Supplementary material for:

FTIR as a rapid tool for monitoring molecular weight distribution during enzymatic protein hydrolysis of food processing by-products

Sileshi Gizachew Wubshet[a*], Ingrid Måge[a], Ulrike Böcker[a], Diana Lindberg[a], Svein H. Knutsen[a], Anne Rieder[a], Diego Airado Rodriguez[a] and Nils Kristian Afseth[a]

[a]Nofima AS - Norwegian Institute of Food, Fisheries and Aquaculture Research, PB 210. N-1431 Ås, Norway

[*]Corresponding author. Tel.: +47 909 17 126

E-mail address: sileshi.wubshet@nofima.no (Sileshi G. Wubshet).

Table S1: Retention times of standards (bovine serum albumin, albumin from chicken egg white, carbonic anhydrase from bovine erythrocytes, lysozyme, cytochrome c from bovine heart, aprotinin from bovine lung, insulin chain B oxidized from bovine pancreas, renin substrate tetradecapeptide porcine, angiotensin II human, bradykinin fragment 1-7, [D-Ala^2]-leucine encephalin, Val-Tyr-Val, and tryptophan) analyzed on BioSep2000 column for molecular weight calibration. Values from triplicate (*i*, *j* and *k*) measurements are presented

	Molecules	Molecular weight(g/mol)[a]	RT (*i*)	RT (*j*)	RT (*k*)	MeanRT	LogM_W	SD
1	Bovine albumin	66000	6.037	6.037	6.044	6.039	4.820	0.004
2	Albumin from chicken egg white	44287	6.108	6.107	6.109	6.108	4.646	0.001
3	Carbonic anhydrase	29000	6.132	6.126	6.132	6.130	4.462	0.003
4	Lysozyme	14300	6.702	6.702	6.704	6.703	4.155	0.001
5	Cytochrome c from bovine heart	12327	6.432	6.430	6.430	6.431	4.091	0.001
6	Aprotinin from bovine lung	6511	7.315	7.309	7.315	7.313	3.814	0.003
7	Insulin Chain B Oxidized from bovine pancreas	3496	9.049	9.037	9.037	9.041	3.544	0.007
8	Renin Substrate Tetradecapeptide porcine	1759	8.698	8.690	8.692	8.693	3.245	0.004
9	Angiotensin II human	1046	9.305	9.293	9.301	9.300	3.020	0.006
10	Bradykinin fragment 1-7	757	9.732	9.724	9.727	9.728	2.879	0.004
11	[D-Ala^2]-Leucine enkephalin	570	11.792	11.778	11.785	11.785	2.756	0.007
12	Val-Tyr-Val	379	11.334	11.327	11.332	11.331	2.579	0.004
13	Tryptophan	204	12.184	12.024	12.032	12.080	2.310	0.090

[a] Rounded to the nearest whole number

RT: retention time; M_W: molecular weight; SD: standard deviation

Table S2: Weight average molecular weight (M_w) of CF hydrolysates calculated form SEC. Samples were acquired from 6 hydrolysis experiments (CF 1 – CF 6) and at different hydrolysis times (0 – 60 minutes). Values from duplicate (*i* and *j*) measurements are presented. Data was not acquired for 0.5 and 5 min samples of CF 3 and CF 4, respectively.

Hydrolysis time (min.)	**M_w (g/mol) CF 1**		**M_w (g/mol) CF 2**		**M_w (g/mol) CF 3**		**M_w (g/mol) CF 4**		**M_w (g/mol) CF 5**		**M_w (g/mol) CF 6**	
	i	*j*	*i*	*j*	*i*	*j*	*i*	*j*	*i*	*j*	*i*	*j*
0	2752	2756.9	2760.8	2776.7	2825.1	2814.3	4314.9	4320.2	3886.9	3909.2	2148.1	2114.4
0.5	3378.1	3409.5	3454.2	3408.3	-	-	3551.4	3542.9	2973.1	2977.9	2796.8	2763.7
2.5	3212.7	3243.2	3039.1	2977.5	2861.1	2874.8	3229.6	3231.4	2828.3	2833.1	2893.3	2917.5
5	2979.6	2934.6	3049.1	2981.8	2734.3	2752.5	-	-	2694.8	2699.7	2943.2	2924.1
7.5	2826.6	2864.4	2750.5	2673.6	2753.2	2764.2	2902.9	2910.9	2790.4	2796.4	2498	2496.9
10	2703.2	2735.7	2898.5	2855.9	2524.3	2531.6	2578.9	2586.9	2508.5	2520.1	2453	2430.8
15	2745.4	2783.6	2602.8	2539	2468.4	2449.9	2459.1	2459.1	2400.9	2410.2	2299.7	2313
20	2454.7	2477.4	2544.6	2494.3	2375.6	2093.9	2243.8	2252.4	2373.9	2379.6	2269.3	2246.4
30	2291.5	2279.7	2328.1	2272.4	2258.7	2259.8	2088.5	2087.9	2181.3	2187.3	2064.5	2071
40	2187.4	2215.4	2207.7	2163.2	2100.9	2093.9	1969.4	1969	2029.2	2036	2007.1	2015.8
50	2103.5	2064.3	2102.9	2044	1961.8	1969.9	1987.7	1994.9	1983	1986.4	1851.9	1837.1
60	2041.9	2061.1	2020.2	1973.9	1972.8	1981	1976.3	1983.7	2026.1	2030.6	1789.9	1779

