



WORLD BREWING CONGRESS

August 13–17, 2016 • Denver, Colorado, U.S.A.

#ElevateBeer

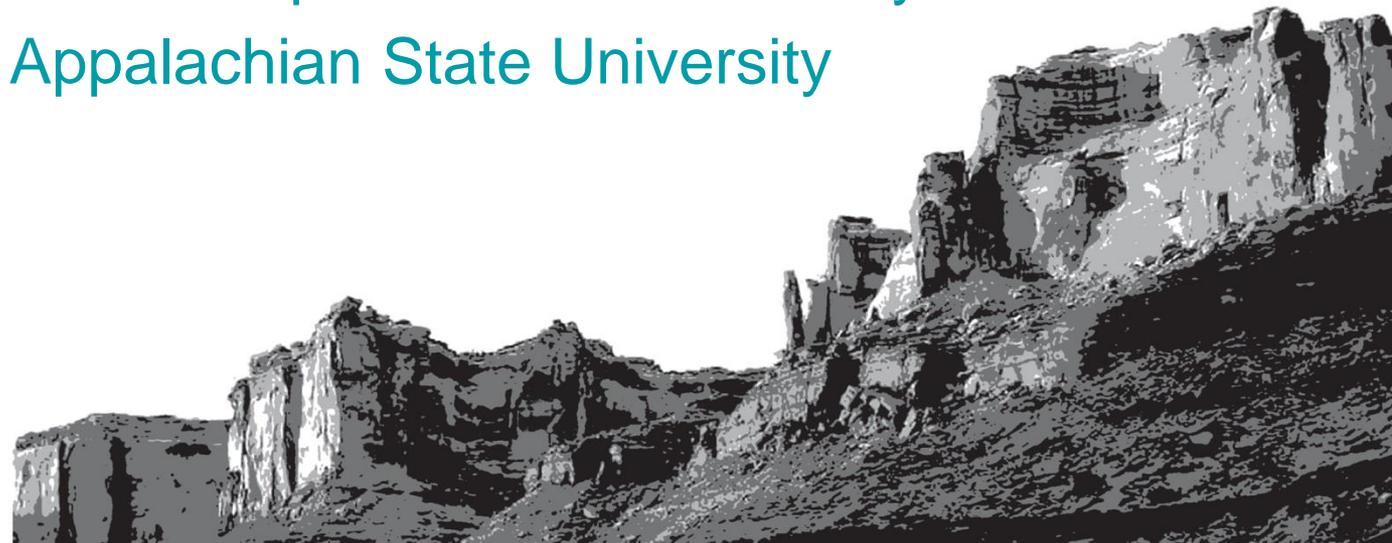


An investigation of process controls and microbes for the degradation of gluten proteins in wort

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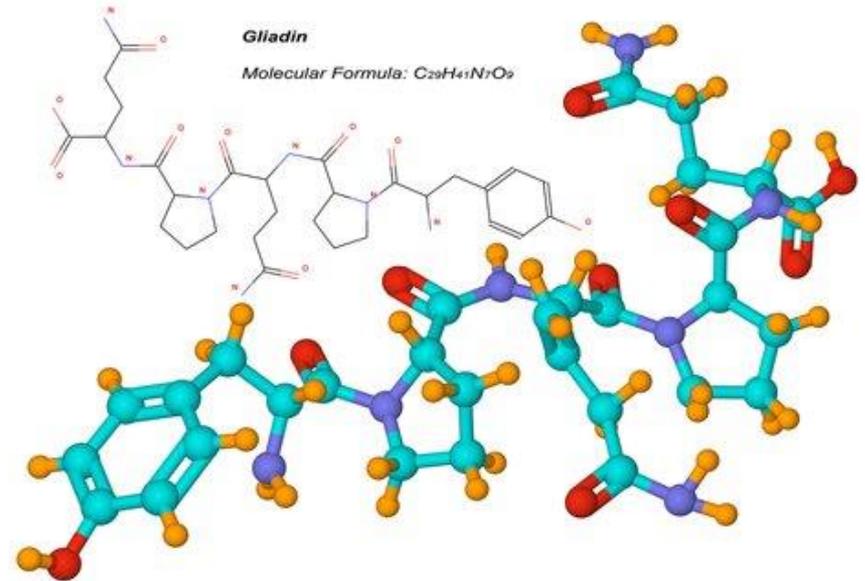
Purpose

The purpose of this study was to investigate the efficacy of using certain lactic acid bacteria to lower gluten concentrations during fermentation as well as how parameters in the brewhouse may be adjusted to lower gluten concentrations in the final product.



What is Prolamin?

- 50% of gluten proteins are monomeric prolamin proteins
- 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”





Gluten Free Beer

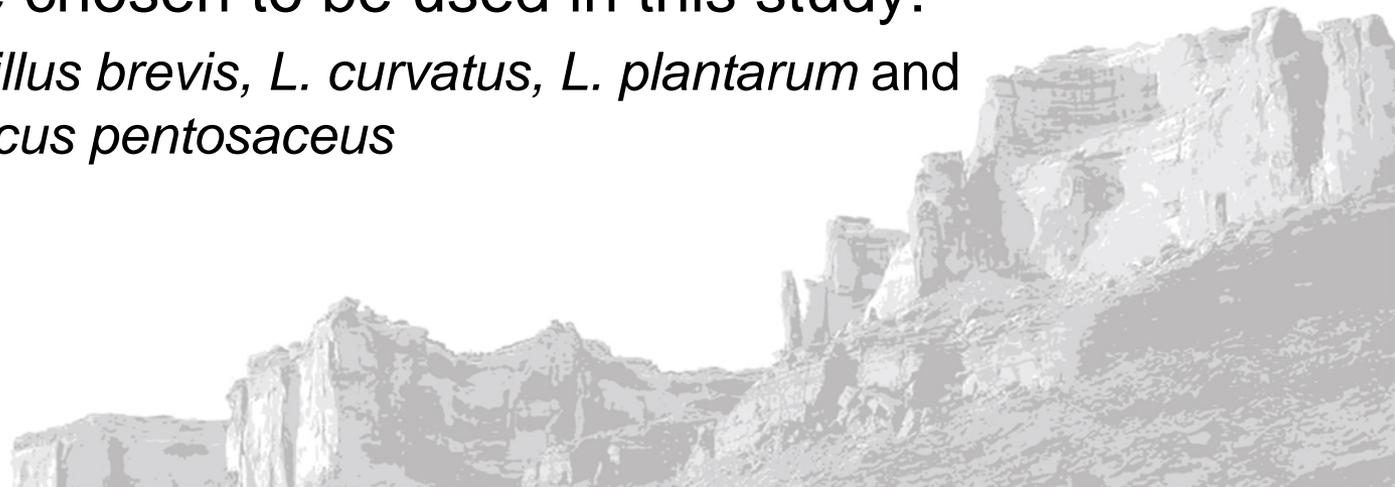
- Two processes to produce gluten free beer:
 - Gluten free grains - sorghum, buckwheat, rice
 - Enzymatic treatment - Brewers Clarex®
- Processes fall under different labeling regulations
- Flavor, foam, mouthfeel and color can be affected





