

Microbiological Quality and Amylase Activity of Weaning Flour Formulated from Taro (*Colocasia Esculenta* (L) Schott), Pigeon Pea (*Cajanus Cajan*) and Malted Maize (*Zea Mays*): Physicochemical and Organoleptic Characteristics of Weaning Mush

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Abstract Exclusive breastfeeding for 4-6 months of life, followed by appropriate complementary feeding, are major factors in the nutritional status of young children. The study aimed to produce infant flour from raw food ingredients available locally in Cote d'Ivoire. Infant flour has been proposed from mixed flours of Taro (*Colocasia esculenta* (L) Schott), legumes (*Cajanus cajan*), malted maize (*Zea mays*) and sucrose in a proportion of 58:18:14:10, respectively. In a first step of the study, the formulated infant flour was evaluated for a microbiological examination such as the total viable count, fecal coliforms, yeasts and molds counts, the detection of *Escherichia coli* and *Salmonella* and amylase activity was studied. Immediately after production, the total viable count (10^4 CFU / g) was below the maximum standard counts (10^5 CFU/g) and the flour was free from pathogens (*Escherichia coli* and *Salmonella*). The count of fecal coliforms (1 CFU/g) and yeasts and molds (1 CFU/g) were significantly lower than standard levels (10^2 and 10^3 CFU/g, respectively). As for the amylase activity, it was 0.400 μmol reducing sugars / minute the first week of preparation but after one year of storage, this activity was stabilized at 0.010 μmol reducing sugars/min. A second stage concerned the physico-chemical and organoleptic characteristics of the porridge made from formulated infant flour. The energy density of the porridge prepared from this infant flour was very high (approximately 119 kcal / 100 ml). A fluid consistency was 90 mm / 30 seconds.

Keywords Porridge, Microbiological quality, Amylase activity, Physicochemical characteristics, Organoleptic

1. Introduction

In less developed countries, malnutrition is responsible for 60% of 10.9 million annual deaths of children under 5 years worldwide [1]. More than two thirds of deaths occurred in the early age and mostly resulted from inadequate feeding practices. Less than 35% of infant in the world are exclusively breastfed during the first six months of life [2]. At birth, mother's milk is infant's food par excellence at mammals which could cover the energy's and nutriment needs of young children. But, from fourth to sixth months, the energy and micronutrients contents of milk could be not sufficient to satisfy the need of baby [3].

Normal growth and development of a child required meeting his nutritional needs through a balanced diet [4]. It is

therefore necessary to supplement the infant's diet with certain foods to meet all of the infant's needs as they increase. The complementary foods must be then provided in balanced proportions, the major nutrients such as proteins, lipids and carbohydrates [5]. The foods initially received by infants, in addition to breast milk, are very often porridge prepared in fluid form. Thus, complementary flours, based on local products responding to consumption habits, have been developed in certain African countries by processing units [6-8]. The raw materials, generally used for these flours, are cereals (rice, millet, sorghum, corn), roots (cassava) or tubers (yam, taro) associated with legumes such as soybeans, beans or cowpeas [5].

In Africa's local language, weaning is merged in breast-feeding substitution by solid food. Therefore, from fourth to sixth months the infant is bound to family meals or to eating gruels made of cereal or tuber flours. In deed on the one hand, the composition in nutrients of baby's food is poor and unbalanced; on the other hand, the richness in starch

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gave a high viscosity to the gruels [9]. So that, the gruels have been accepted for infantile consumption, the consistency of weaning mush must be fluid because of limited gastric capacity in infants. Therefore, the fermentation and germination of cereal grains have interest solutions used to reduce the high viscosity and increase the energy density of gruels [10]. The criteria of WHO recommended to have at least 30 dry matter of gruel, 100 to 120 kilocalories for 100 ml of mush of energy density and the content of protein equals 12-15 g for 100 g [11]. So, to protect the young children from infantile malnutrition, the baby's food needed a mastery of physical, chemical, hygienic and organoleptic characteristics. The aim of this study was to formulate a complementary food from ingredients like taro, leguminous plant, malt maize, available in rural and urban areas of Côte d'Ivoire. Thus, present study has been designed for evaluating microbiological quality and amylase activity of weaning flour. The physicochemical and organoleptic characteristics of gruels prepared from weaning flour were determined and tested of acceptability among children aged 6 to 12 months of Abidjan district.

