The Interaction between Tannins and Gliadin Derived Peptides in a Celiac Disease Perspective

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SUPPLEMENTARY INFORMATION

Experimental

Procyanidin B3, Procyanidin Trimer T1 and Procyanidin Tetramer TT1 Synthesis

Procyanidin B3, procyanidin trimer T1 and procyanidin tetramer TT1 were obtained by hemisynthesis using (+)-taxifolin and (+)-catechin. Briefly, taxifolin and catechin (ratio 2:1) were dissolved in ethanol under argon atmosphere. The mixture was then treated by dropwise addition of sodium borohydride, and left for 15 min under magnetic agitation. The pH was lowered to 4.5 by slowly adding acetic acid/water 50% (v/v) and the mixture was allowed to stand under argon atmosphere for 30 min. After this, the reaction mixture was extracted with ethyl acetate, evaporated and passed through C18 gel, thoroughly washed with water and recovered with methanol. The obtained fraction, after evaporation of methanol, was passed through a TSK Toyopearl HW-40(S) gel column (300 mm x 10 mm i.d., with 0.8 mL.min-1 of methanol as eluent) coupled to a UV-Vis detector (Gilson 115) were several fractions were recovered and analyzed by ESI-MS (Finnigan DECA XP PLUS) yielding procyanidins with varying degrees of polymerization.

MALDI-TOF Mass Spectrometry Analysis of Gliadin Raw Extract

MALDI-TOF-MS was used to obtain the mass spectra of the commercial gliadin raw extract. For this, two microliters of raw sample (0.625 mg/mL), prepared in 62.5% ethanol/ 3.75% acetonitrile/ 0.0125% trifluoroacetic acid (v/v) aqueous solution, was mixed with 2 µL of matrix solution containing 10 mg/mL sinapinic acid in 30% acetonitrile/ 0.1% trifluoroacetic acid (v/v). Afterward, 2 µL of this matrix-sample mixture was applied onto a stainless steel target plate (MTP 394 target plate ground steel BC, Bruker Daltonics) and finally air-dried. The respective mass spectrum was recorded on an Ultraflextreme Mass Spectrometer (Bruker Daltonics) operating in linear positive ion detection mode with laser SmartBeam-III and under FlexCompass 1.4 software control (Bruker Daltonics). The mass spectrum was acquired in a range from 10 to 100 kDa, by accumulation of 500 laser shots with 1000 Hz of frequency.

In vitro Digestion of Gliadin Raw Extract

Briefly, to simulate a gastric digestion, 100 mg of gliadins were weighed and dissolved in 0.01 M hydrochloridric acid followed by incubation in a 37 °C water bath with pepsin (1:100 protease to protein w/w ratio) at pH 2.0 for 30 min. The reaction mixture was then pH adjusted to 7.0 in 50 mM phosphate buffer and added with pancreatin (1:100 w/w) and chymotrypsin (1:100 w/w) at 37 °C for 24 h. After this, the resulting peptide mixture was divided in 2 mL aliquots and centrifuged at 10.5 rpm for 5 min on an Eppendorf Minispin® centrifuge.

Fluorescence Lifetimes Determination

The fluorescence lifetimes of Pep Mix4 and Pep Mix6 were measured on a Fluoromax-4 spectrophotometer, attached to a single photon counting controller (FluoroHub), both from Horiba Jobin-Yvon, at room temperature. The fluorescence excitation was performed with a Horiba Nano LED source of 290 nm, and fluorescence emission was recorded at the maximum wavelength for each peptide mixture (360 nm for both solutions). The lamp profile was recorded by placing a scatter (dilute solution of LUDOX in water) in place of the sample.

Results and Discussion

Analysis of wheat gliadins crude extract by MALDI-TOF-MS

Despite the great heterogeneity and high genetic variability that defines the different classes of wheat gliadin proteins, the obtained mass spectrum clearly shows their protonated mass pattern around 29-42 kDa, as described in the literature (red box).[47,48] In addition, it was also detected the presence of several unknown compounds with molecular weights below 20 kDa in the raw sample. Unable to match a typical profile of any gliadin subtype, those peaks would correspond to other wheat proteins that were co-extracted with gliadins by aqueous alcohol (albumins and globulins, for example), consequently disturbing gliadin analysis.[49]

