

## Effect of Storage Temperature on the Chemical Stability of Enteral Formula

Radamés Baéz, Guadalupe Rojas, Julieta Sandoval-Guillén and M.A. Ángeles Valdivia-López  
Facultad de Química, Departamento de Alimentos y Biotecnología, Universidad Nacional Autónoma de México, México DF 04510, México

**Abstract:** When the only source of nutrition is formula, its composition and the amount administered must satisfactorily meet the patient's energy and nutritional requirements. In this sense, the aim of this research is to evaluate the effect of storage temperature and time on the chemical changes of main micro- and macro-components of a powdered milk formula; understanding these changes would make it possible to establish models for the estimation of the product's shelf life under different temperature conditions. The kinetics of the reactions researched followed a first-order model except for HMF, which was zero order. The activation energy (Arrhenius equation) was 11.220 kJ/mol for HMF, 36.957 kJ/mol for hydroperoxide and 50.536 kJ/mol for the formation of secondary products derived from lipid oxidation, 43.388 kJ/mol for loss of vitamin A, 51.960 kJ/mol for degradation of Vitamin C and 92.361 kJ/mol for the loss of protein solubility. Results showed that prevalent reactions are non-enzymatic browning, followed by lipid oxidation, loss of vitamin A and C and protein solubility. HMF could be used as a good indicator of storage conditions for enteral formulas.

**Keywords:** Enteral formula, hydroxymethylfurfural, kinetics, lipid oxidation, shelf life, storage, vitamin A, vitamin C

### INTRODUCTION

The purpose of enteral nutritional formulations is to cover the specific nutritional requirements of a broad sector of patients suffering from chronic or acute diseases. Poor nutrition or the inability to consume a proper diet usually requires enteral nutrition support. When the only source of nutrition is formula, its composition and the amount administered must satisfactorily meet the patient's energy and nutritional requirements (Henningfield *et al.*, 1995).

The composition of commercial formula is based on proteinic sources incorporated as whole protein, hydrolyzed protein or free amino acids, which bring about polymeric, oligomeric or elemental enteral formulas respectively (García-Baños *et al.*, 2005). Carbohydrates are incorporated in the form of polymers such as glucose, maltodextrins, disaccharides and oligosaccharides and lipids in different quantities that can include mono- and di-glycerides; likewise, most commercially available diets are fortified with minerals and vitamins. Enteral nutrition formulas contain sufficient quantities of hydro- and liposoluble vitamins; hence, the ingestion of 1,500 to 2,000-calories per day supplies the requirements of these nutrients (Frias and Vidal-Valverde, 2001).

The preparation of powdered enteral formula involves several steps, including sterilization and

aspersion drying processes, treatments that can produce different changes in its components and in nutrient reduction bioavailability (García-Baños *et al.*, 2005). In addition, before consumption, the formula is often subjected to varying storage conditions throughout the supply chain during which composition changes occur. Vitamins are affected by several factors, such as type of packaging, storage time and conditions (e.g., exposure to oxygen, light and high temperatures). Vitamin A (all-trans-retinol and 13-cis-retinol) shows stability in nitrogen atmospheres and cool places without light and it is especially sensitive to oxidation by air in the presence of light. Bearing this information in mind, the storage conditions of enteral nutrition formulas could affect vitamin content to some extent (Frias and Vidal-Valverde, 2001). Ascorbic acid (vitamin C) is sensitive to heat, oxygen and light therefore, assessing these conditions is useful in determining how stable it is when subjected to different temperatures (Guerra-Hernandez *et al.*, 2002).

Carbohydrates can undergo different reactions, such as isomerization and degradation (Lowry and Baker, 1989) or can react with proteins (Maillard reaction). In both cases, they can produce HMF as the intermediary of colorful compounds. Enteral formulas are susceptible to these reactions because of the strong presence of reducing carbohydrates (García-Baños *et al.*, 2005).

Caseins are the main ingredient in enteral nutrition formulas. Proteins can undergo severe modifications, especially in the lateral chains of basic amino acids, such as lysine reacting with sugars (Rufian-Henares *et al.*, 2002) or in cross-linked amino acid formation, affecting protein solubility (Boschin *et al.*, 2003).

