J. Dairy Sci. 93:463–472
doi:10.3168/jds.2009-2369

Investigation of the microstructure of milk protein concentrate powders during rehydration: Alterations during storage

A. Mimouni,* H. C. Deeth,* A. K. Whittaker,† M. J. Gidley,‡ and B. R. Bhandari*[1]
*School of Land, Crop and Food Sciences, The University of Queensland, Brisbane 4072, Australia
†Centre for Magnetic Resonance, and Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, Brisbane 4072, Australia
‡Centre for Nutrition and Food Sciences (CNAFS), The University of Queensland, Brisbane 4072, Australia

ABSTRACT

The aim of this work was to use scanning electron microscopy to investigate the microstructure of rehydrated milk protein concentrate powder (MPC) particles. A sample preparation method for scanning electron microscopy analysis of rehydrated MPC particles is described and used to characterize the time course of dissolution and the effects of prior storage on the dissolution process. The results show that a combination of different types of interactions (e.g., bridges, direct contact) between casein micelles results in a porous, gel-like structure that restrains the dispersion of individual micelles into the surrounding liquid phase without preventing water penetration and solubilization of nonmicellar components. During storage of the powder, increased interactions occur between and within micelles, leading to compaction of micelles and the formation of a monolayer skin of casein micelles packed close together, the combination of which are proposed to be responsible for the slow dissolution of stored MPC powders.
Key words: scanning electron microscopy, microstructure, powder, casein micelle

INTRODUCTION

Milk protein concentrate (**MPC**) is a high-protein milk powder containing up to 85% protein. These powders are produced from skim milk by spray-drying after pasteurization, ultrafiltration, diafiltration, and vacuum evaporation (Havea, 2006; Singh, 2006). They are typically used by the food industry as gelling, foaming, or emulsifying ingredients as well as for their nutritional value. For most applications, prior dissolution of MPC in water is needed, preferably at room temperature and ideally as fast as possible with moderate agitation to minimize operating costs. Because the powder does not fully express its functional properties if it remains insoluble, a true solution or complete dispersion of colloidal particles is required (Gaiani et al., 2007; Martin et al., 2007). However, commercial MPC powders, freshly made or stored, are characterized by poor reconstitution properties (Schuck et al., 1994; Singh, 2006; Baldwin and Truong, 2007; Mimouni et al., 2009). The step of dissolution of powder particles, rather than wetting or deagglomeration, has been shown to be the rate-limiting step in rehydration (Mimouni et al., 2009) with storage-induced effects further altering the dissolution kinetics (A. Mimouni, unpublished data). Some form of association of micelles upon storage may be responsible for these alterations (McKenna, 2000; Anema et al., 2006; Havea, 2006). However, further work is needed to understand the structural parameters controlling the dissolution rate of MPC powder particles.

Scanning electron microscopy has been used to study various types of dairy powders by several researchers (Thomas et al., 2004; Gaiani et al., 2007; Tamime et al., 2007b). However, to the best of our knowledge, these investigations were only performed on spray-dried powders in the dry state and did not provide information directly on rehydrated structures. The change in the structure of powder particles during the course of dissolution can provide valuable information on the dispersion mechanism of the colloidal protein particles. Therefore, the aim of this work was to use scanning electron microscopy as a tool to investigate the microstructure of rehydrated MPC powder particles. We present a sample preparation method for electron microscopy analysis that was adapted from different existing protocols. The observed microstructure of MPC powder and its alterations upon storage are described and then discussed in relation to the rehydration properties of the powder.

MATERIALS AND METHODS

Milk Protein Concentrate Powder

The chemical composition of MPC used in this study (MPC85, Murray Goulburn Co-operative Co. Limited,

Received May 11, 2009.
Accepted August 6, 2009.
[1]Corresponding author: b.bhandari@uq.edu.au

Brunswick, Victoria, Australia) was analyzed by the manufacturer using the methods described in Standards Australia (1995). This MPC primarily contains milk proteins (82.4% wt/wt) with the same proportion of casein and whey proteins as in the milk used for the powder manufacture, lactose (4% wt/wt), ash (7.3% wt/wt), and fat (1.6% wt/wt). Immediately after manufacture, the powder was transported to the laboratory where it was stored at 4°C until used. Before rehydration, the powder was removed from the cold room and stored for 2 d at 20°C in plastic desiccators (250-mm diameter) above a saturated potassium acetate solution (equilibrium relative humidity 23%) to ensure moisture content standardization. Changes in powder microstructure occurring during storage were also investigated. For this purpose, MPC85 powder was stored for 2 mo at the same temperature (20°C) and relative humidity (23%).

