Tracking the Fate of Pasta (*T. Durum* Semolina) Immunogenic Proteins by *In Vitro* Simulated Digestion

Gianfranco Mamone[1*], Chiara Nitride[2], Gianluca Picariello[1], Francesco Addeo[1,2], Pasquale Ferranti[1,2], Alan Mackie[3]

[1]Istituto di Scienze dell'Alimentazione, CNR, Avellino, Italy

[2]Dipartimento di Agraria, Università Federico II, Portici (NA), Italy

[3]Institute of Food Research, Norwich Research Park Colney, Norwich, UK

*Corresponding author: Gianfranco Mamone, The Institute of Food Sciences (ISA) of the National Research Council (CNR), via Roma 64 – 83100 Avellino Italy. E-mail: mamone@isa.cnr.it Tel.: +39 0825 299521; Fax: +39 0825 781585.

Table SI: OGD-BBM resistant peptides identified by LC-MS/MS analysis

	Acc #	MH^+	m/z	z	Da	DB Peptide	Variable Mods	Start	End	Score	Protein MW	Species	Protein Name	Epitopes
1	474451266	619.3273	619.3273	1	0.019	FPGIGE		199	204	21.5	58747.8	TRITICUM URARTU	Beta-amylase	
2	71084277	704.3802	704.3802	1	0.019	YPISPQ		271	277	24.0	86054.3	TRITICUM DURUM	HMW glutenin x-type subunit Bx7 precursor	
3	4741697	744.3597	372.6835	2	-0.0039	PQQFPQ		46	51	21.1	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
4	4741697	800.3855	400.6964	2	-0.0041	PQQPPFS		163	169	29.4	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
5	4741697	809.4401	405.2237	2	-0.0057	QQPVLPQ		84	90	24.3	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
6	4741697	814.3583	407.6828	2	-0.0073	QQQPPFS	Gln->pyro-Glu@1	105	111	27.6	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
7	4741697	821.4067	411.2070	2	-0.0043	QQQQPPL	Gln->pyro-Glu@1	58	64	18.3	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
8	4741697	831.3955	416.2014	2	-0.0020	QQQPPFS		67	73	25.4	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
9	34329289	855.3851	428.1962	2	-0.0072	QQFPQPQ	Gln->pyro-Glu@1	47	53	21.9	31471.2	TRITICUM DURUM	gamma gliadin	
10	4741697	918.4527	459.7300	2	-0.0076	QQQPIPPQ	Gln->pyro-Glu@1	172	179	23.7	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
11	4741697	920.4619	460.7346	2	-0.011	QQQPVLPQ	Gln->pyro-Glu@1	83	90	30.9	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
12	4741697	935.4897	468.2485	2	-0.0024	QQQPIPPQ		172	179	19.1	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
13	474451266	941.5317	941.5317	1	0.038	IEVGLGPAGE		176	185	25.3	58747.8	TRITICUM URARTU	Beta-amylase	
14	34329289	965.4637	483.2355	2	0.0080	QTFPHQPQ	Gln->pyro-Glu@1	39	46	26.2	31471.2	TRITICUM DURUM	gamma gliadin	
15	4741697	976.5625	488.7849	2	0.026	QPVLPQQPS	Gln->pyro-Glu@1	85	93	31.9	41660.3	TRITICUM DURUM	low molecular weight glutenin	

