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Characterization of Alternative Grains Used in the Brewing Process and Analysis of Product Parameters

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Appendix C

RESEARCH / CREATIVE PROJECT ABSTRACT / EXECUTIVE SUMMARY
FINAL REPORT FORM

Title of Project
Characterization of alternative grains used in the brewing process and analysis of product parameters

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Abstract
In recent months, an interest has arose in whether or not gluten free beers due in fact have gluten in them. During the first part of my research, a few gluten free beers were tested using an ELIZA (EZ Gluten) test to determine if there was any gluten in the beer (this test would be used later on the grains). The results showed that the gluten free beers did in fact not have gluten in them. After testing the beers, I will utilize alternate grains (sorghum, rice, corn, quinoa) to determine if any gluten is extracted during the fermentation process. During the process, standard tests used during the brewing process will be used on the alternative grain fermentation to analyze that the grains are a plausible alternative to standard grains used (barley, malt, etc.). The tests to be run include hydrometer readings to test specific gravity, a Brix test to determine sugar content and consumption, and a total protein assay to determine the proteins extracted by the fermentation process.

The end product of this project in electronic format has been submitted to the Provost/Vice President for Academic Affairs via the Office of Grants & Sponsored Projects Officer (Maxwell 161, npeterson@winona.edu).

Student Signature ______________________ Date ____________

Faculty Sponsor Signature ______________________ Date ____________

Characterization of alternative grains used in the berwing process and analysis of product parameters

Grant Newton and Francis M. Mann

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Introduction

In recent months, interests have risen in whether or not gluten-free beers do in fact contain gluten in them. Gluten can have a serious effect on patients diagnosed with Celiac Disease because their bodies cannot digest the long, elastic proteins. To be considered gluten-free and safe for consumption for Celiac patients, a product must have a gluten content below 20 ppm. Fermented beverages, such as beer or wine, are advancing in their research to find and create products for everyone to enjoy, including people who cannot have gluten. Typical grains used in the brewing process including barley and wheat, both contain high amounts of gluten. The few gluten-free beers on the market today, use grains such as sorghum and rice, although there are other grains that do not contain gluten that could be used.

The main purpose of the research is to investigate and analyse some alternative grains used in the brewing process. Research was conducted at Winona State University to determine the gluten content in the alternative grains and to determine if they can be a suitable alternative to standard grains that contain gluten. To establish the alternative grains as an acceptable substitute, we analysed the starch and protein consumption of yeast as the samples are fermented. We also wanted to investigate how yeast reacted with the alternative grains, so a growth curve was also created to determine if the yeast reacted positively or negatively in the fermentations. If similarities could be seen, then the alternative grains would be a good substitute for the standard grains that contain gluten.

Methods

Testing of Gluten-Free Beers

- Two gluten free beers, Estrella Damm Daura and New Grist, and one control, Miller Lite, were tested using a simple ELISA or EZ-Gluten test.

Fermentation of Grains

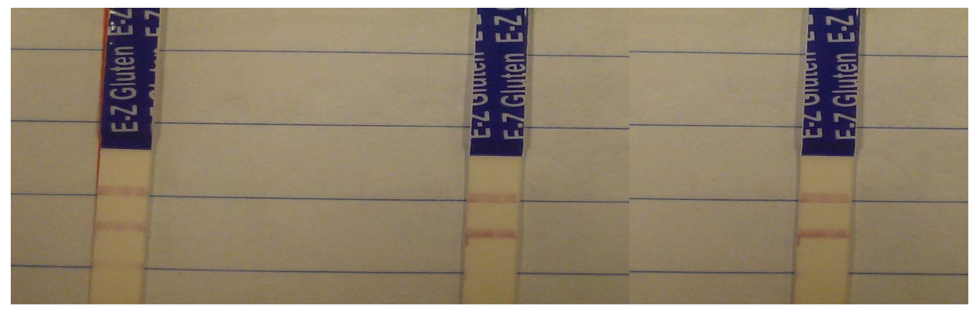
- Three alternative grains assumed to not contain gluten, and one control grain were fermented using a simple Pilsner recipe. The alternative grains were quinoa, rice, and cornmeal. The control was a barley malt.
- The samples were created, and a small portion was collected and frozen to use as a before fermentation sample.
- The rest were put in a 5-gallon carboy and brewer's yeast was added to begin the fermentation.
- Fermentation lasted 8 weeks.

