American Journal of Food and Nutrition, 2022, Vol. 10, No. 1, 16-23
Available online at http://pubs.sciepub.com/ajfn/10/1/2
Published by Science and Education Publishing
DOI:10.12691/ajfn-10-1-2

Over Time Evolution of Nutritional Parameters and Sanitary Quality of Infant Formula Produced in Burkina Faso

Waré Larissa Yacine[1,2,*], Compaoré Hamidou[1], Dao Aboubacar Sidiki[1], Durand Noël[3,4], Hagrétou Sawadogo-Lingani[1], Montet Didier[3,4]

[1]Food Technology Department, Research Institute of Applied Sciences and Technologies, National Center for Scientific and Technological Research (DTA/IRSAT/CNRST); 03 BP 7047 Ouagadougou, Burkina Faso
[2]Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food (LaBESTA), Doctoral School Sciences and Technologies, University Joseph KI-ZERBO; 03, BP 7021, Ouagadougou 03, Burkina Faso
[3]CIRAD, UMR Qualisud, TA B-95/16 ; 73, rue Jean-François Breton, 34398, Montpellier cedex 5, France
[4]Qualisud, University of Montpellier, CIRAD, Montpellier SupAgro, University of Avignon, University of Reunion, Montpellier, France
*Corresponding author: ly.ware@gmail.com

Received February 20, 2022; Revised March 22, 2022; Accepted March 29, 2022

Abstract The infant formula produced aim to cover the nutritional needs of children under 5 years old. Thus, these flours are rich in macronutrients and micronutrients, mainly in carbohydrates, proteins, lipids, iron-zinc and vitamin A. Over the months, contaminants can degrade the macronutrients and micronutrients contained in infant formula. These contaminants can lead to a change in taste, odor and also alter the quality of these foods by the presence of insects and toxins. The aim of this study was to determine the microbiological, physicochemical and nutritional parameters of infant formula at an initial time T1 and at a time T2 6 months later. Then, to make a comparison of the data. Thus, 24 samples of infant formula were collected throughout the city of Ouagadougou. In order to follow the evolution of the parameters over time, 12 samples of infant formula were analyzed at time T1. The other 12 samples were stored at room temperature (35°C-40°C) for 6 months before being analyzed. ISO methods were used for the enumeration of total mesophilic flora, faecal coliforms, yeasts and moulds. The official AOAC methods were used for the determination of energy values of each sample. The determination of Beta-carotene and mycotoxins [(aflatoxins, ochratoxin A and fumonisins (B1+B2)] in infant formula was performed by high performance liquid chromatography (HPLC). In terms of results, the microbiological analysis showed that the bacterial load of the samples remains satisfactory for the germs searched. After 6 months of storage, an increase in the bacterial load of the samples for the desired germs was observed but remains acceptable. The determination of the energy value of the analysed infant formula varied between 390.4±22.8 and 437.2±1.0. After 6 months of storage, the caloric value of the infant formula varied from 328.2±7.6 to 417.4±3.3 which is lower than the Fasonorm recommendation which must be > 420 kcal. Similarly, the Beta-carotene content of infant formula varied between 0 to 596.9±6.1 at time T1. After storage, the contents decrease and are lower than the Fasonorm recommendation which must be > 420 µg. However, mycotoxin levels increase after storage especially for aflatoxin B1 (EU regulation for infant formula: 0.1µg/kg for aflatoxin B1). In conclusion, this study showed statistically there is no significant difference between the studied parameters and the storage time. However, significant differences were observed according to the origin of production of the infant formula (artisanal or semi-industrial). Also, in a global way, we can say that the sanitary quality of the infant formula was unfit for consumption due to the presence of aflatoxin B1 contents.

Keywords: *infant formula, mycotoxins, storage time, nutritional value, contamination, Burkina Faso*

Cite This Article: Waré Larissa Yacine, Compaoré Hamidou, Dao Aboubacar Sidiki, Durand Noël, Hagrétou Sawadogo-Lingani, and Montet Didier, "Over Time Evolution of Nutritional Parameters and Sanitary Quality of Infant Formula Produced in Burkina Faso." *American Journal of Food and Nutrition*, vol. 10, no. 1 (2022): 16-23. doi: 10.12691/ajfn-10-1-2.

1. Introduction

In order to improve the nutrition of young children (0-5 years) in a sustainable way and to contribute to the reduction of the prevalence of malnutrition and micronutrient deficiencies in infants and young children, the NUTRIFASO project was initiated by GRET/IRD [1]. In urban areas, the strategy of the NUTRIFASO project is to support micro and small enterprises in Burkina Faso that produce, or wish to produce, fortified foods based on local raw materials in order to improve the quality of their products (nutritional composition, organoleptic quality, sanitary quality, packaging, etc.), to improve their production processes and to increase the visibility and sales of their products. Thus, since its implementation, several companies have benefited from the project's support and are able to produce infant formula for children aged 6 to 23 months in accordance with NUTRIFASO's specifications [2]. Nowadays, many formulations of infant formula have been developed and all of them are enriched and suitable for nutritional recovery. Indeed, some authors as Kayalto *et al.* (2013) have worked on the nutritional value and hygienic quality of local enriched infant formula of Chad [3]. Zongo (2020) showed that Moringa leaf powder improved the recovery of acutely malnourished children [4]. Kayalto *et al.* (2016) and Dao *et al.* (2021) found that infant flour enriched with Moringa leaves and néré (*Parkia biglobosa*) pulp at a certain content was appreciated and had an energy value close to VITAFORT, a reference infant flour and was very rich in Beta carotenes [5,6]. Sanou *et al.* (2017) showed that the microbiological quality of the analyzed infant formula were satisfactory with the absence of *Staphylococcus aureus* and Salmonella in 1 and 25 g of each sample after culture [7].

