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Effects of heating on the secondary structure of proteins in milk powders using mid-infrared spectroscopy

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ABSTRACT

Milk powder is an important source of protein for adults and children. Protein is very sensitive to heat, which may influence people's usage of nutrients in milk powder. In this study, we describe the temperature-induced secondary structure of protein in milk powders. In this study, whole milk powder containing 24% protein and infant formula containing 11% protein were heated from 25 to 100°C. Attenuated total reflectance (ATR) spectra in the mid-infrared range 400–4,000 cm^{-1} were used to evaluate the heat effect on the secondary structure of protein in these 2 milk powders. The spectral changes as a function of temperature were maintained by difference spectra, second-derivative spectra and Gauss curve-fitted spectra. The secondary structures of protein in the whole milk powder began to change at 70°C and in the infant formula at 50°C. The β-sheet and β-turn structures in the whole milk powder both decreased in the range of 70 to 85°C, whereas α-helix structures increased. The loss of β-sheet and β-turn may contribute to the formation of α-helix in the whole milk powder. In infant formula powder, the β-sheet structure showed a decrease and then increase, whereas the β-turn structure showed an increase and then decrease in the range of 50 to 75°C, and no change was found for α-helix structures. This implies that heating may induce the transformation from β-sheet to β-turn. Overall, whole milk powder had better temperature stability than infant formula powder, probably because of the lower content of lipid in the former than in the latter. These results help us understand the thermal stability of protein in milk powder.
Key words: protein secondary structure, temperature, milk powder, mid-infrared spectroscopy

INTRODUCTION

Milk powder has high nutritional value and some useful functional properties. Milk powder contains several types of proteins, including mucins, caseins, and whey proteins (Malacarne et al., 2002; Haug et al., 2007). The nutrients in milk can be utilized exceptionally well; some of the factors may be the presence of proteins in milk. The proteins provide not only adequate amounts of essential amino acids but also biological activities from antimicrobial effects to immunostimulatory functions (Lönnerdal, 2003).

Milk powder has a long shelf life (up to 12 mo) at room temperature. However, loss of solubility occurs gradually during storage (Anema et al., 2006; Havea, 2006), which may be linked to conformational modification of protein molecules during processing and storage (Kher et al., 2007). It is a common practice to mix milk powder with hot water before feeding or drinking. The effects of hot water on the milk structure and nutrition is unclear.

Milk-based infant formulas, both liquid and powder, are very sensitive to heat damage (Puig et al., 2003). Heat treatment of human milk will influence some of its protective constituents (immunoglobulin, lactoferrin, lysozyme, and others; Ford et al., 1977). When whole milk proteins are heated (Kim and Jimenez-Flores, 1995), β-LG and other milk serum proteins interact with milk fat globule membrane proteins. As the temperature of bovine β-CN increases, the protein secondary structure turns and extended structure are stable, whereas loops and helices are unstable. The β-sheets and β-turns probably form a supporting hydrophobic core (Farrell et al., 2001). The effect of storage temperature on the solubility of milk protein concentrate showed that insolubility could be due to cross-linking of the proteins at the surface of the powder (Anema et al., 2006). The formation of heat-induced whey protein complexes in milk increases the pH of gelation and the firmness of acid milk gels (Morand et al., 2012). The increased capacity of milk proteins to bind curcumin after heat treatment can be attributed to whey pro-

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tein denaturation, as whey proteins bind to the surface of casein micelles with heating (Yazdi and Corredig, 2012).

Mid-infrared spectroscopy (from 400 to 4,000 cm^{-1}) is a vibrational spectroscopy method that can reveal a wealth of information about the constituents in molecules, including proteins (Barth and Zscherp, 2002; Etzion et al., 2004; Barth, 2007). Mid-infrared spectroscopy is an efficient method widely used to predict milk fat, protein, lactose, and more detailed milk composition traits (Soyeurt et al., 2009; Rutten et al., 2011; De Marchi et al., 2014; Pappas et al., 2015; Toffanin et al., 2015; Visentin et al., 2015), as well as energy balance and feed efficiency (McParland et al., 2014). Modern infrared spectrometers are Fourier transform infrared (**FTIR**) spectrometers, which are similar to the Michelson interferometer (Arrondo et al., 1993). Fourier transform infrared spectrometry is a well-established method to probe the secondary structure of protein (Surewicz et al., 1993; Pelton and McLean, 2000; Barth and Zscherp, 2002; Li-Chan, 2007). The amide I band (1,600–1,700 cm^{-1}) of protein is sensitive to changes in secondary structures (Byler and Susi, 1986; Carbonaro and Nucara, 2010; Majzner et al., 2013). The attenuated total reflectance (**ATR**) technique (Goormaghtigh et al., 1999) has advantages in measuring solid samples.