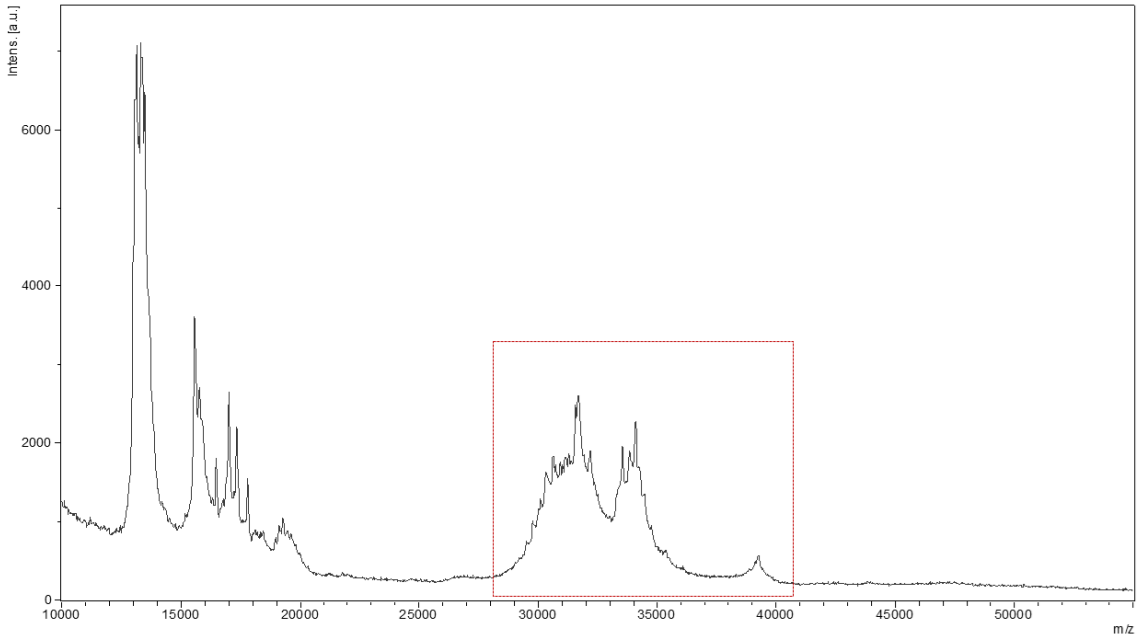

Fig. S1 MALDI-TOF mass spectrum of the wheat gliadins raw extract.

Sequenciation of gliadin derived peptides by ESI-MS/MS

The sequenciation and further identification of gliadin derived peptides after their in vitro digestion were developed by LC-MS/MS analysis. Figure S2 displays the UV and TIC chromatograms for each fraction obtained. As is shown, several differences were found between fractions since they distinguished mainly for their polarity. Given the low peptide concentration needed to verify their identity by mass spectrometry, the signal obtained in UV was very poor. However, the sequenciation, after MS/MS analysis, of the peptides contained in each fraction could be reported since the concentration used was enough to be detected by the mass spectrometer. Thus, Table S1 shows the identification of gliadin derived peptides found in each fraction. The identification of the precursor proteins was achieved using the UniProt Database. For each collected mixture, different peptide colors and asterisks are indicative of a specific protein by which they derived. The peptides containing known epitopes that are recognized by intestinal T cells of celiac disease patients are underlined and highlighted in bold.

