Research Article

Investigation on the Quality of Some Traditional Infant Flour Formulations Used for the Nutritional Recovery of Children in Benin.

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ABSTRACT

In Developing country, infant feeding practices do not always fit with quantity and quality requirements and lead to low growth potential of speech. In order to diversify the sources of protein commonly used in the formulation of infant flours, different formulations were made using locally available food ingredients such as roasted corn, fonio, soybean and fish flour (*Ethmalosa fimbriata*). Physicochemical, microbiological and nutritional analyzes were performed to assess the quality of different infant flours produced. The results indicated that the use of headless fish in the formulation of infant foods significantly reduced ($p<5\%$) microbiological contamination of samples of infant flours produced. The physicochemical and nutritional analyzes indicated that the formulation of infant flour made with roasted corn flour (50%), fonio flour (25%), soybean flour (12.5%) and fish flour (12.5%) provides for infant flour good nutritional values, marked by a high protein content (17.22 ± 0.11%) and minerals such as phosphorus (1.89±0.14%), calcium (2.28±0.17%), magnesium (0.156±0.013%) and iron (344.46±0.22mg/kg). This formulation of infant flour could help to reduce childhood nutritional problems, especially those related to protein and minerals deficiency.

Keywords: Infant flour, cereals, fishes, quality, nutrition, Benin

INTRODUCTION

According to the World Health Organization, malnutrition is the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions[1]. The term protein-energy malnutrition (PEM) applied to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus-kwashiorkor[1]. The most common form of malnutrition in Africa is protein energy deficiency affecting over 100 million people, especially 30 to 50 million children under 5 years of age[2].

In most developing countries, malnutrition is responsible for 60% of 10.9 million annual deaths of children under 5 years worldwide[3].

More than two thirds of deaths occurred in the early age and mostly resulted from inadequate feeding practices. Less than 35% of infants in the world are exclusively breastfed during the first six months of life. Complementary feeding usually starts soon or later with low nutritional and sanitary quality foods[3].

In Benin, infant and child feeding practices do not always fit with quantity and quality requirements, leading to low expression of growth potential[4]. In fact, 43.1% of children of less than 6 months are exclusively breastfed and 68% aged 6 to 8 months receive complementary foods in addition to breast milk[4]. The low trends observed may be explained by household food availability, inadequate maternal care and feeding practices[5].

Some legumes such as soybean, bean, and peanut, are important sources of protein currently used to increase the protein intake of the diet of children. However, these protein sources are sometimes insufficient to cover the nutritional needs of children due to their essential amino acids content. Indeed, according to Musgrove[6] and Benson[7], adult productivity depends to a considerable extent on the contribution of health and nutrition during early childhood. From birth to age 4 months, all the nutritional needs of children are fully covered in milk. But between 4 and 6 months breast milk is not sufficient to cover the needs for energy and protein of the child. This is the period during which nutrients necessary for child growth must supplement the breast milk slurry[8]. Quantitative protein requirements are about 20 g per day between 6 months and 3 years. Ideally, the amino acid composition of these complementary proteins should be identical to that of breast milk that is containing the same proportion of the nine essential amino acids[9]. Fortunately, it is possible to reconstruct a protein mixture composition meeting the needs of the child by mixing cereal flour with legume flour. Amino acids absent in cereal proteins are then supplemented by the amino acids present in legumes. However, these infant's flours are deficient in amino acids from animal origin.

Fish and fish products have an important place in the diet of the people of West Africa[10]. They are rich in proteins and the nutritional value of these proteins is comparable to that of the egg, milk and meat. In Benin, fishing has an important place in the national socio-economic balance as it sustains some 500,000 people and accounts for 3% of GDP[11]. However, the conservation of fish is very difficult due to the lack of adequate conservation system and post-harvest losses are estimated at about 20%[12]. Nowadays, fishes are processed into various products to increase the bioavailability of its proteins. Among these products, fish flours appear to have a high nutritional

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value[13]. Thus, the present study aims to investigate the nutritional potential and the microbiological quality of different infant flour formulations, traditionally used in Benin.

MATERIAL AND METHODS

Preparation of raw materials

Based on traditionally knowledge in the formulation of infant flour in Benin, raw materials such as cereals (primary source of carbohydrate), legumes (essential source of vegetable protein) and fish flour (main source of animal protein) were used.

