Effect of Storage Time on the Oxidative Status of Infant Formula

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Abstract: The aim of this study is to assess the oxidative status evolution of an infant formula product stored at 15°C during and after the shelf life. The initially phase of oxidation was monitored by the peroxide value (PV) and finally stage of oxidation by the thiobarbituric reactive substances (TBARS) test. The storage time has no significant effect on peroxide value (PV) and thiobarbituric acid reactive substances (TBARS).

Keyword: infant formula, oxidation, storage, PV, TBARS, MDA.

INTRODUCTION

Oxidation is the reaction of unsaturated lipids (free fat or free fatty acids) with molecular oxygen. In the first stage of the reaction, free radicals are formed and yield to hydroperoxides, which further react (Celestino et al., 1997). These primary products in turn rapidly decompose, yielding a complex mixture of secondary lipid oxidation products (mainly aldehydes and ketones) such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) (Fernández, et al., 1997 cited by Manglano et al., 2005). Determination of peroxide value is based on oxidation of ferrous ions in an acidic medium and colorimetric detection of ferric ions as ferric thiocyanate, a red-violet complex of strong absorption at 500-510 nm (Dobarganes and Velasco, 2002).

TBARS measures secondary lipid oxidation products, which are also responsible for the rancid taste during storage (Decker et al., 2000). This procedure measures the MDA formed as the split product of an endoperoxide of unsaturated fatty acids resulting from oxidation of a lipid substrate (Fernández et al., 1997 cited by Antolovich et al., 2002). The MDA is reacted with thiobarbituric acid (TBA) to form a pink pigment (TBARS) that is measured spectrophotometrically at its absorption maximum at 532–535 nm (Guillén-Sans and Guzmán-Chozas 1998). Fatty acids with three or more double bonds produce malondialdehyde (MDA), a major product of lipid autoxidation (Janero, 1990 cited by Guy et al., 2006). MDA is the most important secondary product of auto-oxidation (Cesa, 2004). Thus, MDA is commonly used to monitor food and milk products oxidation (Fenaille et al., 2001).

Traditionally, the absorbance at 532 nm of the pink pigment formed in the reaction is measured, even though only a slight amount of this pigment is formed in milk products due to their low content of polyunsaturated lipids. This led Patton and Kurtz (1955) and Jennings et al. (1955) to propose measurement of the more intensively absorbing yellow pigment at 450 nm for dairy products. Since TBARS is a measure of the formation of secondary oxidation products, e.g. carbonyls, being responsible for the sensory impact of lipid oxidation (Hall and Andersson, 1985), this method has been found to correlate well with sensory quality (Stapelfeldt et al., 1992).
Oxidation increases with storage time and, thus, damages the quality of milk powders (Halliwell et al., 1999, cited by Mäkinen, 2002).

The objective of the present study was to analyze the effect of storage time in formation and evolution of primary and secondary oxidation products from infant formula during and after the shelf life. The temperature of storage was 15°C, recommended by producer. Primary oxidation products were assessed by the peroxide value (PV) and secondary oxidation product by the thiobarbituric reactive substances (TBARS).

MATERIAL AND METHODS

The infant formula type taken in study is designed for children’s between 0 and 12 months, contains 13.2% proteins, 26.5% lipids, 55.4% carbohydrates and is supplemented with vitamins and minerals; the shelf life of the product stored at temperature of 15°C is 18 months. The samples were analysed at 3rd, 4th, 5th, 7th, 8th, 15th, 16th, 17th, 18th, 19th, 21st, 28th, 31st, 32nd and 35th months of storage. Beginning with the 19th month of storage the samples were over shelf life.

PV value assessment was done by the protocol described in ISO 3976|IDF 74:2006 and TBARS by the method of King (1962) and modified by Stapelfeldt et al. (1997). PV was determined from the infant formula fat extracted through the method with pentane described by Alabalá-Hurtado et al. (1999) and expressed as mEq O₂/Kg fat. TBARS was readied at 450 nm and 532 nm respectively, and expressed both as absorbance/weight and nM MDA/mL reconstituted milk. For each sample, TBARS number and PV were done in duplicate.

RESULTS AND DISCUSSIONS

![Graph](image)

Fig. 1 represents the evolution of peroxide value in infant formula during storage at the temperature of 15°C in the period and after the validity of the infant formula

The biggest peroxide value was registered in the 8th month of storage (28.73 mEq O₂/Kg fat±0.13) and the lower in the 21st month of storage (14.79 mEq O₂/Kg fat±0.47). From the 3rd until the 8th month of storage can be observed an increase of peroxide value, but not a
significant (P>0.05) one. After the 8th month of storage this parameter registered an irregular behaviour in which increasing and decreasing periods alternate. Those variations can be explained by the fact that peroxides are primary oxidation products; they are instable and quickly change in secondary oxidation products. Fig. 2 and Fig. 3 represent the evolution of TBARS$_{450}$ and TBARS$_{532}$ expressed as absorbance/weight and TBARS expressed as nM MDA/mL reconstituted milk in infant formula on the same product and the same conditions.

