

Fruits and Vegetables Consumption and Assessment of Vitamins A, E, C of the Mature Breast Milk of Women Living in Cote d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Author MDB was the principle investigator, designed the study, performed analysis and wrote the initial manuscript with author GMB. Authors JDN and MA validated data collection and statistical analysis and coordinated the study. All authors contributed to the text, read and approved the final manuscript.

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ABSTRACT

The breast milk is the ideal food of the infant because takes part in his development, and protects it from many diseases. However, the dietary habits of the nursing mother can influence the nutritional value of milk during the period of lactation, and this fact of depriving the infant of certain essential molecules. The objective of this study was to assess vitamins A, E and C of the mature breast milk in partnership with the consumption of fruit and vegetables among women living in Côte d'Ivoire. The study was carried out in Abidjan in two health services maternal and infantile from January

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24th, 2017 to June 28th, 2017. The frequencies of consumption of fruit and vegetables and the taking of mother's milk were realized on the 45th and 105th day after childbirth. Vitamins A and E in milk were carried out by liquid chromatography, and the vitamin C by Spectrophotometry. The statistical analysis of the data was carried out with the software statistical SPSS version 18. Analysis of variance was performed, and the difference was significant for a p value $p < 0.05$. It was noted an irregular consumption of fruit and vegetables. None the women consumed five fruit and vegetables per day. The frequencies of consumption were of 0, 1 and 2 fruit and vegetables per week. The concentrations in vitamins (A, E and C) were weak over the two periods of taking away (45th and 105th day), and given Vitamin C (0.38 ± 0.03 mg/dL with 0.21 ± 0.06 mg/dL); Vitamin E (0.6 ± 0.03 mg/mL with 0.32 ± 0.22 g/mL); Vitamin A (457.97 ± 141.54 μ g/L with 401.53 ± 197.84 μ g/L). This study showed an irregular consumption of the women nursing out of fruit and vegetables and a reduction in the rate of vitamins A, E and C during the period of lactation. The differences observed with other nursing women quoted in the literature require more investigations in order to define the possible origins and consequences of them on the evolution of the immune system of the child up to 6 months.

Keywords: Fruits and vegetables; breast milk; vitamins A, E, C; Côte d'Ivoire.

1. INTRODUCTION

The mother's milk is the ideal food of the infant because it contains very many biologically active factors; proteins, lipids, glucids; minerals and vitamins for the good development of the infant. He also protects it from pathologies infantile like the diarrhoea, pneumonia and long-term against the chronic diseases such diabetes, cancer, arterial hypertension [1,2,3]. However, various studies showed as the content of certain elements of this essential food of the infant would be function of the food supply and the nutritional state of the mother as well during the pregnancy as the period of breast feeding [4,5,6]. Among these elements, we can note vitamins, trace elements and minerals [5,6,7], very important in the defense system against the free radicals [8]. Indeed, the most important vitamins in antioxidant defense are the vitamins A, C and E [9,10]. These vitamins are as important for the adults as for the infants. Indeed, the new-born baby premature or born in the long term is accompanied by an increase in the oxidative stress dependent on the way of the intra-uterine medium low in Oxygen (an oxygen pressure = 20-25 mm Hg) on another hyperoxygenated extra-uterine medium (an oxygen pressure = 100 mm Hg). This oxygen increase at the new-born babies involves a generating hyperoxy of the radicals free [10,11]. Studies showed that the mother's milk offered a better antioxidant power to the infant than infantile milks [11,12,13,14]. These vitamins of the mother's milk play a significant role in the removal of the radicals libreset the maturity of the mechanisms of defence antioxidant and the digestive system of the new-born baby. These exogenous

antioxidants that are the vitamins quoted above are brought in general by regular daily contributions out of fruit and vegetables per day [15,16,17]. In Ivory Coast, a country where the exclusive breast feeding from 0 to 6 months is promoted, little studies was carried out on the content of the vitamins in the mother's milk. The purpose of the present study is to impregnate dietary habits in fruit and vegetables but also to determine the content of vitamins A, E and C in breast milk in women in Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Population

It was about a study, longitudinal and prospective. It was carried out in Abidjan in two health services maternal and infantile which are the National institute of Public health at Adjamé and at the general hospital Attié of Yopougon from January 24th, 2017 to June 28th, 2017. The study included 110 willing mothers who practised the exclusive breast-feeding up to 6 months after their childbirth. The frequencies of the dietary habits were collected and of the taking away of milk were realized on the 45th and 105th day after childbirth, for the assessment of vitamins A, C and E. The mothers should be confined in the long term and practise the exclusive breast-feeding since the childbirth up to 6 months. They should be in good health. The mothers who smoked, and who followed a particular treatment of to acute or chronic disorders (diabetes, hypertension) and those which were supplemented in vitamins were excluded from the study.

