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Physicochemical and sensory characteristics of whey protein hydrolysates generated at different total solids levels

David Spellman[1], Gerard O'Cuinn[2] and Richard J FitzGerald[1]*

[1] Department of Life Sciences, University of Limerick, Republic of Ireland
[2] Department of Life Sciences, Galway-Mayo Institute of Technology, Republic of Ireland

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Whey protein hydrolysates were generated at different total solids (TS) levels (50–300 g/l) using the commercially available proteolytic preparation Debitrase™ HYW20, while enzyme to substrate ratio, pH and temperature were maintained constant. Hydrolysis proceeded at a faster rate at lower TS reaching a degree of hydrolysis (DH) of 16·6% at 300 g TS/l, compared with a DH of 22·7% at 50 g TS/l after 6 h hydrolysis. The slower breakdown of intact whey proteins at high TS was quantified by gel-permeation HPLC. Reversed-phase (RP) HPLC of hydrolysate samples of equivalent DH (~15%) generated at different TS levels indicated that certain hydrophobic peptide peaks were present at higher levels in hydrolysates generated at low TS. Sensory evaluation showed that hydrolysates with equivalent DH values were significantly ($P<0·0005$) less bitter when generated at 300 g TS/l (mean bitterness score=25·4%) than hydrolysates generated at 50 g TS/l (mean bitterness score=39·9%). A specific hydrophobic peptide peak present at higher concentrations in hydrolysates generated at low TS was isolated and identified as β-lactoglobulin f(43–57), a fragment having the physical and chemical characteristics of a bitter peptide.

Keywords: Enzymatic hydrolysis, whey protein, total solids, bitterness, hydrophobicity.

Whey proteins are an important protein source having a wide range of applications due to their excellent functional properties. Whey protein concentrate (WPC) has been used as a functional ingredient in meat products due to its gelation and emulsification properties (El-Magoli et al. 1996; Yetim et al. 2001). WPC can be used in the nitrogen fortification of fruit juices and other beverages due to the good solubility of whey proteins at acid pH (Cayot & Lorient, 1997). Whey proteins are also used to enhance the foaming properties of confectionery products (De Wit, 1989). Whey proteins have a high nutritional value as they contain a relatively high proportion of branched chain and essential amino acids (Ha & Zemel, 2003).

Enzymatic hydrolysis has been extensively used to modify the functional properties of food proteins (Doucet et al. 2001; Flanagan & FitzGerald, 2002; Molina Ortiz & Wagner, 2002). Perhaps the most dramatic improvement of functionality as a result of partial hydrolysis is the increase in solubility (Panyam & Kilara, 1996). Perea et al. (1993) and Mutilangi et al. (1996) showed that proteolysis of whey proteins with various enzyme preparations increased solubility over a wide pH range. Hydrolysis of WPC with papain was found to improve emulsification properties where degree of hydrolysis (DH) values of 2·8 and 3·0% were optimal for improvement of emulsion activity index and emulsion capacity, respectively (Lieske & Konrad, 1996).

Hydrolysis of WPC with Alcalase 0·6 L was shown to increase foam capacity but decrease foam stability in the DH range 0–10%. However, foam capacity was reduced and foam stability was increased at DH values greater than 10% (Perea et al. 1993). Ju et al. (1995) found that the heat-induced gelation ability and gel strength of whey protein isolate (WPI) could be either increased or decreased by hydrolysis, depending on the enzyme, pH of gelation and DH.

The proteins in whey are reported to have a wide range of biological and physiological functions including immunomodulating (Mercier et al. 2003) and anti-cancer activities (McIntosh et al. 1998). Enzymatic hydrolysis of whey proteins has been shown to liberate biologically active peptides (Mullally et al. 1997; Mercier et al. 2003) and reduce allergenicity (Asselin et al. 1988; Ena et al. 1995). The physiological function of food proteins can also be enhanced by enzymatic hydrolysis. The negative

*For correspondence; e-mail: dick.fitzgerald@ul.ie

osmotic effects associated with a high intake of free amino acids makes protein hydrolysates a more suitable nutrient for patients with impaired luminal hydrolysis and other disorders of digestion and amino acid absorption. Small peptides are absorbed more rapidly from the small intestine than free amino acids (Webb, 1990), making protein hydrolysates an ideal supplement in sports nutrition.