Production of fish flour

Fishes (*Ethmalosa fimbriata*) are collected from fishermen of *Agbalilamè Tokpa* in southern Benin. Collected fishes were cleaned by rinsing with water, followed by gutting and salting brine (20g/L). These fish so treated have dried. Two types of drying was made: a solar drying on a site of fish drying located in the town of Ouidah in southern Benin, and an artificial drying by using an oven.

Solar drying conditions are those found on the site of drying: after salting, fishes were simply spread out in the sun on racks raised, allowing water to evaporate from the flesh of fish. In this study, fish spread in the sun are protected by filter cloths to prevent insect infestation that could constitute a source of contamination which can affect the quality of fish during drying. Periodical reversals are performed during drying to facilitate the process by exposing more of the surface of the fish in the air.

After a period of approximately one (01) months of sun exposure, natural drying was replaced by artificial drying by baking with a thermostatic oven. The drying temperature is 50 °C.

Monitor the water content of the fish placed in drying is carried out periodically until relatively constant water content (about 11%). After that, drying fishes were divided into two groups A and B. A first group (A) was carefully headed to serve for the production of fish flour labeled (F1), and fishes of second group (B) were directly used for the production of fish flour (F2). After milling, fish flours were then baked into oven with a temperature of 90 °C for one (01) hours in order to further reduce the water content of the fishes. After this operation, the final water content of fish flours is approximately 7%. Fish flour thus obtained is sieved and packed in sterile packaging. The Figure 1 showed the different stages of the production.

Corn, soybean and fonio flours preparation

Flours, including corn (*Zea mays*) and soybean (*Glycine maxima*) were obtained after unit operation sequences with sorting and cleaning grains, roasting and grinding the roasted grains.

However, for the preparation of soybean flour, crushing followed by gating was performed after roasting the beans. This is to reduce the amount of soybean flour in anti-nutritional factors. Fonio flour (*Digitariaexilis*) was obtained after shelling, winnowing, roasting and grinding grain.

Production of Infant Flour

Flours were produced by mixing in varied proportions, fish, corn, soybean and fonio flours as described by the technological diagram (Figure 2). Based on traditional knowledge used in the formulation of infant flour, three types of formulation have been investigated (Table 1).

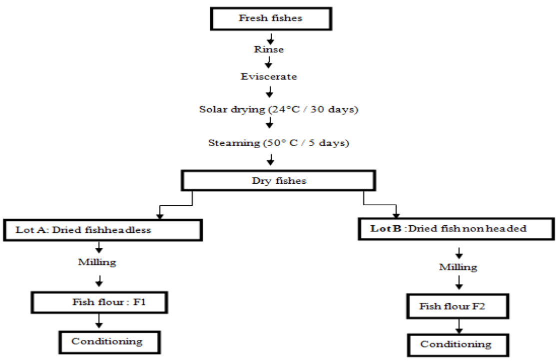

Figure 1: Technological Diagram of fish flours production

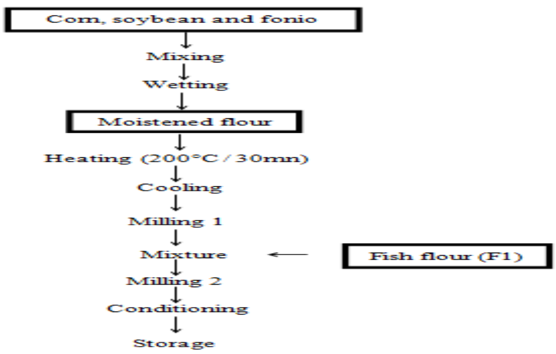

Figure 2: Technological Diagram of infant flours production

Table 1: Formulations of infant flours

Raw materials	*Formulation 1*	*Formulation 2*	*Formulation 3*
Roasted cornflour	50%	50%	50%
Fonio flour	25%	25%	25%
Soybean flour	12.5%	16.67%	8.33%
Fish flour	12.5%	8.33%	16.67%

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Table 2: Physicochemical and nutritional quality of fish flours produced