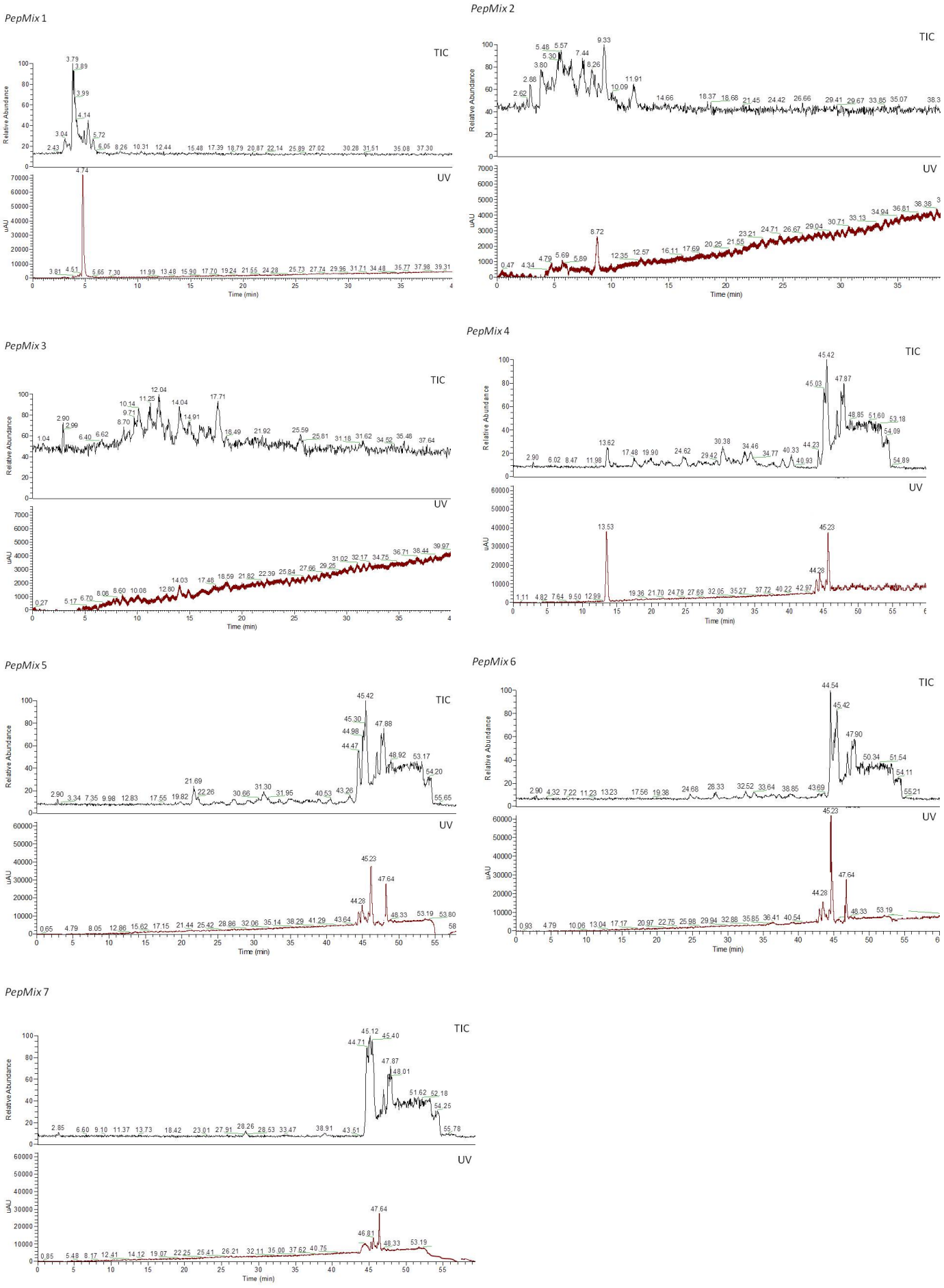

Fig. S2 UV and Total Ion Current Chromatograms of the different peptidic gliadin derived fractions

Table S1 Identification, by LC-ESI-MS/MS, of peptides generated after enzymatic hydrolysis of the wheat gliadins raw extract.

