Resistant peptides to *in vitro* gastrointestinal digestion of casein and whey proteins with brush border peptidases

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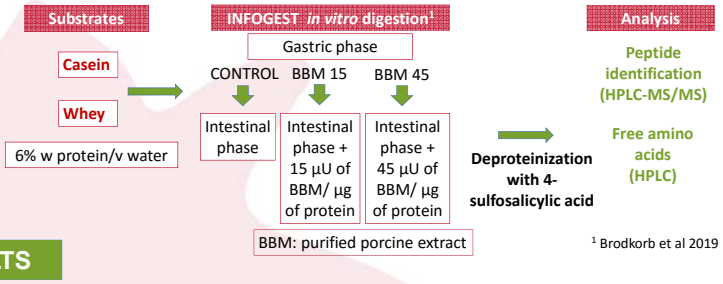
INTRODUCTION

It is known that specific peptides are likely behind several physiological effects observed for dietary proteins and demonstration of their survival in the digestive tract is essential. Moreover, in the context of simulated gastrointestinal digestion, prediction of the *in vivo* bio-accessibility merits attention. *In vivo*, brush border peptidases (BBM) primarily contribute to the profile of the digestion products uptaken at intestinal level and their impact on individual peptides should be evaluated.

The objective of this work is to evaluate the incorporation of BBM of the intestinal epithelium in the *in vitro* gastrointestinal digestion of milk proteins.

EXPERIMENTAL DESIGN



[1] Brodkorb et al 2019

RESULTS

Number and size of peptides

CASEIN	CONTROL	BBM 15	BBM 45
All	1712	703	415
Unique	210	127	88
5 aa's peptides	33%	52%	49%

WHEY	CONTROL	BBM 15	BBM 45
All	855	346	304
Unique	111	68	67
5 aa's peptides	30%	26%	33%

Table 1. Number of peptides found in casein and whey digestion (CONTROL) and with BBM (BBM 15 and BBM 45). All: total identified peptides; Unique: peptides without duplicates; 5 aa's peptides: percentage of pentapeptides.

The incorporation of BBM to the intestinal phase reduces the spectrum of peptides with increased proportion of oligopeptides in the case of casein

Free amino acids[M1]

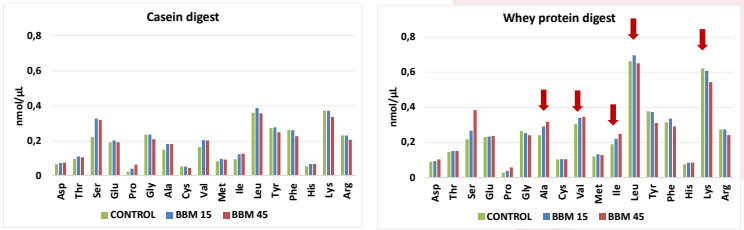


Fig 1. Comparison of free amino acid content after gastrointestinal digestion of casein and whey protein after *in vitro* gastrointestinal digestion and adding two different concentrations of BBM, 15 and 45 µU/mg of protein.

Higher overall concentration of free amino acids in whey digests, with or without BBM, with prominent differences in Ala, Val, Ile, Leu, and Lys

CASEIN	CONTROL	BBM 15		BBM45	
Asp	1,34	1,48	*	1,51	**
Thr	1,97	2,21	*	2,13	*
Ser	4,46	6,54	***	6,40	***
Glu	3,84	4,04	ns	3,85	ns
Pro	0,49	0,83	***	1,29	***
Gly	4,73	4,72	ns	4,19	**
Ala	2,98	3,65	***	3,65	***
Cys	1,07	1,07	ns	0,93	***
Val	3,29	4,08	***	4,05	***
Met	1,67	1,95	**	1,87	**
Ile	1,91	2,47	***	2,55	***
Leu	7,22	7,76	ns	7,16	ns
Tyr	5,47	5,54	ns	5,00	**
Phe	5,24	5,22	ns	4,54	**
His	1,10	1,35	***	1,39	***
Lys	7,46	7,44	ns	6,74	*
Arg	4,63	4,61	ns	4,15	**

