Bio-functional Hydrolysates of Heat-denatured Whey Proteins

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Introduction

The industry-led Food for Health Ireland project aims to determine how milk ingredients can be extracted and used to deliver health benefits for consumers. The protein content of whey can be concentrated as whey protein isolate (**WPI**). The major proteins in WPI are β-lactoglobulin (**β-lg**) and α-lactalbumin (**α-la**). Peptides can be released from the parent protein through hydrolysis (below).

Enzymatic hydrolysis of whey proteins can yield hydrolysates with techno-functional and bio-functional applications. The denaturation of whey proteins may facilitate the release of novel peptides possessing unique functional attributes.

Objectives

The relationship between whey protein structure and enzymatic hydrolysis is studied in order to:

(a) Improve hydrolysis kinetics.

(b) Determine the effect on peptide formation through analysis of hydrolysates.

(c) Understand the effects of variation in substrate quality on peptide release and biological activity.

Methodology

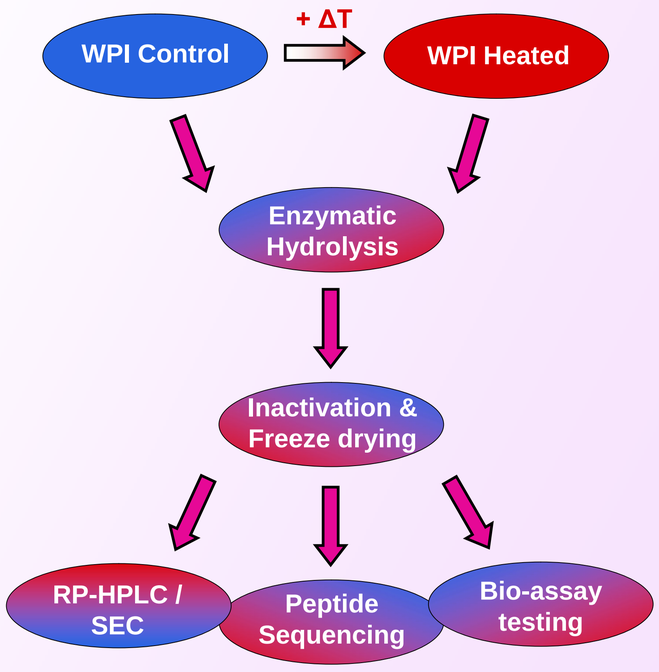
- ***Characterisation of heat-denaturation of WPI***

The conformational state of the whey proteins was determined using reversed-phase high performance chromatography (**RP-HPLC**) and size-exclusion chromatography (**SEC**)

WPI solutions (100 g L^{-1}) were subjected to a heat-treatment of 80 °C for 10 min.

- ***Hydrolysis of control and heat-treated WPI***

Both un-heated control and heat-treated WPI solutions were hydrolysed with a commercial enzymatic preparation to a **degree of hydrolysis (DH)** of 5 %.



Results

- ***Characterisation of heat-denaturation of WPI***

Heat-treatment of the WPI resulted in a loss in native whey proteins (Fig. 1 & 2) e.g. β-lg (Fig. 1) and the formation of large (~ 40 µm [D. v 09]) insoluble aggregates (Fig. 1 insert).

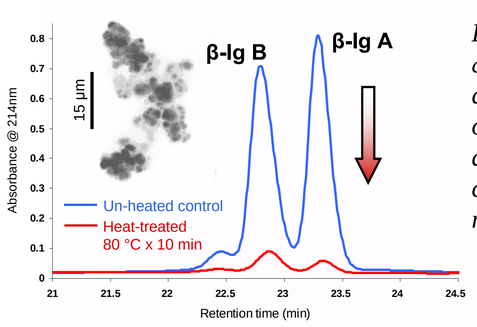


Fig. 1 RP-HPLC chromatogram of β-lg denaturation. Insert is of whey protein aggregate obtained by confocal laser scanning microscopy.

