



# Oligosaccharide Profile of Milk from 60 Mothers Breastfeeding of Infants Aged 45 Days in Abidjan, Ivory Coast

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

**Introduction:** Breast milk contains essential macro and micronutrients for infant maturation. Among these macronutrients are oligosaccharides, considered prebiotics. They are the third most abundant component of breast milk after lipids and lactose, and their concentrations are influenced by various factors, including maternal dietary habits. This study aims to evaluate the effect of two dietary habits on the variation of the oligosaccharide profile in breast milk.

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**Methods:** The study involved 60 mothers of full-term, exclusively breastfed infants. Five (5) ml milk samples were collected from mothers of 45-day-old infants, and a questionnaire on the frequency of consumption of staple foods and fruits was administered. The correlation between the concentrations of N-acetylneuraminic acid (Neu5Ac) and 2'-fucosyllactose (2'-FSL) and the dietary habits of breastfeeding mothers was analyzed using SPSS statistical software.

**Results:** The average concentrations of Neu5Ac and 2'-FSL in mature breast milk were  $2.20 \pm 0.83$  and  $1.19 \pm 0.56$  g/L for mothers consuming rice with peanut sauce (R-PS) and  $2.45 \pm 0.65$  and  $1.76 \pm 0.66$  g/L for those consuming attiéké with fried fish (A-FF). Statistical analyses revealed no significant difference ( $P > 0.05$ ) in oligosaccharide content between the milk of mothers who consumed R-PS and A-FF. However, there was a positive correlation between the frequency of fruit consumption and the occurrence of gastroenteritis in infants ( $r = 0.504$ ) and a significant decrease in the 2'-fucosyllactose content in the milk of A-FF mothers who consumed oranges 3 to 5 times a week ( $P < 0.05$ ).

**Conclusion:** The consumption of R-PS and A-FF, along with moderate fruit consumption, positively influences the oligosaccharide profile of breast milk. Particular attention should be given to nutritional education during lactation due to its potential impact on infant health through microbiota development.

*Keywords: Breast milk; oligosaccharides; peanut sauce; infant health.*

## 1. INTRODUCTION

Breast milk is the sole food source for exclusively breastfed infants up to six months of age and continues to be important until two years of age with dietary diversification [1]. Many studies have shown that breast milk reduces the risk of developing allergies [2], infant diarrheal diseases like necrotizing enterocolitis, gastrointestinal infections [3], and even childhood cancers. In the long term, it decreases the occurrence of chronic diseases such as type 2 diabetes and obesity [4].

These beneficial effects of breast milk are due to its composition of essential macro and micronutrients in appropriate proportions and bioactive factors such as antibodies, hormones, and oligosaccharides [5]. These oligosaccharides are the third most abundant component of breast milk after lactose and lipids [6,7]. They consist of five monosaccharides: glucose (Glc), galactose (Gal), fucose (Fuc), N-acetylglucosamine (GlcNAc), and sialic acid (SA) [8]. The most abundant are 2'-fucosyllactose (2'-FSL) and N-acetylneuraminic acid (Neu5Ac), derived from fucose and sialic acid, respectively [9,10].

SA is a key monosaccharide in cerebral gangliosides and glycoproteins [11]. It is present in neuronal synapses, particularly in the hippocampus, where it plays an important role in synaptic connectivity

modification, memory formation, and learning. Recent clinical studies have shown that high levels of N-acetylneuraminic acid in breast milk correlate with advanced language skills and better cognitive and motor skills in children at 18 months of age [11-13].

As for 2'-FL derived from lactose, it is a prebiotic fiber that promotes a healthy diversity of the infant's microbiota and immunity. This sugar, the most abundant component of breast milk oligosaccharides, reduces the risk of infections caused by pathogens [14,15]. As a prebiotic, this oligosaccharide supports the growth of specific bacterial species such as Bifidobacteria, which are necessary to overcome pathogens, thus preventing infectious diseases [16,17]. It resists acidic pH and enzymatic degradation and reaches the large intestine intact to support microbial growth, ensuring good gastrointestinal health for the infant.

