use in a dark room in the morning where mouse activity is maximal.

The Morris water maze (Morris test): This test consisted in placing the mouse in a circular pool filled with water (T = 24°C), made

opaque by adding milk, in which was a platform hidden 1 cm below the water level. Visible clues as landmarks were placed at four points of the pelvis (proximal index). The animal has learned to use the index to navigate to the platform. Once reached, it was left there for 30 seconds, a period where he stored his immediate environment. Five tests were performed and after 120s, if the mouse had not found the platform, the experimenter deposited the animal on it for 30s. After 24 h, another identical test was performed to assess memory retention. For each test, the time taken to reach the target was recorded. Normally, a rodent performed successive decreasing times in this test, this improved performance reflecting kinetics of animal learning. Since the tests were repeated, the Morris water maze was used to assess working memory of the animal. The repetition of this test the next day was used to assess the long-term memory.

This test was performed on control mice (C) and those fed by formulas (F1 to F5) after 8 weeks of consumption.

Statistical Analysis

Analysis of variance (ANOVA) and Fisher test were used. The level of significance was set at p = 0.05. Statistical analyses were performed using the Stat view 4.5° software (Abacus Concepts).

Results and discussions

Stability of powdered infant foods

Formulations stability was first studied to evaluate possibility of storage by consumers and define preservation boundary conditions.

Dextrose equivalent (DE)

DE increase reflects starch chains hydrolysis. Figure 2 shows DE values of flour stored in different water activities (a_w). In all formulations, DE value increased with relative humidity and storage time. After 6 weeks of storage, a significant starch degradation was demonstrated by a significant increase in DE at high humidity ($a_w = 0.95$). For lower water activities (0 to 0.59), DE slightly changed also showing a little starch hydrolysis of starch over time. After 40 weeks of storage, DE

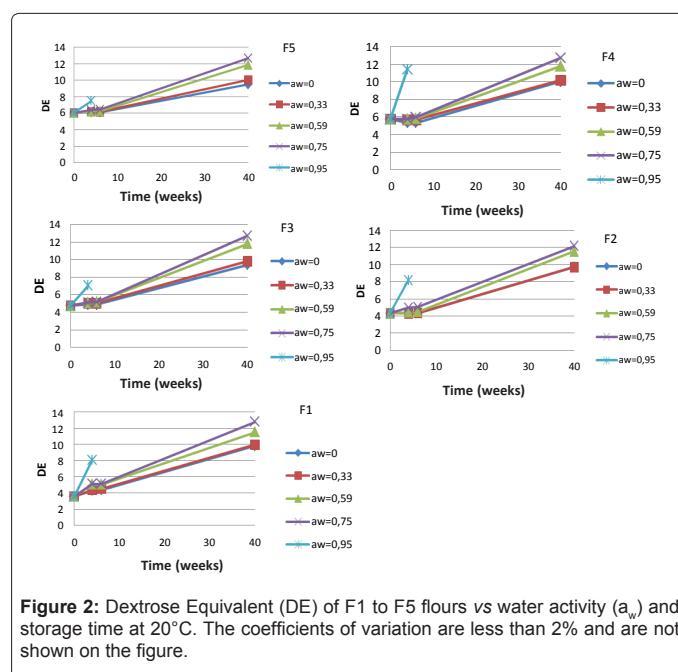


Figure 2: Dextrose Equivalent (DE) of F1 to F5 flours vs water activity (a_w) and storage time at 20°C. The coefficients of variation are less than 2% and are not shown on the figure.

