The final publication is available at

http://doi.org/10.1016/j.foodchem.2007.02.016

Additional Information

Fluorescence and color as markers for the Maillard reaction in milk-cereal based infant foods during storage

Fluorescence and color in infant foods during storage

Lourdes Bosch[a], Amparo Alegría[a], Rosaura Farré[a,*], Gonzalo Clemente[b]

[a] *Nutrition and Food Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, 46100 - Burjassot, Valencia, Spain*

[b] *Department of Statistics, Polytechnic University, Camino de Vera s/n, 46022 - Valencia, Spain*

[*] Author to whom correspondence should be addressed: telephone 34-96-3544950; fax 34-96-3544954; e-mail: rosaura.farre@uv.es

E-mail addresses: lourdes.bosch@uv.es, amparo.alegria@uv.es, gclemente@eio.upv.es

Abstract

Free and total fluorescence compounds and color formation were measured in three different milk-cereal based infant foods stored at 25º, 30º and 37ºC for 9 months to evaluate the advanced and final stages of the Maillard reaction. Milk-cereal infant foods containing honey (B) or fruits (C) had fluorescent values higher than sample (A) without them. This difference could be ascribed to the higher monosaccharide (fructose and/or glucose) content of (B) and (C), which could increase susceptibility to the Maillard reaction. However, for color increase (ΔE), no significant differences ($p < 0.05$) among the three types of samples were found. During the storage period, a gradual increase in fluorescence and color was observed, and statistically significant differences among the three temperatures studied were detected, the values being greater at 37ºC than at 30ºC and 25ºC.

1. Introduction

Cereals in the form of paps prepared with milk are usually one of the first foods added in the diversification of the infant diet from the 5th / 6th month. Milk and cereal based ready-to-eat infant foods are presently available on the market. These products have a long shelf-life and can be consumed for up to one year after manufacture. Due to their composition (protein and high lactose contents and other reducing sugars from ingredients such as honey and fruits), the thermal treatments applied in their manufacture, and their long shelf-life, development of the Maillard reaction (MR) is favoured, protein quality is affected (blockage of lysine and decreased digestibility) and may lead to the development of brown pigments (O´Brien & Morrissey, 1989; Van Boekel, 1998).

In the initial stage of the MR, Amadori compounds are formed, which are not fluorescent, but in advanced MR stages these compounds can form cross-links with adjacent proteins or with other amino groups, giving rise to fluorescent polymeric aggregates or so-called advanced glycation end products (AGEs). The intensity of fluorescence of AGEs is widely used as a marker of the yield of MR. The routes of formation of AGEs are not yet well understood, and most of them remain unidentified, though the typical excitation and emission wavelengths are important properties for characterizing MR fluorescent products (λex 340-370 nm and λem 420-440 nm, except AGEs containing arginine in their structure, which present λex 320 nm and λem 380 nm) (Morales & van Boekel, 1997; Ferrer, Alegría & Farré, 2000; Matiacevich, Santagapita & Buera, 2005). In a recent review (Matiacevich et al., 2005), the range of emission wavelengths of AGEs has been extended up to 470 nm.

Measurements of fluorescence at different wavelengths or the FAST index (Fluorescence of Advanced Maillard products and Soluble Tryptophan) have been used to evaluate the progress of MR resulting from thermal treatments or processing in milk (Morales, Romero & Jiménez-Pérez, 1996; Birlouez-Aragon, Nicolas, Metais, Marchond, Grenier & Calvo, 1998; Siegl, Schwarzenbolz & Henle, 2000; Birlouez-Aragon, Leclère, Quedraogo, Birlouez & Grongnet, 2001; Leclère &

3

Birlouez-Aragon, 2001; Kulmyrzaev & Dufour, 2002; Birlouez-Aragon, Sabat & Gouti, 2002; Gliguem & Birlouez-Aragon, 2005; Guan, Liu, Ye & Yang, 2005) and infant formulas (Ferrer et al., 2000; Birlouez-Aragon et al., 2004; Birlouez-Aragon, Locquet, Louvent, Bouveresse & Stahl, 2005). However, studies on the effects of storage on the formation of fluorescent compounds in infant formulas (Ferrer, Alegría, Farré, Clemente & Calvo, 2005) and milks (Gliguem et al., 2005; Birlouez-Aragon, Sabat, Lutz, Leclère & Nicolas, 1999) are scarce, and there is a lack of reports on the formation of MR products in infant foods, as a consequence of either processing or storage.

In heated milk (Kessler & Fink, 1986; Pagliarini, Vernile & Peri, 1990), stored milk (Rampilli & Andreini, 1992) and infant formulas (Ferrer et al., 2005; Rossi & Pompei, 1991; Guerra-Hernández, Leon, Corzo, García-Villanova & Romera, 2002), changes in color have been used to evaluate the effects of storage. However, only three studies on the evaluation of the effect of thermal treatment or storage on the color of cereal-based infant foods with or without added milk have been found (Fernández-Artigas, Guerra-Hernández & García-Villanova, 1999; Ramírez-Jiménez, Guerra-Hernández & García-Villanova, 2003; Ramírez-Jiménez, García-Villanova & Guerra-Hernández, 2004).

The lack of data on fluorescence and color in infant foods and on the effect of storage upon these parameters, together with the fact that AGEs have been involved in different pathologies (Matiacevich et al., 2005) and their presence in food may constitute a risk factor (Golberg et al., 2004), were the reasons that led us to adapt a method for the measurement of AGEs and color in liquid cereal-milk based infant foods and to apply it to monitor the evolution of these compounds during storage with the final aim of estimating MR yield.

In addition, pentodilysine, an AGE resulting from the reaction between lysine and pentoses or ascorbic acid (Morales et al., 1997) was measured, because in infant formulas a significant increase in this compound during storage has been reported (Ferrer et al., 2005).

2. Material and methods

4

2.1. Samples

Three different (A, B, C) liquid milk-cereal based infant foods commercially available in Spain and containing 88% skimmed milk and 8.8% hydrolyzed 8-cereals flour (wheat, corn, rice, oat, barley, rye, sorghum and millet) were analyzed. All three had the same composition, except for 0.9% honey in B, and 1.1% fruit (banana, orange and apple) in C. The protein contents of the infant foods (A, B, C) were 3.4, 3.5 and 3.5 g/100 g, respectively, the carbohydrate contents were 16.1, 15.7 and 15.8 g/100 g, respectively, and the lipid content was 2.9 g/ 100 g, in all samples. Polyunsaturated fatty acids were 20% of the total fatty acids, and were present in equal proportion in the three different samples. The three products were obtained by the same manufacturing process that included heating at 90ºC and indirect UHT thermal treatment. They were packed in a 250 ml commercial tetra-pack and stored at three different temperatures (25º, 30º and 37ºC). They were analyzed immediately after manufacture (time zero) and after 1, 2, 4, 5, 6, 7, 8 and 9 months.