Mixed Culture Fermentations

- Diverse flora of bacteria and yeast present in many mixed culture fermentations
- In a study by C.L. Gerez *et al.*, bacteria found in sourdough bread were tested to determine if any of the strains would grow on a gluten based medium
- Five strains were capable of growing, four of which were chosen to be used in this study:
 - *Lactobacillus brevis*, *L. curvatus*, *L. plantarum* and *Pediococcus pentosaceus*





Test Kit

Ridascreen® Gliadin competitive enzyme immunoassay

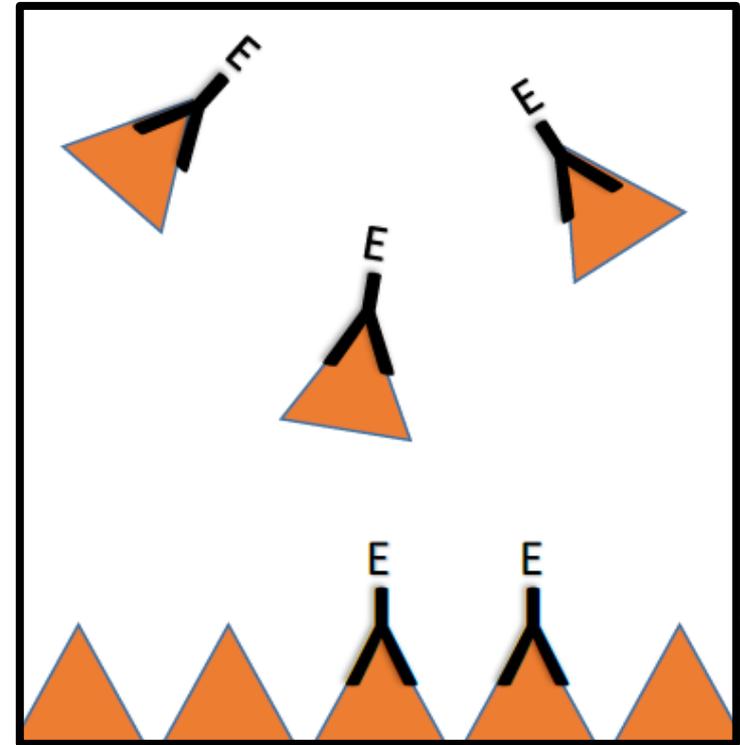
- LOD is 1.36 mg prolamin / kg
- Lower LOQ is 5 mg prolamin / kg
- Upper LOQ is 135 mg prolamin / kg
- Absorbance measured at 450 nm
- Total gluten is calculated with a conversion factor of 2 based on prolamin concentration





Measuring Gluten In Samples

- Uses R5 monoclonal antibody to recognize the sequence QQPFP, which occurs repeatedly in the prolamin molecules
- The enzyme labeled antibody competes for binding to either a solid state antigen that is bound to the walls of microplate wells or to free floating antigen
- A chromagen reacts with bound conjugate to produce color - absorbance measured at 450 nm



Gluten Content Of Selected Beers



Beer	Style	Country	Gluten (ppm)
Cuvee Freddy	Flemish Sour	Belgium	126
Sigma	Dark Sour	Belgium	194
St Louis Peach	Lambic	Belgium	194
Wheat IPA	IPA	US	154
Mad Beach	American Wheat	US	>270
Tell Tale Tart	Slight Sour	US	132
Pilgrims Dole	Wheatwine	US	>270
Oblivion	Sour Red	US	30
Black Angel	Black Sour	US	26
Rolling Rock	American Lager	US	38
The Commodore	American Stout	US	88
Hoptimist IPA	IPA	US	48
St Peters Sorghum	Sorghum	England	<10
Oude Kriek Vieille	Lambic	Belgium	<10
Showdown Rye	Rye IPA	US	<10



Bacterial Ferments

The Beers:

