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Microwave-assisted isomerisation of lactose to lactulose and Maillard conjugation of lactulose and lactose with whey proteins and peptides

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ABSTRACT

Lactose was isomerised to lactulose by microwave heating and purified by a methanolic procedure to a product with approximately 72% lactulose content. Afterwards, lactose and the lactulose-rich product (PLu) were conjugated with either whey protein isolate (WPI) or its antioxidant hydrolysate (WPH) through microwaving. Lactose had a higher Maillard reactivity than PLu, and WPH was more reactive than WPI. The browning intensity of WPI-sugar systems was however higher than that of WPH-sugar pairs. Atomic force microscopy showed larger (up to ≈103 nm) particles for WPI-PLu conjugates compared to WPH-PLu counterparts (up to ≈39 nm). The Maillard conjugation progressively increased the radical-scavenging activity of WPI/WPH-sugar pairs with increasing conjugation time and improved the foaming properties of WPI and WPH. The WPI/WPH-sugar conjugates showed higher solubility and emulsification index than unreacted counterpart pairs. For native WPI, β-lactoglobulin was not degraded by *in vitro* gastric digestion, whereas for WPH-PLu conjugates degraded completely.

1. Introduction

Lactulose (Lu; 4-O-β-D-galactopyranosyl-D-fructofuranose) is a synthetic and prebiotic disaccharide and has been used in the pharmaceutical industry, mainly for treatment of chronic constipation and hepatic encephalopathy (Zokaee, Kaghazchi, Zare, & Soleimani, 2002). Other functionalities of lactulose include reduction of blood ammonia and serum lipids, anti-gallstone formation and anti-endotoxic effects, control of blood glucose and insulin, mineral absorption stimulation, and anti-carcinoma and gastrointestinal infections suppression (Schumann, 2002). This sugar has a low energy value (2.0 kcal g^{-1}), good solubility in water, texturising, stabilising and antiplaque effects, and since the sweetness of lactulose is about 60–80% that of sucrose can be used as a sucrose substitute in the food industry (Schumann, 2002). In the food industry, lactulose is used as a bifidus factor (utilised by *Bifidobacterium*) and prebiotic food additive (Panesar & Kumari, 2011).

Lactulose is produced by isomerisation of lactose (La; 4-O-β-D-galactopyranosyl-D-glucose) by chemical and enzymatic methods. The chemical isomerisation provides higher yields than the enzymatic procedure (Khatami, Ashtiani, Bonakdarpour, & Mehrdad, 2014) and is used exclusively for industrial lactulose production *via* Lobry de Bruyn-Alberda van Ekenstein (LA) rearrangement usually under alkaline conditions (Seo, Park, & Han, 2015). However, the conventional isomerisation process of lactose to lactulose is time-consuming and energy-intensive, which motivated the authors of the present communication to find out an alternative and/or complementary route for rapid lactose to lactulose isomerisation. Microwave heating was employed for this purpose.

Whey proteins are a source of essential amino acids (Martinez-Alvarenga et al., 2014) and their enzymatic hydrolysates have been used extensively for their bioactive and functional properties (Leksrisompong, Gerard, Lopetcharat, & Drake, 2012). Some documented bioactive characteristics of milk protein hydrolysates are anticancer, antihypertension, antithrombosis, mineral carrying, antioxidation and immunomodulation properties (Adjonu, Doran, Torley, & Agboola, 2014). The biologically active peptides may however taste bitter and show some poor techno-functional properties, such as low emulsification and foaming capabilities. It is therefore of interest to derivatise the potentially bioactive peptides, for example, through the Maillard reaction to tune their techno-functional characteristics and generate a class of new bioactive ingredients.

There is no report in the literature dealing with Maillard conjugation of bioactive peptides with nutraceutical sugars. The objective of the present study was therefore to conjugate an *in vitro* antioxidant whey protein hydrolysate with lactose or lactulose and study some of the technological properties and digestibility of conjugates. Microwave heating was employed to increase the

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conjugation rate. Lactulose was obtained *via* microwave-assisted isomerisation of lactose and purified through an inexpensive method.

2. Materials and methods

2.1. Materials

O-Phthaldialdehyde (OPA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and L-leucine were purchased from Sigma–Aldrich (St. Louis, MO). Whey protein isolate (WPI) with at least 90% protein content was a kind gift from Arla Food Ingredients (Viby J, Denmark). The enzyme pepsin with activity of 3000 NFU mg^{-1} was purchased from Bio Basic Canada Inc., Markham, ON, Canada). The enzyme Corolase N was obtained as a sample from AB Enzymes (ABF Ingredients Company, Darmstadt, Germany) with minimum activity of 600 UHb g^{-1}. All other chemicals were of analytical reagent grade and purchased from Merck (Darmstadt, Germany).