Thus, breast milk plays an important role during the first two years of the infant's life due to its oligosaccharide profile [18]. The intestinal microbiota influences the infant's growth, body composition, and long-term protection against chronic diseases. Therefore, focusing on the infant's microbiota through the composition of breast milk is also a means of combating infant malnutrition. Hence, this cross-sectional study aims to evaluate the potential relationship between the levels of 2'-fucosyllactose (2'-FSL) and N-acetylneuraminic acid (Neu5Ac) in breast

milk and maternal nutritional intake 45 days after delivery.

## 2. METHODS

### 2.1 Survey

A consumption frequency questionnaire was administered to sixty (60) breastfeeding mothers to gather information on their dietary habits after informed consent (signing of a consent form). Then, information on the clinical signs presented by the newborns was collected from the mothers.

#### 2.1.1 Survey form

A dietary survey was conducted using a food frequency questionnaire used worldwide to assess food intake [19]. Dietary choices were made based on their effect on antioxidant activity in a previous study [20]. Breastfeeding women were asked to provide detailed descriptions of their basic diet, including consumption frequency, food preparation (ingredients), and cooking method, as detailed as possible since delivery. A basic diet was defined as being consumed at least three (3) to four (4) times per week and repeated until the time of the survey.

### 2.2 Breast Milk Extraction

For each mother, a sample (5 to 10 mL) of milk was collected by manual expression after cleaning the nipple with a sterile solution-soaked compress. Each mother was given a 7 mL sterile container for milk collection, which was then transported to the laboratory and stored in a freezer at -20°C until analysis. Breast milk samples were collected at approximately the same time in the morning (09:00 - 11:00) before the infant's vaccination.

### 2.3 Exclusion and Non-Inclusion Criteria

Women who had given birth to a full-term single pregnancy with a normal birth weight (2.5 kg-4.5 kg), practicing exclusive breastfeeding, and whose newborns were in good health were included in this study.

Exclusion criteria included mothers who met the study's standards but were smokers, suffering from acute and/or chronic illness (diabetes, hypertension), on antibiotics or any medication likely to pass into breast milk. Additionally, women consuming one of the two dishes more or less frequently than the study's criteria or supplemented with fucose or sialic acid.

### 2.4 Materials and Reagents

All reagents were of analytical grade and used as supplied. Standards of 2'-fucosyllactose and N-acetylneuraminic acid were purchased from Simson Pharma Limited. Acetonitrile and methanol (HPLC grade) were obtained from Merck (Darmstadt, Germany). Water was prepared using the Milli-Q system (Millipore, Bedford, Massachusetts, USA).

### 2.5 Derivatization Procedure With 1-Phenyl-3-Methyl-5-Pyrazolone (Pmp) And Removal Of Excess Pmp

The derivatization procedure applied in this study was modified from that reported by Kwon and Kim [21]. The following test procedure was followed: dry saccharide samples (100 g) were dissolved in 100 ml of water. The sample solution (1 ml) was dissolved in a 0.5 M PMP solution in methanol. After adding 1 to 9 ml of 0.3 M NaOH, the mixture was incubated for 10 to 50 minutes between 50 and 70°C. After cooling to room temperature, the mixture was acidified for neutralization by adding 1 ml of an aqueous hydrochloric acid solution (0.3 M). The resulting solution was extracted five times by adding 4 ml of organic solvent (for standards, diethyl ether was used; however, benzene and chloroform were also tried), followed by vigorous mixing and centrifugation at 3,000 g for 5 minutes. The organic layer was carefully removed, and the final aqueous layer was evaporated to dryness and redissolved in 1 ml of water to obtain a standardized final volume. It was filtered through a 0.45 µm filter membrane, and 5 µL of the resulting solution were injected into the analytical column.

### 2.6 High-performance Liquid Chromatography

An HPLC analysis was performed using an Agilent 1100 system (Agilent Technologies Q7, Santa Clara, California, USA) to evaluate the efficiency of the derivatization reaction. The system included a quaternary solvent delivery system, an online degasser, an autosampler, a column temperature controller, and a UV detector coupled with an analytical workstation. The derivatives (samples) were separated on an Agilent Eclipse XDB-C18 column (5 µm, 250 mm x 4.6 mm ID; Agilent Technologies). The mobile phase consisted of two eluents (A and B). Eluent A was a mixture of 0.1 M ammonium

**Table 1. Calibration curve parameters, correlation coefficient, LOD, and LOQ**

Sample	Standard curve	R2	LOD (mg/L)	LOQ (mg/L)
1	$y = 177,89x + 10,14$	0,9949	0,15	0,33
2	$y = 89,27x + 567,10$	0,9967	0,20	0,85

