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

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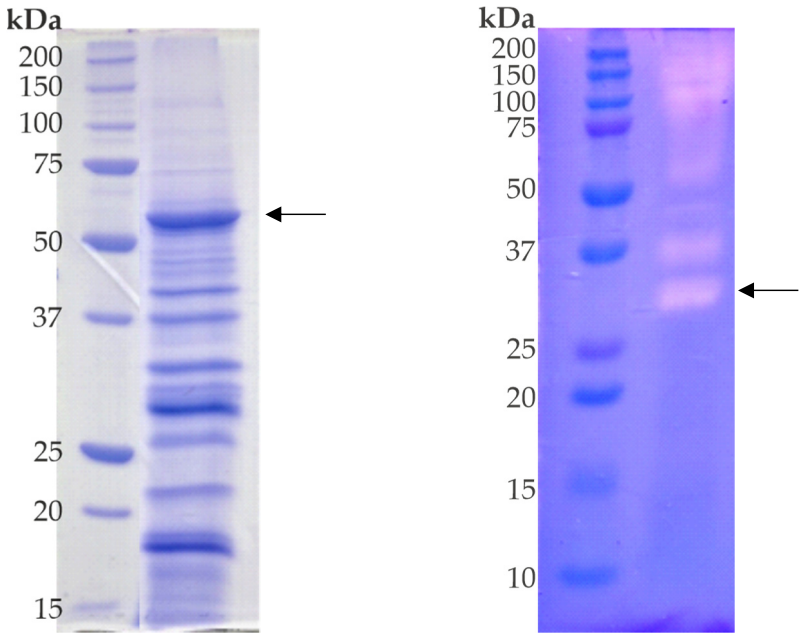
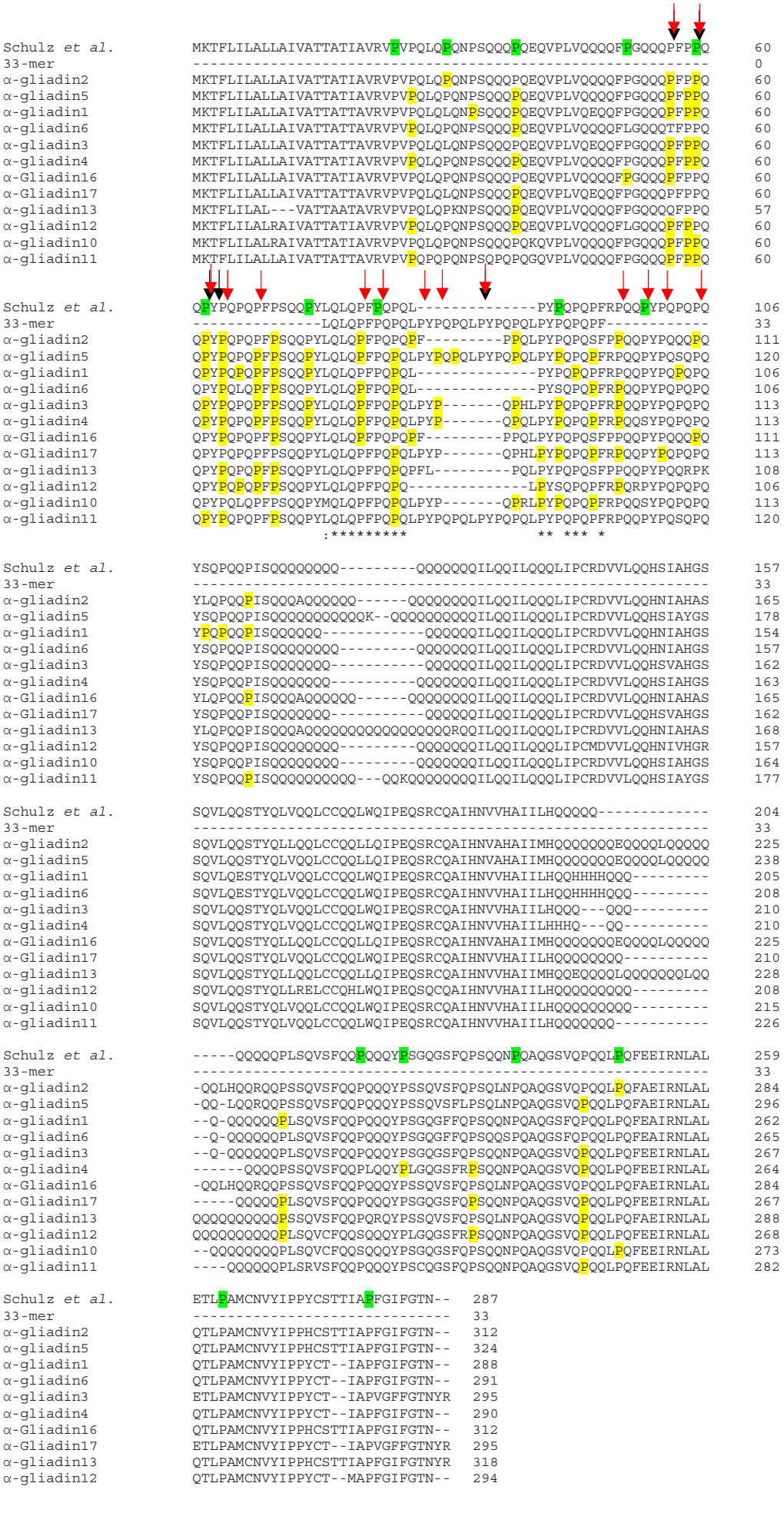
Supplementary Information



