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# Properties of whey protein–maltodextrin conjugates as impacted by powder acidity during the Maillard reaction

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ABSTRACT

Heating the powder of whey protein isolate (WPI)-maltodextrin (MD) mixture, the Maillard reaction, improves thermal stability of WPI, but the effects of powder acidity have not been studied. In this work, solutions with WPI and MD were adjusted to pH 4–7 (m-pH) to obtain spray-dried powder that was glycated at 80 °C and 65% relative humidity for 1–4 h. The conjugates were evaluated for physicochemical properties. A higher m-pH and a longer glycation resulted in a darker color. The m-pH 6 treatment had the highest degree of glycation, lowest surface hydrophobicity, lowest isoelectric point, and highest denaturation temperature, which contributed to the best heat stability evaluated at 5% protein, pH 4–7 and 0–150 mM NaCl by heating at 138 °C for 1 min. The results indicate that adjusting WPI-MD mixture solution to pH 6.0 to prepare powder for glycation can reduce the color of protein ingredients while providing heat stability for transparent beverage applications.

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## 1. Introduction

Whey protein isolate (WPI), containing more than 90% protein, is a common protein ingredient used to manufacture protein beverages. Beverages formulated with WPI are generally highly acidic to receive high clarity and heat stability, and a large amount of sugar is used to mask acid taste, which may cause a number of health problems such as teeth erosion and diabetes (Etzel, 2004; Mettler, Rusch, & Colombani, 2006). Low-acid beverages, with pH above 4.6, may reduce concerns about sugars. Compared to no thermal requirements of the US Food and Drug Administration for acid foods (pH < 4.6), the production of shelf-stable low-acid foods requires thermal processing to ensure microbiological safety, such as ultra-high temperature (UHT) processing of dairy products at 280 °F (138 °C) for at least 8 s (McGarrahan, 1982). This presents a challenge for whey proteins that undergo denaturation and aggregation during heating to cause turbidity and storage instability, and possibly gelation, especially for high protein beverages with more than 4.2%w/v protein (Etzel, 2004).

The folded (native) state of proteins is only slightly more thermodynamically favorable than at the unfolded (denatured) state, and protein denaturation during thermal treatments is thus common (Chi, Krishnan, Randolph, & Carpenter, 2003). The thermally-induced denaturation and aggregation of whey proteins are generally attributed to several molecular forces such as van der Waals, hydrophobic, and electrostatic interactions and intra- and inter-molecular disulfide bonds via sulfhydryl-disulfide interchange, which can be altered by solution pH, protein concentration, ionic strength and temperature (Baier & McClements, 2005; Bryant & McClements, 1998). At neutral pH, the clarity of whey protein solution after heating can be retained by supplementing co-solutes such as sucrose, glycerol, sorbitol, and polysaccharides, as a result of the increased denaturation temperature ($T_d$) of whey proteins (Baier & McClements, 2001; Chanasattru, Decker, & McClements, 2007; Chantrapornchai & McClements, 2002; Kulmyrzaev, Bryant, & McClements, 2000). Preheating whey proteins improves the heat stability and sequential mild preheating and transglutaminase pretreatments can stabilize WPI dispersions after heating at 138 °C and neutral pH for 1–30 min (Wang, Zhong, & Hu, 2013; Zhang & Zhong, 2010; Zhong, Wang, Hu, & Ikeda, 2013). When solution pH is around the isoelectric point (pI) of whey proteins (β-lactoglobulin (β-Lg): 5.2; α-lactalbumin: 4.5–4.8; bovine serum albumin: 4.7–5.1) (Bryant & McClements, 1998), protein aggregation can be extensive because of the weakened electrostatic repulsion at the overall net charge being close to zero.

Glycation of proteins with reducing carbohydrates via the Maillard reaction is an effective method to modify protein functionality and has received much attention in recent years. The ε-amino group of the lysine residues is the primary glycation site (Ames, 1992). Other groups such as the imidazole group of

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histidine, the indole group of tryptophan, and the guanidine group of arginine residues also participate the reaction, but with a lesser extent (Ames, 1992). The Maillard reaction can take place in solutions (Li et al., 2013; Zhu, Damodaran, & Lucey, 2008) and powder (Akhtar & Dickinson, 2003). In aqueous solutions, the glycation rate is low because water inhibits the initial Amadori reaction and subsequent reactions (Liu, Ru, & Ding, 2012; Van Boekel, 2001). In solutions, the Maillard reaction is favored at a higher pH from 4.0 to 12.0, generating undesired darker brown color (Ajandouz & Puigserver, 1999; Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2001; BeMiller & Huber, 2007). Conversely, conjugates produced from powder with neutral acidity improved the thermal stability of whey proteins (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001), showing transparent dispersions after heating samples at pH 3.0–7.0 and 0–150 mM NaCl and $CaCl_2$ (Liu & Zhong, 2012, 2013; Wang & Ismail, 2012). Besides the origin and ratio of substrates (proteins and reducing carbohydrates), reaction temperature and duration, water activity and reaction pH affect the glycation kinetics and thus functionality of reaction products significantly (Sanmartín, Arboleya, Villamiel, & Moreno, 2009). A higher temperature and a longer reaction duration increase the extent of Maillard reactions, which not only increases the degree of glycation (Schiff base) but also generates darkened colors associated with advanced reactions that have caused worldwide concerns about the probable carcinogenicity in humans (BeMiller & Whistler, 1996; Mottram, Wedzicha, & Dodson, 2002). The pathway of forming melanoidins, major pigments responsible for the brown color of Maillard-reaction products, is favored under more alkaline conditions, as being reported for glycation in solutions (Ajandouz et al., 2001). However, impacts of powder acidity on color formation and thermal stability of whey proteins glycated with reducing saccharides at dry conditions have not been studied.

