

A Prolyl Endopeptidase from *Flammulina velutipes* Degrades Celiac Disease-Inducing Peptides in Grain Flour Samples

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Supplementary Information

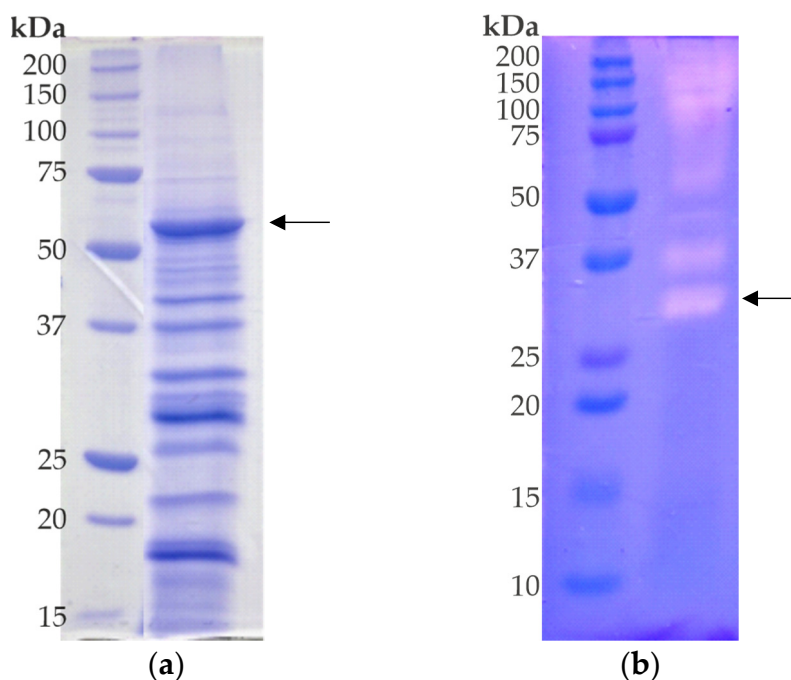


Figure S1. SDS-PAGE-Gel (a) and native PAGE-casein-zymography (b) of the partially purified FvpP. (a) The strong band at about 56 kDa (indicated by arrow) corresponds to FvpP as ascertained through identification by LC-MS/MS. For clarity, a non-related sample between the marker and the FvpP-lane was removed from the figure. (b) White bands indicate peptidase activity. The band indicated by the arrow corresponds to the (native) active FvpP as published earlier [1]. The change in size results from the native gel, as proteins run differently depending on their three-dimensional structure.