However, during the processing and preservation of food products, contamination by pathogens or toxins could occur. Numerous studies have shown that there was a close relationship between the state of nutrition and infectious diseases. Infectious diseases occur more easily and are often fatal for malnourished children, and may be the underlying causes of malnutrition [8]. The malnourished child is less resistant to disease, gets sick more often, and as a result malnutrition worsens [8]. Fortified foods, commonly called complementary foods (infant formula), are based on local raw materials. However, if they are poorly processed and or preserved, these infant formulas may contain certain pathogenic germs or substances that are harmful to health [7,9]. Indeed, it has been reported that the presence of pathogenic germs was the cause of diarrhoea (cholera and dysentery) [10] of children under 5 years old. Work done on cereals, legumes and oilseeds has also shown the presence of mycotoxins (aflatoxins, ochratoxins, fumonisins) especially in maize, millet, sorghum, groundnut, sesame, soybean etc. [11,12,13,14,15]. Chronic exposure to aflatoxin can have a negative impact on health. It has been associated with liver cancer, growth retardation, and weakened immune systems in children [16]. At high levels of concentration, aflatoxin exposure can cause haemorrhagic, oedema, and even immediate death [17].

The conservation of these infant formula is usually done at room temperature and for a long period of time sometime several months on the shelves of the market or at the mother's house who prepares the child's food. These conditions can play on the variation of the quality of the food and the development of microorganisms making the food unfit for consumption. Thus, the objective of this study was to monitor over time the evolution of the nutritional value and health quality of some infant formula from Burkina Faso.

2. Materials and Methods

2.1. Study Areas and Sample Collection Sites

The study was conducted in Ouagadougou, the capital of Burkina Faso, and the analyses were conducted in collaboration with Cirad Montpellier, France.

2.2. Sampling of Infant Formula

- Sample collection was done in duplicate at the different sites: Three samples were taken from Recovery and Nutrition Education Centers (RNEC). The women produce infant formulas in an artisanal manner;
- Three samples were taken from women's associations. These women produce infant formulas in an artisanal manner;
- Six samples were taken from semi-industrial units that produce infant formula.

In total we obtained 24 samples, i.e. 12x2.

Sampling consisted of taking 300 to 500 g of infant formula of different compositions from RNEC. RNEC deals with the recovery of severely and moderately malnourished children. The children are hospitalized in the center in order to take care of the child and ensure a rich, varied and sufficient diet.

The twenty-four (24) samples were collected and were taken in aseptic manner and placed in sterile plastic bags, packed in a cooler with ice. They were sent to the laboratory for analysis within 2 h after collection.

Table 1. Composition of infant formula taken in the city of Ouagadougou

Origin of samples	Composition of infant formula samples
RNEC	- corn, soybean, dry fish, peanut, sugar, salt - millet, soybean, dry fish, peanut, sugar, salt - sorghum, soybean, dry fish, peanut, sugar, salt
Artisanal units	- corn, soybean, dry fish, peanut, sugar, salt - corn, millet, beans, rice, peanuts, sugar - corn, rice, soybean, sugar, milk powder
Semi-industrial units	- millet, sorghum, peanut, soybean, sugar, monkey bread powder, iodized salt, calcium carbonate, vitamins, minerals, enzyme Ban - corn, soybean, dehydrated spinach powder, sugar, milk powder, vitamins and minerals - corn, peanut, soybean, iodized salt, sugar, Mineral and vitamin supplement (CMV), enzymes - millet-wheat, palm oil, sugar, skimmed milk powder, vitamins, trace elements - corn, sugar, peanut paste, skim milk powder, vitamins, trace elements - millet-wheat, palm oil, sugar, milk powder, baking powder, vitamins, trace elements

2.3. Sample Collection and Storage

Upon arrival at the laboratory, each sample in duplicate was split into two parts. One part was used immediately for physicochemical, nutritional and microbiological analyses at time T1. The other part was labelled and stored at room temperature until T2 time 6 months later.

2.4. Analysis of Nutritional and Physico-chemical Parameters of Infant Formula

The nutritional parameters were analyzed at the Department of Food Technology and at the laboratory of plant biology at the University Joseph Ki-Zerbo (Burkina Faso).