		1-3 months	3-6 months	6-9 months	9-12 months	1-3 years
Water	ml/kg/day	150	150	125	100-110	100
Energy	Kcal/kg/day	110	100	95	100	100
Protein	g/kg/day	2	1.8	1.5	1.4	1.2
Energy	Kcal/day	450	600	700	850	1300
protein	g/day	-	-	-	-	16
		0-6 months	6-12 months	>1 year		
Minerals (/day)	mg	400	600	800		
Calcium	mg	300	500	800		
Phosphorus	mg	40	60	80		
Magnesium	mg	8-10	8-10	10		
Iron	mg	5	5	10		
Iodine	µg	40-50	40-50	70-120		
Copper	mg	0.4-0.7	0.4-0.7	0.7-2		
Fluoride1	mg	0.25	0.25	0.5-1		
Selenium	µg	10-15	10-15	20-30		
Vitamins (/day)						
	ER	375-400	375-400	400-700		
	µg	25	25	10		
A2	UI	1000	1000	400		
D3	a-ET	3-4	3-4	6-7		
E4	µg	5-10	5-10	15-30		
K	mg	30-35	30-35	40-45		
C	mg	0.3-0.4	0.3-0.4	0.7-1		
B1	mg	0.4-0.5	0.4-0.5	0.8-1.2		
B2	mg	5-6	5-6	9-13		
niacin	mg	0.3-0.6	0.3-0.6	1-1.7		
B6	mg	25-35	25-35	50-100		
folates	µg	0.3-0.5	0.3-0.5	0.7-1.4		
B12	mg	2-3	2-3	3-5		
B5	µg	10-15	10-15	20-30		
biotin	µg					

Table 2: Recommended dietary allowances for infants (RDA) (Committee on Nutrition of the French Society of Pediatrics).

increased in all formulas. Rate of starch degradation was even more important than the water activity was high.

With the study of humidity effect on color and DE, critically high humidity was defined as 0.75 for flour stability. In fact, due to increasing water content, a significant matrix plasticizing occurred and resulted in starch hydrolysis. For low a_w (0 to 0.33), DE slightly varied since the rate of starch hydrolysis was significantly slowed (glassy state).

Color: In Figures 3, a rapid decrease of lightness (L) is observed during the 5 first days of storage. 5 to 9 Lightness units were lost depending of the formula due to initial surface oxidation and structural changes of powders during the equilibrating period to storage conditions. Figure 4 shows redness continuous increase for all formulas. Figure 5 shows an initial slight decrease of parameter b followed by an overall tendency to yellowing (b increase). After this initial period, two behaviors were observed, stabilization for low water activity, and large increases for 75% and 95%. The storage under 95% RH was stopped after 5 days due to molds development at powder surface for all formulas.

Color measurement was used to assess the degree of non-enzymatic browning [26,27] in formulas. The coloring reflects complexation of amino acids such as lysine and reduction in protein digestibility. It led to modifications in nutritional, organoleptic and toxicological properties. Humidity accelerated non-enzymatic browning kinetics.

Many authors [28-30] found that changes in color (b) depended on formulas composition and moisture content. Indeed, at high humidity, water availability was greater, reactants were mobile and reactions were favored. Non-enzymatic browning was favored by matrix plasticization above glass transition temperature [31]. The glass transition temperature of dry starch is about 130°C and decreases with water content. Water played a plasticizer role, increased chain mobility in amorphous phase and decreased local viscosity. Starch and protein provided by cereals caused brown coloration during storage at high humidity. This is consistent with Nasirpour [32] who showed that proteins and polysaccharides caused a maximum non-enzymatic browning.

This is perfectly correlated with DE increase observed earlier on flour preserved under high water activities. The reducing ends of starch degradation suffered complexation reactions with proteins made by cowpea and peanut. The higher the humidity, the higher the non-enzymatic browning reactions and hydrolysis of starch accelerates, resulting in a change in nutritional and organoleptic quality of formulas.

Concerning storage, DE and color results showed that all formulations had a similar stability and were all compatible with African storage conditions.