Trial 1

2 hL batch

60% Wheat Malt

35% Maris Otter

5% CaraMalt

OG = 1.058

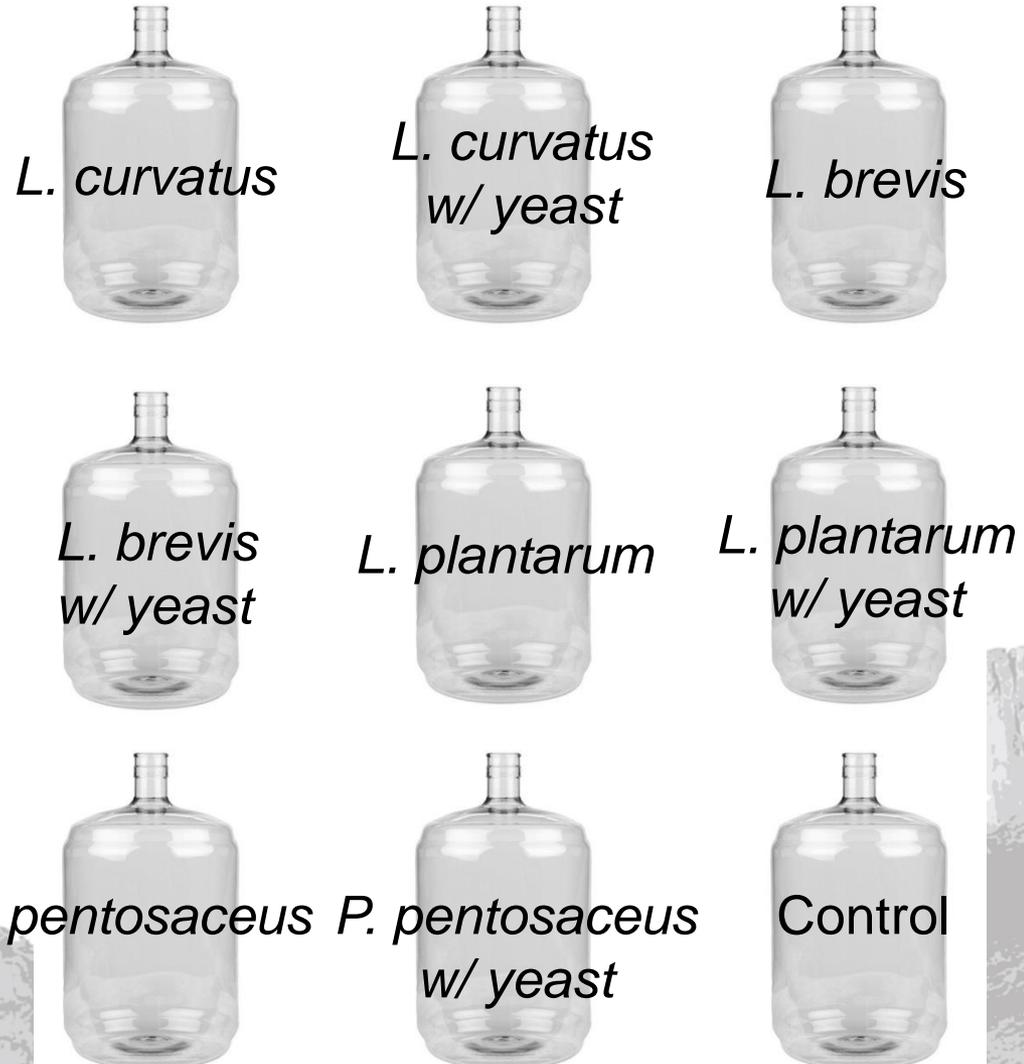
Trial 2

2 hL batch

50% Wheat Malt

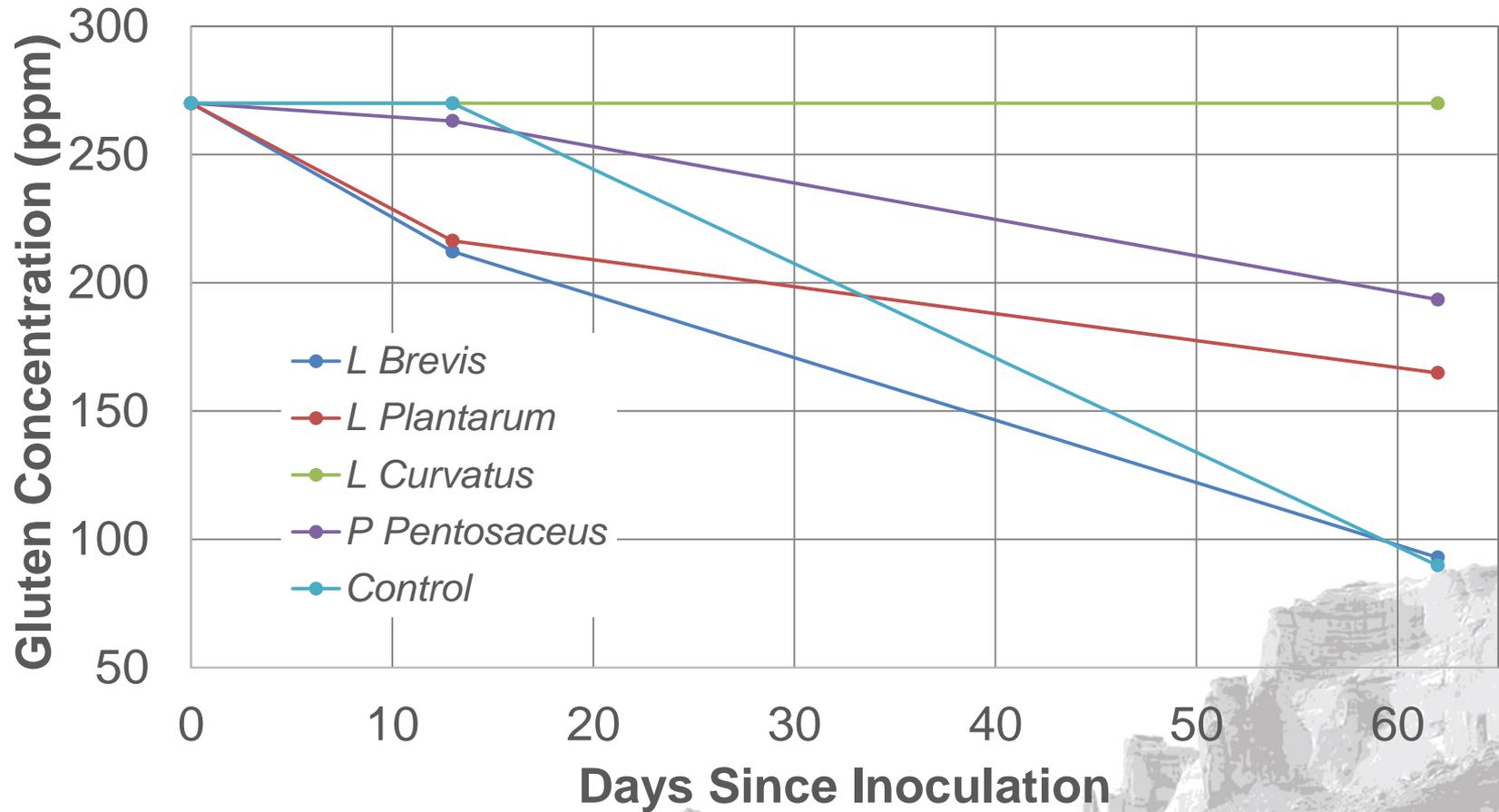
50% Pilsner Malt

OG = 1.063

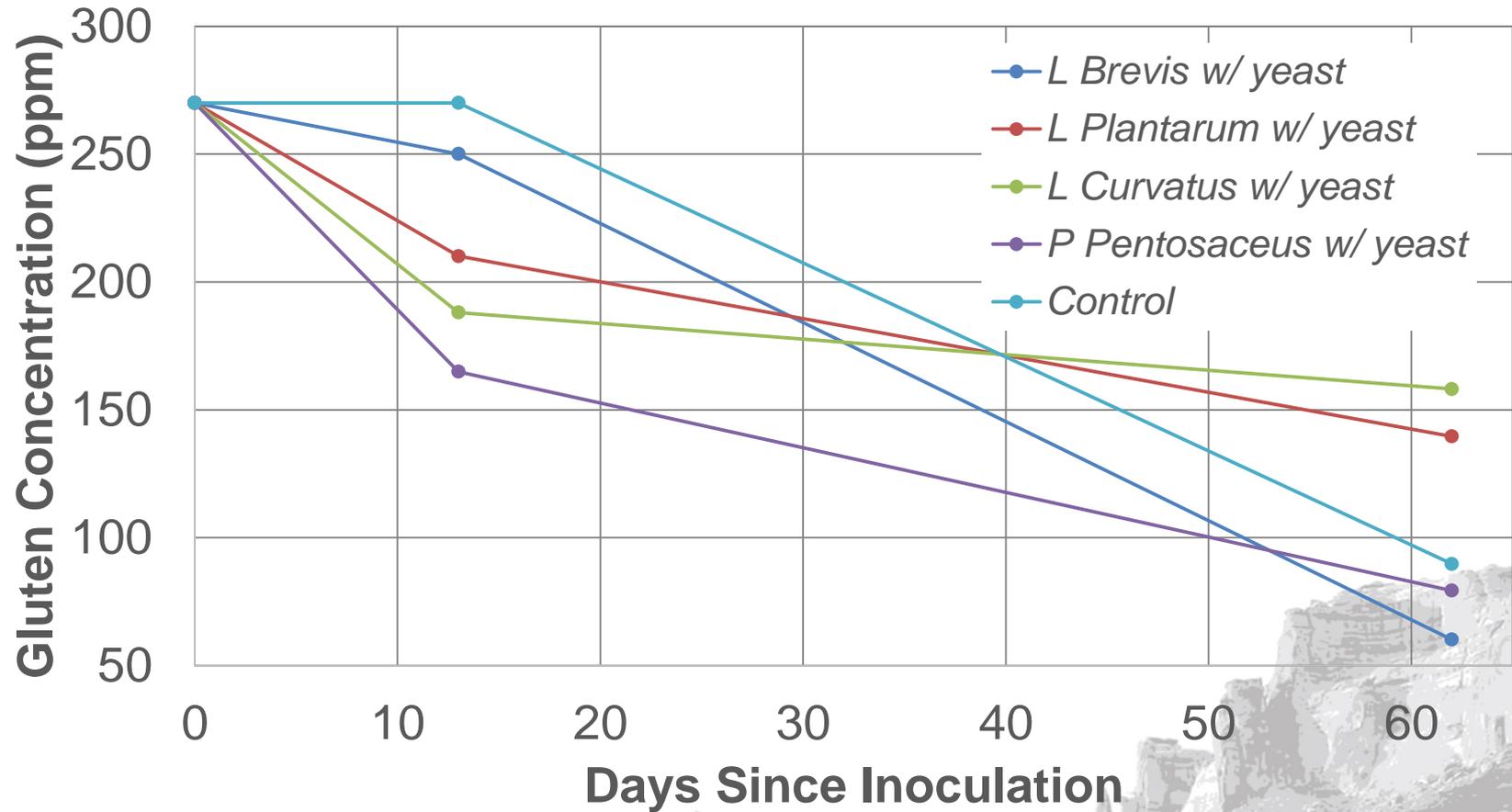




Bacterial Ferments Trial 1

