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The FAST index – A highly sensitive indicator of the heat impact on infant formula model

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ABSTRACT

Infant formulas are highly sensitive to the Maillard reaction during manufacturing, while this reaction induces significant loss in protein nutritional value and safety. The indicators mostly used to monitor the reaction during heat treatment are furosine, carboxymethyllysine and hydroxymethylfurfural, but analysis of these molecules is time-consuming and expensive. The FAST method, based on simple fluorescence measurements on clear milk supernatant, is a good alternative for Maillard reaction monitoring in milk products.

The aim of this study was to determine the respective sensitivity of the various indicators of heat damage to infant formula, including the FAST index. A realistic infant formula model was developed, to compare the reaction kinetics at different temperatures (80–110 °C) for lactulosyllysine, measured as furosine, hydroxymethylfurfural and carboxymethyllysine. By comparing the Arrhenius plots of the three Maillard products to that of the FAST index, the latter was identified as the most sensitive indicator for infant formula quality monitoring during heat treatment.

1. Introduction

Breast milk contains a perfect combination of nutrients, meeting the nutritional requirements of infants, especially when mothers are well-nourished. That is why human milk is considered to be the most appropriate and desirable infant nutrition. However, under some circumstances, substitution with infant formula (IF) is necessary. As production of optimally adapted IF has been a challenge for the food industry, constant progress is being made to bring formula composition as close as possible to that of breast milk. IF is essentially produced by modification of cow's milk, with possible variation in the whey-to-casein ratio, because whey proteins, which are predominant in breast milk (60% whey and 40% casein), are believed to be more easily digested (Morrow, 2004). The combination of different oils (sunflower, palm, soy, coconut, etc.) contributes to obtaining an ideal proportion of essential fatty acids, whereas lactose, minerals and vitamins are added at high levels to compensate for their low content or bioavailability in cow's milk as compared to human milk (Kaup, 1998).

Production of microbiologically safe and stable IF is possible through milk atomisation or heat sterilisation. However, due to the heat processes, major physicochemical changes occur, such as protein denaturation and aggregation, lipid–protein and protein–protein interactions, sugar isomerisation, and a wide range of chemical reactions, especially the Maillard reaction (Rudloff & Lonnerdal, 1992). The numerous neoformed compounds (NFC) which are formed by these reactions affect the product quality, nutritional value and safety (Birlouez-Aragon, Charissou, & Damjanovic, 2006; Henle, Walter, & Klostermeyer, 1991).

The Maillard reaction usually starts with the irreversible reaction between the ε-amino group of lysine and the carbonyl function of lactose, a reducing sugar. The first consequence of this reaction is a significant decrease in lysine bioavailability, through the formation of the Amadori compound lactulosyllysine (LL) (Evangelisti, Calcagno, Nardi, & Zunin, 1999). Up to 30% decrease in lysine bioavailability has been observed in commercial IF (Birlouez-Aragon et al., 2004). Furosine (FUR) analysis is the most common way to quantify early Maillard product LL. FUR concentration depends on the total reducing sugar content (Van Renterghem & De Block, 1996), process time/temperature parameters (Van Boekel, 1998) and storage conditions (Corzo, López-Fandiño, Delgado, Ramos, & Olano, 1994).

At high temperatures, the Amadori product may be further degraded by different processes during the advanced stage of the Maillard reaction. Hydroxymethylfurfural (HMF) is produced by 1,2-enolisation followed by strong dehydration (Ahmed, Dunn, Walla, Thorpe, & Baynes, 1988). In the presence of iron and oxygen, oxidative degradation of LL forms carboxymethyllysine (CML) and

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erythronic acid (Corzo et al., 1994; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000; Leclère, Birlouez-Aragon, & Meli, 2002). HMF shows weak direct genotoxic and mutagenic potential *in vitro,* but only at high millimolar concentrations in food which is already moderately cytotoxic (Janzowski et al., 2000). In contrast, CML could mediate the production of inflammatory cytokines, and induce oxidative stress and lower insulin sensitivity at dietary levels (Birlouez-Aragon, Morales, Fogliano, & Pain, 2010; Hofmann et al., 2002).

Vitamin C is, after previous oxidation, another possible substrate for HMF and CML (Dunn et al., 1990; Leclère et al., 2002; Loscher, Kroh, Westphal, & Vogel, 1991). The presence of high amounts of iron in IF accelerates vitamin C thermoxidation (Gliguem & Birlouez-Aragon, 2005; Leclère et al., 2002; Rosenthal, Rosen, & Bernstein, 1993), inducing the formation of reactive dicarbonyl compounds, which react with lysine residues to form, amongst other products, CML (Birlouez-Aragon et al., 2004; Dunn et al., 1990; Requena & Stadtman, 1999). Similarly, iron-activated PUFA thermoxidation drives the formation of hydroperoxides, aldehydes and other carbonyl compounds possibly generating lipid-derived CML (Fu et al., 1996).