Moisture, ash, protein and fat contents were carried out using the official AOAC methods [18]. Carbohydrate content was estimated using a colorimetric method [19] according to the following formula (see Eq. 1). The energy value was calculated from the conversion coefficients established by Atwater and Benedict (1899) [20]: 4 kcal/g for carbohydrates and proteins and 9 kcal/g for lipids according to the following formula (see Eq. 2).

$$\begin{aligned}&\text{Carbohydrate content}(\%)\\&=100-[\text{Moisture content}(\%)+\text{protein content}(\%)\\&\quad+\text{fat content}(\%)+\text{ash content}(\%)]\end{aligned}\quad(1)$$

$$\begin{aligned}&\text{E}(\text{kcals}/100\text{g})\\&=\left[4(\text{kcal}/\text{g})\,\text{x}\,\%\,\text{Protein}\right]+\left[9(\text{kcal}/\text{g})\,\text{x}\,\%\,\text{Fat}\right]\\&+\left[4(\text{kcal}/\text{g})\,\text{x}\,\%\,\text{Carbohydrate}\right]\end{aligned}\quad(2)$$

The content of Beta (β-carotene) in infant formula was determined using high performance liquid chromatography (HPLC) as described by Somé *et al.* (2004) [21]. The standard solution was prepared by diluting a small amount of the β-carotene standard in 3 mL of hexane to produce the stock solution. The stock solution was diluted 1:10, 1:100 and 1:1000. The optical densities of the diluted solutions were read at 450 nm with a UV-Visible spectrophotometer (CECIL 160-A, United Kingdom). The concentrations of the standard were calculated from the optical densities obtained. The β- carotene in the samples was extracted by weighing 10 mg of infant formula powder in 1 mL ethanol and 4 mL hexane. After stirring with a vortex for 2 min, the preparation was stored at 4°C for 12 to 15 h in a refrigerator (4°C). After bringing to room temperature, 1 mL of a NaCl solution (3M) was added to the preparation. After stirring, the mixture was centrifuged at 3000 rpm at 5°C for 5 min. The supernatant consisting of the hexanic phase was removed and transferred to another tube. Then 3 mL of hexane was added to the pellet, followed by shaking and centrifugation; the supernatant was removed again and added to the first supernatant. This operation was repeated a third time. 1 mL of the extract was then taken for evaporation under a nitrogen jet, and the dry extract was recovered in 1 mL of acetonitrile. The standard solutions were injected into the HPLC and the peak area recorded. Then 60 µL of the extract recovered in acetonitrile after microfiltration was injected. Contents of β-carotene were calculated using HPLC. The HPLC chain was constituted by a JASCO PU 980 pump, a JASCO 975 UV/Visible detector, a C-18 Supelcosil column (Bellefonte, USA), 25 cm long and 4.6 mm in diameter. The mobile phase consisted of acetonitrile/dichloromethane/methanol (70/20/10, v/v/v). The physico chemicals parameters were carried out in the Food Safety Laboratory of the UMR Qualisud, CIRAD in Montpellier/France.

- Quantification of aflatoxins (AFs) and ochratoxin A (OTA)

The quantification of AFs and OTA in Infant formula was determined using HPLC as described by Waré *et al.* (2017) [15]. The sample (25 g) was homogenized with 50 mL methanol/water (80/20, v/v) and 5 g of NaCl at high speed for 2 min with a blender (Waring France). The extract was centrifuged at 6000 rpm for 10 min. Two mL of the filtrate were diluted with 18 mL of PBS buffer. Ten mL of this diluted sample were passed through an immune-affinity column-IAC column (Aflaochraprep, R-Biopharm). The IAC was washed twice with 10 mL of PBS each time before being eluted with 2 mL methanol. The eluting fraction was then evaporated and 1 mL of methanol/water (50/50, v/v) was added. The obtained fraction was collected into a glass bottle, identified by HPLC and quantified by spectrofluorescence (Shimadzu RF 20A, Japan) after post-column derivatization by electrochemical system (Kobra Cell™ R. Biopharm Rhône Ltd, Glasgow, UK). Fluorescence detection for AFs was set at 365 nm excitation and 435 nm emissions and OTA was set at 333 nm excitation and 460 nm emission. The mobile phase A was water/methanol (55/45, v/v), 119 mg of potassium bromide and 350 µL of nitric acid. The mobile phase B was water/methanol (20/80, v/v), 119 mg of potassium bromide and 350 µL of nitric acid. AFs and OTA standard solutions were used for the construction of a five-point calibration curve of peak areas versus concentration (ng/mL). The operating conditions were as follows: injection volume of 100 mL of sample and standard solutions; C18 reverse-phase HPLC column, Uptisphere type, ODS, 5 mm particle size, 5 ODB, 250 x 4.6 mm, with identical pre-column, thermostatically controlled at 40°C, isocratic flow rate of 0.8 mL/min. Mobile phase gradient: mobile phase A: 0% (0-26 min); 65% (26-45 min); 0% (45-50 min); 41% (20-25). The detection and quantification limits of AFs were 0.3 µg/kg and 1 µg/kg, respectively. The detection and quantification limits of OTA were 0.05 µg/kg and 0.1 µg/kg, respectively. The contents were calculated from a calibration curve established with AFs (TSL- 108, Biopharm Rhône Ltd, Glasgow, UK) and OTA standards (TSL-504, Biopharm Rhône Ltd, Glasgow, UK).

