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Research Article

Phytonutrients in Breast Milk and Their Association with Maternal Dietary Intake: A Longitudinal Study in Adjamé

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Abstract

Carotenoids and flavonoids are essential nutrients for infant development. As for today, there is limited data on their availability in breast milk and the associated dietary factors, particularly in Côte d'Ivoire, where the diet of breastfeeding women is largely composed of cooked meals. The primary objective of this study was to determine the levels of carotenoids and flavonoids in breast milk using high-performance liquid chromatography (HPLC) during two lactation periods in Ivorian women who consumed two different diets. Mature breast milk samples were collected from healthy breastfeeding women on the 45th and 105th postpartum days. The mothers' dietary frequencies were obtained using a questionnaire. A final sample of 60 breastfeeding women was selected. Two predominant basic diets were identified: cooked rice with palm nut sauce (R-SG) and attiéké with fried fish in refined palm oil accompanied by a paste of cooked vegetables (onion-red chili-fresh tomato) (A-PF). An overall increase in flavonoid levels was observed in all women's milk on the 105th postpartum day, in contrast to carotenoid levels, where only the β-carotene content increased on the 105th day in the milk of women who consumed the R-SG diet (from 1.82 ± 0.30 µg/L to 2.13 ± 0.6 µg/L). The quercetin content in the milk of women on the A-PF diet (from 10.8 ± 1.6 µg/L to 19.98 ± 3.6 µg/L) significantly increased ($p<0.05$) compared to other flavonoids. Based on our results, consumption of cooked rice with palm nut sauce and attiéké with fried fish in refined palm oil accompanied by cooked vegetable sauce (onion-red chili-fresh tomato) increases the concentrations of quercetin, kaempferol, and epicatechin in milk. These findings can serve as dietary guidelines for breastfeeding mothers to improve β-carotene and flavonoid levels in breast milk, thus promoting infant growth and development.

Keywords

Human Milk, Flavonoids, Carotenoids, Dietary Habits, Maternal And Child Health

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1. Introduction

Newborns experience significant oxidative stress due to the immaturity of their antioxidant defense mechanisms and digestive system. This immaturity exposes infants to various diseases, such as gastroenteritis, bronchopneumonia, allergies, and long-term chronic illnesses. Among the strategies identified to positively impact newborn health, breastfeeding plays a critical role [1-3]. Breast milk contains thousands of components whose combined reactions aim at a singular goal: protecting against oxidative stress, in other words, neutralizing reactive oxygen species in newborns [4, 5]. Human body antioxidants, particularly those in breast milk, consist of active compounds that can be categorized into two main classes: endogenous and exogenous antioxidants. Major endogenous enzymatic antioxidants include glutathione peroxidase, catalase, and superoxide dismutase [6, 7]. Non-enzymatic endogenous antioxidants include glutathione, lipoic acid, uric acid, coenzyme Q10, vitamin D, and intracellular reducing agents like nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Exogenous antioxidants enter the human body solely through food (dietary antioxidants), such as carotenoids; vitamins A1, A2, C, and E; polyphenols; zinc; and selenium [8].

