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Maillard conjugation of lactulose with potentially bioactive peptides

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ABSTRACT

Milk ultrafiltration permeate was heated at 97 °C in the presence of eggshell for 60 min. This decreased the ash content of permeate and converted ≈17% of lactose to lactulose. The isomerized permeate was subsequently purified to a lactulose-rich product (LRP; ≈70% lactulose content to total sugar) through crystallizing lactose out by methanol. The LRP and lactose were then conjugated with either whey protein isolate (WPI) or its antioxidant hydrolysate (WPH) through Maillard reaction at 90 °C. The amount of the Maillard reaction advanced products was higher for WPI–lactose system than WPH–lactose counterpart; whilst, the DPPH· scavenging activities of WPH–sugar conjugates were significantly higher than those of WPI–sugar counterparts. Based on free amino groups content measurement, it was found that lactose is more reactive than LRP for Maillard conjugation with both WPI and WPH. Fourier transform infrared spectroscopy confirmed the bonding of the anomeric region of saccharide configuration of lactulose with WPH.

1. Introduction

Lactose (4-O-β-D-galactopyranosyl-D-glucose) is a major constituent of milk permeate (4.5–4.8%) and may be processed to a variety of non-absorbable compounds, such as lactulose, lactitol and lactobionic acid (Paseephol, Small, & Sherkat, 2008). Lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose) is a synthetic non-digestible disaccharide and has empirical formula, molecular weight and melting point of, respectively, $C_{12}H_{22}O_{11}$, 342.30 g mol^{-1} and 169 °C (Seki & Saito, 2012). This sugar has higher solubility and sweetness than lactose (Zokaee, Kaghazchi, Zare, & Soleimani, 2002). In pharmaceutical industry lactulose is used for treatment of hepatic encephalopathy, chronic constipation, and inflammatory bowel disease. It also lowers blood glucose and insulin levels (anti-diabetic), increases minerals absorption and has purported anti-endotoxin and tumor prevention effects (Panesar & Kumari, 2011), as well as, hypocholesterolemic influence. Lactulose is used as a bifidus factor in food industry and is highly stabile under thermal-acidic conditions, therefore can be used as an excellent ingredient for acidic foods, such as fruit juices (Seki & Saito, 2012).

Lactulose is conventionally synthesized by isomerizing glucose moiety of lactose into fructose under alkaline condition via the Lobry de Bruyn–Alberda van Ekenstein transformation mechanism (Panesar & Kumari, 2011). Alkali catalysts, such as sodium, potassium and calcium hydroxides, tertiary amines, magnesium oxide, and sodium and potassium carbonates are conventionally used for the isomerization reaction (Montilla, Del Castillo, Sanz, & Olano, 2005; Zokaee et al., 2002). By using complexing reagents such as borates and aluminates (Panesar & Kumari, 2011) that form stable complexes with lactulose under alkaline conditions and shift the reaction equilibrium to more lactulose production, high isomerization efficiencies (70–80%) can be achieved. However, large amounts of borate and aluminate are needed; in addition, separation of these reagents from mixture is difficult and tedious (Zokaee et al., 2002).

Eggshell is produced at large amounts by egg processing industries. High quantities of eggshell are still disposed as waste and can affect the environment. Eggshell contains about 96% calcium carbonate, 1% magnesium carbonate, 1% calcium phosphate, organic materials and water (Oliveira, Benelli, & Amante, 2013). Because calcium carbonate is a major component of eggshell, it can be used as a solid calcium carbonate-based catalyst for isomerization of lactose to lactulose. Montilla et al. (2005) reported that optimal yield of lactulose (11.8 mg mL^{-1}) was achieved by 6 mg mL^{-1} eggshell powder at 98 °C and 60 min of reaction. These authors reported that the catalyst removal from mixture was feasible and

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carried out by centrifugation. Paseephol et al. (2008) by heating milk concentrated permeate at 96 °C for 120 min in the presence of oyster shell powder (12 mg mL^{-1}) obtained an isomerization yield of 18–21% corresponding to 7.2–8.4 mg mL^{-1} lactulose.

Milk protein hydrolysates, whey protein hydrolysates and fermented dairy products contain multi-functional biologically active peptide sequences with antioxidative, antimicrobial, antithrombotic, immunomodulatory and antihypertensive activities (Korhonen & Pihlanto, 2006). On the other hand, Maillard reaction products (MRPs) also exhibit antioxidative activity in food systems, through several mechanisms such as metal ions chelation, radical chains breaking, hydrogen peroxide breakdown, and reactive oxygen species scavenging. The reaction initiates via conjugation of carbonyl and amino groups-bearing compounds (Vhangani & Van Wyk, 2013) and occurs when a mixed solution of protein/peptide and reducing sugars is heated. The conjugation of antioxidant (potentially bioactive) peptides with reducing sugars, especially the prebiotic lactulose can result in highly antioxidative and potentially nutraceutical products with diverse applications including at formulation of health-promoting functional foods. There was however no report dealing with peptides and reducing sugars conjugation through the Maillard reaction as a conventional food processing procedure. The objective of the present study was therefore to conjugate lactose and a lactulose-rich product with antioxidant peptides existed in whey protein hydrolysate. Eggshell was employed as an environment-friendly catalyst for permeate lactose isomerization to lactulose.