1 = N-acetylneuraminic acid; 2 = 2'-Fucosyllactose LOD = limit of detection; LOQ = limit of quantification

acetate buffer, and eluent B was acetonitrile. For degassing and sterilization, both eluents were filtered through 0.2 µm polypropylene hydrophilic membrane filters. The mobile phase was delivered at a flow rate of 0.5 to 2.5 ml/min, and the column temperature was set to 30°C. Chromatographic separation of several derivatives and the study of the quantitative performance of the process were carried out under elution conditions. The chromatograms were monitored at 245 nm, and the sample injection volume was 10 µL.

### 2.7 Calibration Curve Preparation and Treatment of Breast Milk Samples

Two standard samples (100 mg) were accurately weighed and dissolved in water to obtain the primary standard solution (1.0 mg/mL). The mother standard solutions (1.0 ml) were transferred to a series of 10 ml volumetric flasks to cover the concentration range of 0.2 to 6.0 µg/mL in a 0.5 M PMP solution in methanol. The calibration curve was constructed by plotting the ratio of peak area to the final sample concentration. Alternatively, the corresponding regression equation was derived.

The breast milk sample was mixed with 70% ethanol in a 1:9 ratio and uniformly shaken. The mixture was left to stand for a while until a white precipitate formed. The supernatant was processed according to the derivatization procedure.

### 2.8 Standard Curves and Recovery

The calibration curve showed excellent linearity within the concentration range studied. The table summarizes the percentage of recovery for LOD and LOQ, with method recoveries ranging from 93.13% to 102.08% and relative standard deviation between 0.96% and 2.48%. This result indicates that the detection efficiency for HPLC using the precolumn method was satisfactory and consistently independent.

### 2.9 Statistical Analysis

Results are presented as arithmetic means and standard deviations. Data were analyzed using

the Student's t-test. Paired t-tests compared oligosaccharide content variations between two means within the same group, while unpaired t-tests compared these oligosaccharide profiles between different groups. Pearson correlation analysis determined the correlation between oligosaccharide profiles and fruit consumption frequency. Differences at a 5% probability level were considered significant. GraphPad Prism 5.0 (San Diego, USA) was used for all analyses.

## 3. RESULTS

### 3.1 Demographic Characteristics of Breastfeeding Mothers

Sixty (60) breastfeeding mothers consented to participate in the study after explanation of procedures. Data were grouped according to two predominant dietary patterns: rice with palm nut sauce (R-PNS, n = 30) and Attiéké with fried fish (A-FF, n = 30). It is also important to note that sauces are consumed with fresh and/or smoked meat or fish, containing mainly three to four vegetables per day (onion, chili, tomato). The mean age of mothers was  $24.71 \pm 4.73$  years; the majority of mothers are civil servants (26.67%), and 33.33% have no formal education (Table 2). All mothers (100%) were multiparous, and their BMI was within the normal range (between 18.5 and 24.9).

### 3.2 Nutritional and Oligosaccharide Profiles of Breastfeeding Mothers

At the end of the study, we observed that twenty-three (23 or 38.33%) breastfeeding mothers who consumed 3 to 5 fruits per week had the same basic diet, namely Attiéké – Fried Fish. The average concentrations of 2'-Fucosyllactose (2-FSL) and N-acetylneuraminic acid (Neu5Ac) in the milk of breastfeeding mothers at 45 days postpartum were  $1.38 \pm 0.70$  g/L and  $2.11 \pm 0.80$  g/L, respectively.

The concentrations for mothers consuming less than 2 fruits per week are summarized in Table 3. The levels showed no significant differences ( $P > 0.05$ ) within the same group or between the two groups.

The consumption of mangoes and orange juice at a frequency of 3 to 5 fruits per week by mothers led to a decrease in oligosaccharide levels. Thus, in maternal milk, the results showed a non-significant decrease between Neu5Ac levels ( $2.45 \pm 0.65$  vs.  $1.89 \pm$

$0.75$  and  $2.24 \pm 0.75$  g/L) ( $p > 0.05$ ). However, consumption of more than three oranges per week significantly reduced 2'-FSL concentrations in maternal milk ( $1.76 \pm 0.66$  g/L vs.  $0.67 \pm 0.12$  g/L) ( $p < 0.05$ ).

