# Fluorescence, Browning Index, and Color in Infant Formulas during Storage

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Free and total fluorescent compounds, browning index, and color formation were measured in milk-based powdered infant formulas (IF) during 2 years of storage at 20 and 37 °C. The excitation spectra from 415 nm emission show three peaks (ex $\lambda_1 = 270$ nm, $\lambda_2 = 325/315$ nm, $\lambda_3 = 350$ nm) and from 347 nm excitation two emission peaks (415 and 520 nm), and no wavelength shifts were observed. Temperature and time of storage exert in general no significant effect on the development of fluorescence emission intensity and browning index. However, an important increase in pentodilysine was recorded—probably because of the iron and ascorbic acid contents of the samples—as well as in browning index in adapted IF. In both IF a color increase ($\Delta E$) throughout storage was observed, this increase being greater in samples stored at 37 °C than in those stored at 20 °C. The increase in color with time fitted a linear regression model. Color appeared to be an indicator of sufficient sensitivity to measure the effect of temperature or storage time.

KEYWORDS: Infant formulas; storage; fluorescence; color; browning index

## INTRODUCTION

Infant formulas (IF) combine factors favoring the development of the Maillard reaction (MR), such as their composition (high lactose and protein contents), the thermal treatment applied, and long storage periods, often under adverse humidity and temperature conditions. The MR affects milk protein quality (blockage of lysine) and gives rise to compounds responsible for color and flavor changes and also to compounds with varied activities (*1*, *2*). In the final stage of the MR, rearrangement of the Amadori products, which undergo dehydration and fission reactions, yields colorless reductones as well as fluorescent compounds—some of which can also be colored (*2–4*).

Given the possible effect of thermal treatment on color, changes in the latter have been evaluated in milk and dairy products (*5–8*) and in milk-resembling systems (*9*, *10*). The effects of storage on the color of milk and dairy products (*11–13*) and model systems (*5*, *10*, *11*) have also been studied. However, studies involving powdered and liquid IF that have investigated the effect of storage conditions on browning index (*14*) or color (*15–18*) are scarce. Recently, a review on the color of milk and milk products has been published (*19*).

Simultaneously to the browning process, Amadori products can form cross-links between adjacent proteins or with other amino groups, giving rise to polymeric aggregates or so-called advanced glycation end products (AGEs) (*1*, *20*, *21*). Such AGEs include a very complex combination of structures, only a few of which have been elucidated. The intensity of fluorescence ($\lambda_{ex} = 340–370$ nm and $\lambda_{em} = 420–440$ nm) of AGEs products is widely used as a marker of the level of AGE-modified protein (*3*, *22*). In this context, some authors have suggested the possibility of using free fluorescent (not bound to protein) compound values as a heat-induced index to evaluate heat damage in processed food—in the same way as the browning index at 420 nm (*21*).

The routes of specific AGE compound formation in milk were reviewed by Van Boekel et al. (*2*), who indicated the need to assess the relevance of cross-linked compounds in foods. A review of AGEs and color in model systems and in milk and milk-resembling systems was published by Ferrer et al. (*4*).

Fluorescence in milk and milk-resembling systems has been measured at $\lambda_{ex} = 347$ nm and $\lambda_{em} = 415$ nm (*3*, *20*, *21*, *23*) and also at other wavelengths in dairy products (*7*, *24*) and model systems (*25–28*).

Studies of the MR, color, and fluorescence in milk and model solutions have mainly focused on the effect of thermal treatment. To our knowledge, studies on the effect of IF storage upon color evolution are scarce, and none address fluorescence changes.

In Spain, infant formulas are mainly used in the powdered form. The adapted ones (infant milks) are used to feed infants up to 4–6 months of life, after which follow-up formulas are

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used and constitute the main liquid food in a progressively diversified diet.

With the ultimate aim of evaluating the development of advanced and final MR, free and total fluorescent compounds, browning index, and color formation were measured in powdered adapted and follow-up IF during 2 years of storage at 20 and 37 °C. An additional study endpoint was to define the best indicator of MR progress during storage, with the aim of proposing it as a routine index.

## EXPERIMENTAL PROCEDURES

**Reagent and chemicals** were obtained as follows: quinine sulfate solution (100 $\mu$g/mL) prepared in 0.1 N sulfuric acid (Merck, Darmstadt, Germany); Pronase E solution (5 units/mg) (Pronase from *Streptomyces griseus*, Fluka, Buchs, Switzerland) prepared in pH 7.2 Tris buffer solution (Aldrich, Buchs, Switzerland; 99.8%); phosphate saline buffer (pH 7), 20 mM, and 15 mM NaCl (Panreac, Barcelona, Spain); 24% (w/v) trichloroacetic acid (Fluka).