Lipid oxidation is well known as the main cause of quality deterioration during the processing or storage of lipid-rich food such as enteral formulas (Manglano *et al.*, 2005; Romeu-Nadal *et al.*, 2007). Lipid oxidation has received a great deal of attention because its implications are undesirable for human health and it contributes to a decrease in the nutritional value of food. The reaction of atmospheric oxygen with unsaturated lipids produces free radicals and hydro peroxides as well as a diversity of secondary compounds, such as volatile carbonyl and dicarbonyl. It has been reported that the development of objectionable flavors in food, such as powdered milk, is caused by the formation of secondary reactions in lipidic peroxidation (Romeu-Nadal *et al.*, 2007).

The aim of this study is to evaluate the effect of storage temperature and time on the chemical changes of main micro- and macro-components of a powdered milk formula. Understanding these changes would make it possible to establish models for the estimation of the product's shelf life under different temperature conditions.

## MATERIALS AND METHODS

**Sampling:** The casein-based powdered enteral formula was expressly prepared for this study at the pilot plant of a company that produces these formulas. They were packaged in 454-g cans. Immediately after production, the enteral formulas were sent to Food and Biotechnology Department of Faculty of Chemistry of National University of Mexico (UNAM), for the shelf study. The samples were analyzed to measure initial composition: Moisture 4.02, Ash 3.12, Fat 12.01, Proteins (N $\times$ 6.38) 18.53 and Carbohydrates 62.30% respectively. This research was conducted for a period of 15 week during year 2010.

**Storage conditions:** The formula was stored (in a chamber with thermal thermostat) at 22, 37 and 45°C for 105 days immediately after production. Each week, lipid oxidation, protein solubility, non-enzymatic browning (HMF), vitamin A and C concentration were monitored, as well as the calorimetric profiles of proteins. Analytical tests were performed in triplicate.

### Lipid oxidation:

**Fat extraction:** An adaptation of the Manglano *et al.* (2005) method was applied. The extracts obtained were combined to evaporate the solvent in a rotary evaporator (Büchi R-205, Flawil, Switzerland) at 35°C.

**Determination of the peroxide index:** A modified ferric thiocyanate method described by Shon and Haque (2007) was used to evaluate hydroperoxide formation in enteral formulas. The Peroxide Values (PV) were expressed as meq/kg.

**Kreis test:** The Kreis Test was used to evaluate the secondary compounds of lipid oxidation. Phloroglucinol reacts with oxidized fat in acid solution and turns red (Gray, 1978). The Kreis Test was expressed as absorbance units per gram of fat.

### Vitamin A and C evaluation:

**Vitamin A evaluation:** All vitamin standards were purchased from Sigma-Aldrich Quimica (Toluca, Mexico). Standard solutions of vitamin A, all trans-retinol (from 1 to 10  $\mu$ g/mL) were prepared in methanol. Methanol and n-hexane were of HPLC grade and obtained from Mallinckrodt (Hazelwood, MO, USA). Vitamin A (retinol) was measured according to Albalá-Hurtado *et al.* (2000). The High-Performance Liquid Chromatography (HPLC) system consisted of a Beckman (Fullerton, CA, USA) System Gold Binary pump, with a 20  $\mu$ L loop injector and a UV detector. Chromatographic data were acquired and analyzed with a System Gold program. The column was an Ultrasphere-ODS C18 column (Beckman, Fullerton, CA, USA), 25 $\times$ 0.46 cm, particle diameter 5  $\mu$ m (Beckman, Fullerton, CA, USA) with a matching guard cartridge. Methanol was used as the mobile phase, working in isocratic mode. The wavelength selected was optimum for the detection of vitamin A (325 nm).

**Vitamin C evaluation:** Vitamin C or ascorbic acid was measured according to Zapata and Dufour (1992). Readings were done in a HPLC, Beckman (Fullerton, CA, USA) System Gold 126 coupled to a UV detector, ( $\lambda$  261 nm), Waters (Milford Massachusetts USA) Symmetry Shield RP 18 5  $\mu$ m 3.9 $\times$ 150 mm column. Mobile phase Methanol/water (5:95) with 5 mM of Cetrimide (hexadecyl trimethyl ammonium bromide) and 50 mM of monobasic potassium phosphate. Twenty  $\mu$ L were injected in the column at a rate of 0.8 mL/min. A calibration curve was obtained in concentrations ranging from 10 to 250  $\mu$ g/mL of ascorbic acid.

### Protein quality:

**Determination of protein solubility:** A modified turbidimetric method described by Vera (1988) was used to evaluate protein solubility.