2. Materials and Methods

This study was carried out in an urban area in the Abidjan district in the south of Cote d'Ivoire. Determination of acceptability test by mothers and their 6 to 12 months old children were the main steps of this study.

Raw material plant

Raw materials used in mixed flours production were constituted of low ground taro (*Colocasia esculenta*), seeds of pigeon pea (*Cajanus cajan*) and malted maize (*Zea mays*).

Production of flours

Taro flour

Taro was peeled, washed, jagged, washing at 90°C during 5 min and then dried in an incubator at 65°C during 5 days. Then, the dried pieces of taro were crushed with a Forplex type grinder. Flour was obtained after sieving with sieve diameter from 180 to 500 µm.

Malted maize flour

Seeds are soaked during 48 hours then spread out on cloth humidified for 3 days. Malted maize is dried, sorted out, degermed and crushed. After sieving from 180 to 500 µm size sieves, malted maize flour was obtained.

Pigeon pea flour

Seeds are sorted, washed, roasted, and then crushed with Forplex type grinder. Flour was obtained after sieving with size sieve diameter from 180 to 500 µm.

Production of infant flour

These ingredients were combined in precise doses: Taro (58%), Pigeon pea (18%), Malt maize (14%) and sucrose (10%). The flow chart of the flour formulation was simplified such that it is reproducible at household level.

Preparation and characterization of weaning mush

Mush was prepared in aluminium saucepan and different proportions of Taro, Pigeon pea and malted maize flours and sucrose were mixed in water (180 ml) [13]. The flow distance (mm / 30 sec) of the gruels obtained was measured using a Bostwick consistometer [13]. Dry matter contents (DM) and consistency of mush were followed; the objective was to establish the best possible formulation.

Biochemical analyses

Determination of the dry matter contents

Dry matter contents (DM) of 1g of sample was measured after flours drying in an oven at 105°C for 24 hrs. Ash content (5 g) was determined by incineration of flour at 500°C in an oven [14].

Determination of the energy density in kilocalorie

Dry matter and energy of the porridge were determined using the formula suggest by Ouédraogo and collaborators [10].

α-Amylase activity

Preparation of rough enzymatic extract from mixed flours

Flour (10 g) was mixed with 20 ml of a sodium chloride solution (NaCl 0,9%). The resulting mixture underwent to a sonication for 10 min and a centrifugation at 5 000 tours /min during 30 min. The supernatant, constituting the enzymatic crude extract was preserved for amylase activity study of prepared flour.

In-vitro digestibility of flour

The starchy substrate used for this test was that of mixed flour. *In-vitro* flour digestibility consisted to a reaction between the enzymatic crude extract and an amount of flour at 1% (w/v). The reaction medium was constituted as follow: 500 µl of acetate buffer (100 mM, pH 5), 100 µl of enzymatic crude extract and 200 µl of 1% of flour. The mixture was then incubated at 37°C for 3 hours in a Marie-bath. Aliquots of the reaction medium were taken every 10 min for reducing sugars [14].

Microbiological quality Preparation of test sample:

Flour (10 g) was diluted in warm (45°C) sterile diluent peptone water solution (90 ml) to make primary dilution (10^{-1}). Then a series up to 10^{-5} was prepared by transferring primary dilution (1 ml) into test tube containing sterile diluent (9 ml) to obtain 10^{-2} dilution and repeating the operations with sterile diluent (9 ml) using the 10^{-2} and further dilutions to obtain 10^{-3} , 10^{-4} and/or 10^{-5} .

Enumeration of total viable count (Colony count technique at 30°C): Total viable counts were enumerated [14].