Fatty acids profiles: Table 4 shows a significant difference between fatty acid profiles of F4 and F5 formulations. F5 was an important source of marine fats rich in polyunsaturated fatty acids in long chains containing several double bonds, such as EPA (C20: 5 n3) and DHA (C22: 6 n3)[33-35]. F4 did not contain EPA and DHA but was rich in arachidonic and palmitic acids. The most highly concentrated fatty acid was C18: 1n9 (oleic acid) and represented 45% of total fatty acids. Essential fatty acids such as C18: 2n6 (linoleic acid, LA) and C18:3n3 (α linoleic acid, ALA) was present in both formulations. They respectively accounted for 25.92% or 531 mg/100 kcal and 1.44% or 29 mg/100 kcal

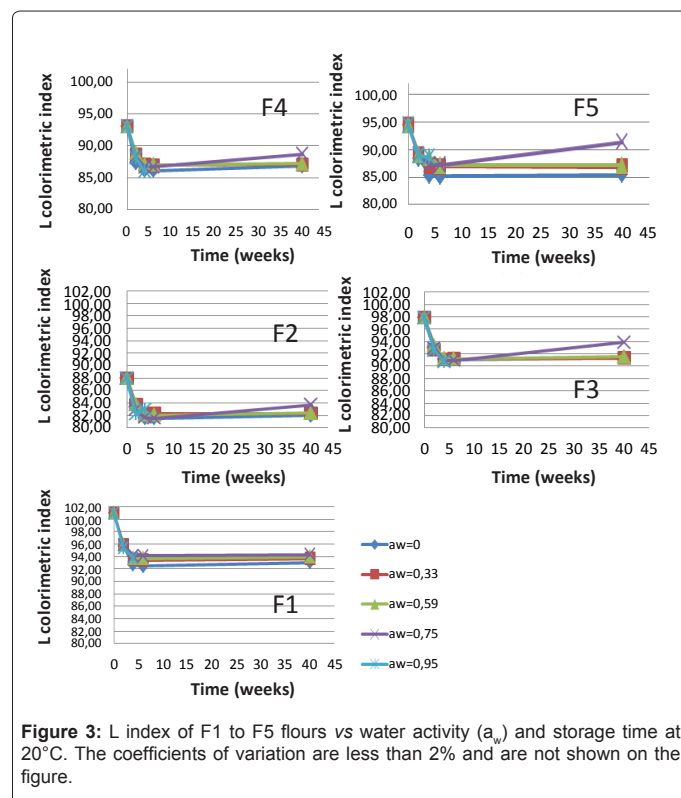


Figure 3: L index of F1 to F5 flours vs water activity (a_w) and storage time at 20°C. The coefficients of variation are less than 2% and are not shown on the figure.

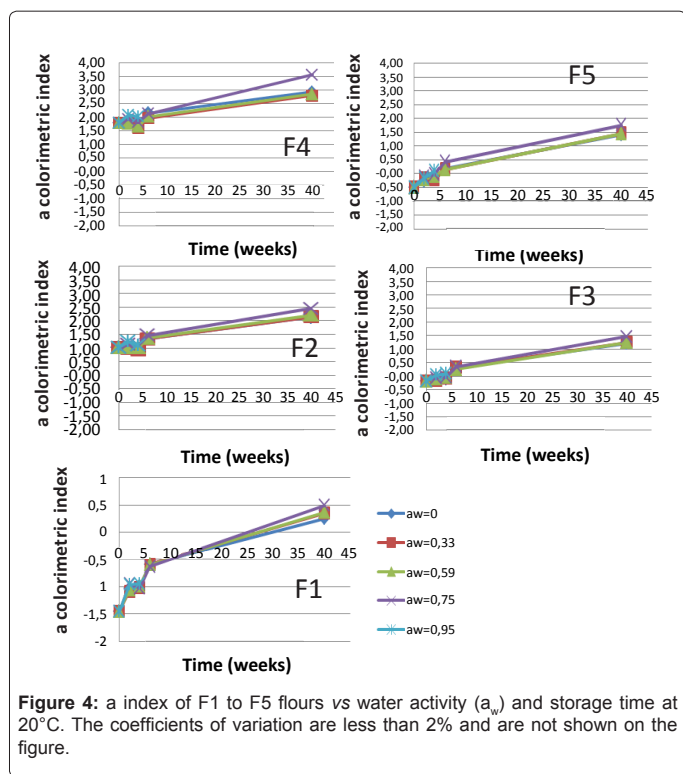


Figure 4: a index of F1 to F5 flours vs water activity (a_w) and storage time at 20°C. The coefficients of variation are less than 2% and are not shown on the figure.