**Table 2. Socio-demographic characteristics of breastfeeding mothers (N=60)**

Parameter	Numbers (%)
<b>Age of mothers</b>	
< 25	30 (50)
> 25	30 (50)
Standard deviation ( $24,71 \pm 4,73$ )	
<b>Residence</b>	
Adjamé	25 (41,67)
Plateau	14 (23,33)
Attécoubé	21 (35)
<b>Occupation</b>	
Homemakers	8 (13,33)
Merchants	12 (20,00)
<b>Civil servants</b>	16 (26,67)
Students	10 (16,67)
Informal sector	14 (23,33)
<b>Level of education</b>	
Primary	12 (20,00)
Secondary	18 (30,00)
Higher	10 (16,67)
None	20 (33,33)
<b>Marital status</b>	
<b>Married/cohabiting</b>	50 (83,33)
Single	10 (16,67)

**Table 3. Effects of dietary patterns on the variation of N-acetylneuraminic acid and 2' Fucosyllactose concentrations in breast milk**

Parameter (g/L)	Numbers of mothers with two dietary habits		P-value
	Rice with palm nut sauce (n=30)	Attikié with fried fish (30)	
N-acetylneuraminic acid	$2.20 \pm 0.83$	$2.45 \pm 0.65$	$P > 0.05$
2'-Fucosyllactose	$1.19 \pm 0.56$	$1.76 \pm 0.66$	$P > 0.05$

Concentrations are expressed as mean  $\pm$  standard deviation. Means in the same row are not significantly different ( $P > 0.05$ ) from each other. In the same column, means are not significantly different ( $P > 0.05$ ) from each other

**Table 4. Effect of consuming 3 to 5 fruits per week on the oligosaccharide concentrations of breastfeeding mothers**

Variables	Concentration (g/L) based on the frequency of fruit consumption			P-value
	> 2fruits per week	3 to 5 Oranges per week	3 to 5 Mangoes per week	
Neu5Ac	$2.45 \pm 0.83$	$2.24 \pm 0.75$	$1.89 \pm 0.75$	
2'-FSL	$1.76 \pm 0.66$	$0.67 \pm 0.12^{**}$	$1.42 \pm 0.77$	0.02

The concentrations are expressed as mean  $\pm$  standard deviation. The mean marked with \*\* is significantly different ( $P < 0.05$ ) from the mean of 30 mothers on the A-PF diet.

**Table 5. Effect of consuming 3 to 5 fruits per week on infant health**

Basic Diet + 3 to 5 Fruits/Week	Numbers of infants by pathology			P-value
	Healthy	Diarrhea	Bloating	
R-PNS (n=30)	29 (96.7%)	1(3.33%)	0	0,000
A-FF (n=30)	7 (2.33%)	18(60%)	4(37.67%)	

### 3.3 Clinical Signs in Infants

Out of a sample of 60 respondents, 24 infants, or 40%, exhibited digestive disorders. Clinical manifestations such as the emission of liquid stools 79.16% (19/24) by children more than 5 times a day and bloating 16.67% (4/24) according to the mothers' reports were noted. A correlation was found between mango consumption and the occurrence of gastroenteritis ( $p = 0.0000$ ).

## 4. DISCUSSION

Breast milk is the biological norm for infant nutrition. Its composition in oligosaccharides, particularly in 2'-fucosyllactoses (2'-FSL) and N-acetylneuraminic acid (Neu5Ac) or sialic acid, is extensively studied for their beneficial effects on infant health [22-27]. These oligosaccharides, influenced by maternal nutrition, protect the newborn against infections and inflammation, contributing to immune and brain maturation, organ development, and microbiome development. In Côte d'Ivoire and most African countries, dietary habits are cultural, especially during breastfeeding periods [28,29]. The staple dishes are intended to provide postpartum care to the mother but also to enrich the milk for better infant health. The levels of N-acetylneuraminic acid or sialic acid ( $2.20 \pm 0.83$  and  $2.45 \pm 0.65$  g/L) and 2'-Fucosyllactose or fucose ( $1.19 \pm 0.56$  and  $1.76 \pm 0.66$  g/L) found in mothers' milk show that the foods consumed by mothers favor the biosynthesis of these two oligosaccharides. Moreover, the levels of N-acetylneuraminic acid (Neu5Ac) and 2'-Fucosyllactose (2'-FSL) at 45 days postpartum are almost similar regardless of the mothers' basic diet. After reviewing the literature, we did not find any major, consistent, and convincing studies on maternal dietary habits affecting the profiles of these two oligosaccharides. However, the average concentrations of 2'-fucosyllactose (2'-FSL) found in our study,  $1.19 \pm 0.56$  and  $1.76 \pm 0.66$  g/L (respectively for R-PNS and A-FF), fall within the range reported in studies by Liu et al. These researchers found average concentrations of 2'-FSL ranging from 0.935 to 2.865 g/L [30].

