

1 SUPPLEMENTARY DATA

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3 **Table S1** Overview of process-relevant characteristics of PepX, PepA and PepN

4 (Stressler *et al.*^{11,13}).

Characteristic	PepX	PepA	PepN
Organism	<i>Lactobacillus helveticus</i>	<i>Lactobacillus debrueckii</i>	<i>Lactobacillus helveticus</i>
His-tag	yes	no	yes
Cleavage	N-terminal X-Pro dipeptide	N-terminal Asp, Glu, Ser	N-terminal AA (unspecific) No hydrolysis before Pro
pH-maximum	6.5	6.0	6.5
Temperature-maximum	50 °C	60 °C	37 °C

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7 **Table S2** Peptidases included in Flavourzyme and their properties (according to Merz *et*

8 *al.*¹⁷).

Peptidase	Abbr.	Max. pH [-]	Max. T [°C]	Size [kDa]	Preferred cleavage site	Specific substrate for determination
Leucin-aminopeptidase A	LAPA	9.0	65	32.5	Leu ¹	H-Ile-pNA
Leucin-aminopeptidase 2	LAP2	9.5	50	51.9	Broad specificity ²	H-Pro-pNA
Dipeptidylpeptidase 4	DPP4	8.5	60	85.1	X-Pro ³	H-Ala-Pro-pNA
Dipeptidylpeptidase 5	DPP5	6.0	65	78.6	n.a.	H-Lys-Ala-pNA
Neutrale protease 1	NP1	7.0	50	42.4	n.a.	Azocasein
Neutrale protease 2	NP2	5 – 5.5	60	19.0	Hydrophobic AA in P1 ⁴	Azocasein
Alkaline protease 1	ALP1	7.0	50	29.0	Broad specificity ⁵	Azocasein

9 n.a. = not available

10 1 MEROPS peptidase M28.006; 2 UniProt: Q2ULM2; 3 UniProt: Q2UH35; 4 UniProt: P46076; 5 UniProt: P12547

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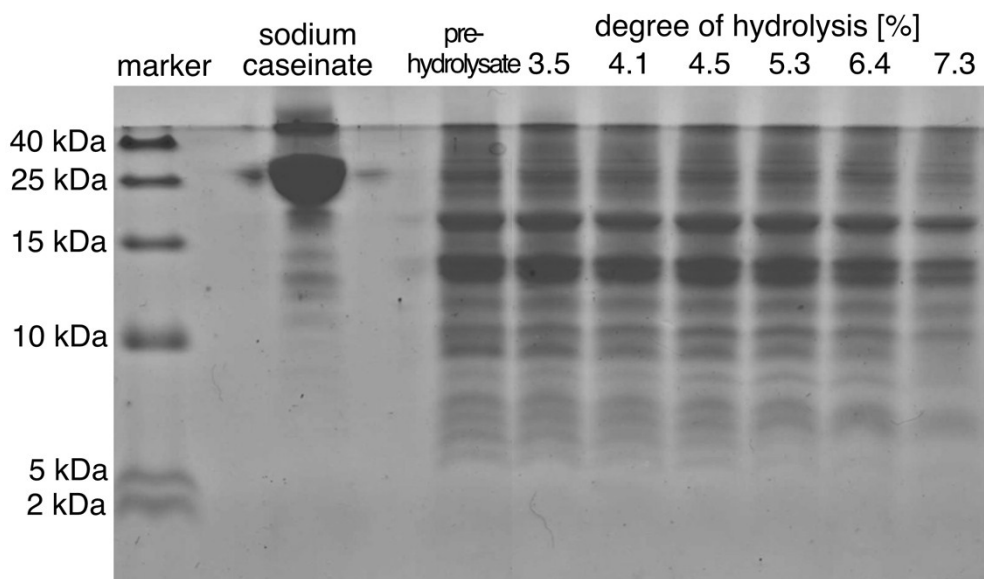
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Table S3 Comparison of the casein hydrolysate and colloidal properties of the two hydrolysate batches (pre-hydrolysates, produced with Sternzym BP 25201) used for the further hydrolysis with single exopeptidases (PepN, PepX, PepA) or Exo-Flavourzyme.