A Pawkit water activity meter (Decagon Devices, Inc., Pullman, Washington, USA) calibrated with 6.0 M sodium chloride was used to measure the water activity (A_w) of samples. A_w and pH of the three infant foods were 1.00±0.01 and 6.59±0.05, respectively, and these values remained stable during the 9 months of storage.

2.2. Chemicals

Trichloroacetic acid was from Fluka (Buchs, Switzerland). Tris (99.9%, Sigma-Aldrich, St. Louis, MO, USA) and hydrochloric acid (sp gr = 1.19, Merck, Darmstadt, Germany) were used for preparing Tris buffer solution (pH 7.2). Pronase solution (pronase from *Streptomyces griseus* 5.1 U/mg, Fluka, Buchs, Switzerland) was prepared in pH 7.2 Tris buffer solution. Phosphate buffered saline (PBS) 20 mM and sodium chloride 15 mM (pH 7) was prepared using sodium di-hydrogen phosphate 1-hydrate, di-sodium hydrogen phosphate 12-hydrate (Panreac, Barcelona, Spain) and sodium chloride (Normapur Prolabo, Fontenay-sur-Bois, France). Quinine sulfate (Guinama,

Valencia, Spain) solution (100 mg/l) was prepared in 0.1N sulfuric acid (Merck, Darmstadt, Germany).

2.3. Fluorescence determination

Samples were diluted with water to obtain a solution containing 0.02 g protein/ml (5.3 g infant food/10 ml).

According to the slightly modified method of Ferrer et al. (2005) for measuring free fluorescence, 24% (w/v) trichloroacetic acid was added to the diluted sample to precipitate the proteins, and prior to measuring total fluorescence, incubation with 24 U pronase at 25ºC for 30 minutes was carried out. Both solutions were centrifuged, and the supernatants were diluted in 20 mM PBS, 15 mM sodium chloride, pH 7 to avoid quenching phenomena.

Fluorescence of samples, blank (PBS 20 mM, sodium chloride 15 mM, pH 7) and standards were measured at: AGEs (λex 347 nm λem 415 nm) and pentodilysine (λex 366 nm λem 440 nm) (Morales et al., 1997; Ferrer et al., 2005). Spectrofluorophotometer (RF-5000 Shimadzu Corporation, Kyoto, Japan) setting was: band width emission 15 nm and excitation 5 nm. Results were expressed as %FI (fluorescence intensity) with respect to the fluorescence of a 0.5 and 7.5 µg/ml quinine sulfate standard for AGEs and pentodilysine, respectively. All analyses were performed in quadruplicate.

To check precision, free and total fluorescent compounds in a sample were measured on three different days and in quadruplicate each day. Inter-day precision values ranged between 4-5%.

2.4. Color determination

Color was measured using a Hunter Labscan II colorimeter (Hunter associates laboratory, INC. Reston, Virginia, USA). Results were expressed according to the CIELAB system with reference to illuminant D65 and a visual angle of 10º. The parameters determined were L* (luminosity or brightness: L*=0 black and L*=100 white), a* (red-green component: −a* =

6

greenness and +a* = redness) and b* (yellow-blue component: −b* = blueness and +b* = yellowness). The difference in color between two samples was given by the expression: $\Delta E* = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$ (Calvo, 2004).

To evaluate color changes, $\Delta E*$ was calculated, where L*, a* and b* values at the considered storage time were considered with respect to those obtained in just manufactured samples (time zero). All analyses were performed in quadruplicate.

2.5. Statistical analysis

Three-factor ANOVA (Statgraphics plus 3.1 statistical software package) was applied to AGEs and pentodilysine values and color increase (ΔE) to detect differences among the three milk-cereal based infant foods (A, B and C), storage temperatures (25º, 30º and 37ºC) and storage times (0, 1, 2, 4, 5, 6, 7, 8 and 9 months). In factors with more than two levels and exhibiting a significant effect ($p < 0.05$), a Tukey test was applied to identify those differing significantly from each other.

Regression analysis were applied to study the effect of storage time, temperature and/or type of sample on AGEs and pentodilysine values and color increase (ΔE).

Partial correlations were carried out to study the relationship between measured variable pairs without considering the possible effect, in one or another sense, of other studied variables, i.e., to establish the possible cause-effect relation between the variable pair. Total correlation was also done to measure the strength of the linear relationship between the variables.

3. Results and discussion

3.1. Optimization of total fluorescence measurement

Taking as starting point the conditions used in infant formulas in a previous study (Ferrer et al., 2005), the amount of pronase and the incubation time at 25ºC required for total fluorescence determination were selected for the analyzed infant foods.

7

The results of the assay of different enzyme amounts (8, 16, 20, 24, 32 and 40 U) showed 24 U to be the amount to be used, because the %FI obtained did not differ ($p < 0.05$) from those obtained with higher enzyme amounts. The selected pronase concentration was higher than that used in infant formulas (Ferrer et al., 2005), but the ratios between total and free fluorescent compounds were also higher in milk-cereal based infant foods (from 8 to 10) than in infant formulas (from 4 to 5).

A wide range of hydrolysis times (30, 60, 90 and 120 minutes) was assayed. Given the lack of significant differences among the hydrolysis times, the shortest (30 minutes) was selected.

3.2. Fluorescence compounds in infant foods

Free and total pentodilysine and AGEs compounds in the infant foods (A, B and C) stored at 25º, 30º and 37ºC for 9 months, expressed as % FI (fluorescence intensity), are reported in Tables 1 and 2.

The wavelengths (λex 347 nm and λem 415 nm) giving the highest peaks in emission and excitation spectra were those used to measure free and total AGEs in milk-cereal based infant foods. These wavelengths have been used to measure AGEs in model systems, milk or infant formulas (Morales et al., 1997; Morales et al., 1996; Ferrer et al., 2005). Nevertheless, other excitation / emission wavelengths have also been reported (Siegl et al., 2000; Leclère, Birlouez-Aragon & Meli, 2002). On the other hand, the FAST method has been proposed to determine fluorescence compounds in milk-resembling systems and heated-treated foods, such as milk and breakfast cereals (Birlouez-Aragon et al., 2001; Leclère et al., 2001; Birlouez-Aragon et al, 2002; Gliguem et al., 2005; Guan et al., 2005).

Fluorescence compounds covalently bound to proteins were higher than free fluorescent compounds, in agreement with the observations of Morales et al. (1997) in heated glucose or lactose/casein systems and of Ferrer et al. (2005) in infant formulas. The partial correlations

8

between free and bound to protein AGEs, and between free and bound to protein pentodilysine were not significant, suggesting different pathways in the their formation, whereby bound to protein compounds would not come from the polymerization of free ones, or bound to protein compounds would not release free compounds. However, important total correlations were found: between free and bound to protein AGEs (r= 0.850), and between free and bound to protein pentodilysine (r= 0.873). These results are reasonable if one considers the increase in variables with the increase in storage time.