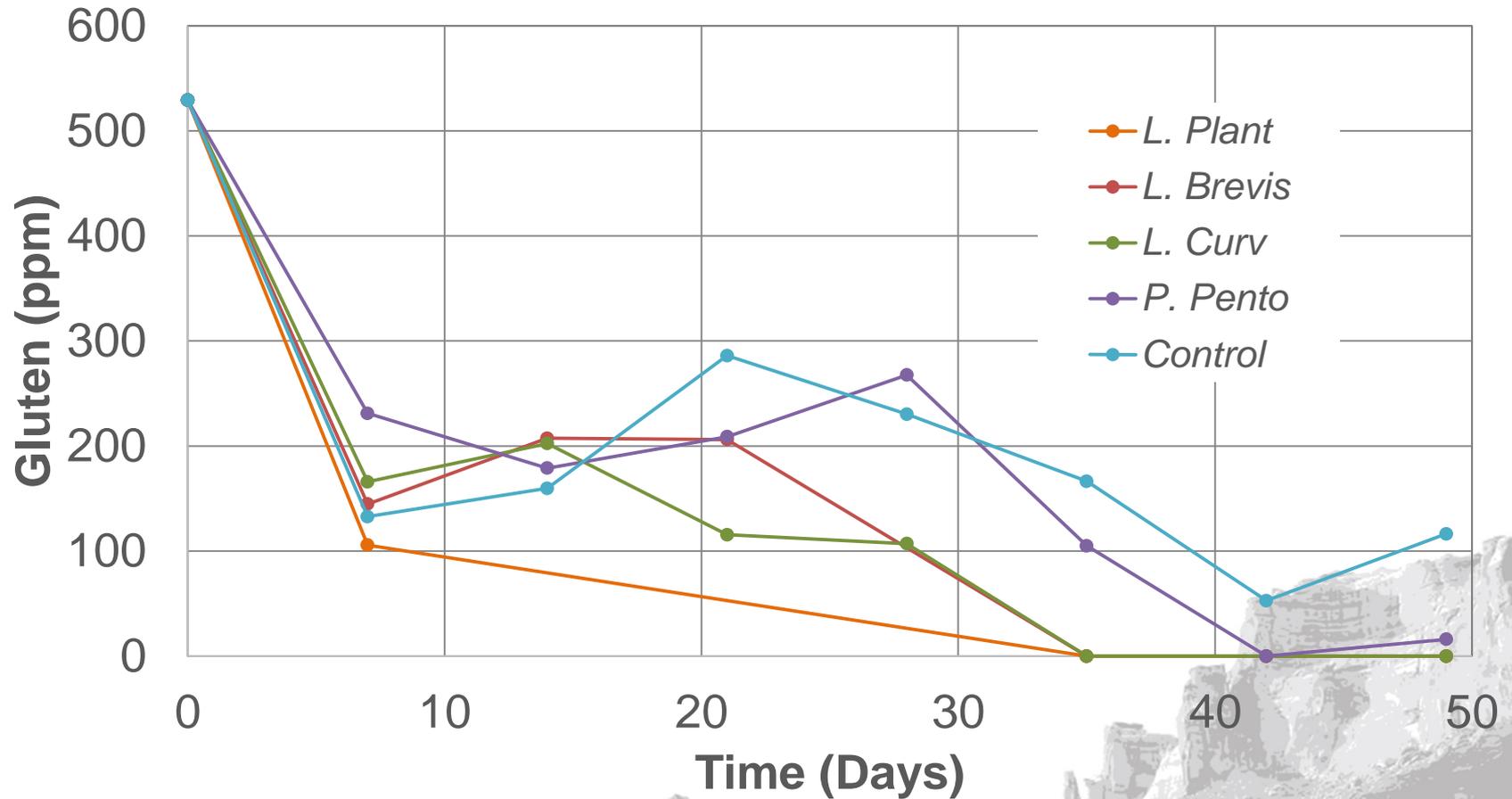


Bacteria and Yeast Trial 1

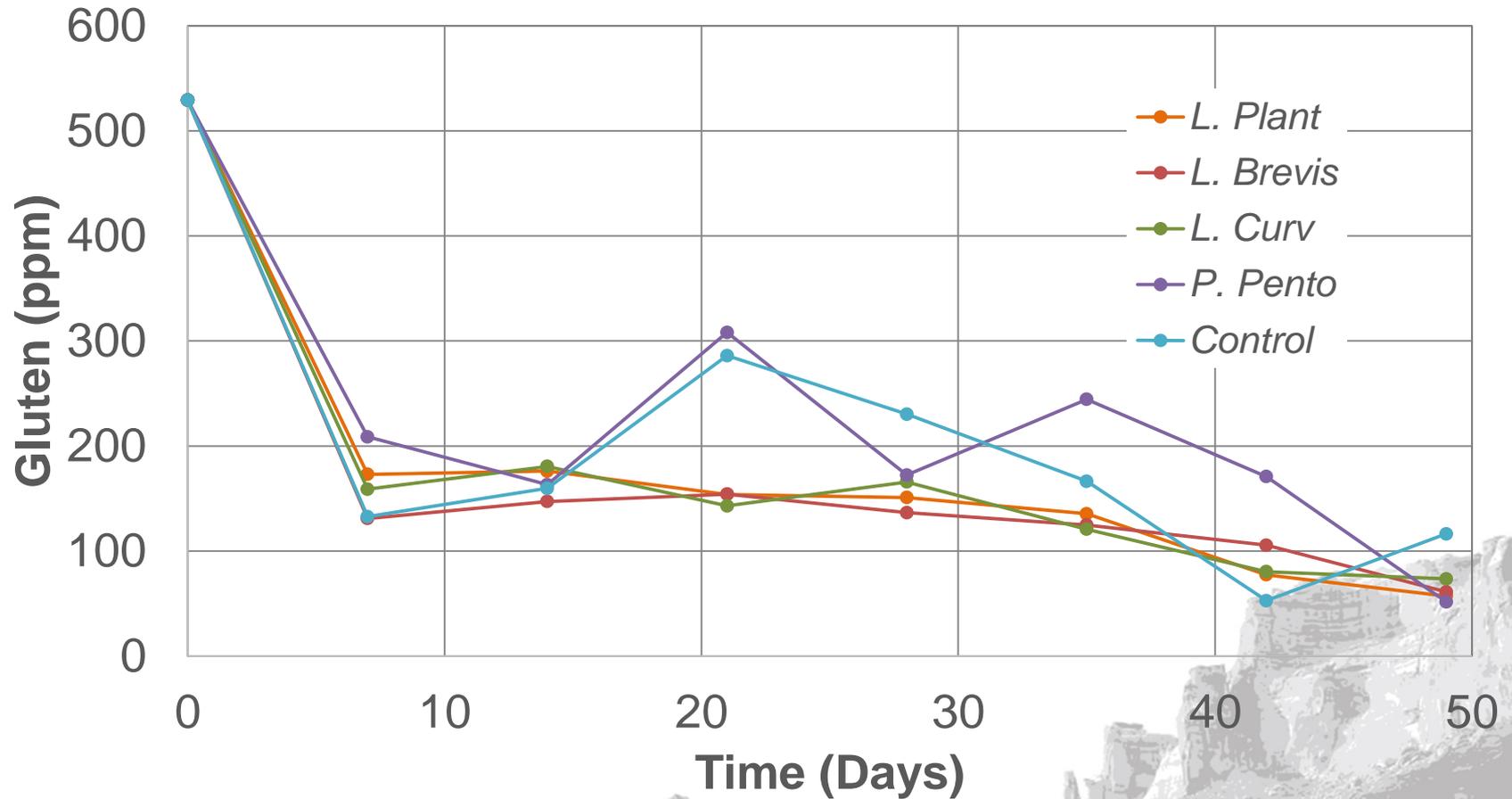




Bacterial Ferments Trial 2



Bacteria and Yeast Trial 2





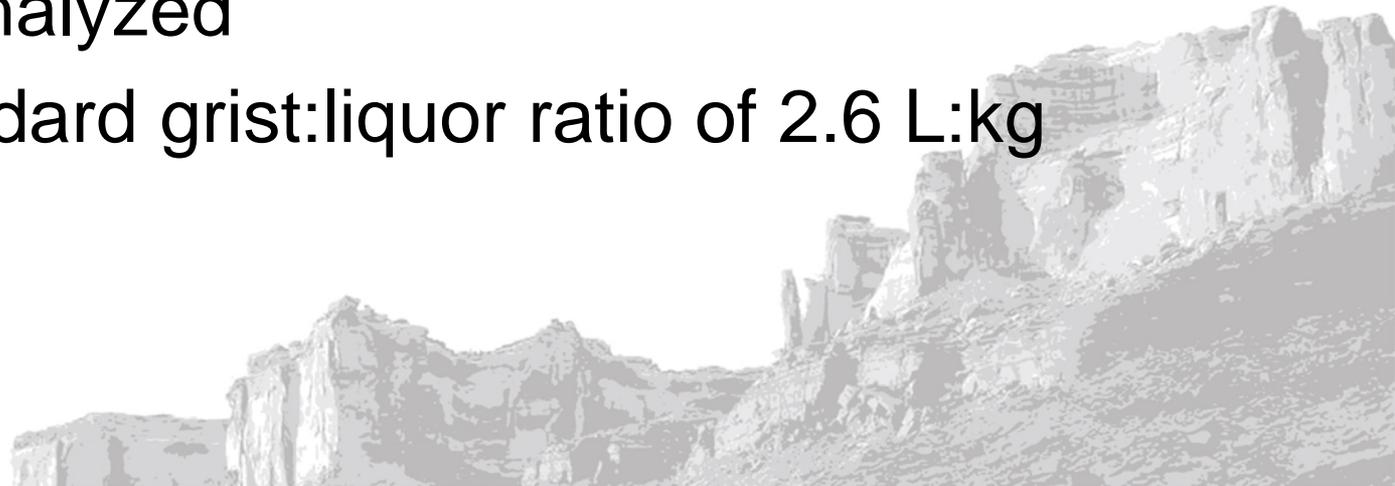
Mixed Fermentation Results

Sample	ABV (%v/v)	Density (g/cm ³)	pH
Initial Wort	-	1.063	5.30
Control	8.59	1.002	4.33
<i>L. plantarum</i>	8.22	1.001	4.31
<i>L. plantarum</i> w/ Yeast	8.33	1.004	4.55
<i>L. brevis</i>	7.91	1.002	4.39
<i>L. brevis</i> w/ Yeast	7.99	1.007	4.39
<i>L. curvatus</i>	7.99	1.003	4.38
<i>L. curvatus</i> w/ Yeast	8.60	1.003	4.51
<i>P. pentosaceus</i>	8.10	1.002	4.30
<i>P. pentosaceus</i> w/ Yeast	8.52	1.003	4.40



