

# Dry heat stress stability of casein hydrolysate peptide mixture

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## INTRODUCTION

Hot melt extrusion (HME) uses a rotating screw and elevated temperature to pump dry, raw materials through a die and into a product of uniform shape. It is currently one of the most widely used processing techniques within the plastics industry and has drawn the attention of the pharmaceutical industry. Several research groups have already demonstrated the possibility of HME in the processing of API's e.g. nifedipine, theophylline, into various pharmaceutical forms including tablets, granulates and implants [1].

Peptides make up a promising class of new therapeutics. Recently, new manufacturing and delivery techniques, combined with intensified research and development programs, resulted in the approval of an anti-HIV peptide drug Fuzeon<sup>®</sup> and an estimated total of 200 peptides in various clinical stages [2]. Depending on the therapeutic application, processing of certain peptides e.g. anti-cancer peptides, into implants with appropriate release rates, would be beneficial for patient compliance and therapeutic effect. Due to the high temperatures reached, HME of peptides is surrounded by skepticism. In order to evaluate the HME processing possibilities of peptides, a number of selected peptides, present in a casein hydrolysate sample, were subjected to dry heat stress stability tests.

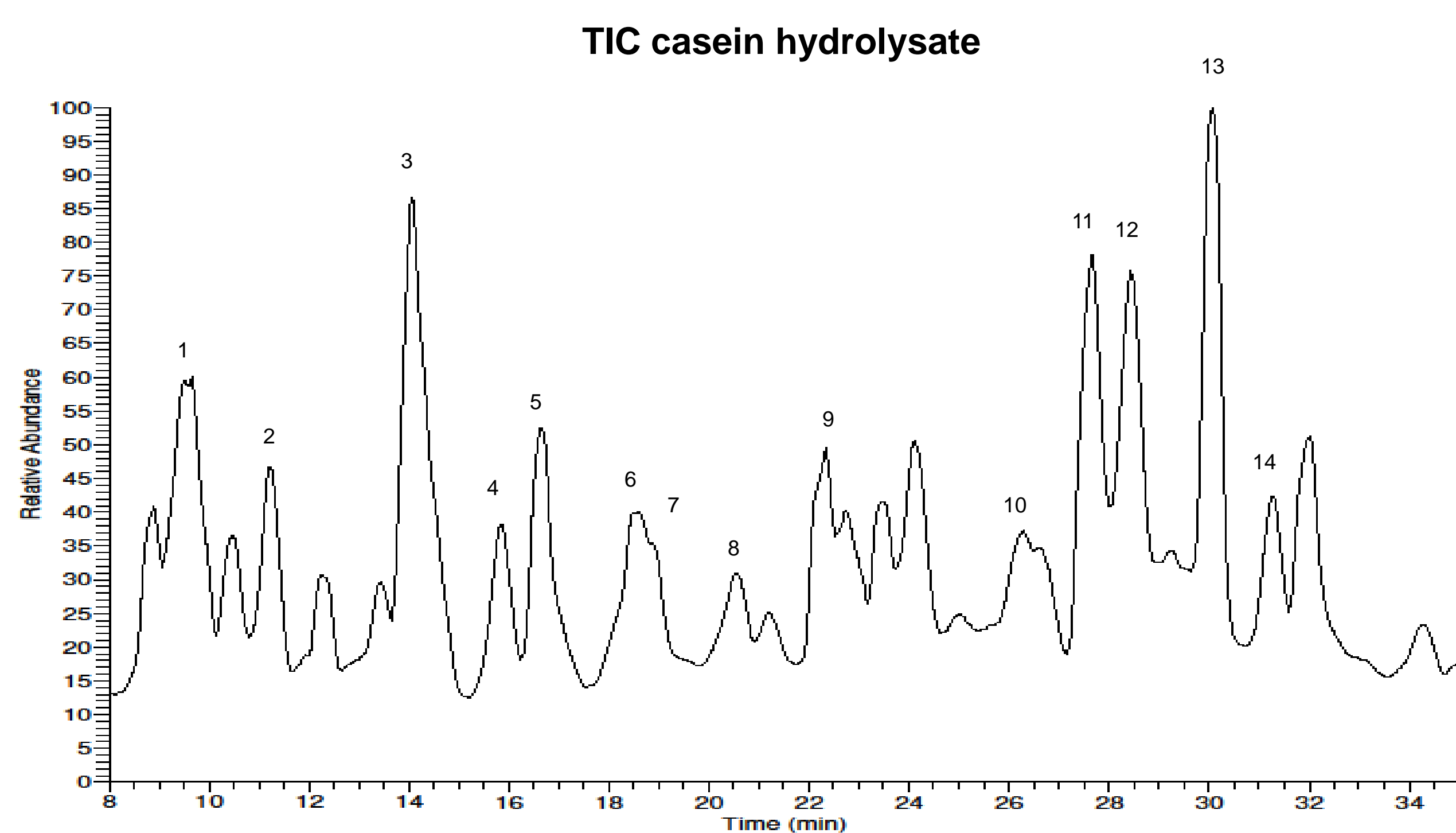
## EXPERIMENTAL

Casein hydrolysate characterization: HPLC-UV/ESI Iontrap MS, peptide C<sub>18</sub> fused-core column (75 mm × 3.0 mm, 2.7 μm; HALO; Achrom), linear gradient (MF<sub>A</sub>: 0.1 % (m/V) FA in H<sub>2</sub>O; MF<sub>B</sub>: 0.1 % (m/V) FA in acetonitrile) T<sub>0 min.</sub>: 95/5 (V/V) A/B; T<sub>40 min.</sub>: 71/29 A/B; 0.6 mL/min., 195 nm.

Experimental design (duplicate): 100 °C – 140 °C – 180 °C  
1 min. – 3 min. – 5 min.

## RESULTS and DISCUSSION

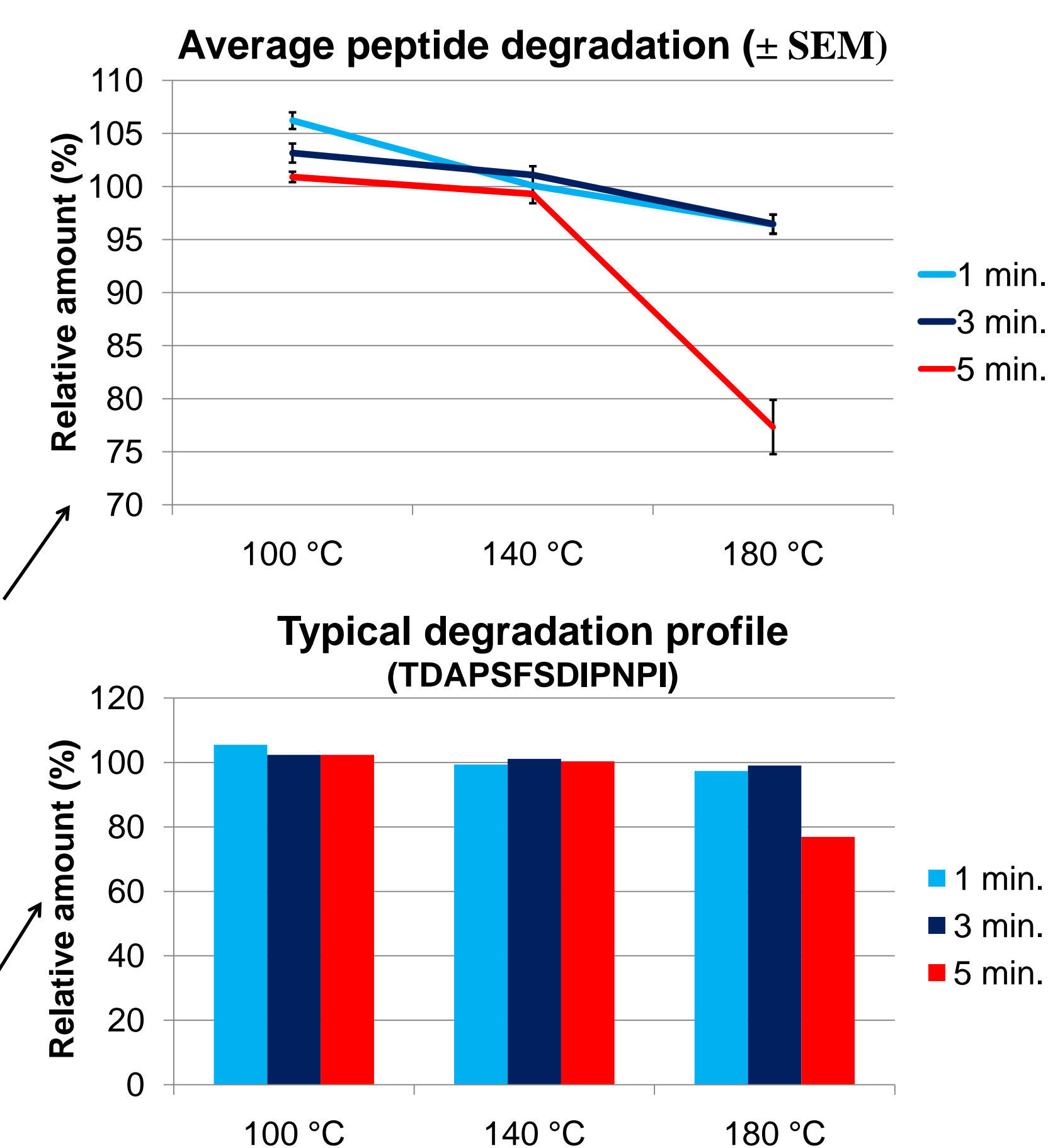
### 1. Model peptide mixture characterization (HPLC-ESI Iontrap MS)