**Samples.** Two milk-based powdered IF (respectively, adapted and follow-up) were analyzed. In the manufacture of both IF, the same raw cow's milk was used and identical thermal treatments were applied (*29*). The compositions of the IF studied, as stated on the labeling, were as follows: adapted IF, 11.6% proteins (casein/serum proteins 40:60), 55% carbohydrates (lactose), 28% lipids; follow-up IF, 16% proteins (casein/serum proteins 80:20), 54% carbohydrates (32.4% lactose + 21.6% maltodextrin), 24% lipids. Samples were stored at 20 and 37 °C in a storage chamber [<10% relative humidity (RH) and temperature controlled]. Color was measured every 3 months during the 2 storage years. Free and total fluorescence and browning index were determined every 3 months during the second storage year. Samples were kept in their airtight containers until analysis.

**Fluorescence and Browning Index Determination.** Powdered IF were reconstituted (20% w/v) to obtain a solution containing 0.02–0.05 g of protein/mL.

To measure free fluorescence and browning index, the proteins of 2 mL of reconstituted sample were precipitated with 2 mL of 24% w/v trichloroacetic acid.

To measure total fluorescence and browning index, a modification of the enzymatic hydrolysis proposed by Palombo et al. (*30*) was applied as follows: to 1.5 mL of reconstituted sample in a stoppered test tube was added 0.4 mL of Pronase solution (20 units/mL), followed by incubation with shaking at 25 °C for 30 min.

Solutions corresponding to free and total AGEs were centrifuged at 13000*g* for 10 min at room temperature, and 100 $\mu$L of the resulting supernatants was diluted in 5 mL of saline buffer (pH 7.2).

Fluorescence values were measured at the following excitation and emission wavelengths, reported by different authors for the different identified AGEs compounds (*3*): AGE ($\lambda_{ex} = 347$ nm; $\lambda_{em} = 415$ nm); pentosidine ($\lambda_{ex} = 335$ nm; $\lambda_{em} = 385$ nm); pentodilysine ($\lambda_{ex} = 366$ nm; $\lambda_{em} = 440$ nm); cross-link ($\lambda_{ex} = 379$ nm; $\lambda_{em} = 463$ nm); pyrropyridine ($\lambda_{ex} = 370$ nm; $\lambda_{em} = 455$ nm); argpyrimidine ($\lambda_{ex} = 320$ nm; $\lambda_{em} = 382$ nm). Spectrofluorometer (fluorometer Shimadzu RF-5000) settings were as follows: slits emission, 15 mm; excitation, 5 mm; integration time scan, 0.1 s; and front-face spectrum.

Excitation ($\lambda_{ex} = 200-400$ nm; $\lambda_{em} = 415$ nm) and emission ($\lambda_{em} = 400-600$ nm; $\lambda_{ex} = 347$ nm) spectra were recorded at different storage time points. Results were expressed as fluorescence–excitation or fluorescence–emission intensity percentage (%FI, respectively) per gram of sample, respectively, with respect to the fluorescence–excitation or fluorescence–emission of a 0.2 $\mu$g/mL quinine sulfate standard.

The browning index was measured at 420 and 520/430 nm (Perkin-Elmer Lambda 2 spectrophotometer).

**Color Determination.** The color of powdered samples was measured using a Hunter Labscan II colorimeter. Results were expressed according to the CIELAB system with reference to illuminant D65 and a visual angle of 10°T.

All analyses were carried out in triplicate.

**Statistical Analysis.** Triple-factor analysis of variance (temperature, time, and type of IF) was performed to analyze the influence of the three factors upon sample fluorescence. For 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.

The possible effect of storage time on browning index was examined by respectively applying simple and multiple regression analysis to color and absorbance values at 420 nm.

