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MASHING LACTOSE INTO A FERMENTABLE ADJUNCT

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Background & Goals

Lactose, the main sugar in milk, is a disaccharide composed of glucose and galactose. Traditional brewer's yeast, *Saccharomyces cerevisiae*, does not have the enzymatic ability to break the beta-1, 4 bond linking the monosaccharides, therefore it can not be used as a sugar source for fermentation. Lactose is commonly used in beer as a non-fermentable sugar to add flavor such as in a milk stout. Supplementation of lactose to the brewing process usually occurs towards the end, after the mashing step. A beta-galactosidase with activity against lactose has been previously isolated from unmalted barley (i, ii). It was shown to rapidly lose activity at typical mash temperatures of $>50^{\circ}\text{C}$ (ii). A previous study also showed that beta-galactosidase was present in other grains such as wheat and rye (i). Incorporating lactose into earlier stages of the brewing process, could allow this enzyme to hydrolyze lactose into glucose and galactose for utilization by *Saccharomyces cerevisiae*. This would allow for the incorporation of lactose as a fermentable adjunct sugar in beer production.

The goals of this study were to:

- ❖ Identify any lactose-hydrolyzing activity in barley grains
- ❖ Optimize mash conditions for lactose hydrolysis
- ❖ Investigate other grains for their enzymatic capabilities

Acknowledgments



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Methods

- 250 ml of a 10% (wt/wt) lactose solution was brought to temperature (40°C or 50°C) in an Erlenmeyer flask placed in a shaking water bath set to 80-90 rpm. Controls consisted of 250 ml of water.
- Once at the desired temperature, the reaction was started by adding 65.9 g of grain (unmalted barley, rye, or wheat) to the flask. An immediate 0' sample was taken.
- Samples taken were placed in a 70°C waterbath for 5 minutes to stop any further enzymatic reaction.
- Samples were taken at 0, 10, 60, 120, and 180 minutes. The pH of the solution was taken at each time point.
- Once heat-treated, samples were centrifuged at 10,000 rpm for 5 minutes and the supernatant was collected.
- Each time point was analyzed in triplicate for glucose concentration via enzymatic assay. Experiments were repeated 3 times.
- The 180 minute time points were analyzed for statistical difference in glucose release to the control via a T-test. Those with a statistical significance ($p < 0.05$) are indicated by *.