The major objective of this work was to characterize and understand thermal stability and color formation of WPI-maltodextrin (MD) conjugates produced by heating spray-dried powder with different acidity at 80 °C and 65% relative humidity for 1–4 h. To study powder acidity, WPI and MD were dissolved in deionized water and adjusted to pH 4–7 before spray drying. Physicochemical bases of powder acidity impacts were studied for the degree of glycation, surface hydrophobicity, isoelectric point, and denaturation temperature of conjugates. Because Maillard reactions impact nutritional quality of proteins such as the loss of lysine and other essential amino acids and the reduced bioavailability (Hurrell, 1990), the secondary objective was to study amino acid compositions and in vitro digestibility of conjugates, with the digestibility evaluated for conjugates before and after the simulated UHT processing.

## 2. Materials and methods

### 2.1. Materials

The WPI was obtained from Hilmar Ingredients (Hilmar, CA). MD with a dextrose equivalent (DE) of 18 was acquired from Grain Processing Corporation (Muscatine, IA). Pepsin, 1-anilino-8-naphtalene-sulphonate (ANS), and pancreatin were purchased from Sigma–Aldrich Corp. (St. Louis, MO). Other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

### 2.2. Production of WPI-MD conjugates

WPI and MD18 were hydrated overnight at room temperature (~21 °C) at 2.5% w/v each in deionized water. The mixture solution was adjusted to pH 4, 5, 6, and 7 using 10 N and 4 N NaOH and was spray-dried at an inlet temperature of 160 °C, an outlet temperature of ca. 90 °C, an air flow rate of 20 $m^3$/h, and a feeding rate of 250 mL/h (model B-290 Mini-Spray Dryer, Büchi Laboratoriums-Technik, Flawil, Switzerland). The spray-dried powder was incubated at 80 °C and 65% relative humidity for 1, 2 and 4 h in a humidity-controlled incubator (model IG420U Environmental chamber, Yamato Scientific America Inc., Santa Clara, CA) for glycation. The powder was then collected and stored at −20 °C in a freezer.

### 2.3. Preparation of conjugate solution and heat stability test

The conjugates were prepared at 5%w/v protein in deionized water and hydrated overnight at ~21 °C. Solutions were measured for pH before adjusting to pH 7 using 4 N and 1 N NaOH to prepare transparent solutions and filtration through a #1 filter paper (particle retention: >11 μm, Whatman, Clifton, NJ) to remove visible particulates such as brush debris resulting from powder collection in sample preparation. Subsequently, samples were adjusted to pH 4–7 using 1 N and 0.25 N HCl or NaOH, 0–100 mM NaCl, and 0 or 5%w/w sucrose. The 1 mL solutions were contained in 4 mL glass vials and were heated in a glycerol bath at 138 ± 1 °C for 1 min to simulate UHT processing that is equivalent to 138 °C for at least 8 s for dairy products (McGarrahan, 1982). The 1 min duration was used to ensure sufficient thermal treatment throughout the vials. The visual appearance of samples was compared by photographing.

### 2.4. Color measurements

The extent of advanced Maillard reactions (brown color) was measured using the UV/Vis spectrophotometer (ThermoScientific, Waltham, MA) at 420 nm (BeMiller & Huber, 2007; Martins, Jongen, & Van Boekel, 2001). The color of protein solutions at pH 7.0 without sucrose and NaCl was measured before and after heating at 138 °C for 1 min using a MiniScan XE Plus Hunter Colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The parameters *L*, *a*, *b* were determined twice each for two independent conjugate replicates and the color intensity (C*) was calculated as follows (Medrano, Abirached, Panizzolo, Moyna, & Añón, 2009).

$$C^* = \left(a^2 + b^2\right)^{1/2} \quad (1)$$

### 2.5. Attenuated total Fourier Transform Infrared (FTIR) spectroscopy

The WPI-MD conjugate structure was studied using a Nicolet Nexus 670 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). WPI and its conjugates were prepared at 2%w/v protein in deuteroxide to reduce the strength of intra-molecular hydrogen bonds (Yost, Tejedor-Tejedor, & Anderson, 1990). A drop of each protein sample solution was placed on the ATR accessory, and over 64 scans at a 4 $cm^{-1}$ resolution were collected and averaged to obtain the spectrum. The original spectrum was smoothed using the OMNIC software. The FTIR spectra at the amide I and II region (1400–1800 $cm^{-1}$) were analyzed to reflect changes in secondary structures of WPI before and after glycation.

### 2.6. Degree of glycation

The degree of glycation was measured for unreacted amines using the 2,4,6-trinitrobenzene sulfonic acid (TNBS) method (Tainturier, Roullier, Martenot, & Lorient, 1992), with some modifications. A fresh working solution of TNBS was prepared prior to assays by diluting the 1% TNBS solution (Geno Technology, Inc., St Louis, MO) in 0.1 M sodium bicarbonate aqueous solution to an overall TNBS concentration of 0.01% w/v. Each conjugate solution

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