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Improving thermal stability of hydrolysed whey protein-based infant formula emulsions by protein–carbohydrate conjugation

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ABSTRACT

Whey protein hydrolysate (WPH) ingredients are commonly used in the manufacture of partially-hydrolysed infant formulae. The heat stability of these emulsion-based formulae is often poor, compared with those made using intact whey protein. The objective of this study was to improve the heat stability of WPH-based emulsions by conjugation of WPH with maltodextrin (MD) through wet heating. Emulsions stabilised by different protein ingredients, whey protein isolate (WPI_E), whey protein hydrolysate (WPH_E), heated WPH (WPH-H_E), and WPH conjugated with MD (WPH-C_E) were prepared and heat treated at 75 °C, 95 °C or 100 °C for 15 min. Changes in viscosity, fat globule size distribution (FGSD) and microstructure, evaluated using confocal laser scanning microscopy (CLSM), were used to monitor the effects of hydrolysis, pre-heating and conjugation on the heat stability of the emulsions. Heat stability increased in the order WPH_E < WPI_E << WPH-H_E <<< WPH-C_E; emulsions WPH_E, WPI_E and WPH-H_E destabilised on heating at 75 °C, 95 °C or 100 °C, respectively. Flocculation and coalescence of oil droplets were mediated by protein aggregation (as evidenced by CLSM) on heat treatment of WPH-H_E emulsion at 100 °C, while no changes in FGSD or microstructure were observed in WPH-C_E emulsion on heat treatment at 100 °C, demonstrating the excellent thermal stability of emulsions prepared with the conjugated WPH ingredient, due principally to increased steric stabilisation as a result of conjugation.

1. Introduction

Human milk is widely accepted as the best source of nutrients required for proper short- and long-term development of infants. The composition of mother's milk is compatible with the infant's digestive system and is known to minimise the risk of gastrointestinal and respiratory infections (Alles, Scholtens, & Bindels, 2004; Exl, 2001; O'Mahony, Ramanujam, Burgher, & O'Callaghan, 2011). However, it is not always possible to provide the infant with mother's milk. Efforts to develop humanised formulae for infant nutrition are focused on many aspects of formula composition and functionality including matching protein content and profile (i.e., whey-dominant protein profile and α-lactalbumin enrichment) (Chatterton, Rasmussen, Heegaard, Sørensen, & Petersen, 2004; Crowley, Dowling, Caldeo, Kelly, & O'Mahony, 2016; Hambraeus, 1977; Ogra & Greene, 1982; O'Mahony et al., 2011), fatty acid profile (Berger, Fleith, & Crozier, 2000), carbohydrate, vitamin and mineral levels to those present in human milk (Pehrsson, Patterson, & Khan, 2014).

Formulae manufactured using whey protein hydrolysate (WPH) ingredients can be categorised based on the degree of hydrolysis of the protein; the main categories are amino acid-based formulae (AAF), where proteins/peptides are hydrolysed to their constituent amino acids; extensively hydrolysed formulae (EHF) containing oligopeptides with molecular weight below 3000 Da and partially hydrolysed formulae (PHF) containing oligopeptides ranging in molecular weight up to 20,000 Da (Exl, 2001; Lowe et al., 2011). While AAF and EHF products are mainly intended for therapeutic purposes in infants suffering from, or with a high risk of cow's milk allergy (CMA), infant nutrition products from the PHF group cannot be used for therapeutic purposes but are recommended for infants at risk of CMA as they have been shown to provide a preventive effect thereon (Chandra, 1997; Exl, 2001; von Berg et al., 2008). Partially hydrolysed formulae are often also referred to as 'pre-digested' formulae based on their improved digestibility and absorption in the gut, helping to reduce gastrointestinal discomfort issues (Hernández-Ledesma, García-Nebot, Fernández-Tomé, Amigo, & Recio, 2014).