The main disadvantage of using protein hydrolysates as food ingredients is the appearance of a bitter taste as the hydrolysis reaction proceeds. Intact food proteins do not contribute significantly to flavour; their molecular size alone suggests that interaction with taste receptors is unlikely (Pawlett & Bruce, 1996). However small hydrophobic peptides released during enzymatic hydrolysis have been shown to be responsible for the bitter taste of protein hydrolysates (Matoba & Hata, 1972; Ney, 1979). Ney (1971) devised a quantitative method to predict peptide bitterness using data reported by Tanford (1962) to calculate an average hydrophobicity value for peptides, which was then used to predict if a peptide would taste bitter. According to Ney (1971), peptides with a molecular mass less than 6000 Da having an average hydrophobicity value greater than 1400 cal mol^{-1} would have a bitter taste.

Many factors such as enzyme to substrate ratio, substrate concentration, pH and temperature are known to affect the rate of enzyme-catalysed reactions (Castro et al. 1996; Margot et al. 1997; Camacho et al. 1998; Marquez & Vazquez, 1999). However, little information is available on the effect of total solids concentration on the rate of food protein hydrolysis or the properties of the resultant hydrolysates. The objective of this study was to determine the effect of total solids concentration on the hydrolysis of WPC with the commercially available proteolytic preparation Debitrase™ HYW20 when pH, temperature and enzyme to substrate ratio were maintained constant. This study focuses on the effect of total solids concentration on the physicochemical properties of the resultant hydrolysates and not on the kinetics of the enzyme-catalysed reaction.

Materials and Methods

Materials

Whey protein concentrate (WPC 75; 739·6 g protein/kg) was purchased from a commercial supplier. Acetic acid, Coomassie brilliant blue R-250, triflouroacetic acid (TFA), HPLC grade acetonitrile, L-leucine and ethanol were obtained from BDH (Poole, Dorset, UK). Sodium dodecyl sulphate, HPLC grade methanol, HPLC grade water, picrylsulphonic acid (TNBS), L-tyrosine hydrochloride, low molecular mass electrophoresis markers, electrophoresis grade glycine, trizma base, glycerol, ammonium persulphate, *N*′*N*′-methylene-bis-acrylamide, acrylamide, tris-HCl, β-mercaptoethanol, bromophenol blue, thyroglobulin, isoamyl alcohol, *N*,*N*,*N*′*N*′ tetramethylethylenediamine (TEMED) and caffeine were from Sigma Chemical Co. (Poole, Dorset, UK). Puradisc™ 25 AS disposable syringe filters (0·2 μm) were from Whatman (Maidestone, UK). Supor® hydrophilic membrane filters (47 mm, 0·2 μm) were from Pall Corporation (Ann Arbor, Michigan, MA). Debitrase™ HYW20 was kindly supplied by Rhodia Ltd. (Cheshire, UK). All other reagents were of analytical grade, unless otherwise stated.

Enzymatic hydrolysis of WPC

Hydrolysis experiments were carried out in a 500 ml sealed reaction vessel (Metrohm, Herisau, Switzerland). Aqueous solutions of WPC 75 were allowed to hydrate for one hour at room temperature with gentle mixing. The protein solution was then equilibrated at 50 °C and the pH adjusted to 7·0 with 2·0 M-NaOH before addition of the enzyme. Debitrase™ HYW20 was added at an enzyme: substrate (E : S) ratio of 10 g enzyme powder/kg protein). The solution was mixed with an over-head stirrer (Heidolph Instruments, Schwabach, Germany) and the pH was maintained constant throughout hydrolysis using a pH stat (718 Stat Titrino, Metrohm, Herisau, Switzerland). Hydrolysate samples were taken at various time intervals and heated at 90 °C for 20 min to inactivate enzyme activity. Samples were stored at −20 °C until required for analysis. Each hydrolysis experiment was performed twice. The average standard deviation between hydrolysis experiments at a particular TS level was less than 0·5% of a DH unit.

Quantification of degree of hydrolysis using TNBS

Degree of hydrolysis (DH, %) of WPC hydrolysates was quantified using the TNBS method of Adler-Nissen (1979), as recently described (Spellman et al. 2003).