**Non-enzymatic browning Hydroxymethylfurfural (HMF):** T-HMF was quantified by reverse phase HPLC and UV detection using the method of Guerra-Hernandez *et al.* (2002). The HPLC analysis was carried out with a Beckman model 110B chromatograph (USA) with a 20  $\mu$ L injection loop, a

Spherisorb ODS (250×4 mm id) column (Waters Milford, Massachusetts USA), a Beckman Model UV/Vis detector (Fullerton, CA, USA) set at 284 nm and a Spectra Physics Integrator 4270 (Mountain View, CA, USA). The mobile phase was water/acetonitrile (95:5) at 1 mL/min.

**Calorimetric profiles of proteins:** DSC (Differential Scanning Calorimetry) was used to characterize interaction patterns of protein in milk-based formulas according to Sánchez del Angel *et al.* (2003). Thermal transitions of protein in milk-based formula were evaluated with a Perkin-Elmer DSC equipped with TAS 7 software package (Perkin Elmer, Waltham, Massachusetts USA), using indium as the standard. The samples were hydrated with deionized water 24 h prior to the calorimetric analysis. Heating rate was 10°C/min temperature range was 30-150°C. Samples of 5 mg in stainless steel capsules were used in all cases.

## RESULTS AND DISCUSSION

The results and its discussion are based on of kinetics parameters obtained for 22, 37 and 45°C storage conditions for all indicators (Table 1).

**Oxidation of lipids:** Hydroperoxides and the Kreis index as primary and secondary oxidation products respectively were measured to determine the effects of storage temperature and time on the oxidative stability of the casein-based formula.

**Peroxides:** The peroxide values, expressed as meq peroxide/kg fat, are summarized in Fig. 1. The peroxide permissible limit value, specified by Joint FAO/WHO (1989) Refined Oil Standards, is 10 meq of peroxides/kg. Lipids of formula showed a value of 1.89 when storage began, which proved satisfactory.

Peroxide evolution at all three temperatures Fig. 1, shows an induction period of 42 days at 22°C and 14 days at 37 and 45°C, period in which the rate of peroxide formation is too low. In this lapse, the rate of peroxide formation is not proportional to the storage time. This behavior can be explained by the presence of antioxidants that increase the induction period in the

product. It is possible to see that the formation of primary oxidation products is favored by increased storage temperature. After the induction period, the samples show an exponential temperature increment; that is, the rate of peroxide formation is greater than decomposition (Gray, 1978), for this reason, there is an observable rise in concentration. The results confirm that storage temperature is a key parameter in the oxidation stability of powdered milk (Romeu-Nadal *et al.*, 2007).

The rate constants obtained show that peroxide formation follows a first-order kinetics model, which is temperature dependent (Table 1).

Activation energy is a measure of temperature sensitivity to reactions responsible for the formation, in this case, of hydroperoxides. The Arrhenius equation shows 36.957 kJ/mol of activation energy, which is similar to the values Subramaniam *et al.* (2008) and Jung-Hyun *et al.* (2007) reported.

**Kreis test:** The values of the Kreis index, expressed in absorbance (absorbance units/g of sample), are shown also in Fig. 1.

Figure 1 show that it is on day 35 that the slope changes for the tests at 45°C, whereas for those at 37 and 22°C, it occurs on day 63. After these dates, the formation of secondary oxidation products is exponential insofar as storage time is concerned, which suggests an evolution pattern that follows a first-order kinetics model.

The rate of peroxide formation and their decomposition explains higher formation of secondary carbonyl compounds. It is possible to establish the direct effect of storage temperature in the reactions involved in oxidative degradation of lipids. This stage is characterized by high concentration of aldehydes, which are the main responsible for objectionable odor and taste in enteral formulas. Furthermore, these aldehydes can react with functional protein groups causing cross-linking and reduction of functional properties, especially solubility. (Romeu-Nadal *et al.*, 2007; Evranuz, 1993)

The fat present in the formula does not show stability against oxidation at any of the temperatures tested.

Table 1: Results of kinetics parameters

Indicators	Temp. 22°C		Temp. 37°C		Temp. 45°C		Ea, (kJ/g molL)
	k	R <sup>2</sup>	k	R <sup>2</sup>	k	R <sup>2</sup>	
Peroxides *	0.00291	0.9022	0.00575	0.7021	0.00877	0.8984	36.957
Kreis index *	0.00691	0.6493	0.01797	0.8763	0.03091	0.9654	50.536
Vitamin A *	0.00192	0.9726	0.00414	0.9449	0.00703	0.9490	43.388
Vitamin C *	0.00094	0.5563	0.00193	0.8724	0.00472	0.8194	51.960
Protein solubility *	0.00008	0.4792	0.00027	0.5752	0.00141	0.8806	92.361
H M F **	0.18045	0.9377	0.22279	0.9065	0.25179	0.9308	11.220