Table S3: Weight average molecular weight (*Mw*) of MCDR hydrolysates calculated form size exclusion chromatography. Samples were acquired from 6 hydrolysis experiments (MCDR 1 – MCDR 6) and at different hydrolysis times (0.5-60 minutes). Values from duplicate (*i* and *j*) measurements are presented. Data was not acquired the 5 min sample of MCDR 4.

Hydrolysis time (min.)	**M_w (g/mol) MCDR 1**		**M_w (g/mol) MCDR 2**		**M_w (g/mol) MCDR 3**		**M_w (g/mol) MCDR 4**		**M_w (g/mol) MCDR 5**		**M_w (g/mol) MCDR 6**	
	i	*j*	*i*	*j*	*i*	*j*	*i*	*j*	*i*	*j*	*i*	*j*
0.5	2743.1	2489	3512.8	2692.7	3254.1	2377.9	5523	5580.2	5256.1	4758	3439.9	4062.4
2.5	2901.9	2905.1	3103.8	2900.6	3590.8	3151.8	5417.3	5272.2	3398.9	2907.1	2901	2947
5	1938.1	1936.9	2385.2	2362.6	4844.5	4330.6	-	-	3179	3074.6	4119	5119.1
7.5	3010.1	3011.1	4185.9	3476.2	4239.2	3458.6	3643.9	3254.4	2073.1	2078.3	2887.7	2864.3
10	2250.1	2264.9	3324.4	2957.8	2042.4	2081	2187.5	2226	2066.2	2071.6	2148.9	2176.2
15	2541.9	2539	2584.8	2389.9	2099.1	2126.3	2026.4	2047.3	1950.6	1944.1	2464.2	2503
20	1899	1901.1	1924.4	1934.1	1939.5	1970.6	2683.8	2721.2	1932.3	1959.4	2934.2	2943
30	1713.2	1698.3	1912.7	1806.8	1803.8	1841.9	1923.3	1970.5	1913.2	1926	1929.6	1954.4
40	1619	1609.4	2095.9	1961.4	1635.9	1641.5	1662.9	1669.7	1662.1	1653.8	1774.4	1796
50	1691.3	1685.8	1722.2	1695.9	1561.9	1593.4	1674	1703.9	1701.3	1702.3	1615.9	1616.5
60	1477.6	1478.2	1628.1	1620.2	1488.6	1512.8	1625	1662.1	1769.2	1778.6	1570.6	1575

Figure S1: Total area of chromatographic traces (214 nm) obtained from SEC analysis of CF (A) and MCDR (B) hydrolysates sampled at different time during a 1 hour enzymatic protein hydrolysis. For hydrolysis of CF, samples collected before addition of the enzyme were used to acquire the $t = 0$ minute data (marked *). Data of four samples (0 and 0.5 min. sample of CF and two 5 min samples, one each from CF and MCDR) is not collected due to technical problems during sampling.

