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Lipid and polyunsaturated fatty acid contents in infant formulas in reference to the *Codex Alimentarius*

Lipídios e ácidos graxos poli-insaturados em fórmula infantil: comparação com o *Codex Alimentarius*

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ABSTRACT

Codex Alimentarius stan-72 (2011) discriminate the adequate values of fatty acids and lipids for infant formula. Total lipids and polyunsaturated fatty acids were quantified in fourteen infant formulas samples and compared the results with the recommended values. Extraction and quantification of lipids followed Roese Gottielb method. Analysis of fatty acid, methylated by Hartman and Lago procedure, was carried out through gas chromatography was performed with the use of internal standard 23:0. In the analyzed samples, at least one parameter was in disagreement with *Codex Alimentarius*.

KEYWORDS: Legislation; Polyunsaturated Fatty Acid; Infant Formula; *Codex Alimentarius*

RESUMO

O Codex Alimentarius stan-72 (2011) preconiza valores adequados para lipídios e ácidos graxos em fórmula infantil. O total de lipídios e ácidos graxos poli-insaturados foi quantificado em 14 amostras de fórmulas infantis e seus resultados comparados com os valores recomendados. A extração e quantificação dos lipídios foi realizado segundo metodologia de Roese Gottielb. Para análise de ácidos graxos, utilizou-se metodologia de metilação segundo Hartman e Lago, injeção em cromatógrafo gasoso e quantificação com padrão interno de 23:0. Todas as amostras analisadas, em pelo menos um parâmetro estava em desacordo com o preconizado pelo Codex Alimentarius.

PALAVRAS-CHAVES: Legislação; Ácidos Graxos Poli-Insaturados; Fórmula Infantil; *Codex Alimentarius*

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INTRODUCTION

Essential fatty acids (EFAs) comprise a class of molecules that are not produced by human beings despite the fact that they are necessary for their proper functioning. This deficiency occurs due to the lack of specific enzymes capable of double bond formation (desaturases), or breakage (hydrogenases), between carbons 3 and or between 6 and 7, of fatty acids (FAs). For example, linoleic (LA, 18:2 ω-6) and α-linolenic (ALA, 18:3 ω-3) acids are synthesized exclusively by members of the plant kingdom. The absence of such nutrients in a diet is associated with many syndromes and diseases[1,2].

EFAs can be modified by mammals in several ways, among which are chain elongation or shortening, through partial beta-oxidation and unsaturation insertion. These modifications give rise to long-chain polyunsaturated fatty acids (LC-PUFAs). However, the ω-3, ω-6, and ω-9 FA families compete for the enzymes responsible for such modifications, which ultimately interferes in the metabolism of EFAs. Excess of LA may reduce the synthesis of ALA metabolites, such as eicosapentaenoic acid (EPA, 20:5 ω-3). Products of metabolism of each EFA, obtained through elongation or desaturation, are always considered part of the same family of their precursor. Therefore, EPA and docosahexaenoic acid (DHA, 22:6 ω-3) are part of the ALA family. Similarly, the LA family includes arachidonic acid (ARA, 20:4 ω-6), which is a product of LA metabolism[2,3,4].

The LC-PUFAs play several metabolic and physiological roles. One role is as structural components of biological membranes, being capable of modifying membrane fluidity and so influencing signal transduction and transcription regulation by the balance on eicosanoid synthesis[2,5].

Infants are not able to produce LC-PUFAs from their precursors due to liver immaturity and should have their requirement supplied by the maternal milk. Human maternal milk contains three times the amount of ARA and DHA present in cow milk, the last one consequently not being appropriate for babies. Yet, when breast-feeding is not possible, the use of infant formula (IF) is presented as an alternative for baby feeding. Despite the advances in technological process, IFs still retain great differences in composition when compared with maternal milk[6,7]. In order to lessen this difference, IFs have been supplemented with LC-PUFAs in the USA since 2002. In Brazil, LC-PUFA- supplemented IFs have been commercialized since the beginning of 2008[8].

LC-PUFAs are exceptionally essential for premature babies having low lipid reserves. Because of their limited caloric reserve, premature babies have to mobilize part of the LC-PUFAs to support their caloric requirement when exogenous intake is inadequate. Besides, premature babies have a nutritional deficit because they do not receive the intra-uterine supplement of ARA and DHA, which occurs in the late phase of gestation. Such nutritional deficiency may contribute to inadequate growth, dermatitis, and a higher susceptibility to infections, among other disorders[7,9,10].

