Analysis of gluten in a wheat gluten incurred sorghum beer brewed in the presence of proline endopeptidase by LC-MS/MS

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Supporting Information

Please see Excel file for Supplementary Table 1. Gluten peptides identified in three out of the four brew replicates of the 200 mg/L wheat gluten incurred sorghum beer for the non-PEP containing beer and beer brewed with the standard dose of PEP (34 µL PEP/L wort), as shown in Figure 1.

Please see Excel file for Supplementary Table 2. Gluten peptides identified in both brew replicates 3 and 4 of the 200 mg/L wheat gluten incurred sorghum beer with their associated gluten protein class for the non-PEP containing beer and beer brewed with either the standard or high dose of PEP, as shown in Figure 4.

Supplementary Table 3. The proline content of each wheat gluten protein class and the average number of peptides (>9 amino acids long) that would be generated from a complete *in silico* digestion with PEP. All UniProt entries for *T. aestivum* (downloaded on 04-04-2017) were filtered according to the following minimum molecular weights, so only full-length sequences were used for the *in silico* digestion: >30 kDa for α-, γ-, and ω-gliadins, >28 kDa for LMW-glutenins, and >70 kDa for HMW-glutenins.

Gluten Protein Class	% Pro	Average # peptides from *in silico* digest with PEP
alpha-gliadin	15	7
gamma-gliadin	17	9
LMW-glutenin	13	11
omega-gliadin	22	4
HMW-glutenin	11	37

Please see Excel file for Supplementary Table 4. Database of 465 known immunogenic sequences with their associated gluten protein class.

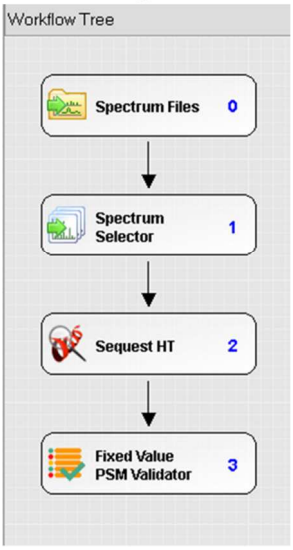
Please see Excel file for Supplementary Table 5. Gluten peptides identified in the four non-PEP containing commercial wheat beers with their associated gluten protein class, as shown in Supplementary Figure 7.

Supplementary Table 6. Number of chymotryptic and hydrolyzed HMW-glutenin peptides identified in the non-PEP containing 200 mg/L wheat gluten incurred sorghum beer and non-PEP containing commercial wheat beers.

	No PEP	Comm 1	Comm 2	Comm 3	Comm 4
Chymotryptic	17	32	67	111	3
Hydrolyzed	0	46	75	112	87

Supplementary Figure 1. PD 2.1 parameters used for data analysis.

Processing Workflow



Processing Node 1

1. General Settings	
Precursor Selection	Use MS1 Precursor
Use New Precursor Reevaluation	True
Use Isotope Pattern in Precursor Reevaluation	True
2. Spectrum Properties Filter	
Lower RT Limit	0
Upper RT Limit	0
First Scan	0
Last Scan	0
Ignore Specified Scans	
Lowest Charge State	0
Highest Charge State	0
Min. Precursor Mass	350 Da
Max. Precursor Mass	10000 Da
Total Intensity Threshold	0
Minimum Peak Count	1
3. Scan Event Filters	
Mass Analyzer	(Not specified)
MS Order	Is MS2
Activation Type	Is HCD
Min. Collision Energy	0
Max. Collision Energy	1000
Scan Type	Is Full
Polarity Mode	(Not specified)
4. Peak Filters	
S/N Threshold (FT-only)	1.5
5. Replacements for Unrecognized Properties	
Unrecognized Charge Replacements	Automatic
Unrecognized Mass Analyzer Replacements	ITMS
Unrecognized MS Order Replacements	MS2
Unrecognized Activation Type Replacements	CID
Unrecognized Polarity Replacements	+
Unrecognized MS Resolution@200 Replacements	60000
Unrecognized MSn Resolution@200 Replacements	30000
6. Precursor Pattern Extraction	
Precursor Clipping Range Before	2.5 Da
Precursor Clipping Range After	5.5 Da