(**a**) (**b**)

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.



```
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].

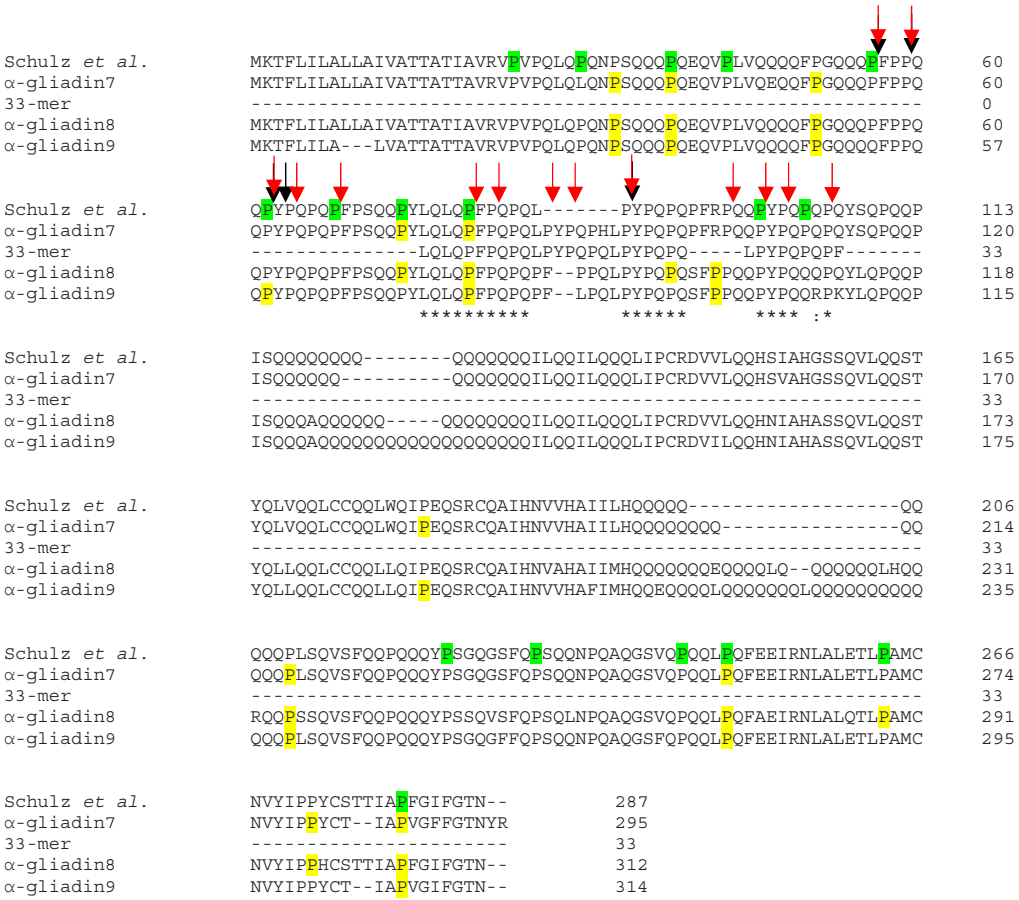


Figure S3. Multiple sequence alignment of all *α*-gliadins cleaved by the An-Pep with identified prolyl-specific cleavage sites marked in yellow. For comparison, the sequence of the CD-active 33-mer peptide and of the *α*-gliadin identified and cleaved (sites marked in green) in Schulz *et al*. [1] was 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      MKTFLIFVLAMTMSIITTARQINPSEQELQSPQQPVPKGQSYPQQSYPSHQPFPTPQQYS   60
secalin4      MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYS   60
secalin3      MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYS   60
secalin1      MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYS   60
CD-active     ------------------------------------------------------------   0

secalin7      PYQPQQPFPQPQQPTPIQPQQPFPQQPQQPFPQPQQQLPLQPQQPFPQPQQPIRQQPQQS   120
secalin4      PYQPQQPFPQPQQPTPIQPQQPFPQRPQQPFPQPQQQLPLQPQQPFPQPQQPIPQQPQQS   120
secalin3      PYQPQQPFPQPQQPTPIQPQQPFPQQPQQPFSQPQQQLPLQPQQPFPQPQQPIPQQPQQS   120
secalin1      PYQPQQPFPQPQQPTPIQPQQPFPQQPQQPFSQPQQQLPLQPQQPFPQPQQPIPQQPQQS   120
CD-active     -------------------------------------------QPFPQPQQPIPQ-----   12
                                                          ********** *

secalin7      FPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQIISQQPFPLQ   180
secalin4      FPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQLISQQPFPLQ   180
secalin3      FPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQIISQQPFPLQ   180
secalin1      FPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQIISQQPFPLQ   180
CD-active     ------------------------------------------------------------   12

secalin7      QQQPFSQPQQPFPQQPGQMIPQQPQQPSPLQPQQPFSQRPQRPQQPFPQQPQQIIPQQPQ   240
secalin4      PQQPFSQPQQPFPQQPGQIIPKQPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQ   240
secalin3      PQQPFSQPQQPFPQQPEQIIPQQPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQ   240
secalin1      PQQPFSQPQQPFPQQPEQIIPQQPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQ   240
CD-active     ------------------------------------------------------------   12

secalin7      QPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQPFSQPQQPFPQQPGQIIPP   300
secalin4      QPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQPFSRSQPPFPQQGGQIIPQ   300
secalin3      QPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFLLQPQQPFSQPQQPFLQQPGQIIPQ   300
secalin1      QPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFLLQPQQPFSQPQQPFLQQPGQIIPQ   300
CD-active     ------------------------------------------------------------   12

secalin7      QPQQPFPLQPQQPFPQQPEQIISQQPQQPFPLQPQQPSPQQPQLPFPLPQQPFVVVV      357
secalin4      QPQQPFPLHPQQPFPQQPEQIISQQPQQPFPLQPQQPSPQQPQLPFPQPQQPFVVVV      357
secalin3      QPQQPFPLQPQQPFPQQPEQIISEQPQQPFLLQPQQPSPQQPQLPFPQPQQPFVVVV      357
secalin1      QPQQPFPLQPQQPFPQQPEQIISEQPQQPFLLQPQQPSPQQPQLPFPQPQQPFVVVV      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 (QPFPQPQQPIPQ) [5] was also aligned. Alignment performed using Clustal Omega [4].