Among the samples (A, B and C), statistically significant differences (p<0.05) were found in free and total AGEs and in pentodilysine values, except between B and C for free pentodilysine values. In all cases, the lowest fluorescence values corresponded to sample A. Considering that the same thermal treatment was applied, and that milk (88% of total composition) of the same quality was used in manufacturing A, B and C, the differences in fluorescence compound contents must be ascribed to differences in the minor components among samples, such as in carbohydrate contents. Samples B and C contained monosaccharides (fructose and / or glucose) from honey and fruits, respectively, with greater possibilities of participating in the MR than disaccharides (O´Brien et al., 1989).

Pentodilysine could result from the reaction between the lysine residues of casein with ascorbic acid, present in the three samples (9.7 mg/100 ml), that also contain added iron (1.1 mg/100 ml) – the latter being able to enhance or catalyze ascorbic acid oxidation to dehydroascorbate and degradation products (treose), yielding high glycating power (Gliguem et al., 2005; Birlouez-Aragon et al., 1999; Leclère et al., 2002). In samples containing fruits (C), pentodilysine could also result from the pentoses (arabinose and xylose) present. Fructose in samples B and C could also favor the formation of pentodilysine, because it is a sugar with a high proportion of the furanoside ring form.

The lack of studies on fluorescence compounds in infant foods with a composition similar to those of the present study precluded comparison of the results obtained. The development of

fluorescence has been monitored in the manufacturing process of milk and infant formulas (Morales et al., 1996; Birlouez-Aragon et al., 1998; Birlouez-Aragon et al., 1999; Siegl et al., 2000; Birlouez-Aragon et al., 2001; Leclère et al., 2001; Leclère et al., 2002; Kulmyrzaev et al., 2002; Birlouez-Aragon et al., 2002; Birlouez-Aragon et al., 2004; Gliguem et al., 2005; Guan et al., 2005; Birlouez-Aragon et al., 2005; Ferrer et al., 2005), but the results obtained in the present study are not comparable because processing temperatures are usually higher than storage temperatures, different excitation and emission wavelengths have been used, and/or results are expressed as IF % with respect to standards of different concentrations.

The three-factor ANOVA applied to evaluate the effect of storage temperature on free and total AGEs and pentodilysine contents shows statistically significant differences (p<0.05) among the three temperatures studied - the highest fluorescence compound values corresponding to the highest storage temperature. The results obtained did not agree with the absence of an effect of storage temperature upon free and total fluorescent AGEs in infant formulas stored for 9 to 24 months at 20ºC and 37ºC, reported by Ferrer et al. (2005) - though the lack of coincidence in the considered storage period has to be taken into account.

In free and total AGEs and pentodilysine, an interaction was found between the temperature of storage and the type of sample, i.e., the enhancer effect of temperature on fluorescence compound formation depends on the infant food involved (A, B or C) (Fig. 1a). The highest increase in fluorescence corresponded to B for a temperature increase from 30º to 37ºC, and can only be justified by the monosaccharides provided by honey. In A, B and C a similar evolution with temperature (parallel, but at a different level) of free AGEs and pentodilysine and of total AGEs and pentodilysine was found.

During storage, a gradual increase in free and total AGEs and pentodilysine was observed (Tables 1 and 2). In agreement with the observed interaction between storage time and temperature, the highest IF values corresponded to 37ºC. The evolution of free and total AGEs and pentodilysine throughout storage was similar at 25, 30 and 37ºC.

In free and total AGEs and pentodilysine an interaction was found between storage time and the type of sample (Fig. 1b): the formation of fluorescence compounds during storage depended on the sample (A, B, C), but it was not possible to ascribe the high or low increase percentages to a given sample.

Storage time had a statistically significant effect on fluorescence values, in agreement with the results reported for infant formulas stored at 20ºC and 37ºC for 9 and 24 months (Ferrer et al., 2005). Nevertheless, in cows' milk and fortified milk stored for 4 months at room temperature, negligible development of the advanced Maillard reaction has been reported by Gliguem et al. (2005), in contrast to the results obtained in the present study, where statistically significant increases ($p<0.05$) were found even at 25ºC from the first storage month onwards. However, the increments begin to be more pronounced from the fourth storage month onwards, corroborating the previously described (Morales et al. 1997; Matiacevich & Buera, 2006) induction period in the development of fluorescent compounds.

Taking into account the significant effect of storage time, storage temperature and type of sample (ANOVA) on fluorescent compound formation, multiple regression analysis was applied to quantify the effect of these factors. Considering that the type of sample is a qualitative variable, dummy variables were used in the analysis, i.e., two auxiliary variables were created (*sample 1 and sample 2*) (Jobson, 1991). The coefficient of variable *sample 1* measures what sample B contents differ on average from those of sample A, taking the latter as reference. In the same way, the coefficient of the variable *sample 2* measures what sample C contents differ on average from those of sample A, taken as reference. Figures 2 and 3 show the observed versus predicted plots for free and total AGEs and pentodilysine, and the corresponding equations. In all cases, the equations explain a high percentage of the variability found in the formation of fluorescent compounds (R^2= 85 and 96%, for free and total AGEs, respectively; R^2= 88 and 95%, for free and total pentodilysine, respectively). The most important effect on fluorescent compound formation is ascribed to the interaction of *temperature* month* variables. The negative sign corresponding to the

11

coefficient of the month variable does not mean a decrease in fluorescent compound content with increasing time. The effect of storage time is jointly described by the variable *month* and the interaction *temperature*month* in such a way that, in all cases, for a given temperature, fluorescent compound content increases with increasing storage time.

3.3. Color in infant foods

Mean L*, a* and b* values and color increase (ΔE*) in the three samples (A, B and C) stored at 25º, 30º and 37ºC for 9 months are reported in Table 3 and Fig. 4, respectively.

Sample luminosity (L*) decreases with the increase in storage temperature and time, due to the formation of brown pigments. Contrarily, values of a* and b* increase with increasing storage temperature and time, denoting the rise of red and yellow characteristics in the system. In the same way, the Chroma value (C*) increases, defined by the equation $C^* = (a^{*2} + b^{*2})^{1/2}$, and indicating the degree of saturation, purity or intensity of visual color (Calvo, 2004). The decrease in L* and the increase in a* and b* with increasing storage temperature and time have been reported elsewhere (Rampilli et al., 1992; Rossi et al., 1991).

No significant differences ($p < 0.05$) among A, B and C were found when three-factor ANOVA was applied to ΔE. However, differences were detected among the three temperatures (25, 30 and 37ºC): the higher the temperature, the greater the color increase (mean values of 2.86, 5.16 and 7.28, at 25, 30 and 37ºC, respectively). The evolution of ΔE with increasing temperature was similar in all three samples (A, B and C), and no interaction between temperature and type of sample was found. During storage, a gradual increase in ΔE depending on the sample type and storage temperature was found (interactions between storage time and type of sample, and storage time and storage temperature being statistically significant $p < 0.05$) (see Figure 4).