Processing Node 2

1. Input Data	
Protein Database	uniprot_RakhiBeer.fasta
Enzyme Name	No-Enzyme (Unspecific)
Max. Missed Cleavage Sites	0
Min. Peptide Length	6
Max. Peptide Length	144
Max. Number of Peptides Reported	10
2. Tolerances	
Precursor Mass Tolerance	10 ppm
Fragment Mass Tolerance	0.03 Da
Use Average Precursor Mass	False
Use Average Fragment Mass	False
3. Spectrum Matching	
Use Neutral Loss a Ions	True
Use Neutral Loss b Ions	True
Use Neutral Loss y Ions	True
Use Flanking Ions	True
Weight of a Ions	0
Weight of b Ions	1
Weight of c Ions	0
Weight of x Ions	0
Weight of y Ions	1
Weight of z Ions	0
4. Dynamic Modifications	
5. Dynamic Modifications (peptide terminus)	
6. Dynamic Modifications (protein terminus)	
7. Static Modifications	

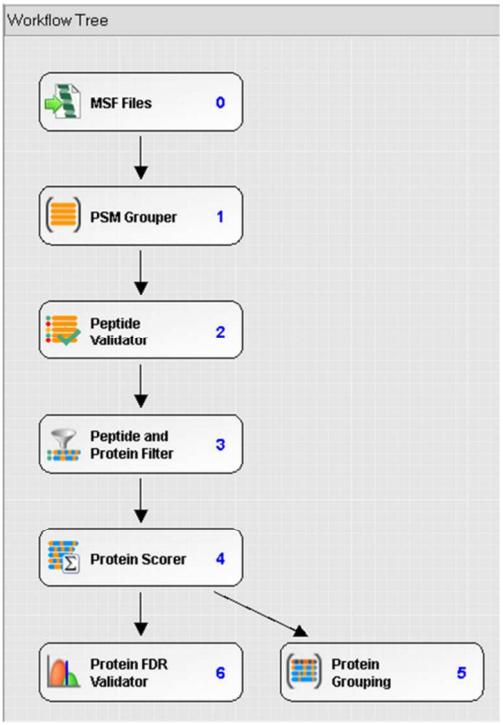
For digested samples only:

7. Static Modifications	
Peptide N-Terminus	None
Peptide C-Terminus	None
1. Static Modification	Carbamidomethyl / +57.021 Da (C)
2. Static Modification	None
3. Static Modification	None
4. Static Modification	None
5. Static Modification	None
6. Static Modification	None

Processing Node 3

1. Input Data	
Maximum Delta Cn	1
Maximum Rank	0

Consensus Workflow



Consensus Node 0

1. Spectrum Storage Settings	
Spectra to Store	Identified or Quantified
2. Merging of Identified Peptide and Proteins	
Merge Mode	Globally by Search Engine Type
File Limit for Automatic Merge.	10
3. FASTA Title Line Display	
Reported FASTA Title Lines	Best match
Title Line Rule	standard
Preferred Accession	
Preferred Taxonomy	
Avoid Expressions	
4. PSM Filters	
Maximum Delta Cn	0.05
Maximum Rank	0
Maximum Delta Mass	0 ppm
1. Score	
1. Threshold	0
2. Score	
2. Threshold	0
3. Score	
3. Threshold	0
4. Score	
4. Threshold	0
5. Score	
5. Threshold	0
6. Score	
6. Threshold	0
7. Score	
7. Threshold	0
8. Score	
8. Threshold	0
9. Score	
9. Threshold	0
10. Score	
10. Threshold	0

Consensus Node 1

1. Peptide Group Modifications	
Site Probability Threshold	75
2. Display Options	
Modification Sites Shown	Best Position

Consensus Node 2

1. General Validation Settings	
Validation Mode	Only PSM level FDR Calculation based on score
Target FDR (Strict) for PSMs	0.01
Target FDR (Relaxed) for PSMs	0.05
Target FDR (Strict) for Peptides	0.01
Target FDR (Relaxed) for Peptides	0.05
2. Specific Validator Settings	
Validation Based on	q-Value
Use Concatenated FDR Calculation for PSM Level FDR Calculation Based on Score	False
Reset Confidences for Nodes without Decoy Search (Fixed score thresholds)	False