\*|k: First order; \*\*|k: Zero order

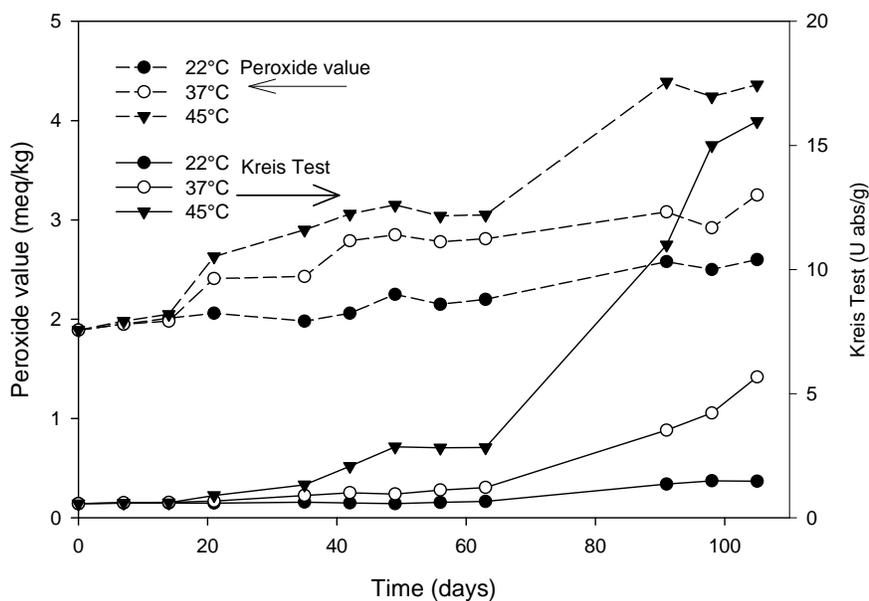


Fig. 1: Lipid oxidation evaluation of enteral formula

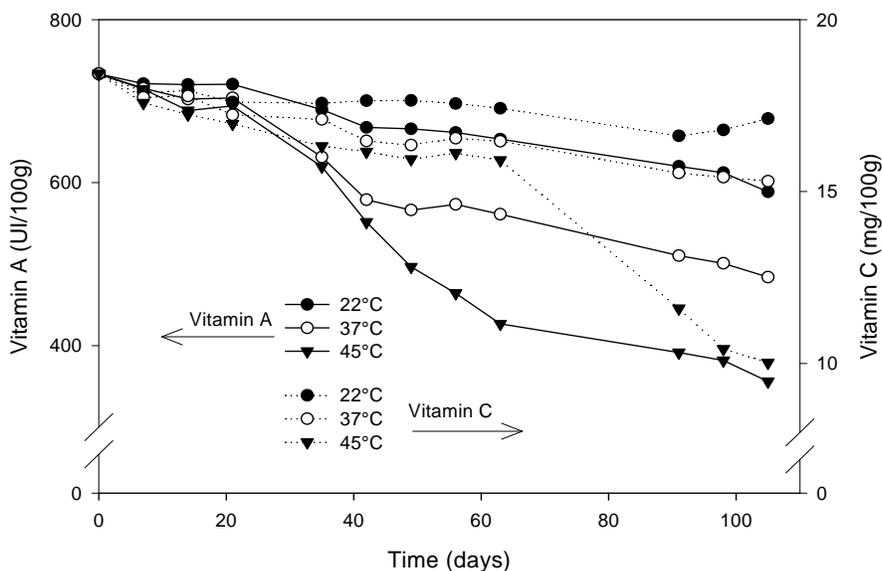


Fig. 2: Vitamins decrease of enteral formula

Rate constants show that the formation of secondary lipid oxidation products is temperature dependent. Using the Arrhenius Law, the activation energy is 50.536 kJ/mol.

**Vitamins evaluation:**

**Vitamin A:** The vitamin A stability is expressed in IU (International Units). Enteral formulas are usually enriched with vitamin A in the form of retinol acetate or retinol palmitate because these molecules are more stable and less susceptible to oxidation than its respective analogs in vegetable oils (Chávez-Servín *et al.*, 2008). Figure 2 shows the results for loss of

vitamin A, whose measurement at zero time was 733.36 UI/100 g, against a reported value of 1,028 UI/100 g on the label.