```
secalin2      MKTLLMLAILAMVTTIATANMQVNPSGQVQCPQQQPFPQPRQSSPQQPQQPFPQQSQQPF   60
secalin5      MKTLLMLAILAMVTTIATANMQVNPSGQVQCPQQQPFPQPRQSSPQQPQQPFPQQSQQPF   60
secalin9      MKTLLMLAILAMATTIATANMQVNPSGQVQCPQQQPFPQPQKSSPQQPQQPFPQQSQQPF   60
CD-active     ------------------------------------------------------------   0

secalin2      PQQPQQSSPQPQQPYPQQPFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQPFPQQPQQPV   120
secalin5      PQQPQQSSPQPQQPYPQQPFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQPFPQQPQQPV   120
secalin9      PQQPQQSSPQPLQPYPQQPFPQQPQQPYPQQPQQPFPQPPQQ------------------   102
CD-active     ------------------------------------------------------------   0

secalin2      PQQPQQQFPQQPQQPVPQQPLQQFPQQPQQSFPQQPQQPVPQQPLQQFPQQPQQPFPQQP   180
secalin5      PQQPQQQFPQQPQQPVPQQPLQQFPQQPQQSFPQQPQQPVPQQPLQQFPQQPQQPFPQQP   180
secalin9      ------------------------------------------------------PFPQQP   108
CD-active     ------------------------------------------------------------   0

secalin2      QQPVPQQSQQPFPQTQQPQQPFPQPQQPQQLFPQTQQSSPQQPQQVTSQPQQPFPQAQPP   240
secalin5      QQPVPQQSQQPFPQTQQPQQPFPQPQQPQQLFPQTQQSSPQQPQQVTSQPQQPFPQAQPP   240
secalin9      QQPVPQQSQQPFPQTQQPQQPFPQPQQPQQLFPQTQQSSPQQPQQVTSQPQQPFPQAQPP   168
CD-active     ------------------------------------------------------------   0

secalin2      QQSSPQSQQPYPQEPQQLFPQSQQPQQPFPQPQQPQQPFPQPQPQTQQSIPQPQQPFPQP   300
secalin5      QQSSPQSQQPYPQEPQQLFPQSQQPQQPFPQPQQPQQPFPQPQPQTQQSIPQPQQPFPQP   300
secalin9      QQSSPQSQQPYPQEPQQLFPQSQQPQQPFPQPQQPQQPFPQPQPQTQQSIPQPQQPFPQP   228
CD-active     ------------------------------------------------------QPFPQP   6
                                                                     ******