Carotenoids are yellow-orange pigments produced by certain plants, algae, fungi, and some bacterial species. They are responsible for the red, orange, and yellow coloration of leaves, fruits (oranges, carrots, mangoes), vegetables (peppers and tomatoes), and flowers of plants, as well as the colors of some birds (flamingos), insects, fish (salmon), and crustaceans [9]. Carotenoids are divided into two groups: carotenes, including β-carotene, α-carotene, and β-cryptoxanthin, and xanthophylls, mainly lycopene, lutein, and zeaxanthin [9]. A-Carotene, β-carotene, and γ-carotene are active provitamins: their oxidation leads to the formation of vitamin A (retinol) [10]. The β-carotene molecule has a unique characteristic, as two molecules of vitamin A can be formed from this single molecule [11]. The provitaminic properties of β-carotene and its oxidative conversion to vitamin A are common in humans and animals, justifying its important role in eye protection [12, 13]. In nature, 600 carotenoids have been identified, but only a few are absorbed in sufficient quantities to be detectable in human plasma: these include β-carotene, lutein, lycopene, α-carotene, β-cryptoxanthin, and zeaxanthin, which are also found in breast milk [14-16]. B-carotene is a red-orange pigment found in plants and fruits and protects human skin against infrared radiation [17]. Lutein and zeaxanthin are not precursors to vitamin A, but these carotenoids are particularly beneficial for eye health. They accumulate in the macula of the eye, helping filter harmful high-energy blue light and protecting against oxidative damage [18]. They protect the retina from oxidative damage caused by these rays, reducing the risk of cataracts and age-related macular degeneration [19]. Lutein, zeaxanthin, and lutein are also present in the skin, where they act as filters to block harmful blue wavelengths and reduce the effects of UV-induced inflammation. They decrease skin roughness [20, 21]. Lycopene is considered the most effective singlet oxygen quencher in the carotenoid family [20].

The most important class of polyphenols is flavonoids, which include flavonols, isoflavones, flavanones, and flavanols or flavan-3-ol, as well as anthocyanins. These are powerful natural antioxidants involved in cell defense against oxidative damage [22, 23]. They are phytonutrients found in plants, fruits, vegetables, grains, chocolate, roots, stems, flowers, tea, and wine [23]. Flavonoids play other important roles in preventing chronic diseases due to their anti-inflammatory properties, which are beneficial in type 2 diabetes, cardiovascular diseases, strokes, and cancer prevention. It has been shown that breast milk contains seven flavonoids: naringenin, kaempferol, hesperetin, quercetin [14].

These phenolic compounds and carotenoids are derived from the mothers' diets [16, 15]. Since the phytonutrient composition of breast milk depends on several factors, including the mother's diet, the objective of this study was to assess the levels of flavonoids and carotenoids and investigate how dietary intake affects their concentrations in breast milk.

2. Materials and Methods

2.1. Study Subjects

This is a prospective study conducted from February 24 to May 28, 2024, in the maternal and child health (MCH) services of the National Institute of Public Health (INSP) in Adjamé, located in the Abidjan district (Côte d'Ivoire).

Eligibility criteria included women practicing exclusive breastfeeding, as well as their healthy newborns with a normal birth weight (2.5 kg-4.5 kg).

Exclusion criteria included smoking women, those suffering from acute or chronic conditions (such as diabetes or hypertension), those undergoing antibiotic treatment or taking any medication that could pass into breast milk. Additionally, women following a vegetarian diet or those with significant food allergies or intolerances that limit the consumption of certain food groups were excluded.

2.2. Study Design

2.2.1. Dietary Data and Sample Collection

Before sampling, breastfeeding women were interviewed by a trained investigator using a structured questionnaire.

This questionnaire collected information on age, geographic location, marital status, educational level, professional activity, and an assessment of the participants' diet.

2.2.2. Dietary Intake Assessment

The dietary survey was conducted using a food frequency questionnaire, commonly used worldwide to assess dietary intake [24]. In this study, breastfeeding women provided a detailed description of their basic diet, including consumption frequency, food preparation (ingredients), and cooking methods (such as frying, boiling, grilling, and steaming) as detailed as possible over the course of a week. A basic diet was defined as being consumed at least three to four times per week and repeated throughout the study period.

2.2.3. Milk Samples

Mature milk samples were collected in the morning using a breast pump from breastfeeding women on the 45th and 105th postpartum days, corresponding to the scheduled dates of the first and third newborn vaccination appointments. A sample of 20 to 35 mL was collected from each participant in a sterile glass container. Immediately after collection, each sample was divided into 5 mL aliquots in test tubes, consistently protected from light, and transported on ice to the laboratory, where they were stored at -20°C until analysis.

2.3. Quantification of Quercetin, Kaempferol, Isoflavone, and Epicatechin

Breast milk samples were extracted and deconjugated according to the method of Song et al. (2013) with a modified extraction solvent.

