Food Science and Nutrition Studies ISSN 2573-1661 (Print) ISSN 2573-167X (Online) Vol. 3, No. 2, 2019 www.scholink.org/ojs/index.php/fsns

# Short Research Article

# Fat Content and Fatty Acids Profile in Follow-on Formulas

# Commercialized in Côte d'Ivoire

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Received: March 11, 2019	Accepted: March 17, 2019	Online Published: April 6, 2019
doi:10.22158/fsns.v3n2p54	URL: http://dx.doi.org/10.22158/	fsns.v3n2p54

## Abstract

This study evaluates the follow-on formula for infants. These products are available under several brands in the Ivorian market. In order to verify their conformity to the WHO standards a post-market control by gravimetric method and gas chromatography with mass spectrometry is executed to evaluate the quantity and quality of fat products contained in the milks of brands available in Côte d'Ivoire. Out of the nine brands of milks analyzed, only four of them were close to the values revealed by their manufacturers, whereas the other products had their values below their respective indications.

## Keywords

milk, follow-on formulas, fats, control

## 1. Introduction

A follow-on formula is food obtained from the natural cow milk with some additional ingredients. These formulas are appropriate to support growth and development of 6 to 12 months children (WHO, 2001). A follow-on formula is produced using some physical means. So it is preciously packaged to prevent spoilage and contaminations caused all normal conditions of handling, storage and distribution in the country where the product is sold (Codexalimentarius FAO-WHO, 1987).

WHO recommends that follow-up formula be proposed to complete or replace human milk to children

of at least 6 months to ensure that all essential nutrients such as, proteins, lipids, carbohydrates, minerals and vitamins cover their nutritional needs (WHO, 2015, 2018).

Follow-on formula are becoming increasingly important in Africa, particularly in C de d'Ivoire. Several imported brands are available pharmacies, supermarket and other small places of distribution (Pereira, Ford, Feeley, Sweet, Badham, & Zehner, 2016; Champeny et al., 2016).

Due to strict standards aimed to maintain the quality of these products, follow-on formula should contain fat content and particularly essential fatty acids similar to those of human milk (Suthutvoravut et al., 2016; Koletzko et al., 2005). As in breastmilk, saturated and unsaturated fatty acids as well as essential fatty acids must be present in these formulas to ensure growth in children (Vaysse et al., 2011; Bernard & Annie, 1995). As part of a post-marketing quality control of formulas, a study was initiated to evaluate total fat content on and fatty acid profile in follow-on formulas commercialized in Abidjan (Côte d'Ivoire).

### 2. Material and Method

#### 2.1 Samples and Chemicals

The samples of follow-on formulas were collected in pharmacies of Abidjan. Nine brands were found and selected. They were numerically labelled from 1 to 9 for the purpose of the study. Four boxes per brand were purchased for analysis.

All chemicals and solvents were analytical grade from different suppliers. Hydrochloric acid, sodium chloride, dichloromethane, hexane, ethanol, methanol, Sodium hydroxide, Chloroform from Sigma-Aldrich (Missouri, USA).

#### 2.2 Instrument

The analysis performed on Agilent Model 7890A gas chromatograph coupled to Agilent S975C mass spectrometer (Agilent, CA, USA) operating in the electron impact (EI) and selected ion monitoring (SIM) modes. Samples (1  $\mu$ L) were injected at 250 °C in split mode. A capillary column (30 m×0.25 mm I.D., 0.25 m Agilent J&W capillary columns, CA, USA) was used.

## 2.3 Fat Content Determination

The fat in the milk samples was extracted by the Folch lipid extraction method (Suthutvoravut et al., 2016).

For each sample, 5g of formula were weighted and put into a 250 ml flask. Then 40 ml of chloroform and 20 ml of methanol were successively added. The mixture was homogenized on a magnetic stirrer at 400 rpm for 30 minutes before being filtered on a Whatman filter paper (70 mm, 8  $\mu$ m). The filtrate was recovered in a 500 mL separatory funnel. Thus, 0.73% sodium chloride solution was deposited in the separating funnel at 20% the volume of the recovered organic solvents. The organic phase obtained was placed in the flask and evaporate till it dried on rotary evaporator at 300 rpm for 40 minutes. Then the flask was dried in the oven at 104 °C for 30 minutes. The fat content was thus weighed after cooling the flask in a desiccator.

## 2.4 Fatty Acids Profile

## 2.4.1 Preparations

Formula of the samples were prepared in dichloromethane to obtain concentrated solutions at 1 mg/ml. Then 1 mL of solution was vortexed in a 15 mL test tube with 1 mL of 0.5 N methanolic solution of sodium for 30 seconds. The tube was then placed in a water bath at 70  $^{\circ}$ C for 30 minutes of saponification. Methylation was carried out by the addition of 1 mL of methanolic solution of hydrochloric acid, before reheating the tube in a water bath at 70  $^{\circ}$ C for 30 min.

The fat was extracted by the successive mixture of 4 ml of 9 mg/mL NaCl and 4 mL of hexane. The mixture was allowed to settle for 5 minutes before recovering the organic phase in a test tube.