secalin2      QQPFPQSQEQFPQVHQPQQPSPQQQQPSIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSK   360
secalin5      QQPFPQSQEQFPQVHQPQQPSPQQQQPSIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSK   360
secalin9      QQPFPQSQEQFPQVHQPQQPSPQQQQPSIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSK   288
CD-active     QQPIPQ--------------------------------------------------------   12
              ***:**

secalin2      IFPQSECQVMQQQCCQQLAQIPQQLQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVG   420
secalin5      IFPQSECQVMQQQCCQQLAQIPQQLQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVG   420
secalin9      IFPQSECQVMQQQCCQQLAQIPQQLQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVG   348
CD-active     ------------------------------------------------------------   12

secalin2      QGALAQVQGIIQPQQLSQLEVVRSLVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH   479
secalin5      QGALAQVQGIIQPQQLSQLEVVRSLVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH   479
secalin9      QGALAQVQGIIQPQQLSQLEVVRSLVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH   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 (QPFPQPQQPIPQ) [5] was also aligned. Alignment performed using Clustal Omega [4].

```
secalin8      MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYS   60
secalin6      MKTFLIFVLAMTMSIITTARQLNPSEQELQSPQQPVPKEQSYPQQPYPSHQPFPTPQQYS   60
CD-active     ------------------------------------------------------------   0

secalin8      PYQPQQPFPQPQQPTPIQPQQPFPQRPQQPFPQPQQQLPLQPQQSFPQPQHPIPQQPQQS   120
secalin6      PYQPQQPFPQPQQPAPIQPQQPFPQQPQQPFPQPQQQLPLQPQQPFPQPQQPIPQQPQQS   120
CD-active     ----------------------------------------QPFPQPQQPIPQ-----   12
                                                      * *****:****

secalin8      FPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRPFAQQPEQIISQQPFPLQ   180
secalin6      FPQQPQRPEQQFPQQPQQIIPQQTQQPFPLQPQQPFPQQPQRSFAQQPKQIISQQPFPLQ   180
CD-active     ------------------------------------------------------------   12

secalin8      PQQPFSQPQQPFPQQPGQIIPQQPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQ   240
secalin6      PQQPFSQPQQPFPQQPGQIIPQQPQQPSPLQPQQPFSQQPQRPQQPFPQQPQQIIPQQPQ   240
CD-active     ------------------------------------------------------------   12

secalin8      QPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQPFSQPQQPFPQQPGQIIPQ   300
secalin6      QPFPLQPQQPVPQQPQRPFGQQPEQIISQRPQQPFPLQPQQPFSQPQQPLPQQPGQIIPQ   300
CD-active     ------------------------------------------------------------   12

secalin8      QPQQPFPLQPQQPFPQQPEQIISQQPQQPFPLQPQQPSPQQPQLPFPQPQQPFVVVV      357
secalin6      QPQQPFPLQPQQPFPQQSEQIIPQQPQQPFPLQPQQPSPQQPQLPFPQPQQPFVVVV      357
CD-active     ------------------------------------------------------------   12
```