From the results in Fig. 2, it is possible to establish that deterioration evolution shows similar induction periods at all three temperatures tested. This behavior is justified by the presence of the antioxidant in the product, which protects the vitamin from co-oxidation due to the isoprenoid structure. On the other hand, the propagation period shows different deterioration patterns at each temperature. The treatment of these results led to a first-order kinetics model.

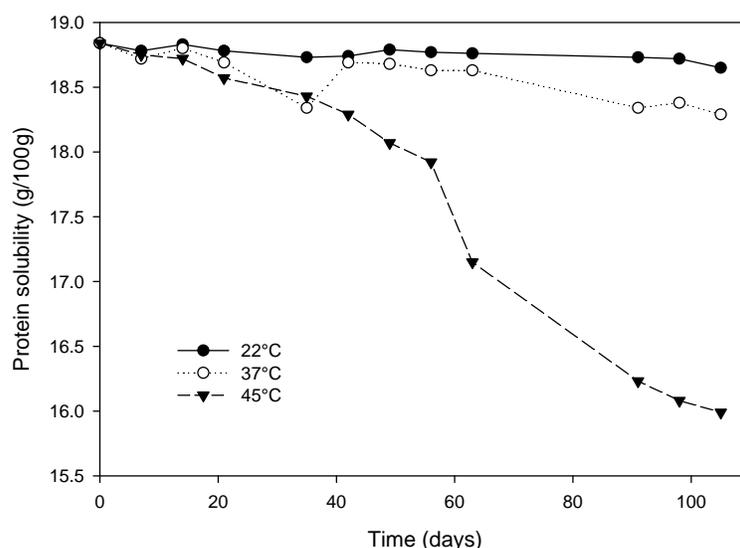


Fig. 3: Protein changes in enteral formula

At the end of the study, the loss of vitamin is 19.72, 34.01, 51.45% at 22, 37 and 45°C, respectively. Values which are much higher than Chávez-Servín *et al.* (2008) reported; who found that the loss of vitamin A in a children's milk formula was 30% at 40°C after 18 months, whereas Frias and Vidal-Valverde (2001) reported 75% vitamin loss after 6 months at 30°C in enteral nutrition formulas.

Rate constants show that the vitamin A degradation is temperature dependent. Using the Arrhenius Law, the activation energy is 43.388 kJ/mol, which is lower than 96 kJ/mol Laohasongkram and Thamjariyapan (1995), reported.

**Vitamin C:** Vitamin C monitoring results are shown in Fig. 2 also. The quantity of vitamin at zero time is 18.43 mg/100 g.

Vitamin C shows good stability in relation to storage and it is the only nutriment that does not change significantly when the temperature remains under 30°C. By the end of the study, the loss of vitamin C is 7.11, 16.98 and 45.66% at 22, 37 and 45°C, respectively.

Excessive vitamin C degradation could be damaging to other aspects of nutritional quality. Gliguem and Birlouez-Aragon (2005), reported the specific effects of products of vitamin C degradation on Maillard reaction development and initiation of radical reactions by vitamin C mixtures.

The marked slope change of the sample stored at 45°C could be because this temperature favors peroxide formation and secondary oxidation products. In which vitamin C can participate due to its ability to form free radicals, which in turn are capable of intervening in the non-enzymatic browning of a complex matrix such as the powdered enteral formula (García-Baños *et al.*, 2005).

Once the reaction order was known, its temperature function is determined by using the Arrhenius expression. The activation energy for vitamin C degradation was 51.960 kJ/mol, a value slightly lower than Burdurlu *et al.* (2006) reported (56-106 kJ/mol); and higher than that reported by Toralles *et al.* (2008) (45 kJ/mol); Scala and Crapiste (2008) (26.9 kJ/mol) and Dhuique-Mayer *et al.* (2007) (35.9 kJ/mol).

#### Protein quality:

**Protein solubility:** Monitoring results are shown in Fig. 3, which indicate that up to day 21, the loss of protein solubility is not proportional to storage time at 22, 37 and 45°C.

Loss of protein solubility shows considerably differentiated patterns. Results from the samples stored at 22 and 37°C suggest an induction period of more than 105 days of monitoring, whereas the sample at 45°C, the induction period was about 56 days.

At the end of the study, the loss of protein solubility is 1.01, 2.92 and 15.13% at 22, 37 and 45°C, respectively.

Loss of protein solubility can lead to a reduction in digestibility and/or bioavailability of some essential amino acids because of the formation of cross-links between 2 different parts of the protein chain (Boschin *et al.*, 2003) and because the formation of Schiff base type compounds within carbonyl groups derived from lipid oxidation with protein amino groups.