The aim of this work was to precisely characterise the impact of heat treatment on the formation of Maillard-derived NFC in a liquid IF model using a kinetic approach. The above described indicators of the reaction advancement FUR, CML and HMF, were monitored during the formula heat treatment, and reaction kinetics and activation energies were calculated. The FAST index, a simple and sensitive indicator of the advanced Maillard reaction (Birlouez-Aragon, Sabat, & Gouti, 2002), was also measured, to provide a comprehensive view on the reaction development. At the same time, vitamin C degradation and protein denaturation were monitored. The heat sensitivity of each indicator was compared, in order to determine the one with the highest sensitivity, which could then be proposed as a suitable marker for heat treatment optimisation.

2. Materials and methods

2.1. Reagents

Whey proteins isolate PROLACTA 90 was purchased from Lactalis (Laval, France). Ingredients and formulation recipe for IF were provided by the dairy SME, Laiterie de Montaigu (Rennes, France). Sodium acetate, metaphosphoric acid, glacial acetic acid, sodium chloride, dichloromethane, methanol and ethanol were from Fisher Scientific (Fair Lawn, NJ); potassium hexacyanoferrate (II), *o*-phenylenediamine and thionyl chloride from Fluka (Buchs, Switzerland); HMF, ascorbic acid, trifluoroacetic acid and cycloleucin from Sigma–Aldrich Inc. (St. Louis, MO); hydrochloric acid from Prolabo (Fontenay sous Bois, France); FUR and CML standards from NeoMPS (Strasbourg, France).

2.2. Experimental design

We chose to prepare IF with whey proteins only. From a technological point of view, caseins play a key role in buffering the pH of IF during heat processing and, therefore, their presence has an influence (either positive or negative) on the heat-induced chemical reactions in milk. However, our previous results indicate that whey-enriched formulas are more prone to the Maillard reaction than cow's-milk-based formulas (Birlouez-Aragon et al., 2010). The whey protein isolate "Prolacta 90" was chosen because its preparation requires minimum heat-processing, and because the whey is decontaminated by microfiltration and spray dried using an Extra Low Heat technology. Prolacta composition was as follows: 92 g of total protein per 100 g Prolacta, with a mean protein composition of 63 g of β-lactoglobulin, 20 g of α-lactalbumin, 5 g of immunoglobulin, 3 g serum albumin, 1 g lactoferrin, 95 μg TGF beta.

The IF recipe (Table 1) was proposed by an industrial partner and was based on a basic formulation, which is actually commercialised. Accordingly, the whey-based IF model (1 l) was prepared by mixing 140 g of an ingredients mixture and 900 ml of redistilled water as follows: while maintaining the mineral solution at 50 °C, the protein (17.92 g Prolacta – whey protein isolates 90%), lactose (81.62 g, food grade) and a vitamin mix in accordance with the regulations regarding IF (0.28 g) were added one by one, and the mixture was strongly homogenised using an Ultra-Turrax 25 (IKA, Staufen, Germany) under nitrogen, to limit oxidation during homogenisation. The oil mixture was prepared separately by adding ascorbyl palmitate and α-tocopherol to coconut and palm oil, allowed to melt at 60 °C before adding the other two unsaturated vegetable oils. The oil mixture set at 50 °C was added to the formula and homogenisation was prolonged up to 30 min at 50 °C, until the air bubbles completely disappeared. The final pH of the IF was 7.35–7.40.

Aliquots (10 ml) of homogenised IF were heated in Pyrex tubes equipped with Teflon stoppers, using an oil bath, after nitrogen headspace saturation. The samples were immersed in a thermostated oil bath and incubated at various temperatures, 80, 87, 95, 100 and 110 °C, at holding times of 0, 2, 3.5, 5, 6.5, 8 and 9.5 min. The samples were taken out from the oil bath at different times and immediately cooled on ice to stop the reactions. A similar tube equipped with heat sensors allowed monitoring of the time–temperature profiles by data logger.

Table 1
The infant formula recipe.

Ingredients	kg/100 kg IF powder	Amount (g) in 5 l of reconstituted IF (14%)
Lactose	58.3	408.1
Prolacta 90	12.8	89.60
Palm oil	8.1	56.70
Soybean oil	6.75	47.25
Coconut oil	5.325	37.28
Sunflower oil	4.825	33.78
Tricalcium diphosphate	0.65	4.550
Dipotassium hydrogen phosphate	0.55	3.850
Disodium hydrogen phosphate	0.45	3.150
Magnesium chloride hexahydrate	0.38	2.660
Calcium carbonate	0.476	3.332
Potassium chloride	0.25	1.750
Soybean lecithin	0.24	1.680
Potassium hydrogen carbonate	0.305	2.135
Vitamin mixture of 13 vitamins	0.2	1.400
Choline bitartrate	0.14	0.980
Trisodium citrate	0.1	0.700
Sodium chloride	0.1	0.700
Iron(II) sulphate heptahydrate	0.031	0.217
Zinc sulphate heptahydrate	0.018	0.126
α-Tocopherol	0.0065	0.0455
Ascorbyle palmitate	0.0065	0.0455
Copper(II) sulphate pentahydrate	0.0013	0.0091
Manganese(II) sulphate monohydrate	0.0001	0.0007
Volume of water (litres) for reconstitution	0.643	4.5