Supporting Information

Identification of the Peptide PyroQ-βCasein$_{194-209}$ as a Highly Specific and Sensitive Marker to Differentiate between Ultrahigh-Temperature Processed (UHT) Milk and Mildly Heated Milk

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Table S1. Evaluation of the Endogenous Milk Peptide *m/z* 1589.9 as Internal Standard for the Analysis of Heated Milk Samples

The synthesized peptide *m/z* 1558.8 was added to the milk samples and its intensity was set as 100%. Relative quantities of the peptide *m/z* 1589.9 in different raw (n=3), pasteurized (n=4), ESL (n=4) and UHT (n=4) milk samples were compared. Raw milk samples were heated at 99 °C for 0 min (n=3), 15 min (n=3), 30 min (n=3) and 45 min (n=3). Mean relative intensity values (RI, %) and standard deviations (SD) of the signals for peptide *m/z* 1589.9 are shown. Different commercial milk samples, different heating durations, and different raw milk samples were compared by one-way ANOVA and Tukey's HSD multiple comparison tests (95% confidence of interval) and different letters indicate statistically significant differences.

commercial samples		heating of raw milk		raw milk samples	
type	RI ± SD	heating time	RI ± SD	sample	RI ± SD
pasteurized	23.86 ± 5.53[a]	0 min	15.08 ± 1.56[b]	raw milk 1	15.11 ± 1.80[b]
ESL milk	24.36 ± 4.60[a]	15 min	15.65 ± 2.16[b]	raw milk 2	14.57 ± 1.57[b]
UHT milk	19.32 ± 2.79[ab]	30 min	16.89 ± 2.19[b]	raw milk 3	17.50 ± 1.96[b]
		45 min	16.18 ± 0.57[b]		

Table S2. Relative Quantification of the Detected Peptides in Mildly Heated and UHT Milk Samples of the Training Set Using the Peptide *m/z* 1589.9 as Internal Standard

Commercially available mildly heated [pasteurized (n=20), microfiltered-ESL (n=13), heated-ESL (n=16)] and UHT (n=29) milk samples from different manufacturers were analyzed in triplicate. Mean relative intensities (RI, %) and standard deviations of the peptides are shown. All peptides of the recorded peptide profile were quantified and peptides, in which the intensities in all mildly heated groups were statistically different compared to the UHT group, are displayed. Different types of milk samples were compared by one-way ANOVA and Tukey's HSD multiple comparison tests (95% confidence of interval) and different letters indicate statistically significant differences between types of milk samples.

higher RI in mildly heated samples					
		mildly heated			UHT
No	*m/z*	pasteurized	microfiltered-ESL	heated-ESL	
1	1718.0	32.53 ± 6.51[a]	32.45 ± 8.25[a]	24.73 ± 7.23[a]	16.79 ± 15.19[b]
2	2331.3	217.67 ± 91.01[a]	293.78 ± 111.24[a]	208.99 ± 144.63[a]	86.44 ± 29.97[b]
3	2381.2	8.64 ± 2.82[a]	9.11 ± 1.71[a]	8.89 ± 3.79[a]	6.08 ± 2.66[b]
4	2437.3	6.71 ± 2.48[a]	7.29 ± 2.72[a]	6.95 ± 3.64[a]	4.48 ± 1.73[b]
5	2586.5	8.45 ± 3.18[a]	9.07 ± 2.82[a]	8.61 ± 4.26[a]	5.45 ± 1.99[b]
6	2616.5	120.50 ± 49.12[a]	137.34 ± 50.67[a]	114.69 ± 76.97[a]	14.09 ± 16.52[b]
7	3215.8	18.33 ± 9.20[a]	23.83 ± 9.78[a]	19.83 ± 14.01[a]	10.09 ± 4.25[b]
higher RI in UHT milk					
		mildly heated			UHT
No	*m/z*	pasteurized	microfiltered-ESL	heated-ESL	
1	997.6	4.16 ± 1.92[b]	4.26 ± 1.69[b]	4.09 ± 1.05[b]	14.09 ± 9.40[a]
2	1001.6	3.07 ± 1.65[b]	3.28 ± 2.26[b]	3.26 ± 1.71[b]	6.31 ± 4.22[a]
3	1012.7	5.88 ± 2.41[b]	5.22 ± 3.65[b]	4.98 ± 2.80[b]	10.00 ± 6.38[a]
4	1092.6	3.64 ± 1.85[b]	4.54 ± 2.90[b]	6.63 ± 3.61[b]	98.41 ± 95.99[a]