WHEY	CONTROL	BBM 15		BBM45	
Asp	1,77	1,86	ns	2,05	***
Thr	2,88	3,00	ns	3,01	*
Ser	4,90	5,31	**	7,67	***
Glu	4,61	4,66	ns	4,73	ns
Pro	0,54	0,73	***	1,13	***
Gly	5,27	5,04	**	4,81	***
Ala	4,80	5,81	***	6,34	***
Cys	2,04	2,09	ns	2,06	ns
Val	6,08	6,78	***	6,91	***
Met	2,38	2,63	***	2,55	***
Ile	3,79	4,41	***	4,95	***
Leu	13,22	13,92	ns	12,99	ns
Tyr	7,54	7,45	ns	6,19	***
Phe	6,28	6,70	*	5,80	ns
His	1,48	1,66	**	1,68	***
Lys	12,42	12,14	ns	10,86	***
Arg	5,46	5,47	ns	4,82	***

Table 2. Statistical analysis of control, BBM 15 and BBM 45 amino acid means calculated in nmol. (*): p < 0,05; (**): p < 0,01; (***): p < 0,001.

Significant increase in most amino acids with the addition of BBM, with higher contribution of Pro, Ala, Val, and Ile in both substrates.

Identification of peptides

CONTROL		Exclusively generated by BBM	
Protein (range)	Sequence	Protein (range)	Sequence
β-CN (1-6)	**RELEEL**	β-CN (1-5)	ELEEL
β-CN (6-12)	**LNVPGEI**	β-CN (6-11) β-CN (7-11)	**LNVPGE** **NVPGE**
β-CN (36-44)	**EEQQQTEDE**	β-CN (35-40)	SEEQQQ
β-CN (39-44) β-CN (39-45) β-CN (39-47)	QQTEDE QQTEDEL QQTEDELQD	β-CN (40-45) β-CN (41-45)	QTEDEL TEDEL
β-CN (58-66)	**LVYPFPGPI**	β-CN (60-65)	**YPFPGP**
β-CN (67-72)	HNSLPQ	β-CN (67-71)	HNSLP
β-CN (108-113)	**EMPFPK**	β-CN (109-113)	**MPFPK**
β-CN (137-143)	LPLLQSW	β-CN (137-142)	LPLLQS
β-CN (170-175)	**VLPVPQ**	β-CN (170-174)	**VLPVP**
β-CN (193-201)	**YQEPVLGPV**	β-CN (195-201)	**EPVLGPV**
α_{s1}-CN (8-13)	**HQGLPQ**	α_{s1}-CN (8-12)	**HQGLP**
α_{s1}-CN (25-30)	**VAPFPE**	α_{s1}-CN (26-30)	**APFPE**
α_{s1}-CN (173-178)	YTDAPS	α_{s1}-CN (174-178)	**TDAPS**
Ƙ-CN (106-111)	MAIPPK	Ƙ-CN (107-111)	AIPPK
Ƙ-CN (125-131)	IASGEPT	Ƙ-CN (126-131)	ASGEPT
Ƙ-CN (142-148)	TVATLED	Ƙ-CN (142-146)	TVATL
α-lactalbumin (48-55)	TEYGLFQI	α-lactalbumin (49-55)	EYGLFQI
β-lactoglobulin (110-116)	**SAEPEQS**	β-lactoglobulin (111-116) β-lactoglobulin (112-116)	AEPEQS **EPEQS**
β-lactoglobulin (127-135)	**EVDDEALEK**	β-lactoglobulin (130-134) β-lactoglobulin (130-135) β-lactoglobulin (131-135)	**DEALE** DEALEK **EALEK**

Table 3. Sequences generated by BBM from longer sequences identified in the control *in vitro* gastrointestinal digestion. **In bold**: sequences previously identified in human jejunum digests.

Occurrence of derived smaller peptides from all proteins. Good correspondence with sequences previously identified in human jejunum digests

CONCLUSION

The incorporation of BBM to the *in vitro* gastrointestinal digestion allows the identification of short peptides that have been demonstrated relevant to the human situation.

Since the amount of identified peptides is reduced,[M2] it should be considered a complementary tool when the total picture of peptides needs to be determined.

ACKNOWLEDGEMENTS

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