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Original Article

Shelf Life Prediction of Infant Formula by Using Rancidity Test

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Abstract

Infant formula has a fatty acid composition that meets the needs of the neonates for unsaturated fatty acids.These fatty acids are of major importance during this period of life in which the brain and retina are developing, and will therefore have an influence upon visual acuity and learning abilities.

Oxygen reacts readily with unsaturated fatty acids, so that every time these compounds are handled there is a danger of contaminated with oxidative products. Oxidative stability is an important parameter in characterization of fats and oils.

The purpose of this study was to predict the shelf life of an infant formula using accelerated stability test (rancimat), to save time. The Rancimat method is based on conductometric determination of volatile degradation products and features automatic plotting of the conductivity against time. The evaluation was performed graphically after completion of the experiments.

The stability of a commercially available infant formula in Tehran, Iran was measured using rancimat method at temperatures of 60, 80, 90, 100, 110, 120 and 130 °C.

Equations have been derived by which the shelf life can be predicted on the basis of the Rancimat method, thus avoiding the time-consuming long-term studies. The shelf life of the commercial infant formula studied was estimated as 534 days (approximately 18 months).

Keywords: Shelf life; Infant formula; Rancimat method; Rancidity test; Fatty acids.

Introduction

Human milk is the optimum food for neonates (1); however, infant formula is the main complementary food for the infants who can't use the breast milk (2), so its quality and safety need attention. The shelf life of infant formula is based on maintenance of the nutritional value and the nutrients such as essential amino acids and fatty acids. Lysine is one of these amino acids and is highly susceptible to temperature and high content of carbohydrates. The loss of lysine availability, because of blockage, and the decrease in protein digestibility are the main nutritional consequences of Millard reaction during heat treatment and storage of foods (3). Infant formulas contain fatty acids which supply

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the needs of the neonates for unsaturated fatty acids. These are of major importance during the early period of life in which the brain and retina are developing, and will therefore have an influence upon visual acuity and learning abilities (1, 2, 4, 5).

The main cause of deterioration of lipids and lipid containing foodstuffs such as infant formulas is the lipid autoxidation (6, 7, 8). The autoxidation process is initiated by radical reaction involving unsaturated fatty acids. The primary products are hydroperoxides, which then break down in series of complex reactions. The exact nature of the initiation step is not fully understood, although it is known that initiation can be encouraged by suitable radicals, including those produced by a metal-catalyzed decomposition of preformed hydroperoxides. Hydroperoxides are frequently present in unsuitably stored oils and fats. The secondary products include alcohols and carbonyl compounds. These can be oxidized further to carboxylic acids (9, 10). The degree of lipid oxidation can be measured by medical and /or physical methods as well as accelerated stability tests, which measure the stability of oil under conditions that force the normal oxidation process.

Rancimat was developed as a rapid automated method, which agrees well with the Active Oxygen Method (AOM) (11). This method differs from the ambient storage condition, in using a flow of air and high temperature to accelerate the oxidation. The rancimat method is based on conductometric determination of the volatile degradation products. These volatile components become trapped in distilled water, a feature which is the base for plotting conductivity against time. The resulted curve, as a representative for the process, shows the progress of oxidation wish virtually parallels the development of the peroxide value. The induction time (point of greatest inflection) is determined graphically after completion of the experiment (tangential intersection point). In reality the induction period is measured as the time required to reach an end point of oxidation corresponding to either a level of detectable rancidity or a sudden change in the rate of oxidation (11).

Rancimat is a good alternative method for the determination of oxidative stability, owing to the appreciable saving in labor. The apparatus requires no supervision during the course of an experiment, e.g. overnight (11).

The purpose of this study was to predict the shelf life of an infant formula by using the accelerated stability test of rancimat to save time. The stability of a commercially available infant formula in Tehran, Iran was measured at temperatures of 60, 80, 90, 100, 110, 120 and 130 °C.

Experimental

Reagents and apparatus

All reagents were of analytical-reagent grade and bidistilled-water was used throughout the study. Nitric acid 65%, absolute ethanol and petroleum ether were obtained from Merck (Darmstadt, Germany).

The commercial infant formula used for this study as well as the sunflower oil were obtained from markets and drug stores in Tehran.

Rancimat instrument 743 Metrohm (Switzerland) and Centrifuge Heraeus Biofuge 28RS were used in this study.

Fat extraction

Approximately 50 g of infant formula was weighed into a 500 ml Erlenmeyer flask, and 500 ml of distilled water and 20 ml of nitric acid 65% were added. The mixture was shaken and then centrifuged at a speed of 10000 rpm/ min for 10 min. The solid phase was separated and 40 ml of absolute ethanol was added. The mixture was then shaken vigorously. The sample was placed in a water bath at 50 °C for 10 min. Then 100 ml of petroleum ether was added and the mixture was shaken vigorously for 2-3 min and centrifuged at a speed of 10000 rpm/min for 10 min. The organic phase was separated and after evaporating the petroleum ether, the pure fat was extracted. The fat extraction was repeated 3 times for each sample (4).

