International Dairy Journal 34 (2014) 146–152

Contents lists available at ScienceDirect

International Dairy Journal

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

Pilot-scale production of hydrolysates with altered bio-functionalities based on thermally-denatured whey protein isolate

I.B. O'Loughlin [a,b], B.A. Murray [a], R.J. FitzGerald [b], A. Brodkorb [a], P.M. Kelly [a,*]

[a] Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland
[b] Life Sciences Department, University of Limerick, Limerick, Ireland

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form
6 June 2013
Accepted 10 July 2013

ABSTRACT

Whey protein isolate (WPI) solutions (100 g L^{-1} protein) were subjected to a heat-treatment of 80 °C for 10 min. Unheated and heat-treated WPI solutions were hydrolysed with Corolase® PP at pilot-scale to either 5 or 10% degree of hydrolysis (DH). Hydrolysates were subsequently processed via cascade membrane fractionation using 0.14 μm, and 30, 10, 5 and 1 kDa cut-off membranes. The compositional and molecular mass distribution profiles of the substrate hydrolysates and membrane processed fractions were determined. Whole and fractionated hydrolysates were assayed for both angiotensin-I-converting enzyme (ACE) inhibitory activity and ferrous chelating capabilities. A strong positive correlation ($P < 0.01$) was established between the average molecular mass of the test samples and the concentration needed to chelate 50% of the iron (CC_{50}) in solution. The lowest ACE inhibition concentration ($IC_{50} = 0.23$ g L^{-1} protein) was determined for the 1 kDa permeate of the heat-treated 10% DH hydrolysate.

1. Introduction

The functional properties of proteins are related to their structural characteristics. Processing steps, such as heat-treatment, can induce structural alterations in whey proteins that may affect protein techno- and bio-functional attributes. These alterations modify the physico-chemical characteristics (O'Loughlin, Murray, Kelly, FitzGerald, & Brodkorb, 2012) and susceptibility to enzymatic hydrolysis (Boye, Ma, & Harwalker, 1997). The enzymatic release of bio-functional peptides from whey proteins has resulted in the generation of ingredients with potential to, for example, control hypertension (Tavares et al., 2011) and enhance mineral binding (Kim et al., 2007b).

Hypertension induces blood vessel constriction and a reduction in elasticity as well as hypertrophy and collagen deposition within the vascular system resulting in increased risk of cardiovascular diseases (Dézsi, 2000). Upon renin cleavage of angiotensinogen to release angiotensin I, the glycoprotein angiotensin-I-converting enzyme (ACE) converts angiotensin I to the potent vasoconstrictor angiotensin II. Much research has been conducted into bio-functional ingredients that possess ACE-inhibitory activity for incorporation into food products to aid, or reduce the reliance on, anti-hypertensive drugs, such as Captopril®. Numerous reports of whey protein derived inhibitory peptides exist in the literature (Morris & FitzGerald, 2008; Mullally, Meisel, & FitzGerald, 1997; Norris & FitzGerald, 2013).

Iron deficiency affects an estimated 2 billion people worldwide (Zimmermann & Hurrell, 2007). The inter-conversion of iron to its different oxidation states is utilised by biological systems for electron transfer, ligand binding, cell growth and differentiation. Iron needs to be in a soluble format to be effectively absorbed. Direct fortification of food products with iron is problematic due to its reactivity and ability to generate reactive oxygen species (ROS) and low solubility (Fidler et al., 2004). Thus, interest in mineral-binding hydrolysates has focused on their ability to reduce the reactivity and enhance the solubility of iron in fortified foods (Ait-Oukhatar et al., 2002).

For these reasons iron-binding hydrolysates and fractions derived from whey protein substrates have garnered significant interest of late. Iron-binding peptide complexes have been produced through hydrolysis, e.g., alcalase hydrolysis of β-lactoglobulin (Zhou et al., 2012). From this study an optimum mass ratio for complex formation at neutral pH was determined to be 40 (hydrolysate):1 (iron, as $FeCl_3$). Peptic hydrolysis of lactoferrin can liberate 'lactoferricin', f(17–41), which has been shown to exhibit iron-binding capacity (Vegarud, Langsrud, & Svenning, 2000; Wakabayashi, Takase, & Tomita, 2003). Fractionated hydrolysates of heated (100 °C, 10 min) whey protein concentrate (WPC) high in

* Corresponding author. Tel.: +353 25 42222.
E-mail address: phil.kelly@teagasc.ie (P.M. Kelly).

