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Interfacial properties of whey protein and whey protein hydrolysates and their influence on O/W emulsion stability

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ABSTRACT

Protein hydrolysates are commonly used in high-tolerance or hypoallergenic formulae. The relation between the physicochemical properties of hydrolysed proteins (*i.e.,* size, molecular weight distribution, charge, hydrophobicity), and their emulsifying properties is not fully understood. In this work, the emulsion forming ability (*i.e.,* the equilibrium between droplet formation and coalescence during emulsification), the gravitational stability, the adsorption kinetics and the interfacial dilatational rheology of whey proteins and whey protein hydrolysates were investigated. More extensive hydrolysis resulted in a progressive decrease of the surface hydrophobicity of the emulsifiers (*i.e.,* whey protein or whey protein hydrolysates). Whey protein was able to form smaller emulsion droplets at low concentrations (<1 wt%) compared to whey protein hydrolysates (WPH). When the concentration of WPH was in excess (>2 wt%), similar minimum droplet sizes were obtained due to the adsorption of large peptides. Whey protein-stabilised interfaces showed the lowest interfacial tension and ζ-potential, which both increased with increasing degree of hydrolysis. Whey protein produced stronger oil-water interfacial layers (*i.e.,* high dilatational moduli and non-linear behavior) and had higher protein surface coverage compared to WPH. Small whey protein peptides (<5 kDa) formed a weak oil-water interfacial film, which led to unstable emulsions. In whey protein-stabilised emulsions, β-lactoglobulin showed preferential interfacial adsorption over α-lactalbumin. In emulsions containing WPH, large peptides (>5 kDa) were preferentially adsorbed over small peptides. Emulsion physical stability was strongly influenced by the oil droplet size, and by the formation of an inter-connected viscoelastic film at the oil droplet interface which was observed only for whey protein and peptides with high molecular weight (>5 kDa). These results should be considered when formulating specialized nutrition emulsions.

1. Introduction

Many food products for specialized nutrition (*i.e.,* clinical and infant nutrition) are formulated as oil-in-water (O/W) emulsions. Whey protein is often used in such products because of its high nutritional quality and its ability of providing physical stability by acting as an emulsifier. As an alternative to whey protein, whey protein hydrolysates can be used (*e.g.,* in hypoallergenic or high-tolerance formulae). Hydrolysis may change the technological functionality of proteins, and reduce their physical stabilising effect at the oil-water interface (Drapala, Auty, Mulvihill, & Mahony, 2016; García-Moreno, Guadix, Guadix, & Jacobsen, 2016; Lacou, Léonil, & Gagnaire, 2016). The relation between the physicochemical properties of whey protein peptides (i.e., size, molecular weight distribution, charge, hydrophobicity), and their emulsifying properties is not fully understood. It has been shown that whey protein hydrolysates with low or high degree of hydrolysis (DH) (4–10% or 27–35%) have poorer emulsifying ability compared to native whey protein, whereas whey protein hydrolysates with an intermediate DH (20–27%) showed improved emulsion forming ability (Euston, Finnigan, & Hirst, 2001). More than the DH, the peptide size distribution has been reported as crucial for emulsion stability (Van der Ven, Gruppen, De Bont, & Voragen, 2001). Coalescence during emulsion formation could be prevented when appropriate concentrations of peptides with a molecular weight higher than 2 kDa were used (*e.g.,* 3–4 kDa). Furthermore, Agboola, Singh, Munro,

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List of abbreviations

ADT	Automated drop tensiometer
ANSA	8-Anilino-1-naphtalenesulfonic acid ammonium salt
BCA	Bicinchoninic acid
BPB	Bromophenol blue
BSA	Bovine serum albumin
DH	Degree of hydrolysis
GFC	Gel filtration chromatography
MCT	Medium chain triglycerides
PSH	Protein surface hydrophobicity
SDS	Sodium dodecylsulphate
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
WPH	Whey protein hydrolysate
WPI	Whey protein isolate