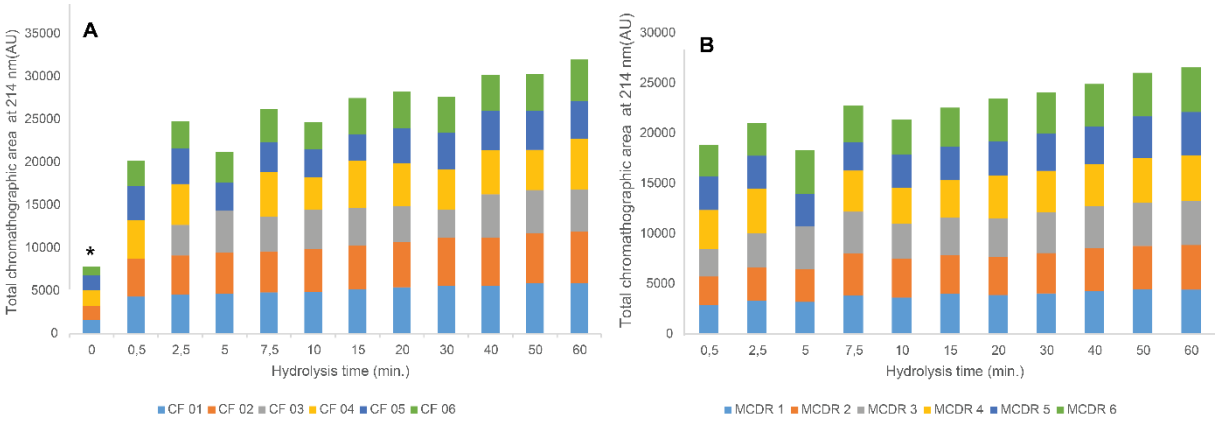

Figure S2: A presentation of the spectral pre-processing steps used before the multivariate calibration. The raw absorption spectra (A) and the corresponding second derivatives before (B) and after (C) EMSC correction are presented. The selected example of spectra are acquired from a single batch of hydrolysis (at different hydrolysis time) of CF.

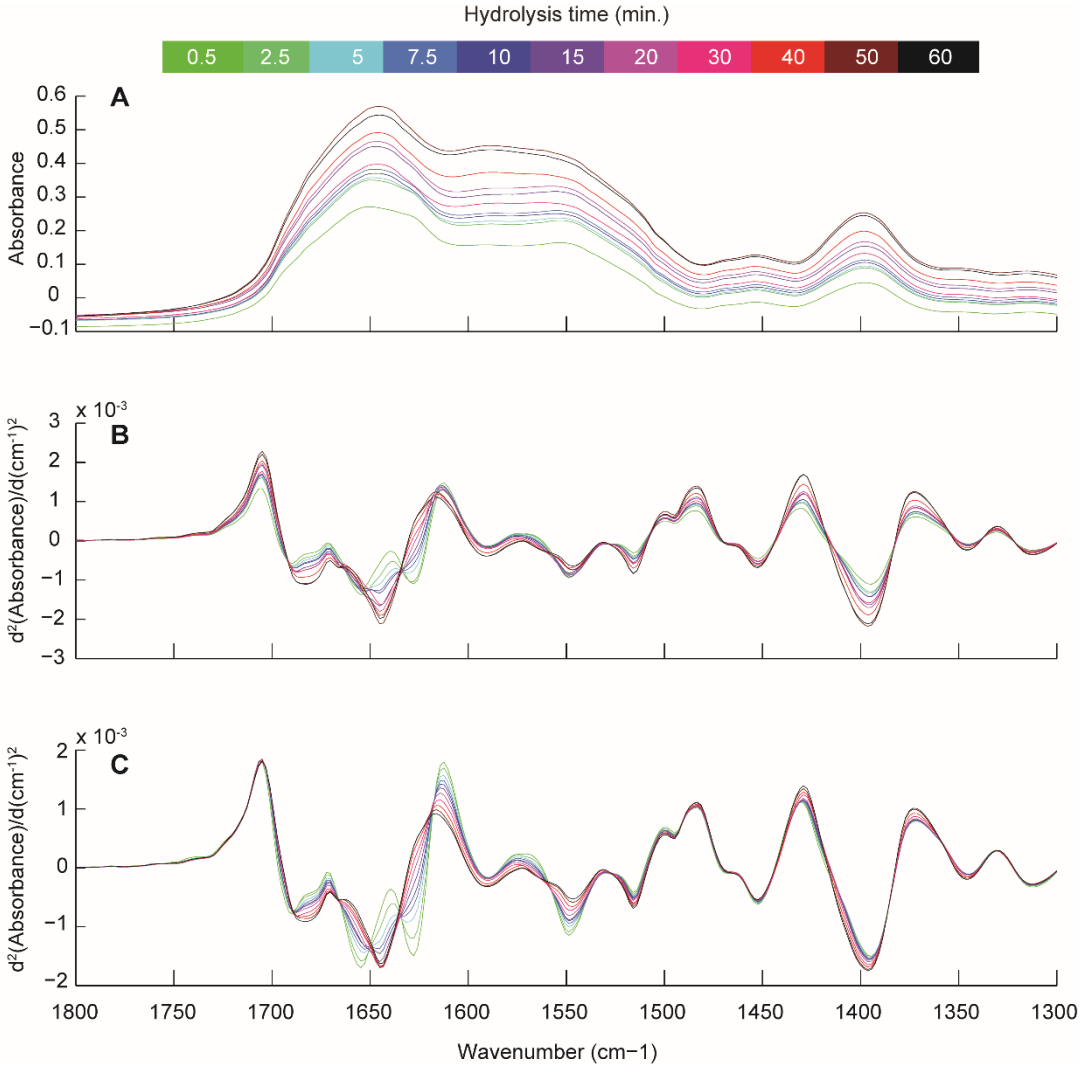

Figure S3: The protein backbone region of second-derivative FTIR spectra (1300 cm^{-1}-1800 cm^{-1}) of samples from hydrolysis experiments of CF and MCDR. Approximately 11 spectra acquired for samples collected at different hydrolysis time (0.5 to 60 min) are overlaid.