Schulz et al.	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
33-mer		0
α-gliadin2	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin5	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin1	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin6	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin3	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin4	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-Gliadin16	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-Gliadin17	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin13	MKTFLILAL--VATTAATAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	57
α-gliadin12	MKTFLILALRAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin10	MKTFLILALRAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
α-gliadin11	MKTFLILALLAIVATTATIAVRVVPQLQPNPSQQQEQEVPLVQQQFGQQQPF	60
Schulz et al.	QYFPQPFPSQQLQLQPFQLPQL-----PYQPFPRPQYFPQPF	106
33-mer		33
α-gliadin2	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	111
α-gliadin5	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	120
α-gliadin1	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	106
α-gliadin6	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	106
α-gliadin3	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	113
α-gliadin4	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	113
α-Gliadin16	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	111
α-Gliadin17	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	113
α-gliadin13	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	108
α-gliadin12	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	106
α-gliadin10	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	113
α-gliadin11	QYFPQPFPSQQLQLQPFQLPQLPYPQQLPYPQQLPYPQQLPYPQPF	120
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Schulz et al.	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	157
33-mer		33
α-gliadin2	YLQPFQISQQQAQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	165
α-gliadin5	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	178
α-gliadin1	YLPQPFISQQQAQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	154
α-gliadin6	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	157
α-gliadin3	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	162
α-gliadin4	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	163
α-Gliadin16	YLQPFQISQQQAQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	165
α-Gliadin17	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	162
α-gliadin13	YLQPFQISQQQAQQQQQQQQQQQQQQQQQQQQQQILQQILQQQLIPCRDVLQHQSHAGS	168
α-gliadin12	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCDVLQHQSHVHGR	157
α-gliadin10	YSQPQPISQQQQQQQ-----QQQQQQILQQILQQQLIPCRDVLQHQSHAGS	164
α-gliadin11	YSQPQPISQQQQQQQ-----QQKQQQQQQQILQQILQQQLIPCRDVLQHQSHAGS	177
Schulz et al.	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQ-----	204
33-mer		33
α-gliadin2	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQEQEQQLQQQQQ	225
α-gliadin5	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQEQEQQLQQQQQ	238
α-gliadin1	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQHHHHQQ-----	205
α-gliadin6	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQHHHHQQ-----	208
α-gliadin3	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQ-----QQ-----	210
α-gliadin4	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHHHQ-----QQ-----	210
α-Gliadin16	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQEQEQQLQQQQQ	225
α-Gliadin17	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQQ-----	210
α-gliadin13	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQEQEQQLQQQQQQQLQ	228
α-gliadin12	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQQ-----	208
α-gliadin10	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQQ-----	215
α-gliadin11	SQVLQSTYQLVQQLCCQQLWQIPEQSRCQAIHNVVHAILHQQQQQQ-----	226
Schulz et al.	----QQQQPLSQVSFQQQQYSGQGSFQPSQNNQAQGSVQPQQLQFEEIRNLAL	259
33-mer		33
α-gliadin2	---QQLHQRRQPSSQVSFQQPQQYPSQVSFQPSQLNPAQGSVQPQQLQFAEIRNLAL	284
α-gliadin5	--Q--LQRRQPSSQVSFQQPQQYPSQVSFQPSQLNPAQGSVQPQQLQFAEIRNLAL	296
α-gliadin1	--Q--QQQQQLSQVSFQQPQQYPSGQGFQPSQQNPAQGSFQPQQLQFAEIRNLAL	262
α-gliadin6	--Q--QQQQQLSQVSFQQPQQYPSGQGFQPSQQSQAQGSFQPQQLQFAEIRNLAL	265
α-gliadin3	--Q--QQQQQLSQVSFQQPQQYPSGQGSFQPSQQNPAQGSVQPQQLQFAEIRNLAL	267
α-gliadin4	-----QQQPSSQVSFQQPLQYELGQGSFRBSQQNPAQGSVQPQQLQFAEIRNLAL	264
α-Gliadin16	---QQLHQRRQPSSQVSFQQPQQYPSQVSFQPSQLNPAQGSVQPQQLQFAEIRNLAL	284
α-Gliadin17	-----QQQQQLSQVSFQQPQQYPSGQGSFQPSQQNPAQGSVQPQQLQFAEIRNLAL	267
α-gliadin13	QQQQQQQQQLSQVSFQQPQRQYPSQVSFQPSQLNPAQGSVQPQQLQFAEIRNLAL	288
α-gliadin12	QQQQQQQQQLSQVCFQSQSQYPLGQGSFRBSQQNPAQGSVQPQQLQFAEIRNLAL	268
α-gliadin10	--QQQQQQQLSQVCFQSQSQYPSGQGSFQPSQQNPAQGSVQPQQLQFAEIRNLAL	273
α-gliadin11	----QQQQQLSRVSFQQPQQYPSQGSFQPSQQNPAQGSVQPQQLQFAEIRNLAL	282
Schulz et al.	ETLAMCNVYIPPYCSTTIAFGIFGTN--	287
33-mer		33
α-gliadin2	QTLPAMCNVYIPPHCSTTIAPFGIFGTN--	312
α-gliadin5	QTLPAMCNVYIPPHCSTTIAPFGIFGTN--	324
α-gliadin1	QTLPAMCNVYIPPYCT--IAPFGIFGTN--	288
α-gliadin6	QTLPAMCNVYIPPYCT--IAPFGIFGTN--	291
α-gliadin3	ETLPAMCNVYIPPYCT--IAPVGFGTNYR	295
α-gliadin4	QTLPAMCNVYIPPYCT--IAPFGIFGTN--	290
α-Gliadin16	QTLPAMCNVYIPPHCSTTIAPFGIFGTN--	312
α-Gliadin17	ETLPAMCNVYIPPYCT--IAPVGFGTNYR	295
α-gliadin13	QTLPAMCNVYIPPHCSTTIAPFGIFGTNYR	318
α-gliadin12	QTLPAMCNVYIPPYCT--MAPFGIFGTN--	294

α -gliadin10	ETLPAMCNVYIPPYCT--IAPVGIFGTN--	299
α -gliadin11	ETLPAMCNVYIPPYCT--IAPVGIFGTN--	308

Figure S2. Multiple sequence alignment of all α -gliadins cleaved by the FvpP with identified prolyl-specific cleavage sites marked in yellow. For comparison, the sequence of the CD-active 33-mer peptide and of the α -gliadin identified and cleaved (sites marked in green) in Schulz *et al.* [1] was also aligned. Black arrows indicate cleavage sites described by Shetty *et al.* [2], red arrows sites from Stepniak *et al.* [3]. Alignment performed using Clustal Omega [4].