Analysis of Samples

- The first analysis was an ethanol percentage test. 250 mL of each sample before and after fermentation was put into a tall graduated cylinder. Using a hydrometer, the ethanol percentage was calculated by determining the specific gravity of the sample.
- The second test performed was a Bradford Assay, a standard assay in determining protein concentrations in samples. A standard curve was created using a protein standard at concentrations of 0, 5, 10, 12.5, 15, and 20 µg/mL in 250 mM Tris-Hcl buffer. The samples were then diluted using a dilution factor of 1:50. All samples and the standard curve were measured at 595 nm.
- The third test was a Starch concentration assay to determine the amount of soluble starch within the fermented samples. First, a standard curve using a 0.5% starch solution was created by taking specific amounts of the standard solution (0, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.5, 3.5, 5 mL) and diluted to 5 mL. A standard iodine solution was created by taking 2g KI, 40 drops of 3% H_2O_2, and 16 mL HCl (1M). 25 µL of the iodine solution was added to each tube, and the absorbance was measured at 610 nm. 5 mL of each sample was taken, and 25 µL of the iodine solution was added. Again, the absorbance was measured at 610 nm, and the concentration of starch in the sample was determined using the standard curve.
- A yeast growth curve was created to determine the growth of yeast within the sample over a 24 hour period. Each of the samples before fermentation were autoclaved to sterilize the sample. One mL of each sample was added to a microcentrifuge tub, and 0.5 µL of a yeast solution was added. The samples were allowed to sit, and at each time interval (0.5, 1, 2, 4, 6, 8, 24 hours), the sample for that time was put in liquid nitrogen to top yeast growth.
- The last test performed was the ELISA test as performed on the market products.

ELISA (EZ-Gluten) Test on Market Products

Miller Lite Lakefront New Grist Estrella Damm Daura



The first attempt in the ELISA test failed because the extraction solution included in the kit was not used as we followed other instructions we had found elsewhere, not included with the kits. This caused all tests to show up positive. The second attempt we were able to successfully complete the test and prove that the market beers we tested that claimed to be gluten-free were in fact gluten free.

Results

After fermenting the grains for 8 weeks, the first test to be ran was to test the ethanol percentage.

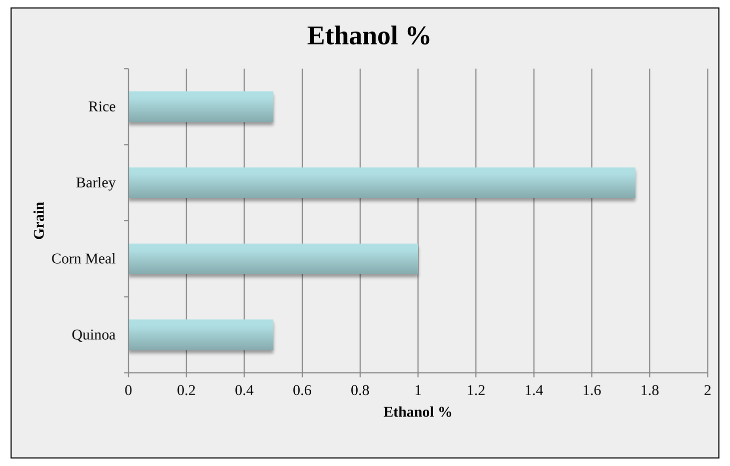


Figure 1: Ethanol Percentage Graph
From the data, the barley (control) created the most ethanol out of the 4 grains.

After performing the ethanol percentage tests, we saw that the percentage of the control was the highest at 1.75%. The three alternative grains had less ethanol with percentages of 0.5%, 1.0%, and 0.5% for rice, Cornmeal, and Quinoa respectively. From the ethanol percentages found, the alternative grains had a smaller percentage of ethanol, and may not be a suitable substitution for grains used in the market today.

After observing the low percentages in the alternative grains, a Bradford assay as well as a Starch assay were performed to investigate the numbers.

Sample w/o yeast	Protein Concentration (Diluted)	Protein Concentration	Amount Consumed
Quinoa	13.80	690.00	626.67
Barley	17.80	890.00	93.33
Rice	7.13	356.67	206.67
Cornmeal	22.20	1110.00	466.67
Sample w/ yeast			
Quinoa	1.27	63.33	
Barley	15.93	796.67	
Rice	3.00	150.00	
Cornmeal	12.87	643.33	

Figure 2: Bradford Assay Data

Using the Bradford standard curve, we were able to calculate the amount of protein consumed during the fermentation process. During fermentation, Barley only consumed 93.33 mg/mL of protein, whereas cornmeal, quinoa, and rice all consumed 466.67, 626.67, and 206.67 mg/mL respectively.

Looking at the Starch standard curve, barley had a starch concentration of 0.211 mg/mL. All three alternative grains all had a starch concentration of below 0.05, over 4 times lower than that of the control. Due to the low amounts of starch in the alternative grains, it's possible to conclude that the yeast began to consume the protein instead of the soluble starch within the grains to produce ethanol.