- Fumonisins quantification

The quantification of fumonisins in Infant formula was determined using HPLC as described by Waré *et al.* (2017) [15]. The sample (25 g) was homogenized with 50 mL of methanol/water (80/20, v/v) and 5 g of NaCl at high speed for 2 min with a blender (Waring France). The extract was centrifuged at 6000 rpm for 10 min. Ten mL of filtrate was diluted with 40 mL of PBS buffer. Ten mL of this diluted sample was passed through an IAC column (Fumoniprep, R-Biopharm), followed by washing with

10 mL of PBS buffer. The IAC column was washed twice with 10 mL of PBS for each time before being eluted with 1.5 mL of methanol and 1.5 mL of water. The eluate was collected and derivatized with O-phthaldialdehyde (OPA) prior to analyze by HPLC and quantified by spectrofluorescence (Shimadzu RF 20A, Japan). Fluorescence detection for fumonisins was set at 335 nm excitation and 440 nm emission. The mobile phase A was: acetonitrile/acetic acid (99/1, v/v), and the mobile phase B was water/acetic acid (99/1, v/v). The derivatized sample was prepared as follow: 100 mL of eluate was mixed with 100 mL of OPA (o-Phthaldialdehyde). The operating conditions were as follows: injection volume of 100 mL of derivatized sample; C18 reverse-phase HPLC column, Uptisphere type, ODS, 5 mm particle size, 5 ODB, 250 x 4.6 mm, with identical pre-column, thermostatically controlled at 35°C; isocratic flow rate of 1 mL/min. Mobile phase gradient: mobile phase A: 41% (0-9 min); 61% (9-16 min); 100% (16-20 min); 41% (20-25 min). The detection and quantification limits were 5 µg/kg and 20 µg/kg, respectively. The contents were calculated from a calibration curve established with fumonisin standard solutions (TSL-202, Biopharm Rhône Ltd, Glasgow, UK).

- **Microbiological analyses of Infant Formula**

These parameters were analyzed at the Laboratory of Molecular Biology, Epidemiology and Surveillance of Bacteria and Viruses Transmitted by Food in University Joseph Ki-Zerbo.

Microbiological analysis was carried out in accordance with the requirements of the International Organization for Standardization (ISO). The stock solution was obtained according to ISO 4833:2003. Ten grams (10 g) of infant formula were collected aseptically and placed in a 100 mL flask containing 90 mL of sterile Buffered Peptone Water (BPW). The mixture was homogenized with a stirrer and diluted using successive decimal.

Total Aerobic Mesophilic Flora (TAMF): was determined according to the NF ISO 4833:2003. One hundred microliters (100 µL) of each dilution were used to inoculate in duplicate on Petri dishes containing Mueller Hinton medium (MH; Liofilchem Diagnostic, Italy) and incubated in an incubator at 37°C. Colonies were counted on the plates after 18 to 24 h of incubation.

Thermotolerant coliforms were determined by the NF V 08-017:1980. The seeding of 100 µL by spreading was performed in duplicate on Petri dishes containing Eosine Methylene Blue Levine medium (L-EMB; Liofilchem Diagnostic-ITALY) and incubated at 44°C for 24 h. Characteristic bluish colonies with a dark brown center, flattened, which occasionally have a metallic luster were counted.

Yeasts and moulds were determined by the NF ISO 7954:1987. Thus, 100 µL of each dilution were seeded in duplicate by plating on Petri dishes containing Sabouraud + chloramphenicol 0.5 g/L medium (Sab., Liofilchem Diagnostic-Italy) and incubated at 25°C for 3 to 5 days. Yeasts and moulds were enumerated using the international standard [22] and the results were expressed as Colony Forming Units per gram of sample (CFU/g). The number N of microorganisms present in the test sample as a weighted average of two successive decimal dilutions was calculated according to the following equation (see Eq. 3).

$$N = \sum C / \left(V \times 0.1 \times d \right) \quad (3)$$

The result of the enumeration of total aerobic flora, total coliforms, yeasts and moulds was interpreted according to a three-class plan.

2.5. Statistical Analysis

All collected data were subjected to an analysis of variance with the Xlstat 2018 statistical software and the means of the variables were compared using the Student Newman-Keuls (SNK) test at the 5% probability threshold

3. Results and Discussion

Research and enumeration of germs in infant formula

1. Interpretation of the microbiological contamination of infant formula

Table 2 and Table 3 presents the results of the microbiological analyses of infant formula. The results were compared to the threshold values proposed by Fasonorm [23] at time T1 of sample collection and at time T2 6 months.

Table 2. Microbiological results for infant formula (IF) according to units

Treatment	T1			T2		
	TAMF(CFU/g)	T coli. (CFU/g)	Yeasts & moulds (CFU/g)	TAMF(CFU/g)	T coli. (CFU/g)	Yeasts & moulds(CFU/g)
Artisanal unit	2734.8 ± 689.6^{a}	40 ± 43.4^{a}	153.7 ± 69.8^{a}	6428 ± 3501.8^{a}	71.7 ± 57.8^{a}	2116.2 ± 1858.4^{a}
Semi-industrial unit	3318.6 ± 1736.1^{a}	0 ^{b}	91.7 ± 86.6^{a}	4431.8 ± 2933.5^{a}	8.3 ± 16^{b}	918 ± 1158.1^{a}
Significant level	NS	0.047	NS	NS	0.027	NS

Table 3. Microbiological results for infant formula (IF) according to the storage time

Treatment	Artisanal unit			Semi-industrial unit		
	TAMF(CFU/g)	T coli. (CFU/g)	Yeasts & moulds (CFU/g)	TAMF(CFU/g)	T coli. (CFU/g)	Yeasts & moulds(CFU/g)
T1	2734.8 ± 689.6^{b}	40 ± 43.4^{a}	153.7 ± 69.8^{b}	3318.6 ± 1736.1^{a}	0^{a}	91.7 ± 86.6^{a}
T2	6428 ± 3501.8^{a}	71.7 ± 57.8^{a}	2116.2 ± 1858.4^{a}	4431.8 ± 2933.5^{a}	8.3 ± 16^{a}	918 ± 1158.1^{a}
Significant level	0.03	NS	0.03	NS	NS	NS