Pep Mix 1	**Peptides**	GNLGG, AGQGPQ, GQASPQ, TLSASQ, RPGCST, MCSVSV, QQQGLG, RPSQQNPQ, QSRQGQQPGQ
	Proteins derived	Alpha/beta-gliadin [M8A3U4_TRIUA]
Pep Mix2	**Peptides**	GQGLPG, TIPSAG, SPVAGQ, ADIGGQ*, GQVPLA, SYDVAG, GSVQPQ, MATTTT, AANMQV**, VLTTLP, GQVLCH, EGRDAL, PQGECQ, QDQQPG, HVSVEH, EHPVPH, PQLQNPS, SQQQQPV*, AQGSVQPQ, GQWHQPGQ, QQEQQGIQ, LSGQGQRPGQ, SHHQQQQPV**, THHQQQQPI*, LQPKNPSQQQPQ
	Proteins derived	Alpha/beta-gliadin clone PW8142 [GDA7_WHEAT] *Gamma-gliadin [Q94G97_WHEAT] **Gamma-gliadin [R9XUA5_WHEAT] High molecular weight glutenin subunit 1Dx [S4U495_9POAL] *Low-molecular-weight glutenin subunit (Fragment) [R4JBG2_WHEAT] **Low molecular weight glutenin subunit D3-3 [D3U328_WHEAT]
Pep Mix3	**Peptides**	VGIGGQ**, PPDTGG, DVGTGV**, TYPGGA, AAQLPA*, ALGRAP*, VQGQGI**, VAGIGGQ*, GVGTGVGG*, SIGTGVGG, YPTSPQ*, IPSVATY, GGSFYPGA*, NIQVDPS*, RPQQPY*, QPRQPY*, QDQQSGQ*, NMQVDPS*, SQPQQPI*, SQPQHPI**, YYPTSPQ*, TSIALHNL, QTFPHQPQ*, QPRQPYPQ*, LQELCCQQL*, RGEQHSSCQT, MNIQVDPSSQV**, GSFQPSQQNPQ, LQPQNPSLQQPQ, AEGEASGQLQCERG**, QPRQPFLQPQQP*, SQQPGQGQQGYYSGS**
	Proteins derived	*Alpha-gliadin [D2X6D2_TRITD] **Alpha-gliadin protein [X2KVH9_WHEAT] Alpha/beta-gliadin [I0IT51_WHEAT] *Gamma-gliadin [R9XV87_WHEAT] **Gamma-gliadin [Q8L6B3_WHEAT] *HMW-glutenin subunit Glu-1Stx1 [L0G7U5_WHEAT] **High molecular weight glutenin x-type [B8XU61_TRIMO] *Low-molecular-weight glutenin subunit group 3 type II (Fragment) [Q8W3W5_WHEAT] **Low-molecular-weight glutenin subunit (Fragment) [F5A655_WHEAT]
Pep Mix4	**Peptides**	NGAHAI, AIHGVV**, HQAAGL, MATTIA*, GIIQPQ**, VSFQPS, QQQPPS, YPISPQ, VRVPVPQ, QPSHQQP, SHQQQPF, RQPQQPF*, QRPQQPF**, GIIQPQQPA*, LLQQCRPV, YSQPQQPI, HLQPQQPI, QSRCNVMQ, QQPSYSQQ, GQPQQQVPV, LQQQQFPQ, SQQQQPPFS, QQNPQAQGSV**, SHIPGLEKPS, VQGQGIIQPQ, SHIPGLERPS, QQAGQWQRP, SQQQQPVIPQ, DVSPECQPVVV, SQQQPPFSQQ, QQPPQQFPQP**, **TQQPQQPFPQ**, QQGYDNPYHV, EQQPGQGQEGY, ECCSRLGQMPP, QPERGCSGESTA, SFQSSQQNPQA, QSGQGQQGHHSA, SFPQSKQPQQP, SQQQQQPVLPQ, QQPEQGQPGYY, DSPYYVSAEHQ, QQQPGQGQQGHC, QPEQGQEGYYP, VQGQGIIQPQQPA**, PQQYSPYQPQQ, QPIPQPQQPQQP**, SKQPQQPFPQPQ**, **TQQPQQPFPQQP**, SQQQQPVLPQQPS, CSPTPYVQSQMWQ, QQGQQPGQGQQGQQP, SRQQPLPPQQTLSH, **SQQPQQPFPQPQQPQ**, SHIPGLERPSQQQPLPPQQT
	Proteins derived	Alpha-gliadin [Q5NDA5_TRITD] *Gamma-gliadin [B6UKP8_WHEAT] **Gamma-gliadin [B6UKQ2_WHEAT] High molecular weight glutenin x-type [B8XU61_TRIMO] LMW-GS [R9XVA5_WHEAT] Gamma-hordein-3 [M7ZUH8_TRIUA]