2.2 Reagents

The reagents used were of an analytical nature and concerned: The rétinol pure acetate 99,87% (Sigma); pure œ-tocopherol 95% (Sigma); rétinyl-acetate (Merck); pure ethanol (Merck); hexane (Merck); methanol HPLC (Merck) for the preparation of the ranges standards and the phosphoric acid méta to 6% (Merck); sulphuric acid 12 M (Merck); 2,4-dinitrophényl hydrazine (Merck); thiourea in solution at 5%; copper sulfate solution to 0.6%; trichloroacetic with 5% (Merck), reference solution of vitamin C to 0.05 mg/L.

2.3 Preparation of the Milk Samples

Biological equipment was the mature mother's milk. Taking away of mature milk were carried out on the 45th and 105th day after childbirth using a car manual milk. The milk samples collected in bottles out of glass and sterile were immediately taken in tubes test wrapped beforehand of aluminum foil, then conveyed at the Laboratory of Nutrition in refrigerators and preserved at - 20°C.

2.4 Determination of the Vitamins A and E

The assessment of vitamins A and E was made using a HPLC of SHIMADZU brand. A spectrophotometer. The technique of extraction used is that of Rodas et al. [18]. The milk samples were brought back to room temperature, safe from the light to be defrosted. A quantity of 100 µL of solution of internal standard (0.95 µg/mL) and 100 µL of absolute ethanol were added to 100 milk µL. After agitation during 30 seconds on vortex, hexane (400 µL) were added; the mixture obtained was again agitated 2 times during 30 seconds on Vortex. The whole was centrifuged (3500 rpm ,15 minutes), then 300 µL of the supernatant hexanic were taken and evaporated dry under nitrogen current to a pressure equivalent to 0.5 bar. The residue was dissolved in 100 µL of methanol and this solution constituted the liposoluble vitamin extract and 10 µL was injected on to C18 reversed phase chromatographic column in isocratic mode with a flow rate of 1.8 mL/mn. Assessments were carried out in isocratic mode; the mobile phase consisted to the methanol mixture/water distilled in the proportions (98.5: 1.5, v/v); the temperature of the column was of 20°C. The reading was made to the wavelength : 290 nm in UV for the simultaneous assessment of the two

vitamins. The determination of the concentration of the samples in rétinol (µg/l) and α-tocopherol (mg/l) was made starting from average measurements of surfaces of the respective peaks of the rétinol, α-tocopherol and the internal standards (rétinyl acetate).The solutions mothers of retinol and retinyl-acetate were solutions with 1 mg/mL in absolute ethanol; that of α-tocopherol stay to 10 mg/mL in absolute ethanol. All these solutions mothers were stored with the refrigerator with + 4°C, safe from the light. The various solutions mothers were used to prepare solutions of concentrations increasing for the calibration line. The concentrations varied as follows: rétinol and rétinyl-acetate (1 mg/mL; 0,5 mg/mL; 0,25 mg/mL; 0.125 mg/mL and 0,0625 mg/mL); tocopherol and of tocopherol acetate (10 mg/mL; 5 mg/mL; 2.5 mg/mL; 1,25 mg/mL; 0,625 mg/mL).

2.5 Determination of the Vitamin C

The determination of the ascorbic acid (vitamin C) in the mother's milk was made according to the method of Dogar et al. [19], using UV-visible brand SCHIMADZU. Each sample was defrosted and centrifuged at 3000 trs/rpm for 10 mn; then 0.3 ml of the aqueous phase were taken in a tube test wrapped of aluminum foil to which 1.2 ml of trichloroacetic acid were added. The whole was homogenized then centrifuged again with 3000trs/mn during 10 mn. A volume of 0.9 ml of the supernatant and 0.4 ml of DTCS were added (copper thiourea hydrazine sulphate 2.4 dinitrophényl). The solution was incubated with 60°C during 1:00 with the bain-marie and cool in ice-cold water. A volume of 1.6 ml of sulphuric acid 65% was added gradually and the solution was at rest during 30 mn with room temperature. The absorbance was read to 520 nm against a white only made up of copper thiourea hydrazine sulphate 2.4 dinitrophényl. The range was prepared starting from a standard solution of vitamin C to 0.05 mg/mL. In 5 test tubes, respectively containing following volumes of the standard solution: 0.5; 1; 2; 4 and 6 mL; of acid méta phosphoric 6% was added there in order to obtain the following final concentrations: 0.001; 0.002; 0.004; 0.008 and 0.012 mg/mL.