In all the 12 samples of infant formula collected, we noted a growth of microorganisms for the germs investigated from T1 to T2. However, statistically there was a significant difference (p = 0.03) when we made the comparison between artisanal unit and conservation among time for two germs. There was also a significant difference when comparing the samples and the quantity of CFU for thermotolerant coliforms both at T1 (p = 0.047) and T2 (p = 0.027). These statistical results indicate that the microbiological quality of artisanally and semi industrially produced infant formula will be unfit for consumption if kept for a certain period without be processed against microorganisms such as Total Aerobic Mesophilic Flora (TAMF), thermotolerant coliforms and yeasts and moulds. Investigations conducted in Burkina Faso on infant flour had shown that they were satisfactory but the characterization of strains had highlighted the presence of pathogenic germs such as *Klebsiella* spp., *Enterobacter* spp. and *Cronobacter* spp [9]. Similarly, in Libya the microbiological quality of infant foods were unsatisfactory for infant consumption because *Cronobacter sakazakii* had been identified [25]. Their presence in IFs can cause gastroenteritis.

2. Results of the nutritional and physico-chemical parameters of the infant formula

The nutritional quality of the infant formula was determined at sampling time T1 and at time T2 (6 months). It consisted in determining ash, energy value and dosage of Vitamin A content of infant formula.

a) Determination of the energy value of infant formula

In order to determine the energy value of infant formula, the determination of moisture, carbohydrates, lipids and proteins was carried out for each sample at time T1 and T2. Then the formula of calculation of the energy value was applied and was the results of the ANOVA who represented in Table 4, Table 5, Table 6 and Table 7.

Moisture content of infant formula must be less than 8% set by Fasonorm [23]. The average moisture content for the artisanally produced infant formula is 4.9 ± 1%. Semi-industrial infant formulas had 5.4 ± 1.3 %. However, the infant formulas tend to become wet under certain storage conditions. Indeed, an increase in water content was observed in all infant formulas after 6 months of storage (Table 4, Table 5, Table 6, Table 7). After storage their average had increased to 9.1 ± 1.2 % for artisanal units. Their average had increased to 9.2 ± 4.2% for semi-industrial units.

The average ash content for the artisanal and semi-industrial units were 2.6 ± 0.8 and 2.2 ± 0.5% respectively. After 6 months of storage, average ash content has not changed much.

The protein contents in the samples were all below the value recommended by Fasonorm [23] (> 12.7%)

The lipid content of the infant flours produced in the artisanal units are in conformity with the Fasonorm threshold [23] (> 8.5 %). For those produced by the semi-industrial units the contents are lower than the Fasonorm threshold [23] (> 8.5 %). After 6 months of storage, the lipid contents have the same trend in each group of units. The carbohydrate content according to the Fasonorm recommendation for infant formula was 64 [23]. The contents found in the infant formulas submitted to our analysis were higher than this reference at time T1 and at time T2.

Fasonorm [23] recommends an energy value above 420 kcal/100g. Our analyses show that the flours produced by the artisanal units have an average caloric value (419.4±12) equal to the recommendation. The flours produced by the semi-industrial units have an average caloric value (408.4±17.2) lower than the recommendation. After conservation, the caloric value of the infant flours, whatever the units, are lower than the recommendation.

Table 4. Results of the energy values content of infant formula at T1

Treatment	Carbohydrate	Protein	Fat	Moisture	Ash	Energy value (kcal/100g)	Beta-carotene (µg/100g)
Artisanal Unit	73.2 ± 3.4^a	9.5 ± 1.2^a	9.9 ± 2.3^a	4.9± 1^a	2.6 ±0.8^a	419.4± 12^a	378,1 ± 5^a
Semi-industrial unit	75.7 ± 7.9^a	9.1 ± 4.2^a	7.7 ± 3.6^a	5.4± 1.3^a	2.2± 0.5^a	408.4 ± 17.2^a	271,3 ± 25.3^a
Significant level	NS	NS	NS	NS	NS	NS	NS

Table 5. Results of the energy values content of infant formula at T2

Treatment	Carbohydrate	Protein	Fat	Moisture	Ash	Energy value (kcal/100g)	Beta-carotene (µg/100g)
Artisanal Unit	70.6 ± 1.1^a	10.1 ± 1.9^a	8.2 ± 1.9^a	9.1 ± 1.2^a	2.1 ±0.5^a	395.8 ±11.3^a	332,8±12.5^a
Semi-industrial unit	72.5 ± 7.7^a	9.5 ± 4.5^a	6.8 ± 3.2^a	9.2 ± 4.2^a	1.9 ± 0.6^a	389.5 ± 32.5^a	242,3±17^a
Significant level	NS	NS	NS	NS	NS	NS	NS

Table 6. Results of the energy values content of infant formula for Artisanal Units

Treatment	Carbohydrate	Protein	Fat	Moisture	Ash	Energy value (kcal/100g)	Beta-carotene (µg/100g)
T1	73.2 ± 3.4^a	9.5 ± 1.2^a	9.9 ± 2.3^a	4.9± 1^b	2.6 ±0.8^a	419.4± 12^a	378,1 ± 5^a
T2	70.6 ± 1.1^a	10.1 ± 1.9^a	8.2 ±1.9^a	9.1 ± 1.2^a	2.1 ±0.5^a	395.8 ±11.3^b	332,8±12.5^a
Significant level	NS	NS	NS	<0.001	NS	0.006	NS