0958-6946/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.idairyj.2013.07.009

alanine, phenylalanine and lysine with improved iron-binding capabilities have been reported (Kim et al., 2007a). Also, digestion of α-lactalbumin-enriched formulae has exhibited increased absorption of iron using infant rhesus monkeys as an animal model (Kelleher, Chatterton, Nielsen, & Lonnerdal, 2003). Hydrolysates may be subsequently formulated into products demonstrating enhanced mineral absorption characteristics, similar to, for example Capolac® (Arla Food Ingredients).

While recent investigations into dual functionalities and thermal pre-treatments of whey proteins have been published (Adjonu, Doran, Torley, & Agboola, 2013), there remains little literature available on the kinetics of pilot/semi-pilot scale whey protein isolate (WPI) hydrolysis and the compositional and bioactive properties of, and consequences for, resulting hydrolysates. The primary objectives of this study were two-fold. Firstly, the scale-up of a high viscosity, aggregated WPI dispersion with limited solubility from a previous thermally-denatured WPI laboratory hydrolysis process (O'Loughlin et al., 2012). Secondly, investigation of the bio-functional, namely; anti-hypertensive and ferrous chelation, attributes of these hydrolysates and membrane processed fractions of heat-treated and unheated control WPI dispersions. Concomitant relationships between selective process treatments and bio-functionalities were also investigated.

2. Materials and methods

2.1. Materials

Whey Protein Isolate (Isolac®) was provided by Carbery Food Ingredients (Ballineen, Co. Cork, Ireland). The powder contained 89.3% (w/w) protein by Kjeldahl ($N \times 6.38$; Merrill & Watt, 1973). The level of denaturation of the starting material was 6.8% (w/w) determined via isoelectric precipitation at pH 4.6 (Parris & Baginski, 1991). The digestive-enzyme complex Corolase® PP was from AB Enzymes GmbH (Darmstadt, Germany) and is compositionally described elsewhere (Mullally, O'Callaghan, FitzGerald, Donnelly, & Dalton, 1994). Corolase® PP possesses generally regarded as safe (GRAS) status and high proteolytic activity in whey producing non-bitter hydrolysates (Svenning, Brynhildsvold, Molland, Langsrud, & Vegarud, 2000).

All other reagents and chemicals were procured from Sigma–Aldrich (Dublin, Ireland) unless otherwise stated. All experiments were determined in duplicate unless otherwise stated.

2.2. Pilot-scale heat-treatment and enzymatic hydrolysis of WPI

WPI was reconstituted to 107 g L^{-1} on a protein basis with reverse-osmosis water (RO) in a jacketed 316-grade stainless steel vessel fitted with a gated scrape surface agitator. The WPI dispersions (at pH 6.4) were adjusted to pH 8.0 with 4 M NaOH and then diluted to 100 g L^{-1} protein with RO water to a final volume of 400 L. Heat-treated WPI dispersions were brought to 80 °C for 10 min by indirect steam injection before cooling to 50 °C utilising chilled water.

