



Determination of
Prolamin Concentrations
in Malt Beverages using
the RIDASCREEN®
Gliadin Competitive
Enzyme Immunoassay

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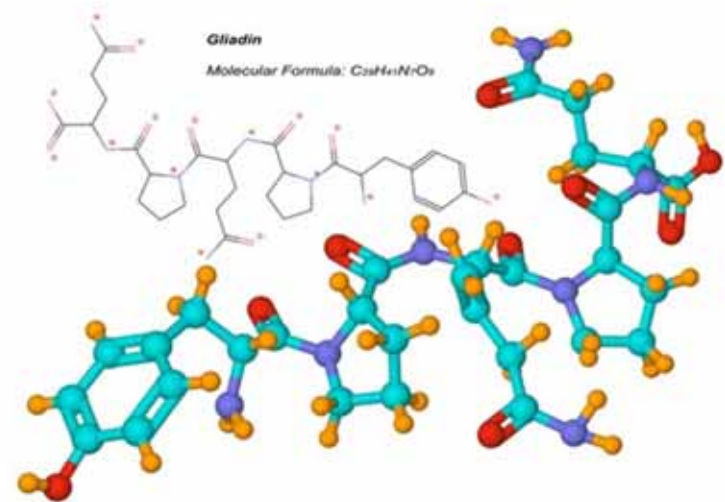
PURPOSE

The purpose of this study was to quantify gluten content in beers made with standard and gluten-free grains using the RIDASCREEN® Gliadin competitive enzyme immunoassay and to determine the suitability of gluten-free grains for fermentability and flavor characteristics.



WHAT IS PROLAMIN?

- 50 % of gluten proteins are prolamins
- Called gliadin in wheat; hordein in barley
- Molecules are partially degraded into small toxic peptide fragments



FDA REGULATIONS

20 ppm or less:

- “gluten-free”
- “free of gluten”
- “without gluten”

Certified



Gluten-Free



TEST KIT

Ridascreen® Gliadin competitive enzyme immunoassay

- limit of detection is 1.36 mg prolamin / kg food
- lower limit of quantification is 5 mg prolamin / kg food
- upper limit of quantification is 135 mg prolamin / kg food.
- Absorbance measured at 450 nm
- Total gluten is calculated with a conversion factor of 2 based on prolamin concentration

TESTED FOR PROLAMIN

Gluten-containing beers:

- Light lager
- IPA
- Wheat beer

Gluten-free beers:

- Red lager (sorghum)
- Blonde ale (sorghum)
- Pale ale (enzymatically degraded barley)



TESTED FOR PROLAMIN

Ferments produced in lab:

- Sorghum with enzyme cocktail
- Sorghum without enzyme cocktail
- Coarsely ground barley
- Finely ground barley



METHOD

Fermentation:

- 1.25 qts water/lb grain
- 2.5 kg Onda Pro enzyme/1000 kg grain
- 1-hour mash at 65 °C
- 30-min boil with Warrior hops
- 11.5 g Safale US-05 yeast/20 L water



METHOD

Sample Preparation:

- 1.00 mL dissolved in 60 % ethanol solution containing 10 % fish gelatin liquid.
- Centrifugation
- Supernatant added to sample diluent

