Supplementary data

Supplementary Table S1. Details of the hydrolysis parameters used to generate the hydrolyzate samples, including the parameters used in the experimental design (hydrolyzates highlighted in grey) applied during α-lactalbumin (α-La) hydrolysis with elastase.

Experiment	Hydrolysis conditions with elastase		
	pH	Temperature (°C)	E:S (%)
H1	7.0 (-1)	37 (-1)	1.0 (-
H2	7.0 (-1)	37 (-1)	2.0
H3	8.5 (1)	37 (-1)	1.0 (-
H4	8.5 (1)	37 (-1)	2.0
H5	7.8 (0)	44 (0)	1.5
H6	7.0 (-1)	50 (1)	1.0 (-
H7	7.0 (-1)	50 (1)	2.0
H8	8.5 (1)	50 (1)	1.0 (-
H9	8.5 (1)	50 (1)	2.0

Supplementary Table S2. Results of the *in silico* digestion of α-lactalbumin (α-La) with pepsin, trypsin, chymotrypsin and glutamyl endopeptidase.

Enzyme*	Peptide sequence**	Previously identified DPP-IV inhibitor
PEPS	EQL	
PEPS	TKCEV	
PEPS	RE	✓
PEPS	LKD	
PEPS	LKG	
PEPS	YGGVSL	
PEPS	PEW	
PEPS	VCTT	
PEPS	HTSG	
PEPS	DTQAIVQNNDSTE	
PEPS	QINNKI	
PEPS	WCKDDQNPHSSNICNISCDK	
PEPS	LDDD	
PEPS	TDDIMCVKKILDKVGIN	
PEPS	AHKALCSEK	
PEPS	DQ	✓
PEPS	CEKL	
PEPS2	EQL	
PEPS2	TKCEV	
PEPS2	RE	✓
PEPS2	LKD	
PEPS2	LKGYGGVSL	
PEPS2	PEWVCTT	
PEPS2	HTSGYDTQAIVQNNDSTEYG	
PEPS2	QINNKIWCKDDQNPHSSNICNISCDK	
PEPS2	LDDD	
PEPS2	TDDIMCVKKILDKVGINYW	
PEPS2	AHKALCSEK	
PEPS2	DQW	
PEPS2	CEKL	
TRYPS	EQLTK	
TRYPS	CEVFR	
TRYPS	ELK	
TRYPS	DLK	
TRYPS	GYGGVSLPEWVCTTFHTSGYDTQAIVQNNDSTEYGLFQINNK	
TRYPS	IWCK	
TRYPS	DDQNPHSSNICNISCDK	
TRYPS	FLDDDLTDDIMCVK	
TRYPS	ILDK	
TRYPS	VGINYWLAHK	
TRYPS	ALCSEK	
TRYPS	LDQWLCEK	
CH-HI	EQLTKCEVF	
CH-HI	RELKDLKGY	
CH-HI	GGVSLPEW	
CH-HI	VCTTF	
CH-HI	HTSGY	

CH-HI	DTQAIVQNNDSTEY	
CH-HI	GLF	
CH-HI	QINNKIW	
CH-HI	CKDDQNPHSSNICNISCDKF	
CH-HI	LDDDLTDDIMCVKKILDKVGINY	
CH-HI	LAHKALCSEKLDQW	
CH-HI	LCEKL	
CH-LO	EQL	
CH-LO	TKCEVF	
CH-LO	REL	
CH-LO	KDL	
CH-LO	KGY	
CH-LO	GGVSLPEW	
CH-LO	VCTTF	
CH-LO	TSGY	
CH-LO	DTQAIVQNNDSTEY	
CH-LO	GL	✓
CH-LO	QINNKIW	
CH-LO	CKDDQNPH	
CH-LO	SSNICNISCDKF	
CH-LO	DDDL	
CH-LO	TDDIM	
CH-LO	CVKKIL	
CH-LO	DKVGINY	
CH-LO	AH	✓
CH-LO	KAL	
CH-LO	CSEKL	
CH-LO	DQW	
CH-LO	CEKL	
GLUEND	QLTKCE	
GLUEND	VFRE	
GLUEND	LKDLKGYGGVSLPE	
GLUEND	WVCTTFHTSGYDTQAIVQNNDSTE	
GLUEND	YGLFQINNKIWCKDDQNPHSSNICNISCDKFLDDDLTDDIMCVKKILDKV GINYWLAHKALCSE	
GLUEND	KLDQWLCE	
GLUEND	KL	✓

*PEPS: pepsin pH 1.3; PEPS2: pepsin pH > 2; TRYPS: trypsin; CH-HI: chymotrypsin high specificity; CH-LO: chymotrypsin low specificity; GLUEND: glutamyl endopeptidase.