Enumeration of yeasts and moulds counts (Colony count technique at 25°C): yeasts and moulds count were enumerated [14].

Enumeration of faecal coliforms counts (Colony count technique at 30°C): Add 50 g or 50 ml of test sample to 200 ml peptone water, then blend in a stomacher for 1 minute at

medium speed. Make -1 to -3 dilutions in saline. Make duplicate plates on VRBL or EMB medium of each dilution by spread plate technique. Incubate for 18-24 hrs at 44°C, count red colonies with diameter greater than 0.5 mm and calculate the number of fecal coliforms per gram or milliliter of sample.

Subculture on EMB plates to confirm *E. coli*: Both positive and negative organisms control should be included with each batch of samples. Confirmation tests: Indol, ethyl red, Voges-Proskauer, Citrate, Catalase, oxidase, glucose and lactose fermentation.

Detection of *Salmonella*: Add 25 g of test sample to 225 ml, of peptone water or selenite cystine broth, blend in stomacher for one minute at medium speed and, incubate for 16 hours at 37°C. Isolation of *Salmonella*: add 0.1 ml of culture to 10.0 ml of Rappaport medium and incubate for 24 hours at 42°C. Add 2.0 ml of culture to 20.0 ml of selenite cystine medium and incubate for 24 hours at 37°C.

Acceptability test

The acceptability test was a sensory evaluation of porridge prepared with the infant flour. Prior to this, a survey was performed in target households identified through the help of medical staff of health centers in the study area. The aim of the survey was to generate information about complementary feeding practices in the study area to help for estimating the amount of porridge to be to the children. Mothers were provided information on the test procedure and their assessment of the porridge was recorded. They were asked not to feed their children with any food including breast milk before coming for the test. Mothers and their children were separated from each other to avoid interference. The children were fed with a specific amount of gruel a standard bowl. Indeed, all children were fed in bowls of same size and color. Two repetitions inside an episode were offered to each child. When a child had consumed the whole amount of gruel offered during the first repetition, the same amount was immediately offered to him in order to continue the episode. On the other hand, when the child did not consume the first repetition, the remaining amount was offered to him at the first repetition of the following episode, after a five minute-break [15]. A child could then have one or two repetitions inside an episode and also had to make three episodes in order to confirm his attitude (denial or acceptance to continue the gruel consumption). During the acceptability test, each child was followed by an observer whose function was to give the gruel to the mother, to ensure that the child was not encouraged or forced to eat; and to fill out the questionnaire. The acceptability test always took place early in the morning between 7 and 8 am for three consecutive days. Number of episodes, gruel's quantity consumed by episode and period of consumption were recorded. Other variables such as total quantity of gruel consumed and speed of the gruel ingestion were also calculated [15]. Mother's assessment of gruel was a relevant indicator for further steps in the programme implementation. Mothers were asked to assess the taste, consistency and color

of the gruel using a five level Likert scale ranging from unpleasant to very pleasant.

Sampling for acceptability tests

A group of 123 children aged 6 to 12 months were identified from a list provided by health centers. Children's age was reported from health books or birth certificates in the village health centre. Where these documents were unavailable, social or national events was used for age estimation. A questionnaire was administered to mothers on infant feeding habits.

Several criteria were used for sampling: age (6-12 months), feeding mode (spoon or cup), usual time of first meal (7-8 am) and parents' agreement. After analyzing the data collected based on the questionnaire, sixty-five mothers and their children (of which there was a twin) who fulfilled the selection criteria and accepted to participate to the study were enrolled [15].