5	1094.7	3.41 ± 1.59^{b}	3.32 ± 1.41^{b}	4.58 ± 2.02^{b}	38.36 ± 24.54^{a}
6	1108.6	2.14 ± 1.51^{b}	2.34 ± 1.28^{b}	2.61 ± 1.08^{b}	20.67 ± 17.64^{a}
7	1124.6	1.78 ± 1.15^{b}	2.35 ± 1.32^{b}	1.95 ± 0.93^{b}	7.30 ± 7.27^{a}
8	1128.6	4.44 ± 3.36^{b}	3.30 ± 1.21^{b}	4.43 ± 1.48^{b}	7.34 ± 4.21^{a}
9	1130.6	2.98 ± 1.77^{b}	3.17 ± 1.07^{b}	3.66 ± 1.14^{b}	8.56 ± 4.30^{a}
10	1237.7	5.45 ± 3.19^{b}	5.02 ± 2.12^{b}	5.15 ± 2.09^{b}	7.75 ± 3.56^{a}
11	1270.7	4.75 ± 2.72^{b}	4.56 ± 2.25^{b}	4.19 ± 1.56^{b}	7.01 ± 2.35^{a}
12	1284.7	13.11 ± 10.94^{b}	13.63 ± 16.04^{b}	48.11 ± 61.53^{b}	415.80 ± 304.21^{a}
13	1306.8	1.78 ± 1.36^{b}	1.86 ± 0.92^{b}	2.25 ± 1.03^{b}	10.06 ± 6.80^{a}
14	1422.8	2.43 ± 1.52^{b}	1.95 ± 1.00^{b}	2.37 ± 1.06^{b}	8.72 ± 6.85^{a}
15	1460.9	4.19 ± 4.07^{b}	7.11 ± 3.45^{b}	13.56 ± 14.34^{b}	111.53 ± 53.40^{a}
16	1572.0	2.46 ± 0.93^{b}	2.07 ± 1.37^{b}	2.05 ± 1.32^{b}	7.93 ± 3.67^{a}
17	1574.9	1.17 ± 0.64^{b}	1.40 ± 0.98^{b}	2.05 ± 1.22^{b}	50.84 ± 54.77^{a}
18	1592.9	9.72 ± 1.50^{b}	10.19 ± 1.67^{b}	11.18 ± 2.01^{b}	40.82 ± 41.98^{a}
19	1608.9	2.76 ± 1.10^{b}	3.61 ± 1.93^{b}	2.89 ± 0.88^{b}	20.64 ± 11.56^{a}
20	1624.9	2.29 ± 0.99^{b}	2.63 ± 1.21^{b}	4.24 ± 3.63^{b}	35.14 ± 18.42^{a}
21	1633.9	4.07 ± 1.86^{b}	3.55 ± 1.32^{b}	3.66 ± 1.49^{b}	6.19 ± 2.36^{a}
22	1668.9	2.53 ± 1.06^{b}	2.99 ± 1.56^{b}	2.95 ± 1.51^{b}	33.52 ± 35.17^{a}
23	1689.9	2.41 ± 1.54^{b}	2.74 ± 2.03^{b}	6.57 ± 3.70^{b}	82.03 ± 69.53^{a}
24	1701.0	3.78 ± 1.10^{b}	5.92 ± 1.67^{b}	8.28 ± 5.58^{b}	62.53 ± 41.07^{a}
25	1705.9	1.30 ± 0.80^{b}	1.64 ± 0.36^{b}	2.15 ± 1.11^{b}	13.29 ± 7.68^{a}
26	1712.9	2.45 ± 1.26^{b}	2.29 ± 1.31^{b}	2.59 ± 0.95^{b}	10.40 ± 4.54^{a}
27	1782.0	2.24 ± 0.89^{b}	2.18 ± 1.15^{b}	2.46 ± 1.25^{b}	15.76 ± 11.28^{a}
28	1825.0	2.15 ± 0.88^{b}	2.13 ± 1.20^{b}	2.07 ± 0.94^{b}	8.13 ± 4.28^{a}
29	1895.1	4.38 ± 1.46^{b}	4.66 ± 1.05^{b}	6.59 ± 4.59^{b}	16.76 ± 8.26^{a}
30	1905.0	1.68 ± 0.89^{b}	2.06 ± 0.91^{b}	2.26 ± 1.71^{b}	12.96 ± 5.07^{a}
31	1973.1	3.33 ± 1.40^{c}	5.13 ± 3.94^{bc}	13.14 ± 11.65^{b}	30.10 ± 11.30^{a}
32	1991.1	15.84 ± 5.72^{b}	15.39 ± 3.27^{b}	13.75 ± 3.95^{b}	38.02 ± 16.25^{a}
33	1994.1	227.71 ± 99.03^{b}	326.41 ± 82.52^{b}	330.25 ± 226.76^{b}	766.32 ± 442.97^{a}
34	2014.0	6.56 ± 2.58^{b}	8.78 ± 4.07^{b}	14.68 ± 6.14^{b}	75.50 ± 50.40^{a}
35	2032.1	3.16 ± 1.81^{b}	4.14 ± 1.61^{b}	4.36 ± 3.17^{b}	12.33 ± 6.56^{a}
36	2107.2	134.80 ± 56.35^{b}	187.11 ± 47.99^{b}	178.23 ± 89.41^{b}	297.61 ± 151.46^{a}
37	2207.2	3.59 ± 1.30^{b}	4.21 ± 2.09^{b}	4.33 ± 1.95^{b}	13.50 ± 6.03^{a}