Results

~ Barley ~

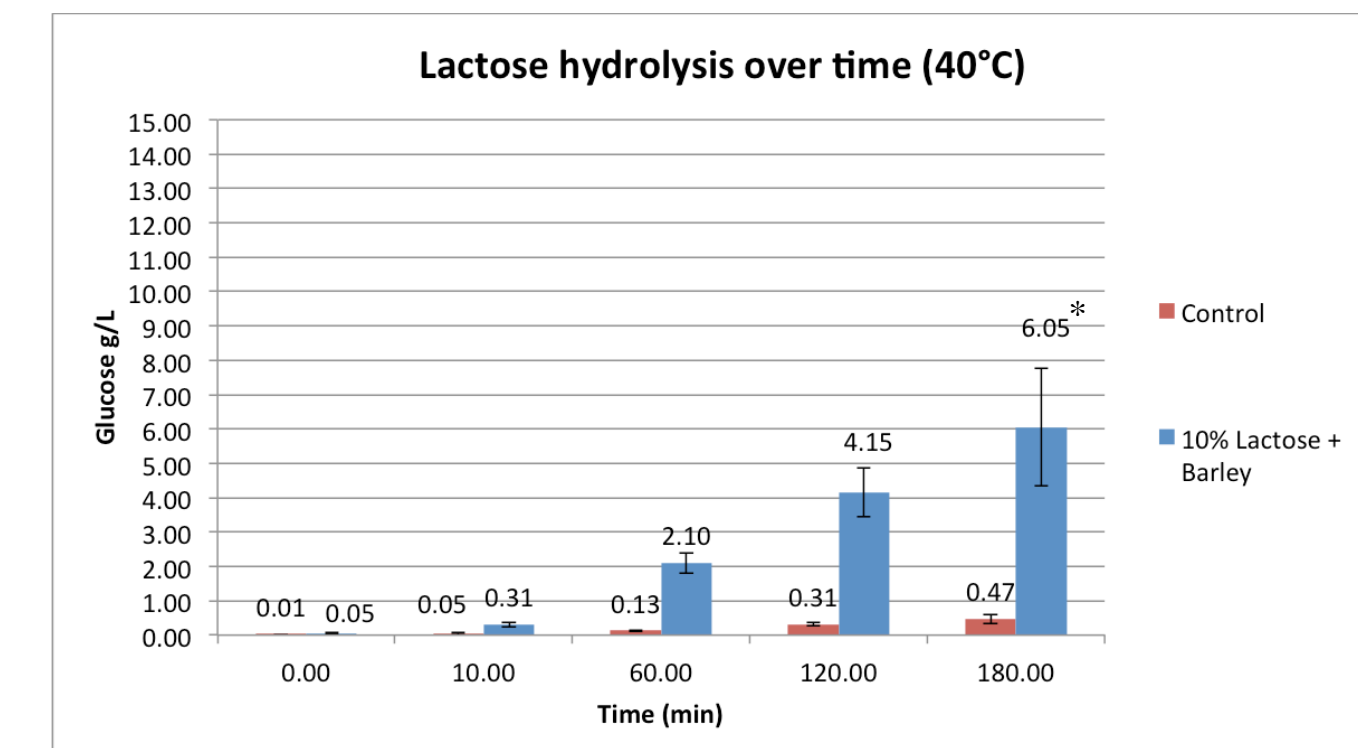


Figure 1: Glucose levels in mash of unmalted barley with 10% lactose over a time period of 3 hours at 40°C as measured via enzymatic assay.

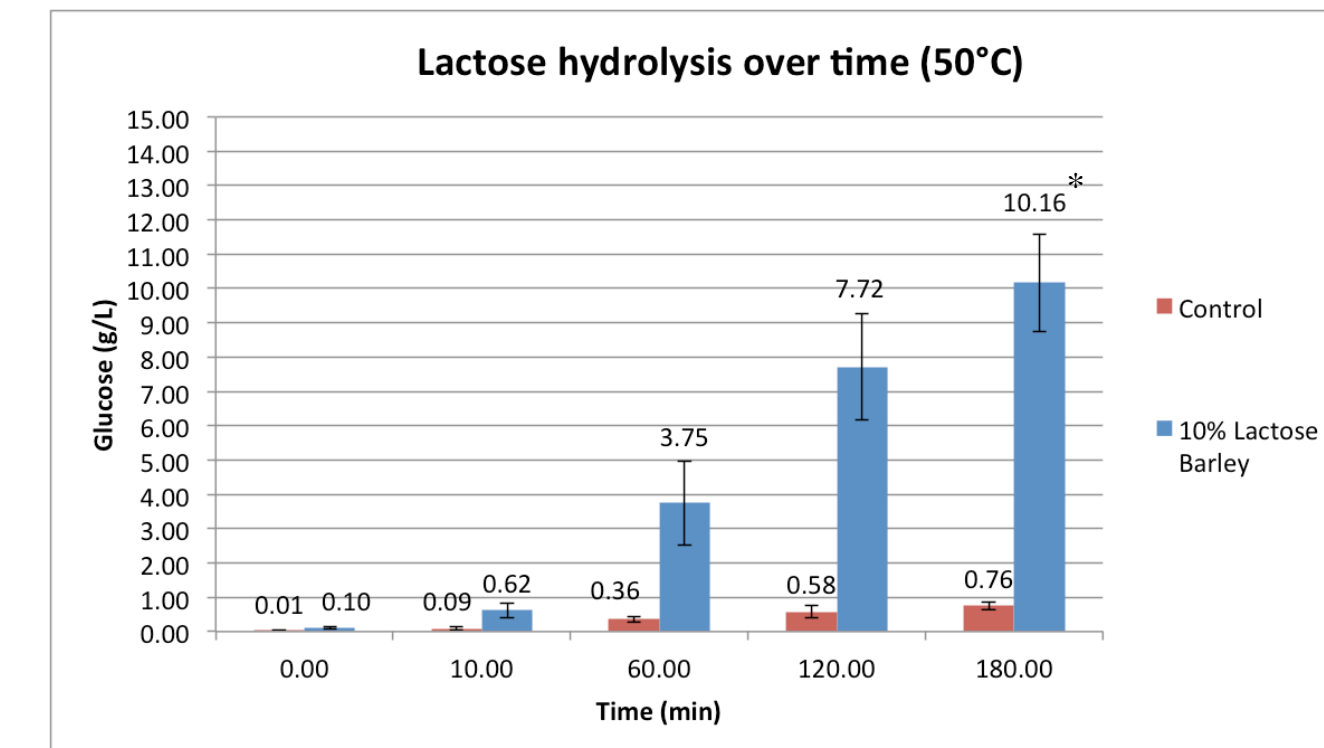


Figure 2: Glucose levels in mash of unmalted barley with 10% lactose over a time period of 3 hours at 50°C as measured via enzymatic assay.