To 1 mL of breast milk, 3 mL of hexane were added. This operation was repeated twice. After lipid removal, 50 µL of 2.7 mmol/L L-ascorbic acid and 2.2 mmol/L Na2-ethylenediaminetetraacetic acid (EDTA) in water were added. The samples were incubated in water bath for 15 minutes at 37°C with gentle shaking. After incubation, the mixture's pH was adjusted to 4.5 with 1.0 N NaOH, and 3.85 kU of β-glucuronidase were added. The samples were incubated for an additional 45 minutes at 37°C in a water bath with gentle shaking. After enzymatic deconjugation, flavonoids were extracted with 3 mL of ethanol diluted in water (50%) (v/v) and transferred into 5 mL vials. The vials were vortexed for 15 seconds, then sonicated for 15 minutes. After resting for 5 minutes at room temperature, the extract was topped up to the calibration mark with the ethanolic solution and then filtered through Wattman No. 4 paper and a 0.45 µm Millipore membrane (CARL ROTH, Karlsruhe, Germany). A 2 mL volume of the filtrate was taken, to which 2 mL of methanol were added for the simultaneous quantification of flavonoids.

2.4. Standard Solution

A stock solution was prepared with a pure mix of 50 µg of quercetin, 50 µg of kaempferol, and 50 µg of epicatechin. In a 5 mL vial, all weighed standards were combined, and 3 mL of a hydroethanolic solution (50%) were added. The vial was sonicated for 15 minutes. After resting for 5 minutes at room temperature, the homogenate was topped up to the calibration mark with the hydroethanolic solution. The vial's contents were then filtered using Wattman No. 4 paper and a 0.45 µm Millipore membrane (CARL ROTH, Karlsruhe, Germany). Finally, 1 mL of the filtrate was diluted with 2 mL of methanol, and for simultaneous quantification, 1 mL was used in a chromatography vial. Flavonoids were then quantified by HPLC under the conditions described below.

2.4.1. Chromatographic Analysis Conditions

A simple HPLC method was used for the simultaneous determination of the three flavonoids in breast milk. The method showed good linearity over a range of 5 to 50 µg/mL for the five standards used (r>0.996).

Molecule elution was carried out in gradient mode with ultra-pure water and methanol, both acidified with 1% acetic acid (CH3COOH), as summarized in the table. Separation was performed at 1 mL/min at 25°C, and the detection wavelength was 360 nm. The column used was a C18 Alltima (150 × 4.6 mm ID, 5 µm, Alltech, Deerfield, USA).

The gradient conditions for analysis are as follows: Time (min) | % Solvent A (H2O 1% CH3COOH) | % Solvent B (CH3OH 1% CH3COOH) | Table 1.

Table 1. *Conditions of the Chromatographic Gradient Analysis.*

Time (min)	% Solvent A (CH3COOH/ H2O)	%Solvent B (CH3COOH/MeOH)
0 – 5	95	5
5 – 15	80	20
15 – 30	50	50
30 – 40	20	80

2.4.2. Quantification of Lutein, β-Carotene, and Lycopene

The technique based on the method of Song et al. (2013) [16] was used for the simultaneous evaluation of lycopene, lutein, and β-carotene.

Carotenoids were extracted from milk using hexane after protein precipitation and removal.

In a test tube, 0.3 mL of 30% methanolic KOH was added to 0.75 mL of milk, then left to sit for 15 minutes at room temperature. One mL of hexane was added, and the tube was vortexed and centrifuged for 10 minutes at 3500 rpm for 15 minutes. The extraction was repeated three times for the same tube, and the hexane phases were transferred to another tube to evaporate the solvent under a nitrogen stream. Finally, the dry residue was reconstituted with 1 mL of the mobile phase for the simultaneous quantification of carotenoids at 450 nm for lutein and β-carotene, and 470 nm for lycopene.

A standard range was prepared with 1 mg of lutein, 1 mg of lycopene, and 1 mg of β-carotene under the same conditions as the samples. Dilutions were made for a six-point range: 0.1, 0.5, 1, 1.5, 2, and 2.5 µg/L by serially diluting the standard mixture solution with methanolic KOH.