The balance between ω-3 and ω-6 FA families is important for the maintenance of health and normal development. As the participation of ω-6 FAs grows on occidental diets, ARA-derived metabolites, i.e., eicosanoids, tend to be formed in a greater amount than metabolites derived from the ω-3 FA family, specifically EPA. ARA-derived eicosanoids are normally biologically active in very small amounts. If they are formed in a large proportion, they may contribute to the formation of blood clots and atheromata, advent of inflammatory and allergic disorders, and cellular proliferation. The ω-6:ω-3 proportion should be preserved at 5:1 to maintain equilibrium in the formation of eicosanoids and the correspondent neurotransmitters and prostaglandins, which are vital for normal cerebral function. For infants, the ratio between ω-6 and ω-3 FAs may vary from 5:1 to 15:1[11,12,13].

The *Codex Alimentarius* stan 72[14] presents the identity standard for IFs, with recommended contents for total lipids and PUFAs. Some definitions of the standard are described below:

> *Infant formula means a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding.*
>
> *Follow-up infant formula consists of any product, in liquid or powdered form, used as substitute for maternal milk to feed babies after the sixth month of age, when prescribed, and infants.*

In Table 1, the reference values of lipids and FAs in IFs for infants are presented, according to *Codex Alimentarius* stan 72[14].

The objective of this work was to quantify the contents of total fat and PUFAs in IFs commercialized in the State of São Paulo (Brazil) and evaluate them with respect to the Normative references of the *Codex Alimentarius* stan 72[14].

MATERIAL AND METHOD

Samples

Fourteen IF samples were analyzed. Seven were commercial IFs indicated to 0- to 6-month-old babies (*IF 1, IF 3, IF 4, IF 6, IF 8, IF 11, and IF 13*); five were IFs for 6- to 12-month-old babies (*IF 2,*

Table 1. Recommendations of lipids and fatty acids in infant formula for infants, according to *Codex Alimentarius* stan 72[14].

	Minimum value	Maximum value	Guidance upper level (GUL)
Total fat (TF) (g. 100 kJ^{-1})	1.05	1.4	
Linoleic Acid (LA) (mg.100 kJ^{-1})	70	-	330
α-Linolenic Acid (ALA) (mg.100 kJ^{-1})	12	-	
Ratio LA : ALA	5:1	15:1	
Docosahexaenoic Acid (DHA)	-	0.5% of total fatty acid content	

IF 5, IF 7, IF 12, and IF 14) and two were IFs for premature babies (*IF 9 and IF 10*). All the analyzed IFs were based on cow milk. On the word of the manufacturers, samples *IF 1, IF 2, IF 9, IF 10,* and *IF 13* were supplemented with ARA and DHA, and sample *IF 14* was supplemented only with DHA. The analyzed IFs were obtained from markets in the State of São Paulo and they were produced in Brazil, México, the Netherlands, and Argentina. The formulations of the analyzed IFs displayed several sources of lipids, the most common being skimmed milk, palm oil, sunflower oil, soybean oil, coconut oil, canola oil, corn oil, and fish oil, among others.

Reagents and standards

Solvents and reagents used at the fat extraction and methyl ester preparation stages were of analytical grade: petroleum ether, ethyl ether, 95% ethanol, NH_4OH, KOH, Na_2SO_4, and NaOH. *n*-Hexane and methanol were of chromatographic grade.

Two methyl esters of FAs 13:0 and 23:0 (Sigma, high-purity grade) were used as internal standards for the chromatographic analyses.

In order to identify the components of the IF samples, two mixtures of FA methyl esters and methyl esters of individual FAs were used. The first mixture consisted of certified amounts of methyl esters of 37 different FAs, varying from 4:0 to 24:0 (Supelco Inc. Bellefonte, PA, USA). The second was a mixture of α-linolenic (ALA – 18:3) and *cis-trans* isomers of LA (LA – 18:2) (Sigma Chemical Co, St Louis, MO, USA). Individual FA standards (Sigma Chemical Co, St Louis, MO USA) were as follows: elaidic acid (18:1 9*t*); vaccenic acid (18:1a 11*c); trans*-vaccenic acid (18:1 11*t*, 18:1 7*c*, and 18:1 12*c*); conjugated LAs (CLA – 18:2 9*c*,11*t*, and 18:2 10*t*,12*c*); palmitoelaidic acid (16:1 9*t*), palmitic acid (16:0); linolelaidic acid (18:2 9*t*,12*t*); EPA (20:5 5*c*, 8*c*, 11*c*, 14*c*, and 17*c*); ARA (20:4 5*c*, 8*c*, 11*c*, and 14*c*); and DHA (20:6 4*c*, 7*c*, 10*c*, 13*c*, 16*c*, and 19*c*).