Regarding the values of Neu5Ac found in this study, they are higher than those reported by Xie et al. In mature breast milk of 33 Chinese mothers, where concentrations ranged from  $514.64 \pm 55.2$  mg/L to  $297.20 \pm 20.78$  mg/L at 30 and 90 days postpartum, respectively [31]. In that study, sialic acid levels were reported on the 2<sup>nd</sup>, 7<sup>th</sup>, 30<sup>th</sup>, and 90<sup>th</sup> days postpartum, unlike ours. The average concentrations of Neu5Ac in the milk samples in this study are also higher than those reported in Tanzanian women living with HIV ( $0.45 \pm 0.11$  g/L) [32]. Another study by Li et al. On breast milk samples collected from 277 Chinese mothers showed that the Neu5Ac and 2'-FSL profile in breast milk was positively correlated with vitamin A intake [33]. However, no significant increase in Neu5Ac concentration was observed in the milk of mothers on the R-PNS diet compared to those on the A-FF diet. Indeed, according to Kouakou et al., palm nut sauce is rich in provitamin A and should therefore increase levels in mothers who consume it [34]. Study was performed by Urrutia-Baca and al. [35], on 70 samples of breast milk collected from Mexican mothers and showed that the frequency of consumption of certain food groups, such as fruits, vegetables, and meats, can increase the concentrations of 2'-FL and 6'-S. This could certainly explain this result. Indeed, rice has certainly reduced the impact of the carotenoids contained in the R-PNS diet.

Studies by Urrutia et al., reported Neu5Ac levels were significantly lower by women consuming large amounts of fruit. [35], Also, consumption of more than 3 to 5 mangoes and/or oranges per week in addition to A-FF led to a non-significant decrease in Neu5Ac concentration ( $2.45 \pm 0.65$  vs.  $1.89 \pm 0.75$  g/L) and 2'-FSL ( $1.76 \pm 0.66$  vs.  $0.67 \pm 0.12$  g/L and  $1.42 \pm 0.77$  g/L) compared to those who consumed less than 2 fruits per week ( $2.20 \pm 0.83$  and  $2.45 \pm 0.65$  g/L;  $1.19 \pm 0.56$  and  $1.76 \pm 0.66$  g/L). Thus, the correlation between the occurrence of gastroenteritis in infants of mothers on the A-FF diet associated with consumption of more than 3 to 5 mangoes per week is likely due to decreases in sialic acid and fucose concentrations. Indeed, 2'-FSL improves the infant's defense mechanism by

strengthening the intestinal barrier. These oligosaccharides inhibit infection by numerous pathogenic germs, intestinal mucosal inflammation, intestinal inflammation, induction of inflammatory signaling molecules, and a reduction in diarrhea.

Given that infants of mothers on the Attié-ké-fried fish with refined palm oil base diet consumed 3 to 5 fruits per week and presented with gastroenteritis, one might question the number of fruits to consume during lactation. This study has a limitation regarding maternal dietary intake during breastfeeding, and it would be difficult to formulate nutritional advice for breastfeeding mothers based on these results.

## 5. CONCLUSION

The consumption of R-PNS and A-FF positively influences the oligosaccharide profile of breast milk. Furthermore, reducing the number of fruits in the postpartum period would be beneficial for infant intestinal maturity. Special attention should be paid to nutritional education during lactation due to its potential impact on infant health through microbiota development. However, the oligosaccharide profile and the impact of other basic diets as well as the frequency of fruit consumption need further investigation with broader coverage.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## CONSENT

Women gave their consent to participate in the study after an explanation of the procedures. All procedures were duly approved by the relevant department heads.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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