2.4.3. Chromatographic Quantifications

The concentrations of the different carotenoids were determined by reverse-phase liquid chromatography after liquid-liquid extraction. The mobile phase consisted of acetonitrile, pure water, acetic acid buffer (pH 6.2), and triethylamine in proportions (45/50/1/4) (v/v/v/v), operating isocratically using a Waters Carotenoid C30 column (2.0 × 150 mm).

2.5. Statistical Analysis

The results were expressed as mean ± standard deviation. Statistical analyses were performed using Epi Data and Excel for data processing, and GraphPad Prism 5 for histogram representations. SPSS 18 variance analysis, Student's t-test, and Pearson's test were used for these statistical analyses. Pearson's test was used to determine correlations between two parameters, and Student's t-test was used to compare means for paired and unpaired tests at day 45 and day 105. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Nutritional Profile and Demographic Characteristics of Breastfeeding Women

Ninety-seven breastfeeding women gave their consent to participate in the study after an explanation of the procedures. Thirty-seven participants belonging to several baseline diet groups, with small sample sizes (fewer than 5 participants per diet group), were subsequently excluded from the analysis to avoid interference with statistical analyses. Consequently, the study was limited to a final sample of sixty breastfeeding women. The data from these sixty breastfeeding women were grouped into two predominant baseline diets: rice with seed sauce (palm oil seed juice) (R-SG, n = 30) and attiéké (cassava semolina) with fried fish in refined palm oil, accompanied by a vegetable paste (boiled onion, fresh pepper, and fresh tomato, n = 30). It is also important to mention that the R-SG dish was consumed with meat and/or smoked fish, with fresh pepper and onion in the sauce. Additionally, the breastfeeding women did not consume more than two fruits per week, regardless of their diet.

The demographic data of the selected mothers are summarized in Table 2. The age of the women participating in the study ranged from 19 to 47 years, with an average of 24.71 ± 4.73 years. Forty-eight women, or 80%, lived with their partner, 26.67% (16/60) of the women were civil servants, while 33.33% (20/60) were uneducated.

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

Parameters	Count (%)
Mother’s Age	
< 25	30 (50)
> 25	30 (50)
Average ± SD (24,71± 4,73)	
Place of residence	
Adjamé	25 (41,67)
Plateau	14 (23,33)
Attécoubé	21 (35)
Occupation	
Housewives	8 (13,33)
Traders	12 (20,00)
Civil servants	16 (26,67)
Students	10 (16,67)
Informal Sector	14 (23,33)
Education level	
Primary	12 (20,00)
Secondary	18 (30,00)
High education	10 (16,67)
None	20 (33,33)
Marital status	
Maried/cohabiting	50 (83,33)
Single	10 (16,67)

3.2. Nutritional Profile

The study revealed that 91.67% of breastfeeding women ate three meals per day: breakfast, lunch, and dinner. The figure below shows the various foods consumed by breast-feeding women each morning during the study.

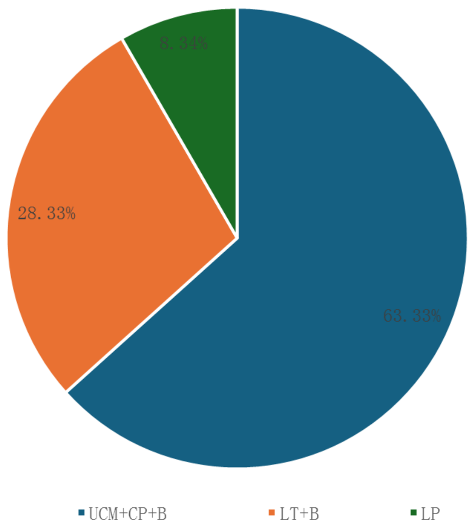

Figure 1. *Distribution of mothers by breakfast diet.*

UCM+CP+B: Unsweetened condensed milk + Cocoa powder + Bread
Lt+B: Lipton tea + Bread
LP: Local porridge (millet, rice, maize)