38	2278.2	7.74 ± 2.59[b]	8.01 ± 2.06[b]	10.31 ± 4.06[b]	14.15 ± 5.75[a]
39	2454.2	3.15 ± 1.35[b]	4.45 ± 2.19[b]	4.14 ± 1.85[b]	15.28 ± 7.77[a]
40	2510.4	4.47 ± 1.55[b]	4.99 ± 2.03[b]	4.86 ± 1.81[b]	11.91 ± 5.08[a]
41	2618.3	39.53 ± 19.02[b]	42.22 ± 14.36[b]	47.63 ± 29.31[b]	124.43 ± 98.19[a]
42	2634.4	2.12 ± 1.24[b]	2.18 ± 0.82[b]	3.62 ± 2.30[b]	11.47 ± 6.69[a]
43	2696.4	2.16 ± 1.33[b]	2.52 ± 1.11[b]	3.56 ± 2.89[b]	52.05 ± 53.89[a]
44	2699.4	14.05 ± 7.69[b]	11.24 ± 3.77[b]	14.19 ± 6.63[b]	22.23 ± 12.00[a]
45	2712.5	3.00 ± 1.00[b]	2.63 ± 1.28[b]	2.62 ± 1.66[b]	8.18 ± 5.30[a]
46	2784.5	2.55 ± 1.13[b]	2.82 ± 1.21[b]	3.94 ± 2.26[b]	10.80 ± 5.89[a]
47	2795.4	3.91 ± 1.70[b]	4.33 ± 2.45[b]	4.44 ± 1.68[b]	16.19 ± 8.88[a]
48	2995.5	3.49 ± 1.53[b]	3.84 ± 1.90[b]	3.32 ± 1.56[b]	11.67 ± 8.42[a]

Table S3. Relative Quantification of Peptides in Heated Raw Milk Samples Using the Peptide *m/z* 1589.9 as Internal Standard