Components	Formulas				
	F1	F2	F3	F4	F5
Millet	80%	-	33%	30%	31.5%
Maize/Corn	-	80%	33%	30%	31.5%
Cowpea	16%	16%	30%	30%	32%
Peanuts	4%	4%	4%	10%	-
Oil	-	-	-	-	5%

Table 3: F1 to F5 formulation of infant cereals.

for F4. Similarly, they accounted for 24.30% or 472mg/100Kcal and 0.6% or 11.6 mg/100 kcal for F5.

Richness of saturated fatty acid (C16: 0) in F4 was explained by the fact that it was mainly composed of maize (30%) and peanut (10%) oils rich in palmitic acid, respectively 10.9 % and 9.5% [36]. The presence of essential fatty acids resulted from vegetable oils. LA and ALA are precursors of long chain PUFA, essential constituents of lipid bilayer in cell membranes. Bourre et al. [37] reported that ALA deficiency caused abnormalities in various cell types and organelles of nervous system such as neurons, myelin, and endoplasmic reticulum. Thus, current regulation establishes thresholds for fatty acids incorporation in infant formulas. It is recommended for LA, a minimum quantity of 300mg /100kcal for the ALA, a minimum quantity of 50 mg/100kcal and a report ω_6 / ω_3 between 5 and 15. In F4 and F5 formulas, ω_6/ω_3 ratios were respectively 40 and 18. These high ratios came from abundance of plants with LA and scarcity of ALA in nature. This fatty acid content decreased in the formula F5 as salmon oil brought mostly EPA and DHA [13].

During storage, contents of EPA and DHA did not vary in F5. We conclude that fats contained in our formulas were not oxidized during storage. This can be explained by oil penetration into flour particles interstices and pores which isolated fatty acids from oxygen, but also by the presence of natural antioxidants such as vitamin E in formulas.

Formulas enriched in essential fatty acids (EPA and DHA) could favor of somatic development and psychomotor without significant deterioration during storage.

Nutritional and behavioral study

Weight management: Animal weights were initially equal (Figure 6). The adaptation required to new diet led to weight loss during the first days for all formulation, with a dramatic weight decrease for F2 and F3 (-10%). From day 8 (D8), a significant difference ($p < 0.05$) in weight was observed between the control group and the F2 and F3 groups. Initially, mice lost weight compared to control group and, even if the weight gain slope is equal for all groups after D8, the initial weight deficiency was not reduced. Groups F1, F4 and F5 had the same evolution as control group ($p < 0.05$). The same phenomenon was observed until D24. From D24 to D36, F2 and F3 groups gained weight and no significant difference was observed up to D40 where F2 and F3 groups showed weight stabilization. F1, F4 and F5 groups stayed close to the control group until the end of test (D52), with a 5 to 10% lower weight. Knowing that the control feed was a nutritionally complete and on-calorie formula, F1, F4 and F5 formulas were considered to have acceptable nutritional values.

We can therefore assume that F2 and F3 formulas were less nutritious than control food. F2 was composed mainly of maize compared to F1 consisting of millet which had no adverse effect on weight. F3 was not fortified with vitamins and minerals such as F4, and poor weight gain was observed in F3 group probably due to a deficit in these nutrients. F5 enriched with fish oil (EPA and DHA) showed good weight gain. These results support the recommendation of supplementation with vitamins, minerals and essential fatty acids preparations for children by international organizations such as WHO, EU, or EFSA. They also confirmed the importance of fatty acids contribution in infant formulas to favor growth in height and weight [38-40]. In Europe, although more than 50% of children do not receive breast milk unlike African children, malnutrition caused by diet deficiency is almost nonexistent. Even before food diversification, infant milks are designed to replace for mother's milk. At the time of diversification, follow-on formulas