Increases in ΔE with temperature and storage time have been reported for indirect UHT semi-skimmed milk stored at 20ºC and 32ºC for 90 days (Rampilli et al., 1992); rice-based infant cereals stored at 32ºC and 55ºC for 12 months (Ramírez-Jiménez et al., 2003); and infant formulas

12

stored at 20ºC and 37ºC for 24 months (Ferrer et al., 2005) and at 4, 20 and 38ºC (Rossi et al., 1991) for 18 months. In contrast, no increase with the lengthening of storage time was found in an infant cereal with rice, corn, soy and powdered milk (40%) stored at 32ºC for 12 months (Ramírez-Jiménez et al., 2004).

Given the lack of statistically significant differences among A, B and C, a multiple regression analysis ($p < 0.01$) was carried out to evaluate the effect of storage time, temperature and the interaction storage time-temperature (3 independent variables) on ΔE (dependent variable). A multiple linear regression model was obtained to describe the relationship that explains 93% of ΔE variability. The fitted model presented the following equation: $\Delta E = -1.169 + 0.088$temperature $- 1.052$month $+ 0.059$temperature.month.

AGEs, pentodilysine and color determinations are useful for monitoring MR development during the storage of milk-cereal based infant foods. However, the determination of choice would be AGEs, because it includes all fluorescent compounds formed in advanced stages of the MR, and is more sensitive to small changes in sample composition (such as the presence of 0.9% of honey) than color. The pronounced increase in fluorescent compound content from the fourth storage month onwards denotes the end of the induction period in MR development. Thus, considering that the shelf-life of the product is one year, the control of storage temperature and time is of interest to maintain the good quality of the product.

Acknowledgements

References

Birlouez-Aragon, I., Nicolas, M., Metais, A., Marchond, N., Grenier, J., & Calvo, D. (1998). A rapid fluorimetric method to estimate the heat treatment of liquid milk. *International Dairy Journal*, 8, 771-777.

Birlouez-Aragon, I., Sabat, P., Lutz, B., Leclère, J., & Nicolas, M. (1999). L'addition de lactose et de vitamine C dans les laits de croissance diminue la qualité nutritionnelle des protéines. *Lait*, 79, 595-606.

Birlouez-Aragon, I., Leclère, J., Quedraogo, C. L., Birlouez, E., & Grongnet, J. F. (2001). The FAST method, a rapid approach of the nutritional quality of heat-treated foods. *Nahrung/Food*, 45 (3), 201-205.

Birlouez-Aragon, I., Sabat, P., & Gouti, N. (2002). A new method for discriminating milk heat treatment. *International Dairy Journal,* 12, 59-67.

Birlouez-Aragon, I., Pischetsrieder, M., Leclère, J., Morales, F. J., Hasenkopf, K., Kientsch-Engel, R., Ducauze, C. J., & Rutledge, D. (2004). Assessment of protein glycation markers in infant formulas. *Food Chemistry*, 87, 253-259.

Birlouez-Aragon, I., Locquet, N., Louvent, E. D., Bouveresse, D. J. R., & Stahl, P. (2005). Evaluation of the Maillard reaction in infant formulas by means of front-face fluorescence. *Annals of the New York Academy of Sciences,* 1043, 308-318.

Calvo, C. (2004). Optical properties. In L. M. L. Nollet, *Handbook of food analysis. Physical characterization and nutrient analysis*, vol. 1 (pp.1-19). New York: Marcel Dekker, Inc.

Fernández-Artigas, P., Guerra-Hernández, E., & García-Villanova, B. (1999). Browning indicators in model systems and baby cereals. *Journal of Agricultural and Food Chemi*stry, 47, 2872-2878.

Ferrer, E., Alegría, A., & Farré, R. (2000). Advanced glycosilation end products (AGEs) and colour in milk and milk resembling systems. A review. *Recent Research Developments in Agricultural & Food Chemistry,* 4, 269-290.

14

Ferrer, E., Alegría, A., Farré, R., Clemente, G., & Calvo, C. (2005). Fluorescence, browning index, and color in infant formulas during storage. *Journal of Agricultural and Food Chemistry,* 53, 4911-4917.

Gliguem, H., & Birlouez-Aragon, I. (2005). Effects of sterilization, packaging, and storage on vitamin C degradation, protein denaturation, and glycation in fortified milks. *Journal of Dairy Science*, 88, 891-899.

Guan, R., Liu, D., Ye, X., & Yang, K. (2005). Use of fluorometry for determination of skim milk powder adulteration in fresh milk. *Journal of Zhejiang University SCIENCE*, 6B (11), 1101-1106.

Guerra-Hernández, E., Leon, C., Corzo, N., García-Villanova, B., & Romera, J. M. (2002). Chemical changes in powdered infant formulas during storage. *International Journal of Dairy Technology*, 55, 171-176.

Jobson, J.D. (1991). Multiple Linear Regression. In *Applied multivariate data analysis*, vol.1 (pp.314-317). New York: Springer-Verlag.

Kessler, H.G., & Fink, R. (1986). Changes in heated and stored milk with an interpretation by reaction kinetics. *Journal of Food Science*, 51, 1105-1111.

Kulmyrzaev, A., & Dufour, E. (2002). Determination of lactulose and furosine in milk using front-face fluorescence spectroscopy. *Lait*, 82, 725-735.

Leclère, J., & Birlouez-Aragon, I. (2001). The fluorescence of advanced Maillard products is a good indicator of lysine damage during the Maillard reaction. *Journal of Agricultural and Food Chemi*stry, 49, 4682-4687.

Leclère, J., Birlouez-Aragon, I., & Meli, M. (2002). Fortification of milk with iron-ascorbate promotes lysine glycation and tryptophan oxidation. *Food Chemistry*, 76, 491-499.

Matiacevich, S. B., Santagapita, P. R., & Buera, M. P. (2005). Fluorescence from the Maillard reaction and its potential applications in food science. *Critical Reviews in Food Science and Nutrition,* 45, 483-495.

Matiacevich, S.B., & Buera, M. P. (2006). A critical evaluation of fluorescence as a potential marker for the Maillard reaction. *Food Chemistry,* 95, 423-430.

Morales, F. J., Romero, C., & Jiménez-Pérez, S. (1996). Fluorescence associated with Maillard reaction in milk and milk-resembling systems. *Food Chemi*stry, 57 (3), 423-428.

Morales, F. J., & van Boekel, M. A. J. S. (1997). A study on advanced Maillard reaction in heated casein/sugar solutions: fluorescence accumulation. *International Dairy Journal*, 7, 675-683.