Data analysis

Nutritional and microbiological characteristics of the infant flour were compared to Codex Alimentarius standards through a conformity test of means. After ensuring that required conditions were met, analysis of variance was used to examine difference in gruel intake between three consecutive days of test and within child day-to-day variations. Proportion of gruel consumed by the child during the test, duration of the test and average speed of gruel consumption were calculated for each child. Statistical Package for Social Sciences software (SPSS 16.0, SPSS Inc., USA) was used for statistical analyses. The statistical significance for all analyses was set at 5% and all tests were two-sided. Data collected during mother's acceptability test was analyzed in a descriptive way. Representatives of the Ministry of the Health in the Abidjan district gave their verbal approval for the implementation of the study. Administrative local authorities such as Prefect and Mayor were informed about the study and gave their verbal consent. Selected mothers were informed about their duties and right in participation in the study.

3. Results

Weaning flour formulated

The combination of ingredients used in weaning flour formulated was 58% of taro, 18% of pigeon pea, 14% of malted maize and 10% sucrose, the best combination among all those were tried out (Table 1).

Table 1. The best formula of weaning flour basis of Taro

Ingredients of infant flour	Proportion (%)
Taro	58
Pigeon pea	18
Malted maize	14
Sucrose	10

Characteristics of the weaning flour formulated

Microbial populations present in weaning flour are presented in Table 2. Total viable bacterial count (TVBC) of the infant flour averaged 10^4 CFU.g⁻¹ were lower than from the recommended values (10^5 CFU.g⁻¹, $p=0.199$). Faecal

coliforms were encountered at level of 1 CFU.g⁻¹ and were significantly lower than the 100 CFU/g recommended by Codex Alimentarius ($p=0.002$). Yeasts and moulds count averaged 1 CFU/g. No pathogens (*Escherichia coli* and *Salmonella*) were found in this flour.

Table 2. Count of aerobic mesophilic germs, faecal coliform, yeasts, moulds and pathogens germs in weaning flour formulated

Germs counted	Flour just after production	Flour after one year	Criteria of FAO/OMS
Aerobic mesophilic germs (CFU/g)	10^4	10^5	10^5
Faecal coliform (CFU/g)	1	10	10^2
<i>E. coli</i> (CFU/g)	0	0	10
Yeasts and moulds (CFU/g)	1	10^3	10^3
Salmonella	absence	absence	absence

