Journal of Food Engineering 178 (2016) 39–46

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Local modifications of whey protein isolate powder surface during high temperature storage

Jennifer Burgain [a, b, **], Racha El Zein [c], Joël Scher [b], Jeremy Petit [b], Eve-Anne Norwood [a, d], Gregory Francius [c], Claire Gaiani [b, *]

[a] CNIEL, 42 rue de Châteaudun, 75314 Paris Cedex 9, France
[b] Université de Lorraine, LIBio, Laboratoire d'Ingénierie des Biomolécules, 2 av de la Forêt de Haye, TSA 40602, 54518 Vandoeuvre-lès-Nancy, France
[c] Université de Lorraine, LCPME, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement, UMR 7564, 54600 Villers-lès-Nancy, France
[d] Agrocampus Ouest, INRA, UMR1253, Science et Technologie du Lait et de l'Œuf, F-35000 Rennes, France

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form
5 January 2016
Accepted 6 January 2016
Available online 8 January 2016

Keywords:
AFM
Dairy powders
Surface characterization
ToF-SIMS
WPI

ABSTRACT

Dairy powders are stable ingredients that are frequently stored prior to use. In fact, during powder manufacture, precautionary measures are taken to ensure optimal technological and nutritional functionalities, but during storage, changes in physico-chemical and functional properties of whey protein isolate (WPI) powders were extensively outlined. In the present study, WPI powders were stored at 60 °C and 0.2 water activity for one month and local modifications on particle surface were explored. Atomic force microscopy (AFM), specifically in chemical force microscopy (CFM) mode, was used to follow surface modifications during storage. An increase in surface hydrophobicity was noticed when powders were stored at high temperatures. Complementary techniques such as microscopy and Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) showed a crackled surface and a decrease in the presence of amino acid at surface. Finally, powder browning and fluorescence of components on particle surface suggested that Maillard reaction is the main phenomenon responsible for powder alteration during storage.

1. Introduction

Dairy powders are ingredients of great interest commonly used by manufacturers and consumers. In the past thirty years, new added-value products were developed by the dairy industry, among them WPI (whey protein isolate) powders. Whey proteins are commonly used as ingredient in formulated foods (*e.g.*, dairy foods, bakery products, beverages, meat products). Dairy powders storage induces changes in their physico-chemical properties, particularly under unfavorable conditions (*e.g.*, high temperature and humidity) (Thomas et al., 2004). High-protein dairy powders functionality is known to be strongly affected by surface properties (Gaiani et al., 2013); that is the reason why exploring particle powder surface modification upon storage is the main concern of the current study. For example, complete rehydration is normally a prerequisite to every industrial use; unfortunately, for dairy powders of high protein content and where casein is the dominant constituent, difficulties to rehydrate are observed even after extended rehydration times (Gaiani et al., 2007b). Number of methodologies are available to characterize powder particle surface (*i.e.*, structure, shape, roughness, composition, energy, etc.) (Gaiani et al., 2013). These techniques are so well developed that they can now be regularly used by researchers and industrials. For example, X-ray photoelectron spectroscopy (XPS) was frequently used to determine the surface composition of dairy powders (Fyfe et al., 2011b; Gaiani et al., 2006, 2010, 2011b, 2007a; Kim et al., 2002; Millqvist-Fureby et al., 2001; Nikolova et al., 2015a, 2015b). Other microscopy techniques, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), were employed to explore food powders suprastructure and organization (Murrieta-Pazos et al., 2012). Regarding dairy powders, dents on particle surface were widely registered (Gaiani et al., 2006; Kim et al., 2009; Millqvist-Fureby and Smith, 2007) and during storage of casein powders, surface pores were noticed (Gaiani et al., 2009). It is interesting to underline the fact that some surface analysis techniques employed for studying pharmaceutical powders remain

* Corresponding author.
** Corresponding author.Université de Lorraine, LIBio, Laboratoire d'Ingénierie des Biomolécules, 2 av de la Forêt de Haye, TSA 40602, 54518 Vandoeuvre-lès-Nancy, France.
E-mail address: claire.gaiani@univ-lorraine.fr (C. Gaiani).

http://dx.doi.org/10.1016/j.jfoodeng.2016.01.005
0260-8774/

unapplied to food powders (Murrieta-Pazos et al., 2012). For example, time-of-flight secondary ion mass spectrometry (ToF-SIMS) was poorly used for food powders (Belu et al., 2003), although it was successfully combined to XPS in order to better understand the influence of spray-drying conditions on surface properties of dairy powders (Nikolova et al., 2014). In the same work, authors used atomic force microscopy (AFM) in topographical mode to qualify surface roughness. Previously, AFM was used to characterize dairy powders surface having low and high fat surface coverage (Murrieta-Pazos et al., 2011). The authors demonstrated the ability of the technique to characterize the powder surface, as AFM images were in agreement with those obtained by SEM: skim milk powder presented a flat surface, while the surface of whole milk powder was rougher. Finally, AFM was used to study surface composition and structural changes in milk protein particles during storage (Fyfe et al., 2011b). The authors succeeded to make a link between powder functional properties (decrease in solubility) and surface modifications (increase in surface hydrophobicity). The latter study reinforces the idea that particle surface strongly influences powder functional properties and confirms that AFM, with its high spatial resolution, is an interesting method for better understanding surface changes during storage, as it is the only method providing local information, while others take into account the entire particle surface or even the surface of several particles. In addition to the production of three dimensional images of analyzed surfaces, AFM can be used in force mode for measuring the adhesive interactions between AFM tip and sample surface. More specifically, chemical force microscopy (CFM) (Butt et al., 2005; Frisbie et al., 1994) is a powerful technique in which the AFM tip is functionalized with well-defined chemical groups. For example, CFM can be used to quantify and map hydrophobic and electrostatic interactions at nanoscale.