The Statgraphics plus 3.0 statistical package was used throughout.
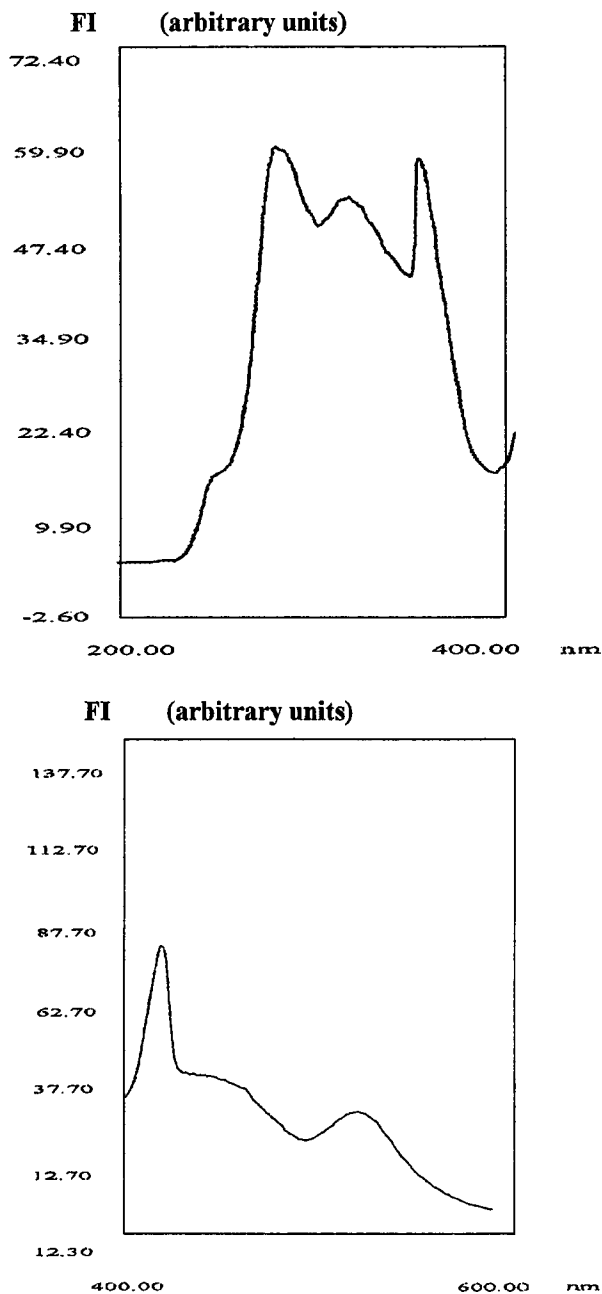
## RESULTS AND DISCUSSION

**Optimization of Total Fluorescence and Browning Index Measures.** Taking as starting point the method described by Palombo et al. (*30*), the amount of Pronase and the incubation time to be used in total fluorescence and browning index determination were selected for the IF analyzed. Enzyme amounts of 8, 16, 20, and 24 units yielded similar fluorescence intensities and optical densities; we thus selected the lowest Pronase amount (8 units).

The incubation temperature was reduced from 45 to 25 °C, and a wide range of hydrolysis times (30, 40, 60, 80, and 100 min and 2, 4, 6, 8, and 10 h) was assayed to ensure the release of all possible fluorescent compounds or pigments bound to proteins. Similar fluorescence intensities and optical densities were obtained with all assayed times; an incubation time of 30 min was therefore selected.

The method applied used a lower temperature and half the incubation time reported by Palombo et al. (*30*) for dehydrated dairy powders, although the studied adapted and follow-up IF had protein (11.6 and 16%, with casein contributions of 40 and 80%, respectively) and lactose (55 and 32.4%, respectively) contents within the wide ranges of protein (8–26%) and sugar (38.2–76.35%) contents described by Palombo et al. (*30*). Differences in the manufacturing processes could justify the lower temperature and incubation time required for the release of fluorescence and color compounds in our assay. It has been reported that the extent of fluorescent compound formation is mainly related to the intensity of the thermal process applied—temperature and heating time being important factors in this sense (*21*).

**Free and Total Fluorescence.** Excitation and emission spectra corresponding to free and total fluorescence in the adapted and follow-up IF are similar. In **Figure 1** are shown excitation and emission spectra corresponding to free fluorescence in the adapted IF. These spectra show three excitation peaks from emission of 415 nm, $\lambda_1 = 270$ nm, $\lambda_2 = 325/315$ nm, and $\lambda_3 = 350$ nm, and two emission peaks from excitation of 347 nm, a high peak at 415 nm and a lower one at 520 nm. No wavelength shifts were observed as a consequence of either storage time or storage temperature, in contrast to Morales and Van Boekel (*3*), who in milk-resembling systems (lactose or glucose with casein) reported a shift in the emission spectra of the free fluorescent compounds with increasing heating time— this shift being attributable to changes in fluorogenic structures. In our study differences in storage temperature were relatively low (20 and 37 °C)—a fact that could explain the absence of a shift. To obtain changes in fluorogenic structures, higher temperatures are probably required.