The biggest TBARS$_{532}$ number expressed as absorbance/weight was registered in the 8th month of storage (0.230±0.004) and the lowest in the 7th month of storage (0.032±0.001).
The difference between the higher and the smaller value of TBARS\textsubscript{532} expressed as nM MDA/mL reconstituted milk is 6.9 units. The biggest value was 8.07 nM MDA/mL±0.15 (in the 8\textsuperscript{th} month of storage) and the lowest was 1.17 nM MDA/mL±0.02 (in the 7\textsuperscript{th} month of storage). In the case of TBARS\textsubscript{450} number the biggest value was registered in the 28\textsuperscript{th} month of storage (0.110±0.001) and the lowest in the 7\textsuperscript{th} month of storage (0.068±0.005).

Considering that between TBARS\textsubscript{532} and TBARS\textsubscript{450} coefficient of correlation was 0.2740 (P>0.05) and coefficient of determination 0.07510 it showed that between the two parameters does not exists a direct correlation.

From the Fig. 2 can be observed that TBARS\textsubscript{532} number has an irregular behaviour but TBARS\textsubscript{450} is maintained relative constant. In the case of TBARS\textsubscript{532} the differences between the higher and the lower value are 0.198 units and in the case of TBARS\textsubscript{450} are 0.042 units. Thus, it can be concluded that during infant formula oxidation MDA is formed in a higher quantity that other secondary oxidation products, e.g. hexanal and other saturated aldehydes that absorb at 450 nm. As well as in the case of peroxide value, from the 3\textsuperscript{rd} until the 8\textsuperscript{th} month of storage it can be observed an increase of TBARS\textsubscript{532} number, but not a significant (P>0.05) one. After the 8\textsuperscript{th} month of storage this parameter has an unpredictable variation as well like peroxide value for which was noticed alternative increasing and decreasing periods.

<table>
<thead>
<tr>
<th>Correlation between parameters</th>
<th>r</th>
<th>R\textsuperscript{2}</th>
<th>P</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS\textsubscript{532} number expressed as absorbance/weight</td>
<td>1</td>
<td>1</td>
<td>P&lt;0.0001</td>
<td>***</td>
</tr>
<tr>
<td>expressed as nM MDA/mL reconstituted milk</td>
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<tr>
<td>PV</td>
<td>0.6130</td>
<td>0.3758</td>
<td>0.0151</td>
<td>*</td>
</tr>
<tr>
<td>storage time</td>
<td>-0.3229</td>
<td>0.1042</td>
<td>0.2405</td>
<td>NS</td>
</tr>
<tr>
<td>MDA/mL reconstituted milk</td>
<td>-0.1457</td>
<td>0.02122</td>
<td>0.6045</td>
<td>NS</td>
</tr>
</tbody>
</table>

r – correlation coefficient
R\textsuperscript{2} – coefficient of determination
P – Probability
S – Significance of effect

Between the values of TBARS\textsubscript{532} number expressed as absorbance/weight and nM MDA/mL reconstituted milk there is a perfect correlation, the correlation coefficient (r) and the coefficient of determination being 1. Thus, in the case of a time course study in which is needed the assessment of oxidative status of a product, it can be used by choice one of the parameters mentioned before; in this case is more easy to use TBARS\textsubscript{532} number because is not required a calibration curve. If the study is not made during storage time and on the same matrix is indicated to use a calibration curve of malondialdehyde (MDA) to express the results. Correlation coefficients of storage time with PV and TBARS respectively, show that time has not a significant effect on PV and TBARS at this storage temperature. Correlation (P≤0.05) founded between the TBARS\textsubscript{532} and PV shows a small dependence between the two parameters.

CONCLUSIONS

From our study we can concluded that the storage time has not a significant effect on the evolution of oxidative status of infant formula stored at 15°C, the temperature which is recommended by the producer in order to maintain the product shelf life. Both PV and
TBARS had an irregular variation, conclusion attained also by Manglano et al. (2005) and McCluskey et al. (1997) in their studies on their matrixes. Thus, the time of storage for assessment is very important considering that peroxides are formed and then split in secondary oxidation products; the last one after they are formed could react with some of the matrix compounds. To evaluate the oxidative status of one matrix, both PV and TBARS should be determined because the first one is an indicator of primary phase of oxidation and the second one of secondary phase of oxidation. This could be the reason of the lack of maximum limits for PV and TBARS in infant formula.

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REFERENCES