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# Influence of storage conditions in evolution of fatty acids profile from infant formula

**Cristina Semeniuc, Mihaela A. Rotar, C. Guş, C. Bele, Sonia A. Socaci, C. Laslo**

*Sciences and Veterinary Medicine, Faculty of Agriculture, 3-5 Mănăştur str., 400372 Cluj-Napoca, Romania*

***Abstract***

In this study was followed how storage temperature and storage time affect fatty acids profile of infant formula. Storage conditions chosen were: room temperature and 15°C. Samples of infant formula were deposited during 18 months, which correspond to infant formula shelf life; the samples were analyzed at obtaining and after 3, 6, 9, 12, 15, and 18 months of storage at conditions mentioned before. For statistical analysis were used One-way ANOVA with Tukey's Multiple Comparison post Test (for the effect of time on fatty acids profile during storage) and with Paired t Test (for the effect of temperature).

***Keywords***: Infant formula, Storage, Fatty Acids Profile, Temperature, Shelf life.

## 1. Introduction

Infant formula is a product based on milk of cows or other animals and/or other edible constituents of animal (including fish) or plant origin, which have been proved to be suitable for infant feeding. The product shall contain PUFA (expressed as linoleic acid) at a level of not less than 300 mg per 100 available calories [7].

Lipids comprise an important part of infant formula; they are a source of n-3 and n-6 fatty acids necessary for the intestinal absorption of fat-soluble vitamins [6].

Milk is pored in PUFA and therefore infant formula is usually supplemented with oils rich in these fatty acids, but in the same time makes it vulnerable to oxidation [3, 5].

The extent of oxidation can be measured beside of peroxide value and analysis of hexanal also by the loss of substrate (PUFA) [4].

PUFAs, in particular, are easily attacked by free radicals that react with their double bonds, thereby yielding products, such as short-chain aldehydes. The major susceptibility of fatty acids to oxidation is thought to be directly dependent on their degree of unsaturation [1].

Being a dehydrated product the shelf life is between 1.5 and 3 years, depending on storage conditions and packaging. Since in retail trade (stores, pharmacies and supermarket) it is stored at room temperature, the fatty acids can be oxidized, giving rise to a loss of nutritive value, the generation of highly toxic compounds (peroxides, aldehydes and ketones) and also the appearance of off-flavor.

The aim of this study was to follow the effect of storage temperature and storage time on fatty acids profile of infant formula during the shelf life by monitoring the fatty acids losses.

## 2. Materials and Method

*The Sampling and storage*

Infant formula is an instant dry dairy product for healthy infants use (0-12 months), obtained by spray-drying of a mix that contains cow milk, demineralised

* Corresponding author: *e-mail address*: cristinasemeniuc@yahoo.com

whey, vegetable oil, vitamins and minerals in a such proportion that assures similarity with human milk. The product has a high content of fat (24%).

Samples were analyzed at obtaining (0) and after 3, 6, 9, 12, 15 and 18 months of storage in different temperature conditions: room temperature and 15°C (which is the maximum temperature admitted by the Romanian standard).

IF(0) was the symbol for infant formula analyzed after obtaining and IF1(3)… IF1(18) for infant formula stored at room temperature during 3…18 months.

***Fatty acids analysis***

Fatty acid profiles were obtained by GC analysis after derivatisation to FAMEs. Fat from IF samples were extracted after modified Folch method, 1957.

For the fatty acid analysis, a GC-17 A Shimadzu gas chromatograph equipped with a flame ionization detector was used. The fatty acid methyl esters (FAMEs) were separated on a capillary column AT-WAX (Alltech) of 30 m with 0.25 mm I.D., and coated with polyethylene glycol of 0.20 µm film thickness. The carrier gas was Helium at a pressure of 147 kPa. Injection was in split mode, with a split ratio of 1:28. The injection volume was 1 µL.

The injector and detector temperatures were kept at 260°C. The temperature program was as follows: initial temperature 70°C (holding for 2 min.), increasing at 10°C/min. until 150°C (holding for 3 min.), and then increasing at 4°C/min until 235°C (holding for 5 min.). Data acquisition and processing were performed with the Shimadzu software for GC.

Fatty acids profile was determined at obtaining (0) and after 3, 6, 9, 12, 15 and 18 months.

***Statistical analysis***

Statistical interpretation of data was performed with One-way ANOVA with Tukey's Multiple Comparison post Test (for the effect of time during storage) and with Paired t Test (for the effect of temperature during storage), using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com".

## 3. Results and Discussion

The major saturated fatty acid (SFA) was C16:0, in the range 26.04–26.26 %; the major monounsaturated fatty acid (MUFA) was C18:1 *n*-9 in the range 24.72–25.46 %; and the major LC-PUFA was C18:2 *n*- 6 in the range 13.88–14.68 %.

A small difference was noticed between fatty acids over time (Table 1) and similar with the study of Chávez-Servín et al., 2009 [2]. Only two fatty acids studied exhibited significant decreases at the end of the study, the first being C18:2 *n*-6 (from 14.68 % to 13.88 %) and the second C18:3 *n*-3 (from 1.05 % to 1.02 %).