Parameters	***Moisture (%)***	***Protein (%)***	***Ash (%)***	***Iron (mg/kg)***	***calcium (%)***	***magnesium (%)***	***phosphorus (%)***
Fish flour *(F1)*	7,00±0,51^{a}	53,78±0,04^{a}	12,26±0,18^{a}	194,86±0,06^{a}	0,26±0,04^{a}	0,09±0,02^{a}	0,48±0,03^{a}
Fish flour *(F2)*	7,33±0,84^{a}	53,84±0,09^{a}	13,24±0,09^{a}	246,82±0,05^{b}	3,02±0,08^{b}	0,18±0,05^{b}	1,83±0,01^{b}

Values are mean (n=3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 3: Microbiological quality of fish flours produced (ufc/g)

	Total bacterial Count	Total coliforms count	Faecal coliforms count	*E.coli*	*Staphylococcus aureus*	A.S.R spores count	Mould and yeast count
Fish flour *(F1)*	5.10^{4}	<10	<10	*00*	*00*	00	02
Fish flour *(F2)*	8.10^{6}	1.10^{3}	4.10^{2}	6.10^{1}	*00*	08	5.10^{2}
European Union criteria (2005)	-	10	10	10	-	Absence/10 g	Absence/10 g

A.S.R: Anaerobic Sulfito-Reducer

Table 4: Physico-chemical and nutritional parameters of infant flour

Infant flours	**Moisture (%)**	**Proteins (%)**	**Ash (%)**	**Phosphorus (%)**	**Calcium (%)**	**Magnesium (%)**	**Iron (mg/Kg)**
Formulation 1	3.90±0.11^{a}	17.22±0.11^{a}	4.076±0.173^{a}	1.89±0.14^{a}	2.28±0.17^{a}	0.156±0.013^{a}	344.46±0.22^{a}
Formulation 2	4.18±0.35^{a}	17.07±0.46^{a}	2.548±0.102^{b}	0.43±0.07^{b}	0.35±0.04^{b}	0.085±0.004^{b}	182.05±0.43^{b}
Formulation 3	4.29±0.57^{a}	16.82±0.31^{a}	2.076±0.155^{b}	0.38±0.02^{b}	0.31±0.07^{b}	0.083±0.011^{b}	176.36±0.27^{b}

Values are mean (n=3) ± SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests

Table 5: Microbiological quality of infant flours

Infant flours	**Total bacterial Count**	**Total coliforms count**	**Faecal coliforms count**	***E. coli***	***Staphylococcus aureus***	**A.S.R spores count**	**Mould and yeast count**
Formulation 1	5.10^{4}	<10	<10	*00*	*00*	00	00
Formulation 2	7.10^{4}	<10	<10	00	00	00	00
Formulation 3	$4.\ 1\ 10^{4}$	<10	<10	00	00	00	00
European Union criteria (2005)	-	10	10	10	-	Absence/10 g	Absence/10 g

A.S.R: Anaerobic Sulfito-Reducer

Physicochemical and Nutritional Analysis

Moisture content of fish flour was determined by desiccation using the method of De Knegt and Brink[14]. A clean platinum dish was dried in an oven and cooled in a desiccator and weighed. From each sample, 5 g was weighed and spread on the dish, the dish containing the sample was weighed. It was then transferred into the air oven at 105°C to dry until a constant weight was obtained and the loss in mass was determined. Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 and fat content was obtained by Soxhlet extraction as described by Pearson[15]. Ash was determined according to the standard methods described by the Association of Official Analytical Chemists[16]. Minerals were analyzed by the method reported by Oshodi[17]. Minerals were analyzed by dry-ashing 1 g of the sample at 550°C in a furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Minerals content were determined using atomic absorption spectrophotometer (Perkin Elmer, Model 403).

Microbiological Analysis

To 25 g of each sample, 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total Coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the Faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using EMB (Eosine Methylene blue). Tryptone Sulfite Neomicin Agar was used for Anaerobic Sulfito-Reducer (ASR) count and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked using a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by

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calculation, bearing in mind the factors of dilution (Singh, 1991). The isolation of fungi from samples was performed using dilution plating method. 10 g of each sample were separately added to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10–1 dilution. Further 10-fold serial dilutions up to 10^{-4} were made. One milli litre of each dilution was separately placed in Petri dishes, over which 10 to 15 ml of Potato Dextrose Agar with 60 µg/ml of chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days (Rampersad, 1999).

