JOURNAL OF FOOD TECHNOLOGY
MEDWELL PUBLICATIONS

Spectrophotometric Measurement of Available Lysine and Protein Carbonyls in Commercial Infant Formulas and Milk Products

Israel Olusegun Otemuyiwa and Olalekan Olabisi Yahya
Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

Key words: Investigated, quality, milk products, measurement, carbonyls

Corresponding Author:
Israel Olusegun Otemuyiwa
Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

Page No.: 9-13
Volume: 14, Issue 2, 2016
ISSN: 1684-8462
Journal of Food Technology

Abstract: This study investigated the impact of storage on protein quality in infant formulas and milk products through measurement of available lysine and formation of protein carbonyls. The samples manufactured by dietetic company were collected from open market as displayed on the shelves, these consists of four brands of powdered milk, three of evaporated tin milk and two of infant cereal products.. Available lysine and protein carbonyls were measured after derivatization with 1-fluoro-2-4-dinitrobenzene (FDNB) and 2, 4-dinitro-phenylhydrazine (DNPH) respectively, using spectrophotometer. The results showed that moisture content, protein, available lysine and protein carbonyl ranged from 2.04-37.81%, 9.23-29.07%, 7.24-24.57 g^{-1} 100 g protein and 0.45-18.96 nmol/mg proteins, correspondingly. Protein carbonyl increases whereas available lysine decreases as storage period is prolonged. The study concluded that though infant formulas and milk products was said to a have prolonged shelf-life, the products in the open markets especially in the tropics should be periodically analyzed to ensure that the protein quality did not deteriorate before the product is consumed.

INTRODUCTION

Ten million children under the age of 5 years old die each year. More than half of the deaths occur because of malnutrition. If adequate health systems were in place nearly 2/3 of the deaths could be prevented (Bryce *et al*., 2005). Part of the health systems picture is to promote appropriate feeding practices for infants and young children. The most vulnerable period for developing under-nutrition remains the transition from breast feeding to family foods. Nutritional status in children is most vulnerable during the weaning stages when both macro and micronutrients may be insufficient to maintain growth and development. Protein-energy malnutrition and micronutrient under-nutrition occur together. It is an important part of weaning strategies to optimize nutritional status and to tackle under-nutrition-related problems as a group for maximum effectiveness (Allen *et al*., 1991).

Weaning is the process of transition from a breast feeding to a semi solid diet for the infant. The weaning period is crucial for the maintenance and continued growth and development of the child and yet it is often the time when foods are given to provide the volume necessary to keep the child from being hungry without regard to the nutritional quality of the transitional foods.

Poor quality of weaning foods and improper weaning practices predispose infants to malnutrition, growth retardation, infection, diseases and high mortality (Onofiok and Nnanyelugo, 2005).

Infant formulas and milk products are designed to be substitutes for mother's milk when breast feeding is not possible (Behrooz *et al*., 2008). In the past, Nigerian infants were given cereal-based food that was processed at home by toasting and boiling cereal flours. Nowadays, conventional formulas are based on cow's milk products which are processed by dietetic product manufacturers in large-scale factories. The process of manufacture of infant formulas includes the blending of components, homogenization, pasteurization and spray-drying. The application of heat during some steps to obtain powdered infant formulas inevitably gives rise to several chemical reactions of which Maillard reaction is the most important. The ε-amino group of protein-bound lysine can react with glucose, maltose or lactose to form Amadori products which are not susceptible to attack by proteolytic enzymes during digestion (Finot *et al*., 1981). Loss of available lysine which is the most negative nutritional consequence of the Maillard reaction, is particularly significant in cereals, in which this amino acid is limiting (O'Brien and Morrisey, 1989) and is undesirable in these products as it can produce a loss of nutritive value (Guerra-Herna´ndez *et al*., 1999).

Infant cereals have been reported to have a long shelf-life and can usually be consumed until two years after their manufacture. However, storage duration and conditions, as well as the composition of these cereals, affect the progress of the Maillard reaction that is initiated during their processing (Ferna´ndez-Artigas *et al*., 1999; Guerra-Herna´ ndez *et al*., 1999).

Various research efforts have been carried out to investigate the impact of heat processing and storage conditions on the availability or loss of lysine in milk and cereal products (Ferna´ndez-Artigas *et al*., 1999), report is scanty on the stability or loss of lysine in infant cereals and milk products displayed on retailer's shelves in the open market. The main concern is making sure that there is no gap between nutrient requirements and what a child is able to consume, absorb and utilize. Since, lysine is an essential amino acid necessary for human health but cannot be made by the body, infant formulas can be the sole source of lysine for babies, hence, the level of damage of lysine and protein quality has to be ascertained. Therefore, the present study was undertaken to study the lysine damage and formation of protein carbonyl in commercial infant formulas and milk products displayed for more some months on the shelves in Nigerian market.