SDS-PAGE of protein hydrolysates

SDS-PAGE was performed in vertical-slab gels using a Protean II system (Biorad Laboratories, California, US), as described by Laemmli (1970). A stacking gel concentration of 40 g acrylamide/l and a separating gel concentration of 120 g/l were used. Hydrolysate samples were applied at a protein equivalent of 40 μg protein per lane. A current of 10 mA per gel was applied until the dye front had migrated past the stacking gel after which the current was increased to 20 mA per gel until the dye front reached the end of the separating gel. After electrophoresis, the gels were stained with 2 g coomassie brilliant blue R-250/l (in a 10 : 40 : 50 solution of acetic acid : methanol : water) and de-stained with a 10 : 40 : 50 solution of acetic acid : methanol : water.

Reversed-phase HPLC of whey protein hydrolysates

Analytical reversed-phase (RP-)HPLC was carried out on whey protein hydrolysate samples using a Waters HPLC system, comprising a Model 1525 binary pump, a Model 717 Plus autosampler and a Model 2487 dual λ absorbance

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detector interfaced with a Breeze™ data-handling package (Waters, Milford, MA, USA). The column used was a Phenomenex Jupiter (C18, 250 × 4·6 mm ID, 5 μm particle size, 300 Å pore size) separating column (Phenomenex, Cheshire, UK) with a Security Guard™ system containing a C18 (ODS) wide pore cartridge (4 × 3 mm ID, Phenomenex, Cheshire, UK). The column was equilibrated with solvent A (0·1% TFA) at a flow rate of 1·0 ml min^{-1} and peptides were eluted with an increasing gradient of solvent B (0·1% TFA, 80% acetonitrile). Detector response was monitored at 214 nm. Hydrolysate samples were diluted to 7·5 g protein equivalent/l in distilled H_2O, filtered through 0·2 μm syringe filters and 20 μl was applied to the column.

Semi-preparative RP-HPLC was performed using the same HPLC system fitted with a Phenomenex Jupiter column (C18, 250 × 15 mm ID, 10 μm particle size, 300 Å pore size) with a Security Guard™ system containing a C18 (ODS) wide pore cartridge (10 × 10 mm ID) (Phenomenex, Cheshire, UK). The column was equilibrated with solvent A (0·1% TFA) at a flow rate of 10·0 ml min^{-1} and peptides were eluted with an increasing gradient of solvent B (0·1% TFA, 80% acetonitrile). Detector response was monitored at 214 nm. Hydrolysate samples were diluted to 22·0 g protein equivalent/l in distilled H_2O, filtered through 0·2 μm syringe filters and 500 μl applied to the column. Fractions collected by semi-preparative RP-HPLC were evaporated using a centrifugal evaporator (Jouan RC 10.22, Jouan Inc., Winchester, Virginia, USA) connected to a refrigerated solvent trap (Jouan RCT 90) and a Javac model DD40 vacuum pump (Javac Pty. Ltd., Victoria, Australia). Samples were dried at temperature setting three using pulsed ventilation-evaporation until all liquid had been evaporated.

Gel permeation HPLC of whey protein hydrolysates

Gel permeation HPLC (GP-HPLC) was performed using the Waters HPLC system previously described. Hydrolysate samples were diluted to 7·5 g protein equivalent/l in H_2O, filtered through 0·2 μm syringe filters and 20 μl applied to a TSK G2000 SW separating column (600 × 7·5 mm ID) connected to a TSKGEL SW guard column (75 × 7·5 mm ID) (Smyth & FitzGerald, 1997). Separation was by isocratic elution with a mobile phase of 0·1% TFA in 30% acetonitrile, at a flow rate of 1·0 ml min^{-1}. Detector response was monitored at 214 nm. A calibration curve was prepared from the average retention times of standard proteins and peptides (Smyth & FitzGerald, 1998). The void volume (V_o) was estimated with thyroglobulin (600 000 Da) and the total column volume (V_t) was estimated with L-tyrosine.HCl (218 Da).

Peptide sequencing

Dried samples from semi-preparative RP-HPLC were dissolved in 50% acetonitrile, containing 0·1% formic acid. Samples were loaded into thin walled glass nano-vials, mounted on a nano-spray device and sprayed under atmospheric pressure into a Waters Micromass QTOF-2 mass spectrometer (Waters, Milford, MA, USA). Data corresponding to intact peptide masses was collected. Selected peptides were further fragmented in a collision cell and daughter ion spectra were interpreted using MassLynx software incorporating BioLynx packages to assist in sequence interpretation (Waters Micromass).