**Peptide sequence abbreviated with the one letter amino acid code.

Supplementary Table S3. Peptide sequences identified by LC-MS within the α-lactalbumin (α-La) H9 and its associated simulated gastrointestinal digest (SGID) sample, which present structural features of known dipeptidyl peptidase IV inhibitory peptides (i.e., W at the N-terminus, P/A at position 2 or P at the C-terminus [1-3]). These peptides have not previously been evaluated for their DPP-IV inhibitory potential, therefore, their DPP-IV inhibitory properties are not known.

Feature	Peptide sequence*	Parent protein**	Fragment	Identified in	
				H9	SGID of H9
N-terminal W	WGAVLL				✓
	WGLF				✓
P at position 2	EPMLGV				✓
	HPHAY			✓	
	KPTPE	β-Lg	f(47-51)		✓
	KPTPEGDL	β-Lg	f(47-54)		✓
	KPTPEGDLE	β-Lg	f(47-55)		✓
	KPTPEGDLEL	β-Lg	f(47-56)		✓
	KPTPEGDLEI	β-Lg	f(47-56)		✓
	MPEATLGAAFKF			✓	
	MPEGGKKPM			✓	
	MPFSPPRA			✓	
	MPKEQDSVY				✓
	MPPEV				✓
	MPVVCLSQM			✓	
	PPPVSLPADE				✓
	QPQSQ			✓	
	SPPSVT			✓	
	TPEVD	β-Lg	f(125-129)		✓
	TPEVDD	β-Lg	f(125-130)		✓
	TPEVDDE	β-Lg	f(125-131)		✓
	TPEVDDEA	β-Lg	f(125-132)		✓
	TPEVDDEAL	β-Lg	f(125-133)		✓
	TPEVDDEALE	β-Lg	f(125-134)		✓
	TPEVDDEALEK	β-Lg	f(125-135)		✓

	TPLPL				✓
	VPYPQ	β-CN	f(178-182)		✓
	YPSYG	κ-CN	f(35-39)	✓	
	NPWDQV	α_{s2}-CN	f(107-112)	✓	✓
A at position 2	AAAFKALAEAGL			✓	
	AANTVYPL				✓
	AATVALL				✓
	CAGLDLQ			✓	
	DAGLLV				✓
	DAFLGSFLYEYSR	BSA	f(329-341)	✓	
	DAIPENLPPLTA	BSA	f(301-312)	✓	
	DALNEN	β-Lg	f(85-90)		✓
	DAQSAPL	β-Lg	f(33-39)		✓
	EAAVK			✓	
	EAGLV				✓
	EALPH				✓
	EASVPE				✓
	FAGKN				✓
	FASQY			✓	
	LAPHCDKL			✓	
	LAPPQEEL				✓
	LAQSVT				✓
	LAQSVTLNSYVQLGVLPR				✓
	MAGGGSGLV				✓
	MAGSASPK			✓	
	MAGSGGRL			✓	
	MALPPK	κ-CN	f(106-111)	✓	
	MALRN			✓	
	MAVLVL			✓	
	RATDATAL			✓	
	SAEPEQS	β-Lg	f(110-106)		✓
	SAPFPS			✓	
	SAPSVT			✓	
	SASSPLLSTK			✓	
	TASSGLT			✓	