Rehydration Procedure

Rehydration was carried out at 5% (wt/wt) powder concentration in water. The MPC85 powder (10 g) was added to 190 g of water in a 250-mL glass beaker. The suspension was maintained at constant temperature using a water bath at 24°C. Stirring was performed for 10 min (short-term rehydration) and 80 min (long-term rehydration) at constant speed (200 rpm) using an electric overhead mixer (RW20, IKA, Staufen, Germany) and a 4-bladed propeller stirrer of 50-mm diameter (R 1342, IKA; Mimouni et al., 2009).

Sample Preparation for Scanning Electron Microscopy and Imaging

The MPC powder was rehydrated following the procedure presented above. To capture powder particles in suspension, particles were adhered to a silicon chip wafer (ProSciTech, Kirwan, Queensland, Australia) coated with poly-L-Lys (Sigma, Castle Hill, New South Wales, Australia), which creates electrostatic bonding between the powder particles and the substrate (McMahon and Oommen, 2008). A few drops of poly-L-Lys solution were deposited on the silicon wafer and allowed to air-dry at room temperature (i.e., approximately 20°C) in a dust-free environment. One or two drops of MPC suspension were then deposited and left for 5 min before draining and rinsing the wafer with 100 m*M* phosphate buffer (pH 7).

A solution of 3% glutaraldehyde in 100 m*M* phosphate buffer (pH 7) was then applied for 15 min to achieve chemical fixation of the rehydrated protein material. After fixation, the samples were washed in deionized water (3 times) and dehydrated using a graded ethanol series: 50, 70, 90, 95 (2 times), and 100% (3 times). The elapsed time per solution was 5 min (Dalgleish et al., 2004). Samples were then dried using CO_2 in a critical point dryer (CPD 010, Balzers Union Ltd., Liechtenstein). The silicon wafer was subsequently mounted onto electron microscopy stubs by placing it on a carbon double-sided adhesive tape. Protein powders suffer from extensive charge buildup under the electron beam, and hence they need to be coated with conductive material. Samples were platinum-coated for 2 min (to about 5 nm thick) and examined with a field emission scanning electron microscope (6300, Jeol, Tokyo, Japan) operating at 2.5, 5, 10, or 15 kV. For analysis of the spray-dried powder, the powder samples were placed on the carbon double-sided tape mounted onto electron microscopy stubs. The images were produced in the Centre for Microscopy and Microanalysis of the University of Queensland (St Lucia, Queensland, Australia).

RESULTS AND DISCUSSION

Microstructure of Fresh Powder Particles

Effects of Short-Term Rehydration. Figure 1 shows the difference in surface microstructure between spray-dried (A) and rehydrated (B) milk protein powder particles rehydrated for 10 min. Before rehydration (Figure 1A), the surface of the particle was essentially smooth, without any visible substructures on the surface. An increase in magnification did not reveal additional structural details. Note that the scanning electron microscope can achieve a very high resolution with spray-dried materials. Some holes and roughness on the surface of the particles measuring less than 500 nm were visible, which suggests that the smooth surface observed was not the consequence of a lack of resolution but seems to represent the true surface of spray-dried powder particles. A smooth surface appearance was demonstrated in previous work (Tamime et al., 2007b) and was attributed to the strong compaction and shrinkage of the protein material, especially the casein micelles, during air-drying. In contrast, the hydration of powder particles (i.e., the presence of water) considerably modified the 3-dimensional organization of the material on the surface of the powder particles. The microstructure of a powder particle (Figure 1, panels B, C, and D) was porous and characterized by a loose assembly of spherical nanoparticles grouped in small clusters separated by pores of various sizes. The diameter of these spheres was in the range of 40 to 200 nm, which strongly suggests that these particles are casein micelles. The pore diameter as well as the size of the casein micelle clusters were both in the approxi-