2.6 Statistical Analysis

Data were processed using the software statistical SPSS version 18. The Analysis of variance was performed using the ANOVA test ; the difference was significant for a p value p<0.05.

3. RESULTS

3.1 Sociodemographic Characteristics

The median age of the mothers was of 26,68±6,76 years with a minimum of 15 years and a maximum of 43 years with a population made up of 71.82% (79) mothers were Ivorian and 28.18% (31) non Ivorian. Among the Ivorian mothers, we recorded: 48.1% of Akan origin (38); 21.5% of Krou origin (17); 17.7% of Mande origin (14); 12,7% of Gour origin (10); the results were represented by Fig. 1 & 2. The study with the professional plan comprised 35% of housewives, 22.7% of trader, 20% of the informal sector, 13.6% of students and 8.2% of official; the results were represented in Table 1.

Table 1. Distribution of mothers according to their professional activity

Profession	Number	Percentage
Housewife	39	35.5
Trader	25	22.7
Student	15	13.6
Official	9	8.2
Informal sector	22	20.0
Total	110	100.0

3.2 Dietary Habits of the Nursing Mothers

None the mothers consumed five fresh fruit and vegetables per day besides its food mode containing starchy food, cereals and others. However referring to the catch of fruit and vegetables consumed per week, three groups could be revealed of which those which did not

consume fruit at all, those which consumed with more the one fruit per week and those which consumed some to more both per week. In addition, all the mothers consumed only vegetables cooked in the sauces on average 2 with times 3 per day.

It was noted that on the 45th day (J45), none the mothers consumed five fresh fruit and vegetables per day. However, 27.28% consumed two fruits per week; 11.82% a fruit per week and 60.90% of the mothers of the study, any fruit and vegetable fresh. The vegetables, very cooked in sauces, were made up mainly of tomato, pepper, onion and the vegetables sheets. The consumed fruits were primarily orange. On the 105th day (J105), the mothers continued still not to consume five fresh fruit and vegetables per day. However, about ten mothers who did not consume fruits with the first taking away it did. Thus, at J105, 43,64% of the nursing mothers of the study still did not consume fresh fruit and vegetables; 31,82% consumed two fruits per week and 24,54 % consumed one fruit week. The vegetables were the same than at J45 and always cooked in sauce; the consumed fruits were the mangos. All these characteristics were consigned in Table 2.

3.3 Rate of Vitamins A, E and C in Mature Milk

The results showed a fall of the contents vitamins A, E and C of the mother's milk on the 45th and 105th day. Indeed the percentage of reduction of the contents was of 0,12% for

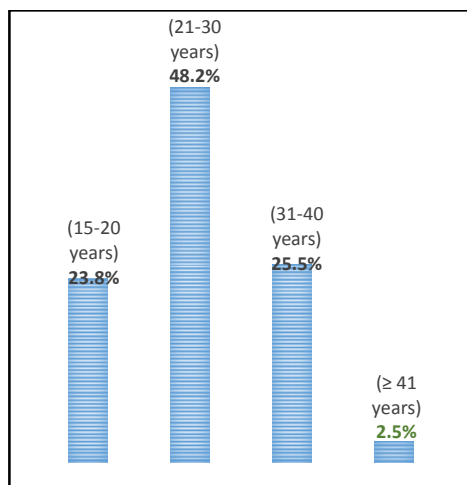


Fig. 1. Distribution of mothers by age group

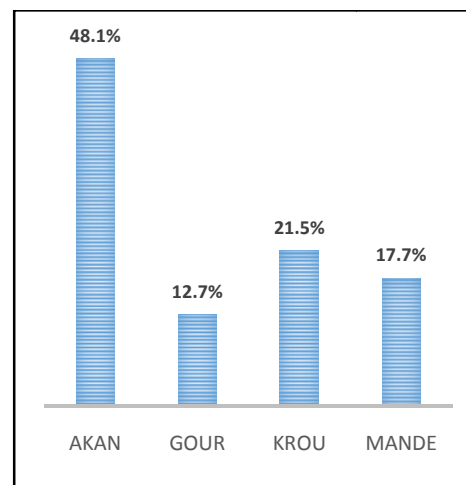


Fig. 2. Distribution of mothers according to their ethnic group

Table 2. Summary of the dietary habits of the mothers of the study

Percentage of nursing mothers		
Quantity of fruits and vegetables consumed per week	45th day after childbirth (J45)	105th day after childbirth (J105)
0	67 (60.90 %)	48 (43.64 %)
1	13 (11.82 %)	27 (24.54 %)
2	30 (27.28 %)	35 (31.82 %)