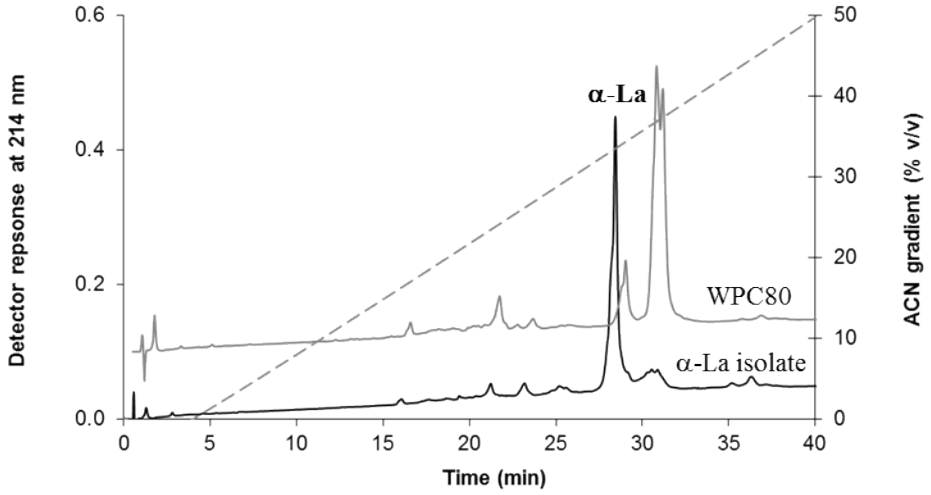
	TAVQGSKP			✓	
	VAGTK			✓	
	VAPFDPLFG				✓
	VAPFPEV	α_{s1}-CN	f(25-31)		✓
	VAPFTVSNAG				✓
	VAYGGV				✓
	YAASSYLSLTSSDWK			✓	
	YAKPA	κ-CN	f(61-65)	✓	
C-terminal P	AVGEPLP				✓
	EMPFKP			✓	
	ESGPSLVSKP				✓
	FLAAFMP				✓
	FNTP			✓	
	FYDP				✓
	HKKP			✓	✓
	HLPLP	β-CN	f(134-138)		✓
	HYTALP			✓	
	LFPGNTSVKP				✓
	LGGPGLP				✓
	LVVHP				✓
	LVYPFPGLP				✓
	MHQHP			✓	
	NKPALAGMP			✓	
	NMMDVGVMP				✓
	SELSALP				✓
	SVYPFPGLP			✓	
	TAVQGSKP			✓	
	TFDLP				✓
	TMTLGTFP				✓
	VSGGLP			✓	
	VTHFP				✓
	VVAGALLPP			✓	
	SLELYP			✓	

*Peptide sequences abbreviated with the one letter amino acid code.
**β-Lg: β-lactoglobulin; BSA: bovine serum albumin; CN: casein