In the present work, CFM was used to map and quantify the evolution of surface hydrophobicity of WPI powders at nanoscale during their storage. Complementary microscopic and surface analysis methods would give additional information on how storage conditions influence the particle powder surface.

2. Material and methods

2.1. WPI powders: production and storage

The whey protein isolate (WPI) was provided as a concentrate by a dairy company (Lactalis, France). It was obtained by ultrafiltration and diafiltration of milk microfiltrate, followed by a spray-drying at Bionov (Rennes, France) in a 3-stage pilot-plant spray-dryer (GEA, Niro Atomizer, St Quentin en Yvelines, France). WPI powders were packaged in sealed tins ($a_w = 0.23$; moisture content of 8.4%) and were stored under a controlled temperature of 4 °C (further named as “reference powder”) and 60 °C (further named as “aged powder”) for one month. This high temperature was chosen to enhance and accelerate phenomena observed during powder storage.

2.2. Surface observation

2.2.1. Scanning electron microscopy observation

Scanning electron microscopy (SEM) was used to observe the morphology and surface structure of WPI powders. To this end, analyzed powders were set on a double-sided adhesive tape and fixed to SEM stubs. Excess particles were removed by gentle tapping. Samples were then coated under partial vacuum with gold-palladium for 2 × 100 s (Polaron SC7640, Thermo VG Scientific, England). Finally, the samples were observed with a Stereoscan 240S/N (Léo, Rueil-Malmaison, France) operating at 15 kV.

2.2.2. AFM: surface topography and roughness

Dairy powders were fixed onto a circular glass thanks to epoxy glue. AFM measurements were performed at room temperature using an Asylum MFP-3D atomic force microscope (Santa Barbara, CA, USA) with IGOR Pro 6.04 operation software (Wavemetrics, Lake Osewego, OR, USA). All images were acquired in liquid media, more particularly in ethanol to avoid powder rehydration. Topography images were obtained in contact mode at 1 Hz scan rate. The scanned surface area was of 40 × 40 μm^2 corresponding to 512 points × 512 lines.

2.2.3. Light microscopy

WPI particles were examined without further staining on an Olympus Provis microscope with an Olympus DP70 digital camera. Fluorescence was revealed using a DAPI filter ($\lambda_{excitation}$ = 340–380 nm; $\lambda_{emission}$ = 425 nm).

2.3. Surface characterization

2.3.1. XPS

Elemental composition of WPI powder surface (up to 5–6 nm depth) was measured by X-ray Photoelectron Spectroscopy (XPS). Spectra were obtained with a KRATOS Axis Ultra X-ray photoelectron spectrometer (Kratos Analytical, Manchester, UK) equipped with a monochromated Al Kα X-ray ($h\nu$ = 1486.6 eV) operated at 150 W. Spectra were collected at normal take-off angle (90°), and the analysis area was 700 × 300 μm^2.

2.3.2. ToF-SIMS analysis

Measurements were performed in both positive and negative ion modes with TOF-SIMS 5 (IonTOF GmbH, Münster, Germany). The analysis was performed using a bismuth liquid metal ion gun (LMIG, Bi_3^+ ions, 25 keV). Images of WPI powder surface were acquired with a field of view of 200 × 200 μm^2 corresponding to 512 × 512 pixels, leading to a pixel size of 0.4 μm. The fluence (also called primary ion dose density) was maintained to 1.0×10^{12} ions/cm^2 that is below the so-called static SIMS limit (Vickerman and Briggs, 2001). The data acquisition and processing software was SurfaceLab 6.3 (ION-TOF GmbH, Münster, Germany).

2.3.3. Chemical force microscopy

Gold-coated AFM tips (NPG-10, Bruker AXS, Palaiseau, France) were functionalized by immersing the tip for 15 h in a solution containing 2 mM 1-dodecanethiol (≥98%, 471364 SIGMA). The process for tip functionalization consists in the immobilization of thiol-based self-assembled monolayer on a gold-coated tip. This results from an oxidative addition of the thiol bond to the gold surface, concurrently with hydrogen removal. The functionalization of AFM tips with alkane-thiol monolayers is useful for mapping the spatial arrangement of hydrophobic chemical groups on powder surface by measuring adhesive forces. A glass substrate was coated with chromium through the use of a sputter coater (EMITECH, K575 Turbo, United Kingdom) and was further covered with a top gold layer. Gold-coated glass surfaces were immersed in a solution containing 1-dodecanethiol and rinsed with ethanol. The gold surfaces are used as references to control the correct functionalization of AFM tips (Alsteens et al., 2007).

Interaction measurements were performed in liquid to avoid capillary forces.

The spring constant of functionalized tips was determined using the thermal calibration method (Levy and Maaloum, 2002). The usual calibration process was employed to transform the experimental cantilever deflection curves as a function of the vertical scanner displacement Δz into force–distance curves. In the contact region, the slope of the retraction deflection curve was used to