A raw milk sample was heated at 99 °C for 0 min, 15 min, 30 min and 45 min. Mean relative intensities (%) and standard deviations of the peptides at different time points are shown. All peptides of the recorded peptide profile were quantified and peptides showing a significant decrease or increase are displayed. Different heating times were compared by one-way ANOVA and Tukey's HSD multiple comparison tests (95% confidence of interval) and different letters indicate statistically significant differences between heating times.

peptides increasing under heat exposure					
No	*m/z*	0 min	15 min	30 min	45 min
1	1092.6	6.75 ± 2.78^{b}	18.90 ± 5.22^{b}	55.80 ± 17.81^{a}	77.49 ± 27.35^{a}
2	1094.7	6.65 ± 2.20^{b}	21.63 ± 5.68^{b}	55.47 ± 18.06^{a}	75.41 ± 20.89^{a}
3	1108.6	4.83 ± 0.87^{b}	7.78 ± 2.99^{b}	12.86 ± 4.18^{ab}	24.88 ± 14.73^{a}
4	1197.6	5.51 ± 1.13^{c}	54.36 ± 18.93^{bc}	108.80 ± 42.49^{a}	86.18 ± 27.59^{ab}
5	1460.9	5.02 ± 2.15^{d}	41.75 ± 3.52^{c}	120.90 ± 13.20^{b}	206.42 ± 14.19^{a}
6	1624.9	5.09 ± 2.02^{c}	22.75 ± 7.71^{c}	117.42 ± 57.90^{b}	178.68 ± 21.09^{a}
7	1689.9	3.80 ± 0.38^{b}	33.77 ± 11.41^{b}	103.54 ± 45.33^{a}	116.86 ± 41.30^{a}
8	1701.0	4.66 ± 2.07^{c}	12.81 ± 3.70^{c}	30.27 ± 3.48^{b}	52.00 ± 8.84^{a}
9	1705.9	4.06 ± 2.60^{c}	10.42 ± 5.01^{bc}	28.73 ± 12.11^{ab}	40.30 ± 18.74^{a}
10	1905.0	10.95 ± 3.90^{c}	9.87 ± 1.93^{c}	36.92 ± 10.24^{b}	57.08 ± 12.32^{a}
11	1973.1	6.97 ± 2.45^{c}	85.17 ± 26.39^{b}	158.64 ± 24.09^{a}	171.06 ± 35.72^{a}
12	1991.1	44.76 ± 14.12^{b}	44.76 ± 15.82^{b}	85.90 ± 12.94^{a}	115.91 ± 23.06^{a}
13	2014.0	6.96 ± 2.99^{c}	44.02 ± 16.25^{b}	76.19 ± 20.29^{ab}	78.01 ± 25.50^{a}
14	2216.1	5.97 ± 0.59^{c}	24.60 ± 5.41^{b}	39.79 ± 9.77^{a}	47.46 ± 6.59^{a}