Table 7. Results of the energy values content of infant formula for Semi-industrial units

Treatment	Carbohydrate	Protein	Fat	Moisture	Ash	Energy value (kcal/100g)	Beta-carotene (µg/100g)
T1	75.7 ± 7.9^a	9.1 ± 4.2^a	7.7 ± 3.6^a	5.4± 1.3^a	2.2± 0.5^a	408.4 ± 17.2^a	271,3 ± 25.3^a
T2	72.5 ± 7.7^a	9.5 ± 4.5^a	6.8 ± 3.2^a	9.2 ± 4.2^a	1.9 ± 0.6^a	389.5 ± 32.5^a	242,3±17^a
Significant level	NS	NS	NS	NS	NS	NS	NS

Fasonorm [23] recommends a beta-carotene content for infant formula above 420 µg/100g. It is noticeable that the IF produced by the artisanal units have an average beta-carotene content lower than the recommendation. The same is true for the IF produced by the semi-industrial units. However, here too, after the storage period, the values decrease considerably in all the IF. Vitamin A plays a role in the good development of the young child. In the course of time, these already low contents in certain samples had decrease considerably. The beta-carotene content in infant formula produced by artisanal units was higher than that of semi-industrial units. This can be explained by the fact that the ingredients of which they were composed were rich in beta-carotene.

Statistically there was logically no significant difference between the samples and the storage time for the nutritional parameters. It was the same for the comparison between the semi-industrial units and the nutritional parameters carried out. However, we noted a significant difference between artisanal units and moisture ($p<0.001$), between artisanal units and energy value ($p = 0.006$). In fact, the results obtained above already showed differences between artisanal and industrial units. The nutritional value of semi-industrial infant formulas was satisfactory even after storage for a long time at room temperature (between 35-40°C). This was not the case for the infant formulas produced in a traditional way. Also, the type of packaging could explain the dehumidification of infant formulas after conservation. Indeed, the packaging if it is of type PE (polyethylene; required thickness: 80 to 100µm) can allow during the storage an air and water exchange between outside and the product. The major disadvantage of packaging type PE is that let fats pass through and then low shelf life, the IF, rancid and thus favour the development of mould [26]. This type of packaging is also permeable to odours that attract rodents. Consequently, the infant formulas get wet and the microorganisms multiply. However, our samples from the artisanal units were all packaged in PP and PE type bags and stored at room temperature (condition in supermarkets where infant formulas are sold) for six months.

The development of moulds was linked to the humidification of the product. However, some moulds were responsible for the production of mycotoxins, particularly Aflatoxins [15], which have to be avoided in food. Producers need to review the types of packaging. Those recommended for these types of foods are BOPP20/PE20/Al7/PE30, BOPP30/Al7/PE60, PET12/PE25/Al7/PE30, PET20/Al7/PE50 and PVC (200 µm). All of these can be stored one year, have good presentation, have good conservation and impermeable to odours and light [26].

b) Determination of the mycotoxin content in infant formula

The determination of the mycotoxin contents was carried out during storage and they are given in Table 8 and Table 9. The infant formulas were contaminated with AFB1, OTA and F (B1 + B2). The fumonisin levels obtained were lower than the EU regulation 1881/2006 [24] (200µg/kg). For OTA at T1, only 2 samples of baby food had levels above the standard [24] (0.5 µg/kg); the rest of the samples were not contaminated. At T2, three samples of infant formula had levels above the standard [24] (0.5 µg/kg); For AFB1, at T1, all samples of infant formula had levels above the standard [24] (0.1 µg/kg). At T2, the levels increased again. On the whole it can be noticed that the infant formulas produced in the artisanal units were more contaminated by mycotoxins than those produced in the semi-industrial units.

For the mycotoxin contamination of the infant flours, a predominance of aflatoxin B1 was noted. Statistically there was no significant difference between the samples of infant formulas and the content of the different mycotoxins during the conservation among time; however, we was noted predominance of aflatoxin B1 in the sample of the infant formulas produced artisanally. The predominance of aflatoxin B1 was also obtained by [15], with more than 80% of infant formulas with a content 900 times higher than the norm. The predominance of aflatoxin B1obtained in our case in infant formulas produced artisanally indicated that the contamination could come from the ingredients used, notably peanut, soy, dry fish that could be incriminated or because of the good conservation practices were not respected. The statistical analysis carried out by Waré [27] showed that an infant formula composed of cereals (corn or rice), peanut or soy, have more chance to be either contaminated with very high content of aflatoxins or fumonisins. The presence of aflatoxin B1 in infant formula lead to malnutrition of the child, weakening of the immune system and increased risk of cancer in the long term.