secalin7	MKTFLIFVLAMTMSIITARQINPSEQELQSPQQPVPKGQSYPPQSYPSHQPFPTPQQYS	60
secalin4	MKTFLIFVLAMTMSIITARQLNPSEQELQSPQQPVPKQSYPPQSYPSHQPFPTPQQYS	60
secalin3	MKTFLIFVLAMTMSIITARQLNPSEQELQSPQQPVPKQSYPPQSYPSHQPFPTPQQYS	60
secalin1	MKTFLIFVLAMTMSIITARQLNPSEQELQSPQQPVPKQSYPPQSYPSHQPFPTPQQYS	60
CD-active	-----	0
secalin7	PYQFQQPFQQQQPTPIQPQQFQQQQPQQFPQQQLPLQPQQFPQQPIRQQPQQS	120
secalin4	PYQFQQPFQQQQPTPIQPQQFQQQQPQQFPQQQLPLQPQQFPQQPIRQQPQQS	120
secalin3	PYQFQQPFQQQQPTPIQPQQFQQQQPQQFPQQQLPLQPQQFPQQPIRQQPQQS	120
secalin1	PYQFQQPFQQQQPTPIQPQQFQQQQPQQFPQQQLPLQPQQFPQQPIRQQPQQS	120
CD-active	-----QPFPPQQPIPQ-----	12
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secalin7	FPQQPQRPEQQFPQQPQQIIPQQTQQFPLQPQQFPQQPQRPFQAQQPEQIISQQPFPLQ	180
secalin4	FPQQPQRPEQQFPQQPQQIIPQQTQQFPLQPQQFPQQPQRPFQAQQPEQLISQQPFPLQ	180
secalin3	FPQQPQRPEQQFPQQPQQIIPQQTQQFPLQPQQFPQQPQRPFQAQQPEQIISQQPFPLQ	180
secalin1	FPQQPQRPEQQFPQQPQQIIPQQTQQFPLQPQQFPQQPQRPFQAQQPEQIISQQPFPLQ	180
CD-active	-----	12
secalin7	QQQFQSQPQQPFQQPGQMIPQQPQQFSPQLPQQPFSQRPQRPPQQFPQQPQQIIPQQPQ	240
secalin4	PQQPFSQPQQPFQQPGQIIPKQPQQPSPLQPQQPFSQQPQRFPQQFPQQPQQIIPQQPQ	240
secalin3	PQQPFSQPQQPFQQPEQIIPQQPQQPSPLQPQQPFSQQPQRPPQQFPQQPQQIIPQQPQ	240
secalin1	PQQPFSQPQQPFQQPEQIIPQQPQQPSPLQPQQPFSQQPQRPPQQFPQQPQQIIPQQPQ	240
CD-active	-----	12
secalin7	QPFPLQPQQVPQQPQRPFQGQQPEQIISQRPPQQPFLQPQQPFSQPQQPFPQQPGQIIP	300
secalin4	QPFPLQPQQVPQQPQRPFQGQQPEQIISQRPPQQPFLQPQQPFSRSQPPFFQQGGQIIPQ	300
secalin3	QPFPLQPQQVPQQPQRPFQGQQPEQIISQRPPQQPFLQPQQPFSQPQQPFLQQPGQIIPQ	300
secalin1	QPFPLQPQQVPQQPQRPFQGQQPEQIISQRPPQQPFLQPQQPFSQPQQPFLQQPGQIIPQ	300
CD-active	-----	12
secalin7	QPQQPFPLQPQQPFQQPEQIISQQPQQPFPLQQQPSPQQQLPFLPQQPFVVVV	357
secalin4	QPQQPFPLHPQQPFQQPEQIISQQPQQPFPLQQQPSPQQPQLPFPQQPQPFVVVV	357
secalin3	QPQQPFPLQPQQPFQQPEQIISEQPQQPFLQPQQPSPQQPQLPFPQQPQPFVVVV	357
secalin1	QPQQPFPLQPQQPFQQPEQIISEQPQQPFLQPQQPSPQQPQLPFPQQPQPFVVVV	357
CD-active	-----	12

Figure S4. Multiple sequence alignment of all ω -secalins cleaved by the An-Pep (secalins 1 and 3) or FvpP (secalins 4 and 7) with identified prolyl-specific cleavage sites marked in yellow (An-Pep) or green (FvpP). For comparison, the sequence of the CD-active peptide (QPFPPQQPIPQ) [5] was also aligned. Alignment performed using Clustal Omega [4].

secalin2	MKTLLMLAILAMVTTIATANMQVNPSPGQVQCPQQQ B FPQPRQSS P QQ P QQ P FPQSSQQ P F	60
secalin5	MKTLLMLAILAMVTTIATANMQVNPSPGQVQCPQQQ B FPQPRQSS P QQ P QQ P F B QQSSQQ P F	60
secalin9	MKTLLMLAILAMATTIATANMQVNPSPGQVQCPQQQ B FPQPRQSS P QQ P QQ P FPQSSQQ P F	60
CD-active	-----	0
secalin2	PQQ B QQSSPQQPYPPQQPFPQQPQQPYPPQQPQQPFPQQPQQPYPPQQPQQPFPQQPQQPV	120
secalin5	PQQ B QQSS B QQQQ B YPQQ B FPQQPQQ B YPQQPQQPFPQQPQQPYPPQQPQQPFPQQ B QQPV	120
secalin9	PQQ B QQSSPQ B LQPYPPQQPFPQQPQQPYPPQQPQQPFPQQPPQQ-----	102
CD-active	-----	0
secalin2	PQQPQQQFPQQPQQPVPQQPLQQFPQQ P QQSF B QQPQQPVPQQPLQQFPQQPQQPFPQQP	180
secalin5	PQQ B QQQFPQQPQQPVPQQPLQQFPQQPQQSF B QQPQQPVPQQPLQQFPQQPQQPFPQQP	180
secalin9	-----PFPQQP	108
CD-active	-----	0
secalin2	QQPVPQQSSQQPFPQTQQPQQPFPQPQQ P QQLF B QTQQSSPQQPQQVTSQPQQPFPQAQPP	240
secalin5	QQPVPQQSSQQPFPQTQQPQQPFPQPQQ P QQLF B QTQQSSPQQPQQVTSQPQQPFPQAQPP	240
secalin9	QQPV B QQSSQQPFPQTQQ B QQPFPQPQQPQQLFPQTQQSSPQQPQQVTSQPQQPFPQAQPP	168
CD-active	-----	0
secalin2	QQSSPQSQQ P YPQE B QQLFPQSQQPQQPFPQPQQPQQPFPQPQTQSSIPQPQQPFPQP	300
secalin5	QQSSPQSQQ P Y B QEPQQLFPQSQQPQQPFPQPQQPQQ B FPQPQ B QTQSSIPQPQQ B FPQP	300
secalin9	QQSS B QSQQ B Y B QEPQQLFPQSQQPQQPFPQPQQPQQPFPQPQTQSSIPQPQQ B FPQP	228
CD-active	-----QFPFPQ *****	6
secalin2	QQPFPQSQE B QF B QVHQPPQPS B QQQQ B SIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSK	360
secalin5	QQPFPQSQE B QF B QVHQPPQPS B QQQQ B SIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSK	360
secalin9	QQPF B QSQE B QF B QVHQPPQPS B QQQQ B SIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSK	288
CD-active	QQPIPQ----- ***:***	12
secalin2	IFPQSECQVMQQCCQQLAQIPQQLQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVG	420
secalin5	IFPQSECQVMQQCCQQLAQIPQQLQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVG	420
secalin9	IFPQSECQVMQQCCQQLAQIPQQLQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVG	348
CD-active	-----	12
secalin2	QGALAQVQGIIPQQLSQLEVVRSLVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH	479
secalin5	QGALAQVQGIIPQQLSQLEVVRSLVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH	479
secalin9	QGALAQVQGIIPQQLSQLEVVRSLVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH	407
CD-active	-----	12