Identification and quantification of lipids and PUFAs

The extraction and quantification of lipids from the IF samples were performed as described by Roese Gottielb, following the official method for this type of food in accordance with the *Method Compendium of the Association of Official Analytical Chemistry*[15]. The methyl ester derivatization of FAs was performed in accordance with the procedure initially described by Hartman and Lago[16] and subsequently modified by Maia and Rodrigues-Amaya[17]. Methyl esters were separated on a fused silica capillary column with a cyanopropyl polysiloxane stationary phase (SP™-2560, 100 m x 0.25 mm inner diameter, 0.20 µm film thickness; Supelco Inc., Bellefonte, PA, USA), in a Shimadzu gas chromatograph (GC 17A model) with a flame ionization detector (FID), under temperature and pressure conditions described by Kramer et al.[18] The separated components were identified by coinjection of standards and subsequent comparison of absolute and relative retention times in relation to the internal standard. Quantification of PUFAs was accomplished by means of addition of an internal standard, the methyl ester of FA 23:0, and theoretical response correction factors of the FID in comparison to the internal standard, according to the proposed methodology by Kus et al[19].

All samples were analyzed in triplicate, and the results are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Quantification values for the amounts of lipids, LA, ALA, ARA, and DHA present in commercial IF samples are displayed in Table 2.

Among the analyzed IFs for 0- to 6-month-old babies and those for 6- to 12-month-old babies, considering the values in reference to the *Codex Alimentarius*[14], namely, a minimum of 1.05 g/100 kJ and a maximum of 1.4 g/100 kJ, no IF had total lipid values within its suggested contents range.

Similar case was verified by Zunin et al.[20]. They analyzed 32 Ifs in Italy, and the values varied between 0.796 g·kJ^{-1} to 0.606 g·kJ^{-1}, with an average of 0.693 g·kJ^{-1}. As observed in this present work, the contents were lower than the levels recommended by the *Codex Alimentarius* (2007).

Considering the values for LA content, all IFs complied with the recommended ranges of Normative *Codex Alimentarius*[14], i.e., LA content greater than 70 mg·100 kJ^{-1}. Only six samples

Table 2. Quantification values for lipids, linoleic acid (LA), α-linolenic acid (ALA), arachidonic acid (ARA), and docosahexaenoic acid (DHA) present in commercial infant formula samples.

Sample	Lipids (g·100 kJ^{-1})	LA (mg·100 kJ^{-1})	ALA (mg·100 kJ^{-1})	ARA (% fatty acids)	DHA (% fatty acids)
IF1	0.721 ± 0.017	126.8 ± 5.2	12.73 ± 0.12	0.545 ± 0.005	0.270 ± 0.004
IF2	0.541 ± 0.015	104.9 ± 8.6	10.02 ± 0.84	0.665 ± 0.039	0.320 ± 0.009
IF3	0.522 ± 0.008	87.5 ± 6.2	10.22 ± 0.79		
IF4	0.534 ± 0.001	138.1 ± 2.7	8.71 ± 0.11		
IF5	0.445 ± 0.001	137.6 ± 5.2	7.39 ± 0.38		
IF6	0.646 ± 0.006	96.2 ± 8.7	9.50 ± 0.07		
IF7	0.533 ± 0.018	75.3 ± 2.3	7.96 ± 0.02		
IF8	0.513 ± 0.024	79.7 ± 5.3	9.10 ± 0.60		
IF9	0.612 ± 0.046	102.0 ± 0.8	8.20 ± 0.05	0.122 ± 0.005	0.307 ± 0.077
IF10	0.654 ± 0.003	76.0 ± 1.3	8.15 ± 0.42	0.382 ± 0.008	0.287 ± 0.002
IF11	0.594 ± 0.005	101.3 ± 8.2	10.72 ± 0.35		
IF12	0.444 ± 0.003	66.8 ± 7.7	1.94 ± 0.24		
IF13	0.654 ± 0.072	97.6 ± 8.9	9.70 ± 0.87	0.180 ± 0.002	0.179 ± 0.002
IF14	0.510 ± 0.042	75.8 ± 0.9	8.89 ± 0.16	0.015 ± 0.004	0.153 ± 0.004

Mean ± SD (triplicate); *IF1, IF3, IF4, IF6, IF8, IF11,* and *IF13*: IFs recommended for infants 0-6 months old; *IF2, IF5, IF7, IF12,* and *IF14*: continued IFs recommended for infants 6–12 months old; *IF9 and IF10*: IFs recommended for premature babies.

displayed values around the established inferior limit. The *Codex Alimentarius* defines a guidance upper level (GUL) content of 330 mg/100 kJ because high LA ingestion can induce undesirable effects on lipoprotein metabolism, immune response, eicosanoid balance, and oxidative stress[21]. Riva et al.[22] verified, in a study comprising 16 IF samples, that LA content values were in accordance with the recommendation by the European Union[23], which correspond to the values of the *Codex Alimentarius*[14].