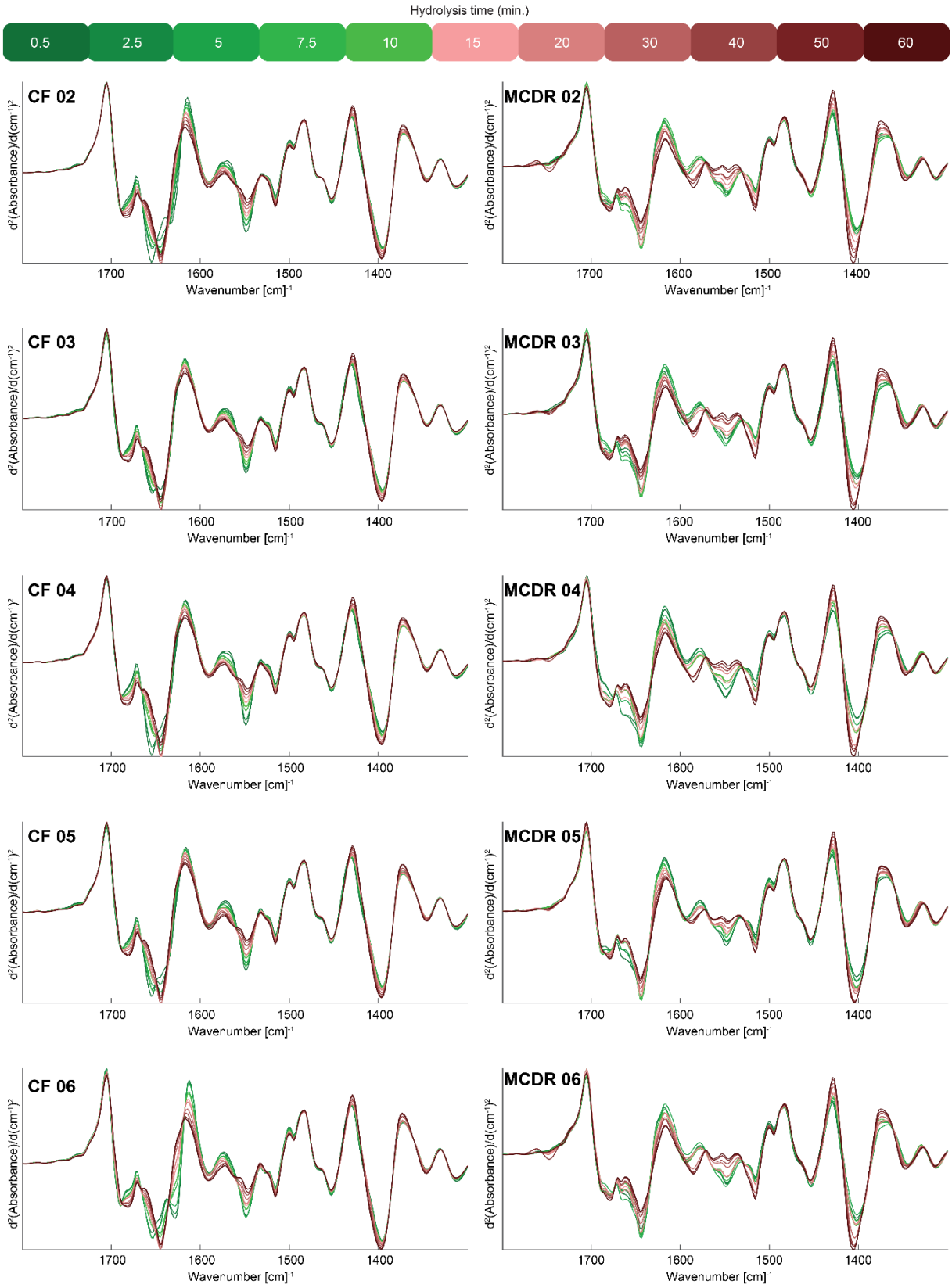

Figure S4: A zoomed region (1430-1510 cm^{-1}) of the second derivative spectra of CF 01 showing the change in absorption bands of the amino acid side chains as a function of hydrolysis time.