In both IF the fluorescence–excitation and fluorescence–emission intensities corresponding to total fluorescence spectra was between 3 and 5 times higher (depending on the spectra and peak) than those corresponding to free fluorescence spectra. Fluorescence–emission intensity values due to total fluorescence spectrum were between 3 and 4.5 higher in each peak of the emission spectrum, and fluorescence–excitation values were 5

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**FI**   **(arbitrary units)**



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**Figure 1.** Excitation ($\lambda_{ex}$ = 200–400 nm; $\lambda_{em}$ = 415 nm) and emission ($\lambda_{em}$ = 400–600 nm; $\lambda_{ex}$ = 347 nm) spectra. FI = fluorescence intensity. Free fluorescence–excitation and free fluorescence–emission in free adapted IF precipitated with TCA and diluted with saline buffer (pH 7.2) are shown.

times higher in the three excitation spectrum peaks versus those of free fluorescence.

In model systems (lactose/casein) heated for 2, 5, 10, 15, and 20 min, Morales and Van Boekel (*3*) obtained free AGE spectra ($\lambda_{em}$ = 300−600 nm and $\lambda_{em}$ = 347 nm) with a fluorescence maximum at $\lambda$ < 400 nm, which increased with storage time. In total AGE spectra reported a maximum at $\lambda$ = 415 nm, regardless of the heating time. In fluorescence spectra of $\beta$-lactoglobulins stored for 2 and 10 days with lactulose or lactose, only one peak at 415 nm was reported (*26*). In the same way, in our study a peak at $\lambda$ = 415 nm in the emission spectra and one additional peak ($\lambda$ = 520 nm) not reported in the lactose−casein model system were obtained.

To progress in the interpretation of the changes at final stage MR, fluorometric measurements were carried out at wavelengths

reported in the literature (*3*), corresponding to different individual compounds, and also at 347/415 nm—which would correspond to all fluorescent compounds.

As can be seen in **Figure 2**, different fluorescent products covalently bound to proteins (total fluorescence) are quantitatively much more prevalent than free fluorescent compounds.

Temperature did not affect free fluorescence AGE values ($p$ < 0.05); however, total fluorescence AGE values were higher at 37 °C than at 20 °C, although the differences were of little significance as inferred from the following values: AGEs (9029 > 8517% IF/g of sample), cross-link (3336 > 3130% IF/g of sample), and argpyrimidine (7601 > 7063% IF/g of sample).

Between the adapted and follow-up IF, statistically significant differences ($p$ < 0.05) were found in only free fluorescence values—the latter being greater in follow-up than in adapted IF. This could be attributed to the higher protein and casein contents in follow-up IF, because use was made of the same milk and of a similar manufacturing process. In model systems the presence of casein favors the formation of fluorescence compounds when compared to systems containing only sugars such as lactose (*25*) or only glucose (*28*), in which fluorescence development is lower.

On the other hand, the fact that differences in only free fluorescent compounds among formulas were observed could be explained by the two different reactions proposed to account for the accumulation of fluorescence compounds in milk (*21*). From a quantitative point of view, the most important route is MR, but small amounts of compounds also originate from Lobry de Bruyn-Alberda van Ekenstein transformation or lactose isomerization. In this sense, Moreaux and Birlouez (*27*) found significantly higher fluorescence in high-lactose systems than in low-lactose formulations. Thus, free fluorescent compounds would be formed by MR, with limitations imposed by the casein content. In our study adapted and follow-up IF differed in casein content (lower in the former, which on the other hand had a higher lactose content). Fluorescent compounds originating from lactose could be formed, although they would remain bound to proteins, would be measured as total fluorescence, and would be higher in adapted IF richer in lactose than in follow-up IF.

A statistically significant effect of storage time on fluorescence values, with the exception of free fluorescence measured for pentosidine and argpyrimidine, was found. However, only two facts stand out. First, an increase (albeit moderate) was observed in free and total AGEs measured at $\lambda_{ex}$ = 347 nm and $\lambda_{em}$ = 415 nm (see **Figure 2**)—the total AGE increase being higher than the free AGE increase.

Second, during the considered storage period, an important increase in free and total fluorescent compounds (measured at $\lambda_{ex}$ = 366 nm and $\lambda_{em}$ = 440 nm, corresponding to pentodilysine) was observed (see **Figure 2**). Given the marked evolution of total pentodilysine fluorescence over time, a second-degree polynomial in one variable model was obtained to describe the relationship between pentodilysine ($y$-dependent variable) and time ($x$-independent variable). This fitted a model represented by the equation $y = 14669 - 1808x + 62x^2$ (correlation coefficient = 0.991). The statistic $R^2$ indicates that the model as fitted explains 98% of the variability in pentodilysine.