The organic solvent was evaporated using a rotary evaporator at 75  $^{\circ}$ C at 60 rpm. Then 100  $\mu$ L of absolute ethanol was added to remove all traces of water and evaporated on the rotary evaporator. A solution of volatile methylated fatty acids was obtained by adding 1.2 mL of hexane before being analyzed by gas chromatography (GC).

2.4.2 Analysis Conditions

GC temperature program was as follows:  $150 \,^{\circ}$ C for 2 min, followed by temperature ramp at 1,3  $^{\circ}$ C/min to 200  $^{\circ}$ C, then temperature ramp at 40  $^{\circ}$ C/min to 250  $^{\circ}$ C, and hold for 5 min.

Full scan data was acquired under the following conditions: mass range 50-550 m/z, scan time 1 s, solvent delay 13.5 min. Quantitation of the analytes was carried out in the SIM mode, once their characteristic masses were selected from their full spectra. The electron energy was 70 eV, and the electron multiplier was operating at 200 to 300 V (Kuo & Ding, 2004). Analyses of each brand of milk were repeated 6 times.

## 3. Results and Discussion

The analysis in the follow-on formula marketed in Abidjan revealed fat content between 5.01 g and 20.82 g per 100 g of milk powder. Very low values of standard deviation have been obtained (Table 1).

Designation	Fat content (g/10	0g of milk)					
	Mean	Standard deviation (SD)	Content reports on packaging (g/100 g of milk)				
1	16.40	0.22	22				
2	21.32	0.70	22				
3	15.19	0.35	21.85				
4	21.23	0.06	21.70				
5	20.82	1.84	23.50				
6	15.48	0.36	25.40				
7	20.72	0.77	21.10				
8	21.68	0.33	21.80				
9	5.01	0.23	21.50				

Table 1. Average Total Fat Content of Follow-up Formula Brands

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Among the nine brands of milks analyzed, only four brands showed content were close to the values reported by the manufacturers, whereas the other five brands (labelled 2, 4, 7, 8) presented low values, particularly brand 9. This last result could indicate a non-compliance of the analyzed batch. Besides brand 9, these milks were found to be most concentered in fat than breast milk (Kent, Mitoulas, Cregan, Ramsay, Doherty, & Hartmann, 2006). In all samples analyzed, 07 fatty acids were identified which include 04 saturated fatty acids, 02 monounsaturated fatty acids and only 01 polyunsaturated fatty acids (Table 2).

Group	Fatty acids detected	Proportion of fatty acids identified in follow-up formula (%)								
		1	2	3	4	5	6	7	8	9
Fatty acids	C 12 :0 (lauric acid)	0,49	7,45	4,97	6,95	5,15	7,74	7,61	7,23	3,93
saturated	C 14 :0 (myristic acid)	0,63	6,05	3,16	3,83	3,42	3,92	4,16	4,13	2,53
	C 16 :0 (palmitic acid)	26,88	27,79	22,46	22,76	25,59	23,37	21,7	19,29	24,53
	C 18 :0 (st éaric acid)	3,71	5,40	3,96	3,90	4,40	3,56	3,67	3,89	3,89
Mono unsaturated	C 18 :1n-9 (oleic acid)	46,69	32,12	44,82	43,15	43,22	43,03	47,19	48,57	45,41
fatty acids	C 18 :1n-7 (elaidic acid)	2,11	1,70	2,00	1,85	1,82	1,88	2,12	2,19	2,52
Polyunsaturated	C 18 :2n-6 (linoleic acid)	19,50	19,49	18,62	17,57	16,39	16,49	13,55	14,77	17,18
fatty acids										

#### Table 2. Fatty Acid Profile of Milk Analyzed

Oleic acid is the fatty acid found in greater proportion in all analyzed brands while elaidic acid is the one that is generally in the lowest proportion except in the sample of brand 1. Only one essential fatty acid has been identified, linoleic acid whereas packaging reported the presence of two essential fatty acids: linoleic acid and alph-linolenic acid (Hansen, Haggard, Boelsche, Adam, & Wiese, 1958). Further investigation must be realized as it seems that  $\alpha$  linolenic acid content could not be detected with the proposed method while the latter plays an important role in growth and development in infants.

Setting up a post-marketing control system for follow-on formula is necessary. These regulatory measures would aim to ensure the quality of follow-on formula made available on the market in C ôte d'Ivoire and remove non-compliant batch and or brands.

## 3. Conclusion

Post-marketing quality control of follow-on formulas for infants marketed in C  $\hat{\alpha}$ te d'Ivoire constitutes a contribution to the nutrition monitoring of infant's formulas commercialized. This study evaluated the fat content of these formulas and the fatty acids profile. The results showed fat content close to the one reported on packaging for 4 out of nine analyzed brands. Fat content was also higher than those

reported in human milk by literature. Only one essential fatty acid was identified in these formulas. Further investigation must be pursued as regarding the importance of these fatty acids in growth and development infants.

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