Making Gluten-Free beers with Barley Malt and a Proline-Specific Endoprotease

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Code	Beer Type	Gliadin (ppm)	RSD%
A	Light American lager*	<5	NA
B	Light American lager*	<5	NA
C	Light American lager*	6	10
D	American lager*	<5	NA
E	American lager*	<5	NA
F	American lager*	12	16
G	GF beer non barley*	<5	NA
H	GF beer non barley	<5	NA
I	Amber Ale	55	15
J	Amber Ale	127	22
K	Amber Ale	65	10
L	Brown Ale	34	17
M	Brown Ale	149	6
N	Brown Ale	285	14
O	Brown Ale	158	6
P	IPA	126	11
Q	IPA	17	20
R	IPA	9	19
S	IPA	56	12
T	IPA	40	9
U	Pale Ale	71	14
V	Hefeweizen	801	12
W	Porter	139	8
X	Porter	55	12
Z	Red Ale	43	16
AA	Red Ale	93	17
BB	Maybock	114	9
CC	Strong Ale	40	29
DD	Triple	235	8

Table 1: Gliadin content of American beers available commercially. * Non craft beers.

Background

- Gluten is a storage protein found in wheat, barley and rye composed of two fractions: prolamins and glutenins.
- The prolamin fraction (called hordein in barley and gliadin in wheat) is rich in amino acids proline and glutamin.
- Toxic gluten peptides cause unwanted reactions in people who are sensitive or intolerant to gluten, which may result in serious damage to people who have celiac disease.
- The only therapy is a lifetime avoidance of products containing gluten.
- Typically beers are made with barley or wheat so they contain gluten(1).
- Most GF beers are made with raw materials that are free of gluten (sorghum, rice, buckwheat); the flavor profile is different compared to standard barley beers.
- A proline-speficic endo protease (PSEP) hydrolyzes proline-rich proteins (haze sensitive proteins and gluten) and cleaves celiotoxic epitopes (Fig 1).
- Gluten can be reduced to undetectable levels (as measured with the R5 competitive ELISA method) by treating wort in fermentation with PSEP (assuming the dosage and contact time are appropriate) (Fig 3) (2).

Materials and Method

A selection of beers available commercially (craft and non-craft by US Brewers Association definition) were tested for gluten content using a ELISA competitive assay with R5 antibody from R-Biopharm (Dramstadt, Germany). This method is approved by the ASBC and AACCI.
Each beer sample was extracted 2 times according to the kit manufacturer and measured 3 times in a microplate reader. The average gliadin content is expressed in ppm and the standard deviation of the 6 readings is stated. It is recommended to double the gliadin concentration to achieve gluten content; however in case of barley hordeins the factor of two largely overestimates the content therefore the results are displayed in gliadin levels. The limit of quantification of the kit is 5ppm gliadin.
The classification of gluten-free beer (orange in the table) is based on a content of gluten < 20ppm as recommended by the Codex Alimentarius.

Code	Beer Type	Gliadin (ppm)
EE	Pale Ale*	<5
FF	American Lager*	<5
GG	Brown Ale	<5
HH	Amber Ale	<5
II	European Lager*	<5
JJ	European Lager*	<5
KK	European Lager*	<5

Table 2: American and European Beers available commercially where PSEP is used in the beermaking process. Dosage is proprietary. *Non-craft beers