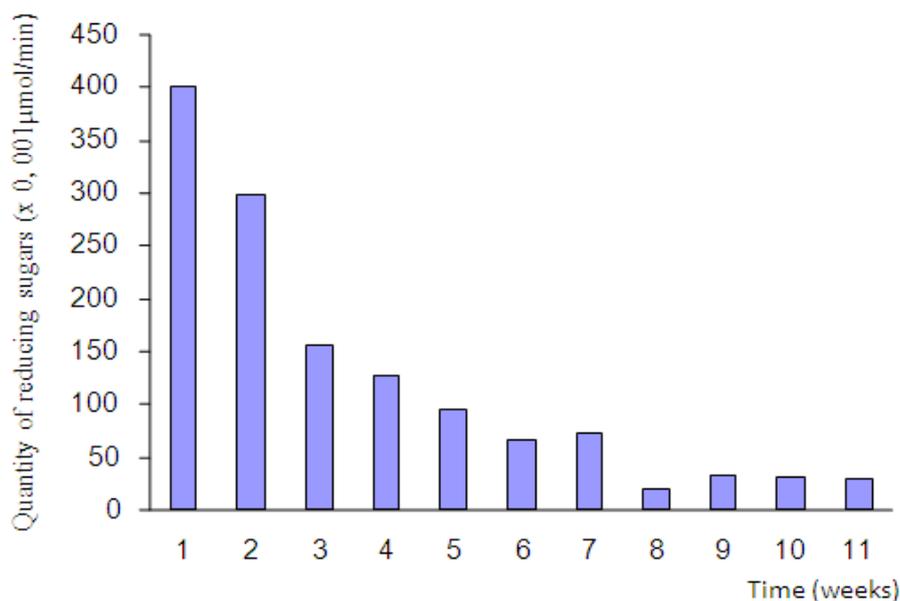


Figure 1. α-amylase activity of infant flour during a conservation time

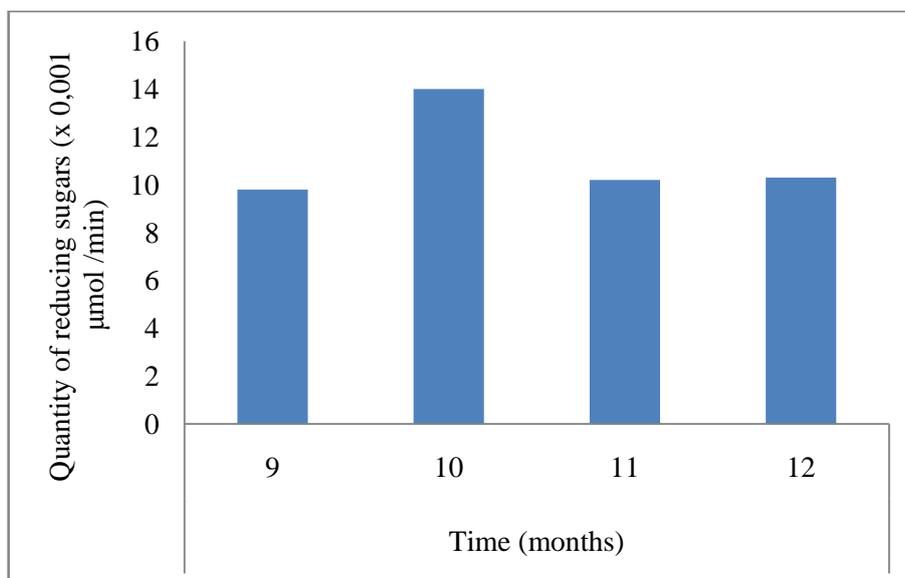


Figure 2. α-amylase activity of infant flour during one year of conservation

Changes in α -amylase activity of infant flour formulated during his conservation are presented in Figures 1 and 2. The strongest activity was observed in flour at the first week (0,400 $\mu\text{mol}/\text{min}$ of reducing sugars). The activity was decreased during the second at the eleventh week (0,300 at 0,050 $\mu\text{mol}/\text{min}$ of reducing sugars). The hydrolytic enzymes activities were present up to one year (0,010 $\mu\text{mol}/\text{min}$ of reducing sugars) but lower to first month activities.

Characteristics of gruels prepared from infant flour formulated

The gruel prepared from weaning flour had 30.9 g /100 g of dry matter. The mush fluid consistency was 90 mm/30 s and its density energy of 119 kcal /100 g. The protein content was 15.75% (Table 3).

Table 3. Physicochemical characteristics of weaning mush

Physicochemical characteristics	Weaning mush
Dry matter (g /100 g)	30.90
Density energy (kcal /100 g)	119
Fluid consistency (mm /30 seconds)	90
Protein content (g /100 g)	15.75

Acceptability test with children

Table 4 presented that the organoleptic attribute scores associated with porridges made from weaning flour formulated and commercial complementary food (control). The mean sensory scores of the control porridge and that of formulated flour showed no significant difference except in their odour characteristic acceptability ($p \geq 0.05$). The quantity of gruel consumed by the baby at time of sensory evaluation is presented in Table 5. The children have consumed the formulated flour mush (315 \pm 10 g) and gruels of marketed flour. The children accepted the gruel.

Table 4. Average trained panel scores (n=20) of various organoleptic characteristics of mushes prepared from mixed flour and marketed flour

Characteristics	Weaning mush	
	Gruel of mixed flour	Gruel of marketed flour
Taste	4,2 \pm 1,21 ^a	4,6 \pm 0,39 ^a
Color	4,4 \pm 0,39 ^a	4,7 \pm 0,36 ^a
Odor	4,7 \pm 0,36 ^b	4,5 \pm 0,39 ^a

*Results are the medium of 20 panelists mother-baby who have tasted
Medium values of lines indexed of same letters are not different threshold of $p \geq 0,05$.