MATERIALS AND METHODS

Samples: Infant cereals and milk products produced by dietetic product manufacturer were purchased as displayed on the market shelves from Odo Ogbe Market in Ile-Ife, Nigeria. The samples of various brands differing in manufacturing batches were procured. The samples consist of: Powdered milk (Peak, Cowbell, Milksi, Peak 123 infant formula, Cowbell Chocolate), Evaporated tin milk (Peak, Three Crowns and Coast) and Infant Cereals (Cerelac and Nutrend).

Reagents: 1-fluoro-2-4-dinitrobenzene (FDNB), N-ε-2-4-DNP-L-lysine-HCl, 2,4-dinitro-phenyl hydrazine (DNPH), tri-chloro acetic acid (TCA) (Sigma Aldrich Chemical, Germany).

Analytical procedure: The moisture content was determined by gravimetric method and protein by Kjeldahl method as outlined by AOAC Method (2000).

Determination of the available lysine: The DNP-lysine was determined using the method of Ramirez-Jimerez *et al*. (2004). The sample (0.20-0.50 g based on protein content) was placed in a glass ampoule; to this was added 1.0 mL of 8% $NaHCO_3$ solution and 1.5 mL of 1fluoro -2-4-dinitrobenzene (FDNB) solution in 3% ethanol. The tubes were shaken mechanically for 3 h at room temperature and the ethanol content was evaporated by immersing in a 95°C water bath. The FDNB modified protein was hydrolyzed with 3.0 mL of 8.1M HCl, the content was boiled for 30 min in water bath to remove carbon-dioxide and the glass ampoule was then sealed with Bunsen burner flame and suspended with a string in an oven at 110°C for 24 h. The hydrolyzed solution was filtered, put into 25 mL standard flask and made up to mark with distilled water. 2.0 mL of the solution was extracted with 2.0 mL diethyl-ether. The ether layer was removed and the aqueous layer was diluted to 10.0 mL. The absorbance was measured at 415 nm. The derivatized N-ε-2-4- DNP-L-lysine HCl (Sigma Chemical Co.) was used as standard and the content of available lysine was calculated from the equation:

$$C = \frac{Ws \times Au \times \text{volume of filtrate} \times 1000}{Wp \times Abs\ (\text{standard}) \times A \times Cp}$$

Where:

C = Content as g lysine/16 g N
Ws = Weight of standard expressed as μg lysine
Wp = Weight of sample
Au = Net absorbance of sample
As = Net absorbance of standard
A = Aliquot of the filtrate, 2 cm^3 recommended
Cp = Crude protein

Determination of protein carbonyls: The protein carbonyl was determined using method of Levine *et al.* (1990). An aliquot of aqueous milk (0.5 mL solution or 0.5 g) corresponding to about 2 mg protein) was incubated with 5 mL of 10 mM of 2,4-dinitro-phenylhydrazine (DNPH) in 2M Hydrochloric acid for 30 min at room temperature. Milk proteins were then precipitated with 2mL of 10% trichloroacetic acid, the precipitate was recovered by centrifugation for 5 minutes at 1677×g. Protein pellets were washed with 5 mL of ethanol/ethyl acetate (50:50) to remove unreacted free DNPH reagent, washing was carried out twice and the precipitate was dissolved in 10 mL of 6 M urea. The solution was filtered and the absorbance was measured at 370 nm using a Lambda Bio 20 spectrophotometer (Perkin-Elmer, Rotlkreuz, Switzerland). The protein carbonyl content was calculated from extinction coefficient. The extinction coefficient (ε) for DNPH is 22,000 M-1cm^{-1}.

Protein carbonyl (nmol/mg protein) =
(absorbance×106/22,000)/mg protein =
absorbance×45.45/mg protein

Statistical analysis: Statistical analysis was carried out by the use of Graph-Pad Instat-3 Packages (Graph Pad software Inc, USA). All analyses were carried out in triplicate and the results presented as mean and standard deviation. Analysis of variance was used to assess and compare results.

RESULTS AND DISCUSSION

The result of available lysine and protein carbonyls is presented in Table 1. The sample batches analyzed were stored between three to eleven months from manufactured date to date of analysis. The moisture content of powdered milk and cereal product ranged from 2.04-6.34% while evaporated tin milk recorded moisture between 35-37%. The protein content (g/100 g Protein dry weight basis) ranged from 9.23 in Nutrend to 29.07 in Cowbell milk; the result indicated that powdered milk recorded higher protein than evaporated tin milk while protein was higher in Cerelac than Nutrend. The protein content reported in this work agrees with the protein content displayed in the product's label.