O´Brien, J., & Morrissey, P. A. (1989). Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Critical Reviews in Food Science and Nutrition*, 28 (3), 211-248.

Pagliarini, E., Vernile, M., & Peri, C. (1990). Kinetic study on color changes in milk due to heat. *Journal of Food Science,* 55, 1766-1767.

Ramírez-Jiménez, A., Guerra-Hernández, E., & García-Villanova, B. (2003). Evolution of non-enzymatic browning during storage of infant rice cereal. *Food Chemistry*, 83, 219-225.

Ramírez-Jiménez, A., García-Villanova, B., & Guerra-Hernández, E. (2004). Nonenzymatic browning during storage of infant cereals. *Cereal Chemistry*, 81 (3), 399-403.

Rampilli, M., & Andreini, R. (1992). Evaluation of colour components in sterilized milk. *Italian Journal of Food Science*, 4, 285-291.

Rossi, M., & Pompei, C. (1991). Changes in liquid milk-based infant formulas during long-term storage. *Italian Journal of Food Sci*ence, 1, 49-57.

Siegl, T., Schwarzenbolz, U., & Henle, T. (2000). Irreversible casein oligomerisation and formation of fluorescent crosslink amino acids in dairy products. *Czech Journal of Food Science*, 18, 72-73.

Van Boekel, M. A. J. S. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry,* 62 (4), 403-414.

Table 1. Free and total pentodilysine[1] in the three (A, B, C) milk-cereal based infant foods analyzed, expressed as % FI (fluorescence intensity) with respect to the fluorescence of 7.5 µg/ml quinine sulfate standard.

	month	A			B			C		
Free	0	10.7 ± 0.1^a			12.3 ± 0.8^a			11.8 ± 0.2^a		
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	11.7 ± 0.4^b	11.5 ± 0.7^b	12.2 ± 0.3^b	13.0 ± 0.1^b	13.6 ± 0.2^b	14.9 ± 0.8^b	13.8 ± 0.3^b	14.1 ± 0.6^b	15.4 ± 0.4^b
	2	11.0 ± 0.3^b	11.8 ± 0.1^b	12.2 ± 0.1^b	12.7 ± 0.1^b	13.5 ± 0.2^b	15.0 ± 0.5^b	13.5 ± 0.6^b	14.8 ± 0.4^b	15.7 ± 0.1^b
	4	10.6 ± 0.3^c	13.0 ± 0.5^c	13.4 ± 0.3^c	14.0 ± 0.2^c	15.5 ± 0.3^c	18.5 ± 0.9^c	13.8 ± 0.3^c	16.6 ± 0.8^c	17.3 ± 0.3^c
	5	12.4 ± 0.5^d	14.6 ± 0.4^d	16.2 ± 0.3^d	13.0 ± 0.2^d	15.7 ± 0.2^d	18.1 ± 0.4^d	14.1 ± 0.8^d	16.9 ± 0.7^d	18.5 ± 0.7^d
	6	13.4 ± 0.8^e	16.8 ± 0.5^e	19.9 ± 0.9^e	13.9 ± 0.2^e	16.7 ± 0.4^e	20.4 ± 0.5^e	15.1 ± 0.6^e	18.5 ± 0.3^e	21.5 ± 0.5^e
	7	11.4 ± 0.6^d	14.4 ± 0.1^d	17.0 ± 0.5^d	12.4 ± 0.3^d	15.1 ± 0.4^d	18.7 ± 0.3^d	12.6 ± 0.2^d	16.5 ± 0.2^d	19.3 ± 0.3^d
	8	10.2 ± 0.2^f	13.3 ± 0.2^f	16.8 ± 0.2^f	14.7 ± 0.3^f	18.4 ± 0.6^f	22.2 ± 0.5^f	14.2 ± 0.6^f	17.7 ± 0.2^f	21.0 ± 0.4^f
	9	$11.8\pm0.6^{e,f}$	$14.3\pm0.3^{e,f}$	$18.5\pm0.4^{e,f}$	$14.5\pm0.2^{e,f}$	$18.5\pm0.3^{e,f}$	$23.0\pm0.3^{e,f}$	$14.0\pm0.6^{e,f}$	$16.3\pm0.5^{e,f}$	$21.0\pm0.4^{e,f}$
Total	0	106.4 ± 2.8^g			142.7 ± 5.5^g			118.4 ± 2.7^g		
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	121.1 ± 3.7^h	127.0 ± 2.0^h	153.8 ± 6.7^h	180.6 ± 2.4^h	197.1 ± 3.6^h	215.5 ± 3.0^h	142.7 ± 1.5^h	149.1 ± 6.2^h	167.9 ± 5.7^h
	2	115.6 ± 2.6^h	130.5 ± 2.8^h	160.7 ± 5.6^h	167.5 ± 5.3^h	183.2 ± 7.1^h	207.6 ± 6.1^h	133.5 ± 3.7^h	152.6 ± 2.3^h	182.6 ± 9.3^h
	4	140.6 ± 5.5^i	181.6 ± 5.6^i	210.9 ± 3.9^i	166.9 ± 4.1^i	211.9 ± 5.1^i	246.6 ± 3.2^i	136.8 ± 2.3^i	190.1 ± 2.0^i	208.1 ± 3.6^i
	5	124.4 ± 1.3^i	178.9 ± 1.6^i	206.6 ± 2.6^i	168.0 ± 1.4^i	226.5 ± 10.4^i	275.1 ± 7.2^i	139.5 ± 2.5^i	204.4 ± 6.7^i	232.0 ± 3.0^i
	6	131.9 ± 2.2^j	186.9 ± 5.6^j	232.6 ± 5.8^j	190.2 ± 2.8^j	257.5 ± 4.4^j	308.2 ± 7.8^j	146.6 ± 1.2^j	226.3 ± 5.9^j	260.7 ± 6.2^j
	7	156.7 ± 4.9^k	217.2 ± 4.7^k	260.0 ± 7.3^k	194.7 ± 3.2^k	265.4 ± 5.5^k	339.5 ± 13.0^k	151.0 ± 4.2^k	225.1 ± 3.9^k	271.6 ± 6.2^k
	8	159.6 ± 10.6^l	220.0 ± 5.4^l	273.0 ± 3.1^l	205.3 ± 7.5^l	280.2 ± 9.2^l	354.7 ± 6.7^l	188.2 ± 13.5^l	243.0 ± 11.1^l	291.2 ± 12.0^l
	9	$145.1\pm10.0^{k,l}$	$206.3\pm4.6^{k,l}$	$267.6\pm8.6^{k,l}$	$210.6\pm13.2^{k,l}$	$281.6\pm12.1^{k,l}$	$347.7\pm19.7^{k,l}$	$169.6\pm6.6^{k,l}$	$231.3\pm11.0^{k,l}$	$289.0\pm11.6^{k,l}$

[1]Mean± standard deviation (n=4)

A: milk-cereal based infant foods; B: milk-cereal based infant foods with 0.9% honey; C: milk-cereal based infant foods with 1.1% fruits.