The activation energy for the loss of protein solubility is 92.361 kJ/mol.

**Non-enzymatic browning Hydroxymethylfurfural (HMF):** Non-enzymatic browning is a significant cause of food quality loss because it diminishes the nutritional value of proteins and modifies the organoleptic properties of enteral formulas. The specific composition of enriched formulas can explain their high

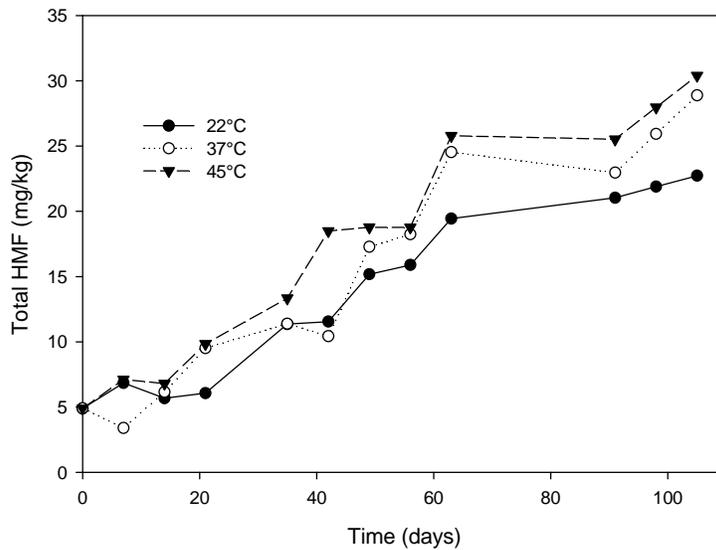


Fig. 4: Total HMF evolution in enteral formula

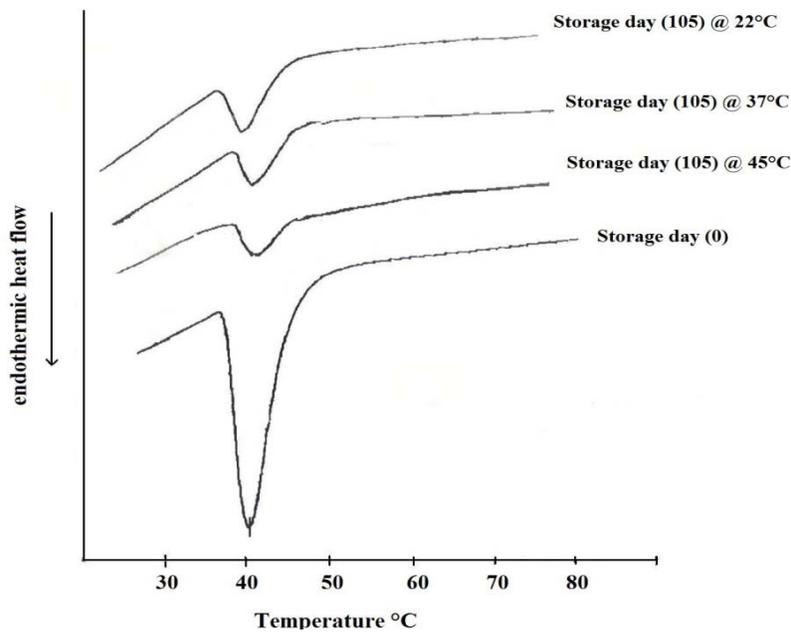


Fig. 5: DSC thermograms in enteral formula

susceptibility to form Maillard reaction products, especially due to the high lactose content, the presence of proteins with many reactant lysine residues and presence of vitamin C (Gliguem and Birlouez-Aragon, 2005).

Figure 4 indicates the effects of storage temperature on HMF formation. The initial HMF (5 mg/kg) content is noticeably higher at all temperatures with kinetics patterns suggesting a zero-order model, which agrees with Gentry and Roberts (2004). Toward the end of the study, the HMF values are 22.73, 28.89 and 30.34 mg/kg at 22, 37 and 45°C, respectively.

The Arrhenius result shows the activation energy as 11.220 kJ/mol, a value much lower than those reported by Gentry and Roberts (2004) 27.3 kJ/mol; Burdulu *et al.* (2006) 181.71 kJ/mol; Tosi *et al.* (2002) 226 kJ/mol; Morales *et al.* (1995) 118.5-95.04 kJ/mol and Sahai *et al.* (1992) 104-76.7 kJ/mol.