Enzymatic hydrolysis at 50 °C was initiated by the addition of Corolase® PP. The pH was maintained at pH 8.0 throughout the reaction by manual titration with 4 M NaOH using a Sandpiper® air-operated double-diaphragm polypropylene pump (Warren Rupp Inc., OH, USA) and a DEM JCS-60 floor scales (DEM Machines Ltd., Naas, Ireland) to monitor base addition. The degree of hydrolysis (DH) was determined from the volume of base consumed (Adler-Nissen, 1986; O'Loughlin et al., 2012). The enzyme:substrate ratio (E:S) employed varied depending on the target DH. An E:S of 2:300 (0.67%, w/w) on a protein equivalent basis was utilised to generate hydrolysates at a DH of 5%, whereas for 10% DH hydrolysates an E:S of 1:100 (1.0%, w/w) was employed. The reaction was terminated by heating the hydrolysate solutions 85 °C for 25 s in a Unison H17 109 plate-and-frame 53 L heat-exchanger plant (Unison Engineering Services Ltd., Limerick, Ireland). A 50 L sample of the heat-inactivated hydrolysate was withdrawn, evaporated (to ~40% total solids) on a Tetra Scheffers™ falling-film single-stage evaporator (Tetra Pak, Gorredijk, The Netherlands) and spray dried using a pilot scale Anhydro Lab 3 spray drier (SPX Flow Technology A/S, Soeborg, Denmark). Inlet and outlet temperatures during spray drying ranged from 185 to 190 and 85 to 90 °C, respectively.

2.3. Microfiltration and ultrafiltration of WPI hydrolysates

The remaining hydrolysate sample (~350 L) was subjected to cascade membrane fractionation utilising a GEA Model F unit (GEA Process Engineering A/S, Skanderborg, Denmark). For hydrolysates at 5% and 10% DH that were derived from heat-treated WPI, initial fractionation was via microfiltration (MF). MF was accomplished with three Tami Isoflux™ ceramic membranes (25 × 1178 mm, 23 channels, Tami Industries, Nyons Cedex, France) having a nominal cut-off of 0.14 μm and a total membrane area of 1.05 m^2. MF was carried out at 50 °C to a volume concentration factor (VCF) of 8. A feed recirculation rate of 1500 L h^{-1} at 1 bar and membrane inlet pressure of 4.2 bar were maintained throughout processing. For hydrolysates to 5% DH of unheated control substrates two 30 kDa cut-off Koch KMS HFK™-328 spiral wound membranes (96 × 965 mm, Koch Membrane Systems, Wilmington, MA, US) were used as the initial fractionation step. For control hydrolysates to the 10% DH level two 10 kDa cut-off Koch spiral wound membranes were utilised.

In all cases, the subsequent permeate stream (~300 L) prepared above was then subjected to ultrafiltration (UF) using the same GEA model F unit fitted with two Koch KMS HFK™-328 spiral wound membranes. These membranes have a nominal cut-off of 5 kDa and a total membrane area (TMA) of 11.2 m^2. The 5 kDa permeate stream (~300 L) was then processed (at 50 °C) on the GEA model F plant fitted with two 1 kDa cut-off Alpha Laval UF-ETNA spiral wound membranes (TMA 11.2 m^2, 95 × 965 mm, Alpha Laval AB, Lund, Sweden) to a VCF of 3, whereupon feed volume was returned to 300 L with RO and UF was carried out to a final VCF of 7. A feed recirculation rate of 1500 L h^{-1} at 1 bar and membrane inlet pressure of 5 bar were maintained throughout processing. All process streams, with the exception of 1 kDa retentates and permeates, were dehydrated in a pilot scale Anhydro Lab 3 spray drier using the conditions as described above. The 1 kDa retentates and permeates were further concentrated (to ~35% total solids) before spray drying, as outlined above, in an Anhydro F1 Lab single effect falling-film evaporator. As an example, the process flow diagram for the production of fractionated powders from hydrolysed (to 10% DH) heat-treated WPI is shown in Supplementary material (Fig. S1). Final volumes prior to concentration and total solids levels are provided in Supplementary material Fig. S2 for all four processes.

2.4. Particle size, chromatography and compositional analysis of control and heated-treated WPI and hydrolysates

Particle size analysis was carried out using a Malvern Mastersizer MSS (Malvern Instruments Ltd., Worcestershire, U.K.) according to the protocol of O'Loughlin et al. (2012). Size-exclusion chromatography (SEC) on samples was carried out according to the protocol of O'Loughlin et al. (2013b).

Protein content was determined by micro-Kjeldahl on a Foss Kjeltec™ 8400 (Foss, Hillerød, Denmark). The procedure was modified from Koops, Klomp, and Elgersma (1975) where a conversion factor of 6.38 was used in accordance with Merrill and Watt