Figure S5. Multiple sequence alignment of all 75k γ -secalins cleaved by the An-Pep (secalin 2) or FvpP (secalins 5 and 9) with identified prolyl-specific cleavage sites marked in yellow (An-Pep) or green (FvpP). For comparison, the sequence of the CD-active peptide (QPFPPQQPIPQ) [5] was also aligned. Alignment performed using Clustal Omega [4].

secalin8	MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPPQQPYPSHQPFPTPQQYS	60
secalin6	MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPPQQPYPSHQPFPTPQQYS	60
CD-active	-----	0
secalin8	PYQPPQPFPPQPPPTPIQPQQPFPQRPQQPFPQQQLPLQPQQSFPPQPHPIPPQPPQS	120
secalin6	PYQPPQPFPPQPPPAPIQPQQPFPQQPQQPFPQPPQQQLPLQPQQPFPQPPQPIPPQPPQS	120
CD-active	-----QPFPPQQPIPPQ----- * * * * * : * * * *	12
secalin8	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPPLQPQQPFPQQPQRPFQAQQPEQIISQQPFPLQ	180
secalin6	FPQQPQRPEQQFPQQPQQIIPQQTQQPFPPLQPQQPFPQQPQRPFQAQQPKQIISQQPFPLQ	180
CD-active	-----	12
secalin8	PQQPFSQPQQPFPQQPGQIIPQQPQQPSPLQPQQPFSQQPQRPPQPPFPQQPQQIIPQQPQ	240
secalin6	PQQPFSQPQQPFPQQPGQIIPQQPQQPSPLQPQQPFSQQPQRPPQPPFPQQPQQIIPQQPQ	240
CD-active	-----	12
secalin8	QPFPLQPQQPVPQQPQRPFQQQPEQIISQRPPQPFPLQPQQPFSQPQQPFPQQPGQIIPQ	300
secalin6	QPFPLQPQQPVPQQPQRPFQQQPEQIISQRPPQPFPLQPQQPFSQPQQPLPQQPGQIIPQ	300
CD-active	-----	12
secalin8	QPQQPFPPLQPQQPFPQQPEQIISQQPQQPFPLQPPQSPQQPQLPFPQPQQPFVVVV	357
secalin6	QPQQPFPPLQPQQPFPQQSEQIIPQQPQQPFPLQPQQPSPQQPQLPFPQPQQPFVVVV	357
CD-active	-----	12

Figure S6. Multiple sequence alignment of all Sec1 precursors cleaved by the FvpP with identified prolyl-specific cleavage sites marked in green. For comparison, the sequence of the CD-active peptide (QPFPPQQPIPPQ) [5] was also aligned. Alignment performed using Clustal Omega [4].

secalin11	MKTLMLAILAMATTIATANMQVNPSGQVQCPQQQPF	PQPQQSSP	QQPQQPFPQSSQQPF	60
secalin10	MKTLMLAILAMATTIATANMQVNPSGQVQCPQQQPF	PQPQQSSP	QQPQQPFPQSSQQPF	60
CD-active	-----			0
secalin11	PQPQQSSPQPQQPYPQQ	FFPQQPQQ	PYPQQPQQPFPQQPQQPYPQQPQQQF	PQPQQPV 120
secalin10	PQPQQSSPQPQQ	PYPQQP	FFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQQF	PQPQQPV 120
CD-active	-----			0
secalin11	PQPQLQQFPQQPQQPFPQQPLQQFPQQPQQPF	QQPQQPVPQQSQQFPQTQQ	PQPF	180
secalin10	PQPQLQQFPQQPQQPFPQQPLQQFPQQPQQPF	QQPQQPVPQQSQQFPQTQQ	PQPF	180
CD-active	-----			0
secalin11	PQPQLLF	PQTQQSSPQQPQQVTSQPQQPFPQAQFPQQSS	PQSQQPY	QEPQQLFPQSQQ 240
secalin10	PQPQLLF	PQTQQSSPQQPQQVTSQPQQ	FFQAQFP	QQSSPQSQQPYPQEPQQLFPQSQQ 240
CD-active	-----			0
secalin11	PQPFPQPQQPQQPFPQPQPQTQQSIPQPQC	FFPQPQQPFPQSQEQFPQVHQPQQPS	PQQ	300
secalin10	PQPFPQPQQPQQPFPQPQPQTQQSIPQPQC	FFPQPQQPFPQSQEQFPQVHQPQQPSPQQ		300
CD-active	-----		-QPFPQPQQPIPQ-	12
			*****; **	
secalin11	QQ	SIQLSLQQQLNPCKNVLLQQCSPVALVSSLSRSKIFPQSECQVMQQCCQQLAQIPQQ		360
secalin10	QQPSIQLSLQQQLNPCKNVLLQQCSPVALVSSLSRSKIFPQSECQVMQQCCQQLAQIPQQ			360
CD-active	-----			12
secalin11	LQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVGQGALAQVQGI	IQPQQLSQLEVVR		420
secalin10	LQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVGQGALAQVQGI	IQPQQLSQLEVVR		420
CD-active	-----			12
secalin11	LVLQNLPATMCNVYVPRQCSTIQAPFASIVTGIVGH			455
secalin10	LVLQNLPATMCNVYVPRQCSTIQAPFASIVTGIVGH			455
CD-active	-----			12