Parameter	<i>Pre-hydrolysate used for PepN, PepX and PepA</i>		<i>Pre-hydrolysate used for Exo-Flavourzyme</i>	
	Sodium caseinate	+Sternzym	Sodium caseinate	+Sternzym
DH [%]	0 ^a	2.3 ± 0.1 ^b	0 ^a	2.4 ± 0.04 ^b
Protein particle size [µm]	1.3 ± 0.3 ^a	43.2 ± 3.3 ^b	1.1 ± 0.18 ^a	36.2 ± 1.9 ^b
Rel. hydrophobicity [%]	100 ± 1.8 ^a	92 ± 0.8 ^b	100 ± 2.4 ^a	91 ± 0.7 ^b
Foam				
Overrun [%]	273 ± 14 ^a	188 ± 4 ^b	292 ± 13 ^a	201 ± 13 ^b
Drainage [%]	83.4 ± 0.5 ^a	75.3 ± 0.2 ^b	85.3 ± 2.5 ^a	75 ± 1.1 ^b
t _{1/2} (foam) [min]	22.4 ± 1.6 ^a	30.3 ± 1.4 ^b	21.6 ± 3.1 ^a	33.3 ± 3.0 ^b
Emulsion				
Oil droplet size [µm]	18.2 ± 1.8 ^{ab}	16.9 ± 1.0 ^{ab}	19.5 ± 1.1 ^a	17.0 ± 0.9 ^b
ζ-potential [mV]	-62.6 ± 2.9 ^a	-46.0 ± 0.6 ^b	-58 ± 4.0 ^a	-39 ± 3.7 ^c
t _{1/2} (emulsion) [h]	0.8 ± 0.1 ^a	2.4 ± 0.1 ^b	0.8 ± 0.1 ^a	2.7 ± 0.3 ^b
Bitterness				
Relative bitterness	2.1 ± 2.1 ^a	9.1 ± 2.4 ^b	1.7 ± 1.3 ^a	8.8 ± 1.4 ^b

different superscripted letters indicate a significant difference ($p < 0.05$) between the samples in a row.

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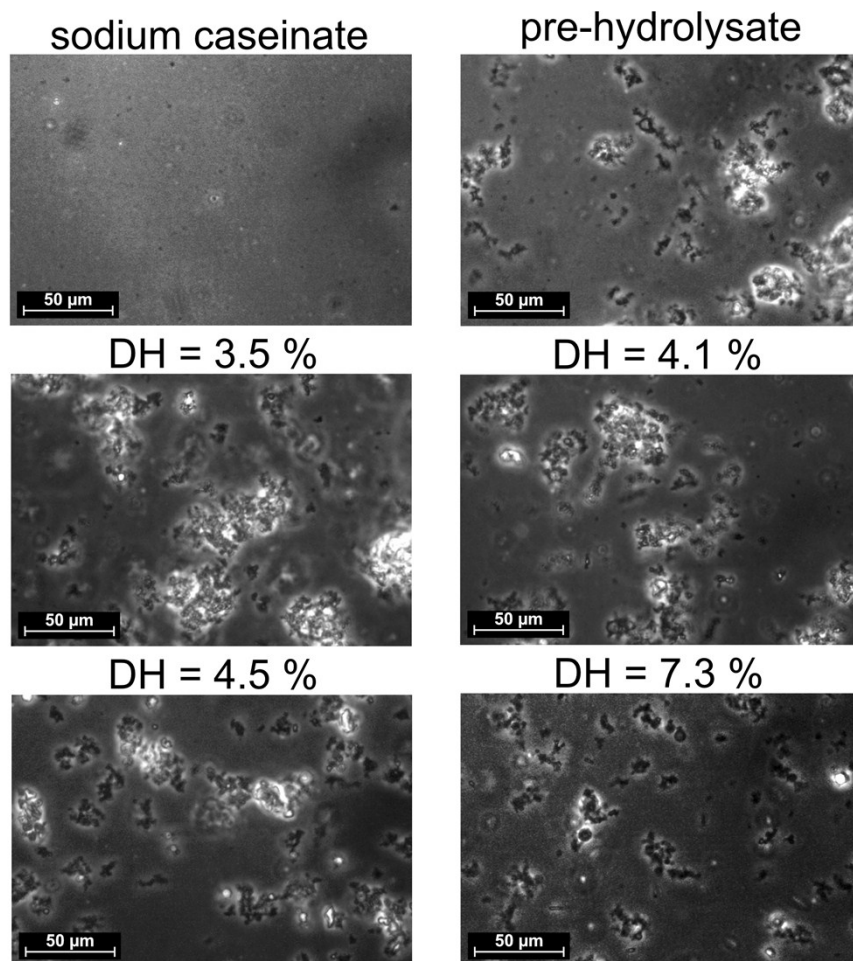


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Fig. S1 Tricine-SDS-PAGE of the sequential hydrolysis of sodium caseinate with, firstly, Sternzym BP 25291 (pre-hydrolysate) and, subsequently, Exo-Flavourzyme (M = protein marker, 15 µg_{protein} per lane loaded).

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29 **Fig. S2** Microscopic images of sodium caseinate, the pre-hydrolysate produced with
 30 Sternzym BP 25201, and the products of the further hydrolysis with Exo-Flavourzyme
 31 (DH 3.5 – 7.3 %). Microscopic images were taken at a 40x magnification.