The content of available lysine in g/100 g protein and g/100 g sample ranged from 7.24-24.57 and. 1.46-4.78, respectively. The available lysine in cowbell milk 3, 5, 6 and 8 months were 16.48, 15.06, 12.40 and 12.15 showing percentage reduction range of between 8.62 and 26.27%. Milksi recorded lysine content of 9.05 at 5 month and 7.24 at 7 month, about 20% reduction. The evaporated milk samples peak, three crowns and coast milk recorded 28.22, 22.06 and 12.79% lysine loss between 3-10 months of manufacture. Peak 123 and Peak Chocolate recorded 3.45 and 2.51% lysine loss between 6-11 and 3-5 months, respectively whereas the loss of lysine between 5 and 10 months in Cerelac was 9.32% and Nutrend recorded 0.44% loss at 11-15 months. The loss of lysine was unexpectedly higher in all brands of powdered milk, although these were dried products with very low water activity (2.04-6.34% water), it could be seen that not only water activities but also the length of storage could contribute to loss of lysine. The high loss of lysine in evaporated tin milk could be adduced to high moisture content, higher water activities and length of storage have been reported to influence loss of lysine even at room temperature (Ramiraz-Jimenez *et al*., 2004). Water activity is sometimes defined as "free", "bound" or "available water" in a system. A portion of the total water content present in a product is strongly bound to specific sites on the chemicals that comprise the product. These sites may include the hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins and other polar sites. Water is held by hydrogen bonds, ion-dipole bonds and other strong chemical bonds. Some water is bound less tightly but is still not available (as a solvent for water-soluble food components). Many preservation processes attempt to eliminate spoilage by lowering the availability of water to microorganisms. Reducing the amount of free--or unbound--water also minimizes other undesirable chemical changes that occur during storage.

Protein carbonyl which is a measure of protein oxidative changes showed consistent increase as the storage period increases. The values were higher in powdered milk and infant cereals than liquid evaporated tin milk. For instance, Cowbell powdered milk at 3 months was 6.65 but the value increased to 18.96 nmol mg^{-1} protein after 5 months. This is an indication of susceptibility of peptides in powdered milk to oxidative modifications. Values obtained in this study falls within the range of 1.9-60.9 nmol mg^{-1} protein obtained for powdered infant milk (Fenaille *et al*., 2005). Storage duration and conditions as well as the particular composition of these cereals, affect the progress of the Maillard reaction that is initiated during their processing (Fernandez-Artigas *et al*., 1999; Guerra-Hernandez *et al*., 1999).

The findings of this study revealed the possibility of progression of Maillard reaction in the products analyzed during the process of handling until it gets to the consumers. As such, since chemical reactions did not take place in isolation, other food components apart from amino acid and protein may equally be affected. Hence, the nutrient quality of the products may have deteriorated to a level that would not support a healthy growth and development among infants by the time it is consumed.

Table 1: Available lysine, protein carbonyls and percentage loss of lysine in commercial infant formulas and milk products (dry weight)

Samples	Sample Code	No. of months	Moisture	Protein	Available lysine (g/100 g sample)	Available lysine (g/100g protein)	Lysine loss (%)	Protein carbonyl (nmol mg^{-1} protein)	Increase in protein carbonyl (%)
Dry powdered milk									
Cowbell milk	1A	3	3.88±0.01	29.07±0.01	4.78±0.12[a]	16.48±0.43[a]	____	6.65±0. 36	-
	1B	5			4.36±0.98[b]	15.06±0.34[a]	8.62	18.96±0.68	185
	1C	6			3.59±0.27[c]	12.40±0.95[b]	24.76	3.09±0.82	-
	1D	8			3.53±0.39[d]	12.15±0.13[b]	26.27	17.15±0.36	157
Miksi	2A	5	6.34±0.02	20.80±0.00	1.89±0.15a	9.05±0.71a	_____	14.22±0.19	-
	2B	7			1.51±0.06b	7.24±0.28b	20.00	16.17±0.91	13.7
Peak123	3A	6	2.04±0.00	18.44±0.00	1.87±0.01a	10.14±0.04a	___	10.45±0.83	-
	3B	11			1.80±0.01b	9.79±0.07b	3.45	11.37±0.92	8.8
Cowbell chocolate	4A	3	4.54±0.00	9.87±0.00	1.57±0.01[a]	15.94±0.14	____	2.85±0.09	-
	4B	4			1.54±0.01[b]	15.58±0.13	2.26	3.29±0.29	15.4
	4C	5			1.53±0.01[c]	15.54±0.13	2.51	5.20±0.009	82.45
Evaporated tin milk									
Peak milk	7A	3	35.04±0.01	11.48±0.01	2.62±0.25[a]	22.82±0.22[a]	___	4.03±0.04	-
	7B	6			1.88±0.11[b]	16.38±0.92[b]	28.22	6.25±0.30	55
Three crowns	8A	3	37.81±0.01	11.14±0.00	2.74±0.18[a]	24.57±0.17[a]	___	6.71±0.11	-
	8B	6			2.13±0.11[b]	19.15±0.10[b]	22.06	7.04±0.41	4.9
Coast milk	9A	4	37.81±0.02	11.14±0.01	2.33±0.26[a]	20.88±0.23[a]	___	6.41±0.04	-
	9B	10			2.03±0.16[b]	18.21±0.15[b]	12.79	7.18±0.34	12
Cereal Product									
Cerelac	5A	5	3.36±0.00	19.11±0.00	2.28±0.01[a]	11.91±0.03[a]	____	1.89±0.007	-
	5B	10			2.06±0.08[b]	10.80±0.44[b]	9.32	4.48±0.36	137
Nutrend	6A	11	2.08±0.00	9.23±0.01	1.47±0.04	15.89±0.33	____	0.66±0.14	-
	6B	15			1.46±0.03	15.82±0.24	0.44	0.45±0.007	46