This is a product of the reaction between lysine residues and pentoses or ascorbic acid (*3, 31, 32*), and given that the analyzed IF did not contain pentoses, the formation of pentodilysine by MR was unlikely, so pentodilysine had to originate from different routes. The analyzed samples had added ascorbic acid; pentodilysine thus could originate from the reaction between ascorbic acid degradation products and lysine residues. Besides
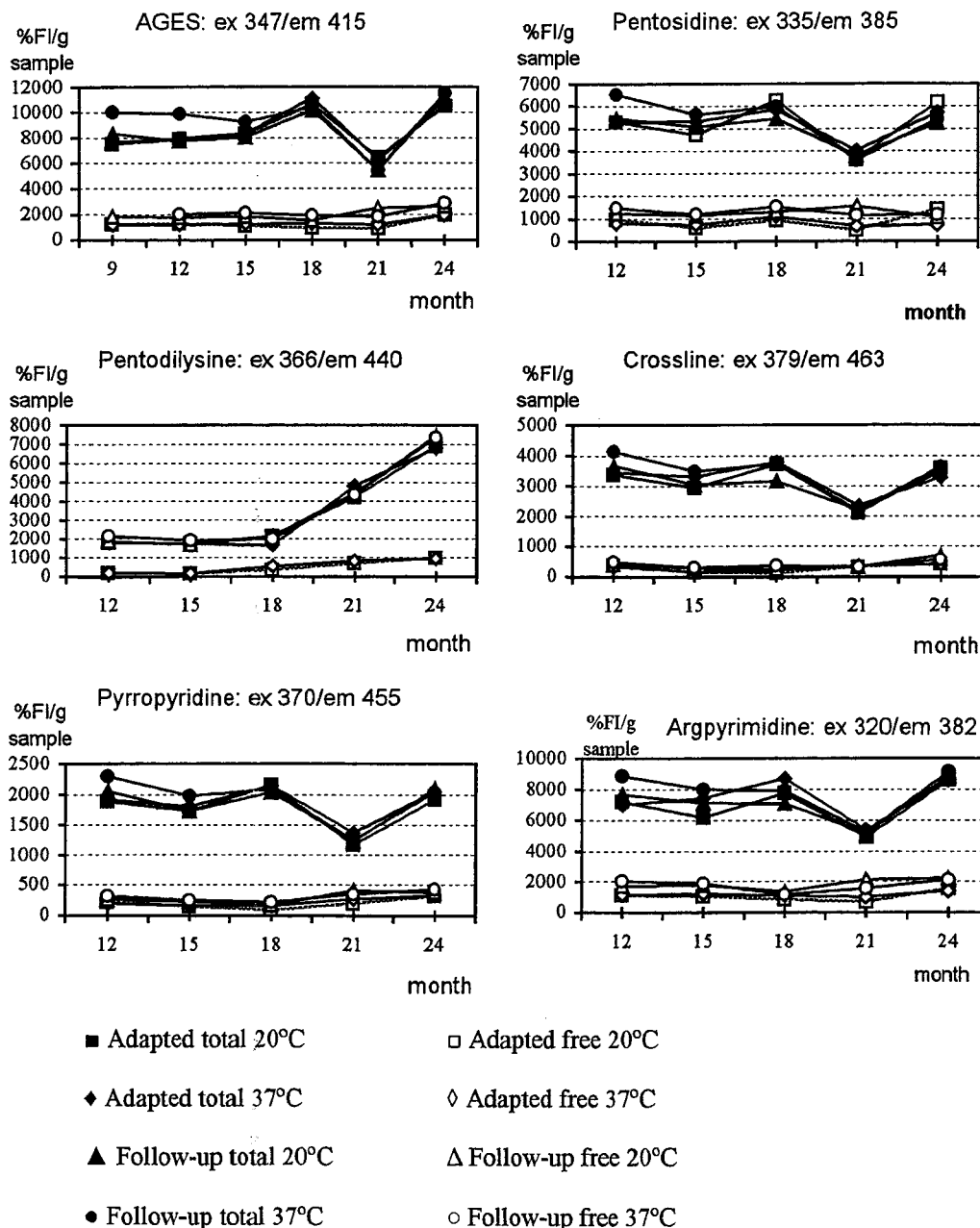
**Figure 2.** Fluorescence—emission (%IF/g of sample) of free and total different fluorescent compounds in adapted and follow-up IF stored at 20 and 37 °C.