Hydrolysis causes alteration to the functional properties of proteins and hydrolysate functionality is ultimately dependent on a number of factors including enzyme type and specificity, hydrolysis conditions and method of enzyme inactivation (Panyam & Kilara, 1996; Tavano, 2013). Generally, moderate hydrolysis improves the surface activity of proteins/peptides as the hydrolysate fractions migrate rapidly to

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surfaces/interfaces which can give rise to improved functional properties such as foaming and emulsification (Agboola & Dalgleish, 1996a,b; Banach, Lin, & Lamsal, 2013; Foegeding & Davis, 2011; Kilara & Panyam, 2003). Moderate hydrolysis of globular proteins (i.e., whey proteins) improves their heat stability as a result of the diminished secondary structure; however, this improvement does not always translate directly to more complex systems such as emulsions made using hydrolysed whey protein, where heat stability has been shown to be negatively affected by hydrolysis of whey protein (Singh & Dalgleish, 1998; Ye & Singh, 2006). Responsibility for the poor heat stability of hydrolysed whey protein-based emulsions is related to reduced steric hindrance (Ye, Hemar, & Singh, 2004) and increased number of available (i.e., exposed) reactive sites on protein/peptide molecules at the oil globule surface and in the serum phase of the emulsion (Euston, Finnigan, & Hirst, 2000; Hunt & Dalgleish, 1995).

Conjugation of proteins with carbohydrates using the Maillard reaction has been shown to be effective in modifying protein functionality (Liu, Ru, & Ding, 2012; Oliver, Melton, & Stanley, 2006; O'Regan & Mulvihill, 2010a,b). Extensive research documenting the beneficial effects of protein modification through conjugation is available in the scientific literature; improved functional properties of proteins including solubility, emulsification, encapsulation and emulsion stability (Akhtar & Dickinson, 2003; Kasran, Cui, & Goff, 2013a,b; Lei, Wang, Liang, Yuan, & Gao, 2014), thermal stability (Jimenez-Castano, Lopez-Fandino, Olano, & Villamiel, 2005; Kato, Aoki, Kato, Nakamura, & Matsuda, 1995; Liu et al., 2012; O'Regan & Mulvihill, 2010a; Wang & Zhong, 2014) or foaming and gelation properties (Campbell, Raikos, & Euston, 2003; Martínez & Pilosof, 2013) as a result of conjugation are well documented. However, published scientific reports on the properties and functionality of hydrolysed whey protein ingredients modified by Maillard conjugation appear to be limited; the authors are not aware of any published studies reporting on the performance of such ingredients in oil-in-water emulsion systems, particularly in infant formula (IF) systems. The current study aims to investigate and report on the performance of ingredients produced by conjugation of hydrolysed whey protein with maltodextrin in comparison with that of intact whey protein in production and stabilisation of model IF emulsions.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) and whey protein hydrolysate (WPH; 8% degree of hydrolysis; DH) were obtained from Carbery Food Ingredients Ltd. (Ballineen, Co. Cork, Ireland). Composition of WPI and WPH ingredients was determined using standard International Dairy Federation (IDF) methods and molecular weight profile of the protein ingredients was determined using size exclusion chromatography as detailed by Drapala, Auty, Mulvihill, and O'Mahony (2015). The composition, DH and molecular weight profile of the WPI and WPH ingredients are shown in Table 1. Maltodextrin (MD) was obtained from Corcoran Chemicals Ltd. (Dublin, Ireland) and had moisture and ash contents of <5.0% and <0.2%, respectively. Soybean oil was obtained from Frylite Group Ltd. (Strabane, Co. Tyrone, Northern Ireland). All other chemicals and reagents used in the study were of analytical grade and sourced from Sigma-Aldrich (Arklow, Co. Wicklow, Ireland).