Sample w/o yeast	Concentration (mg/mL)	Starch Consumed
Quinoa	0.015	0.01
Barley	0.21	0.05
Rice	0.05	0.01
Cornmeal	0.04	-0.26
Sample w/ yeast		
Quinoa	0.00	
Barley	0.17	
Rice	0.03	
Cornmeal	0.30	

Figure 3: Starch Assay Data

Further investigating the low percentages of ethanol formation in the alternative grains, we performed a yeast growth curve for each sample to determine how the yeast reacted within the sample. Each sample was autoclaved and 1 mL was added into a microcentrifuge tube along with 0.5 µL yeast solution. After incubating, the following graph was created.

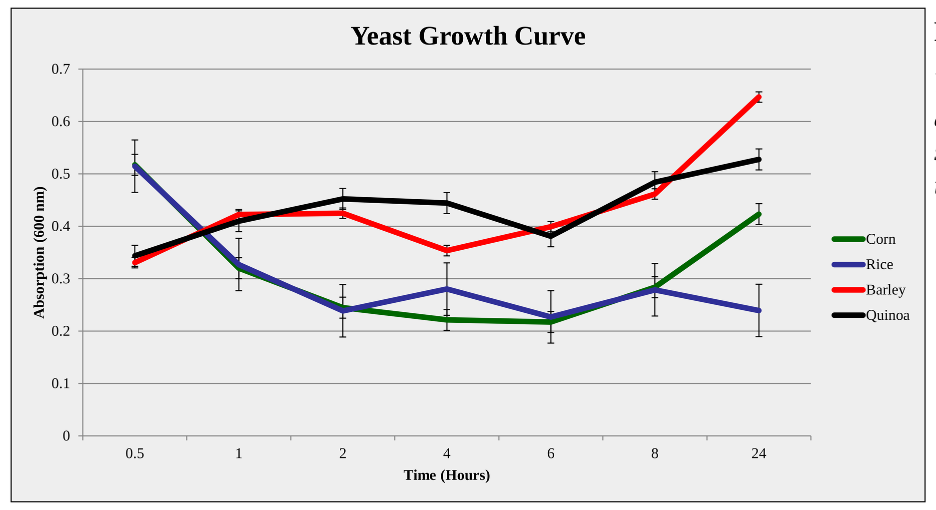


Figure 4: Yeast Growth Curve

Yeast growth curve created from taking samples, after being autoclaved, and adding 0.5 µL yeast solution and incubating for a certain period of time.

When looking at the graphs and trendlines, all of the yeast cell counts in the samples tended to rise overtime except in the rice sample. Rice had the lowest protein concentration and one of the lowest starch concentrations of the samples. It's possible that there wasn't enough food for the yeast to consume to grow compared to the other samples. When taking out the outliers, the trendline for the cornmeal would also decrease, and the quinoa levels roughly stayed the same. With the low food sources for the yeast in the three alternate grains, the ethanol production of the yeast wasn't present compared to the control, barley. Barley had a strong increase in yeast cell count, and had a starch concentration 4 times larger than the other grains. Strong cell growth equals larger ethanol formation, making barley an ideal grain for fermenting.

Lastly, we performed the ELIZA test on each sample after fermentation.

Barley Quinoa Rice Cornmeal

As we predicted, the rice and quinoa samples both came up negative for gluten, whereas the barley showed a very high gluten level in the sample. The cornmeal however, showed that it did in fact have gluten. Before we started the experiment, we researched grains that would not have gluten, and research showed that corn had gluten, but cornmeal did not. Cornmeal has the husk and kernel removed before drying and crushing, so the gluten may be found in these parts of the corn. The cornmeal we purchased may not have had all of the husks and kernels removed, allowing for the possibility of gluten being in the cornmeal.

Conclusion

- The market beers we tested that claimed they were gluten-free were in fact gluten-free.
- The ethanol percentage of the alternative grains was lower than the control.
- The Bradford Assay showed the protein consumption levels to be much higher in the alternative grains compared to the control.
- The Starch Assay results showed that the control consumed much more starch than the alternative grains.
- From both tests, we can conclude that the alternative grains had little starch for a food source, so they began to consume proteins instead. This caused a much lower yield in the ethanol percentage.
- In the ELIZA test, both rice and quinoa had results we expected with a negative result for gluten. The barley had gluten, as we expected with our control.
- The cornmeal contained gluten, contradicting our hypothesis. It is possible the gluten came from the kernel or the hull not being fully removed before grinding.

Future Work

- Quinoa and Rice fermentations had a potent smell. We could test for ammonia that has been created during the fermentation process, instead of ethanol.
- Investigate the cornmeal further, to determine where exactly gluten is present in corn.
- Determine if the type of rice may affect the ethanol percentage at the end. Investigate brown rice vs. white rice.
- Investigate other gluten-free alternatives to barley such as sorghum, potatoes, etc.

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