Evaluation of flavonoid and carotenoid content

The separation and detection of aglycones of flavonoids analyzed in breast milk were determined from the chromatograms of the standard solution. These chromatograms identified the presence of kaempferol, epicatechin, and quercetin. Quercetin, kaempferol, and epicatechin were detected in all samples, and their concentrations increased during the lactation period. These increases revealed a significant difference in the quercetin and epicatechin levels among breastfeeding women who consumed R-SG and A-PF from day 45 to day 105 ($p < 0.05$) within the same diet. However, between different groups, the differences were not significant. The results are presented in Table 3.

Table 3. *Flavonoid content of milk according to mothers' diets.*

Regimes/periods	Flavonoid Contents (µg/L)		
	Kaempferol	Quercetin	Epicatechin
R-SG D45	11,9 ± 2,3	11,53 ± 5,9	20 ± 3,8
D105	13, 1 ± 3,9	18, 74 ± 3,7	33,56 ± 4,1**
A-PF D45	8,9 ± 3,8	10,8± 1,6	18,49 ± 5,2
D105	10 ± 0,9	19,98 ± 3,6**	34,51 ± 9,6**

The values are expressed as mean ± SD. Comparison between day 45 and day 105.
** represents significance between day 45 and day 105 (P <0.05).

The separation and detection of carotenoids analyzed in breast milk were determined from the standard solution chromatograms. All three carotenoids were detected in the samples, and their concentrations are shown in Table 4. The carotenoid levels decreased from day 45 to day 105 ($p > 0.05$), except for an increase in β-carotene in the R-SG diet group, though this difference was not significant ($p > 0.05$).

Table 4. *Lycopene, β-carotene, and lutein content in mature breast milk according to mothers' diets.*

Regimes/periods	Carotenoid content (µg/L)		
	Lutein	Lycopene	β-carotene
R-SG D45	0,90 ± 0,60	0,40 ± 0,2	1,82 ± 0,30
D105	0,11 ± 0,01**	0, 24 ± 0,3	2,13 ± 0,6
A-PF D45	0,33 ± 0,10	0,38 ± 0,12	1,09 ± 0,20
D105	0, 22 ± 0,10	0,28 ± 0,10	0,51 ± 0,10**

The values are expressed as mean ± SD. Comparison between day 45 and day 105.
** represents significance between day 45 and day 105 (P <0.05).

4. Discussion

Breastfeeding remains a crucial tool for protection against free radicals, reactive oxygen species, and oxidative stress. This study quantified the levels of three flavonoids (quercetin, epicatechin, and kaempferol) and three carotenoids (lutein, lycopene, and β-carotene) in the mature breast milk of 60 breastfeeding women based on their diet. After consuming Rice with Seed Sauce and Attiéké with Fried Fish accompanied by cooked vegetable paste (fresh tomato, fresh pepper, and onion), flavonoid concentrations, unlike carotenoid levels, increased in women's milk from day 45 to day 105 postpartum. The observed consistency between dietary habits and the quality of breast milk is supported by the impact of the consumed foods on polyphenol concentrations at day 105. Flavonoid and β-carotene levels evaluated in the breast milk samples suggest that these compounds were present in the foods and transferred from the maternal blood into the milk at significant levels [25, 26].

The concentrations of flavonoids, particularly epicatechin on day 105 (33.56 ± 4.1 µg/L and 34.51 ± 9.6 µg/L), in the milk of the two groups of Ivorian women, may be due to the high levels of this flavonoid in cocoa powder and Lipton tea, the primary components of the mothers' breakfast during the study period. Zhou et al., 2021 [26], demonstrated that the levels of quercetin and epicatechin, two flavonoids in breast milk, were strongly correlated with the consumption of tea and vegetables by breastfeeding women, suggesting that these flavonoids are absorbed and transferred into breast milk from the mother's diet. Additionally, onions contain two main flavonoids, quercetin and kaempferol, present in all onion varieties (yellow, red, or white) [27], which would explain the increase in these flavonoid concentrations in mothers' milk at day 105. The consistently high levels of these three flavonoids during both periods in the present study suggest that the accumulation of these compounds in breast milk is not affected by the stage of lactation but rather reflects the daily consumption of flavonoid-containing foods by the mothers [14]. The experimental study by Fujiwara et al. conducted on mice is the first to experimentally demonstrate that a diet rich in quercetin would lead to the accumulation of the aglycone of this phytonutrient in the breast milk of mother mice [28].