Sensory evaluation of whey protein hydrolysates

Hydrolysate samples, at a protein equivalent of 22·0 g/l, were randomly presented in quadruplicate to a 10-member sensory panel, which had been trained to detect and quantify bitterness using caffeine. Panellists were trained to assign bitterness scores to unknown solutions based on a 0–100% scale, where a 100% bitter solution was taken to have a bitterness value equivalent to 1 g caffeine/l. Non-sparkling mineral water was used as the 0% bitterness standard.

At each sitting, panellists were firstly presented with solutions of 0·00, 0·25, 0·50, 0·75 and 1·00 g caffeine/l, which had been labelled as 0, 25, 50, 75 and 100% bitter. Panellists then assigned bitterness scores to the test hydrolysates on the basis of the caffeine standards they had tasted. Between samples, panellists were asked to eat a piece of un-salted cracker and rinse their mouths thoroughly with non-sparkling mineral water.

Apparent viscosity

Apparent viscosity (η_{app}) was measured using a Brookfield programmable DV-II+ viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) fitted with an ultra-low (UL) adaptor. The UL adaptor was connected to a Brookfield circulating water bath (model TC-500) by an ULA-40Y water jacket. Apparent viscosity values were measured at 25 °C, pH 7·0 at various shear rates.

Statistical analysis

One-way analysis of variance (ANOVA) and independent-samples t-tests were performed on sensory data comparing samples generated at the different total solids levels using SPSS, version 11.0.

Results and Discussion

DH values obtained were significantly different ($P<0·0005$) for WPC hydrolysates generated at different TS levels, with a lower DH being achieved as the TS was increased (Fig. 1). After 6 h hydrolysis at 300 g TS /l a DH value 16·6% was achieved compared with a DH value of 22·7% after 6 h hydrolysis at 50 g TS/l. Therefore, the rate and extent of proteolysis was slower at higher TS levels. A possible

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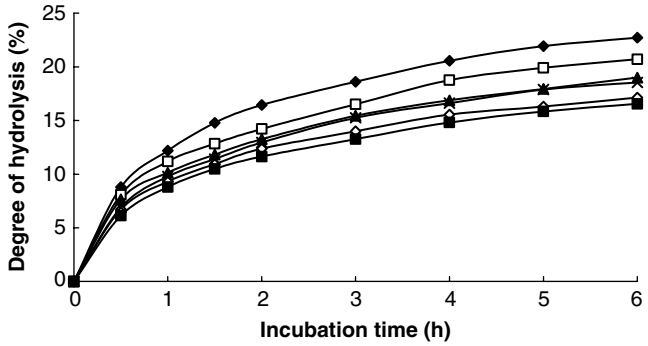

Fig. 1. Degree of hydrolysis (DH, %) values obtained using the trinitrobenzene sulphonic acid method during the hydrolysis of whey protein concentrate with Debitrase™ HYW20 at 50 (◆), 100 (□), 150 (▲), 200 (×), 250 (◇) and 300 (■) g/l total solids (pH 7·0, 50 °C, E : S = 10 g/kg).

explanation may be that a 300 g TS/l solution of WPC was found to have η_{app} of 57·60 mPa (shear rate = 9·78 s^{-1}) compared with η_{app} of 1·50 mPa (shear rate = 85·6 s^{-1}) for a 50 g TS/l solution. It was not possible to compare η_{app} values at equivalent shear rates due to the large difference in viscosity between the low TS and high TS solutions. Increasing solution viscosity is known to affect the reaction rates of several enzymes including carboxypeptidase A (Gavish & Werber, 1979), subtilisin (Ng & Rosenberg, 1991) and an H^+-ATPase from *Kluyveromyces lactis* (Uribe & Sampdero, 2003). Debitrase™ HYW20 is a blend of microbial proteinase and exopeptidase activities (Rhodia Inc. Technical Bulletin: TB DEB 99-11.0).