this, the samples also had added iron, and the latter is known to accelerate ascorbate oxidation, yielding dehydroascorbate and degradation products with a strong glycating effect (*28*). The measurement of fluorescence ($\lambda_{ex} = 345$ nm; $\lambda_{em} = 412$ and 425 nm) in whey protein samples incubated with lactose (60 °C, 0−140 h) in the presence and absence of iron ascorbate yielded values 1.5-fold higher in ascorbylated whey proteins than in lactosylated whey proteins (*28*). A fluorescent condensation product formed between the ε-amino group of lysine and two molecules of threose (one of the major degradation products of ascorbate) has been identified (*33*).
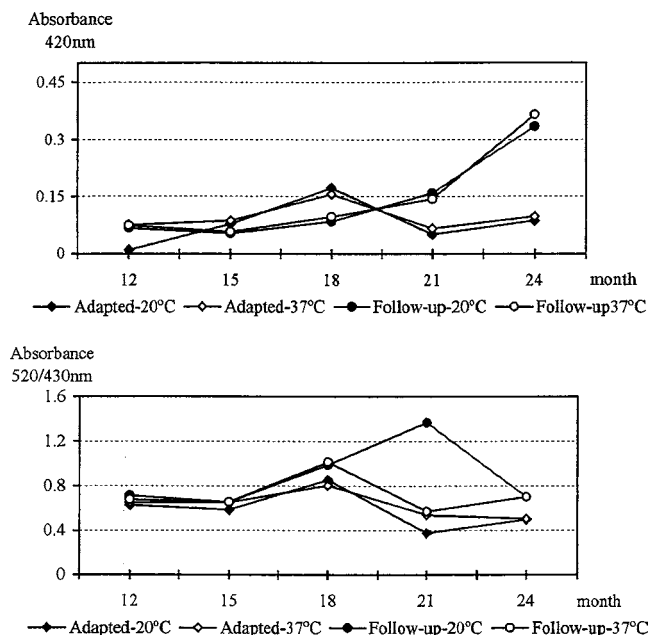
Pentosidine (see **Figure 2**) is a fluorescent cross-link molecule (a 5-carbon sugar covalently linking lysine to arginine). Pentosidine has been identified in systems including pentoses such as ribose and in small amounts in the presence of glucose, fructose, ascorbate, Amadori compounds, and 3-deoxyglucosone. It has been found in milk in very small amounts (*2*, *34*, *35*) and in milk subjected to a thermal treatment, originating from the

decomposition of the Amadori product, 3-deoxyosone pathway—an example of 1,2-enolization that occurs mainly at pH values of <7. This route is nonspecific for disaccharides and occurs mainly under acidic conditions; because milk pH is close to neutral, and the major milk sugar is lactose, pentosidine contents in milk will be low.

Siegl et al. (*24*) recorded in dairy products an increase in fluorescence intensity related to the thermal treatment and carbohydrate content and attributed it to cross-link formation with amino acids. However, in our study no noticeable changes in cross-links (see **Figure 2**) were found depending on storage time, temperature, or formula type—thus suggesting that these factors did not induce the formation of cross-links between amino acids during storage.

A review of the literature yielded no studies on pyrropyridine and argpyrimidine for the comparison of results. The lowest fluorescence intensity obtained corresponded to pyrropyridine, a product resulting from the interaction of 3-deoxyglucosone

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**Figure 3.** Browning index in adapted and follow-up IF stored at 20 and 37 °C.



**Figure 4.** Change of color through storage time in adapted and follow-up IF stored at 20 and 37 °C.

**Table 1.** Mean Values of $L^*$, $a^*$, and $b^*$ Color Parameters[a] for Adapted and Follow-up Infant Formula Storage, at 20 and 37 °C, for 2 Years