**Calorimetric profiles of proteins:** One of the main practical applications of DSC in processed food has been ingredient quality control as well as component modifications in processing operations-freezing, drying and mixed operations (Dollimore, 1992; Harwalkar and

Man, 1990). In this research, DSC is used to characterize enteral formula interaction patterns.

Figure 5 shows the DSC thermograms for the enteral formula. Only one endothermic peak at 40-45°C can be observed at all three storage temperatures. These peaks are due to denaturation of proteins (Delben and Stefancich, 1997).

The temperature-induced denaturation mechanism is highly complex and primarily involves destabilization of the major non-covalent interactions. Hydrogen bonding, electrostatic and van der Waals interactions are enthalpy-driven and exothermic in nature. Therefore, they are destabilized at high temperature but stabilized at low temperature. In contrast, hydrophobic interactions are entropy-driven and endothermic; they are stabilized at high temperature (60 to 70°C) but destabilized at low temperature. The enthalpy change results from the combination of exothermic events, such as hydrogen bond breakage and endothermic processes, including protein aggregation and breaking up of hydrophobic interactions. The thermograms show loss of peak definition and of symmetry degree. Enthalpy diminishes as storage time increases, which shows the effect of storage temperature on the energy requirements for unfolding proteins.

This enthalpy change is likely due to loss of protein solubility from cross-linking after the denaturation process resulting from storage conditions.

## CONCLUSION

It is evident from this study that the main chemical changes of the powdered milk formula are non-enzymatic browning, lipid oxidation and loss of vitamins A and C. The results found using thermal transitions are in accordance with the loss of protein solubility, vitamin content, increased HMF formation and the formation of oxidation products. The study provides information that allows establishing conditions of temperature and storage time, which ensure chemical quality of the formula.

## REFERENCES

- Albalá-Hurtado, A.S., M.T. Veciana-Nogués, M.C. Vidal-Carou and A. Mariné-Font, 2000. Stability of vitamins A, E and B complex in infant milks claimed to have equal final composition in liquid and powered formulas. *J. Food Sci.*, 65: 1052-1055.
- Boschin, G., A. D'Agostina, A. Rinaldi and A. Arnoldi, 2003. Lysinoalanine content of formulas for enteral nutrition. *J. Dairy Sci.*, 86: 2283-2287.
- Burdurlu, H.S., N. Koca and F. Karadeniz, 2006. Degradation of vitamin C in citrus juice concentrates during storage. *J. Food Eng.*, 74(2): 211-216.
- Chávez-Servín, J.L., A.I. Castellote, M. Rivero and M.C. López-Sabater, 2008. Analysis of vitamins A, E and C, iron and selenium contents in infant milk-based powered formula during full shelf life. *Food Chem.*, 107: 1187-1197.
- Delben, F. and S. Stefancich, 1997. Interaction of food proteins with polysaccharides I: Properties upon mixing. *J. Food. Eng.*, 31: 325-346.
- Dhuique-Mayer, C., M. Tbatou, M. Carall, C. Caris-Veyrat, M. Domier and M.J. Amiot, 2007. Thermal degradation of antioxidant micronutrients in citrus juice: Kinetics and newly formed compounds. *J. Agr. Food Chem.*, 55: 4209-4216.
- Dollimore, D., 1992. Thermal analysis. *Anal. Chem.*, 64: 147-153.
- Evranuz, E.Ö., 1993. The effects of temperature and moisture content on lipid peroxidation during storage of unblanched salted roasted peanuts: Shelf life studies for unblanched salted roasted peanuts. *Int. J. Food Sci. Tech.*, 28(2): 193-199.
- Frias, J. and C. Vidal-Valverde, 2001. Stability of thiamine and vitamins E and A during storage of enteral feeding formula. *J. Agr. Food Chem.*, 49: 2313-2317.
- García-Baños, J.L., M.D. Del Castillo, M.L. Sanz, A. Olano and N. Corzo, 2005. Maillard reaction during storage of power enteral formulas. *Food Chem.*, 89: 555-560.
- Gentry, T.S. and J.S. Roberts, 2004. Formation kinetics and application of 5-hydroxymethylfurfural as a time-temperature indicator of lethality for continuous pasteurization of apple cider. *Innov. Food Sci. Emerg.*, 5: 327-333.
- Gliguem, H. and I. Birlouez-Aragon, 2005. Effects of sterilization, packaging and storage on vitamin C degradation, protein denaturation and glycation in fortified milks. *J. Dairy Sci.*, 88: 891-899.
- Gray, J.I., 1978. Measurement of lipid oxidation: A review. *J. Am. Oil. Chem. Soc.*, 55: 539-546.
- Guerra-Hernandez, E., C. Leon Gomez, B. Garcia-Villanova, N. Corzo Sanchez and J.M. Romera Gomez, 2002. Effect of storage on non-enzymatic browning of liquid infant milk formulae. *J. Sci. Food Agri.*, 82: 587-592.
- Harwalkar, M.A. and C.Y. Ma, 1990. Thermal Analysis of Foods. Elsevier Applied Food Science Series, USA.
- Henningfield, M.F., S.P. Smith, P.A. Reynolds, S.E. Garcia and J.H. Baxter, 1995. Protein quality of enteral nutrition products is consistent with label claims during shelf life and beyond expiration date. *J. Am. Diet. Assoc.*, 95: 46-52.
- Joint FAO/WHO, 1989. Food Standard Program Recommended International Standards. London.
- Jung-Hyun, Y., C. Yong-Jun and L. Dong-Sun, 2007. Storage stability and shelf life characteristics of Korean savory sauce products. *J. Food Sci. Nutr.*, 12(4): 242-250.