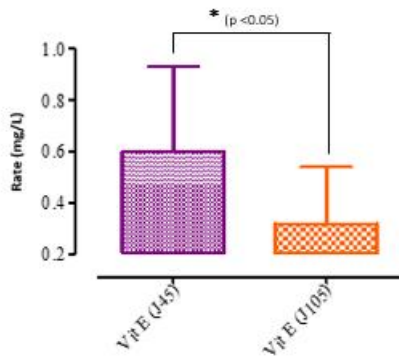


Fig. 3. Rate of Vitamin E (J45 et J105)

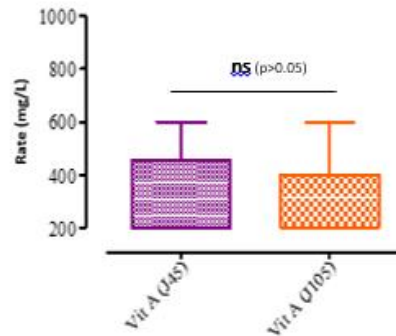


Fig. 4. Rate of Vitamin A (J45 et J105)

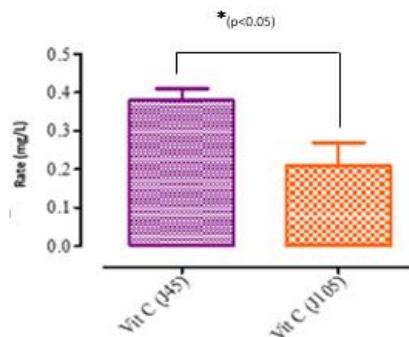


Fig. 5. Rate of Vitamin C (J45 et J105)

the rétinol (vitamin A), 46,66% pour-α-tocopherol (vitamin E) and of 44,73% for the L-ascorbic acid (vitamin C). For the two periods, no significant difference was not noted for vitamin A ($p > 0,05$); however, it was raised a significant difference for the vitamins C and E ($p < 0,05$). (Fig. 3. 4. and 5.)

4. DISCUSSION

This study aimed to evaluate the contents of vitamins A, C and E in the mature mother's milk of the mothers nursing exclusively babies from 0 to 6 months. For this purpose, taking away of milk were carried out on 110 mothers agreeing over two periods: the first at 45th days after delivery (J45) and the second 105th day after

delivery (J105). The majority of the mothers had an age ranging between 21 and 30 years was 48.2%. None the surveyed mothers consumed a fruit per day and of fresh vegetables for different reasons from/to each other and that in spite of the educational level from 57% for the primary education; 28% for the superior and 15% for the secondary. Thus, one could allot the low contents of vitamins raised in this study, with a prolonged cooking of food or an irregular and fruit supply [20, 21,22].

Indeed, all breastfeeding mothers surveyed during this period consumed vegetables that were very well cooked in sauces. However, the question of whether they consumed the cooking water and the cooking time was not asked.

The results of this study showed that the average levels of α -tocopherol (vitamin E) decreased between the 45th and 105th day. These results corroborate those of Yzydorczyka et al. [11] and Martysiak et al. [23] who also found in their work that the vitamin content decreased during the period of lactation. However, the values of this study are low compared to those of 60 mothers in Canada with an average of α -tocopherol content in mature milk of 2.32 ± 0.11 mg/L [12]. Another study on the determination of vitamin E content in mature breast milk was also found in a study of 48 mothers in Poland with mean levels of α -tocopherol ranging from 2.92 ± 0.84 mg/L at 30 days post partum and 2.07 ± 0.66 mg/L 90 days post partum [23]. Otherwise, higher values of vitamin E have been revealed by other works in Greece. Indeed, these authors found the mean values of 3.575 ± 1.464 mg/L with a population of 64 mothers at 30 days post partum and $3,489 \pm 1,809$ mg/L with 39 mothers at 120 days post partum [24]. The decrease in vitamin E levels in mothers' mature milk is due, on the one hand, to the increase in fat globules as milk matures [25], and on the other hand, this low in the mothers of our study would be due to a consumption of foods low in this vitamin E. Indeed, after the first days of lactation, the synthesis of triglycerides in mammary gland and its secretion in milk start to increase which is not proportional to the secretion of the other components of the membrane of the fatty globule. Consequently, there is a significant reduction of the quantity of these components of the fatty globule, including α -tocopherol, since the major part of the vitamin E is secreted like constituent membrane of these globules [25]. The same observations were made with vitamin C, the levels of which were very low compared to that reported in the literature, which gives vitamin C levels in mature breast milk, between 10 mg/dL and 4 mg/dL [1,26]. These levels can be increased to 11.5 mg/dL for breastfeeding mothers who are supplemented [26]. However, researchers, after a study of mothers in Bangladesh, found mean vitamin C levels of 3.03 ± 0.67 mg/dL in the mature breast milk of 26 mothers two weeks postpartum and 3.49 ± 0.53 mg / dL one month after [27]. In our study, mean vitamin C levels were 0.38 ± 0.03 mg/dL at day 45 and 0.21 ± 0.06 mg/dL at day 105. The low levels of ascorbic acid were due to the fact that the mothers cooked the vegetables too much, and on the other hand they had a diet low in fruit, the highest number of which was two per week; but also in fresh vegetables. Indeed, vegetables cooked in large quantities of boiling water for a long time on a large fire would lose 75% of

