Food Hydrocolloids 44 (2015) 453–460

Contents lists available at ScienceDirect

Food Hydrocolloids

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

High temperature-short time glycation to improve heat stability of whey protein and reduce color formation

Gang Liu, Qixin Zhong*

Department of Food Science and Technology, The University of Tennessee, Knoxville, USA

ARTICLE INFO

Article history:
Received 1 May 2014
Accepted 10 October 2014
Available online 4 November 2014

Keywords:
Whey protein isolate
Maillard reaction
High temperature-short time glycation
Heat stability
Color
5-Hydroxymethyl-2-furaldehyde

ABSTRACT

Glycation of whey protein isolate (WPI) improves functionalities such as heat stability. Conventionally, glycation is achieved by heating at <~90 °C for hours up to several days, which favors the formation of Maillard reaction products such as 5-hydroxymethyl-2-furaldehyde (HMF) that is linked to undesirable color and flavor. In this work, we report for the first time that glycating WPI with lactose or maltodextrin at 130 °C for <30 min and 79% relative humidity simultaneously reduced the color formation and improved the heat stability. Maltodextrin was less reactive than lactose, as a longer (30 vs. 20 min) glycation at 130 °C was needed to obtain heat stability at all acidity (pH 3.0–7.0) and ionic (0–150 mM NaCl or $CaCl_2$) conditions. However, WPI glycated with maltodextrin had a lighter color and a lower content of HMF. Our findings indicate high temperature-short time glycation favors the industrial production of high quality WPI ingredients.

1. Introduction

Whey proteins isolates (WPI) are commercially available ingredients with high contents of essential amino acids and versatile functionalities applicable for the production of transparent beverages (Vardhanabhuti, Yucel, Coupland, & Foegeding, 2009). Thermal pasteurization or sterilization is required to ensure microbiological safety and quality of protein beverages. Conversely, protein denaturation during thermal processing can cause protein aggregation that results in quality defects of turbidity, dispersion instability, and possibly gelation in beverage products. The heat instability is particularly problematic at acidity near the isoelectric point (pI) of whey proteins, about pH 4.8–5.2 (Hickstein & Peuker, 2008; Nakano & Ozimek, 2000), which however is within the acidity range of many beverage products (Etzel, 2004; Mettler, Rusch, & Colombani, 2006).

Thermal stability of proteins can be improved by glycation with a reducing saccharide via the Maillard reaction (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Jimenez-Castano, Villamiel, & López-Fandiño, 2007). Heat stability of ovalbumin, evaluated at pH 7.0 and 0.1% protein concentration, was much improved after glycation with glucose or glucuronic acid at 65% relative humidity and 50 °C for up to 3 d (Aoki et al., 1999). Recently, we reported that thermal stability of WPI at pH 3.0–7.0 and 0–150 mM NaCl or $CaCl_2$ was improved after glycation with maltodextrin (MD) at 60 °C and 80% relative humidity for 72 h (Liu & Zhong, 2012). Dispersions of glycated WPI constituted with 7% protein remained transparent after heating at 88 °C for 2 min and 138 °C for 1 min, with the former simulating the hot-fill process used for acidic (pH < 4.6) beverages (Etzel, 2004) and the latter exceeding ultrahigh temperature processing requirement of dairy products (at 138 °C for at least 8 s) (McGarrahan, 1982). Similar improvement of heat stability was observed for WPI glycated with dextran at a mass ratio of 1:4 at 50, 55, and 60 °C for 48–144 h (Wang & Ismail, 2012). The much reduced thermal gelation properties of WPI were observed after glycation with dextran at 60 °C and 63% relative humidity for 2–9 days (Spotti et al., 2014). Glycation in solutions with 10% WPI and 30% dextran at pH 6.5 and 60 °C for 48 h was also studied as an approach to improve thermal stability of whey proteins (Zhu, Damodaran, & Lucey, 2010). Pulsed electric field was another approach to glycate WPI with dextran in solutions to improve the heat stability (Sun, Yu, Zeng, Yang, & Jia, 2011). In our other study, WPI glycated with lactose or maltodextrin at a higher temperature (80 °C) for a much shorter time (2 h) showed similar heat stability improvement, and the dispersions had a much lighter color than those glycated with MD at 60 °C for 72 h (Liu & Zhong, 2013). Lactose was more reactive than maltodextrin, showing better heat stability of glycated WPI and a darker color. These two studies suggest that high temperature short time (HTST) glycation can be

* Corresponding author. Department of Food Science and Technology, The University of Tennessee, 2510 River Drive, Knoxville, TN 37996, USA. Tel.: +1 865 974 6196; fax: +1 865 974 7332.
E-mail address: qzhong@utk.edu (Q. Zhong).

http://dx.doi.org/10.1016/j.foodhyd.2014.10.006
0268-005X/

further studied to improve the quality and functionality of glycated WPI.