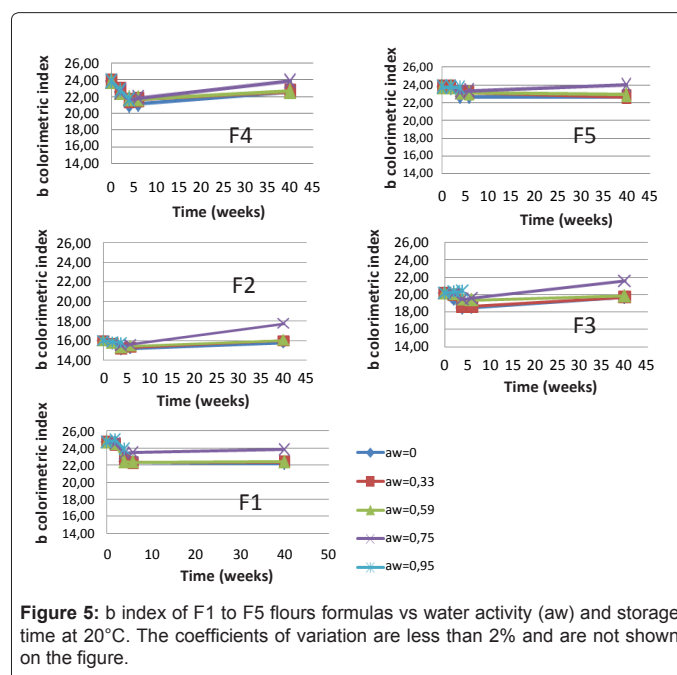


Figure 5: b index of F1 to F5 flours formulas vs water activity (a_w) and storage time at 20°C. The coefficients of variation are less than 2% and are not shown on the figure.

Formula	F4				F5			
	t=0	After 10 months of storage			t=0	After 10 months of storage		
Water activity		0	0.59	0.75		0	0.59	0.75
C14	0.03	0.04	0.04	0.00	2.13	2.41	2.39	0.00
C16	10.94	12.40	12.85	13.93	10.13	12.67	13.82	16.84
C16.1	0.00	0.00	0.00	0.00	7.26	3.27	3.19	3.20
C18:0	9.96	10.26	11.29	13.27	5.59	5.59	5.53	8.90
C18:1n9(OA)	45.30	45.25	44.83	44.32	30.71	33.19	32.57	31.91
C18:2n6(LA)	25.92	25.46	24.43	23.30	26.91	26.55	25.95	23.70
C18:3n6	1.62	1.99	2.03	1.46	5.12	5.10	5.17	4.81
C18:3n3(ALA)	1.44	1.18	1.16	1.15	0.67	0.59	0.58	0.48
C20:1n9	1.09	0.96	0.95	0.84	5.49	5.10	5.44	3.58
C20:4n6 (AA)	2.39	1.70	1.65	1.56	0.61	0.50	0.49	0.30
C20.4n3	0.00	0.00	0.00	0.00	5.38	5.02	4.87	3.95
C22:0	1.32	0.75	0.76	0.87	0.00	0.00	0.00	0.00

Table 4: Evolution of the percentage of fatty acids in F4 and F5 formulas related to water activity (a_w) during storage.

are enriched, according to the regulation, with modified proteins to overcome shortfalls in essential amino acids and avoid constipation by reducing casein content in formulas. They are also rich in vitamins, minerals and polyunsaturated fatty acids such as long-chain EPA, DHA or AA to take the child to a satisfactory state of health, proper growth and harmonious development [4,41]. F1, F4 and F5 formulas responded to these nutritional requirements despite being poorer than control food and baby food in European market. Cereals formulas were nutritionally tested as effective when supplemented with vitamins, minerals and essential fatty acids.