When the effect of storage temperature was followed, respective storage time between the entire fatty acids profile of infant formula stored at room temperature and infant formula stored at 15°C, the differences registered were not significant ($P>0.05$).

Figure 1 shows unsaturated fatty acid (UFA) losses for stored IF. When UFA losses were plotted against storage time, the following correlation coefficients were achieved: -0.952 for $IF_1$, and -0.912 for $IF_2$. This indicates that unsaturated fatty acids decrease with storage time.

UFA losses in IF occurred in both studied conditions, especially at room temperature storage. Major UFA losses were found in samples stored for 18 months at room temperature.

The correlation coefficients between UFA/SFA ratio and storage time (-0.9541 for $IF_1$, and -0.9080 for $IF_2$), between PUFA/MUFA ratio and storage time (-0.9937 for $IF_1$ and -0.9941 for $IF_2$) and between C18:2/C18:3 ratio and storage time (-0.9394 for $IF_1$, and -0.9591 for $IF_2$) were also calculated.

**Table 1.** Evolution of fatty acids profile during storage

| Fatty Acid | $IF_{(0)}$ | $IF_{1(3)}$ | $IF_{2(3)}$ | $IF_{1(6)}$ | $IF_{2(6)}$ | $IF_{1(9)}$ | $IF_{2(9)}$ | $IF_{1(12)}$ | $IF_{2(12)}$ | $IF_{1(15)}$ | $IF_{2(15)}$ | $IF_{1(18)}$ | $IF_{2(18)}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | | | | | | | | | | | | |
| C8:0 | 0.52 | 0.53 | 0.52 | 0.54 | 0.53 | 0.55 | 0.54 | 0.55 | 0.54 | 0.56 | 0.55 | 0.56 | 0.55 |
| C10:0 | 1.86 | 1.87 | 1.86 | 1.87 | 1.86 | 1.88 | 1.87 | 1.89 | 1.87 | 1.89 | 1.88 | 1.91 | 1.89 |
| C12:0 | 2.98 | 2.98 | 2.98 | 2.99 | 2.98 | 3.00 | 2.99 | 3.00 | 2.99 | 3.01 | 3.00 | 3.02 | 3.01 |
| C14:0 | 8.66 | 8.66 | 8.66 | 8.67 | 8.66 | 8.67 | 8.66 | 8.68 | 8.67 | 8.68 | 8.67 | 8.69 | 8.68 |
| C15:0 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.29 | 1.28 | 1.29 | 1.28 | 1.28 |
| C16:0 | 26.26 | 26.24 | 26.26 | 26.21 | 26.26 | 26.16 | 26.21 | 26.13 | 26.16 | 26.07 | 26.11 | 26.04 | 26.08 |
| C16:1 | 1.19 | 1.19 | 1.19 | 1.19 | 1.18 | 1.19 | 1.18 | 1.19 | 1.18 | 1.18 | 1.17 | 1.16 | 1.15 |
| C17:0 | 1.03 | 1.03 | 1.03 | 1.03 | 1.03 | 1.03 | 1.03 | 1.04 | 1.04 | 1.04 | 1.04 | 1.04 | 1.04 |
| C18:0 | 11.46 | 11.46 | 11.46 | 11.48 | 11.46 | 11.50 | 11.48 | 11.55 | 11.53 | 11.62 | 11.59 | 11.65 | 11.63 |
| C18:1 *n*-9 | 24.72 | 24.92 | 24.82 | 25.01 | 24.92 | 25.11 | 25.02 | 25.28 | 25.21 | 25.37 | 25.29 | 25.46 | 25.39 |
| C18:1 is *n*-7 | 4.30 | 4.30 | 4.30 | 4.30 | 4.30 | 4.30 | 4.30 | 4.29 | 4.30 | 4.29 | 4.30 | 4.29 | 4.29 |
| C18:2 *n*-6 | 14.68 | 14.48 | 14.58 | 14.38 | 14.48 | 14.28 | 14.38 | 14.08 | 14.18 | 13.98 | 14.08 | 13.88 | 13.98 |
| C18:3 *n*-3 | 1.05 | 1.05 | 1.05 | 1.04 | 1.05 | 1.04 | 1.05 | 1.03 | 1.04 | 1.03 | 1.03 | 1.02 | 1.03 |
| TOTAL | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| UFA | 45.94 | 45.94 | 45.94 | 45.92 | 45.93 | 45.92 | 45.93 | 45.87 | 45.91 | 45.85 | 45.87 | 45.81 | 45.84 |
| SFA | 54.06 | 54.06 | 54.06 | 54.08 | 54.07 | 54.08 | 54.07 | 54.13 | 54.09 | 54.15 | 54.13 | 54.19 | 54.16 |
| MUFA | 30.21 | 30.41 | 30.31 | 30.50 | 30.40 | 30.60 | 30.50 | 30.76 | 30.69 | 30.84 | 30.76 | 30.91 | 30.83 |
| PUFA | 15.73 | 15.53 | 15.63 | 15.42 | 15.53 | 15.32 | 15.43 | 15.11 | 15.22 | 15.01 | 15.11 | 14.90 | 15.01 |
| PUFA/MUFA | 0.5207 | 0.5107 | 0.5157 | 0.5056 | 0.5109 | 0.5007 | 0.5059 | 0.4912 | 0.4959 | 0.4867 | 0.4912 | 0.4820 | 0.4869 |
| C18:2/C18:3 | 13.9810 | 13.7905 | 13.8857 | 13.8269 | 13.7905 | 13.7308 | 13.6952 | 13.6699 | 13.6346 | 13.5728 | 13.6699 | 13.6078 | 13.5728 |
| UFA/SFA | 0.8498 | 0.8498 | 0.8498 | 0.8491 | 0.8495 | 0.8491 | 0.8495 | 0.8474 | 0.8488 | 0.8467 | 0.8474 | 0.8454 | 0.8464 |