Figure S7. Multiple sequence alignment of all secalin precursors cleaved by the FvpP with identified prolyl-specific cleavage sites marked in green. For comparison, the sequence of the CD-active peptide (QPFPQPQQPIPQ) [5] was also aligned. Alignment performed using Clustal Omega [4].

hordein5	-----QQPVSRQ-----PQQIIPQQP-	16
hordein11	MKTFLIFALLAIVATSTIAQQQPPYPQQPQPPFQQQIPQQPQPPFQQPQPPFQQ-----	53
hordein6	MKTFLIFALLXIAATSTIAQQQPPFQQPXP-----QQPQPYPPQQPQPYPPQQPFQP---	50
hordein7	MKTFLIFALLAIVATSTIAQQQPPYPQQPQPPFQQQIPQQPQPPFQQPQPPFQQQIPQQPQ	60
hordein4	-----	0
hordein9	-----QQPVSRQ-----PQQIIPQQP-	16
hordein5	-----QQP-----FPLQPPQPPQPPQPPVPQQPQPPYPPQPPQPPFQQPPFCQQKPPF	62
hordein11	-----PFPSPRQPPF	62
hordein6	-----QQPFPPQQTIPQQPQPYPPQQPQPYPPQPPF-----QQAFFPQQPPFWPQQPPF	97
hordein7	PYPQQPQPPFSQQPIPPQQPQPYPPQQPQPPFQQPIPPQQPQPYPPQPPFQQP-FPSQQPPF	119
hordein4	-----	0
hordein9	-----QQPF-----PLQPPQPPQPPQPPIPQQPQPPYPPQPPQSFPQQPXPFSQQPPF	62
hordein5	QQPFFGLQQPILSQQQPCTPQQTPLPQGQLYQTLQLQIQYVHPSILQQLNPKVFLQQQ	122
hordein11	QQPFFWQQQPVLSSQQQPCTQDQTPLLQEQQDQMLLVQVIPFVHPSILQQLNPKVFLQQQ	122
hordein6	QQPFFGLQQPILSQQQPCTPXQTPLPQGQLYQTLQLQIPNVXPSILQQLPCKVFLQQQ	157
hordein7	QQPFFWQQQPILSQQQPCTPQQTPLPQGQDQMLLVQVIPFVHPSILQQLNPKVFLQQQ	179
hordein4	-----	0
hordein9	QQPFFWQQQPVLSSQQQPCTQDQTPLLQEQQDQMLLVQVIPFVHPSILQQLNPKVFLQQQ	122
hordein5	CSPVPVPQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQFRHEAIRAIVYSIFLQEPPQQL	182
hordein11	CSPVAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQIRHEAVRAIVYSIVLQEPPQL	182
hordein6	CSPVRMQQLIARSQMLQQSSCHVLQQQCCQQLPQIPEQFRHEAIRAIVYSIFLQEPPQQS	217
hordein7	CSPVAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQLRHEAVRAIVYSIVLQEPPQL	239
hordein4	-----	0
hordein9	CSPVAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQLRHEAVRAIVYSIVLQEPPQL	182
hordein5	VEGVSPQQQLWPQQVGQCSFQQPQPQQVGQQQVPQSAFLQPHQIAQLEATTSIARLTL	242
hordein11	VQGVSPQQQSQQQVGQCSFQQPQPQQ-GQQQVPQSVFLQPHQIAQLEATTSIARLTL	241
hordein6	VQGVSPQQQLQQEQVGQCYFQQPQPQQLGQPQVPQSVFLQPHQIAQLEATTSIARLTL	277
hordein7	VQGVSPQQQSQQQVGQCSFQQPQPQQ-GQQQVPQSVFLQPHQIAQLEATTSIARLTL	298
hordein4	-----QQQVPQSVFLQPHQIAQLEATTSIARLTL	30
hordein9	VQGVSPQQQSQQQVGQCSFQQPQPQQXGQQQVPQSVFLQPHQIAQLEATTSIARLTL	242
* * * * * . * * * * * : * : * * * *		
hordein5	PMMCNVNPLYRILRXGVGPSVG	266
hordein11	PRMCNVNPLYDIMPDPFWH----	261
hordein6	PTMCNVNPLYDIMPFGVGRVGV	301
hordein7	PTMCNVNPLYRIVPLAIDTRVGV	322
hordein4	PTMCNVNPLYRIVPLAIDTRVGV	54
hordein9	PTMCNVNPLYRIVPLAIDTRVGV	266
* * * . * * * * * * * :		

Figure S8. Multiple sequence alignment of all B-hordeins cleaved by the FvpP with identified prolyl-specific cleavage sites marked in yellow. The ,X' at position 54 in hordein9 was marked as prolyl-specific cleavage site, as some of the identified peptides contained a proline at that position. Alignment performed using Clustal Omega [4].