Table 8. Quantities of mycotoxins in infant formula samples per Units

Treatment	T1			T2		
	AFB1(µg/kg)	OTA(µg/kg)	F (B1+B2) (µg/kg)	AFB1(µg/kg)	OTA(µg/kg)	F (B1+B2) (µg/kg)
Artisanal Unit	0.5±0.4[a]	0.1±0.2[a]	3.2±5.9[a]	1.8±1.7[a]	0.4±1[a]	14.3±22.5[a]
Semi-industrial unit	0.2±0.1[a]	0.1±0.2[a]	1.6±3.1[a]	0.8±0.6[a]	0.5±1.1[a]	7.5±11.5[a]
Significant level	NS	NS	NS	NS	NS	NS

Table 9. Quantities of mycotoxins in infant formula samples at time T1 and T2

Treatment	Artisanal Unit			Semi-industrial unit		
	AFB1(µg/kg)	OTA(µg/kg)	F (B1+B2) (µg/kg)	AFB1(µg/kg)	OTA(µg/kg)	F (B1+B2) (µg/kg)
T1	0.5±0.4[a]	0.1±0.2[a]	3.2±5.9[a]	0.2±0.1[a]	0.1±0.2[a]	1.6±3.1[a]
T2	1.8±1.7[a]	0.4±1[a]	14.3±22.5[a]	0.8±0.6[a]	0.5±1.1[a]	7.5±11.5[a]
Significant level	NS	NS	NS	NS	NS	NS

4. Conclusion

According to the germs and the parameters investigated over time, it appears that the contamination rate increases with time. However, the statistical analysis showed significant differences for the artisanally produced infant formula. This has an impact on the quality of infant formula and confirms that storage has an impact on infant flours if parameters such as humidity, type of packaging, ingredients and process are not well controlled. The presence of aflatoxin B1 in all samples at levels above the standards (EU Regulation 1881/2006) makes all infant formula unfit for consumption. it appears that the infant formula are satisfactory because the ingredients used are important for the development of the infant and the young child. From a nutritional point of view, the infant formulas produced by craftsmen have a very high energy value and are very rich in beta-carotene, however the microbiological quality, the presence of aflatoxin B1 makes their quality unfit for consumption. It also emerges from the statistical analysis that it appears to be more judicious to consume quickly and not to preserve the infantile formula produced artisanally over a long time following the date of production of the product. While waiting for innovative solutions, we recommend to the producers of the units to respect rigorously the good practices of hygiene, the good practices of manufacture, the good practices of conservation and to apply the HACCP method along the chain of production This proposed precaution could reduce the risks that young children run during their growth.

Acknowledgements

This work was supported by the French Embassy of Ouagadougou (Scolarship N° 0185BFAB14005) whom granted the first author for her travel and scholarship to Cirad (France) to carry part of this work. Thanks, for the RNEC and the women's associations that produce and sell infant formula for providing the samples. The authors would like to express profound gratitude to the personnel of Center for Research in Biological Sciences, Food and Nutrition, University of Joseph Ki-Zerbo, and the personnel of Cirad for providing the necessary facilities to carry out the research work and for their assistance.

Conflict of Interest

All of the authors declare no conflict of interest in this article.

Abbreviations

CMV: Mineral and Vitamin Supplements; GHP: Good Hygiene Practices; GMP: Good Manufacturing Practices; HACCP: Hazard Analysis Critical Control Point; RNEC: Recovery and Nutrition Education Centers; TAMF: Total Aerobic Mesophilic Flora; AFs: aflatoxins; AFB1: aflatoxin B1; OTA: ochratoxin A; F(B1+B2): fumonisins; (OPA): o-Phthaldialdehyde; β-carotene: beta-carotene; ISO: International Organization for Standardization; IF: Infant formula; PE: Polyéthylène; PP: Polypropylène; BOPP: Polypropylène bi-orienté; Al: Aluminum; PET: Polytéréphtalate d'éthylène glycol; PVC: Polychlorure de vinyle; NS: Not significant.

References

[1] Gret, Dossier « Les farines infantiles », in Bulletin du réseau TPA (Technologie et partenariat en agroalimentaire), TPA, 211-213.

[2] Gret/IRD, Cahier des charges d'une farine infantile souhaitant être labellisée, Projet Nutrifaso: volet NUTRIFASO– Qualité, Ouagadougou, 2007, p. 9.

[3] Kayalto, B., Zongo, C., Compaore, R.W., Savadogo, A., Otchom, B.B. and Traore A.S, Study of the nutritional value and hygienic quality of local infant flours from chad, with the aim of their use for improved infant flours preparation. Food and Nutrition Sciences, 4, 59-68, July 2013.

[4] Zongo, U, Etude de l'évolution des paramètres cliniques et biologiques des enfants vulnérables dont le régime alimentaire inclut le Moringa, Document de thèse de doctorat Unique soutenu à l'Université Joseph Ki-Zerbo, Ouagadougou, 2020, 152 pages.

[5] Kayalto, B., Otchom, B.B., Zongo, C., Kam, L. and Traore, A.S, Local and improved infant flours in vitamin A and iron, using powdered Moringa oleifera leaves and Parkia biglobosa's pulps powder, International Journal of Scientific and Research Publications, 6 (4), 27-37, April 2016.

[6] Dao, A.S., Parkouda, C., Traoré, M.A.E., Guissou, A.W.D.B., Vinceti, B., Termote, C., Manica M. and Bassolé, I.H.N, Comparison of chemical composition of fruit pulp of Parkia biglobosa (Jacq.) Benth from differents ecoregions. African Journal of Food Science, 15 (1), 26-32, January 2021.

[7] Sanou, A., Tapsoba, F., Zongo, C., Savadogo, A. and Traoré Y, Etude de la qualité nutritionnelle et microbiologique des farines infantiles de quatre unités de production : CMA saint Camille de Nanoro, CSPS Saint Louis de Temnaoré, CM Saint Camille de Ouagadougou et CHR de Koudougou, Nature & Technology Journal, Vol. B : Agronomic & Biological Sciences (17), 25-39, May 2017.