													subunit	
16	56709482	1031.5739	516.2906	2	0.011	LPQFEEIR		249	256	37.3	33157.8	TRITICUM DURUM	alpha-gliadin precursor	
17	56709482	1034.6119	517.8096	2	0.041	QPFPQP	Gln->pyro-Glu@1	79	87	25.1	33157.8	TRITICUM DURUM	alpha-gliadin precursor	
18	56709482	1065.5599	533.2836	2	0.012	QLQPFPQPQ	Gln->pyro-Glu@1	77	85	26.4	33157.8	TRITICUM DURUM	alpha-gliadin precursor	α-I (or α-9)
19	56709482	1080.5327	540.7700	2	0.011	QQQPFPPQQ	Gln->pyro-Glu@1	53	61	27.2	33157.8	TRITICUM DURUM	alpha-gliadin precursor	
20	34329289	1080.5707	540.7890	2	0.030	QQPQQPFPQ	Gln->pyro-Glu@1	76	84	26.4	31471.2	TRITICUM DURUM	gamma gliadin	γ -II (or glia-γ30)
21	4741697	1086.6325	543.8199	2	0.027	SIILQEQQQ		267	276	25.4	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
22	474451266	1097.5625	549.2849	2	0.023	HPEWEFPR		225	232	29.2	58747.8	TRITICUM URARTU	Beta-amylase	
23	474451266	1097.7135	549.3604	2	0.059	EVGLGPAGELR		177	187	32.0	58747.8	TRITICUM URARTU	Beta-amylase	
24	4741697	1104.5507	552.7790	2	-0.0089	QQPVLPQQPS	Gln->pyro-Glu@1	84	93	31.4	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
25	56709482	1142.6413	571.8243	2	0.029	QLPQFEEIR	Gln->pyro-Glu@1	248	256	27.6	33157.8	TRITICUM DURUM	alpha-gliadin precursor	
26	4741697	1152.5849	576.7961	2	-0.0079	SQQQQPVLPQ		155	164	27.1	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
27	4741697	1169.6285	585.3179	2	0.017	LPQQPPFSQQ		162	171	25.1	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
26	29150726	1171.6583	586.3328	2	0.029	QYEQTVVPPK	Gln->pyro-Glu@1	87	96	32.1	77343.7	TRITICUM DURUM	HMW glutenin subunit 1By8	
29	71084277	1174.6363	587.8218	2	0.013	YEQQPVVPSK		65	74	28.4	85233/8.7	TRITICUM DURUM	HMW glutenin x-type subunit Bx7 precursor	
30	34329289	1198.6495	599.8284	2	0.032	TQQPQQPFPQ		85	94	26.9	31471.2	TRITICUM DURUM	gamma gliadin	γ -II (or glia-γ30)
31	34329289	1208.5851	604.7962	2	0.0079	QQFPQPQQPQ	Gln->pyro-Glu@1	47	56	30.1	31471.2	TRITICUM DURUM	gamma gliadin	
32	34329289	1225.6111	613.3092	2	0.0076	QQFPQPQQPQ		47	56	30.7	31471.2	TRITICUM DURUM	gamma gliadin	
33	34329289	1226.6861	613.8467	2	0.045	FPQPQQTFPH		34	43	32.6	31471.2	TRITICUM DURUM	gamma gliadin	
34	4741697	1234.6589	617.8331	2	0.0061	QLPPFSQQLPP	Gln->pyro-Glu@1	128	138	24.3	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
35	34329289	1247.6961	624.3517	2	0.029	QGQGIIQPQQPA	Gln->pyro-Glu@1	220	231	26.1	31471.2	TRITICUM DURUM	gamma gliadin	γ-VIIa
36	56709482	1275.6989	638.3531	2	0.013	QLQPFPQPQLP	Gln->pyro-Glu@1	77	87	24.7	33157.8	TRITICUM DURUM	alpha-gliadin precursor	α-I (or α-9)
37	34329289	1322.6723	661.8398	2	0.012	QQPFPQPQQPQ		99	109	25.5	31471.2	TRITICUM DURUM	gamma gliadin	
38	56709482	1335.6895	668.3484	2	0.0046	RPQQPYPQPQP		95	105	26.6	33157.8	TRITICUM DURUM	alpha-gliadin precursor	Glia-α-20