Psychomotor activity and short-term memory (Y-maze): Significant disparities were found between groups in total triplets number (Figure 7). A significant increase in psychomotor activity with F3, F4 and F5 formulas was observed compared to the control group. Moreover, in Y labyrinth, F2 formula consisting mainly of corn caused a deficit in short-term memory and a significant difference was observed with ANOVA and Fisher tests (Figure 8). The lower number of triplets could slightly influence the lower percentage of alternation. Nevertheless, no significant reduction of alternation% was observed for the control but was only appeared for F2. These results on F2 formula led to believe that corn had a deficit in at least one memory essential element. This can be correlated with corn poor protein quality that has been subject of numerous studies [42,43]. Analysis of maize amino acid composition by the FAO/WHO, shows a deficit in lysine and tryptophan. As demonstrated by Choi et al [44], brain proteins nature depends on dietary protein quality. Many epidemiological studies have shown that malnutrition seriously affects brain development and functioning [45]. Two particular brain structures associated with memory processes such as hippocampus and hypothalamus appeared to be particularly sensitive [46]. We can therefore associate impaired short-term memory of group F2 and low protein quality. The mixture of millet had a positive effect on psychomotor activity. Indeed, on the one hand, no increase was observed in psychomotor activity on the F1 and F2 formulas where millet and maize are not associated. On the other hand, F5 without peanut had an effect on psychomotor activity, and excluded peanut effect on psychomotor activity. Mixture of millet and maize appeared to be an efficient stimulant. Combination of millet and maize could change protein quality by compensating limited amino acids value and render corn formulation more nutritive.

Long-term memory(Morris Pool): Compared to the control during the 3 last consecutive tests (Table 5), no significant improvement

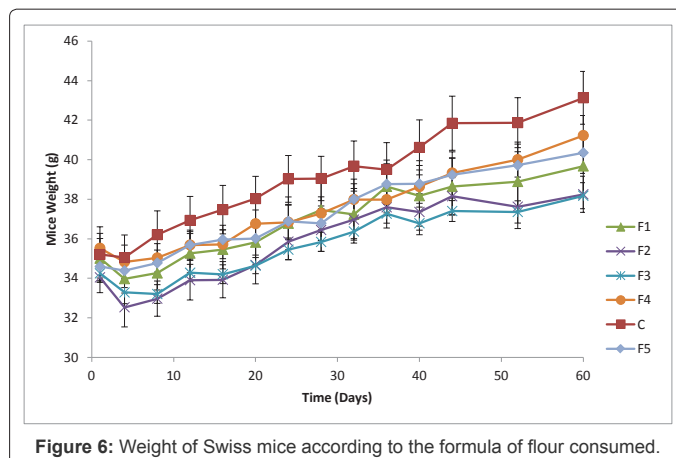


Figure 6: Weight of Swiss mice according to the formula of flour consumed.

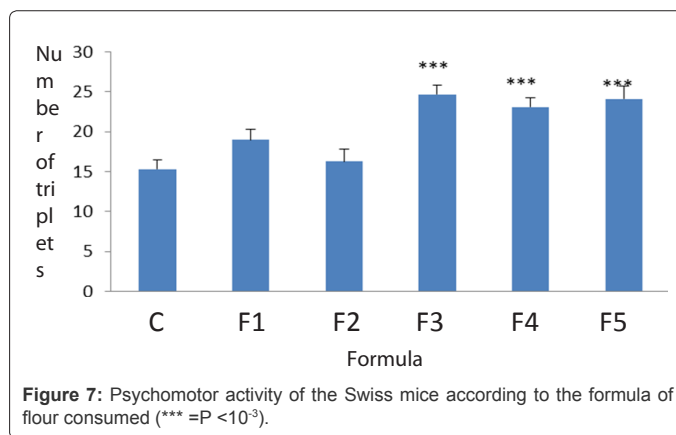


Figure 7: Psychomotor activity of the Swiss mice according to the formula of flour consumed (***)= $P < 10^{-3}$.