Consensus Node 3

1. Peptide Filters	
Peptide Confidence At Least	High
Keep Lower Confident PSMs	False
Minimum Peptide Length	6
Remove Peptides Without Protein Reference	False
2. Protein Filters	
Minimum Number of Peptide Sequences	1
Count Only Rank 1 Peptides	True
Count Peptides Only for Top Scored Protein	False

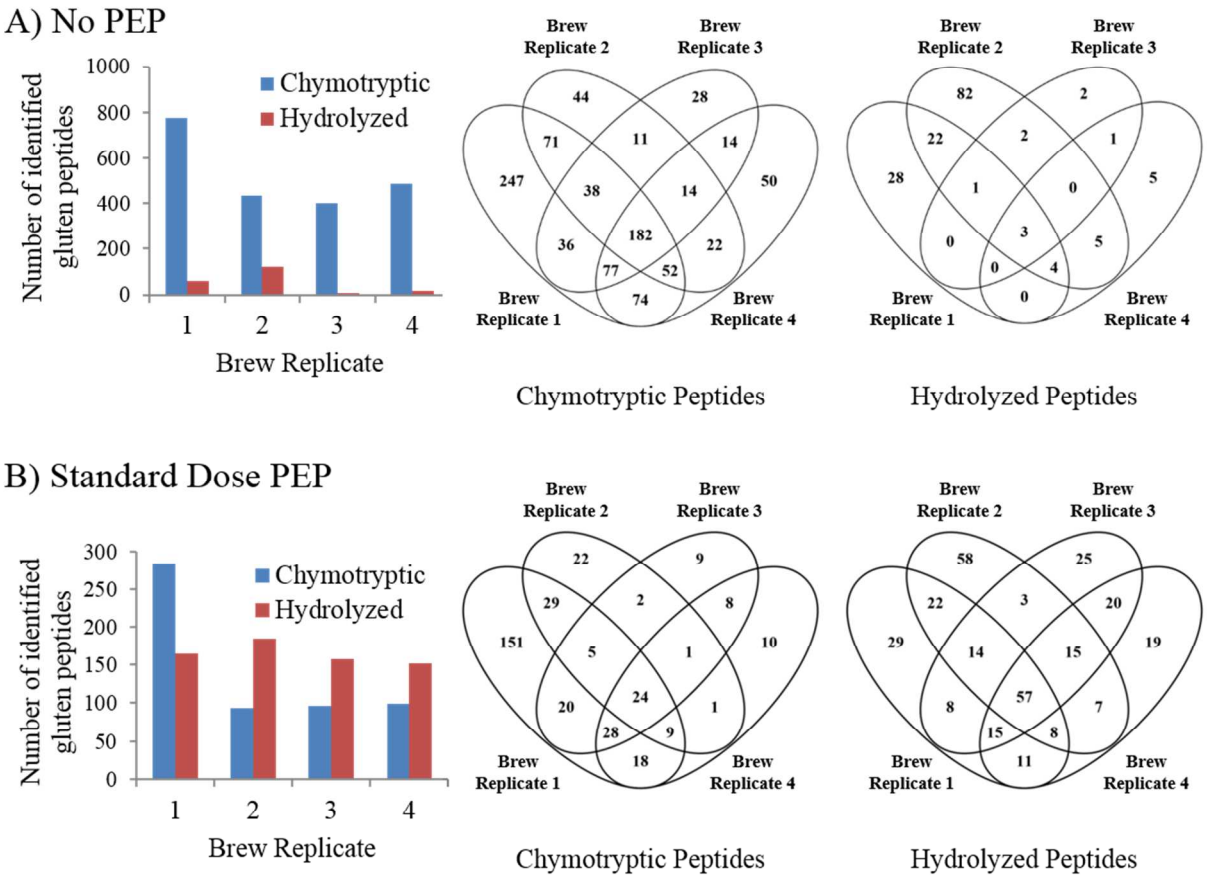
Consensus Node 5

1. Protein Grouping	
Apply strict parsimony principle	True

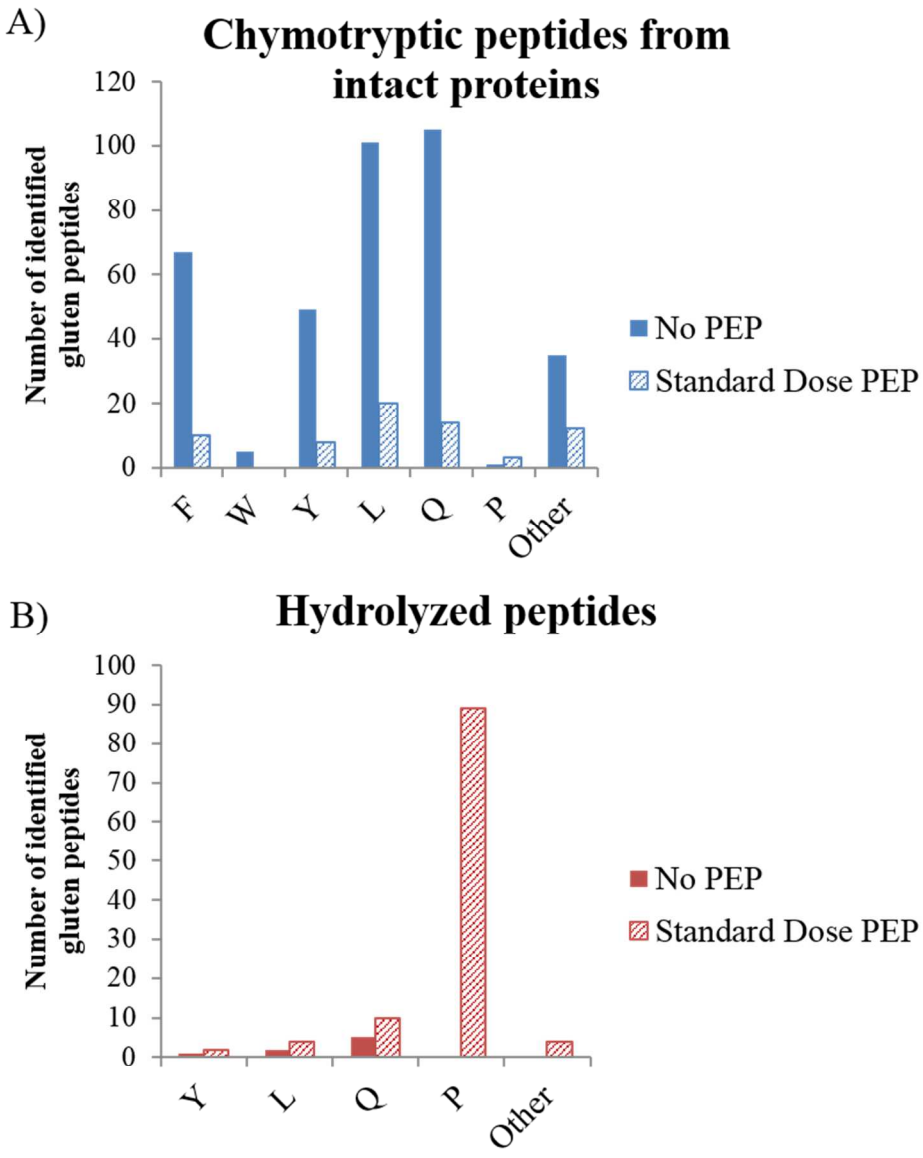
Consensus Node 6

1. Confidence Thresholds	
Target FDR (Strict)	0.01
Target FDR (Relaxed)	0.05