39	4741697	1348.7883	674.8978	2	0.049	QQPVLPQQPPFS	Gln->pyro-Glu@1	158	169	28.2	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
40	4741697	1360.7535	680.8804	2	0.034	QQQQPVLPQQPS	Gln->pyro-Glu@1	112	123	28.4	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
41	29150726	1368.7621	684.8847	2	0.038	SLQQPGQGQQIGQ		187	199	26.5	77343.7	TRITICUM DURUM	HMW glutenin subunit 1By8	
42	29150726	1395.7169	698.3621	2	0.038	QQPGQGQQGHYPA		408	420	24.5	77343.7	TRITICUM DURUM	HMW glutenin subunit 1By8	
43	34329289	1435.7707	718.3890	2	0.037	PQQPYPQQPQQP		70	81	24.1	31471.2	TRITICUM DURUM	gamma gliadin	
44	4741697	1464.8257	732.9165	2	0.041	SQQQQPVLPQQPS		111	123	31.1	41660.3	TRITICUM DURUM	low molecular weight glutenin subunit	
45	34329289	1530.8099	765.9086	2	0.038	QPQQPFPQPQQPQ	Gln->pyro-Glu@1	97	109	25.3	31471.2	TRITICUM DURUM	gamma gliadin	
46	34329289	1579.7683	790.3878	2	0.0016	FPQPQQTFPHQPQ		34	46	32.9	31471.2	TRITICUM DURUM	gamma gliadin	
47	34329289	1675.8815	838.4444	2	0.031	QPQQPFPQPQQPQQ		97	110	28.1	31471.2	TRITICUM DURUM	gamma gliadin	
48	34329289	1803.8849	902.4461	2	0.0019	QIFPQPQQTFPHQPQ	Gln->pyro-Glu@1	32	46	25.4	31471.2	TRITICUM DURUM	gamma gliadin	
49	34329289	1812.9617	604.9921	3	0.028	PHQPQQQFPQPQQPQ		42	56	24.2	31471.2	TRITICUM DURUM	gamma gliadin	
50	34329289	1820.9019	910.9546	2	-0.003	QIFPQPQQTFPHQPQ		32	46	30.0	31471.2	TRITICUM DURUM	gamma gliadin	
51	29150726	2004.837	668.9507	3	-0.025	QQPGQGQQPEQGQQPGQGQ		542	560	22.1		TRITICUM DURUM	HMW glutenin subunit 1By8	
52	34329289	2149.0595	717.0247	3	0.0045	QPQQPFPQTQQPQQPFPQ		77	94	26.4	31471.2	TRITICUM DURUM	gamma gliadin	γ -II (or glia-γ30)
53	34329289	2189.2037	730.4061	3	0.050	QTFPHQPQQQFPQPQQPQ		39	56	27.0	31471.2	TRITICUM DURUM	gamma gliadin	
54	34329289	2317.2611	773.0919	3	0.050	QTFPHQPQQQFPQPQQPQQ		39	57	26.0	31471.2	TRITICUM DURUM	gamma gliadin	
55	34329289	2320.2559	1160.6316	2	0.042	PFPQPQQPQQSFPQQQPSLI		101	120	25.7	31471.2	TRITICUM DURUM	gamma gliadin	γ-I
56	34329289	2374.3125	1187.6599	2	0.052	PQQPQQPFPQTQQPQQPFPQ		75	94	25.4	31471.2	TRITICUM DURUM	gamma gliadin	γ -II (or glia-γ30)
57	56709482	2406.1859	802.7335	3	-6.3e-4	LGQQQPFPPQQPYPQPQPFPS		51	71	24.1	33157.8	TRITICUM DURUM	alpha-gliadin precursor	31-43; 31-49
58	34329289	2559.3815	853.7987	3	0.040	QQPFPQPQQPQQSFPQQQPSLI	Gln->pyro-Glu@1	99	120	28.4	31471.2	TRITICUM DURUM	gamma gliadin	γ-I
59	34329289	2575.4141	859.1429	3	0.061	QPQQPFPQPQQPQQSFPQQQPS		97	118	28.5	31471.2	TRITICUM DURUM	gamma gliadin	γ-I
60	34329289	2576.4092	859.4746	3	0.040	QPFPQPQQPQQSFPQQQPSLIQ		100	121	28.6	31471.2	TRITICUM DURUM	gamma gliadin	γ-I
61	56709482	2922.4154	974.8100	3	0.0019	LGQQQPFPPQQPYPQPQPFPSQQPY		51	71	24.1	33157.8	TRITICUM DURUM	alpha-gliadin precursor	31-43; 31-49; 43-55; 31-55