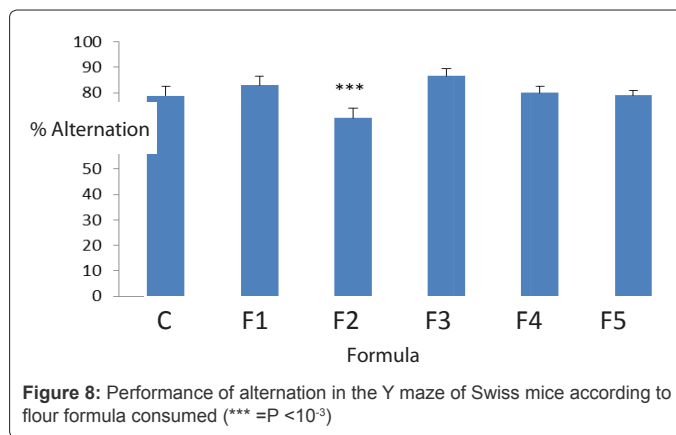


Figure 8: Performance of alternation in the Y maze of Swiss mice according to flour formula consumed (***)= $P < 10^{-3}$

of long term memory was observed for F1,F2, F3 and F4. The best result was obtained with F5. Our formulas have thus enabled earning as well as the standard formula richly selected (considered as "ideal"), but addition of salmon oil led to noticeable improvement of long term memory.

The Morris water maze showed that formulas did not induce deficiencies that could lead to learning deficits. However, among all formulas, F5 simply enriched in EPA and DHA led to the lowest learning and retention times. These results confirmed those of Bourre et al. [47] who showed that these fatty acids can control neurosensory functions and higher functions as learning. These results are also in line with

Formula	Morris Test: Learning					Average t_3-t_5	Significance
	t_1 (s)	t_2 (s)	t_3 (s)	t_4 (s)	t_5 (s)		
C	118 ± 3	94 ± 5	76 ± 3	68 ± 4	71 ± 5	72 ± 4	a, b
F1	114 ± 4	84 ± 4	85 ± 5	80 ± 5	70 ± 5	78 ± 5	a
F2	110 ± 3	101 ± 5	89 ± 5	69 ± 5	55 ± 4	71 ± 5	a, b
F3	108 ± 2	84 ± 2	88 ± 4	64 ± 5	50 ± 4	67 ± 4	b
F4	103 ± 4	95 ± 3	86 ± 6	64 ± 3	54 ± 3	68 ± 4	b
F5	105 ± 4	79 ± 5	74 ± 3	59 ± 3	48 ± 2	60 ± 3	c

C=control; different letter in the last column traduces significant difference between the formulas ($p < 0.05$).

Table 5: Learning time (Morris test).

McCann and Ames [48] who showed that increase in brain DHA content was associated with better cognitive performance and behavior in rodents and primates.

Conclusion

In this study, our main interest was to propose five formulations of infant flours based on local cereals to fight against children malnutrition which causes half of overall deaths of infants according to WHO. Five different flours were formulated according to international standards to meet child nutritional requirements. These flours were shown very stable as long as they are stored at low relative humidity (<75% RH).

The nutrition and behavior of mice was studied by comparing a control group that received standard nutritive food, and groups receiving F1 to F5 formulas. The results showed that F3, F4 and F5 formulas increased psychomotor activity compared to food control. Millet and maize combination seemed effective in stimulating psychomotor functions. All formulas, except F2 consisting mainly of corn, showed learning and memory abilities comparable to those of the standard food. F4 and F5 formulas appeared to be the most appropriate for efficient growth in height and weight, increased psychomotor activity and normal cognitive development. F5, the only formulation enriched with PUFA, gave better long term memory results than F4 enriched with vitamins and minerals.

F4, supplemented with vitamins and minerals, and F5, enriched in PUFA (EPA and DHA), equaled performance of enriched Western formulations. The fight against child malnutrition could be initiated by a variety of formulas enriched in fish oils depending on raw materials availability for each area of Africa. To start, F4 formula enriched with fish oil available in Africa could probably maximize nutritional benefits.

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