Epitope Glia 31-43

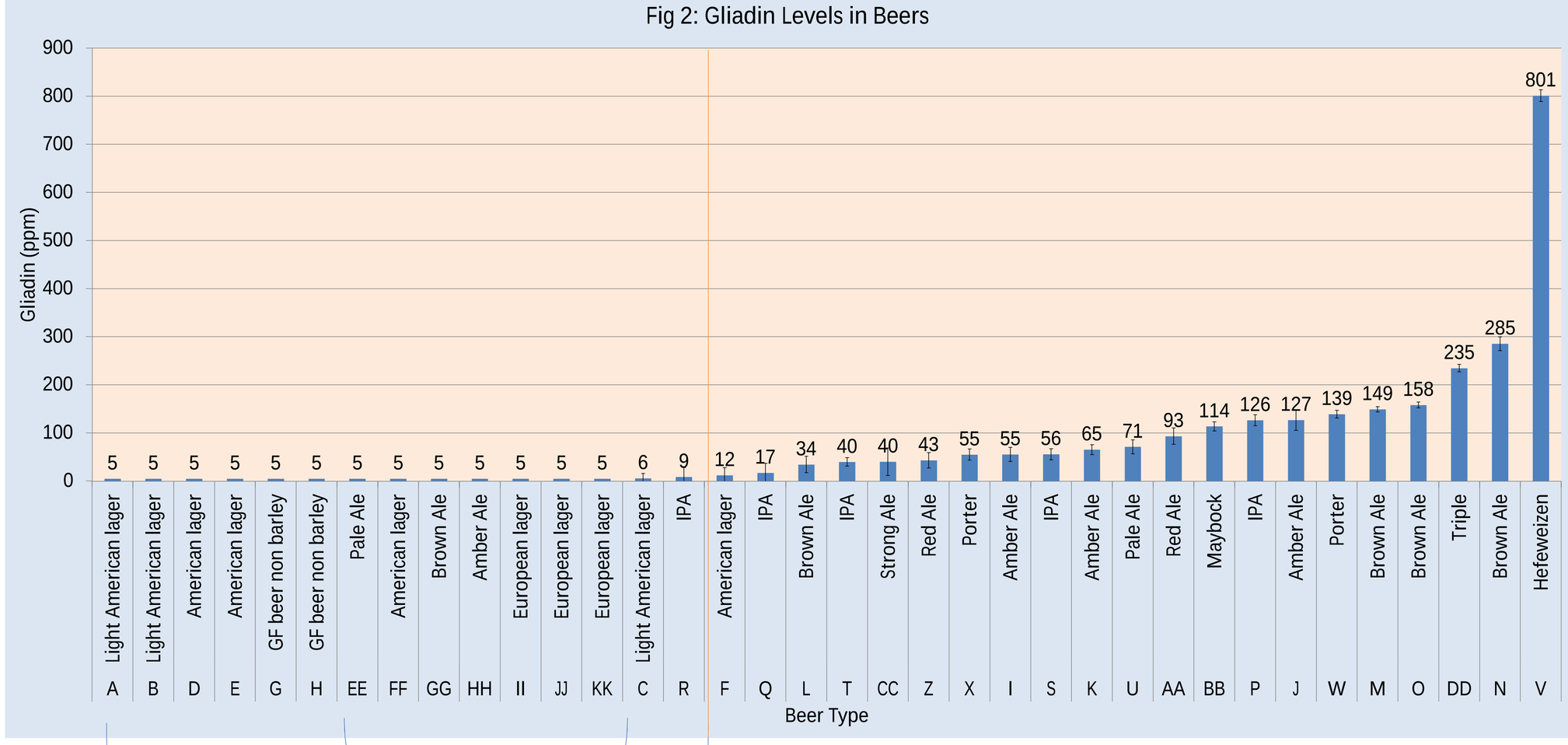
LGQQQP↓FPPQQP↓YP↓QPQPF

PSEP Cleavage site = ↓

QQPFP = target for the R5 antibody

Fig 1: Cleavage site of PSEP in a T cell stimulatory gluten peptide with repetitive motive QQPFP (3).

Fig 2: Gliadin Levels in Beers



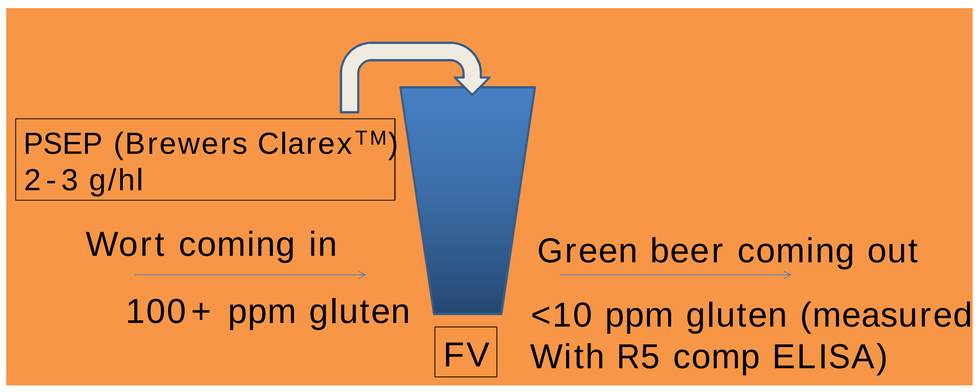


Fig 3: PSEP (Brewers Clarex™) usage during the brewing process

Results and Discussion

Levels of gliadin were measured in a selection of American beers craft and non craft (Table 1). Industrial light American lagers and 2 of the American lager tested displayed less than 10ppm gliadin and therefore qualify to be gluten free under the Codex Alimentarius recommendations of <20ppm gluten (assuming gluten = 2X gliadin); typically those beers contain only 60% barley malt. The two GF beers made with sorghum scored undetectable gliadin (<5ppm) with the R5 ELISA comp method. All beers treated with PSEP were similarly under the quantification limit (LOQ) of the kit (Table 2). For the beers that scored above 10ppm gliadin – the levels varied depending on the style of beer and the brewery that produced it (Figure 2). Indeed, the brewing process influences the levels of protein that will remain in the beer at the end and levels of gluten will vary according to the factors described below. This would explain the wide variation in gliadin between brown ale N (285ppm), brown ale M (149ppm) and brown ale L (34ppm). The hefeweizen beer V scored the highest amount of gliadin (801ppm); this is due to the content of gluten being higher in wheat compared to barley.
Factors influencing gluten levels:

- % malt in recipe (more malt more gluten)
- Type of malt (some malts have lower gluten levels)
- The original gravity of the beer (the higher the OG the more gluten)
- The use of stabilizers (tannins, SHG, PSEP will reduce the amount of gluten)
- The use of wheat in recipe (there is more gluten in wheat than barley)
- The use of process equipment that reduce proteins (centrifuge, whirlpool, filtration)

References
(1) Guerdrum & Bamforth 2011
(2) Guerdrum & Bamforth 2012
(3) Stepniak *et al* 2006

Conclusions

PSEP offers an opportunity to produce a variety of GF beers which meet the flavor expectations of the consumer. Dosage and usage during the brewing process need to be optimized and levels of gluten carefully monitored with the R5 Competitive ELISA method.