2. Materials and methods

2.1. Materials

Adhering albumen of fresh eggshells was removed through washing with water; subsequently the eggshells were dried at 105 °C for 12 h, grounded and sieved by 80 mesh screen (mesh size, 177 μm). The obtained eggshell powder was stored at ambient temperature (Montilla et al., 2005). Whey protein isolate (WPI) was a kind gift from Arla Food Ingredients (Vibyj, Denmark) with at least 90% protein content. The enzyme Corolase N (with minimum activity of 600 UHb g^{-1}) was obtained as a sample from AB Enzymes (ABF Ingredients Company, Darmstadt, Germany). O-phthaldialdehyde (OPA) reagent was purchased from Sigma–Aldrich Co. (St Louis, MO, USA). Other chemicals and reagents used were of analytical grade and purchased from Merck (Darmstadt, Germany).

Milk ultrafiltration permeate was prepared by dissolving milk permeate powder (Pegah Dairy Co., Tehran, Iran) in distilled water (4.80% wt/v) for 15 min at 400 rpm and 30 °C.

2.2. Isomerization reaction

Conventional isomerization was carried out following the method of Montilla et al. (2005). Briefly, eggshell at concentration of 12 mg mL^{-1} was added to 100 mL milk ultrafiltration permeate at a flat bottom flask. The flask was placed in an oil bath and heated up to 97 °C within minutes. Eggshell-permeate mixture was stirred throughout the isomerization process for 60 min. Then, the flask was cooled down rapidly with tapping water to room temperature for stopping isomerization. This was followed by centrifuging mixture at 1520×*g* for 10 min at room temperature in order to sediment eggshell powder. Supernatant was collected and stored at 4 °C for less than 12 h before analyses.

Sedimented eggshell powder was collected, washed and dried. The dried powder was reused to isomerize fresh permeate by the above mentioned procedure. Eggshell powder was reused for four rounds; after each time, isomerized permeate was analyzed for determination of pH, total reducing sugars, ash content and conversion yield of lactose to lactulose.

2.3. Purification of isomerized milk permeate

Lactulose in isomerized milk permeate was purified according to the method of Montañés, Olano, Ibáñez, and Fornari (2007) with some modifications. For this purpose, eggshell-free centrifugal supernatant was dried by rotary vacuum evaporator at 50 °C and then a known amount of dried sample was weighed in 15 mL plastic vials. Subsequently, 10 mL methanol was added to the sample and the vials were incubated at 30 °C for 48 h while being shaken at 150 rpm. Later, samples were kept for 24 h without shaking and after separation of crystallized lactose by centrifugation at room temperature (1520×*g*, for 10 min), supernatant was collected and heated under vacuum for methanol evaporation. This procedure yielded a lactulose-rich product (LRP) that was subsequently dissolved in distilled water for analyses.

2.4. WPI hydrolysis

WPI powder was hydrated (40 mg mL^{-1}) and stirred at 500 rpm for 2 h at ambient temperature; sodium azide (50 ppm) was added for preventing microbial growth. The protein solution was stored overnight at 4 °C to warrant complete hydration. Next day, the solution was heated at 80 °C for 15 min, cooled rapidly to 30 °C and pH was adjusted on 7.7 by 2.0 M NaOH. The proteolytic enzyme corolase was added to whey protein solution at the enzyme to protein ratio of 1:100 and hydrolysis progressed for 2 h at 55 °C while the solution was being stirred at 100 rpm. The whey protein hydrolysate (WPH) was heated at 90 °C for 15 min to inactivate the enzyme and cooled rapidly to ambient temperature.

2.5. Maillard reaction

Maillard reaction was progressed by heating either lactose or lactulose-rich product (3 mg mL^{-1}) with WPI solution and WPH (3 mg mL^{-1}) at 90 °C for up to 45 min at pH 6.0. Preliminary experiments indicated that heating at higher pH values (7.0 and 8.0) degraded lactulose to organic acid products and decreased pH. A known amount of mixed samples was removed from solutions at times 0, 15, 30 and 45 min of heating and cooled rapidly in ice-water bath for subsequent analyses.

2.6. Analysis

2.6.1. Chemical analysis of permeate

Permeate samples (before and after isomerization, and after purification) were analyzed for determination of pH (at 20 °C), ash content (dry ash method; AOAC, 1997), protein content (Kjeldahl method; AOAC, 1997), and total reducing sugars (Lane and Eynon method; James, 1995).

2.6.2. Lactulose content determination

Lactulose content of isomerized permeate was measured by Seliwanoff's reagent according to the method of Amine, Moscone, and Palleschi (2000) with some modifications. Briefly, 2 mL of Seliwanoff's reagent (4 M HCl containing resorcinol 0.05%) was added to 0.5 mL isomerized milk permeate, followed by heating at 90 °C for 10 min. Samples were cooled and filtered through disposable filter (0.22 μm porosity) and absorbance was measured at 398 nm by using a UV/Visible spectrophotometer (CE2502, Cecil Instrument Ltd., England). The blank was prepared with the same procedure, but distilled water was used instead of isomerized permeate. Standard curve of lactulose was plotted at concentration range of 0.5–5 mM.