Chromatographic and mass spectrometry analysis of wheat flour prolamins, the causative compounds of Celiac Disease

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SUPPLEMENTARY INFORMATION

This paper aims to contribute for the better knowledge about wheat prolamins, the main responsible in celiac disease (CD) onset. After prolamin extraction from commercial wheat flour, the extracts were characterized by MALDI-TOF in order to analyze the intact proteins profile. After that, and given the high complexity of each extract, the sample was fractionated before analysis. The study was focused on gliadin extract (GLI) given its strong relationship with CD, and it was fractionated by chromatographic methods. Each GLI fraction was further characterized by MALDI-TOF to establish the proteomic profile. The composition of proteins of each fraction was further verified by nano-LC-MS/MS following a shotgun proteomics approach. This supplementary information comprises the results obtained after digestion ofthe richest GLI fractions, further analysis by nano-LC-MS/MS and MS/MS spectra treatment by using the Proteome Discoverer software as described in the experimental section. Some additional information was included in this document to better understand the results presented herein.

MATERIALS AND METHODS

Wheat Gluten extraction. 1 g of wheat flour was sequentially extracted with buffered salt solution, 0.4 M NaCl (Sigma, UK), 50mL 70% (v/v) aqueous ethanol (Sigma, UK) and 50% (v/v) 1-propanol (Merck, Germany) with 1% (w/v) dithiothreitol (DTT) (Sigma, UK) and Tris–HCl at pH 7.5, in order to extract the water-soluble albumins (ALB) and salt-soluble globulins (GLO), ethanol-soluble prolamins (GLI) and ethanol-insoluble prolamins (GLU), respectively. A 20:1 (w/v) solids-to-liquid ratio was used for the extractions, which were initiated by vortexing for 2 min at room temperature and continued with magnetic stirring during 1h. Ethanol-insoluble prolamins were extracted at 60ºC under nitrogen. Extractions for each sample were done in duplicate and the supernatants were collected by centrifugation (10000×g for 15 min). In the case of sodium chloride, an additional wash with deionised water was performed in order to remove the residual salt.

Fractionation of gliadins fractions. GLI fraction was submitted to a dialysis cleanup process before fractionation. Two different cut-off membranes (Spectrum Laboratories, Inc., Canada) were assayed, 3.5kDa and 12-14kDa. The dialyzed GLI at 12-14kDa were further separated into a preparative chromatographic system (Krauer K-1001 pump coupled to an Elite Lachrom L-2420 UV vis detector, Agilent Technologies). Sample was pumped at 4mL/min into a Purospher C18 (250x25mm, 5µm). The mobile phases used were (A) water (0.1%TFA) and (B) acetonitrile (0.1%TFA) from 24%B to 56%B in 140min. The chromatograms were obtained at 214nm through the EZChrom Elite software (Agilent Technologies). The protein elution was monitorized and collected by measuring absorbance at 214nm. After isolated proteins from GLI (12-14kDa) were collected, these fractions were lyophilized and further characterized by mass spectrometry.

RESULTS

Identification of gliadin sub-fractions by nano-LC-MS/MS. Protein fractions resulting from GLI fractionation were submitted to enzymatic digestion. The hydrolysates were further analyzed by tandem mass spectrometry. Each fingerprint peptide was, therefore, used to sequence the peptides and further identify their percursor protein. The results are described in the following table.

Table S1. Identification of the richest gliadin sub-fractions from FIII by nano-LC-MS/MS and further data treatment in Proteome Discoverer