- Laohasongkram, K. and S. Thamjariyapan, 1995. Kinetics of vitamin A degradation in pork liver puree during heat processing. *Thai. J. Agr. Sci.*, 28: 229-236.
- Lowry, K.R. and D.H. Baker, 1989. Effect of storage, carbohydrate composition and heat processing on protein quality and amino acid bioavailability of a commercial enteral product. *J. Food. Sci.*, 54(4): 1024-1030.
- Manglano, P., M.J. Lagarda, M.D. Silvestre, C. Vidal, G. Clemente and R. Farré, 2005. Stability of the lipid fraction of milk-based infant formulas during storage. *Eur. J. Lipid. Sci. Tech.*, 107: 815-823.
- Morales, F.J., C. Romero and S. Jimenez-Perez, 1995. New methodologies for kinetic study of 5-(hydroxymethyl)-furfural formation and reactive lysine blockage in heat treated milk and model systems. *J. Food Protect.*, 58: 310-315.
- Romeu-Nadal, M., J.L. Chávez-Servín, A.I. Castellote, M. Rivero and M.C. López-Sabater, 2007. Oxidation stability of the lipid fraction in milk powder formulas. *Food Chem.*, 100: 756-763.
- Rufian-Henares, J.A., E. Guerra-Hernández and B. García-Villanova, 2002. Maillard reaction in enteral formula processing: Furosine, loss of o-phthaldialdehyde reactivity and fluorescence. *Food Res. Int.*, 35: 527-533.
- Sahai, D., Adhikari, A.K. and O.N Mathur, 1992. Formation Kinetics of total hydroxyl methylfurfural during manufacture of khoa. *Food Sci. Technol. German*, 25: 146-149.
- Sánchez del Angel, S., E. Moreno Martínez and M.A. Valdivia López, 2003. Study of denaturation of corn proteins during storage using differential scanning calorimetry, *Food Chem.*, 83(4): 531-540.
- Scala, K., G. Di and G. Capriste, 2008. Drying kinetics and quality changes during drying of red pepper. *Food Sci. Technol. German*, 41: 789-795.
- Shon, J. and Z.U. Haque, 2007. Antioxidative ability of native and thermized sour whey in oxidation-catalysed model systems. *Int. J. Dairy Tech.*, 60(2): 143-148.
- Subramaniam, S., J. Huang and W. Prinyawiwatkul, 2008. Thermal properties and applications of the arrhenius equation for evaluating viscosity and oxidation rates of unrefined pollock oil. *J. Food Eng.*, 84(2): 187-193.
- Toralles, R.P., J.L. Vendruscolo, C. Tondo-Vendruscolo, F.A. Burkert-del-Pino and P.L. Antunes, 2008. Determination of reaction rate constants for ascorbic acid degradation in peach puree: Effect of temperature and concentration. *Food Sci. Technol. Portuguese*, 28: 18-23.
- Tosi, E., M. Ciappini, E. Re and H. Lucero, 2002. Honey thermal treatment effects on hydroxymethylfurfural content. *Food Chem.*, 77: 71-74.
- Vera, J.C., 1988. Measurement of microgram quantities of protein by a generally applicable turbidimetric procedure. *Anal. Biochem.*, 174: 187-196.
- Zapata, S. and J.P. Dufour, 1992. Ascorbic, dehydroascorbic and isoascorbic acid simultaneous determinations by reverse phase ion interaction HPLC. *J. Food Sci.*, 57: 506-511.