Figure S6. Multiple sequence alignment of all Sec1 prescursors 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].

```
secalin11       MKTLLMLAILAMATTIATANMQVNPSGQVQCPQQQPFPQPQQSSPQQPQQPFPQQSQQPF  60
secalin10       MKTLLMLAILAMATTIATANMQVNPSGQVQCPQQQPFPQPQQSSPQQPQQPFPQQSQQPF  60
CD-active       ------------------------------------------------------------  0

secalin11       PQQPQQSSPQPQQPYPQQPFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQQFPQQPQQPV  120
secalin10       PQQPQQSSPQPQQPYPQQPFPQQPQQPYPQQPQQPFPQQPQQPYPQQPQQQFPQQPQQPV  120
CD-active       ------------------------------------------------------------  0

secalin11       PQQPLQQFPQQPQQPFPQQPLQQFPQQPQQPFPQQPQQPVPQQSQQPFPQTQQPQQPFPQ  180
secalin10       PQQPLQQFPQQPQQPFPQQPLQQFPQQPQQPFPQQPQQPVPQQSQQPFPQTQQPQQPFPQ  180
CD-active       ------------------------------------------------------------  0

secalin11       PQQPQQLFPQTQQSSPQQPQQVTSQPQQPFPQAQPPQQSSPQSQQPYPQEPQQLFPQSQQ  240
secalin10       PQQPQQLFPQTQQSSPQQPQQVTSQPQQPFPQAQPPQQSSPQSQQPYPQEPQQLFPQSQQ  240
CD-active       ------------------------------------------------------------  0

secalin11       PQQPFPQPQQPQQPFPQPQPQTQQSIPQPQQPFPQPQQPFPQSQEQFPQVHQPQQPSPQQ  300
secalin10       PQQPFPQPQQPQQPFPQPQPQTQQSIPQPQQPFPQPQQPFPQSQEQFPQVHQPQQPSPQQ  300
CD-active       ------------------------------QPFPQPQQPIPQ------------------  12
                                               *********:**

secalin11       QQPSIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSKIFPQSECQVMQQQCCQQLAQIPQQ  360
secalin10       QQPSIQLSLQQQLNPCKNVLLQQCSPVALVSSLRSKIFPQSECQVMQQQCCQQLAQIPQQ  360
CD-active       ------------------------------------------------------------  12

secalin11       LQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVGQGALAQVQGIIQPQQLSQLEVVRS  420
secalin10       LQCAAIHSVVHAIIMQQEQREGVQILLPQSHQQHVGQGALAQVQGIIQPQQLSQLEVVRS  420
CD-active       ------------------------------------------------------------  12

secalin11       LVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH 455
secalin10       LVLQNLPTMCNVYVPRQCSTIQAPFASIVTGIVGH 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       MKTFLIFALLAIVATSTIAQQQPYPQQPQPFPQQPIPQQPQPFPQQPQPFPQQ-------  53
hordein6        MKTFLIFALLXIAATSTIAQQQPFPQQPXP-------QQPQPYPQQPQPYPQQPFQP---  50
hordein7        MKTFLIFALLAIVATSTIAQQQPYPQQPQPFPQQPIPQQPQPYPQQPQPFPQQPIPQQPQ  60
hordein4        ------------------------------------------------------------  0
hordein9        --------------------------------QQPVSRQ-----------PQQIIPQQP-  16

hordein5        ----------QQP----FPLQPQQPQPFPQQPVPQQPQPYPQQPQPFPQQPPFCQQKPFP  62
hordein11       ------------------------------------------------PFPSRQPFP  62
hordein6        -----QQPFPQQTIPQQPQPYPQQPQPYPQQPFPP--------QQAFPQQPPFWPQQPFP  97
hordein7        PYPQQPQPFSQQPIPQQPQPYPQQPQPFPQQPIPQQPQPYPQQPQPFPQQP-FPSQQPFP 119
hordein4        ------------------------------------------------------------  0
hordein9        ----------QQPF----PLQPQQPQPFPQQPIPQQPQPYPQQPQSFPQQPXFPSQQPFP  62

hordein5        QQPPFGLQQPILSQQQPCTPQQTPLPQGQLYQTLLQLQIQYVHPSILQQLNPCKVFLQQQ 122
hordein11       QQPPFWQQQPVLSQQQPCTQDQTPLLQEQQDQMLLQVQIPFVHPSILQQLNPCKVFLQQQ 122
hordein6        QQPPFGLQQPILSQQQPCTPXQTPLPQGQLYQTLLQLQIPNVXPSILQQLXPCKVFLQQQ 157
hordein7        QQPPFWQQQPILSQQQPCTPQQTPLPQGQQDQMLVQVQIPFVHPSILQQLNPCKVFLQQQ 179
hordein4        ------------------------------------------------------------  0
hordein9        QQPPFWQQQPVLSQQQPCTQDQTPLLQEQQDQMLVQVQIPFVHPSILQQLNPCKVFLQQQ 122

hordein5        CSPVPVPQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQFRHEAIRAIVYSIFLQEQPQQL 182
hordein11       CSPVAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQIRHEAVRAIVYSIVLQEQPLQL 182
hordein6        CSPVRMQQLIARSQMLQQSSCHVLQQQCCQQLPQIPEQFRHEAIRAIVYSIFLQEQPQQS 217
hordein7        CSPVAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQLRHEAVRAIVYSIVLQEQSLQL 239
hordein4        ------------------------------------------------------------  0
hordein9        CSPVAMSQRIARSQMLQQSSCHVLQQQCCQQLPQIPEQLRHEAVRAIVYSIVLQEQSLQL 182

hordein5        VEGVSQPQQQLWPQQVGQCSFQQPQPQQVGQQQQVPQSAFLQPHQIAQLEATTSIALRTL 242
hordein11       VQGVSQPQQQSQQQQVGQCSFQQPQPQQ-GQQQQVPQSVFLQPHQIAQLEATTSVALRTL 241
hordein6        VQGVSQPQQQLQQEQVGQCYFQQPQPQQLGQPQQVPQSVFLQPHQIAQLEAXTSIALRTL 277
hordein7        VQGVSQPQQQSQQQQVGQCSFQQPQPQQ-GQQQQVPQSVFLQPHQIAQLEATASIALRTL 298
hordein4        ----------------------------QQQQVPQSVFLQPHQIAQLEATTSIALRTL 30
hordein9        VQGVSQPQQQSQQQQVGQCSFQQPQPQQXGQQQQVPQSVFLQPHQIAQLEATTSIALRTL 242