Molecular mass distribution profiles, obtained by GP-HPLC, also showed a slower rate of proteolysis at higher TS (Fig. 2). After 6 h hydrolysis at 300 g TS/l and 50 g TS/l, 21·8% and 10·8% of the peptide material in the sample was >10 kDa, respectively (Fig. 2a). Consequently, there was a much faster increase in the percentage of low molecular mass peptide material (<0·5 kDa) in hydrolysates generated at low TS (Fig. 2b). After 6 h hydrolysis at 50 g TS/l, 47·9% of peptide material was <0·5 kDa compared with 36·1% at 300 g TS/l. The faster breakdown of the main whey proteins (β-lactoglogulin (β-lg) and α-lactalbumin) at low TS was also observed by SDS-PAGE (results not shown).

The results presented in Figs. 1 and 2 show that for the hydrolysis of WPC 75 with Debitrase™ HYW20, the reaction proceeded at a much slower rate at higher total solids concentrations, when all other factors were maintained constant. Therefore, longer incubation times would be required to reach a particular DH at higher TS levels. For example, a DH of 15% would be achieved after 1·5 h hydrolysis at 50 g TS/l but it would take 4 h to achieve the same DH at 300 g TS/l.

Hydrolysate samples with equivalent DH values (~15%) were analysed by analytical RP-HPLC to determine if the same peptide profiles were generated at high and low TS. It was observed that although there were some similarities

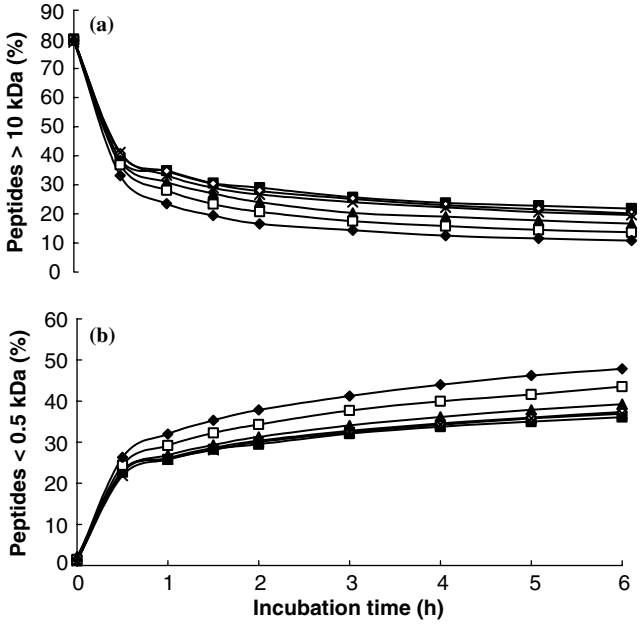

Fig. 2. Percentage of peptide material (a) greater than 10 kDa and (b) less than 0·5 kDa in Debitrase™ HYW20 hydrolysates of whey protein concentrate generated at 50 (◆), 100 (□), 150 (▲), 200 (×), 250 (◇) and 300 g/l (■) total solids (pH 7·0, 50 °C, E : S = 10 g/kg). Values shown represent areas within a defined molecular mass range, expressed as a percentage of the total area of the chromatogram at 214 nm.

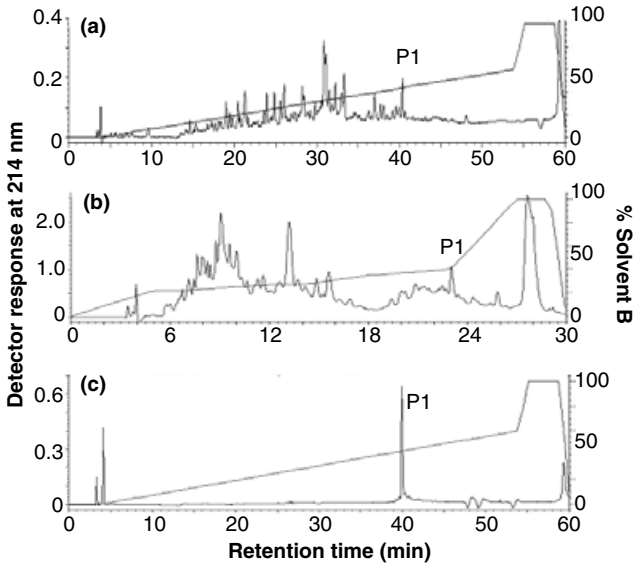

Fig. 3. Summary of the isolation of the hydrophobic peptide P1 by reversed-phase (RP) HPLC. Fig. 3(a) analytical RP-HPLC of a 15% DH hydrolysate generated at 50 g/l total solids (pH 7·0, 50 °C, E : S = 10 g/kg). Fig. 3(b) semi-preparative RP-HPLC of the same sample. Fig. 3(c) analytical RP-HPLC of the isolated peptide fraction.