hordein3	MAKRLVLFVAVIVALVALTTAEREINGNNIFLDSRSRQLQCERELQESSLEACRRVVDQQ	60
hordein2	MAKRLVLFVAVIVALVALTTAEREINGNNIFLDSRSRQLQCERELQESSLEACRRVVDQQ	60
hordein1	MAKRLVLFVAVIVALVALTTAEREINGNNIFLDSRSRQLQCERELQESSLEACRRVVDQQ	60
hordein12	MAKRLVLFVAVIVALVALTTAEREINGNNIFLDSRSRQLQCERELQESSLEACRRVVDQQ	60

hordein3	LVGQLPWSTGLQMCCCQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL	120
hordein2	LVGQLPWSTGLQMCCCQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL	120
hordein1	LVGQLPWSTGLQMCCCQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL	120
hordein12	LVGQLPWSTGLQMCCCQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL	120

hordein3	QQGGWWTGSVKWYYPDQTSSQQSWGQQGYHQSVTSSQQPGQQGQGSYPGSTFPQQPGQG	180
hordein2	QQGGWWTGSVKWYYPDQTSSQQSWGQQGYHQSVTSSQQPGQQGQGSYPGSTFPQQPGQG	180
hordein1	QQGGWWTGSVKWYYPDQTSSQQSWGQQGYHQSVTSSQQPGQQGQGSYPGSTFPQQPGQG	180
hordein12	QQGGWWTGSVKWYYPDQTSSQQSWGQQGYHQSVTSSQQPGQQGQGSYPGSTFPQQPGQG	180

hordein3	QQPGQRQPWSYPSATFPQQPGQGQQGQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	240
hordein2	QQPGQRQPWSYPSATFPQQPGQGQQGQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	240
hordein1	QQPGQRQPWSYPSATFPQQPGQGQQGQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	240
hordein12	QQPGQRQPWSYPSATFPQQPGQGQQGQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	240

hordein3	GQQEYPPIATSPHQPGQWQQPGQGQQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	300
hordein2	GQQEYPPIATSPHQPGQWQQPGQGQQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	300
hordein1	GQQETYPPIATSPHQPGQWQQPGQGQQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	300
hordein12	GQQETYPPIATSPHQPGQWQQPGQGQQGYYPGATSLLPQGQQGQGPYQSATSPQQPGQG	300
**** *****:*****		
hordein3	GQGQQPGQQGQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQQGQGNPSGTSTQQPGQVQ	360
hordein2	GQGQQPGQQGQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQQGQGNPSGTSTQQPGQVQ	360
hordein1	GQGQQPGQQGQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQQGQGNPSGTSTQQPGQVQ	360
hordein12	GQGQQPGQQGQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQQGQGNPSGTSTQQPGQVQ	360

hordein3	QLGQQQQGYYPPIATSPQQPGQQQLGQQQQPGHGQQLVQGGQQGQQGQGHYPSMTSPHQT	420
hordein2	QLGQQQQGYYPPIATSPQQPGQQQLGQQQQPGHGQQLVQGGQQGQQGQGHYPSMTSPHQT	420
hordein1	QLGQQQQGYYPPIATSPQQPGQQQLGQQQQPGHGQQLVQGGQQGQQGQGHYPSMTSPHQT	420
hordein12	QLGQQQQGYYPPIATSPQQPGQQQLGQQQQPGHGQQLVQGGQQGQQGQGHYPSMTSPHQT	420

hordein3	GQGQKGYYPISAI SPQQSGQQGQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ	480
hordein2	GQGQKGYYPISAI SPQQSGQQGQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ	480
hordein1	GQGQKGYYPISAI SPQQSGQQGQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ	480
hordein12	GQGQKGYYPISAI SPQQSGQQGQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ	480

hordein3	GLGSLYYPGAYTQQKPGQGYNPGGTSPLHQGGGFGGGLTTEQPQGGKQPFHCQQTTVS	540
hordein2	GLGSLYYPGAYTQQKPGQGYNPGGTSPLHQGGGFGGGLTTEQPQGGKQPFHCQQTTVS	540
hordein1	GLGSLYYPGAYTQQKPGQGYNPGGTSPLHQGGGFGGGLTTEQPQGGKQPFHCQQTTVS	540
hordein12	GLGSLYYPGAYTQQKPGQGYNPGGTSPLHQGGGFGGGLTTEQPQGGKQPFHCQQTTVS	540

hordein3	PHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVS	600
hordein2	PHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVS	600
hordein1	PHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVS	600
hordein12	PHRGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVS	600
** : *****		
hordein3	PHPGQQTTVSPHQGQQTTVSPHPGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVS	660
hordein2	PHPGQQTTVSPHQGQQTTVSPHPGQQTTVSPHQGQQTTVSPHQGQQTTVSPHQGQQTTVS	660
hordein1	PHPGQQTTVSPHQGQQTTVSPHPGQQTTVSPHQGQQTTVSPHQGQ-----QTTVS	650
hordein12	PHPGQQTTVSPHQGQQTTVSPHPGQQTTVSPHQGQQTTVSPHQGQ-----QTTVS	650
***** *****		
hordein3	PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYGSPYHV	720
hordein2	PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYGSPYHV	720