Supplementary Figure 2. Total number of gluten peptides identified in each brew replicate 1-4 of the 200 mg/L wheat gluten incurred sorghum beer for the non-PEP containing beer (A) and beer brewed with the standard dose of PEP (34 µL PEP/L wort) (B).

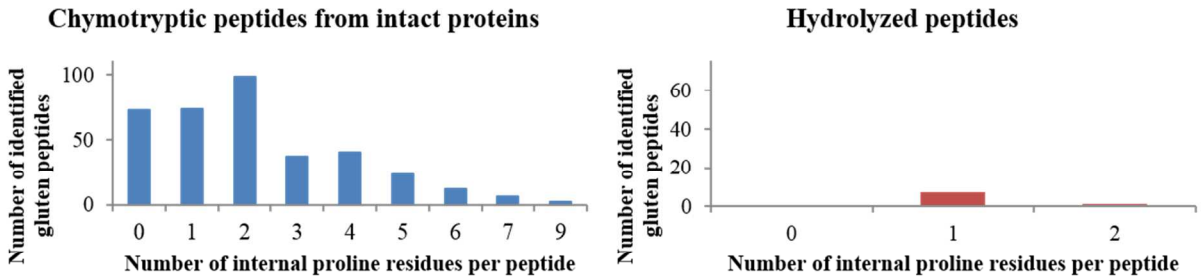


Supplementary Figure 3. A plot of the C-terminal amino acid for each chymotryptic gluten peptide (A) and hydrolyzed gluten peptide (B) from Figure 1, which shows the cleavage specificity observed for fermentation alone in the non-PEP containing beer and fermentation and PEP treatment in the beer brewed with the standard dose of PEP.