                                           * ******.************ :*:*****

hordein5        PMMCSVNVPLYRILRXGVGPSVGV    266
hordein11       PRMCNVNVPLYDIMPPDFWH----    261
hordein6        PTMCNVNVPLYDIMPFGVGTRVGV    301
hordein7        PTMCSVNVPLYRIVPLAIDTRVGV    322
hordein4        PTMCSVNVPLYRIVPLAIDTRVGV    54
hordein9        PTMCSVNVPLYRIVPLAIDTRVGV    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        LVGQLPWSTGLQMQCCQQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL  120
hordein2        LVGQLPWSTGLQMQCCQQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL  120
hordein1        LVGQLPWSTGLQMQCCQQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL  120
hordein12       LVGQLPWSTGLQMQCCQQLRDVSPECRPVALSQVVRQYEQQTEVPSKGGSFYPGGTAPPL  120
                ************************************************************

hordein3        QQGGWWGTSVKWYYPDQTSSQQSWQGQQGYHQSVTSSQQPGQGQQGSYPGSTFPQQPGQG  180
hordein2        QQGGWWGTSVKWYYPDQTSSQQSWQGQQGYHQSVTSSQQPGQGQQGSYPGSTFPQQPGQG  180
hordein1        QQGGWWGTSVKWYYPDQTSSQQSWQGQQGYHQSVTSSQQPGQGQQGSYPGSTFPQQPGQG  180
hordein12       QQGGWWGTSVKWYYPDQTSSQQSWQGQQGYHQSVTSSQQPGQGQQGSYPGSTFPQQPGQG  180
                ************************************************************

hordein3        QQPGQRQPWSYPSATFPQQPGQGQGQQGYYPGATSLLQPGQGQQGPYQSATSPQQPGQGQ  240
hordein2        QQPGQRQPWSYPSATFPQQPGQGQGQQGYYPGATSLLQPGQGQQGPYQSATSPQQPGQGQ  240
hordein1        QQPGQRQPWSYPSATFPQQPGQGQGQQGYYPGATSLLQPGQGQQGPYQSATSPQQPGQGQ  240
hordein12       QQPGQRQPWSYPSATFPQQPGQGQGQQGYYPGATSLLQPGQGQQGPYQSATSPQQPGQGQ  240
                ************************************************************

                                 ↓       ↓   ↓           ↓
hordein3        GQQEPYPIATSPHQPGQWQQPGQGQQGYYPSVTSPQQSGQGQQGYPSTTSPQQSGQGQQL  300
hordein2        GQQEPYPIATSPHQPGQWQQPGQGQQGYYPSVTSPQQSGQGQQGYPSTTSPQQSGQGQQL  300
hordein1        GQQETYPIATSPHQPGQWQQPGQGQQGYYPSVTSPQQSGQGQQGYPSTTSPQQSGQGQQL  300
hordein12       GQQETYPIATSPHQPGQWQQPGQGQQGHYPSVTSPQQSGQGQQGYPSTTSPQQSGQGQQL  300
                **** ***********************:**********************************

hordein3        GQGQQPGQGQQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQGQQGYNPSGTSTQQPGQVQ  360
hordein2        GQGQQPGQGQQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQGQQGYNPSGTSTQQPGQVQ  360
hordein1        GQGQQPGQGQQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQGQQGYNPSGTSTQQPGQVQ  360
hordein12       GQGQQPGQGQQGYPSATFPQQPGQWQQGSYPSTTSPQQSGQGQQGYNPSGTSTQQPGQVQ  360
                ************************************************************

                ↓                   ↓
hordein3        QLGQGQQGYYPIATSPQQPGQGQQLGQGQQPGHGQQLVQGQQQGQGQQGHYPSMTSPHQT  420
hordein2        QLGQGQQGYYPIATSPQQPGQGQQLGQGQQPGHGQQLVQGQQQGQGQQGHYPSMTSPHQT  420
hordein1        QLGQGQQGYYPIATSPQQPGQGQQLGQGQQPGHGQQLVQGQQQGQGQQGHYPSMTSPHQT  420
hordein12       QLGQGQQGYYPIATSPQQPGQGQQLGQGQQPGHGQQLVQGQQQGQGQQGHYPSMTSPHQT  420
                ************************************************************

hordein3        GQGQKGYYPSAISPQQSGQGQQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ  480
hordein2        GQGQKGYYPSAISPQQSGQGQQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ  480
hordein1        GQGQKGYYPSAISPQQSGQGQQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ  480
hordein12       GQGQKGYYPSAISPQQSGQGQQGYQPSGASSQGSVQGACQHSTSSPQQQAQGCQASSPKQ  480
                ************************************************************

hordein3        GLGSLYYPSGAYTQQKPGQGYNPGGTSPLHQQGGGFGGGLTTEQPQGGKQPFHCQQTTVS  540
hordein2        GLGSLYYPSGAYTQQKPGQGYNPGGTSPLHQQGGGFGGGLTTEQPQGGKQPFHCQQTTVS  540
hordein1        GLGSLYYPSGAYTQQKPGQGYNPGGTSPLHQQGGGFGGGLTTEQPQGGKQPFHCQQTTVS  540
hordein12       GLGSLYYPSGAYTQQKPGQGYNPGGTSPLHQQGGGFGGGLTTEQPQGGKQPFHCQQTTVS  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        PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYYGSPYHV  720
hordein2        PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYYGSPYHV  720
```

```
hordein1        PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTASLHHGQQSNELYYGSPYHV  710
hordein12       PHQGQQTTVSPHQGQQTTVSPHQGQQPGEQPCGFPGQQTTVSLHHGQQSNELYYGSPYHV  710
                ************************************.*******************

hordein3        SVEQPSASLKVAKAQQLAAQLPAMCRLEGGGGLLASQ        757
hordein2        SVEQPSASLKVAKAQQLAAQLPAMCRLEGGGGLLASQ        757
hordein1        SVEQPSASLKVAKAQQLAAQLPAMCRLEGGGGLLASQ        747
hordein12       SVEQPSASLKVAKAQQLAAQLPAMCRLEGGGGLLASQ        747
                ***********************************
```