Among the quality defects of glycated whey proteins are sensory attributes such as color, flavor and taste (Martins & Van Boekel, 2005). Maillard reaction is a series of complex reactions leading to a mixture of reaction products through different pathways (Moreno, Molina, Olano, & López-Fandiño, 2003). The reaction is initiated by formation of covalent bonds between the carbonyl group of a reducing saccharide and an amino group of a protein, i.e., the Amadori reaction (Martins & Van Boekel, 2005). The formed N-substituted glycosylamine is unstable and rearranges to form ketosamines that can undergo various reactions, producing intermediate Amadori rearrangement products that can react further to form final glycation products (Martins & Van Boekel, 2005). Dependent on the reaction temperature, humidity and duration, as well as the structure and mass ratio of protein and reducing saccharides, both intermediate products such as furfurals and reductones and final products of brown polymers (melanoidins) can be present in glycated proteins (Ajandouz, Tchiakpe, Ore, Benajiba, & Puigserver, 2006; Moreno et al., 2003). The brown pigments are needed in processes such as baking and coffee roasting as they are associated with sensory characteristics of these products, but are undesirable in most dairy products (Martins & Van Boekel, 2005). In addition to impacting color and flavor, some Maillard reaction products have negative effects on health, e.g., 5-hydroxymethyl-2-furfural (HMF) that has been investigated extensively for its potential mutagenicity, toxicity, carcinogenicity (Capuano & Fogliano, 2011; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000; Surh & Tannenbaum, 1994), and adverse effects on human blood cells (Rufian-Henares & de la Cueva, 2008). Therefore, minimizing the formation of HMF and color is needed to produce functional glycated whey proteins.

The objective of the present study was to investigate the possibility of HTST glycation of WPI and lactose or maltodextrin at 130 °C for up to 30 min to improve heat stability and reduce color and HMF formations. The molecular structures, heat stability, HMF content, and color of the HTST-glycated WPI were characterized and were compared to those prepared at 80 °C and 79% relative humidity for 2 h which were used in our previous study (Liu & Zhong, 2013).

2. Materials and methods

2.1. Materials

WPI and MD (with a dextrose equivalent of 18) were acquired from Hilmar Ingredients (Hilmar, CA) and Grain Processing Corp. (Muscatine, IA), respectively. Precast 4–20% gradient polyacrylamide gels and protein markers were purchased from Bio-Rad Laboratories Inc. (Hercules, CA). Other chemicals were from either Thermo Fisher Scientific Inc. (Pittsburgh, PA) or Sigma–Aldrich Corp. (St. Louis, MO).

2.2. Preparation of glycated whey protein

WPI and saccharide (MD or lactose) were hydrated at a mass ratio of 1:2 in distilled water overnight on a stir plate operating at 300 rpm and room temperature (21 °C). The dispersion mixture was adjusted to pH 7.0 with 1.0 N NaOH and spray-dried using a Mini-Spray Dryer (model B-290, Büchi Laboratoriums-Technik, Flawil, Switzerland). The spray-drying process was carried out at following operational conditions: an inlet air temperature of 160 °C, an outlet temperature of ca. 65 °C, an air flow rate of 32 m^3/h, and a feed flow rate of about 4 mL/min. The resultant powder was applied in a container to a layer thickness of about 1 mm. The container was then placed on a perforated plate in an emptied desiccator. A saturated KBr solution was placed under the perforated plate to pre-equilibrate the powder to 79% relative humidity (Akhtar & Dickinson, 2007). The desiccator with sample was then incubated in an oven at 130 °C for 10, 15, 20 and 30 min or at 80 °C for 2 h.

2.3. Amino acid composition

The amino acid composition analysis was determined by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University (New Haven, CT). Detailed procedures were provided in our earlier paper (Liu & Zhong, 2013). The assay involved hydrolysis of proteins by 6.0 N HCl at 115 °C for 16 h. The hydrolyzed sample was separated using a Hitachi 2622SPH ion-exchange column (4.6 mm ID × 60 mm) equipped on a Hitachi L-8900 PH amino acid analyzer (Tokyo, Japan). The assay conditions partially destroy tryptophan and cysteine and their contents are not reported.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS–PAGE was carried out using a precast polyacrylamide gel obtained from Bio-Rad Laboratories, Inc. (Hercules, CA). The 7% w/v protein solutions were diluted 25 folds with an SDS-PAGE sample buffer (catalog number MB01015, GenScript Corp., Piscataway, NJ) and then heated at 95 °C for 5 min. Five μL of each sample was loaded into each well of the gel. Electrophoresis was conducted at 200 V until the indicator dye reached the gel bottom. Afterwards, the gel was stained with Coomassie Blue for 10 min and destained until bands became visible.

2.5. Analytical ultracentrifugation (AUC)

AUC was conducted using a Beckman XL-I analytical ultracentrifuge (Beckman Coulter, Inc., Palo Alto, CA). The overall protein concentration of solutions at pH 3.0 was adjusted to 0.12% w/v. The UV absorbance data at 280 nm was obtained every 4 min using the standard double sector cell, which was performed at 50,000 rpm and 25 °C. Data were analyzed according to the reference (Liu & Zhong, 2012, 2013). The number of saccharide (lactose or MD) molecules (N) glycated to one whey protein molecule was obtained by the following equation:

$$N = (M_2 - M_1)/M_3 \quad (1)$$

where M_1, M_2, and M_3 represent the average molecular weight (MW) of WPI conjugate, WPI, and saccharide (lactose or MD), respectively. The monomeric MW of WPI and its conjugate was used in calculations.

2.6. Heat stability test

Heat stability was assessed at 88 °C for 2 min, simulating a hot-fill process in the beverage industry (Etzel, 2004). Samples were prepared at protein concentration of 7% w/v and adjusted to pH 3.0–7.0 and 0–150 mM NaCl or $CaCl_2$. One mL of final dispersions was contained in 4 mL glass vials sealed with caps, before heating in a water bath.

2.7. Color determination

The instrument was a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Color parameters L^*, a^*, and b^* were measured on a layer of 10 mL samples with 7% w/v protein. Duplicate analyses were carried out for each sample.