Storage condition

The extracted fat was kept in covered vials which were capped tightly and stored at -20 °C until analysis. Exposure to high temperature and bright light were avoided throughout the entire

Table 1. Repeatability of the fat extraction method used in this study.

	n	Induction time (h) (mean ± SD)	RSD (%)
Sunflower oil	10	25.5 ± 0.84	3.3
Sunflower oil [after extraction from a mixture of starch (15 g) + sunflower oil (5 g)]	10	25.1 ± 1.72	6.8

process, since they could induce oxidative decay of unsaturated fatty acids (6, 11).

Tests conducted

The fat components extracted from infant formula were subjected to seven temperatures in the rancimat method (60, 80, 90, 100, 110, 120 and 130±1.6 °C). Temperatures above or below this range did not show good results.

Antioxidant activity was evaluated by measuring the length of the induction time determined by Rancimat test. Rancimat test has gained acceptance due to its ease of use and reproducibility. In Rancimat test, an oil sample is heated under atmospheric pressure, and air is allowed to bubble through the oil at a selected temperature. Under these conditions, the lipoperoxidative process reaches its final steps, and the short-chain volatile acids produced are recovered and measured conductimetrically in distilled water. The time required to produce a sudden increase in conductivity, due to the formation of volatile acids, determines an induction time which can be defined as a measure of the stability of a fat or oil. Evaluations were performed with a 743 Rancimat (Metrohm, Herisau, Switzer land). Samples (5 g of each) were weighed out the vessel and air of supplied (20 ml/min) into the vessel. The induction time decreased, as the measuring temperature increased. The natural logarithms of the induction time varied linearly with temperature, for the same source of oil.

For controlling the extraction method and method validation, a sample oil (sunflower oil) was tested at 100 °C in the rancimat before and after extraction (n=10) (12).

Results and Discussion

The average induction time was 25.5±0.84 h for 10 sunflower oil samples, of without extraction, and was 25.1±1.72 h after fat extraction from a mixture of starch (15 g) and sunflower oil (5 g). Repeatability of this method was expressed by relative standard deviation (%RSD) (Table1). The accuracy of the extraction method is represented by the recovery, which was 98% (Table 2).

As found in this study, the extraction process did not have a significant effect on the oxidative stability of the oil, hence the method seems to be suitable.

Five grams of the fat, extracted from the infant formula, were evaluated at 60, 80, 90, 100, 110, 120 and 130 °C by rancimat. Each examination was repeated three times. Table 3 shows the induction times of the fat extracted from the commercial infant formulas.

A curve is obtained, when different temperatures are plotted against the logarithms of the Oxidative Stability Indices (OSI) (13). The equation that best fit the curve, taking into account the fact that at low temperatures OSI values increase rapidly, was:

$$(T+T_{ref}) = A \exp. [-B (\log (OSI))]$$

Table 2. Accuracy of the extraction method used in this study.

	Extracted oil (g) (n=10)	Added oil (g)	Next extracted oil (g)	Recovery (%)
Starch	0	5	4.89	98

Table 3. The induction times of (T_i) the infant formula.

[illegible]	T_i (h)
[illegible]	192.65
[illegible]	[illegible]
[illegible]	[illegible]
[illegible]	11.53
110	6.51
[illegible]	[illegible]
[illegible]	2.[illegible]

In this egnation T is temperature (°C), and T_{ref} is a constant parameter that was determined for different values (the best numeric results were found for a T_{ref} value of 20). A and B were calculated using a linear regression equation, which was deduced from the stated equation, when neperian logarithm was applied as shown below:

$$Ln (T+ T_{ref}) = Ln (A) – B \log (OSI)$$

Therefore, the curve equation will be as follows:

$$t = [A/(T +T_{ref})]^{2.30258/B}$$

The shelf life of the commercial infant formula at 20 and 25 °C were predicted as 25920 h (1080 days) and 12816 h (534 days), respectively. The shelf life determination of this product by the blocked-lysine method (unpublished data) shows that it is not suitable for shelf life prediction below 30°C. This is because while the essential fatty acids had been degradated at certain temperatures, the lysine was still available. This is due to the fact that Millard reaction will occur well above 40 °C (14). Therefore, it is more useful for quality control in production process, during the stability tests.

The use of rancimat method seems to be suitable for predicting the shelf life of the infant formula. This also saves a lot of time, which is very precious in quality control. as well as research and development laboratories.