Table 5. Quantity of gruel consumed by the baby at the time of sensory evaluation

Type de bouillie	Quantity of mush consumed (g) by 20 babies		
	1 ^{ère} Showing	2 ^e Showing	Medium
Mush of formulated flour	320 \pm 12	310 \pm 8	315 \pm 10
Mush of marketed flour	315 \pm 9	322 \pm 5	318 \pm 7

4. Discussion

The development of a healthy and quality complementary food that met international criteria required technical skills and expertise to take into account several technological, microbiological and nutritional parameters. This required applying the appropriate formulation methods [16]. Infant flour should be contained the macronutrients and micronutrients in quantity and quality necessary for the harmonious growth of the child [16]. In the present study, the principle of mixing various ingredients is useful to meet this requirement. The development of a nutrient-balanced flour meeting WHO standards [17] guided in this study. Indeed, each ingredient provided in the formula must be contributed to solving a nutritional requirement. Thus, in the formulation, the taro flour was a source of energy and the basis of weaning flour while the pigeon pea provided the proteins and the malted corn the enzymes.

Raw food ingredients used for the formulation of the infant flour (taro, pigeon pea, malted maize and sucrose) and their respective proportion (58%, 18%, 14% and 10%) were similar to the ones used for Bitamin flour (millet: 67%, Bean: 20% and pulp baobab's fruit: 3%) promoted in Republic of Niger [18]. Millet and baobab pulp were used as cereal and minerals and vitamins sources respectively whereas in the Benin infant flour, maize and water from boiled leafy vegetable were used [19].

Mush had a high energy density (119 kcal/100 ml of mush) true to international criteria of WHO/UNICEF (1998) [20]. This energy could be sufficient to satisfy the energy needs of young children. Moreover, the supply of malted maize has improved the consistency of mush flour infant. The children would eat easily that gruel.

When porridge is viscous and heavy, it is indigestible, and children after consuming a small amount feel full. These types of traditional porridge are therefore unsuitable for children as they cannot meet their nutritional needs. The incorporation of low doses of sprouted corn flour (source of alpha amylases) in heavy and viscous porridge results in an increase in flow rate associated with an increase in dry matter and energy density [21]. Prepared porridge is liquid and fluid. This is due to the hydrolytic action of alpha amylases which break down large starch molecules into smaller molecules (maltodextrins) with reduced swelling capacity. Sprouted corn flour therefore predigests starch in order to make porridge more digestible and easier to consume. The present results corroborate those obtained with Zannou-Tchoko et al. [22] who used small amounts of germinated millet flour (5 to 15%) to reduce the viscosity of weaning porridges made from cassava and its derivative, attiéké. The low dose of 14% of sprouted corn flour, flows of 90 mm/30 s, a dry matter of 30%, an energy density of 119 Kcal / 100 ml of porridge and a protein content of 15.75 g/100 g corresponding to FAO/WHO standards [23] according to the work of Kouassi et al. [24]. These values highlight the quality and ability of

sprouted corn flour to predigest starch. The results of the present study are in agreement with those of several authors having similar values for the same parameters with different cases [16,22,24].

The aerobic mesophilic flora that was found complies with the criteria defined by the FAO/WHO since no result exceeded the limit of 10^5 CFU/g. Also, total coliforms count ranged from 1 to 10 CFU of fecal coliforms in 1 g of the sample; however, *Escherichia coli* has not been identified. In the samples analyzed (respectively just after the production of the flour and one year after the production of this flour), yeasts and molds in the flour did not exceed the recommendation which is 10^3 CFU/g. Salmonella is absent in our sample. In summary, in the presence of micro-organisms indicative of general hygiene and in the absence of identified potentially pathogenic germs, it can be concluded that the consumption of this infant flour does not present a danger to health. These results are consistent with some studies [25-29].

5. Conclusions

Weaning mush prepared from mixed flours showed that this infantile flour could cover the energy's needs and nutriment needs of young children. Therefore, the mush weaning had a good consistency, a good content of dry matter and high energy density sufficient for children.

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