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Structural markers of the evolution of whey protein isolate powder during aging and effects on foaming properties

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ABSTRACT

The market for dairy powders, including high added-value products (e.g., infant formulas, protein isolates) has increased continuously over the past decade. However, the processing and storage of whey protein isolate (WPI) powders can result in changes in their structural and functional properties. It is therefore of great importance to understand the mechanisms and to identify the structural markers involved in the aging of WPI powders to control their end use properties. This study was performed to determine the effects of different storage conditions on protein lactosylations, protein denaturation in WPI, and in parallel on their foaming and interfacial properties. Six storage conditions involving different temperatures (θ) and water activities (a_w) were studied for periods of up to 12 mo. The results showed that for $\theta \leq 20$°C, foaming properties of powders did not significantly differ from nonaged whey protein isolates (reference), regardless of the a_w. On the other hand, powders presented significant levels of denaturation/aggregation and protein modification involving first protein lactosylation and then degradation of Maillard reaction products, resulting in a higher browning index compared with the reference, starting from the early stage of storage at 60°C. These changes resulted in a higher foam density and a slightly better foam stability (whisking) at 6 mo. At 40°C, powders showed transitional evolution. The findings of this study will make it possible to define maximum storage durations and to recommend optimal storage conditions in accordance with WPI powder end-use properties.

Key words: whey protein, storage, lactosylation, denaturation, foam

INTRODUCTION

Milk and by-products are increasingly dried these days to extend their stabilization to up to 3 yr due to the lowering of water activity ($\mathbf{a_w}$), and to reduce product volume by a factor of 5 to 15 to reduce both storage facility needs and transport costs. Powder processing represents a significant global market share with 10 million tons of milk powder produced per year on a global scale, corresponding to 20% of world milk production. High protein powders with targeted functionalities have been developed in the past decades, including whey protein concentrates (**WPC**) and isolates (**WPI**) that have generated increased interest.

Whey protein isolate consists of >90% protein (Burin et al., 2000), with about 70% β-LG and 20% α-LA (McClements et al., 1993). Whey proteins in their native state have biological and functional properties that appear to have undeniable advantages in many food applications. They are recognized for their nutritional role, solubility, and ability to form gels, emulsions, and foams. Whey proteins form viscoelastic layers at the air/water interface when they are adsorbed, which can lead to a resistant and cohesive interfacial network under certain conditions, improving foam stability (Martin et al., 2002). Their surface activity properties allow the formation of a wide range of foamed products such as bread, meringues, and ice creams. In fact, proteins are often used as foaming agents in food because they contribute both to foam formation and foam stability, and controlling these properties is therefore of great interest for the food industry.

However, dairy powders can be subjected to extremely variable conditions during the period of preservation (storage, export). Leinberger (2006) showed that 40% of shipments were subjected to temperatures above 45°C, sometimes reaching 57°C inside the most exposed containers during continental or inter-continental delivery over periods of up to 3 mo. As a result, studies have shown that these WPI powders evolve during storage from a functional point of view, thereby affecting their commercial value (Anema et al., 2006; Gaiani et al.,

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2006; Havea, 2006). It is therefore of utmost importance to characterize these changes and link them to structural modifications to identify markers of change for early detection and to recommend a range of conditions to ensure their end-use properties.

In this study, 2 WPI powders were produced from a fresh whey protein ultra-diafiltrate to achieve a_w close to industrial storage conditions or as a result of a poor control of the drying parameters (0.23 and 0.36, respectively). These powders were stored at temperatures representative of storage conditions [i.e., from 4°C (reference) to 60°C]. Then the change of protein structures was followed in the powder state for periods of up to 12 mo and the foaming properties after powder rehydration.

MATERIALS AND METHODS

WPI Powders

The WPI powders were obtained by spray drying of a whey protein ultra-diafiltrate obtained from the ultrafiltration and diafiltration of milk microfiltrate. This whey protein ultra-diafiltrate contained 27.8% DM (wt/wt) including approximately 90% milk proteins. The spray drying was carried out at a constant flow rate of around 100 $L \cdot h^{-1}$, and the inlet and outlet temperatures were fixed at 169 and 66°C, respectively, to obtain a powder a_w of 0.23, and at 146 and 54.3°C to obtain a powder a_w of 0.36. After drying, WPI powders presented water content of 7.5 and 10.1% at a_w of 0.23 and 0.36, respectively. Both were packed under air in 400-g airtight tins.

Storage of WPI Powders

The WPI powder tins were stored in chambers heated at 4, 20, 40, and 60°C for periods of up to 12 mo. One tin per temperature and a_w condition was brought out after 0.5, 1, 3, 6, 9, and 12 mo of storage to analyze the structural and functional properties of the powders.

Powder Composition

The native forms of β-LG and α-LA were quantified in stored powders by reverse-phase chromatography after precipitation at pH 4.6 using a 300A 8 μ*M* 150 × 2.1 MM PLRP-S column (Polymer Laboratories, Amherst, MA). The latter was connected to a HPLC made of a separation system Waters 2695, a double wavelength detector Waters 2487 and an acquisition and Empower data processing software (Milford, MA). The elution flow was 0.2 $mL \cdot min^{-1}$ using a gradient of acetonitrile obtained by an appropriate combination of buffer solution A (trifluoroacetic acid, **TFA**, 0.1%) and buffer solution B (80% acetonitrile and 0.1% TFA). The column was first equilibrated with 35% buffer solution B and then linear gradients of buffer solution B moving from 35 to 44% in 3 min, from 44 to 48% in 11 min, from 48 to 53% in 12 min, and finally from 53 to 62% in 14 min were used to elute the proteins. Proteins were detected at 214 nm.

Quantification of Free Thiol Groups

The thiol groups exposed on the protein surface were quantified using Ellman's method (Ellman, 1959). Briefly, 100 μL of a 10 $g \cdot L^{-1}$ solution of rehydrated stored WPI powder were mixed with 900 μL of Tris glycine buffer (50 m*M*, pH7.0) and 25 μL of a 2.2′-Dinitro-5.5-dithiodibenzoate solution (8 m*M*; **DTNB**, Merck, Darmstadt, Germany). After 2 h at room temperature, absorbance was measured at 412 nm and the accessible DTNB thiol groups were determined according to $\varepsilon_{412nm} = 13{,}600\ M^{-1} \cdot cm^{-1}$.

Level of β-LG Lactosylation

The percentages of lactosylated β-LG were determined by mass spectrometry after protein separation on reverse-phase HPLC using a C4 Vydac column (214TP5215, 150 × 2.1 mm; Grace, Columbia, MD). The analysis was carried out with 15 μL of a 0.5% (wt/wt) solution reconstituted in milliQ water 2/3 diluted with a 0.212% buffer solution of TFA. The flow applied was 0.25 $mL \cdot min^{-1}$ with an acetonitrile gradient. At the end of the column, a fraction of the eluate entered a QSTAR XL mass spectrometer (MDS Sciex, Toronto, Ontario, Canada) at a flow rate of 75 $\mu L \cdot min^{-1}$. Proteins were ionized with an ion sprayer source before determining their mass with a TOF scanner (MDS Sciex) previously calibrated with β-CN peptide from β-casein f(193–209). The mass acquisition was carried out in the mass range of m/z 500 to 3,000 at 5 kV.

The percentage of lactosylation was determined using the following calculation:

$$\frac{I(\text{lact})}{I(\text{NP}) + I(\text{lact})} \times \%\text{NP},$$

where I(lact) is the intensity of the lactosylated protein, I(NP) is the intensity of the native protein, and %NP is the % native protein amount determined from reverse-phase HPLC.