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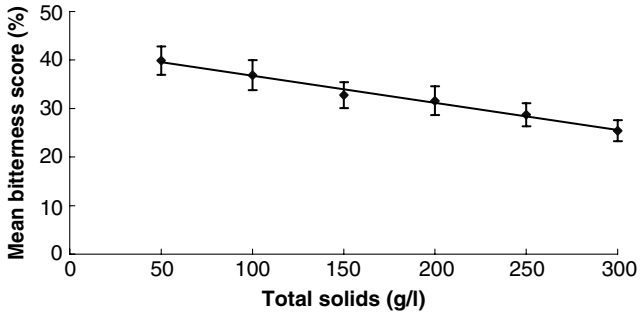

Fig. 4. Mean bitterness scores ± SED (%) for Debitrase™ HYW20 hydrolysates of WPC 75 generated at different total solids concentrations (pH 7·0, 50 °C, E : S = 10 g/kg). All hydrolysates had degree of hydrolysis values of approximately 15%.

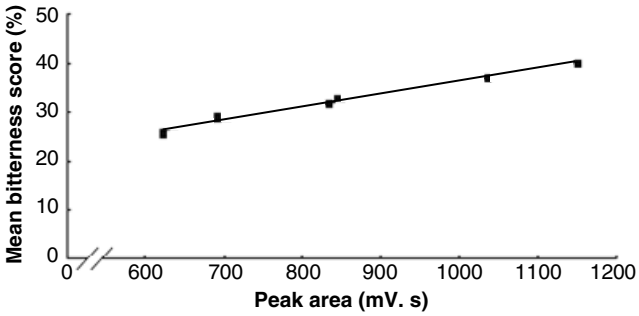

Fig. 5. Mean bitterness scores ± SED (%) for Debitrase™ HYW20 hydrolysates of WPC 75 generated at various total solids concentrations (pH 7·0, 50 °C, E : S = 10 g/kg) as a function of the peak area of the hydrophobic peptide peak P1. All hydrolysates had degree of hydrolysis values of approximately 15%.

in the peptide profiles of 15% DH hydrolysates generated at the six different total solids levels, there were also some distinct differences. For example, the peptide peak labelled P1 in Fig. 3a (retention time (Rt) ~40 min, ~40% solvent B) was present in much higher concentrations in the 50 g TS/l hydrolysate than in the 300 g TS/l hydrolysate. Furthermore, the concentration of this peak increased as the TS concentration decreased over the range of TS values studied.

The peptide peak P1 was the most prominent hydrophobic peak in the RP-HPLC chromatographs. Given the relationship between bitter taste in protein hydrolysates and the presence of hydrophobic peptides, the 15% DH hydrolysates generated at different TS levels were presented to a sensory panel trained to quantify bitterness. An inverse linear relationship was observed between hydrolysate bitterness and the TS level at which the hydrolysate was generated (Fig. 4). An independent samples t-test confirmed that the 300 g TS/l hydrolysate (mean bitterness score = 25·4%) was significantly ($P < 0{\cdot}0005$) less bitter than the 50 g TS/l hydrolysate (mean bitterness score = 39·9%) while analysis of variance (ANOVA) showed a significant difference ($P = 0{\cdot}003$) between the bitterness of the 15% DH hydrolysates across the range of total solids concentrations studied.