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].

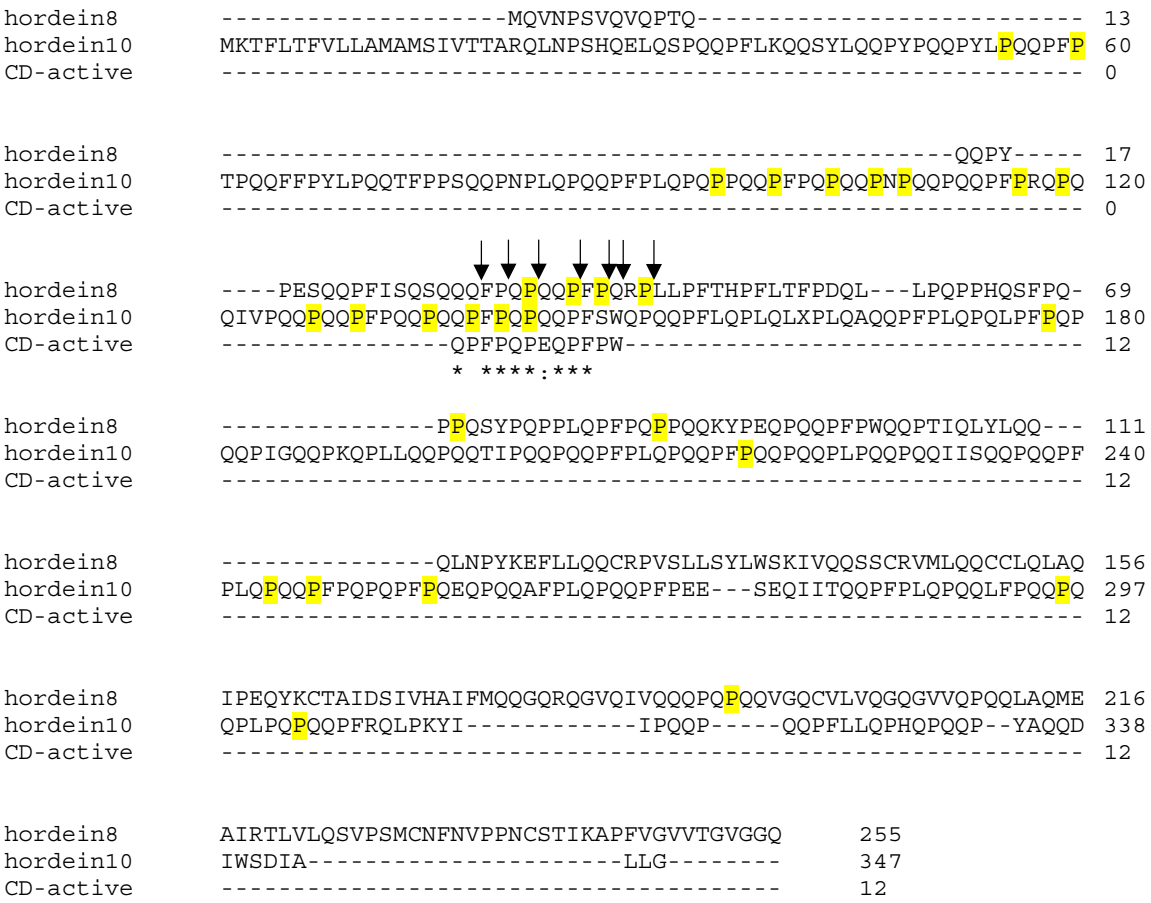


Figure S10. Multiple sequence alignment of the remaining hordeins cleaved by the FvpP with identified prolyl-specific cleavage sites marked in yellow. For comparison, the CD-active peptide QPFPQPEQPFPW was also added. Black arrows indicate cleavage sites described by Shetty *et al*. [2]. Alignment performed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