No coincidence in the superscript letters of the same column indicates significant differences (p<0.05) with storage time.

Table 2. Free and total AGEs compounds[1] in the three (A, B, C) milk-cereal based infant foods analyzed, expressed as % FI (fluorescence intensity) with respect to the fluorescence of 0.5 µg/ml quinine sulfate standard.

|	month	A			B			C		
Free	0	8.7 ± 0.1^a			10.0 ± 0.3^a			9.7 ± 0.2^a		
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	9.9 ± 0.4^b	9.4 ± 0.1^b	9.9 ± 0.1^b	10.0 ± 0.1^b	10.6 ± 0.4^b	11.1 ± 0.3^b	10.2 ± 0.4^b	10.3 ± 0.2^b	10.8 ± 0.3^b
	2	9.1 ± 0.3^b	9.5 ± 0.2^b	9.8 ± 0.1^b	10.2 ± 0.3^b	10.7 ± 0.1^b	11.7 ± 0.4^b	10.5 ± 0.5^b	11.4 ± 0.3^b	12.0 ± 0.4^b
	4	8.9 ± 0.2^c	10.7 ± 0.5^c	10.9 ± 0.3^c	11.7 ± 0.1^c	12.6 ± 0.2^c	14.8 ± 0.7^c	11.0 ± 0.2^c	12.8 ± 0.5^c	13.7 ± 0.3^c
	5	9.7 ± 0.1^c	11.1 ± 0.4^c	12.4 ± 0.1^c	10.7 ± 0.2^c	12.9 ± 0.6^c	14.1 ± 0.1^c	11.0 ± 0.7^c	12.8 ± 0.3^c	14.1 ± 0.5^c
	6	11.0 ± 0.4^d	13.5 ± 0.5^d	15.8 ± 0.4^d	11.2 ± 0.7^d	12.8 ± 0.3^d	15.5 ± 0.4^d	12.0 ± 0.2^d	14.4 ± 0.2^d	16.9 ± 0.3^d
	7	9.4 ± 0.2^c	11.8 ± 0.2^c	13.9 ± 0.3^c	9.6 ± 0.9^c	11.1 ± 0.3^c	13.7 ± 0.2^c	9.1 ± 0.2^c	12.1 ± 0.1^c	14.2 ± 0.2^c
	8	$7.4\pm.0.2^e$	9.9 ± 0.2^e	12.7 ± 0.1^e	11.5 ± 0.2^e	14.4 ± 0.4^e	17.8 ± 0.3^e	11.6 ± 0.1^e	14.6 ± 0.2^e	17.3 ± 0.3^e
	9	10.1 ± 0.6^f	12.3 ± 0.4^f	15.8 ± 0.4^f	11.4 ± 0.2^f	14.5 ± 0.2^f	17.9 ± 0.2^f	12.9 ± 0.8^f	15.1 ± 0.2^f	19.4 ± 0.3^f
Total	0	64.7 ± 0.8^g			82.7 ± 4.5^g			72.8 ± 1.7^g		
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	72.2 ± 2.5^h	74.9 ± 1.5^h	87.3 ± 5.8^h	93.3 ± 2.2^h	100.3 ± 1.7^h	114.5 ± 4.7^h	82.3 ± 1.6^h	85.3 ± 3.2^h	94.0 ± 1.4^h
	2	65.7 ± 1.7^h	72.5 ± 2.2^h	89.0 ± 6.2^h	88.6 ± 3.8^h	95.0 ± 3.4^h	107.7 ± 2.8^h	75.8 ± 2.9^h	86.3 ± 1.4^h	98.9 ± 5.5^h
	4	69.7 ± 4.1^i	87.8 ± 2.8^i	101.6 ± 2.0^i	99.1 ± 1.6^i	125.7 ± 4.1^i	148.0 ± 2.4^i	81.8 ± 2.3^i	110.0 ± 2.1^i	117.4 ± 2.5^i
	5	73.2 ± 0.7^j	100.2 ± 1.5^j	116.0 ± 2.0^j	101.8 ± 1.1^j	133.6 ± 6.7^j	162.0 ± 4.3^j	85.5 ± 1.9^j	121.8 ± 7.1^j	135.2 ± 3.2^j
	6	77.9 ± 3.0^k	103.9 ± 3.0^k	126.5 ± 3.0^k	102.8 ± 0.9^k	133.6 ± 5.9^k	160.1 ± 5.4^k	91.1 ± 1.4^k	132.1 ± 3.9^k	152.9 ± 4.2^k
	7	82.6 ± 2.8^l	107.7 ± 4.3^l	133.7 ± 6.2^l	110.2 ± 5.1^l	145.6 ± 4.0^l	185.8 ± 7.3^l	89.7 ± 3.6^l	127.7 ± 4.1^l	156.4 ± 4.1^l
	8	96.1 ± 6.1^m	128.6 ± 4.1^m	156.7 ± 1.8^m	115.6 ± 5.6^m	153.8 ± 6.2^m	192.9 ± 7.4^m	114.1 ± 11.7^m	145.4 ± 8.9^m	174.8 ± 7.9^m
	9	94.1 ± 7.1^m	130.0 ± 4.2^m	171.1 ± 7.3^m	129.8 ± 8.5^m	167.5 ± 6.4^m	211.1 ± 14.3^m	101.3 ± 4.8^m	134.3 ± 7.0^m	166.5 ± 8.1^m

[1]Mean± standard deviation (n=4)

A: milk-cereal based infant foods; B: milk-cereal based infant foods with 0.9% honey; C: milk-cereal based infant foods with 1.1% fruits

No coincidence in the superscript letters of the same column indicates significant differences (p<0.05) with storage time.

Table 3. Mean values (n=4) of L*, a* and b* color parameters for the three (A, B, C) milk-cereal based infant foods analyzed.