Semi-preparative RP-HPLC was used to isolate the peptide present in P1 from a 15% DH hydrolysate generated at 50 g TS/l (Fig. 3). Fragmentation data from electrospray ionisation MS/MS analysis indicated that the fraction contained a peptide with a molecular mass of 1681·9 Da having an amino acid sequence corresponding to residues 43 to 57 of bovine β-lg. An average hydrophobicity (Q) value of 1466 cal mol^{-1} for the isolated peptide was calculated using the hydrophobicity values for the individual amino acid side chains (Tanford, 1962). According to the 'Q-rule' devised by Ney (1971), this peptide should display a bitter taste as it had an average hydrophobicity greater than 1400 cal mol^{-1} and a molecular mass less than 6000 Da. The peptide sequence (VEELKPTPEGDLEIL) also contains two proline residues in central positions. This is reported to increase peptide bitterness due to the conformational alteration of the peptide caused by the imino ring of proline (Ishibashi et al. 1988). Intense bitterness is associated with the presence of at least two hydrophobic residues at the C-terminus and the presence of leucine in a peptide, particularly at the C-terminus, increases peptide bitterness (Shinoda et al. 1985; Ishibashi et al. 1987). Fragment β-lg f(43–57) has two hydrophobic residues at the C-terminus (isoleucine and leucine) with leucine as the C-terminal amino acid residue.

The mean bitterness scores of the 15% DH hydrolysates generated at various total solids concentrations were plotted as a function of the peak area of P1 from RP-HPLC of the hydrolysate samples (Fig. 5). A linear relationship ($R^2 = 0{\cdot}987$) existed between the area of the peak designated P1 and the bitterness of hydrolysates generated at various TS levels. Therefore, these results indicate that the increased bitterness of Debitrase™ HYW20 hydrolysates of WPC generated at low TS may, in part, be due to higher concentrations of β-lg f(43–57) present in these samples.

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References

Adler-Nissen J 1979 Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry* **27** 1256–1262

Asselin J, Amiot J, Gauthier SF, Mourad W & Hebert J 1988 Immunogenicity and allergenicity of whey protein hydrolysates. *Journal of Food Science* **53** 1208–1211

Camacho F, Gonzalez-Tello P & Guadix EM 1998 Influence of enzymes, pH and temperature on the kinetics of whey protein hydrolysis. *Food Science and Technology International* **4** 79–84

Castro S, Vasquez Peyronel D & Cantera AMB 1996 Proteolysis of whey proteins by a *Bacillus subtilis* enzyme preparation. *International Dairy Journal* **6** 285–294

https://doi.org/10.1017/S0022029904000688 Published online by Cambridge University Press

Cayot P & Lorient D 1997 Structure-function relationships of whey proteins. In *Food Proteins and their Applications*, pp. 225–256 (Eds S Damodaran & A Paraf). Marcel Dekker Inc.

De Wit JN 1989 The use of whey protein products. In *Developments in Dairy Chemistry – 4*, pp. 323–345 (Ed. PF Fox). Elsevier Applied Science Publishers

Doucet D, Gauthier SF & Foegeding EA 2001 Rheological characterisation of a gel formed during extensive enzymatic hydrolysis. *Journal of Food Science* **66** 711–715

El-Magoli SB, Laroia S & Hansen PMT 1996 Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. *Meat Science* **42** 179–193

Ena JM, Van Beresteijn ECH, Robben AJPM & Schmidt DG 1995 Whey protein antigenicity reduction by fungal proteinases and a pepsin/pancreatin combination. *Journal of Food Science* **60** 104–110

Flanagan J & FitzGerald RJ 2002 Functionality of *Bacillus* proteinase hydrolysates of sodium caseinate. *International Dairy Journal* **12** 737–748

Gavish B & Werber MM 1979 Viscosity-dependent structural fluctuations in enzyme catalysis. *Biochemistry* **18** 1269–1275

Ha E & Zemel MB 2003 Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people. *Journal of Nutritional Biochemistry* **14** 251–258

Ishibashi N, Arita Y, Kanehisa H, Kouge K, Okai H & Fukui S 1987 Bitterness of leucine containing peptides. *Agricultural and Biological Chemistry* **51** 2389–2394

Ishibashi N, Kubo T, Chino M, Fukui S, Shinoda I, Kikuchi E, Okai H & Fukui S 1988 Taste of proline-containing peptides. *Agricultural and Biological Chemistry* **52** 95–98

Ju ZY, Otte J, Madsen JS & Qvist KB 1995 Effects of limited proteolysis on gelation and gel properties of whey protein isolate. *Journal of Dairy Science* **78** 2119–2128

Laemmli UK 1970 Cleavage of structural proteins during the assembly of the head of a bacteriophage T4. *Nature* **227** 680–686