2.2. Conjugate and stock protein solutions

Two unheated stock solutions (5.00 g/100 mL protein) were prepared from WPI and WPH and allowed to hydrate for 18 h at 4 °C and pH was adjusted to 6.8 before being used for emulsion formulation. The protein–carbohydrate conjugate solution was prepared by solubilising required quantities of WPH and MD in ultrapure water for 2 h at 20 °C using a magnetic stirrer to give 5.00 g/100 mL protein and 5.00 g/100 mL carbohydrate. The solution was adjusted to pH 8.2 with 0.5 N potassium hydroxide (KOH) and allowed to hydrate for 18 h at 4 °C, before being readjusted to pH 8.2 with 0.5 N KOH at 20 °C. Aliquots (250 mL) of this solution were placed in 500 mL screw-capped, glass conical flasks and heated at 90 °C for 8 h. After heating for 8 h, the solutions were cooled immediately to 4 °C and stored at that temperature overnight. A control for the heat treatment was prepared in exactly the same way as outlined above with the exception that no MD was added to the WPH. In summary, four stock protein or protein–carbohydrate solutions were prepared and were subsequently used to formulate emulsions that are referred to as whey protein isolate emulsion (WPI_E), whey protein hydrolysate emulsion (WPH_E), heated whey protein hydrolysate emulsion (WPH-H_E) and conjugated whey protein hydrolysate emulsion (WPH-C_E), respectively.

Table 1
Composition, degree of hydrolysis and molecular weight profile of the whey protein isolate (WPI) and whey protein hydrolysate (WPH) ingredients used in the preparation of emulsions.

Composition	WPI	WPH
	% *w*/*w*	
Protein	87.2 ± 0.9	83.7 ± 0.5
Fat	0.72 ± 0.1	0.67 ± 0.1
Carbohydrate[a]	4.21	7.80
Ash	2.76 ± 0.1	2.92 ± 0.1
Moisture	5.11 ± 0.0	4.91 ± 0.1
Degree of hydrolysis	NA[b]	8.00
Molecular weight profile	% of total protein	
Insoluble	0.00	2.00 ± 0.6
>20 kDa	28.0 ± 3.4	12.0 ± 1.6
10–20 kDa	50.5 ± 3.7	24.2 ± 8.8
5–10 kDa	3.90 ± 0.2	9.49 ± 1.9
2–5 kDa	15.6 ± 0.3	11.9 ± 1.6
1–2 kDa	0.92 ± 0.1	9.30 ± 2.0
0.5–1 kDa	0.29 ± 0.0	11.0 ± 1.9
<0.5 kDa	0.83 ± 0.7	20.2 ± 3.0

[a] Carbohydrate content determined by difference.
[b] NA = not applicable.

2.3. Measurement of free thiol groups

The level of free thiol groups in the stock protein solutions was determined following an assay described by Hoffmann and van Mil (1997) with the exception that a Bis-Tris/HCl buffer (pH 6.8) was used in place of the Tris–HCl buffer (as performed by Alting, Hamer, De Kruif, Paques, & Visschers, 2003). Aliquots (0.05 mL) of stock protein solutions (5.00 g/100 mL) were added to 2.70 mL of 0.05 M Bis-Tris/HCl buffer (pH 6.8) before adding 0.25 mL of Ellman's reagent (107.5 mg/100 g of the buffer) (Ellman, 1959). Solutions were vortexed and absorbance was measured using a dual beam UV–visible spectrophotometer (Varian Cary 300, Varian Ltd., Walton-on-Thames, UK) at a wavelength of 412 nm. Measurements were completed in triplicate and the level of thiol groups was calculated using a molar extinction coefficient for 2-nitro-5-mercapto-benzoic acid (i.e., Ellman's reagent) of 13,600 M^{-1} cm^{-1}.

2.4. Preparation of emulsions

Model infant formula emulsions containing 1.55, 3.50 and 7.00 g/100 mL of protein, oil and carbohydrate, respectively, were prepared as follows: stock protein or protein–carbohydrate solutions (see Section 2.2) were diluted with ultrapure water to the appropriate concentration followed by addition of MD as required with continuous mixing using a magnetic stirrer at intermediate speed for 1 h at 22 °C to prepare the aqueous phases of the emulsions. Innate levels of lactose present in the protein powders were taken into account when calculating the requirement for added carbohydrate (i.e., MD). Emulsions were prepared as described by Drapala et al. (2015) except that higher 1st and