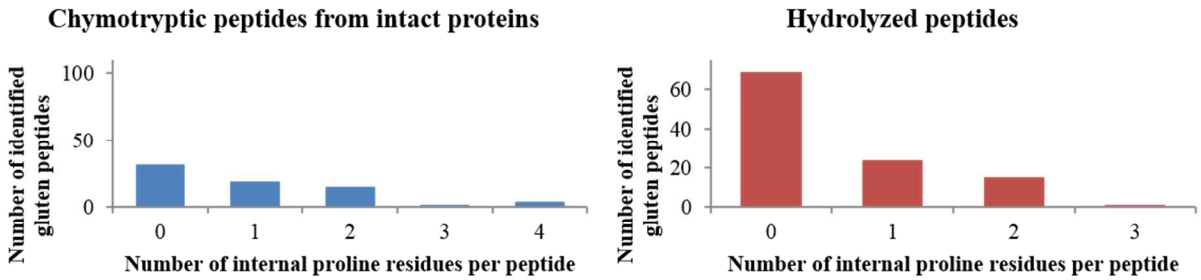


Supplementary Figure 4. The number of internal proline residues per peptide identified in the 200 mg/L wheat gluten incurred sorghum beer for the non-PEP containing beer (A) and beer brewed with the standard dose of PEP (B), as shown in Figure 1.