| | | adapted | | | follow-up | | |
|---|---|---|---|---|---|---|---|
| $T$ (°C) | month | $L^*$ | $a^*$ | $b^*$ | $L^*$ | $a^*$ | $b^*$ |
| 20 | 0 | 92.34 | −0.25 | 12.31 | 90.70 | −0.25 | 14.78 |
| | 3 | 92.06 | −0.36 | 12.67 | 90.51 | 0.29 | 14.97 |
| | 6 | 92.54 | −0.56 | 12.78 | 91.14 | −0.1 | 15.08 |
| | 9 | 92.37 | −0.42 | 12.82 | 90.91 | 0.06 | 15.20 |
| | 12 | 92.36 | −0.43 | 12.99 | 90.74 | 0.11 | 15.07 |
| | 15 | 92.52 | −0.46 | 12.66 | 91.00 | 0.04 | 15.11 |
| | 18 | 92.52 | −0.52 | 13.17 | 91.20 | −0.04 | 15.09 |
| | 21 | 92.49 | −0.59 | 13.17 | 91.18 | −0.16 | 15.22 |
| | 24 | 92.57 | −0.56 | 13.06 | 91.24 | −0.14 | 15.40 |
| 37 | 0 | 92.34 | −0.25 | 12.31 | 90.70 | −0.25 | 14.78 |
| | 3 | 92.08 | −0.39 | 13.29 | 90.55 | 0.11 | 15.60 |
| | 6 | 92.55 | −0.60 | 13.44 | 91.28 | −0.27 | 15.43 |
| | 9 | 92.35 | −0.37 | 13.57 | 91.11 | −0.15 | 15.73 |
| | 12 | 92.09 | −0.27 | 13.86 | 90.76 | −0.08 | 15.79 |
| | 15 | 92.17 | −0.22 | 13.95 | 91.07 | −0.18 | 16.05 |
| | 18 | 92.21 | −0.36 | 14.58 | 91.17 | −0.27 | 16.34 |
| | 21 | 92.27 | −0.41 | 14.45 | 91.20 | −0.38 | 16.59 |
| | 24 | 92.20 | −0.33 | 14.76 | 91.20 | −0.31 | 16.77 |

[a] $L^*$ (0, black; 100, white component, luminosity); $a^*$ (+, red; −, green component); $b^*$ (+, yellow; −, blue component).

with lysine. The 3-deoxyosone pathway is not specific for disaccharides (lactose) and occurs under acidic conditions; it is therefore not so important for milk (*2*). Argpyrimidine is formed by the reaction between methylglyoxal (MR intermediate product) and arginine residues (*3*).

**Browning Index.** Given that no absorbance was found after precipitation of the samples with trichloroacetic acid (free browning index), samples were subjected to Pronase digestion (total browning index), and the absorbances at 420 and 520/430 nm were then measured. Results are reported in **Figure 3**.
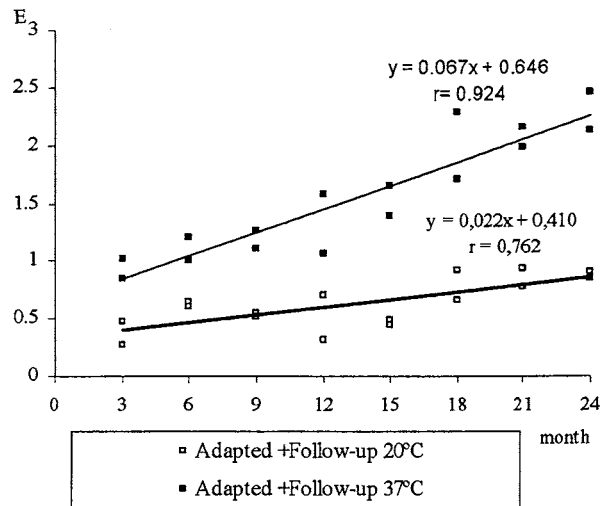
In adapted IF the browning index did not increase with storage time at either 20 or 37 °C. In contrast, in follow-up IF the absorbance at 420 nm increased with storage time, and a second-degree polynomial in one variable model was obtained to describe the relationship between absorbance at 420 nm (*y*-dependent variable) and time (*x*-independent variable). The equation corresponding to the fitted model is $y = 0.782 - 0.099x + 0.003x^2$ (correlation coefficient = 0.997). The $R^2$ statistic indicates that the model as fitted explains 99% of the browning index variability as a measure of absorbance at 420 nm.

As previously mentioned, adapted and follow-up IF differ in protein content, casein/seroprotein ratio, and lactose content, and these differences in composition could explain the different behaviors in browning development—the latter being more accentuated in follow-up IF, which have higher protein and casein contents, than in adapted IF.

From the absorbance values obtained in follow-up IF, no relationship can be established between the two proposed measures of browning index (absorbance at 420 and 520/430 nm).

The values obtained in this study do not coincide with those reported by Guerra-Hernández et al. (*14*), who in liquid IF stored in oxygen or nitrogen atmospheres observed an increase in absorbance at 420 nm after a short (90 days) storage period—although their storage temperature (55 °C) was higher than ours.

**Color.** To evaluate the color changes, the following equation was applied: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (*36*), where $L^*$ (black−white component, luminosity), $a^*$ (+, red; −, green component), and $b^*$ (+, yellow; −, blue component) values at

the considered storage time were taken into account with respect to the values obtained in just manufactured IF.

Values of $E$ obtained for adapted and follow-up IF stored at 20 and 37 °C for 2 years and the corresponding linear regression model are presented in **Figure 4**. Mean $L^*$, $a^*$, and $b^*$ values are reported in **Table 1**.