A: milk-cereal based infant foods

B: milk-cereal based infant foods with 0.9% honey

C: milk-cereal based infant foods with 1.1% fruits

|	month	A			B			C		
L*	0		80.67			77.63			77.25	
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	80.57	80.29	79.83	77.43	76.82	76.31	77.61	77.57	76.81
	2	80.36	79.77	79.10	77.22	76.80	76.01	77.66	77.27	76.66
	4	79.95	79.02	78.32	76.66	75.98	74.39	77.23	76.56	75.78
	5	79.87	78.50	77.79	76.50	74.92	73.70	76.93	76.08	75.10
	6	79.54	78.12	76.97	76.16	74.66	73.20	76.76	75.69	74.71
	7	78.38	76.64	75.34	75.57	74.11	72.11	75.78	74.66	73.43
	8	78.13	75.89	74.68	75.30	73.04	70.90	75.68	74.26	73.02
	9	78.63	76.57	75.08	75.20	72.89	71.71	76.22	75.17	73.42
a*	0		1.75			3.37			1.44	
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	2.75	2.96	3.37	4.25	4.67	5.19	2.54	2.73	3.33
	2	2.86	3.39	4.09	4.44	4.86	5.33	2.51	2.83	3.64
	4	2.76	3.58	4.19	4.33	5.02	6.11	2.42	3.26	4.04
	5	2.70	3.70	4.28	4.15	5.72	6.13	2.43	3.62	4.19
	6	2.89	3.92	4.84	4.83	5.91	6.93	2.50	3.71	4.49
	7	2.88	4.06	5.05	4.15	5.24	6.51	2.49	3.74	4.57
	8	3.11	4.65	5.62	4.62	6.00	7.32	2.78	4.24	5.23
	9	3.21	4.67	5.45	5.19	6.06	7.08	3.00	3.89	5.19
b*	0		18.90			21.59			16.44	
		25ºC	30ºC	37ºC	25ºC	30ºC	37ºC	25ºC	30ºC	37ºC
	1	20.68	21.11	22.55	23.34	23.56	25.18	18.45	19.25	20.67
	2	20.68	21.28	23.08	23.70	24.34	25.10	18.64	19.35	21.39
	4	21.33	23.21	24.65	24.50	25.71	28.17	19.25	21.44	23.40
	5	21.88	23.96	25.53	25.10	26.56	29.55	19.54	22.48	24.23
	6	21.68	23.80	25.93	24.06	26.23	28.00	19.70	23.13	25.07
	7	21.42	24.76	26.70	23.92	26.33	28.64	19.52	23.07	24.96
	8	21.44	24.56	26.75	24.06	26.25	29.38	19.72	23.02	24.67
	9	22.48	25.72	27.96	24.85	28.54	30.71	20.32	23.24	26.03

Fig. 1. Free and total AGEs and pentodilysine interactions temperature-type of sample (A, B and C) (a) and storage time-type of sample (b).
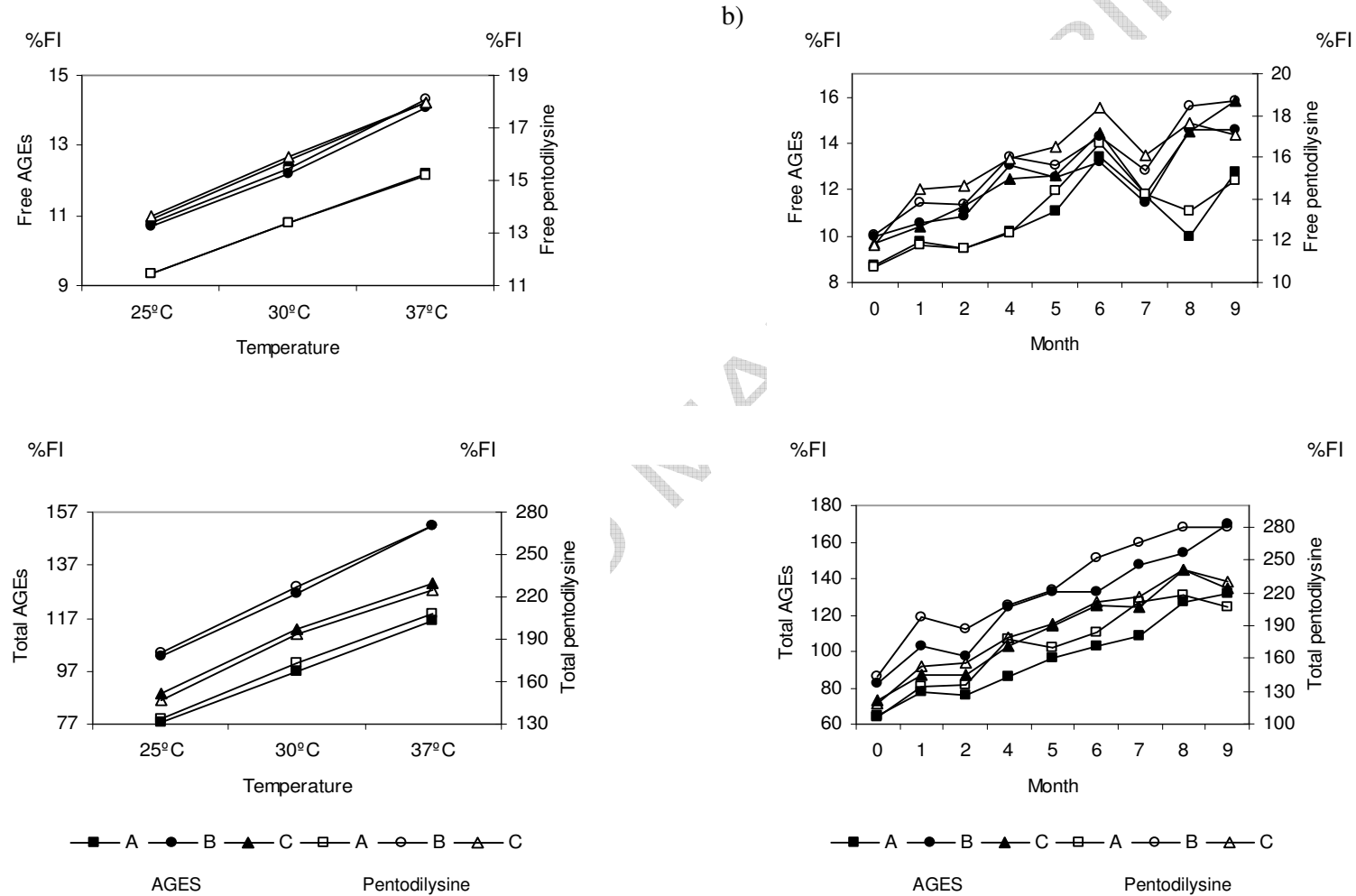
A: milk-cereal based infant foods; B: milk-cereal based infant foods with 0.9% honey; C: milk-cereal based infant foods with 1.1% fruits
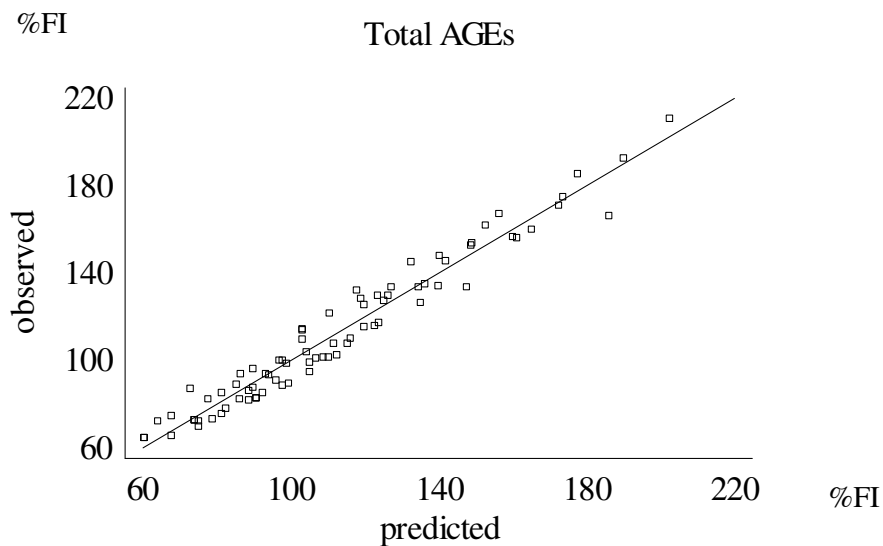
FI: Fluorescence intensity