UFA – Unsaturated Fatty Acids
SFA – Saturated Fatty Acids
MUFA – Monounsaturated Fatty Acids
PUFA – Polyunsaturated Fatty Acids

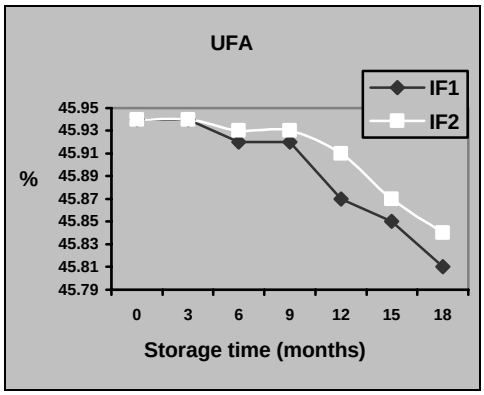


**Figure 1.** Unsaturated fatty acid losses during storage

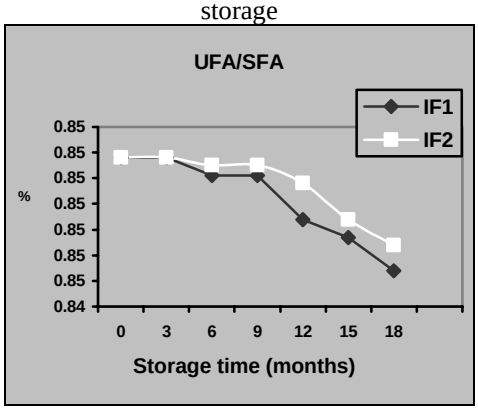


**Figure 2.** UFA/SFA ratio during storage

These indicate a decrease of ratios between UFA/SFA (fig. 2), PUFA/MUFA (fig. 3) and respective C18:2/C18:3 (fig. 4) with storage time.

The higher decreases for UFA/SFA ratio, respective PUFA/MUFA ratio are in the case of $IF_1$.

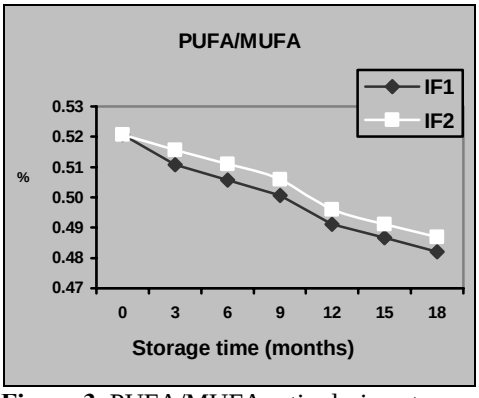


**Figure 3.** PUFA/MUFA ratio during storage

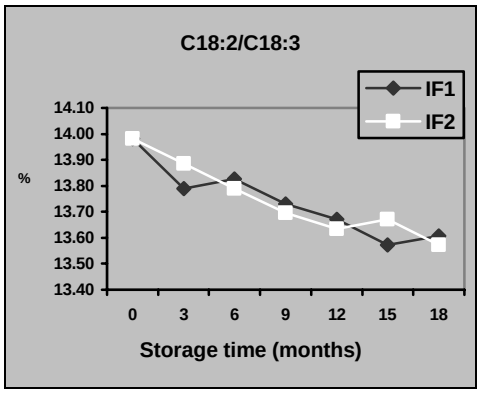


**Figure 4.** C18:2/C18:3 ratios during storage

## 4. Conclusion

Unsaturated fatty acids decrease with storage time. C18:2 *n*-6 and C18:3 *n*-3 were the fatty acids which registered the biggest losses. Being unsaturated fatty acids, they were the most susceptible to oxidation and this is directly dependent on their degree of unsaturation.

Fatty acids profile was not significantly affected either by the storage temperature, or by the storage time. This may be a result of the small differences between room temperature (average of 25°C) and storage temperature recommended by the Romanian standard (15°C) chosen in this study.

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