	Glutenins								Gliadins																Avenins			
	HMW				LMW				alpha				beta				gamma				omega							
	Code	MW	Cover	Score	Code	MW	Cover	Score	Code	MW	Cover	Score	Code	MW	Cover	Score	Code	MW	Cover	Score	Code	MW	Cover	Score	Code	MW	Cover	Score
1	Q94IJ7	86,299	99.8	104	Q9XGF0	42,242	86.90	32	A5JSA7	33,177	100.00	71	M7ZZV2	23,558	95.82	91	R9XT02	37,261	87.65	56					D2KFH2	11,325	85.29	123
	S4U1S7	90,417	76.5	69					R9XW75	34,418	87.14	97	I0IT55	33,489	98.95	83	Q94G96	38,634	91.23	87								
									Q9M4M0	32,211	98.58	71																
2	V9TP40	77,371	64.3	128	Q9XGF0	42,242	78.61	56	Q9M4M0	32,211	100.00	94													G9I0S9	32,352	80.82	56
	Q41553	88,476	58.9	121					K7X1I3	34,588	98.66	87																
	E4W506	70,677	87.1	154					Q41529	36,145	100.00	78																
3	G4Y3Y1	85,243	69.11	116	R4JB56	36,458	70.66	110	X2KWL3	32,109	79.74	60	I0IT57	33,392	91.19	97	B6UKM1	31,933	100.00	65	R9XVE2	30,862	87.65	45	D2KFG9	22,371	77.51	97
	Q52JL2	75,691	68.92	33	Q0GQX1	33,978	70.57	124	Q41533	29,996	100.00	75	I0IT54	33,308	98.34	48	B6DQB3	35,618	100.00	32	D6QY51	40,493	74.35	56				
	S4U142	90,336	67.43	41	Q9XGF0	42,242	70.27	106	R9XU81	32,884	100.00	15	I0IT55	33,489	96.38	65	D0ES81	35,990	100.00	23								
					Q0GQX0	33,436	69.11	25	A0A023WGX2	23,220	82.89	91	D2T2K3	32,668	100.00	86	R9XV56	32,959	100.00	10								
					O49958	39,791	68.92	45	K7X1I9	35,444	93.90	75	I0IT53	33,611	99.10	72	Q30DX7	34,124	100.00	9.7								
					B8Y0L6	39,840	67.43	67	B8XU32	34,132	97.33	72	I0IT52	36,532	86.59	15												
									D2X6C9	34,522	98.20	15																
									M4WFC9	34,262	100.00	67																
									R9XSW3	23,500	92.47	66																
									K7XEB5	33,047	100.00	96																
4	A4URY8	70,038	66.94	101	R4JB56	36,458	75.07	14	R9XW75	34,418	92.61	67	I0IT57	33,392	100.00	87	B8XU40	34,900	100.00	45					D2KFH2	11,325	98.76	45
	Q94IJ6	77,371	66.81	108	R4JAP5	39,950	66.94	29					Q3S4V8	33,485	97.86	65									M8A4Q2	35,103	76.54	54
	D7RT26	91,638	65.76	68	Q9XGF0	42,242	66.81	26																				
5	Q38LF4	48,519	63.18	103	V9P737	34,216	98.65	47	F6M8E7	17,151	95.59	74													D2KFH2	11,325	95.64	98
					R4JAP5	39,950	89.56	46	Q41533	29,996	91.19	71													D2KFG9	22,371	75.64	45
					B2Y2S3	42,008	78.67	11	R9XW75	34,418	98.34	94																
					Q9XGF0	42,242	89.65	64	X2KS61	34,517	96.38	87																
									K7X1I9	35,444	97.39	78																