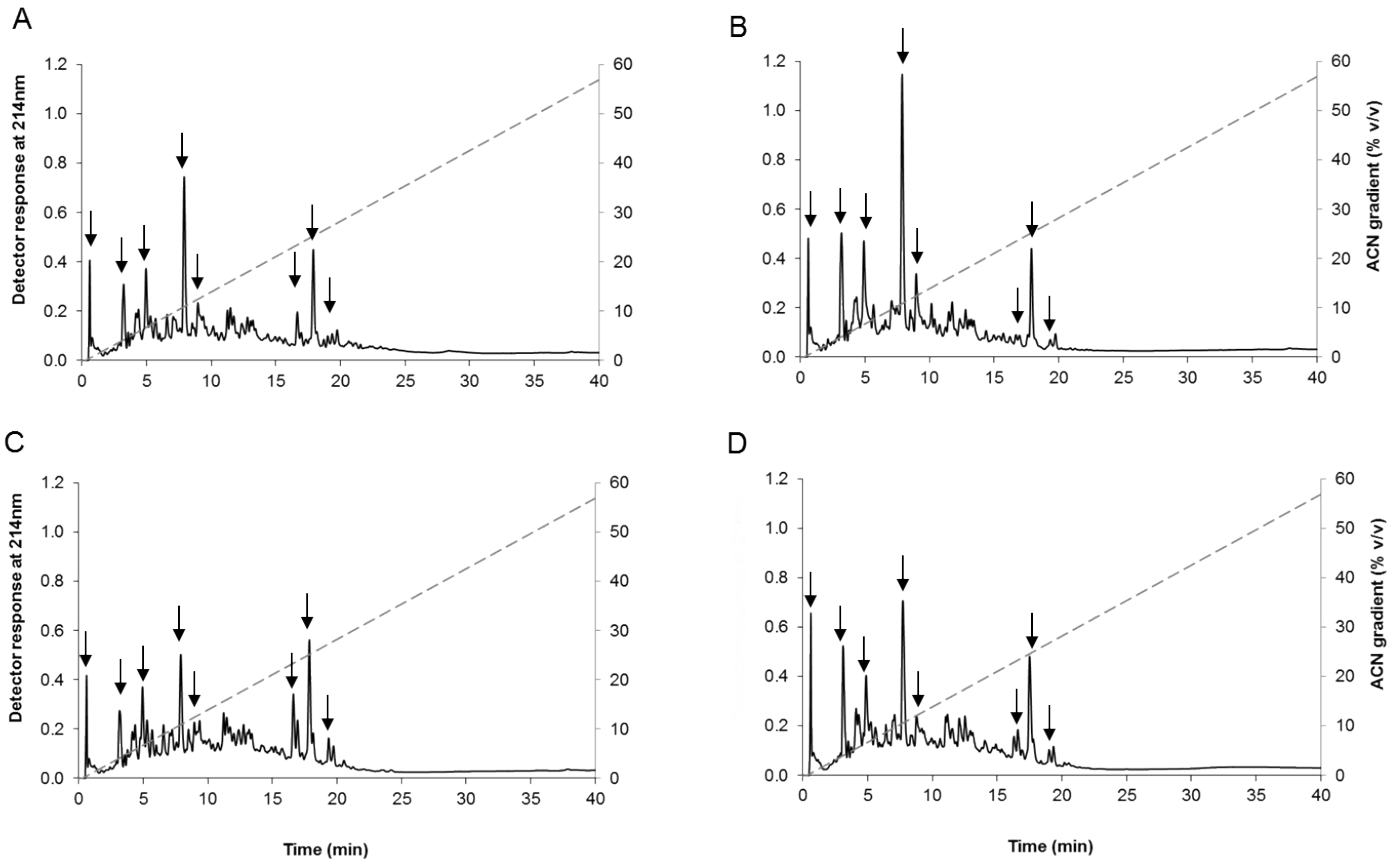
1. G. Tulipano, L. Faggi, A. Nardone, D. Cocchi and A. M. Caroli, *Int. Dairy J.*, 2015, **48**, 62-72.

2. A. B. Nongonierma and R. J. FitzGerald, *Food Chem.*, 2014, **165**, 489–498.

3. A. B. Nongonierma and R. J. FitzGerald, *J. Funct. Foods*, 2013, **5**, 1909–1917.



Supplementary Fig. S1. Reverse phase ultra-performance liquid chromatographic (RP-UPLC) profile of the whey protein concentrate 80 (WPC 80) and the α-lactalbumin (α-La) isolate.



The peptide profiles (RP-UPLC) of H4, H7, H8 and H9 are depicted on Supplementary Fig. S3A-S3D, respectively. Only minor differences were seen on incubation at 37 (H4, Supplementary Fig. S3A) compared to 50 ℃ (H9, Supplementary Fig. S3D). At higher E:S, peaks eluting before 10 min were more intense (H9 vs. H7, Supplementary Fig. S3D and S3C, respectively). Certain peaks eluting before 10 min were more intense at pH 7.0 (H8, Supplementary Fig. S3B), than at pH 8.0 (H9, Supplementary Fig. S3D).

Supplementary Fig. S2. Reverse phase ultra-performance liquid chromatographic (RP-UPLC) profile of the α-lactalbumin (α-La) hydrolyzates generated with elastase at (A) pH 8.5, 37°C, E:S 2% (H4), (B) pH 7.0, 50°C, E:S 2% (H7), (C) pH 8.5, 50°C, E:S 1% (H8) and (D) pH 8.5, 50°C, E:S 2% (H9). Peptide peaks highlighted with an arrow show major differences within the hydrolyzates.