Fig. 2. Observed versus predicted values for free and total AGEs and obtained regression equations of the models.
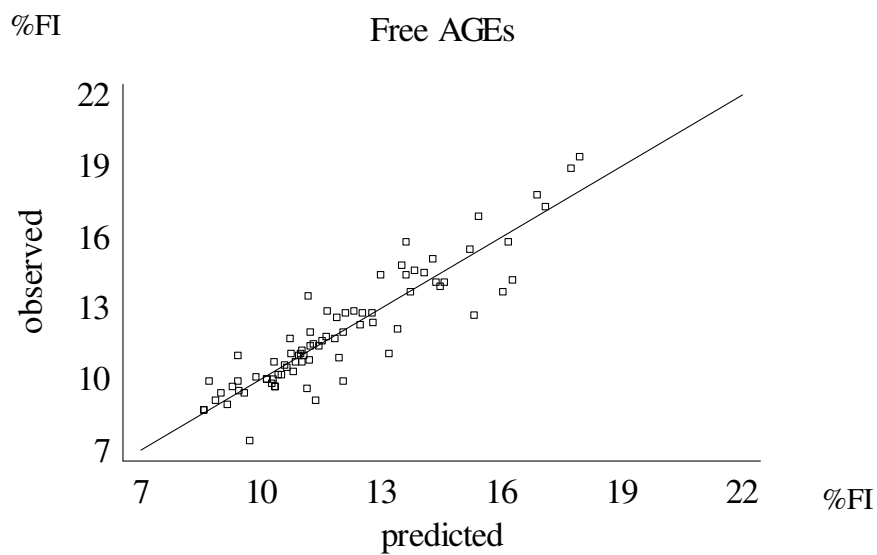
Free AGEs= 8.57 + 0.06temperature.month – 1.31month + 1.57sample 1 + 1.79sample 2
(R^2=84.5%)

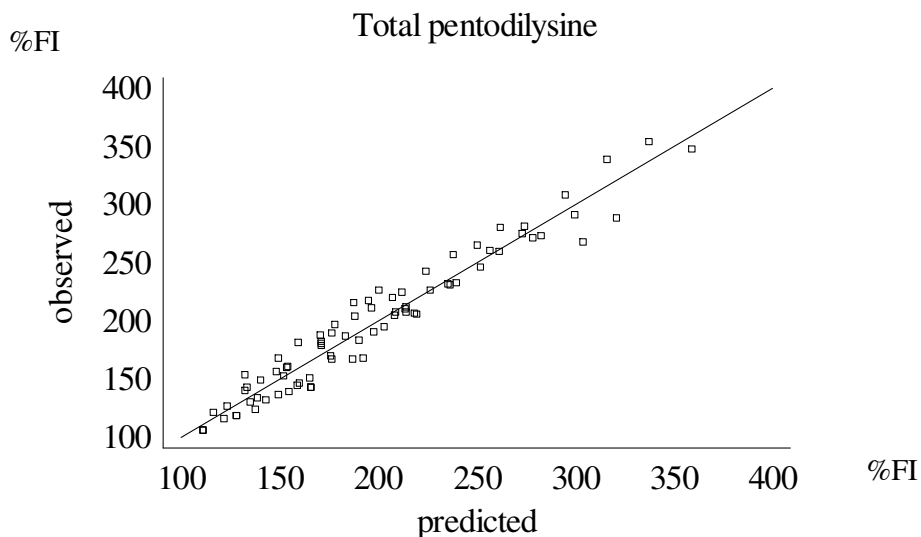Total AGEs= 60.33 + 0.73temperature.month – 14.66month + 30.13sample 1 + 13.55sample 2
(R^2=95.7%)

Fig. 3. Observed versus predicted values for free and total pentodilysine and equations of the model.

Free pentodilysine
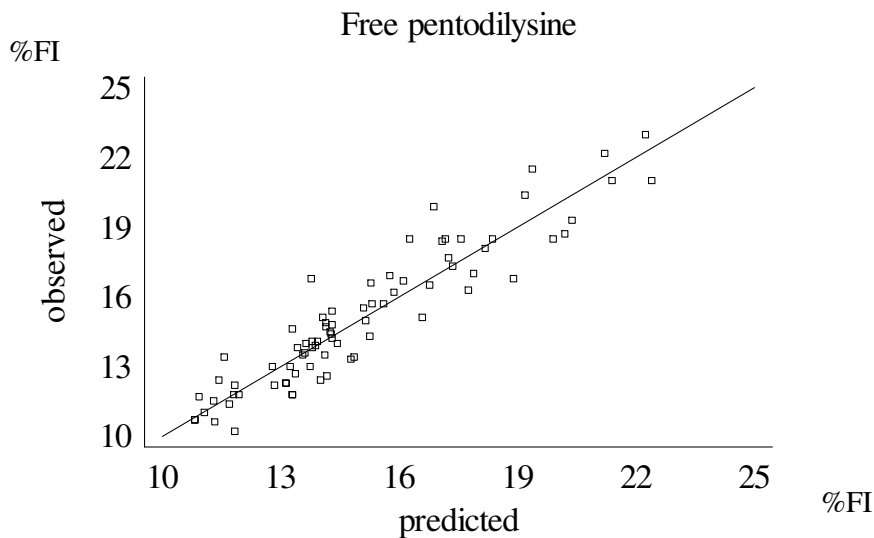
Free pentodilysine = 10.81 + 0.07temperature.month – 1.72month + 2.31sample 1 + 2.49sample 2 (R^2=87.6%)

Total pentodilysine

Total pentodilysine = 111.17 + 1.35temperature.month – 28.35month + 54.90sample 1 + 17.05sample 2 (R^2=94.5%)

Fig. 4. ΔE* in the three (A, B, C) milk-cereal based infant foods analyzed.

A: milk-cereal based infant foods; B: milk-cereal based infant foods with 0.9% honey; C: milk-cereal based infant foods with 1.1% fruits