6	A4URY8	70,038	59.40	1.05	R4JB56	36,458	82.89	100	J7I026	33,958	93.23	67					Q9FS73	32,576	98.76	43					D2KFH2	11,325	76.55	14
	A0A059UHD1	89,835	58.82	1.02	R4JAP5	39,950	93.90	45									Q41547	36,759	93.24	42								
					Q9XGF0	42,2	97.3	102																				
7	A4URY8	70,038	69.11	1.16	R4JAP5	39,950	98.20	34	A5JSA8	33,213	98.00	75	I0IT57	33,392	100.00	56	Q9S8M8	2,240	95.64	56					D2KFH2	11,325	67.89	97
	Q03872	89,819	58.19	1.32	R4JDM1	36,356	100.00	116	J7I026	33,958	90.30	15					B8XU40	34,900	99.10	96					G9I0S9	32,352	56,43	54
	Q1KL95	84,677	58.01	1.13	Q9XGF0	42,242	92.47	89	F6M8E7	17,151	93.61	91																
					A0A068F6W7	33,302	82.89	115	K7XE68	33,552	80.23	92																
					V9P737	34,216	93.90	100	K7X1L1	32,113	100.00	71																
									Q3YFI1	32,893	57.14	84																
									Q9M4L8	32,370	98.58	61																
8	A4URY8	70,038	56.00	57	Q9XGF0	42,242	92.61	101	F6M8E7	17,151	100.00	60	I0IT59	34,114	100.00	93	R9XUT4	37,225	95.43	88					D2KFH2	11,325	99.76	79
	Q03872	89,819	54.67	28	A0A089VMB3	37,548	95.82	121	X2KWL1	35,709	98.66	82					B6DQB2	31,847	73.98	42					G9I0S9	32,352	97.65	45
					D3UAL6	36,283	98.95	144	K7WV42	32,831	100.00	19													D2KFG9	22,371	100.00	67
					B2Y2Q6	42,109	95.59	108	X2KS61	34,517	79.74	18																
					Q0GNF9	32,424	91.19	1.39																				
9					Q5MFH4	34,485	98.34	55	K7WV42	32,831	100.00	89	M7ZZV2	23,558	100.00	56	A7XDG3	16,206	100.00	50					D2KFH2	11,325	94.12	23
	Q94IJ6	77,371	51.97	18	R4JAP5	39,950	96.38	45	Q3YFI1	32,893	82.89	11	Q3S4V7	32,568	98.65	21	B6DQB8	32,449	98.76	96					V5M290	32,309	100.00	45
	B5TM09	79,278	56.60	12	Q00M55	40,031	97.39	54	R9XV20	34,404	93.90	75	I0IT57	33,392	75.96	23	U5UA50	32,691	99.43	83								
	A0MZ38	88,461	55.39	15	F8SGN3	40,071	93.23	102	R9XW75	34,418	97.33	17	P04722	33,661	99.12	65	F2X0K8	33,083	100.00	31								
					B2Y2Q7	41,981	100.00	118	X2KS61	34,517	98.20	20																
					Q9XGF0	42,242	97.86	177																				
10	A5HMG2	79,467	65.79	1 8	R4JAN5	37,763	98.00	104	F6M8E7	17,151	92.47	91	Q3S4V8	33,485	100.00	47	B6DQB4	32,295	96.78	97								
	G3K725	93,648	78.98	110					K7X1L1	32,113	100.00	8 1					B6UKS0	34,439	78.98	43								
									K7WV42	32,831	92.61	16					R9XWD0	37,229	98.34	68								
									X2KS61	34,517	95.82	70																
11					I3QPI2	17,399	89.76	142	Q41528	33,193	98.95	32	I0IT57	33,392	91.65	95	P08453	37,122	97.39	17					D2KFH2	11,325	65.76	90
					B2Y2S2	38,977	97.65	40	Q9M4M3	30,625	100.00	76	I0IT58	30,959	97.65	56	L7R4Z9	31,294	76.54	89	A0A0B5JD20	39,242	100.00	95				
					Q8W3X0	38,818	99.00	43	X2KWE1	35,567	100.00	89					Q6EEX0	31,451	69.87	98								

					V9P757	35,338	100.00	67	A0A023WGB8	32,324	100.00	98	A0A060N479	32,0	96.7	23				
					R4JB46	31,6	89.76	56					R9XSZ2	32,1	100	51				
					Q8GU18	31,1	100	31												
12	Q94IJ6	77,3	67.8	112	B2Y2S2	38,9	69.8	120	B8XU37	32,7	95.5	42					D2KFH2	11,3	63.1	67
	T2HRF3	89,3	96.7	119													P0CZ06	32,4	59.4	53
	D7RT26	91,6	73.9	67																
13	A9QUS3	72,3	92.1	106					R9XV20	34,4	97.3	65	B6UKL3	33,1	98.3	13	D2KFH2	11,3	76.8	82
	T2HRF3	89,3	67.9	15					R9XW75	34,4	93.2	83					P0CZ06	32,4	99.1	75
14	A3RF25	41,9	54.3	47	Q5MFP8	29,9	58.9	34	K7WV42	32,8	100	78	F2X0K8	33,0	100	45				
	Q670Q5	70,3	67.9	21	B8Y0L5	38,2	68.9	56	R9XV20	34,4	97.8	43								
	A5HMG2	79,4	98.7	69																
	G3K725	93,6	100	115																