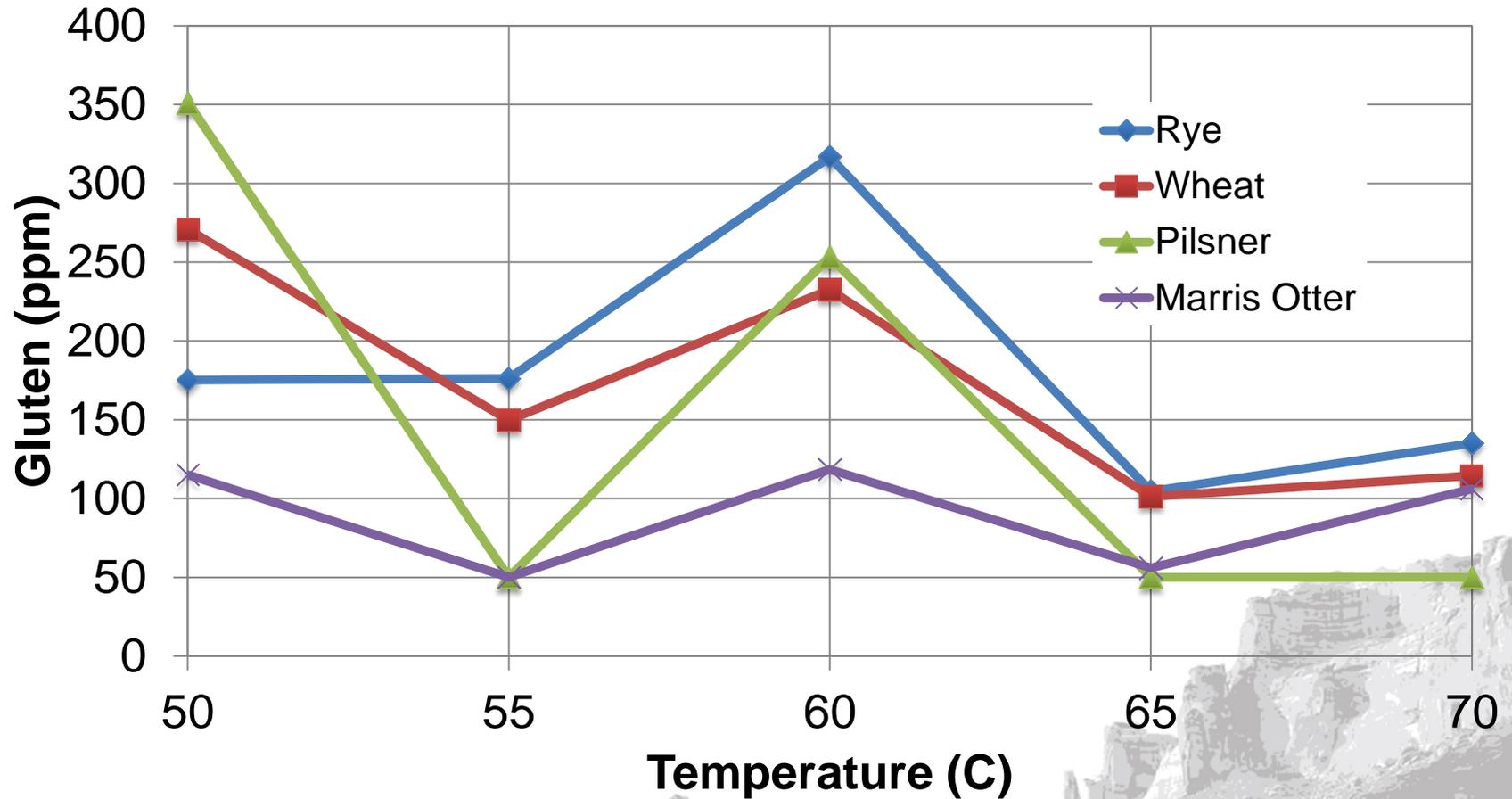
Manipulating Mash Parameters

- pH, temperature, and grist:liquor ratio were all manipulated to determine their effect on gluten in the wort
 - pH and grist:liquor were manipulated for 60 min mash at 65 °C
- Temperatures of 50, 55, 60, 65, and 70 °C were analyzed
 - Standard grist:liquor ratio of 2.6 L:kg