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hordein1      PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTASLHHGQQSNELYGSPYHV 710
hordein12     PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYGSPYHV 710
*****.*****

hordein3      SVEQPSASLKVAKAQQQLAAQLPAMCRLEGGGGLLASQ      757
hordein2      SVEQPSASLKVAKAQQQLAAQLPAMCRLEGGGGLLASQ      757
hordein1      SVEQPSASLKVAKAQQQLAAQLPAMCRLEGGGGLLASQ      747
hordein12     SVEQPSASLKVAKAQQQLAAQLPAMCRLEGGGGLLASQ      747
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Figure S9. Multiple sequence alignment of all D-hordeins cleaved by the FvpP with identified prolyl-specific cleavage sites marked in yellow. No prolyl-specific cleavage sites were obtained for hordein12. Black arrows indicate cleavage sites described by Fiedler *et al.* [6]. Alignment performed using Clustal Omega [4].

hordein8	-----MQVNPSSVQVQPTQ-----	13
hordein10	MKTFLTFLVLLAMAMSIVTTARQLNPSHQELQSPQQPFLKQQSYLQQPYPPQQPYLPQQQFP	60
CD-active	-----	0
hordein8	-----QQPY-----	17
hordein10	TPQQFFPYLPQQTFFPSSQQPNPLQPQQPFPLQPQPPQQPFFQPQQPNPQQPQQPFPRQPQ	120
CD-active	-----	0
hordein8	<div style="text-align: center;"> ↓ ↓ ↓ ↓ ↓ ↓ ↓ -----PESQQPFISQSQQQFPQQPFPQRPLLPTHTPFLTFPDQL--LPQPPHQSFPQ- </div>	69
hordein10	QIVPQQPQQPFPQQPQQPFPQQPFSWQPQQPFLLQLXLPLAQQPFPLQPQLPFPQP	180
CD-active	-----QFPFPQPEQFPFW-----	12
	* ****:***	
hordein8	-----PPQSYQPPLQPFPQPQQKYPEQPQQPFPWQQPTIQLYLQQ-----	111
hordein10	QQPIGQQPKQPLLQQPQQTIPQQPQQPFPLQPQQPFPQQPQQPLPQQPQQIISQQPQQPF	240
CD-active	-----	12
hordein8	-----QLNPYKEFLLQQCRPVSLLSYLWSKIVQQSSCRVMLQQCCLQLAQ	156
hordein10	PLQPQQPFPQPQFPQEQPQQAFLPQPQQPFEE--SEQIITQPFPLQPQLPFPQQPQ	297
CD-active	-----	12
hordein8	IPEQYKCTAIDSIVHAIIFMQQGQRQGVQIVQQQPQPQQVGQCVLVQGQGVVQPQQLAQME	216
hordein10	QPLPQPQQPFRQLPKYI-----IPQQP-----QQPFLQLPHQPQQP--YAQQD	338
CD-active	-----	12
hordein8	AIRTLVLQSVPSMCNFNVPNCSTIKAPFVGVTGVGGQ	255
hordein10	IWSDIA-----LLG-----	347
CD-active	-----	12

Figure S10. Multiple sequence alignment of the remaining hordeins cleaved by the FvpP with identified prolyl-specific cleavage sites marked in yellow. For comparison, the CD-active peptide QPFPQPEQPFPW was also added. Black arrows indicate cleavage sites described by Shetty *et al.* [2]. Alignment performed using Clustal Omega [4].



Figure S11. Superimposed structures of the An-Pep (PDB-ID: 7WAB, blue) and the SWISS-MODEL predicted model of FvpP (black). The catalytically active amino acids were highlighted in red for the An-Pep (S179, D458 and H491) and yellow for the FvpP (S194, D461 and H490). For clarification, the catalytically active amino acids were indicated with arrows of the respective colors. The figure was created using Pymol [8].

Model_01 RLQDGRPHGNAPPPIPVLPVVEVLDsrTEAFQFSGS 35
7wab.1.A -----

Model_01 SI PPYN **V**YVYFDQ **X**IDHDD **A**SPG **G**TFsORYH **E**WEF 70
7wab.1.A ---- **T**GEAY **F**EQ **L**DH **H**N **P**EP **G**TFsORYH **S**TEY 31

Model_01 YQ-PGGP **L**LLTPGEANA **A**PYFGY **I**TNT **D**ITGLIA 104
7wab.1.A **A**WGGPG **S**FPV **E**NPGEVSA **D**GYEGY **I**TND **D**LTGVYA 66

Model_01 **Q**QEHG **A**TIV **E**HRFYGLSNPY **E**LTPE **S**LKYHT **I**Q 139
7wab.1.A **Q**EIQG **V**LL **E**HRYWGDSSPY **E**VLNAE **T**LQY **L**TL **D** 101

Model_01 **Q**AIDDLLEYFANNVALPMPNGTGVT **P**DKAF **H**LLVGG 174
7wab.1.A **Q**SILDMTYFAETV **K**QFD **N**SSRSNAQNAP **V**MMV 136

Model_01 **S**YSGALTSW **T**MVN **K**PGLFW **A**GY **S**AVVEAIT **E** 209
7wab.1.A **S**YSGALTAWTESI **A**PGTFW **Y**H **S**APVEAL **Y** 171

Model_01 **T**YFEP **I**RQ **N**MP **V**NCsADVA **A**VIAYVDET **F**SGENT **T** 244
7wab.1.A **Q**YFYPI **Q**Q **G**MA **Q**NCsKDVSLVAEYVDKIGKN **G**TAK 206