water-soluble vitamins and in this case vitamin C[28].

The low levels of vitamins E and C in this age group can be explained by a habit acquired since childhood. [22]. Also, these low levels could also be explained by a preference for the consumption of foods rich in energy and poor in micronutrients [25]. Thus, among mothers of this age group (adolescent girls), in addition to the requirements of these vitamins for their own growth, should be added those of pregnancy and also breastfeeding [25]. Therefore, when pregnancy and lactation occur in adolescence, the nutritional risks associated with this condition may increase.

The proportioning of the rétinol, whose averages of the contents were of 457.97 ± 441.54 μ g/l against 401.53 ± 197.86 μ g/l were statistically in conformity with those found in the literature [26,29]. Average values of different but lower levels than those of our study have been reported by other studies; the authors worked on 49 Brazilian mothers and found mean values of 399.41 ± 28.53 μ g/L [30]. The work carried out at 196 mothers in Rio de Janeiro and at 60 Brazilian mothers showed mean values of 502.11 μ g/L and 430.76 μ g/L, respectively, in the mothers' milk [30,31]. These contents of rétinol similar to those of other studies carried out on mother's milk in the countries in the process of development, would be certainly related to the dietary habits of these mothers. Indeed, the use of consumable oils enriched with vitamin A, as well as other ingredients of seasoning such as the stocks, Maggi, the consumption of fruit and vegetables such as tomato, eggplant sauce, the palm oil (sauce granulates), the sorrel of Guinea (dah), the amaranth, onion, mango and cassava (foutou banana, placali, attiéké) which is rich foods in carotenes, would provide the totality of rétinol to consumers [32]. In the study of these investigators, crude palm oil would contain 832 to 3757 μ g/g of total carotenoids, 580 to 2390 μ g/g of β -carotene and 864 to 1124 μ g/g of vitamin E per gram of seed. With the birth, the new-born baby has weak reservations of vitamin A in the liver because of the maternal homeostatic control, which controls the transfer placental of vitamin A to the foetus and prevented the transfer of high concentrations. The storage of the liver of the new-born baby can be increased during breast feeding, if the mother who nurses presents a food supply or an adequate reserve in vitamin A, as well as a production of milk to a concentration and an adequate volume [29].

Vitamins being transferred from the mother to the child by placental way during the pregnancy and way of the breast-feeding which is the most important way besides quantitatively after the birth [33]. This study showed that the contents of vitamins A, C and E decreased in the mother's milk with the progression of lactation, and that the problems of quality of this mature milk to answer or not the nutritional needs of the infant up to six months were asked. Consequently, a sensitizing of the mothers in periods of breast feeding aiming at increasing the levels of vitamins in milk would be important.

5. CONCLUSION

This study made it possible to provide the first description of the composition in vitamins A, E and C of the breast milk in Côte d'Ivoire. These data show that the use of the breast milk as only exclusive food of the infant during the first six months of its life is a practice to be encouraged. However, their low contents in milk during lactation and require it more a great attention on the nutritional state of the mothers, who showed an irregular consumption into fruity and vegetables. The differences observed with the literature require additional investigations in order to define the possible origins and consequences of them on the evolution of the immune system of the child up to 6 months. Otherwise, it is important to mention that the basis for the change in the concluded incorrect consumption of fruit and vegetables by feeding mothers should be a health-awareness nutrition education.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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