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

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


PALAVRAS-CHAVES: Legislação; Ácidos Graxos Poli-Insaturados; Fórmula Infantil; Codex Alimentarius
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 diseases1-4.

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 interfere 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 metabolism2,3,4.

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 synthesis5-7.

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 milk8-10. 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 20088.

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 disorders9,10.

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 prosta-glandins, which are vital for normal cerebral function. For infants, the ratio between ω-6 and ω-3 FAs may vary from 5:1 to 15:111,12,13.

The Codex Alimentarius stan 72* 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*.

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

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, IF 5, IF 7, IF 9, and IF 10); and two were IFs for 6- to 12-month-old babies (IF 12, IF 14). The results are presented in Table 1.

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

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Minimum value (g. 100 kJ-1)</th>
<th>Maximum value (g. 100 kJ-1)</th>
<th>Guidance upper level (GUL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat (TL)</td>
<td>1.05</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Linoleic Acid (LA)</td>
<td>70</td>
<td>-</td>
<td>330</td>
</tr>
<tr>
<td>α-Linolenic Acid (ALA)</td>
<td>12</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ratio LA : ALA</td>
<td>5:1</td>
<td>15:1</td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoic Acid (DHA)</td>
<td>-</td>
<td>0.5% of total fatty acid content</td>
<td></td>
</tr>
</tbody>
</table>
IF 5, IF 7, IF 12, and IF 14) and two were IFS for premature babies (IF 9 and IF 10). All the analyzed IFSs 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 IFSs 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 IFSs 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, NH₄OH, KOH, Na₂SO₄, 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 IFS 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 11c); trans-vaccenic acid (18:1 11t, 18:1 7c, and 18:1 12c); conjugated LAs (CLA - 18:2 9c,11t, and 18:2 10r,12c); palmitoleaacid (16:1 9t), palmitic acid (16:0); linoleaacid (18:2 9t,12c); EPA (20:5 5c, 8c, 11c, 14c, and 17c); ARA (20:4 5c, 8c, 11c, and 14c); and DHA (20:6 4c, 7c, 10c, 13c, 16c, and 19c).

Identification and quantification of lipids and PUFAs

The extraction and quantification of lipids from the IFS 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". The methyl ester derivatization of FAs was performed in accordance with the procedure initially described by Hartman and Lago16 and subsequently modified by Maia and Rodrigues-Amaya17. Methyl esters were separated on a fused silica capillary column with a cyanopropyl polysiloxane stationary phase (SP-AQ-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 IFS samples are displayed in Table 2.

Among the analyzed IFSs for 0- to 6-month-old babies and those for 6- to 12-month-old babies, considering the values in reference to the Codex Alimentarius20, namely, a minimum of 1.05 g/100 kJ and a maximum of 1.4 g/100 kJ, no IFS had total lipid values within its suggested contents range.

Similar case was verified by Zunin et al.21. 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 IFSs complied with the recommended ranges of Normative Codex Alimentarius20, i.e., LA content greater than 70 mg·100 kJ⁻¹. Only six samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lipids (g/100 kJ⁻¹)</th>
<th>LA (mg/100 kJ⁻¹)</th>
<th>ALA (mg/100 kJ⁻¹)</th>
<th>ARA (% fatty acids)</th>
<th>DHA (% fatty acids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IF1</td>
<td>0.721 ± 0.017</td>
<td>126.8 ± 5.2</td>
<td>12.73 ± 0.12</td>
<td>0.549 ± 0.005</td>
<td>0.270 ± 0.004</td>
</tr>
<tr>
<td>IF2</td>
<td>0.541 ± 0.015</td>
<td>104.9 ± 8.6</td>
<td>10.02 ± 0.84</td>
<td>0.665 ± 0.039</td>
<td>0.320 ± 0.009</td>
</tr>
<tr>
<td>IF3</td>
<td>0.522 ± 0.008</td>
<td>87.5 ± 6.2</td>
<td>10.22 ± 0.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF4</td>
<td>0.534 ± 0.001</td>
<td>138.1 ± 2.7</td>
<td>8.71 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF5</td>
<td>0.445 ± 0.001</td>
<td>137.6 ± 5.2</td>
<td>7.39 ± 0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF6</td>
<td>0.646 ± 0.006</td>
<td>96.2 ± 8.7</td>
<td>9.50 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF7</td>
<td>0.533 ± 0.018</td>
<td>75.3 ± 2.3</td>
<td>7.96 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF8</td>
<td>0.513 ± 0.024</td>
<td>79.7 ± 5.3</td>
<td>9.10 ± 0.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF9</td>
<td>0.612 ± 0.046</td>
<td>102.0 ± 0.8</td>
<td>8.20 ± 0.05</td>
<td>0.122 ± 0.005</td>
<td>0.307 ± 0.077</td>
</tr>
<tr>
<td>IF10</td>
<td>0.654 ± 0.003</td>
<td>76.0 ± 1.3</td>
<td>8.15 ± 0.42</td>
<td>0.382 ± 0.008</td>
<td>0.287 ± 0.002</td>
</tr>
<tr>
<td>IF11</td>
<td>0.594 ± 0.005</td>
<td>101.3 ± 8.2</td>
<td>10.72 ± 0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF12</td>
<td>0.444 ± 0.003</td>
<td>66.8 ± 7.7</td>
<td>1.94 ± 0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF13</td>
<td>0.654 ± 0.072</td>
<td>97.6 ± 8.9</td>
<td>9.70 ± 0.87</td>
<td>0.180 ± 0.002</td>
<td>0.179 ± 0.002</td>
</tr>
<tr>
<td>IF14</td>
<td>0.510 ± 0.042</td>
<td>75.8 ± 0.9</td>
<td>8.89 ± 0.16</td>
<td>0.015 ± 0.004</td>
<td>0.153 ± 0.004</td>
</tr>
</tbody>
</table>

Mean ± SD (triplicate); IF1, IF3, IF4, IF6, IF8, IF11, and IF13: IFSs recommended for infants 0-6 months old; IF2, IF5, IF7, IF12, and IF14: continued IFSs recommended for infants 6-12 months old; IF9 and IF10: IFSs 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, eosinocaid balance, and oxidative stress\(^1\). Riva et al.\(^2\) verified, in a study comprising 16 IF samples, that LA content values were in accordance with the recommendation by the European Union\(^3\), which correspond to the values of the Codex Alimentarius\(^4\).

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.\(^2\), 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\(^\text{(2011)}\), when an IF is supplemented with LC-PUFAs, it should have no more than 0.5% total FA content of DHA. A similar value should be attributed to

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Agreement</th>
<th>Disagreement</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids</td>
<td>0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>LA</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>ALA</td>
<td>1</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>ARA</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>DHA</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>LA: ALA</td>
<td>11</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

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\(^\text{(2011)}\), 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\(^\text{(2011)}\) 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\(^8,24\) on the composition of IF became effective, contemplating the standards of the Codex Alimentarius\(^\text{(2011)}\). 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.

**REFERENCES**


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