Model_01 **A**IDELKAT **F**GLSDVTH **L**DDAA **G**AL **R**NNLWDWQSLQ 279
7wab.1.A **E**QQELKEI **F**GLGAVE **H**YDDF **A**VL **P**NGPYLWQDND 241

Model_01 **I**NSGPGT **Q**FTKFCDA **L**EVKDGE---VAGEEGWGL 311
7wab.1.A **F**VT-GYS **S**FFQFCDA **V**EGVEAGAAVTPGPEGVGL 275

Model_01 **N**ALASWGGGYWRD **T**Y **S**LLCGD-----ANAE **D**CEG 340
7wab.1.A **K**ALANYANWFNSTI **L**PNYC **A**SYGYWTDEWSV **A**CE **D** 310

Model_01 **S**YNASQ **E**IYNT **I**-DNTW **S**SHFWIVC **N**EV-GY **Q** 373
7wab.1.A **S**YNASS **P**IF **D**TSVGNPVD **Q**NEWFLC **N**EPF **F**W **Q** 345

Model_01 **D**GPPLGV **D**IKPIVTRLVN **V**ET **N**YDARQCQL **N**YQDT 408
7wab.1.A **D**GAP **E**GT--STIV **P**RLVS--**A**SYW **R**Q **C**PLY **F**PE **V** 376

Model_01 **Y**A----SPPLPD **V**ERTINTVY **K**GW **D**V--SIE **R** **L**EE **D** 437
7wab.1.A **A**NG **D**TY **G**SAK **G**KN **S**ATVNSWT **G** **N**MT **R**NT **T**RL **I**WT 411

Model_01 **D**LRDPWR **E**ATMNAEGVE---I-QSTDRC **P**TV **S**D 468
7wab.1.A **D**QYDPWR **D**SGVSS **T**FRPGGPLVSTANEP **V**QIT **P**G 446

Model_01 **G**FHCSDI **D**AARGAS **D**ASIGALHKQALES **M**HNWLA **E** 503
7wab.1.A **G**FHCSDI **D**MEDYYAN **E**GVRKVV **D**NEVKQIK **E**WVE **E** 481

Model_01 **F**SSA 508
7wab.1.A **Y**YA-- 484

Figure S12. Multiple sequence alignment of the An-Pep (7wab.1.A, without signaling and propeptide) and FvpP (Model_01, without signaling peptide). Mismatches are presented in bold in the An-Pep-sequence. (Predicted) Secondary structures are indicated in purple for β -sheets and green for α -helices. Catalytically active amino acids were highlighted with red rectangles. The alignment was generated by SWISS-MODEL [7].

Table S1. gi-numbers of the α -gliadins identified in the wheat samples.

α -gliadin	Enzyme	Substrate	gi-number
1	FvpP	Gluten	147883552
2	FvpP	Gliadins	147883554
3	FvpP	α -Gliadins	401787284
4	FvpP	α -Gliadins	421932462
5	FvpP	α -Gliadins	421932500
6	FvpP	α -Gliadins	513129892
7	An-Pep	α -Gliadins	401787284
8	An-Pep	α -Gliadins	147883554
9	An-Pep	γ -Gliadins	421932488
10	FvpP	Gluten	283476402
11	FvpP	Gliadins	421932480
12	FvpP	α -Gliadins	421932466
13	FvpP	α -Gliadins	401787290
14	An-Pep	Gliadins	147883566
15	An-Pep	α -Gliadins	147883548
16	FvpP	Gluten	147883554
17	FvpP	α -Gliadins	401787284
Schulz et al.	-		21932416 [1]

Table S2. gi-numbers of the secalins identified in the rye samples.

Secalin	Enzyme	Substrate	Kind of secalin	gi-number
1	An-Pep	Prolamins	ω -Secalin	2145025
2	An-Pep	Prolamins	75k γ -Secalin	311554174
3	An-Pep	Prolamins	ω -Secalin	2145025
4	FvpP	Prolamins	ω -Secalin	229610198
5	FvpP	Prolamins	75k γ -Secalin	311554174
6	FvpP	Prolamins	Sec1 precursor	21204
7	FvpP	Prolamins	ω -Secalin	229610190
8	FvpP	Prolamins	Sec1 precursor	21202
9	FvpP	Prolamins	75k γ -Secalin	311554180
10	FvpP	Glutelins	Secalin precursor	11493665
11	FvpP	Glutelins	Secalin precursor	11493665

No secalins were identified in the hydrolysates from the gluteline fraction that were prepared using An-Pep.

Table S3. gi-numbers of the hordeins identified in the barley samples.

Hordein	Enzyme	Substrate	Kind of hordein	gi-number
1	FvpP	Glutelins	D-hordein	326522222
2	FvpP	Glutelins	D-hordein	30421167
3	FvpP	Glutelins	D-hordein	30421167
4	FvpP	Prolamins	B-hordein	530093
5	FvpP	Prolamins	B-hordein	224386
6	FvpP	Prolamins	B-hordein precursor	922538239
7	FvpP	Prolamins	B3-hordein	1779397018
8	FvpP	Prolamins	Putative γ 2-hordein, partial	34365052
9	FvpP	Prolamins	B-hordein	224385

10	FvpP	Prolamins	C-hordein	893242
11	FvpP	Prolamins	Truncated B-hordein	255348356
12	FvpP	Prolamins	D-hordein	392514929

No hordeins were identified in the hydrolysates that were prepared using An-Pep.

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