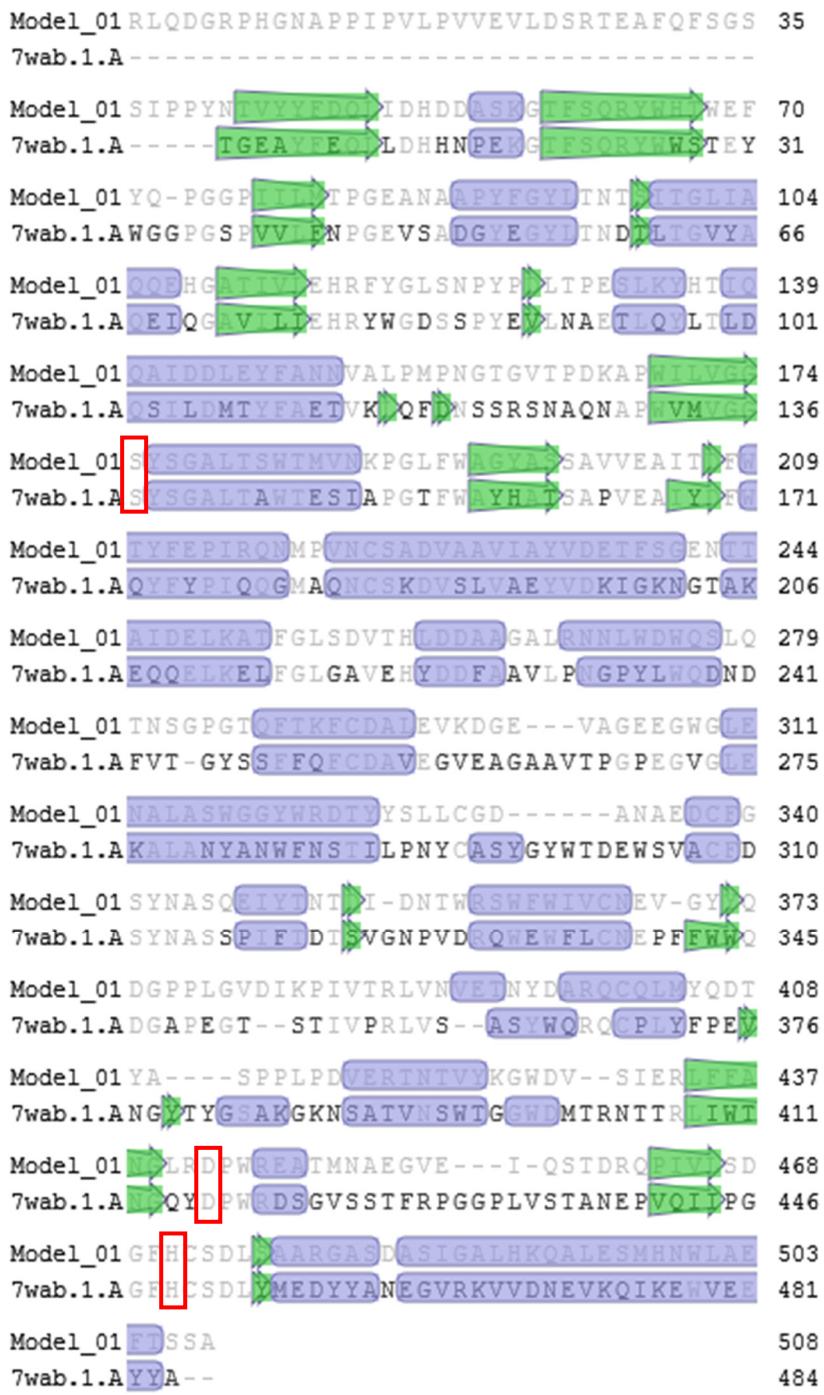


Figure S12. Multiple sequence alignment of the An-Pep (7wab.1.A, without signaling and propeptide) and FvpP (Model_01, without signaling peptide). Mismatches are presented in bold in the An-Pep-sequence. (Predicted) Secondary structures are indicated in purple for *ß*-sheets and green for *α*-helices. 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 γ2-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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