Dalgleish, and Singh (1998) reported that fairly stable emulsions (in which the main destabilisation mechanism was coalescence with no oiling-off) could be formed with highly hydrolyzed whey protein, at high peptide concentration (>2%, in a 4 wt% soy oil-in-water emulsion). However, they showed that the stabilising action of these highly-hydrolysed whey proteins was due to the increased concentration of high molecular weight peptides (>5 kDa) at the interface. It has also been indicated that the hydrophilic/hydrophobic distribution of amino acids in peptides is more important for emulsion forming ability than the length of the peptide (Rahali, Chobert, Haertlé, & Guéguen, 2000). However, size may influence the rheological properties of the interfacial film, which may result in a decreased physical stability (Berton-Carabin, Schröder, Rovalino, Schröen, & Sagis, 2016; Davis, Doucet, & Foegeding, 2005). Likewise, Gauthier, Paquin, Pouliot, and Turgeon (1993) suggested that the presence of intact sequences of hydrophobic amino acids on the peptides are important for emulsion formation. Most of these studies related the physicochemical properties of proteins and peptides to emulsion forming ability (*e.g.,* the droplet size after emulsion production), and they do not consider the interfacial properties of proteins and peptides in relation to the physical stability of the emulsion. It is generally recognized that there is a clear link between the physical instability of protein-stabilised emulsions (*e.g.,* coalescence) and the properties of the protein film at the oil-water interface, such as, the formation of a dense viscoelastic and interconnected network with high elastic moduli (*e.g.*, interfacial rheology) (Dickinson, 1986; Maldonado-Valderrama & Rodriguez Patino, 2010; Mokni Ghribi et al., 2015). However, such interfacial properties have not been investigated for hydrolysed proteins. In the present work, the physicochemical properties of whey protein and peptides (two commercial whey protein hydrolysates and their permeates obtained by 10 kDa cut-off ultrafiltration) were studied in relation to their interfacial properties (adsorption kinetics, interface rheology, surface load and composition), and their ability to form and stabilise O/W emulsions.

2. Experimental

2.1. Materials

Commercial whey protein isolate (WPI), two whey protein hydrolysates (H1 and H2), alpha-lactalbumin (α-LA) and beta-lactoglobulin (β-LG) were supplied by Davisco Foods International Inc. MN, USA. The composition of WPI, H1 and H2 is reported in Fig. 1 and Table 1. Medium chain tryglyceride oil (MCT, Miglyol 812 N) was purchased from Cremer Oleo Division, Germany. All other reagents were purchased from Sigma Aldrich, Merck or Bio-Rad and were of analytical grade.

2.2. Methods

2.2.1. Characterization of whey protein isolate and whey protein hydrolysates

Fractionation of hydrolysates. For some experiments, protein hydrolysates were separated into two fractions depending on molecular weight using 10 kDa cut-off ultrafiltration centrifugal filter tubes (Amicon filter units/Vivaspin® Turbo 15). The tubes were filled with concentrated whey protein hydrolysate (H1 or H2) solution (5 wt%) and centrifuged at 4000 g for 30 min at 20 °C. The permeates (P1 and P2) were recovered and used for further experiments.

Peptide size distribution. The peptide size distribution of hydrolysed whey protein was determined by gel filtration chromatography (GFC) using a 17-5176-01 Superdex™ Peptide 10/300 GL column (GE Healthcare, Buckinghamshire, UK) and a UV-detector. Cytochrome C from bovine heart (MW = 12,327 Da), aprotinin from bovine lung (MW = 6500 Da), adrenocorticotropic hormone from porcine pituitary (MW = 4567 Da), insulin A-chain oxidized ammonium salt from bovine pancreas (MW = 2532 Da),

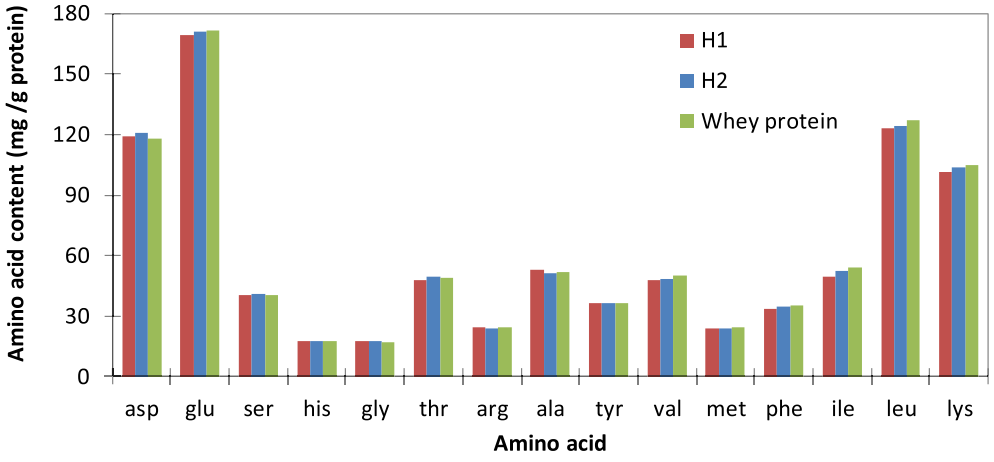

Fig. 1. Amino acid composition of H1, H2 and whey protein determined by HPLC-fluorescence.