peptides decreasing under heat exposure

No	*m/z*	0 min	15 min	30 min	45 min
1	1060.6	21.03 ± 6.78^{a}	14.73 ± 4.49^{ab}	12.74 ± 4.42^{ab}	10.95 ± 3.58^{b}
2	1237.7	30.03 ± 10.85^{a}	9.94 ± 6.30^{b}	10.25 ± 2.99^{b}	7.74 ± 1.92^{b}
3	1679.9	60.19 ± 6.42^{a}	48.97 ± 15.79^{ab}	39.49 ± 9.93^{b}	33.19 ± 1.61^{b}
4	1718.0	26.30 ± 3.33^{a}	11.05 ± 3.80^{b}	11.22 ± 1.41^{b}	12.11 ± 2.61^{b}
5	1863.1	63.58 ± 20.95^{a}	38.29 ± 16.99^{ab}	41.34 ± 12.29^{ab}	26.27 ± 5.29^{b}
6	1881.1	811.03 ± 148.83^{a}	9.79 ± 3.40^{b}	14.99 ± 8.45^{b}	8.99 ± 2.76^{b}
7	2309.3	33.15 ± 11.12^{a}	18.16 ± 3.40^{b}	15.95 ± 1.81^{b}	14.34 ± 2.91^{b}
8	2331.3	338.41 ± 100.59^{a}	218.12 ± 51.54^{b}	192.07 ± 55.50^{b}	149.71 ± 31.67^{b}
9	2347.3	62.83 ± 22.58^{a}	36.18 ± 6.97^{b}	32.76 ± 10.82^{b}	31.47 ± 10.88^{b}
10	2430.3	38.21 ± 11.99^{a}	21.59 ± 6.30^{b}	15.28 ± 3.54^{b}	15.55 ± 4.81^{b}
11	2460.4	832.97 ± 238.97^{a}	389.32 ± 70.95^{b}	353.04 ± 140.17^{b}	291.67 ± 81.86^{b}
12	2616.5	298.81 ± 79.53^{a}	129.48 ± 20.12^{b}	123.18 ± 37.04^{b}	120.09 ± 46.25^{b}
13	2763.6	314.39 ± 110.66^{a}	86.64 ± 27.54^{b}	71.17 ± 34.23^{b}	53.18 ± 17.03^{b}
14	2827.6	45.67 ± 18.07^{a}	26.78 ± 7.40^{ab}	22.54 ± 8.18^{b}	20.36 ± 7.23^{b}
15	3215.8	30.65 ± 6.22^{a}	24.50 ± 9.62^{ab}	17.84 ± 4.60^{bc}	11.41 ± 3.52^{c}
16	3982.2	75.98 ± 29.69^{a}	65.13 ± 29.54^{ab}	31.35 ± 11.26^{bc}	16.48 ± 6.11^{c}

