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Evaluation of milk powder quality by protein oxidative modifications[1]

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ABSTRACT

The objective of the present research was to evaluate commercially available milk powders according to their protein oxidative modifications and antioxidant capacity, and to evaluate if these characteristics are related to physical quality parameters such as dispersibility or stability during storage. Fifteen commercially processed spray-dried milk powders were evaluated: 6 whole milk powders (WMP), 4 skim milk powders (SMP), and 5 infant formula powders (IFP). Protein oxidative status was measured as protein carbonyl (PC) content, dityrosine content, and extent of protein polymerization. The level of PC was slightly lower in SMP than in WMP, whereas IFP had more than twice as much PC as WMP (2.8 ± 0.4, 2.1 ± 0.2, and 6.5 ± 1.3 nmol/mg of protein for WMP, SMP, and IFP, respectively). No differences were detected in dityrosine accumulation. Although all the possible pairs of parameters were tested for correlations, we found that 4 parameters were linked: PC, whey content, protein aggregate level, and dispersibility. After 9 mo of storage at −20°C or room temperature, all milk samples were analyzed to evaluate changes in protein oxidative status (PC, dityrosine, and protein integrity) and related parameters. Compared with the initial condition, PC increased in all tested samples after 9 mo of storage at −20°C or at room temperature. Stored milk powders had increased PC and decreased dispersibility compared with prestorage levels. Our results highlight the importance of protein oxidative status in milk powder and its relationship to other related quality parameters, such as protein integrity and dispersibility. Our findings suggest that the understanding of such relationships could help in developing quality differentiation for different types of milk powders in the product market.
Key words: milk protein, protein oxidation, milk powder, infant formula

INTRODUCTION

Milk off-flavor has often been attributed to lipid oxidation (Biolatto et al., 2007; Lloyd et al., 2009) or to the Maillard reaction with consequent advanced glycation end products (Birlouez-Aragon et al., 2004). However, proteins, peptides, and amino acids are also susceptible to oxidative changes caused by free radicals that are manifested to a greater or lesser degree in different types of milk, depending on the quantity and type of lipids, prooxidants, antioxidants, and storage conditions (Davies, 2005; Fenaille et al., 2006; Scheidegger et al., 2010). Milk protein solubility in water could be closely related to oxidative status because oxidation enhances protein interactions and aggregation (Scheidegger et al., 2010), which lessen milk powder solubility (Thomas et al., 2004). Oligomerization and proteolysis were found in thermal treated milk (Carbonaro et al., 1998; Jovanovic et al., 2007) and UV-irradiated dairy powders (Scheidegger et al., 2010). Milk powder quality parameters are mostly related to solubility (e.g., wettability, dispersibility, and undissolved particles). These parameters are closely related to manufacturing process and storage conditions. Dairy powders are subject to oxidation during manufacturing because of the high temperatures used (Stapelfeldt et al., 1997), and spray-drying technology can induce changes in proteins that influence hydrophobicity, solubility, and denaturation (Singh and Creamer, 1991; Kalapathy et al., 1997; Kurozawa et al., 2009). In the case of infant formula powder (**IFP**), relationships between the oxidative status

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of proteins and quality parameters are more difficult to predict because IFP are prepared by mixing milk, whey proteins (that have been subjected to different thermal treatments and dehydration procedures), lipids, and (sometimes) nucleotides (Fenaille et al., 2006).

The changes that occur during storage of milk powders include protein polymerization and a decrease in solubility, with temperature and relative humidity being the predominant factors involved (Stapelfeldt et al., 1997; Gaiani et al., 2007).

The literature is sparse regarding protein oxidative alterations in milk, and the oxidative status of proteins from milk powder has not been reported because oxidative modifications have been mainly focused on lipids. For example, antioxidants used in milk powders have been evaluated for their capacity to inhibit peroxidation and prevent off-flavor development (Romeu-Nadal et al., 2007; Matumoto-Pintro et al., 2011) but not on protein oxidative modifications such as protein carbonyl (**PC**) or dityrosine bond formation.