Thanks to the minimal labor requirement, the rancimat method is suitable not only for determination of the oxidative stability of fats and oils but also for evaluation of the efficiency of antioxidant. Additional applications such as the investigation of pro-oxidant and investigation of the influence of warehouse condition have also been stated in the literature (7,11,13,15-18). The shelf life of the infant formula tested is 2 years in cool and dry place, based on its label. If the consumer does not keep the product exactly under this condition, the shelf life will decrease to 18 months in room temperature (25 °C). It is necessary to pay more attention to selection of the source of infant formulas imported. The infant formulas should be protected against auto-oxidation during the storage and distribution via controlled storage temperature and better packaging. Every time these products are handled, they should be kept away from air, light and high temperature.

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Table 4. Shelf life prediction of the Infant formula by rancidity and available Lysine methods.

[illegible]	Shelf Life (month)	
[illegible]	[illegible] (available essential fatty acid)	[illegible]* (available lysine)
20	36	40
25	18	37
30	7	31
40	3	15
45	1	3

* [illegible] unpublished data

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References

(1) Sadeghi N, Oveisi MR, Jannat B, Hajimahmoodi M, Bonyani H and Jannat F. Incidence of aflatoxin M1 in human breast milk in Tehran, Iran. Food Control. DOI: 10.1016/j.foodcont.2008.02.005

(2) Oveisi MR, Jannat B, Sadeghi N, Hajimahmoodi M, Nikzad A. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. Food Control. (2007) 18: 1216–1218

(3) Gonzales ASP, Naranjo GB, Malec LS and Vigo MS. Available lysine, protein digestibility and lactulose in commercial infant formulas. International Dairy Journal. (2003) 13: 95-99

(4) Oveisi MR, Sadeghi N, Hajimahmoodi M, Jannat B, Behfar A and Sobhani H. Quantitative determination of fatty acids in infant formula by GC without derivatization. Acta Medica Iranica. (2006) 44 (4): 225-229

(5) Innis SM. Essential fatty acids in growth and development. Prog.Lipid.Res. (1991) 30: 39-103

(6) Gray JI. Measurement of lipid oxidation: a review. J. Am. Oil. Chem. Soc. (1978) 55: 539-546

(7) Shiota M and Tatsumik K. Effect of sucrose ester of fatty acid on the antioxidant activity of milk products on fish oil oxidation. Food. Chem. Toxico. (2002) 67

(8) Bertrand WM. Determination of the oxidative stability of vegetable oils by rancimat and conductivity as chemiluminescence measurements. J. Am. Oil. Chem. Soc. (1996) 73: 1039-1043

(9) Frankel EN. In search of better methods to evaluate natural antioxidants and oxidative stability in food lipid. Trend. Food. Sci. Technol. (1993) 4: 220-225

(10) Reynbout G .The effect of temperature on the induction time of the stabilized oil. J. Am. Oil. Chem. Soc. (1991) 68: 983-984

(11) Laubli M and Bruttle P. Determination of the oxidative stability of fat and oils, comparison between an active oxygen method (Aocscd 12-57) and rancimat method. J. Am. Oil. Chem. Soc. (1986) 63: 792-795

(12) Kaya A, Tekin AR and Oner MD. Oxidative stability of sunflower oil and olive oils: comparison between a modified oxygen method and long term storage. Food. Sci. Technol. (1993) 26: 464-468

(13) Farhoosh R. The effect of operational parameters of the Rancimat Method on the determination of the oxidative stability measures and Shelf-Life prediction of Soybean oil. J. Am. Oil. Chem. Soc. (2006) 84: 205-206

(14) Ferrer E, Alegrý´a A, Farre´ R, Abella´n P and Romero F. Effects of Thermal Processing and Storage on Available Lysine and Furfural Compounds Contents of Infant Formulas. J. Agric. Food Chem. (2000) 48: 1817-1822

(15) Allam SSM and Mohamed HMA. Thermal stability of some commercial natural and synthetic antioxidants and their mixtures. J. Food. Lipids. (2002) 9: 277-293

(16) Valenzuela A, Sanhueza J and Niet S. Effect of synthetic antioxidants on cholesterol stability during the thermal- induced oxidation a polyunsaturated vegetable oil. J. Am. Oil. Chem. Soc. (2002) 79: 325-328

(17) Kowalski B, Gruczynska E and Maciaszek K. Kinetics of rapeseed oil oxidation by pressure differential scanning calorimetric measurements. Eur. J. Lipid. Sci. Tech. (2000) 102: 337-341

(18) Xu Z, Zhang T, PrinyaWiWatkul W and Godber JS. Capabilities of different cooking oils in preventing of cholesterol oxidation during heating. J. Am. Oil. Chem. Soc. (2005) 82: 243-248

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