[8] Cruchot, H, La spiruline, bilan et perspectives. Thèse de pharmacie, université de Franche-Comté, Besançon, 2008, p. 353. online http://lagrandcour.chez.com/images/these_pharma_franche_comte.pdf consulted 27/06/2018

[9] Waré, L.Y., Nikièma, A.P., Meile, J-C., Kaboré, S., Fontana, A., Durand, N., Montet, D. and Barro, N, Microbiological safety of flours used in follow up for infant formulas produced in Ouagadougou, Burkina Faso, AIMS Microbiology, 4 (2), 347–361, May 2018.

[10] Dembélé, R., Huovinen, E., Yelbéogo, D., Kuusi, M., Sawadogo, G., Haukka, K., Bonkoungou, I.J.O., Siitonen, A., Traoré, A.S. and Barro, N, Burden of acute gastrointestinal infections in Ouagadougou, Burkina Faso. Journal of Microbiology and Infectious Diseases, 6 (2), 45-52, June 2016

[11] Ouattara-Sourabié, B.P., Nikièma, A.P. and Traoré, S.A, Caractérisation de souches d'Aspergillus spp isolées des graines d'arachides cultivées au Burkina Faso, Afrique de l'Ouest. International Journal of Biological and Chemical Sciences, 5 (3), 1232-1249, June 2011.

[12] Warth, B., Parich, A., Atehnkeng, J., Bandyopadhyay, R., Schuhmacher, R., Sulyok, M. and Krska, R, Quantitation of mycotoxins in food and feed from Burkina Faso and Mozambique using a modern LC-MS/MS multitoxin method, Journal of Agricultural and Food Chemistry, 60, 9352−9363, July 2012

[13] Ezekiel, C.N., Udom, I.E., Frisvad, J.C., Adetunji, M.C., Houbraken, J., Fapohunda, S.O., Samson, R.A., Atanda, O.O., Agi-Otto, M.C. and Onashile, O.A, Assessment of aflatoxigenic Aspergillus and other fungi in millet and sesame from Plateau State, Nigeria, Mycology: An Intern. J. on Fungal Biol., 5 (1), 16-22, March 2014.

[14] Afolabi, C.G., Ezekiel, C.N., Kehinde, I.A., Olaolu1, A.W. and Ogunsanya, O.M,. Contamination of groundnut in South-Western

Nigeria by Aflatoxigenic fungi and aflatoxins in relation to processing, Journal of Phytopathology, 163, 279-286, september 2014.

[15] Waré, L.Y., Durand, N., Nikiema, P.A., Alter, P., Fontana, A., Montet, D. and Barro, N, Occurrence of mycotoxins in commercial infant formulas locally produced in Ouagadougou (Burkina Faso), Food Control, 73 518-523, Auguest 2016.

[16] Waré, L.Y., Durand, N., Barro, N. and Montet, D, Benefit/risks related to the consumption of infant flours produced in Burkina Faso, Didier Montet, Catherine Brabet, Sabine Schorr-Galindo and Ramesh C. Ray Publishers, Boca Raton, 2021, 175-188.

[17] USAID, Aflatoxine : une synthèse de la recherche en santé, agriculture et commerce, Regional economic growth and integration USAID east Africa, Nairobi, 2012, 82.

[18] Association of Official Analytical Chemists (AOAC) (2005). Official Methods of Analysis of the Association of Official Analytical Chemists, 18th edition. AOAC, Arlington, Va, USA.

[19] Montreuil, J. and Spik, G, Microdosage des glucides. I/Méthode colorimétrique de dosage des glucides totaux. Monographe du labochimie-biol. Fac. Sc. Lille (France). (1969).

[20] Atwater, W.O. and Benedict, F.G, Experiments on the metabolism of matter and energy in the human body, US Department of Agriculture.Washington, D.C., Bulletin 1899, 69, 112.

[21] Somé, I.T., Zagré, M.N., Kafando, P.E., Bendech, M.A., Baker, S.K., Deslile, H. and Guissou, P.I, Validation d'une méthode de dosage des caroténoïdes par CLHP: application à la détermination de teneur en caroténoïdes dans dix variétés de patates douces (Ipomea batata), C. R. Chimie 7 ;1063-1071. 2004.

[22] Internationale, N., Microbiologie des denrées alimentaires et aliments pour animaux-Règles générales relatives aux analyses microbiologiques. ISO 7218, 69, 2007.

[23] Fasonorm, Farines infantiles specifications, Norme Burkinabè NBF 01-198, 7-9, (2014).

[24] Regulation, E. U.; setting maximum levels for certain contaminants in foodstuffs, Official Journal of the European Communities, 20-25, 2006.

[25] Matug, S.M., Aidoo, K.E. and Elgerbi, A.M, Microbiological examination of infant food and feed formula, Emerg. Life Sci. Res., 1, 46-51. 2015.

[26] Gret -TPA, Les farines infantiles : Production semi-industrielle d'une farine infantile à cuire, Céréales – farines ; Dossier d'information pour les PME d'Afrique sur la transformation agroalimentaire CTA, 27 p. 2012.

[27] Waré, L.Y, Évaluation de la qualité sanitaire des farines infantiles produites au Burkina Faso. Thèse de doctorat de l'université Ouaga I Pr Joseph Ki-Zerbo, 107p, 2018.