Protein carbonyl formation is a consequence of the oxidation of some AA residues (mainly lysine, arginine, and proline residues), and PC can be produced via oxidative cleavage of the peptide backbone via the α-amidation pathway or cleavage associated with the oxidation of glutamyl residues (Stadtman and Levine, 2000). Carbonyl derivatives can also be formed as a consequence of secondary reactions of some AA side chains with lipid oxidation products, such as 4-hydroxy-2-nonenal (Stadtman and Levine, 2000). The formation of PC is highly indicative of milk protein oxidation (Fenaille et al., 2006). Moreover, it has been shown that PC and dityrosine levels increase as a consequence of thermal treatments or photo-oxidation in milk samples exposed to UV or fluorescent light (Scaloni et al., 2002; Scheidegger et al., 2010), whereas isolated milk proteins show significant variability in sensitivity to photo-oxidation (Dalsgaard et al., 2007).

The goal of this study was to test the relationships between protein oxidative modifications and antioxidant capacity with physical quality parameters of milk powders such as dispersibility or stability during storage. The understanding of such relationships could contribute to develop quality differentiation between different types of milk powders.

MATERIALS AND METHODS

Guanidine hydrochloride (ultrapure) was obtained from Genbiotech (Buenos Aires, Argentina). Trichloroacetic acid, glacial acetic acid, gallic acid, ethyl acetate, ethyl alcohol, and hydrochloric acid were purchased from Cicarelli (Buenos Aires, Argentina). Acrylamide, 2,4-dinitrophenylhydrazine (**DNPH**), and 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) were purchased from Sigma-Aldrich (Steinheim, Germany). *N*,*N*′-Methylene-bis-acrylamide and acrylamide were from Sigma Chemical Co. (St. Louis, MO). All products were of analytical grade.

Dairy Powders

Fifteen commercially processed spray-dried milk and formula powders were studied. Milks were purchased from SanCor Cooperativas Unidas Limitada (Sunchales, Argentina), La Serenísima Mastellone Hermanos S.A. (General Rodríguez, Argentina), Nestlé Argentina S.A. (Villa Nueva, Argentina), and La Sibila (Nogoyá, Argentina). Table 1 shows the physicochemical characteristics of the individual samples. Fat content was determined by the Rose-Gottlieb method (AOAC International, 2005; method 989.05), protein content by the Kjeldahl method (AOAC International, 2005; method 930.29), moisture by drying at 100°C for 2 h according to AOAC International (2005; method 931.04), and wettability and dispersibility according to FIL Standard (International Dairy Federation, 1979; FIL-IDF 87:1979). Milk powders samples were stored at 24°C (warm storage, **WS**) or −20°C (cold storage, **CS**) for 9 mo in the dark.

Color Measurement

Evaluation of color was carried out using a spectrophotometer (model CM 600d, Konica Minolta, Tokyo, Japan) according to the CIE Lab Scale. For each sample, L (brightness), a (− green to + red component) and b (− blue to + yellow component) parameters were measured for all samples. The instrumental settings were a D65 artificial daylight (10° standard angle). Each reported color value was the mean of 3 determinations at 25°C.

Protein UV-Visible Spectral Analyses

Proteins from reconstituted milk (1 mg of protein) were precipitated with 10% (wt/vol) TCA (final concentration) and recovered by centrifugation for 5 min at 7,500 × *g* (Micromax RF centrifuge, International Equipment Company, Needham, MA). Protein pellets were washed 3 times with 1 mL of ethanol:ethyl acetate 50:50 (vol/vol), and redissolved in 1 mL of 6 *M* guanidine hydrochloride, pH 7. Spectral data were obtained (200–600 nm) using a Mini Spec UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). The ratio of whey protein to total milk protein was calculated using the UV fourth-derivative absorption spectroscopy method (Lüthi-Peng and Puhan, 1999).