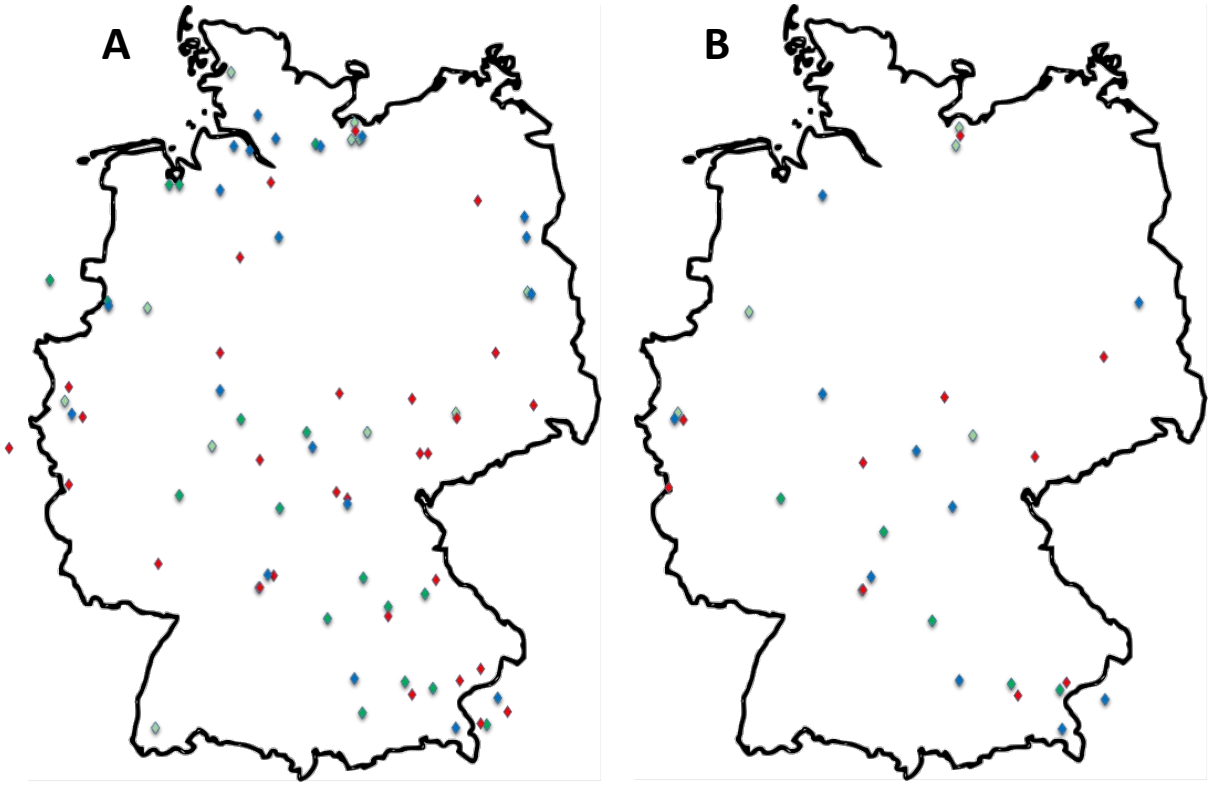

Figure S1. Map of the distribution of training set (A) and test set (B) samples. (A) Commercially available pasteurized (n=20, blue diamond), microfiltered-ESL (n=13, light green diamond), heated-ESL (n=16, green diamond) and UHT (n=29, red diamond) milk samples were produced by dairies mostly located in Germany (72 samples) and neighboring countries like Austria (4 samples), Belgium (1 sample) and The Netherlands (1 sample).

(B) Commercially available pasteurized (n=10, blue diamond), microfiltered-ESL (n=5, light green diamond), heated-ESL (n=5, green diamond) and UHT (n=10, red diamond) milk samples were collected from local supermarkets. The production facilities were localized by the identification marks on the milk packages.

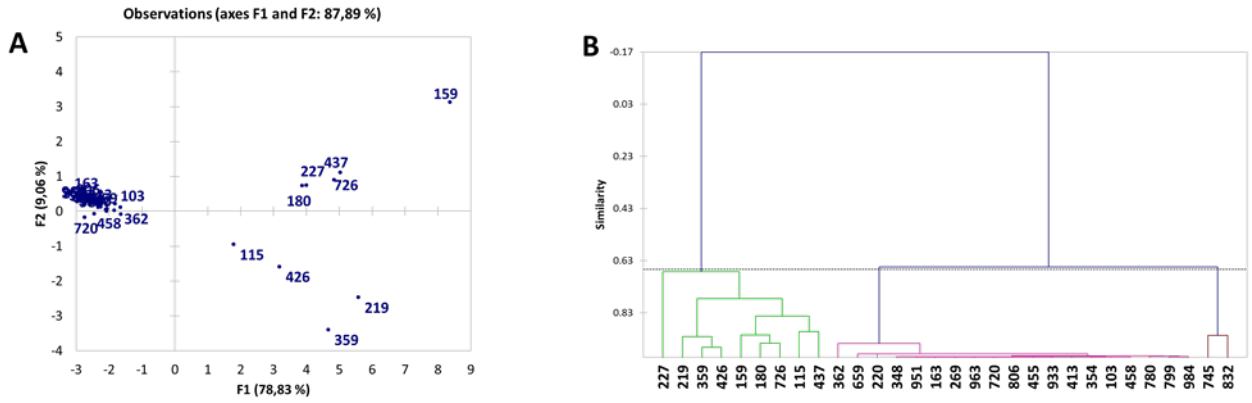

Figure S2. Projection of blind test set samples with multivariate statistical analysis (principal component analysis, PCA and agglomerative hierarchical clustering, AHC) (A) 2D-map visualization of 30 blinded milk samples. The negative side of the x-axis represents mildly heated samples; the positive side of the x-axis corresponds to UHT samples. (B) The similarity dendrogram of 30 blind test set samples. Each color represents a single cluster and samples with the same color share similar characteristics. Green lines represent UHT samples, while purple and brown lines represent mildly heated samples. The dotted line represents the truncation of three main clusters.