Lieske B & Konrad G 1996 Physico-chemical and functional properties of whey protein as affected by limited papain proteolysis and selective ultrafiltration. *International Dairy Journal* **6** 13–31

Matoba T & Hata T 1972 Relationship between bitterness of peptides and their chemical structures. *Agricutural and Biological Chemistry* **36** 1423–1431

Margot A, Flaschel E & Renken A 1997 Empirical kinetic models for tryptic whey-protein hydrolysis. *Process Biochemistry* **32** 217–223

Marquez MC & Vazquez MA 1999 Modeling of enzymatic protein hydrolysis. *Process Biochemistry* **35** 111–117

McIntosh GH, Royle PJ, Le Leu RK, Regester GO, Johnson MA, Grinsted RL, Kenward RS & Smithers GW 1998 Whey proteins as functional food ingredients? *International Dairy Journal* **8** 425–434

Mercier A, Gauthier SF & Fliss I 2003 Immunomodulating effects of whey proteins and their enzymatic digests. *International Dairy Journal* **14** 175–183

Molina Ortiz SE & Wagner JR 2002 Hydrolysates of native and modified soy protein isolates: structural characteristics, solubility and foaming properties. *Food Research International* **35** 511–518

Mullally MM, Meisel H & FitzGerald RJ 1997 Identification of a novel angiotensin-1-converting enzyme inhibitory peptide corresponding to a tryptic fragment of bovine β-lactoglobulin. *FEBS letters* **402** 99–101

Mutilangi WAM, Panyam D & Kilara A 1996 Functional properties of hydrolysates from proteolysis of heat denatured whey protein isolate. *Journal of Food Science* **61** 270–303

Ney KH 1971 Prediction of bitterness of peptides from their amino acid composition. *Zeitschrift für Lebensmittel Untersuchung und Forschung* **147** 64–68

Ney KH 1979 Bitterness of peptides: amino acid composition and chain length. In *Food taste chemistry*, pp. 149–171 (Ed. JC Boudreau). Washington: American Chemical Society (ACS) Symposium Series 115

Ng K & Rosenberg A 1991 Possible coupling of chemical to structural dynamics in subtilisin BPN′ catalyzed hydrolysis. *Biophysical Chemistry* **39** 57–68

Panyam D & Kilara A 1996 Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science and Technology* **7** 120–125

Pawlett D & Bruce G 1996 Debittering of protein hydrolysates. In *Industrial Enzymology*, 2nd edition, pp. 177–185 (Eds T Godfrey & S West). London: Macmillan Press Ltd

Perea A, Ugalde U, Rodriguez I & Serra JL 1993 Preparation and characterisation of whey protein hydrolysates: Applications in industrial whey bioconversion processes. *Enzyme and Microbial Technology* **15** 418–423

Rhodia Inc. 2004 Technical Bulletin: TB DEB 99–11.0

Shinoda L, Fushima A, Kato H, Okai H & Fukui S 1985 Bitter taste of synthetic C-terminal tetradecapeptide of bovine β-casein, H-Pro196-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val209-OH, and its related peptides. *Agricultural and Biological Chemistry* **49** 2587–2596

Smyth M & FitzGerald RJ 1997 Characterisation of a new chromatography matrix for peptide molecular mass determination. *International Dairy Journal* **7** 571–577

Smyth M & FitzGerald RJ 1998 Relationship between some characteristics of WPC hydrolysates and the enzyme complement in commercially available proteinase preparations. *International Dairy Journal* **8** 819–827

Spellman D, McEvoy E, O'Cuinn G & FitzGerald RJ 2003 Proteinase and exopeptidase hydrolysis of whey protein: Comparison of the TNBS, OPA and pH stat methods for the quantification of degree of hydrolysis. *International Dairy Journal* **13** 447–453

Tanford C 1962 Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. *Journal of the American Chemical Society* **84** 4240–4247

Uribe S & Sampdero JG 2003 Measuring solution viscosity and its effect on enzyme activity. *Biological Procedures Online* **5** 108–115

Webb KE 1990 Intestinal absorption of protein hydrolysis products: a review. *Journal of Animal Science* **68** 3011–3022

Yetim H, Muller WD & Eber M 2001 Using fluid whey in comminuted meat products: effects on technological, chemical and sensory properties of frankfurter-type sausages. *Food Research International* **34** 91–101