2.2. Microwave isomerisation of lactose

The lactose solution (40 mg mL^{-1}) was prepared by dissolving a known amount of lactose in distilled water and stirred at 500 rpm for 10 min at room temperature (25 °C). The pH of the solution was adjusted to 9.0 by 2.0 M NaOH and then 30 mL of the solution were poured into a flat-bottomed flask. The flask was sealed and placed in a microwave oven (M245; Butane, Tehran, Iran) and irradiated at 2450 MHz, 600 W power (270 W actual power) for 0, 10, 40, 50 and 60 s. The isomerised solution was rapidly cooled to ambient temperature and analysed for determination of pH (at 25 °C), total reducing sugars (Lane and Eynon method; James, 1995) and lactulose concentration (Leon, 1970).

2.3. Methanolic purification of lactulose from isomerised solution

Purification of lactulose in isomerised solution was carried out by the modified method of Montañés, Olano, Ibáñez, and Fornari (2007). The isomerised solution was dried by rotary vacuum evaporator at 50 °C and then transferred at a known amount (0.8 g) into 15 mL plastic vials, followed by mixing with 10 mL methanol. The vials were stirred in an incubation shaker at 150 rpm and 30 °C for 48 h. Subsequently, the vials were kept for 24 h without shaking, and the crystallised lactose was separated by centrifugation at 1520*g* for 10 min at room temperature. Supernatant was collected and methanol was evaporated by heating under vacuum. A known volume of distilled water was added to the purified lactulose (PLu) which was then analysed for pH, total reducing sugar and lactulose concentration.

2.4. Lactulose content determination

The quantitative determination of lactulose was carried out by the optical rotation method (Leon, 1970) with some modifications. Lactose and lactulose are dextrorotatory and laevorotatory optically active substances with specific rotations of +52.5° and −51.5°, respectively. First, the lactose concentration in isomerised solution was measured by polarimeter (ATAGO, AP-300) at 589 nm and 28 °C according to the following equation:

$$C\ (\%) = \frac{100 \times \alpha}{l \times [\alpha]} \quad (1)$$

where C is lactose concentration, α is the measured optical rotation in degrees, l is the path length in decimetres, and $[\alpha]$ is specific rotation at 28 °C and 589 nm. Then, lactulose concentration was calculated by the following equation:

$$\text{Lactulsoe content}\ (\%) = \frac{\text{Total reducing sugar} - C}{\text{Total reducing sugar}} \times 100 \quad (2)$$

2.5. Hydrolysis of whey protein isolate (WPI) solution

WPI solution was prepared at a concentration of 40 mg mL^{-1} by stirring at 500 rpm for 2 h at room temperature and stored at 4 °C for 12 h. Sodium azide (50 ppm) was added to the solution during stirring as an antimicrobial. The solution was later heated at 80 °C for 15 min and cooled rapidly to 30 °C. Based on our previous experiments and optimisation studies, the protein solution was hydrolysed at pH 7.7 and 55 °C for 2 h with the proteolytic enzyme Corolase N at an enzyme-to-protein ratio of 1:100. The solution was stirred at 100 rpm during hydrolysis. Afterwards, the whey protein hydrolysate (WPH) was heated at 90 °C for 15 min to inactivate the proteolytic enzyme and cooled rapidly to room temperature.

2.6. Preparation and characterisation of Maillard conjugates

2.6.1. Conjugates preparation

Lactose or the purified lactulose were conjugated with WPH or WPI by microwave heating. For this purpose, WPI/WPH solutions were diluted with distilled water to 3 mg mL^{-1}, and used for lactose/lactulose hydration. The sugars concentration was ultimately 3 mg mL^{-1}. The mixed solutions were microwaved in sealed glass containers at 600 W and pH 6.0 for up to 60 s. After heating, each solution was removed and cooled rapidly in an ice-water bath.

2.6.2. Conjugation extent determination

The glycation degree of WPI and WPH was determined by measuring the free amino groups content of conjugates using the OPA colorimetric assay (Zhang et al., 2012). The OPA solution was prepared freshly by dissolving 80 mg of OPA in 2.0 mL of 95% ethanol and this solution was mixed with 50 mL of 100 mM sodium tetraborate buffer (pH 9.75), 200 μL of β-mercaptoethanol and 5.0 mL of 20% (*w*/*v*) SDS solution. Next, the solution was diluted to 100 mL with distilled water and 1.5 mL of OPA solution was added to 100 μL of diluted samples (1 mg mL^{-1}). After incubation at room temperature for 2 min, the absorbance of solution was recorded at 340 nm by using a UV/visible spectrophotometer (CE2502, Cecil Instruments Ltd., Cambridge, UK). The free amino groups content was calculated using L-leucine (0–0.5 mg mL^{-1}) standard curve and the glycation degree (%) was calculated based on the loss of amino groups compared to unreacted protein according to the following equation:

$$\text{Glycation degree}\ (\%) = \left(1 - \frac{A}{B}\right) \times 100 \quad (3)$$

where A and B are the free amino groups content in glycated samples and unreacted proteins, respectively.

2.6.3. Estimation of the Maillard reaction products content

The UV absorbance and browning intensity of WPH-sugar and WPI-sugar conjugates as a function of microwave heating were measured spectrophotometrically at room temperature at 294 nm (for estimating intermediate products content) and 420 nm (for estimating final products content) (Tu et al., 2013). Corresponding non-heated WPI-sugar and WPH-sugar mixtures were used as blanks.