~ Other Grains ~

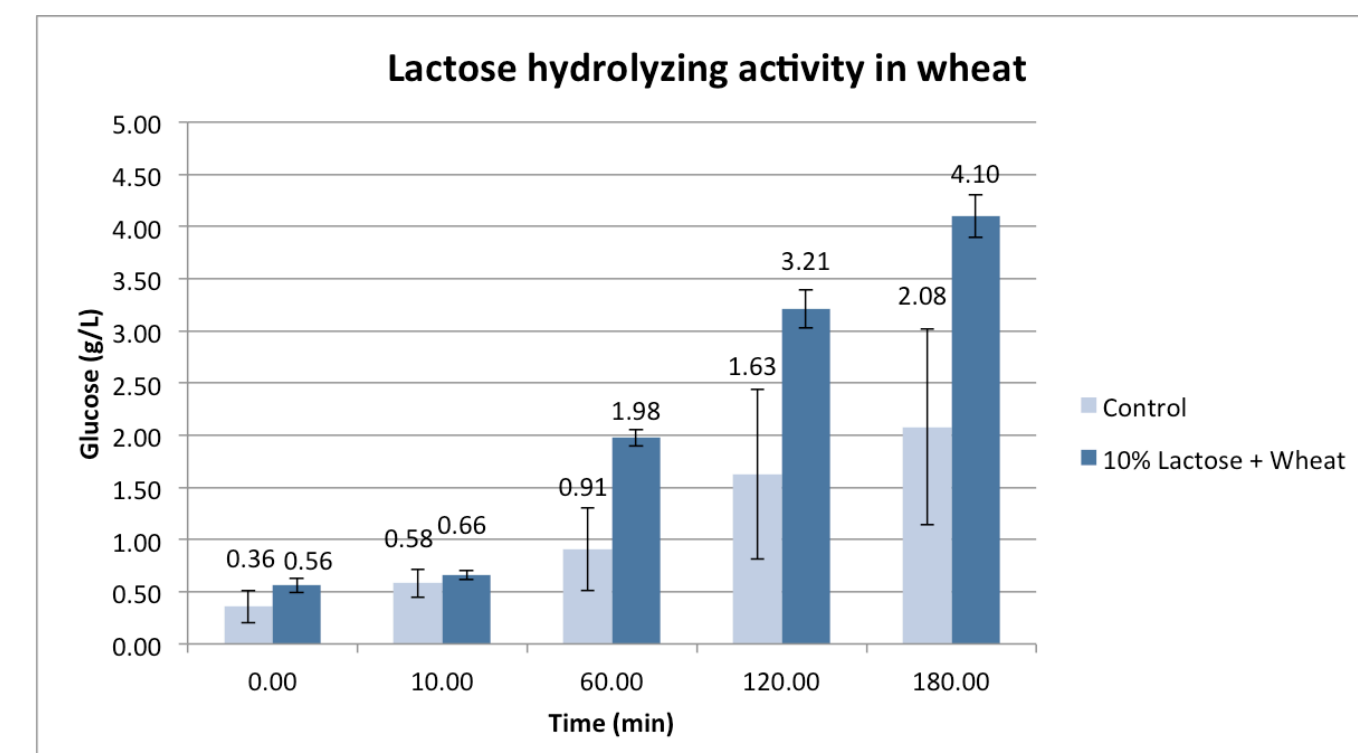


Figure 3: Glucose levels in mash of unmalted wheat with 10% lactose over a time period of 3 hours at 40°C as measured via enzymatic assay.