Out of the 14 analyzed IF samples, just 1 (*IF 1*) had an ALA content value above the advised minimum limit of 12 mg/100 kJ. The remaining 13 displayed values up to 75% lower than the limit. ALA is an indispensable FA, because it is the precursor for DHA synthesis, and its minimum intake is important for infant development[2,21]. A study conducted by Straarup et al.[24] in Denmark demonstrated that in only 4 out of 28 analyzed IFs, ALA levels were above the minimum advised by that country's legislation. The maximum content of ALA is regulated by the LA-to-ALA ratio. Data on such ratios for the analyzed commercial IFs are presented in Figure.

Among all the analyzed IFs, one for 0- to 6-month-old babies (*IF 4*) and two for 6- to 12-month-old babies (*IF 5* and *IF 12*) had LA-to-ALA ratios that were not within the advised range, above the upper limit. Straarup et al.[24] discovered similar results: two IFs had LA-to-ALA ratios of 17:1 and 55:1. Riva et al.[22], however, did not obtain any values nonconforming to the recommended range because they performed only analysis of the nutritional information. Some studies alert us to the fact that high concentrations of LA inhibit the synthesis of DHA from ALA[6,24].

In reference to the *Codex Alimentarius* stan-72[14], when an IF is supplemented with LC-PUFA, it should have no more than 0.5% total FA content of DHA. A similar value should be attributed to

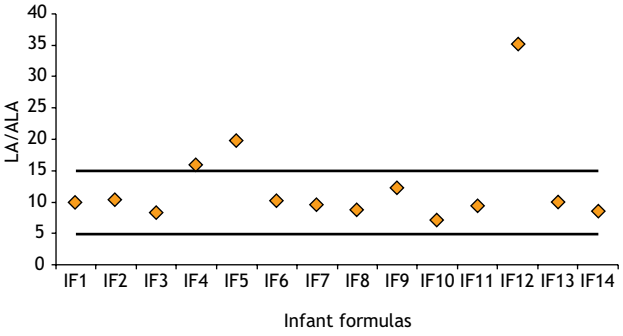

Figure. LA-to-ALA ratios in commercial IFs compared to the limits defined by the *Codex Alimentarius* stan-72 (2011). ◊: LA/ALA; limits advised by the *Codex Alimentarius*.

Table 3. Number of infant formulas in agreement and disagreement with the *Codex Alimentarius* stan-72 (2011), in terms of the content of lipids, linoleic acid (LA), α-linolenic acid (ALA), arachidonic acid (ARA), docosahexaenoic acid (DHA), and linoleic-to-α-linolenic acid ratio (LA : ALA).

Parameter	Agreement	Disagreement	Total
Lipids	0	14	14
LA	14	0	14
ALA	1	13	14
ARA	2	3	5
DHA	6	0	6
LA: ALA	11	3	14

ARA. In case of supplementation with EPA, its content should not surpass the DHA content.

Among the 14 analyzed commercial IF samples, only 6 (*IF 1, IF 2, IF 9, IF 10, IF 13,* and *IF 14*) were supplemented with LC-PUFAs. Three (50%) – *IF 1, IF 2* and *IF 10* – displayed a higher content of ARA in comparison to that of DHA. In two samples (*IF 9* and *IF 14*), the ARA content was lower than the DHA content. Only in sample *IF 13*, the values for both LC-PUFAs were very similar to those advised.

Therefore, when the 14 studied IFs were considered in relation to the contents of lipids, LA, ALA, LA-to-ALA ratio, ARA, and DHA in relation to Normative *Codex Alimentarius* stan-72[14], all presented at least one analyte that was not within the advised range. In Table 3, the number of commercial IFs in agreement or disagreement with *Codex Alimentarius* stan-72[14] is shown for each of the analyzed parameters.

It should be noted that the samples of IFs were analyzed in 2008, and comparisons were made with respect to *Codex Alimentarius* standards revised in 2007, which is the current one. In Brazil, in 2011, a new legislation[25,26] on the composition of IF became effective, contemplating the standards of the *Codex Alimentarius*[14]. Despite the fact that maternal milk is the best feed for a newborn, the use of IF is a common occurrence in Brazil, and control of its entire nutritional contents, not only for lipid contents, should be intensified because adequate child development depends mainly on feeding with appropriate nutrient-bearing foods.

CONCLUSION

All the IFs analyzed in this study, which are sold in the State of São Paulo, have at least one lipid compound (total lipid or PUFA) in disagreement with the *Codex Alimentarius* standard. Commercial IFs should be continuously monitored in regard to lipid and FA contents, beside other components, as recommended by legislation, while the use of such products becomes frequent and their nutritional quality can influence child development.

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