Mean±SD mean and standard deviation of replicate analysis (n = 3); values in different superscripts within the row for each sample are significantly different at $p \geq 0.001$

However, the shortcoming of this study was the brands selected for the analysis might not be from the same production batch and may not have been subjected to same conditions of handlings which may allow some variations in the content of nutrients. This observation notwithstanding, the shelf life of the infant formula (as shown on its label) is two years in cool and dry place, if the consumer does not keep the product exactly under this condition, the shelf life might decrease, it is therefore, necessary to periodically take samples of these products for analysis to be able to know how long it will take to reduce the nutrients to 50% and probably note the time as the product's shelve life. Then there is the need to give proper orientation to retailers on the implication of open display of milk products on nutritional quality and safety of the products.

CONCLUSION

Estimation of lysine loss in commercial infant cereals and milk products provide a direct means of obtaining information about the extent of initial stages of the Maillard reaction and further deteriorative effect of exposure to tropical weather.

REFERENCES

AOAC, 2000. Official Methods of Analysis of Association of Analytical Chemists. 17th Edn., AOAC International, Orowitz.

Allen, J.C., R.P. Keller, P. Archer and M.C. Neville, 1991. Studies in human lactation: Milk composition and daily secretion rates of macronutrients in the first year of lactation. Am. J. Clin. Nutr., 54: 69-80.

Behrooz, J., R.O. Mohammad, S. Naficeh, B. Abdolazim, H. Mannan, J. Forouzandeh and K. Sahar, 2008. Shelf life prediction of infant formula by using rancidity test. Iranian J. Pharm. Res., 7: 269-273.

Bryce, J., C. Boschi-Pinto, K. Shibuya and R.E. Black, 2005. WHO estimates of the causes of death in children. Lancet, 365: 1147-1152.

Fenaille, F., V. Parisod, P. Visani, S. Populaire, J.C. Tabet and P.A. Guy, 2005. Modifications of milk constituents during processing: A preliminary benchmarking study. Int. Dairy J., 8: 208-228.

Fernandez-Artigas, P., B. Garcia-Villanova and E. Guerra-Hernandez, 1999. Blockage of available lysine at different stages of infant cereal production. J. Sci. Food Agric., 79: 851-854.

Finot, P.A., R. Deutsh and E. Bujard, 1981. The extent of the Maillard reaction during the processing of milk. Prog. Food Nutr. Sci., 5: 345-355.

Guerra-Hernandez, E., N. Corzo and B. Garcia-Villanova, 1999. Maillard reaction evaluation by furosine determination during infant cereal processing. J. Cereal Sci., 29: 171-176.

Levine, R.L., D. Garland, C.N. Oliver, A. Amici and I. Climent *et al*., 1990. Determination of carbonyl content in oxidatively modified proteins. Meth. Enzymol., 186: 464-478.

Onofiok, N.O. and D.O. Nnanyelugo, 2005. Weaning foods in West Africa: Nutritional problems and possible solutions. http://archive.unu.edu/unupress/food/V191e/ch06.htm.

Ramirez-Jimenez, A., B. Garcia-Villanova and E. Guerra-Hernandez, 2004. Effect of storage conditions and inclusion of milk on available lysine in infant cereals. Food Chem., 85: 239-244.