In both IF a color increase ($\Delta E$) throughout storage was observed. In all considered storage periods, $\Delta E$ was higher in samples stored at 37 °C than at 20 °C, the increments likewise being higher in samples stored at 37 °C. These color changes were in agreement with the losses of available lysine (MR) obtained in a previous study. During the second storage year a decrease in available lysine contents in adapted and follow-up formulas was found, those corresponding to follow-up formulas being higher. IF stored at 20 °C had higher available lysine contents than those stored at 37 °C (*37*).

To describe color evolution over time, two linear models are proposed, one for each storage temperature, because no significant differences were found between both samples at each

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temperature considered. Thus, a single predictive model was available for each assayed temperature. As can be seen (**Figure 4**), the slope (representative of color change) was greater in samples stored at 37 °C than in samples stored at 22 °C.

These values are in agreement with the kinetic studies carried out in milk heated at different temperatures (90−130 °C) (*6*), thus indicating that browning measured as *E* and as yellowness index (YI = 142.86*b/L*) follows zero-order kinetics, with an intercept at the ordinate and increasing with storage temperature.

The analysis of each of the equation components Δ*E* (*L*\*, *a*\*, *b*\*) (see **Table 1**) shows that the differences are attributable to components *a*\* and *b*\* (yellow/yellow-blue component), which are higher in samples stored at 37 °C than at 20 °C. This would agree with the study by Rampilli and Andreini (*12*), who indicated that storage conditions of sterilized milk can modify all color components—the rates of change being higher for *a* and *b* than for *L*.

In our study no changes in luminosity (*L*\*) were found, in contrast to other reports of an *L*\* decrease in strongly heated samples, attributed to the formation of brown pigments in the advanced stage of MR in sugar−casein mixtures (*10*) or in sterilized milk stored at 20 and 32 °C for 30 and 90 days (*12*).

Rossi and Pompei (*15*) measured Δ*E* in liquid IF stored at 4, 20, and 38 °C for 20 months. In coincidence with our own observations, they recorded an increase in Δ*E* throughout storage, due to the positive increase in component *b*\*. Values reported for samples stored at 4 and 20 °C were comparable to the values obtained in our own study at 20 and 37 °C, respectively. In comparison, much higher values (5−7-fold) have been reported for samples stored at 38 °C. Such discrepancies can be attributed to differences in the technological process applied in liquid IF manufacture (ultrahigh temperature at 140 °C for 24 s; in-container sterilized at 107 °C for 120 s) and also to the fact that liquid IF have a shorter shelf life than powdered formulations.

Values of *E* obtained in IF stored at 37 °C are comparable to those reported by Rampilli and Andreini (*12*) in different types of sterilized milk (direct and indirect UHT, in-bottle sterilized) stored at 20 and 32 °C for 30 and 90 days and in in-bottle sterilized milk stored at 20 °C. In turn, the values obtained are lower than those corresponding to samples kept at 32 °C. Thus, storage conditions (temperature and time) affect all color components.

Albalá-Hurtado et al. (*16, 17*) evaluated visible color changes in liquid and powdered IF and junior milk stored at 20, 30, and 37 °C for 9 months. In samples stored at 20 °C, no changes were reported after 1 year. However, samples stored at 30 and 37 °C showed a yellow-brown color after 5 months of storage, this effect being more intense in samples at 37 °C than in samples at 30 °C.

In samples similar to those of the present study, no significant changes in color were reported during storage at 20 °C in a nitrogen or oxygen atmosphere, although the period considered was much shorter (90 days). However, in the same samples at 55 °C, an increase in *E* value was recorded from 30 days of storage onward (*18*).

Temperature and storage time do not seem to exert a significant effect on the development of the different fluorescent compounds in the adapted and follow-up IF studied, with the exception of pentodilysine formation (probably because of the iron and ascorbic acid content of the samples), or on the browning index in adapted IF.

On the other hand, no correlation was observed between the measurement of browning index at 420 nm and at 520/340 nm.

Although browning index, color, or fluorescent compound measurement have been proposed to evaluate the effect of thermal treatment on the development of MR in milk and dairy products, in the case of powdered milk-based IF storage only color measurement appears to constitute a sufficiently sensitive indicator for assessing the effect of temperature (20−37 °C) or storage time (3 months).

Color changes together with the observed lysine losses (*37*) indicate the need for stricter control of storage temperature—it being advisable not to exceed 20 °C to avoid increased MR development. Minimization of storage time is also advised.

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