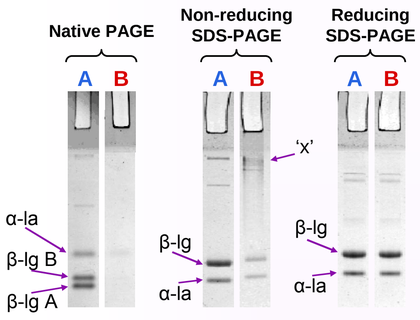
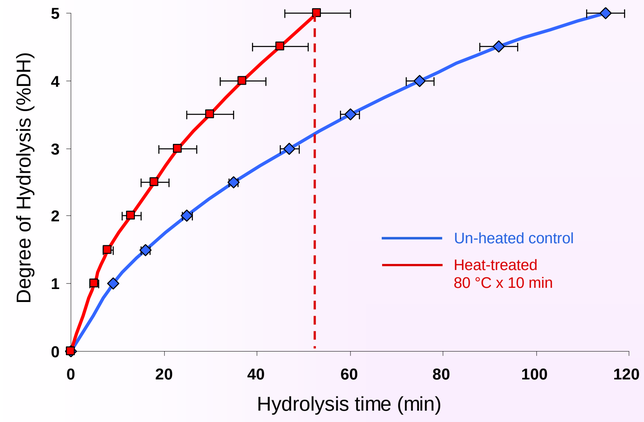


Fig. 2 Electrophoresis patterns of un-heated control (A) and heat-treated WPI (B). Aggregates of whey proteins shown at 'x'.

- ***Hydrolysis of control and heat-treated WPI***

A heat-treatment of 80°C for 10 min approx. halved the reaction time to reach 5 %DH (Fig. 3).

Fig. 3 Hydrolysis curves of control and heat-treated WPI solutions hydrolysed to a degree of hydrolysis of 5 %.

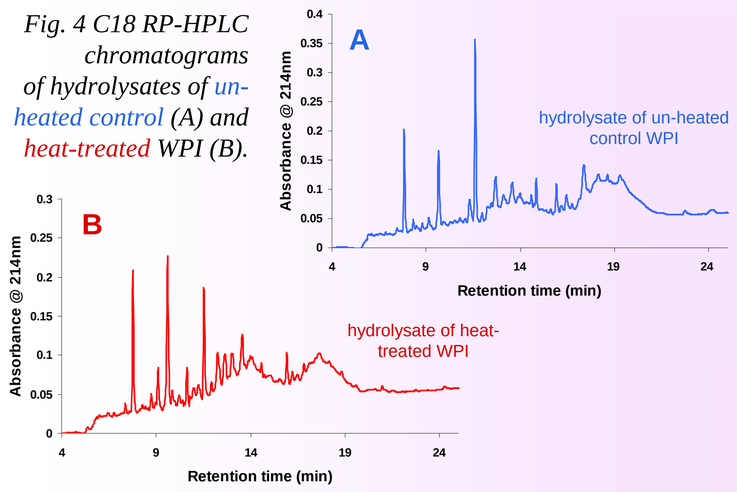


The un-heated control and heat-treated hydrolysates differed in their concentration of certain free amino acids (Table 1) and their peptide profiles (Fig 4).

Table 1 Concentration of free amino acids in hydrolysates of un-heated control and heat-treated WPI hydrolysed to 5 %DH.