Statistical Analysis

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) (19; 20)

RESULTS

Quality of Fish flour

Tables 2 and 3 showed the physico-chemical, nutritional and microbiological quality of different fish flours produced. The results of physicochemical characterization of two samples of fish flour (Table 2) showed that the moisture content of different samples ranged from 7.00±0.51% to 7.33±0.84%. Fish flours are rich in nutrients such as proteins (53.78±0.04-53.84±0.09%) and ash (12.26±0.18-13.24±0.09%). All samples analyzed were rich in minerals such as calcium, magnesium, potassium and iron, with a higher content of calcium (0.26±0.04-3.02±0.08 %). Fat content was very low. However, by comparing the values obtained in the two types of fish flours, there is a significant difference in parameters such as the levels of iron, calcium, magnesium and phosphorus (p<5%). The highest levels were recorded in the flour obtained from non headless fishes.

The evaluation of the microbiological quality of different fish flours produced (Table 3) indicated that the microbial flora of the flours obtained with dried headed fish is very low, with a total flora count of 5.10^{4}ufc/g. The enumeration of total coliforms and faecal coliforms was less than 10cfu/g with an absence of spores of an aerobic sulphite reducers (ASR). Fungal flora was 02ufc/g. However, microbiological results from the flours obtained with non-headless fish, indicated the high microbial count, characterized by a total flora of 8.10^{6}cfu/g. The enumeration of total coliforms and fecal coliforms was high than 10cfu/g with the presence of spores of an aerobic sulphite reducers (ASR). Fungal flora was also high (5.10^{2}ufc / g).

Quality of Infant flour

Tables 4 and 5 showed the results of the evaluation of the physico-chemical and microbiological characteristics of different infant flours. Flours produced have moisture content between 3.90 ± 0.11% and 4.29 ± 0.57%. Nutritional analyzes indicated that flours are high content in protein (16.82 ± 0.31 - 17.22 ± 0.11%) and minerals including iron (344.46 ± 0.22 - 176.36 ± 0.27 mg/kg), calcium (0.31 ± 0.07 - 2.28 ± 0.17%), magnesium (0.083 ± 0.011 - 0.156 ± 0.013%) and phosphorus (1.89 ± 0.14-0.38 ± 0.02%). Comparing the nutritional characteristics of different infant flours produced, there is no significant difference in moisture and protein contents of different infant flour (p <5%). However, significant different are observed on levels of minerals in infant flours. Indeed, the highest levels of minerals are obtained in the infant flour obtained with the *formulation 1* consists with of roasted corn flour (0.5kg/kg), fonio flour (0.25kg/kg) soybean flour (0.125 kg/kg) and fish and flour (0125 kg/kg). The evaluation of the microbiological quality of different infant flours produced (Table 5) indicated that the microbial flora is very low. The enumeration of total coliforms and faecal coliforms was less than 10cfu/g with an absence of spores of anaerobic sulphite reducers(ASR) and fungi.

DISCUSSION

The nutritional potential of fish flour (proteins and minerals content) indicated that the use of small dried fishes as ingredient for making infant flour is an interesting alternative that should help to diversify infant complementary feeding. They are animal proteins of good nutritional quality and digestibility. Mothers who fed their child with infant flour enriched with small dried fishes should be encouraged[21]. Indeed, infants and young children are often regarded as two particularly vulnerable groups in terms of food safety. Furthermore, the requirements for essential nutrients due to rapid growth and development put these groups at risk of deficiencies of essential minerals.

It is therefore essential that products intended for use by infants and young children contain minerals in amounts that satisfy their nutritional requirements without leading to adverse effects. In addition, products must not contain contaminants in amounts that could lead to negative health effects. Then, great attention should be paid to the quality of raw food ingredients used for the formulation of infant flour as done in this study. This has allowed to remarks, by observing the results of physico-chemical, microbiological and nutritional analysis, that despite the high levels of minerals in flour obtained with non-headless fish (F2), there are potential microbiological risks associated with their incorporation into food for children. The presence in high proportion of microorganisms such as *E.coli,* Anaerobic Sulfite Reducer, mould and yeast in fish flour (F2) compared to those obtained with headless fish flour (F1) confirmed that the important source of contamination is localized in the head of the fish. This could be explained by the fact that the gills off is hare important organs in the phenomena of respiratory gas exchange are also richly vascularized and therefore constituted a center of concentration of microorganisms[22]. It would be better to use the headless fish in feed formulation for children as done in this work.