Pep Mix5	**Peptides**	GQGLPG, ISPAPT, GEGAQQ, NAAHAI, GVNVPL, LGVPIL, KAGSFC, PQCSPV, SSCQSM, SGQGNQG, SQQPPF, QPSILR, MNTFLV, TTTSVPF, QPQCSPV, NPQAQGSV, VQQQLPV, PQLQQPL**, PEQEQQP, SQQPPFPQ, QEQQGQQP, GIIQPQQPA*, QQSQQPFP**, FSQQQQTV, HSKQGQQPG, FQQPHQPF**, YYSTPPQQP, QLLYPQPQP, PQLPCPQPQP, DQCCLQLRPV, **TQQPQQPFPQ**, EQQHGQGLSQP, EQQPGQGQEGY, EQPGQWQQGY, QQGYDNPYHV, SFQSSQQNPQA, QPERGCSGESTA, QQQQPSYSQQ, QQQIPVVQPSV, SQQAFPQPQQT, PGQGQQGHCPASQ, AATPNSSTEVMTS, AQMETNCISGLE, QQQPGQGQQGHC, QPEQGQEGYYP, GGSFYPGETTPPQ, VQGQGIIQPQQPA**, VQGQAIIQPQQPA**, **SQQPQQPFPQQP**, **TQQPQQPFPQQP**, **LQQPQQPFPQPQ**, LQPGQGQQGYYPT, QSISQYQQQQPQ, SQQQQPVLPQQPP, YQQPQQTFPQPQ, QPGYYPTSPWQPE, ILQQQQQQQQQQV, QCAAINSVVHSIMMQQE, LQPQNPSQQQPQEQVPL, **TQQPQQPFPQQPQQPFPQ**, SHIPGLERPSQQQPLPPQQT, SQHQQQQQQQQQQQQQQQQI
	Proteins derived	Alpha-gliadin [K7WV57_WHEAT] *Gamma-gliadin [R9XSZ2_WHEAT] **Gamma-gliadin [Q9M6P7_WHEAT] Low-molecular-weight glutenin subunit [Q1ZZT4_WHEAT] Putative puroindoline-like protein [B1GXL7_TRITD]
PepMix 6	**Peptides**	GIGVGV, GGARGL, LSGAIP, DPLGAL, GEGAQQ, APSGIF, GGGGGSQQ, GQVQEP, QQGTFL, SIILPR, SQVCFQ, ALETLPA, HVNTEQ, VQQGTFL, QPSQQNP, KKITLVL, CQQQPQP, GQVQWPQ, QIPEQSR, QQSSYQVL, QLEMMTSI, QQPHQAFPQ, LQQPGREQQ, QQPLQQYPL, QEPGQGQQWY, QEPGQWQQGY, SHIPSLEKPL, EQQPGQGQEGY, SFQSSQQNPQA, VPQPQQPQQPF, PFCQHQQPFY, MQQQCCQQLW, GQPGCDPTSPQQPG, MWQRSSCNVMQ, SSSPQQLGQGQPRY, HPSILQQLDPCKVF, LQPQNPSQQQPQEQVPL, SQQQQSPFSQQQQQQPPF, **PQQPFPQQPQQPYPQQPL**, **TQQPQQPFPQQPQQPFPQ**, **MQLQPFPQPQLPYPQPQL**
	Proteins derived	Alpha-gliadin [R9XV48_WHEAT] High-molecular-weight glutenin [Q41516_WHEAT]
Pep Mix7	**Peptides**	AVGVPV, VSSKAG, GGGGGSQQ, SQHEQV, QGSVQPK, PSRQQPQ, VPGLEKPW, KANLRLATMK, LQLQPFPQPQ, QQGYDSPCHVS, RNNNSPGHNNP, QQGYDNPYHV, EQQPGQGQEGY, YSSTTSMPFSI, QEPGQWQQGY, QQGYDSPCHVS, QQGYDNPYHV, QSRCNVMQQQ, QQQQPSYSQQ, QPERGCSGESTA, QQPEQGQPGYY, QPGQEQQGYYP, QPEQGQQGYYP, QQQPGQGQQGHC, QPEQGQEGYYP, MWQRSSCNVMQ, EHYTPCMTFLQ, GLRVGGNQIAGHIP, LQLQPFPQPQLP, SQQQQPVLPQQPPFS, **LQPQQPFPQQPQQPFPQ**, LQLQPFPQPQPFPPQLPYPQPQ, **TQQPQQPFPQQPQQPFPQQPQQPFPQ**, **QQPFPQQPQQPQQPFSQPQQQLPLQPQQPFP**
	Proteins derived	Alpha-gliadin [Q27HK6_TRIMO] Alpha/beta-gliadin MM1 [M7ZZV2_TRIUA] *Gamma-gliadin [R9XUS6_WHEAT] **Gamma-gliadin (Fragment) [Q9M6P7_WHEAT]