A) No PEP

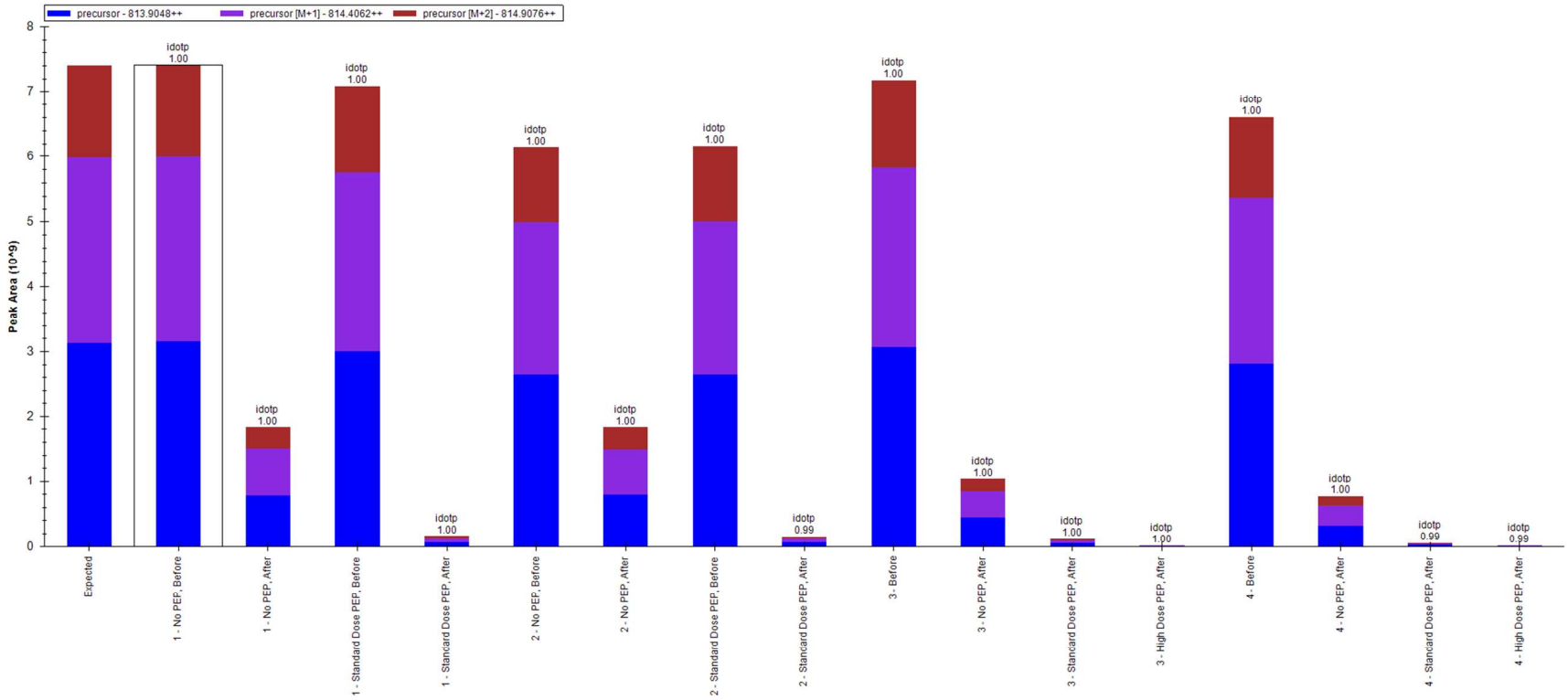


B) Standard Dose PEP

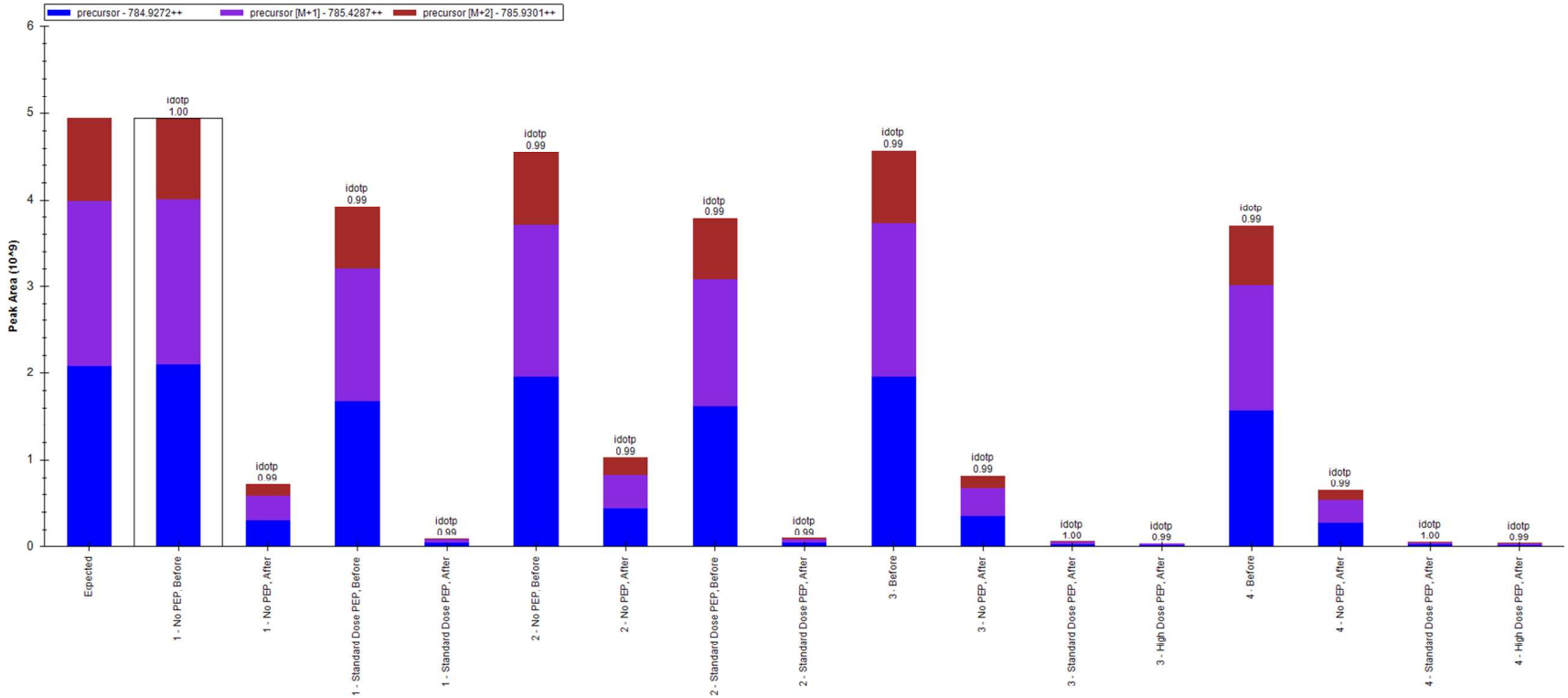


Supplementary Figure 5. MS1 peak areas for two chymotryptic peptides from α/β gliadin, RPQQPYPQPQPQY (A) and LQLQPFPQPQLPY (B), which were used to determine the percent intact gluten in Figure 3. Samples are labeled as before or after fermentation by brew replicate 1-4. The data shown is before normalization to isotopically labeled versions.

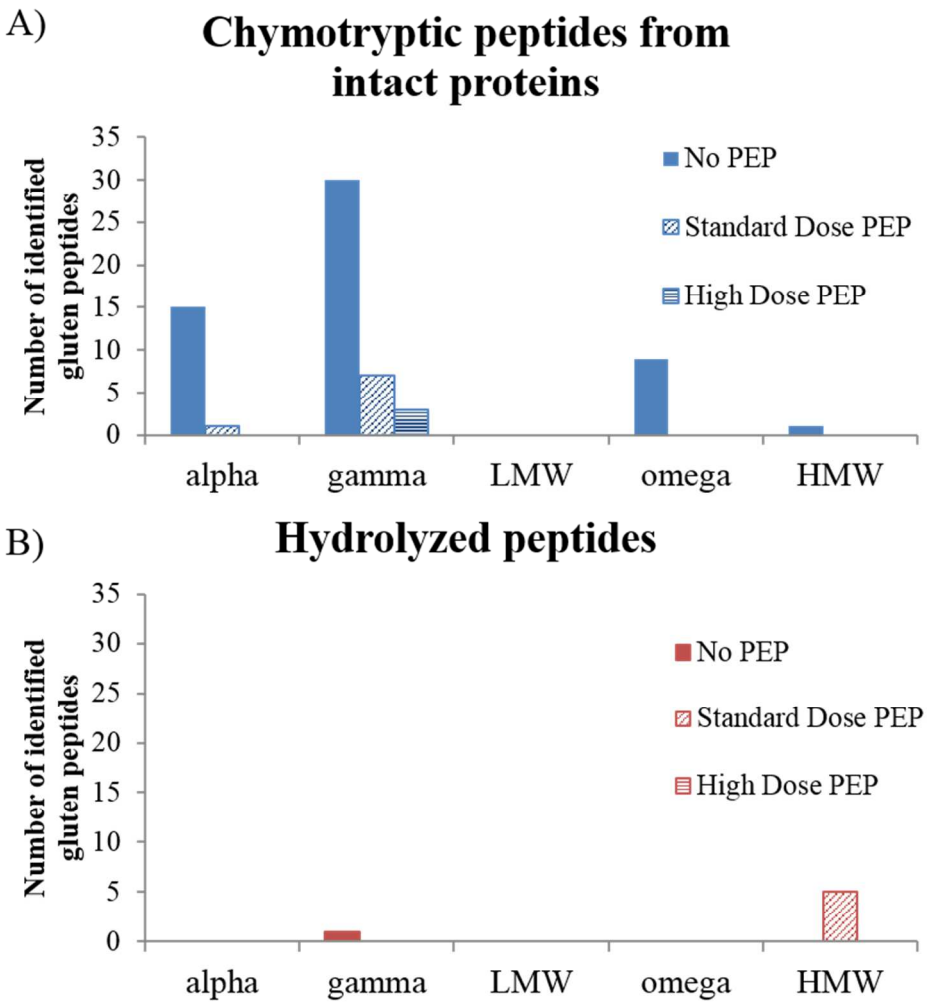
A) RPQQPYPQPQPQY



B) LQLQPFPQPQLPY



Supplementary Figure 6. Gluten peptides identified in the non-PEP containing beer, beer brewed with the standard dose of PEP, and beer brewed with the high dose of PEP that contain known immunogenic sequences, according to their associated gluten protein class.



Supplementary Figure 7. Total number of gluten peptides identified in the non-PEP containing 200 mg/L wheat gluten incurred sorghum beer and four non-PEP containing commercial wheat beers.