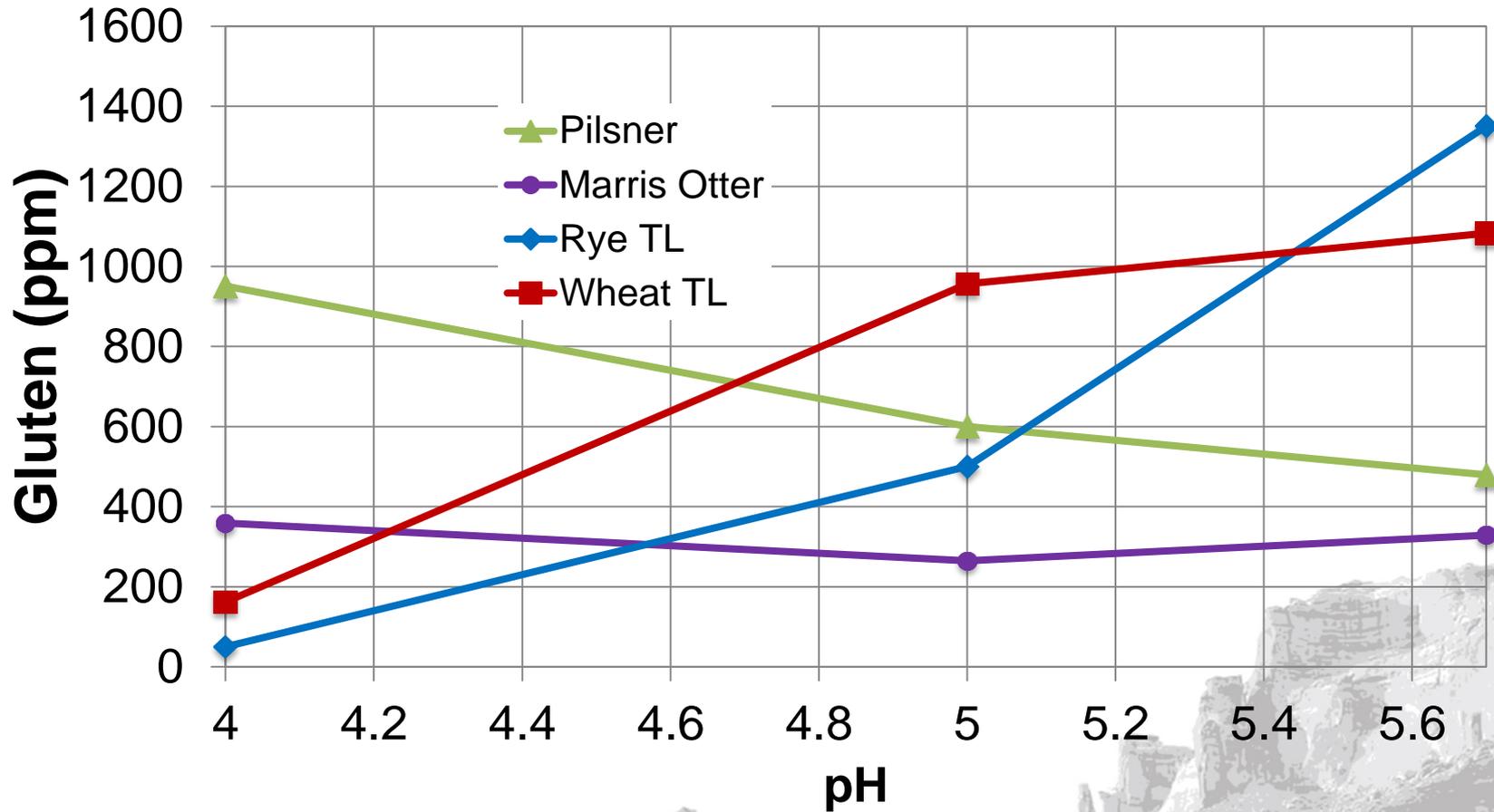


Mash Temperature



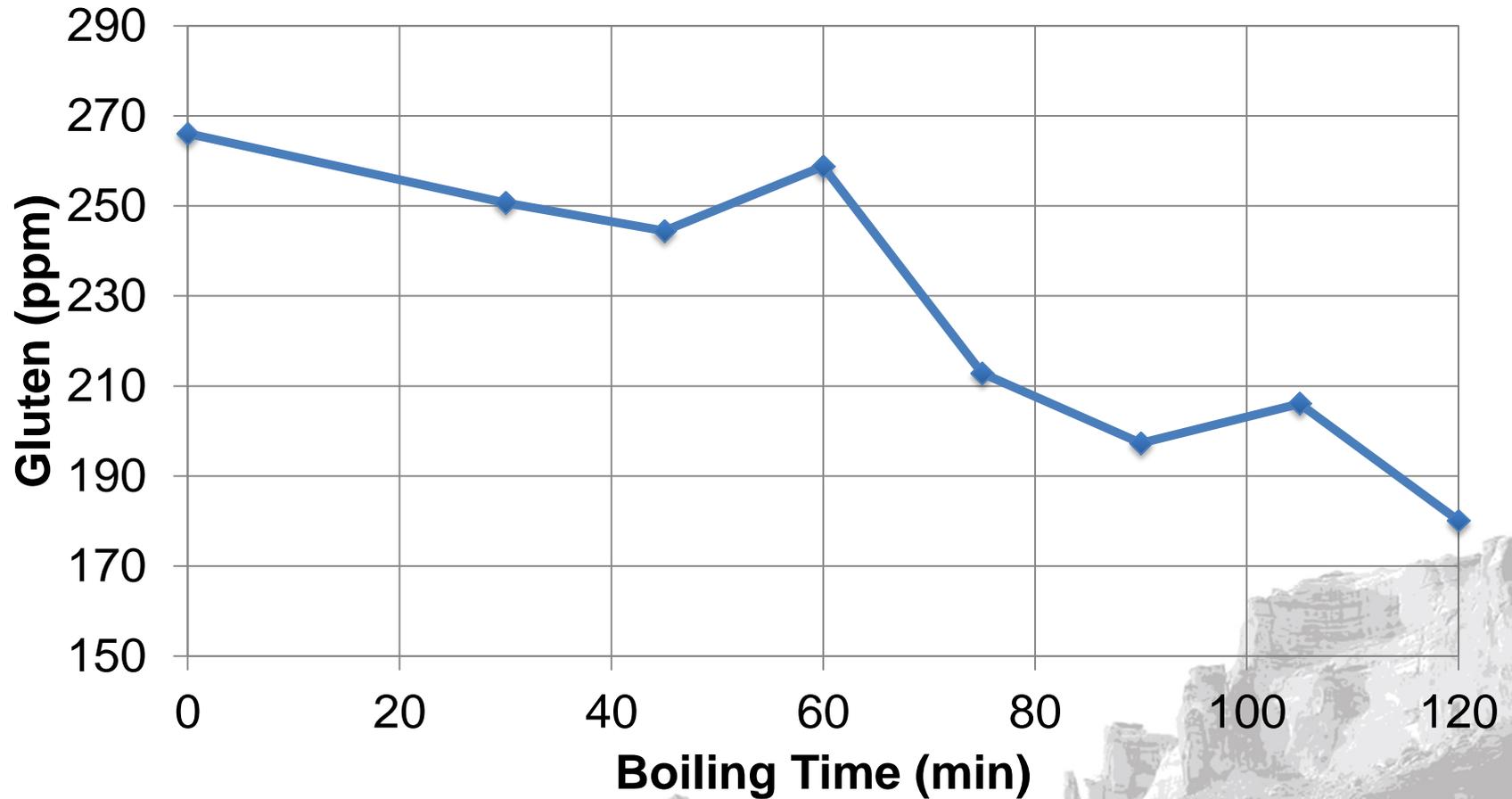


Mash pH



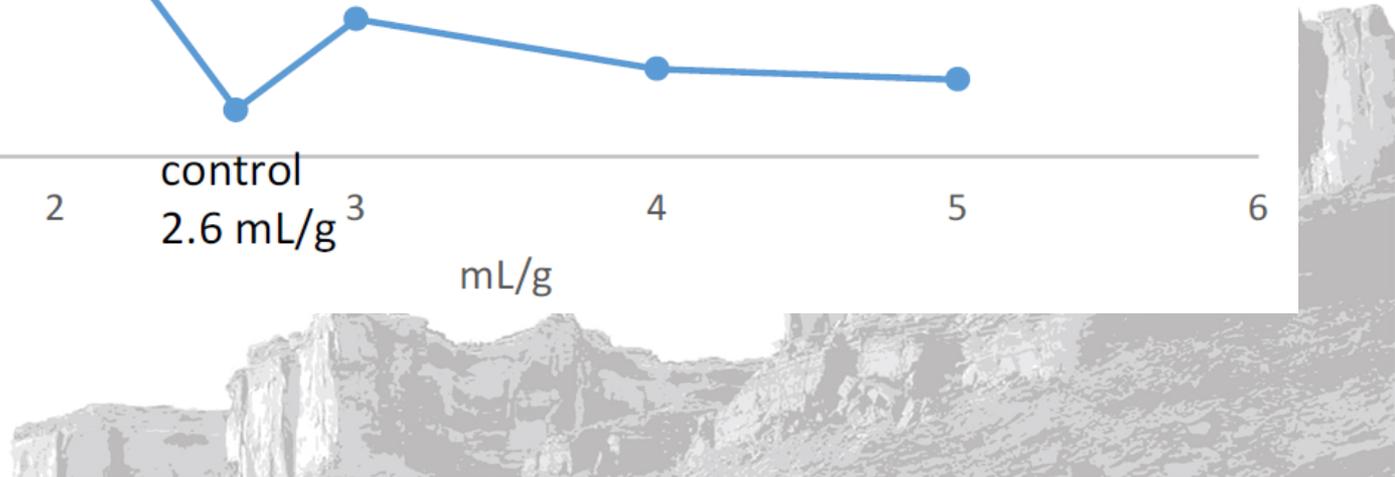
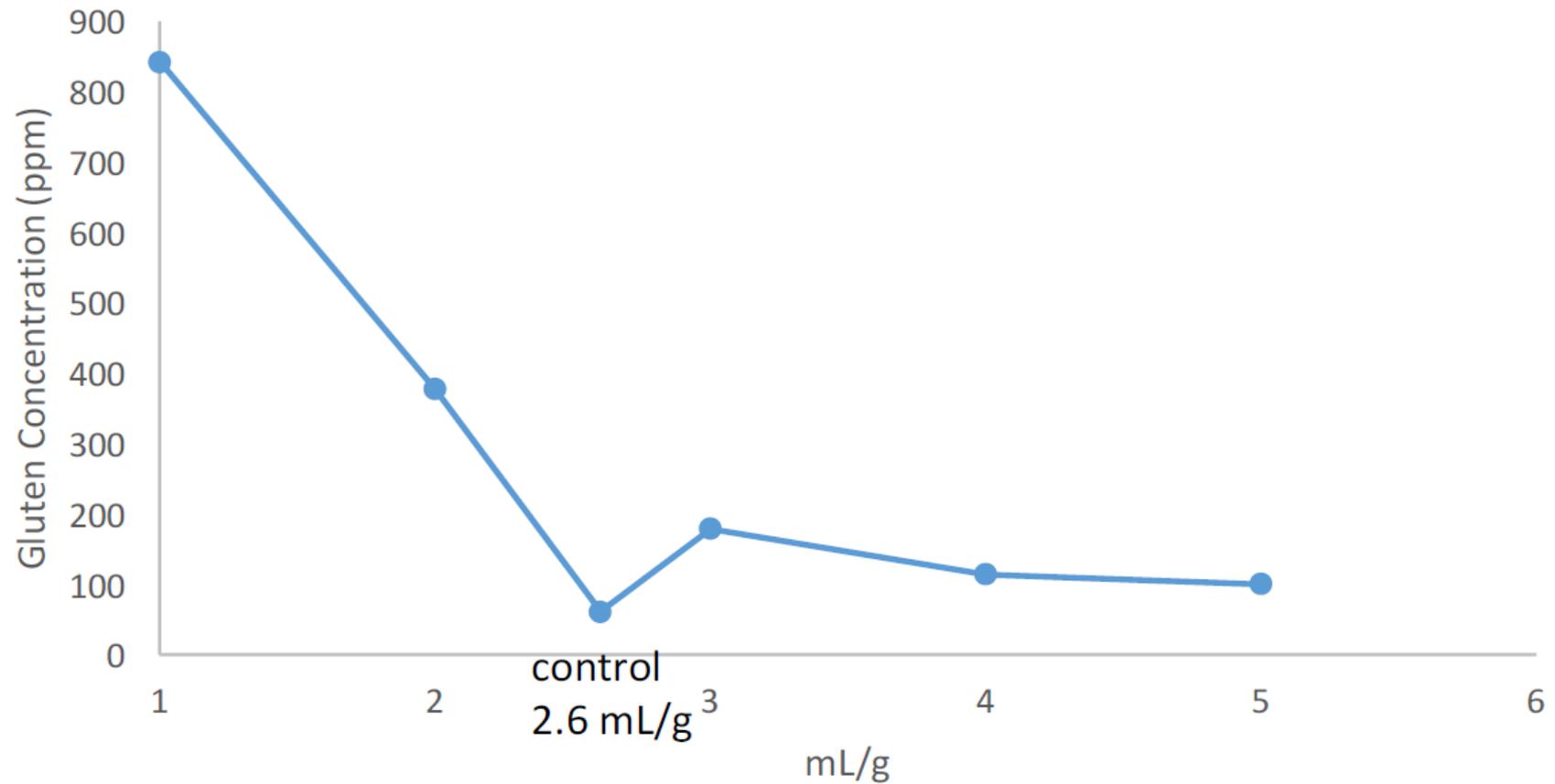


Boiling Time





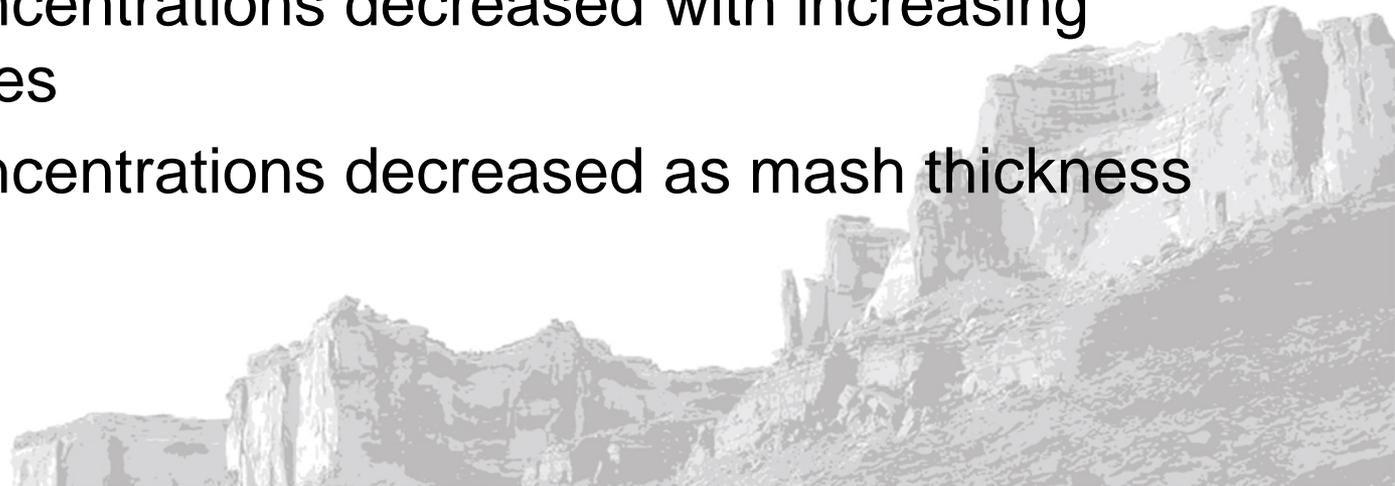
Mash Thickness





Conclusions

- Some beers tested were already reduced gluten
- The bacteria appear successful in lowering the gluten concentration...in second trial
- 55 and 65 °C showed lowest gluten concentrations for all grains
- Gluten increased with pH for wheat and rye and decreased with increasing pH for barley malts
- Gluten concentrations decreased with increasing boiling times
- Gluten concentrations decreased as mash thickness decreased





Acknowledgements

Appalachian
STATE UNIVERSITY
BOONE, NORTH CAROLINA

The people who did the work:
Travis Laws Jake Edwards Taylor Smith



The people who funded the work:

The Office of Student Research,
Appalachian State University