#	Retention time (min.)	Observed [MH] <sup>+</sup>	Amino acid sequence	Origin
1	9.66	631.26	NKKIE	β-casein
2	11.21	604.15	YPVEP	β-casein
3	14.08	652.15	VLPVPQ	β-casein
4	15.87	659.10 727.15	VAPFPE NVPGEIV	αS <sub>1</sub> -casein β-casein
5	16.64	684.14 576.10	LEIVPN HLPLP	αS <sub>1</sub> -casein β-casein
6	18.53	668.10	DIPNPI	αS <sub>1</sub> -casein
7	18.89	755.18	SDIPNPI	αS <sub>1</sub> -casein
8	20.59	686.34	RGPFPI	β-casein
9	22.36	838.27 659.18	PFPGPPIPN APFPEV	β-casein αS <sub>1</sub> -casein
10	26.31	1001.28	YFPFGPIPN	β-casein
11	27.69	1025.33	VVPFLQPE	β-casein
12	28.46	1100.36	VYFPFGPIPN	β-casein
13	30.11	1373.33	TDAPSFSDIPNPI	αS <sub>1</sub> -casein
14	31.34	889.25	VYFPFGPI	β-casein

### 2. Dry heat stress stability tests (HPLC-UV)

Peak information		Relative amount 100 °C (%) <sup>(1,2)</sup>			Relative amount 140 °C (%) <sup>(1,2)</sup>			Relative amount 180 °C (%) <sup>(1,2)</sup>		
RT (min.)	AA sequence	1 min.	3 min.	5 min.	1 min.	3 min.	5 min.	1 min.	3 min. <sup>(3)</sup>	5 min. <sup>(3)</sup>
9.08	NKKIE	102.24	100.73	96.83	97.00	95.52	94.23	<b>88.60</b>	94.37	<b>85.23</b>
10.17	YPVEP	105.06	101.99	102.29	98.55	101.41	99.67	94.63	97.51	<b>78.53</b>
12.91	VLPVPQ	104.64	102.19	100.87	100.94	101.18	100.32	97.29	96.58	<b>78.00</b>
15.50	VAPFPE NVPGEIV LEIVPN HLPLP	104.04	99.58	98.23	99.56	100.36	99.89	94.57	101.44	<b>75.59</b>
17.40	DIPNPI	109.59	105.16	102.50	105.86	105.42	98.03	96.49	101.15	94.26
17.70	SDIPNPI	<b>110.48</b>	109.87	101.30	103.74	98.39	95.01	97.18	93.45	<b>75.04</b>
19.19	RGPFPI	<b>110.03</b>	107.78	101.35	100.52	107.09	105.84	101.24	92.95	<b>71.43</b>
20.88	PFPGPPIPN APFPEV	106.31	104.34	100.85	103.42	102.31	103.03	98.62	98.35	<b>70.52</b>
24.78	YFPFGPIPN	101.90	99.24	102.35	96.53	98.82	98.53	100.65	93.76	<b>62.77</b>
26.16	VVPFLQPE	107.89	104.82	102.38	100.83	102.96	101.72	98.24	98.71	<b>83.32</b>
27.00	VYFPFGPIPN	104.84	99.33	99.21	95.93	100.23	97.89	95.06	95.54	<b>63.96</b>
28.73	TDAPSFSDIPNPI	105.49	102.37	102.39	99.35	101.15	100.36	97.38	99.03	<b>76.90</b>
29.83	VYFPFGPI	108.17	103.55	101.16	98.83	99.18	96.42	93.72	91.24	<b>89.69</b>



<sup>(1)</sup> Calculated using formula: ((Σpeak area stressed) / (Σpeak area unstressed))  
<sup>(2)</sup> Peptide abundance outside [90-110 %] interval relative to unstressed sample are considered significant and indicated in bold  
<sup>(3)</sup> Increase in degradation peaks: RRT: 0.983 (relative to RGPFPI) - [MH]<sup>+</sup>: 730.25; RRT: 0.986 (relative to VYFPFGPI) - [MH]<sup>+</sup>: 1315.40

## CONCLUSIONS

Hot-melt extrusion related dry heat stress stability study performed with a model peptide mixture set, originating from casein hydrolysate, showed peptide extrusion potential up toward 5 minutes at 140 °C and 3 minutes at 180 °C. However, significant degradation, ranging from 10 % to 40 %, was seen during 5 minutes incubation at 180 °C.

## REFERENCES

- [1] M. M. Crowley, F. Zhang, M. A. Repka, S. Thumma, S. B. Upadhye, S. Kumar, J. W. McGinity, C. Martin, Drug Development and Industrial Pharmacy, 2007, 33, 909.  
 [2] P. McGee, Drug Discovery and Development, 2005, 4.