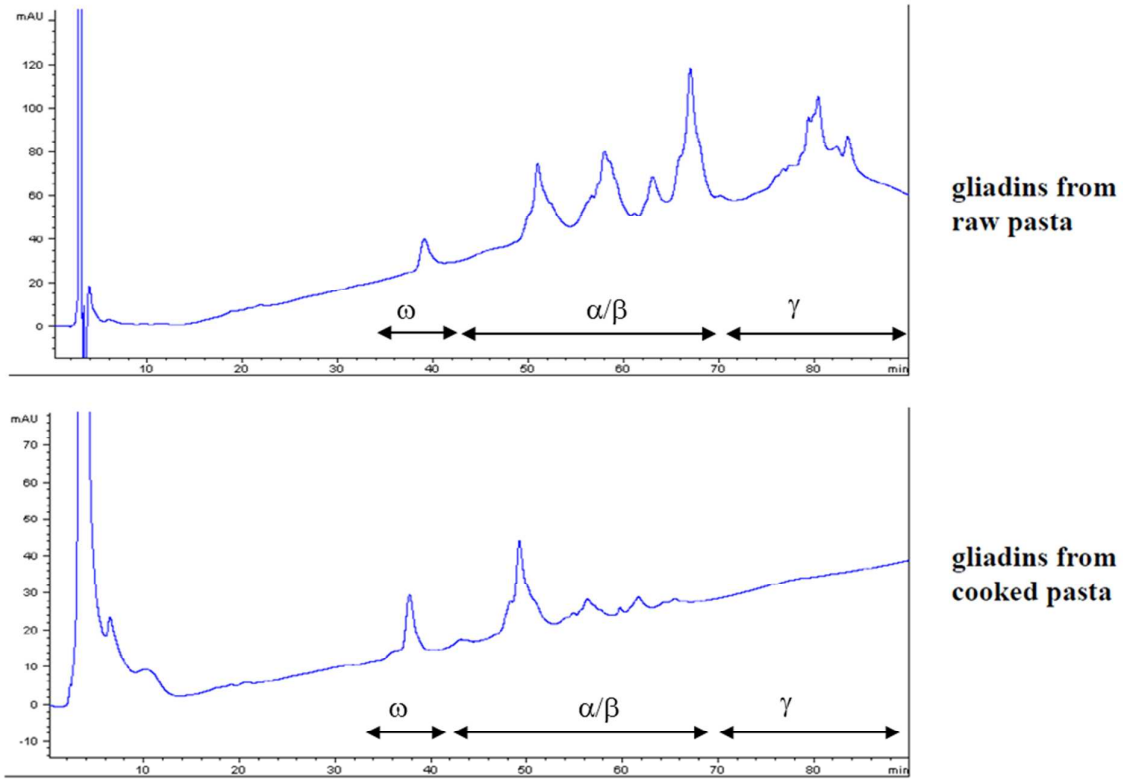

Figure S1: Gliadin proteins were extracted as previously described.[1] 20 µL aliquots of each extract were analysed by HPLC using a C8 reversed-phase column with a flow-rate of 0.2 mL min on a Agilent 1100 system with an integrated diode array detector. The column temperature was 55°C and the eluent monitored at 210 nm. Solvent A was 0.1% trifluoroacetic acid (TFA) in water and solvent B was 0.1% TFA in acetonitrile. Separation of gliadins performed by using a gradient 25±55% B in 100 min. Gliadin type proteins were distinguished into three groups based on their retention time (Rt): the ω- , α/β-, and γ-gliadin.[2]

Reference:

(1) Mamone, G.; Ferranti, P.; Chianese, L.; Scafuri, L.; Addeo, F. Qualitative and quantitative analysis of wheat gluten proteins by liquid chromatography and electrospray mass spectrometry. *Rapid Commun. Mass Spectrom*. **2000**, *14*, 897-904.

(2) Marchylo, B.A.; Hatcher, D.W.; Kruger, J.E.; Kirkland, J.J. Reversed-Phase High-Performance Liquid Chromatographic Analysis of Wheat Proteins Using a New, Highly Stable Column. *Cereal Chem* .**1992**, *69*, 371–378.