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During the first 2 years of life, infants undergo a period of rapid growth. Minerals are particularly important at this time for optimal development and breast milk contains the appropriate balance[23]. Infant formulas need to contain all necessary vitamins and minerals for those babies whose mothers cannot or have chosen not to breast-feed. Results of nutritional potential of infant flours produced, indicated that the combination of raw material ingredients using the *formulation 1* has the best nutritional content, compared to those from literature[24]. This may be explained by the relatively high amount of fish and fonio flours used. It has been reported that proteins content for infant flours produced in Africa fluctuate from 8.2% to 21.3%[25]. It was the case for this study. Differences often observed between flours may be probably due to protein sources and/or processing effects. The presence relatively in high proportion of minerals also constitutes an important factor in the nutritional potential of this infant flour. Indeed, minerals are needed to form body structures and regulate chemical reactions. Like vitamins, minerals are needed in small amounts and do not provide energy. Also much like vitamins, minerals are required to regulate many body processes, such as heart beat, nerve response and reactions, blood clotting, fluid regulation and energy metabolism (release of energy from food). Minerals form part of the structure of bones, teeth, nails, muscles and red blood cells. The body does not function properly unless all are supplied in sufficient quantities.

Calcium is essential for healthy bone growth and for nerve and muscle functions; it may protect against high blood pressure. Calcium cannot be made in the body so it is essential that babies receive the calcium they need from their diet. In their first year, babies double the mass of their skeleton[26]. The structure of the body depends on calcium. About 99% of the body's entire supply is deposited as calcium salts in the bones and teeth (Thomas and Bishop, 2007). During these periods of rapid growth, bone mass will depend on appropriate amounts of calcium being available from dietary sources[27] and has implications for bone health in later life. It is also found in body fluids and tissues where it is important for cell membrane transport and stability[28], and is needed for coagulation[29]; low plasma levels being associated with a reduced ability to form blood clots[30]. Calcium is also involved in digestion and muscle contraction. In childhood and adolescence, it is particularly important to eat and drink calcium rich foods to ensure maximum calcium storage and strong bones.

Babies begin life with their own stores of iron but after 4-6 months they need iron from dietary sources as their stores are depleted[31]. The levels of iron in breast milk are low but are very well absorbed (about 50%) while that in infant formulas is less well absorbed (about 10%) so the latter have higher levels of iron than breast milk[32]. Iron has several roles. It is a component of both hemoglobin in blood and myoglobin in muscle as well as being important for the body's metabolic processes[28]. It is also important in brain function and development[33]. At birth the brain is only about a quarter of its eventual size[34] but grows rapidly up to the age of two. Insufficient iron in the diet during the first two years of life has been linked to cognitive and behavioral problems later in life[35,36].

Appropriate levels of phosphorus in an infant's diet are also important to achieve optimum bone mineralization. 85% of the body's phosphorus is found in the bones[28]. The remainder is within DNA and RNA and in phospholipid membranes. Phosphorus is associated with the release of energy and oxygen to cells associated with energy metabolism[30].

Based on the nutritional potential of this infant flour produced (Formulation1), it should be recommended for use in infant feeding. Indeed, the WHO Global Strategy on Infant and Young Child Feeding emphasizes the importance of infant feeding and promotes exclusive breastfeeding in the first six months of life. In infants who cannot be breast-fed or should not receive breast milk, substitutes are required. These should be a formula that complies with the appropriate Codex Alimentarius Standards or, alternatively, a home-prepared formula with micronutrient supplements (03).

CONCLUSION

The infant flour formulation proposed in this study offers a nutritional potential benefits that can help to improve nutritional status of 6 to 12 month-old children in rural area. Beside this, there is need to integrate personal production of this infant flour in a nutrition and counseling package offered to mothers in order to assess its benefits on nutritional outcomes of targeted children.

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