As for carotenoid levels, only β-carotene concentrations increased in the milk of women who consumed the Rice with Palm Seed Sauce diet. This contrasts with the results of studies by Zhou et al. and Sun et al., which found no correlation between β-carotene intake and carotenoid levels in breast milk [29, 16]. Zhou's team showed that dietary carotenoid intake does not affect the β-carotene profile in breast milk. In our study, palm oil, a key ingredient in the Rice-Palm Seed Sauce diet, was rich in carotenoids, especially β-carotene [30, 31], and would be responsible for the positive variation in β-carotene levels in women's milk. Furthermore, the vegetables like tomatoes and fresh peppers present in both basic diets had no positive effect on lutein and lycopene levels in breast milk. Zhou et al. found that dietary carotenoid and vitamin A intake in 87 breastfeeding women in Hong Kong did not affect β-carotene in breast milk but did influence lutein and lycopene levels [30].

Our results are more similar to those of Dai et al. (2022) [15], who found that carotene levels in milk were linked to carotene intake in the mother's diet and could reflect the transport of carotenoids from maternal plasma to breast milk. Another study by Haftel et al. on the milk of 26 Hebrew mothers found that β-carotene and lycopene concentrations in breast milk were positively correlated with the consumption of fresh carrot paste and fresh tomato paste, two foods rich in carotenoids. Additionally, this author suggests that regular consumption of cooked tomato paste and peppers could increase the anti-inflammatory and antioxidant properties of breast milk and positively affect the infant's health. Indeed, vegetables such as fresh tomatoes and cooked fresh peppers, ingredients found in both basic diets, contain lycopene, the most abundant carotenoid in these fruits [32].

The difference in flavonoid and carotenoid levels in breast milk in these studies is likely due to ethnic or geographical differences. It is also important to mention that the heterogeneity of study methodologies (including sample collection protocol, sample size, study parameters, measurement units, etc.) makes it difficult to establish comparisons between different studies.

5. Conclusion

This study is the first of its kind conducted in our country, exploring the impact of diet on flavonoid and carotenoid levels in breast milk. In our study, the concentrations of quercetin, kaempferol, epicatechin, and β-carotene in the breast milk of women who consumed the Rice with Palm Seed Sauce and A-PF diets accompanied by cooked pepper, tomato, and onions increased after consuming these diets. However, a much larger prospective study, recruiting a larger number of participants, should be conducted to evaluate the effects of several diets on the levels of these phytonutrients in the breast milk of Ivorian women. Furthermore, this study could encourage breastfeeding women to adopt healthy eating habits to optimize the phytonutrient content of their breast milk.

Abbreviations

R-SG	Rice with Sauce (Palm Oil Seed Juice)
A-PF	Cassava Semolina with Fried Fish in Refined Palm Oil Accompanied by a Vegetable Paste (Boiled Onion, Fresh Pepper and Fresh Tomato)
UCM+CP+B	Unsweetened Condensed Milk + Cocoa Powder + Bread
Lt+B	Lipton tea + Bread
LP	Local Porridge (Millet, Rice, Maize)

Author Contributions

Matogoma Digbé Ble: Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft

Francis Béranger Angelo Aka: Formal Analysis, Funding acquisition

Dorothée Marie Koumi: Methodology

N'Bra Koko Alexandre: Methodology, Validation

Joel Bonouma: Methodology, Validation

Conflicts of Interest

The authors declare no conflicts of interest.

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