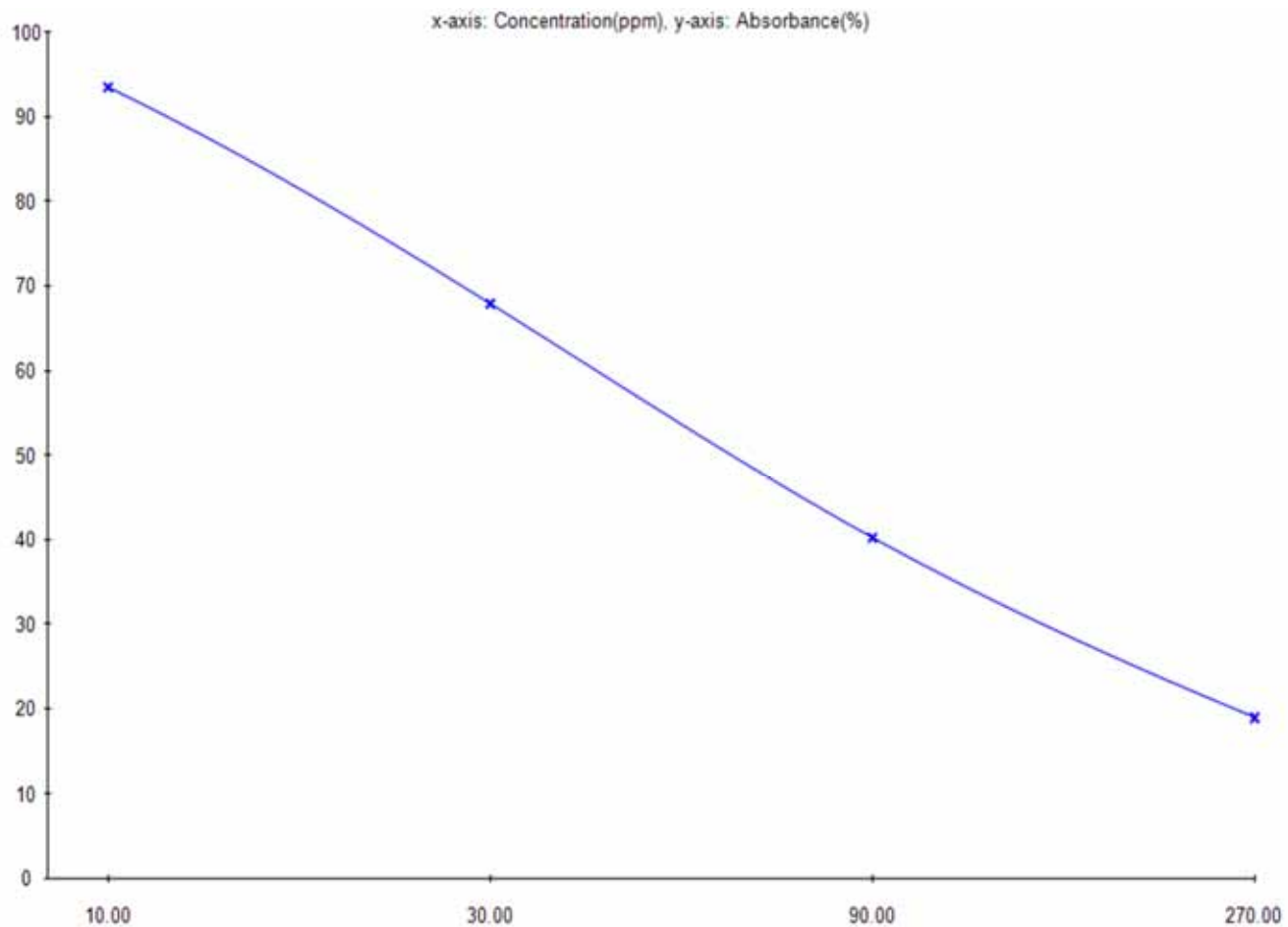


TEST IMPLEMENTATION

- 50 μ L sample/standard
- R5 antibody enzyme conjugate
- 30-minute incubation
- Washing buffer
- Substrate/chromagen
- 10-minute incubation
- Stop reagent



RESULTS



Spline

Standards Conc. (ppm) / A(Mean)

10.00 0.793
(Coeff. of Var.: 0.0%)

30.00 0.576
(Coeff. of Var.: 0.0%)

90.00 0.341
(Coeff. of Var.: 0.0%)

270.00 0.161
(Coeff. of Var.: 0.0%)

50% inhibition: 60.0

RESULTS

Sample	Prolamin Concentration (ppm)	Gluten Concentration (ppm)
Light lager	36.30	72.60
IPA	39.47	78.94
Wheat beer	>135	>135
Gluten-free red lager (sorghum)	6.31	12.62
Gluten-free blonde ale (sorghum)	5.39	10.78
Gluten-free pale ale (barley)	<5	Not quantifiable
Sorghum ferment with enzyme	9.94	19.88
Sorghum ferment no enzyme	8.93	17.86
Coarse grind barley ferment	12.73	25.46
Fine grind barley ferment	105.9	211.8

Nitrogen Combustion Analysis

Local 2-row pale malt

PerkinElmer Series II CHNS/O Analyzer 2400

Nitrogen: 1.95 %

Total Proteins: 12.19 %



Protein Analysis

2-row pale malt ferment: 130 ppm gluten

American IPA: >135 ppm prolamin

- 2-row pale malt: 34.7 %
- Pilsener: 34.7 %
- 6-row lager malt: 10.2 %
- Rye malt: 10.2 %
- Wheat malt: 10.2 %

Gluten-Free Cereal Grains

Ferments produced in lab:

- Millet
- Spelt
- Quinoa
- Black/Forbidden Rice
- Wild Rice
- Sorghum



SPECIFIC GRAVITY

Sample	Specific Gravity				Alcohol (%)
	Pre-Boil	Post-Boil	After 3 days	After 7 days	
Millet	1.0017	1.0265	1.0167	1.01643	0.88
Spelt	1.0087	1.0189	1.0087	1.0074	0.32
Quinoa	1.0062	1.0428	1.0301	1.0290	1.69
Wild Rice	1.0301	1.0343	1.0212	1.0207	1.25
Black Rice	1.0293	1.0371	1.0263	1.0259	1.19
Roasted sorghum	1.0157	1.0266	1.0171	1.0165	0.54

Sensory

Sample	Aroma	Flavors
Millet	Sweet pear, apples	Slightly sour apple cider, pear
Spelt	Honey, flour	Bland, watery
Quinoa	Oak, vanilla	Sweet vanilla with pleasant bitter aftertaste
Wild Rice	Strawberry breakfast bar, spicy	Sweet, minty, strawberry
Black Rice	Raspberry, strawberry, blackberry	Raspberry, fruity
Roasted Sorghum	Roasted coffee, burnt wood	Acidic, roasted coffee

MILLET BEER

- 15-gallon batch
- 25 lbs grain
- Gelatinized at 170 °F for 1 hour
- Added CaCl_2 , enzyme
- Mashed at 149 °F for 1 hour
- Boiled for 90 minutes
 - East Kent Golding Hops added at 60 min



MILLET BEER

- Initial gravity 1.046 (14 April)
- Final gravity 1.023 (28 April)
- Alcohol: 3.2 %
- Visual: cloudy, hazy, pale yellow
- Green apple, pear, cider aromas
- Sweet with sour after taste
- Green apple, pear, sour cider flavors



Future Brews

- Millet with apple for a grain cider
- Wild rice with local strawberry fruit
- Black rice with local raspberry/strawberry
- A mixture of quinoa and roasted sorghum



Conclusions

- All commercially available gluten-free beers contained 20 ppm or less of gluten
- The wheat beer exceeded the upper quantification limit of 135 ppm prolamin
- The two sorghum ferments were gluten free
- The two barley ferments had gluten levels above 20 ppm
- Local 2-row pale malt had high protein and gluten concentration
- Gluten-free grains show promise from a fermentability and sensory standpoint

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