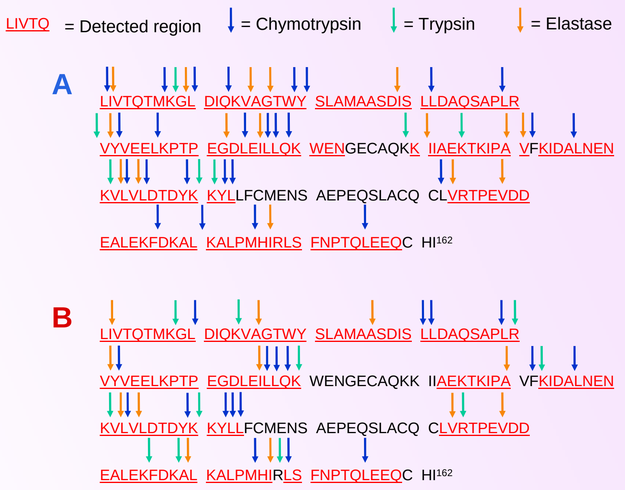
Control hydrolysate		Heat-treated hydrolysate	
Amino acid	mg kg^{-1}	Amino acid	mg kg^{-1}
Cysteine	27.9 ± 1.5	Cysteine	32.1 ± 1.7
Methionine	13.8 ± 1.1	Methionine	21.3 ± 1.2
Aspartate	2.1 ± 1.6	Aspartate	6.2 ± 1.5
Threonine	12.0 ± 1.2	Threonine	11.8 ± 1.6
Serine	1.2 ± 1.0	Serine	0.2 ± 0.6
Glutamate	24.1 ± 1.4	Glutamate	21.1 ± 2.1
Glycine	1.9 ± 0.8	Glycine	1.5 ± 0.7
Alanine	8.5 ± 1.3	Alanine	8.1 ± 2.2
Valine	73.1 ± 1.2	Valine	66.8 ± 1.0
Isoleucine	52.7 ± 1.8	Isoleucine	45.9 ± 1.2
Leucine	166.8 ± 1.4	Leucine	168.8 ± 1.9
Tyrosine	136.4 ± 2.1	Tyrosine	196.7 ± 1.4
Phenylalanine	139.4 ± 1.9	Phenylalanine	290.6 ± 1.3
Histidine	49.8 ± 2.1	Histidine	43.8 ± 1.9
Lysine	24.4 ± 1.6	Lysine	154.5 ± 1.3
Arginine	32.1 ± 1.8	Arginine	88.1 ± 1.7
Proline	3.0 ± 1.2	Proline	1.8 ± 0.7

Fig. 4 C18 RP-HPLC chromatograms of hydrolysates of un-heated control (A) and heat-treated WPI (B).



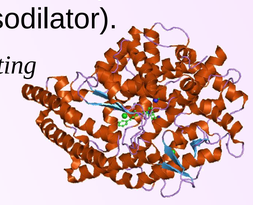
LC-MS/MS analysis revealed differences in the attributed endo-proteolytic pattern (Fig. 5).

Fig. 5 Amino acid sequence of β-lactoglobulin (β-lg) showing detected cleavage points originating from β-lg in control (A) and heat-treated WPI (B) and associated enzymatic activities.



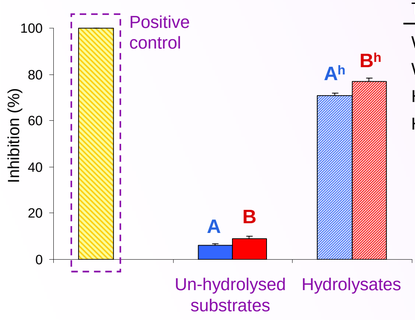
Angiotensin-I-converting enzyme (ACE, Fig. 6) plays a central role in the control of blood pressure in humans through the conversion of angiotensin I to angiotensin II (a potent vasoconstrictor) and the inactivation of bradykinin (a vasodilator).

Fig. 6 Human angiotensin-I-converting enzyme (PDB-1o86).



The WPI substrates and hydrolysates exhibited differences in their inhibition of ACE (Fig. 7) with heat-treatment having a positive effect on inhibition.

Fig. 7 ACE inhibitory activity in un-heated control and heat-treated WPI and respective hydrolysates at 5 %DH determined at 10 mg mL^{-1}. 'Positive control' for inhibition is acid inactivated ACE.



Test sample	IC_{50}*
WPI control (A)	>100
WPI heat-treated (B)	>100
Hydrolysed control (A^h)	6.71
Hydrolysed heat-treated (B^h)	5.39

* *The concentration required to inhibit ACE activity by 50 % (IC_{50}) expressed in mg mL^{-1}.*

Conclusions

Heat-treatment of WPI changes the proteolytic pattern of the hydrolysis reaction resulting in:-

(a) An increased rate of enzymatic hydrolysis.

(b) Hydrolysates with different peptide distributions and free essential amino acids concentration.

(c) Hydrolysates with altered bio-functionalities.

Acknowledgements

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