We used ATR-FTIR to detect the heat-induced changes of 2 milk powders: whole milk powder and infant formula. To avoid the interference of water (Etzion et al., 2004), we used milk powders directly as our experimental samples without mixing with water.

MATERIALS AND METHODS

Apparatus and Materials

The measurements were carried out using an FTIR spectrometer (Tensor 27, Bruker, Bremen, Germany), equipped with globar source, KBr beamsplitter, and deuterated triglycine sulfate detector. The crystal in the ATR attachment for the FTIR (Pike Technologies, Madison, WI) was germanium, and the angle of incidence was 45°. A press was used so that the milk powders could contact the crystal, avoiding trapped air. Infrared spectra were recorded with 16 scans in in 400–4,000 cm^{-1} range with a resolution of 4 cm^{-1}. A vacuum blast-drying oven (DGG-9030A, Shanghai Senxin, Shanghai, China) was used to dry and heat the samples. The whole milk powder and infant formula (0–12 mo) were manufactured by the Inner Mongolia Yili Industrial Group Co. Ltd. (Hohhot, China), a well-known corporation in the Chinese milk powder market and were purchased in a local supermarket.

Methods

Sixteen tubes in parallel, each with a volume of 10 mL and with 20 g of sample each, were put into the vacuum blast-drying oven and then heated at a rate of 5°C/min. The temperature was increased from 25 to 100°C at 5°C intervals (16 samples). One tube was taken out after the temperature had reached each preset cut-off temperature, capped immediately, and stored in a desiccator with silica gel. To guarantee the samples were completely heated, heating was maintained for 5 min. About 2 to 3 g of milk powder from each capped tube was placed on the ATR crystal for measurement.

A linear baseline was subtracted from each spectrum to give a straight baseline in the spectral region of 1,800–2,000 cm^{-1}, and the spectra were smoothed with a 4-point Savitzky-Golay function to remove the possible white noise (Dong et al., 1990). For the secondary structure analysis, deconvolution of each spectrum was performed according to the methods of Fourier self-deconvolution (Kauppinen et al., 1981) and the finite impulse response operator using the software Opus 6.5 (Bruker, Bremen, Germany). A spectrum of a single band that is characteristic of a secondary structure is broadened in the liquid or solid states. The bands overlapped and could not be distinguished from each other. Second derivatives of the amide I (1,600–1,700 cm^{-1}) bands were used to indicate the position of individual component peaks of secondary structure within the amide I envelope. A curve-fitting procedure was used to estimate the area of each component representing secondary structures (Kumosinski and Farrell, 1993). The second derivatization and Gaussian curve fitting in the amide I region were analyzed using the software Origin 8.0 (OriginLab, Northampton, MA).

RESULTS AND DISCUSSION

FTIR

The mid-infrared spectra (400–4,000 cm^{-1}) of whole milk and infant formula powders at 25°C are shown in Figure 1. The characteristic infrared spectral bands of the 2 milk powders were very similar. The absorption bands of 1,630 to 1,680 cm^{-1} and 1,510 to 1,570 cm^{-1} were from protein (Carbonaro and Nucara, 2010) assigned to C=O stretching vibration absorption of amide I and N–H and C–H bending vibration absorption of amide II, respectively. The characteristic peaks of 2,920 cm^{-1}, 2,850 cm^{-1}, and 1,743 cm^{-1} were from lipids in the milk powders, which can be assigned to the bands of antisymmetric CH_2 stretching, symmetric CH_2 stretching, and C=O double-bond stretching, respec-

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