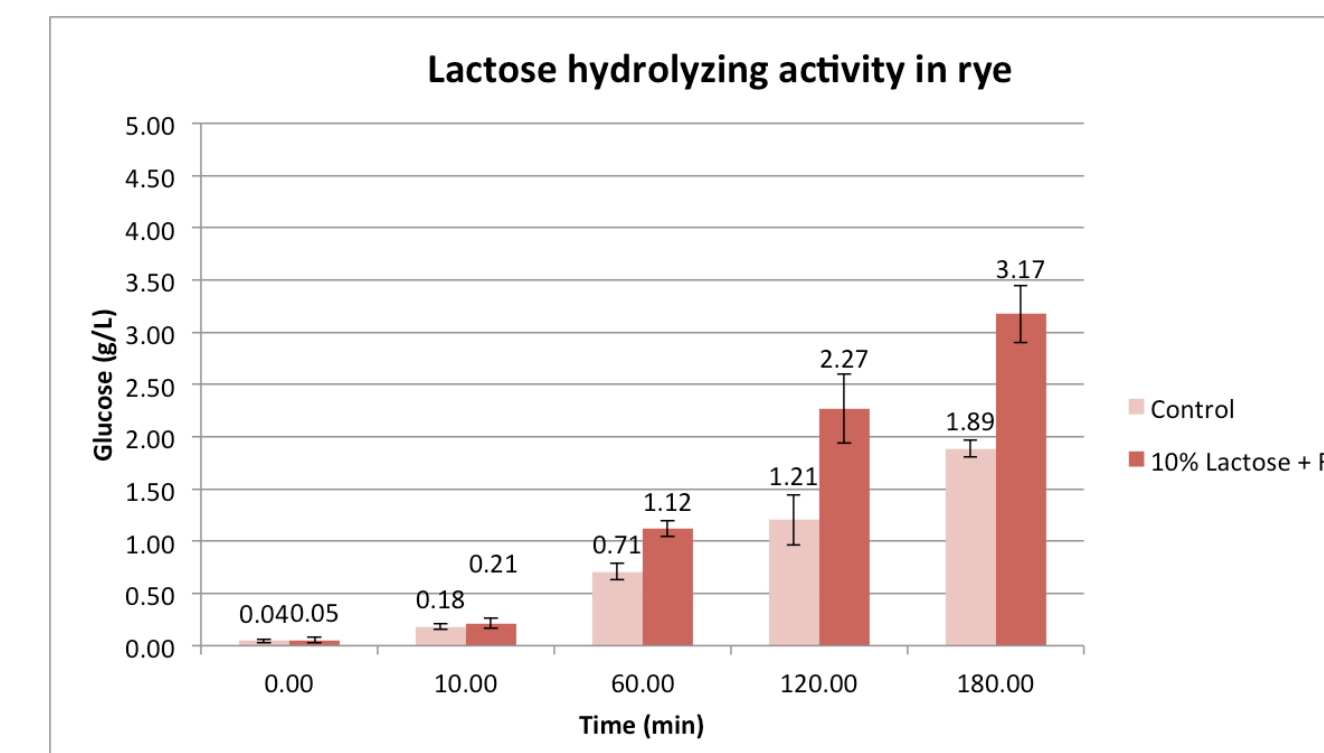


Figure 4: Glucose levels in mash of unmalted rye with 10% lactose over a time period of 3 hours at 40°C as measured via enzymatic assay.

Conclusions

- ❖ Unmalted barley possesses an endogenous enzyme that can sufficiently hydrolyze lactose.
- ❖ Enzyme activity in unmalted barley is *significantly* greater at 50°C than at 40°C .
- ❖ Although not significant, rye and wheat show some levels of enzyme activity.
- ❖ Incorporating lactose into the mash step of brewing can allow for an additional fermentable sugar source.

Future Work

- ❖ Does enzymatic ability vary greatly by barley variety and can we select for varieties that have high lactose hydrolysis activity?
- ❖ Evaluate incorporation of lactose containing substrates (whey, permeate, etc) into a mash.
- ❖ What are the sensory attributes of a beer produced with these substrates and mash profile?

Selected References

- i. Gelman, A. L. (1969). Some β -glycosidases in barley and other cereals. *Journal of the Science of Food and Agriculture*, 20(4), 209-212.
- ii. Simons, G., & Georgatsos, J. G. (1988). Lactose-hydrolyzing β -glycosidases of barley meal. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 967(1), 17-24.