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Analysis of Fermentable Carbohydrates using High Performance Liquid Chromatography in Gluten and Gluten-free Beer

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Abstract

The purpose of this study was to develop a consistent, reliable method to detect and quantify carbohydrates, such as glucose and maltose, throughout the fermentation process of gluten and gluten-free beer. A precolumn derivitization procedure with 1-phenyl-3-methyl-5-pyrazolone (PMP) followed by high performance liquid chromatography (HPLC) was used to separate and quantify the carbohydrates during the fermentation process over a period of two weeks. Determining the composition of glucose and maltose over time is crucial in monitoring fermentation as well as evaluating the quality of the beer. The separation and quantification of the derivitized carbohydrates were performed using a mobile phase of acetonitrile to ammonium acetate buffer (0.1M, pH 5.5) of 22:78 (v/v) at a flow rate of 1.5mL/min. PMP produces strong UV absorbance at 254nm. Both barley and sorghum brews were studied with aliquots removed and analyzed at days 3, 7, 10, and 14 of the fermentation process. Successful separation and quantification of glucose and maltose in wort and aliquots were established using this method.

Materials and Methods

Derivatization of standards with PMP

- A stock solution of glucose and maltose was prepared (0.1M each in 0.5M NaOH).
- 75uL of the carbohydrate stock solution was added to a test tube containing 100uL of 0.5M PMP in methanol.
- The mixture was incubated at 70ºC for 45 minutes.
- The reaction mixture was then neutralized with 75uL of 0.5M HCl.
- 500uL distilled water was added and then a 10 fold serial dilution was performed into two more test tubes (65uL and diluting it to 650uL).
- The mixtures were extracted 4x with 500uL methylene chloride with vigorous mixing and centrifuging at 10,000 rpm for 5 minutes.
- The organic layer was the carefully removed after each extraction.
- The final derivatized standards were centrifuged at 10,000 rpm for 5 minutes and the supernatant was removed and analyzed by HPLC.

Derivatization of fermented samples with PMP

- 200uL of the beer sample was added to 150uL 0.5M NaOH and 100uL 0.5M PMP in methanol in a test tube.
- The mixture was incubated at 70ºC for 45 minutes and then neutralized with 150uL 0.5M HCl.
- The extraction process (4x) was carried out as described above with the standards.

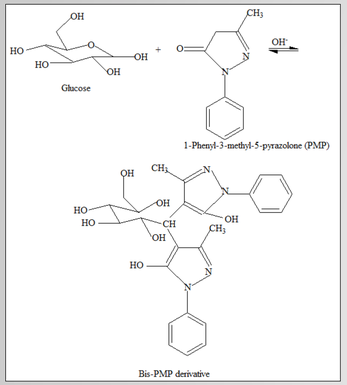
Separation on HPLC

- HPLC analysis was carried out on the standards and samples using a reverse phase C18 column with a Shimadzu HPLC at a constant flow rate of 1.5mL/min and oven temperature of 40ºC.
- The solvent was composed of 22% acetonitrile in 0.1M ammonium acetate buffer (pH 5.0).
- 15uL of sample was injected and detection was carried out at 254nm.

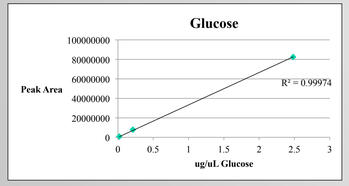
Quantification

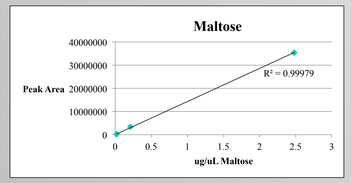
- Standard curves were used to quantitate the amounts of glucose and maltose (ug/uL) in each beer sample using peak areas.

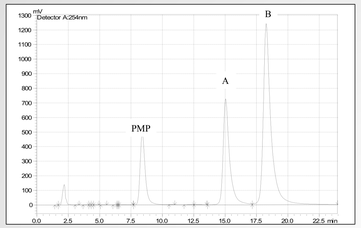
Reaction Scheme



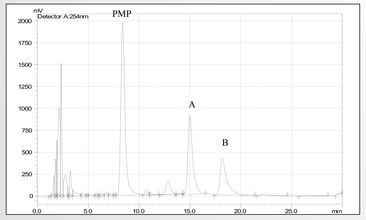
Standard Curves



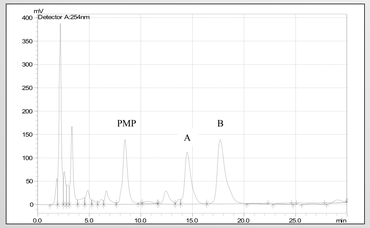




HPLC chromatogram of a standard solution containing glucose and maltose after Detivitization with PMP. Maltose (A); Glucose (B)

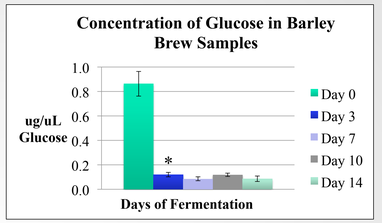


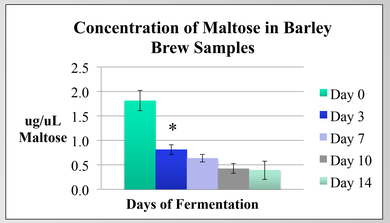
HPLC chromatogram of a barley wort sample after derivitization with PMP. Maltose (A); Glucose (B)



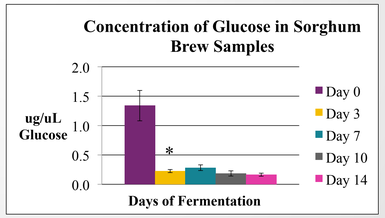
HPLC chromatogram of a sorghum wort sample after derivitization with PMP. Maltose (A); Glucose (B)

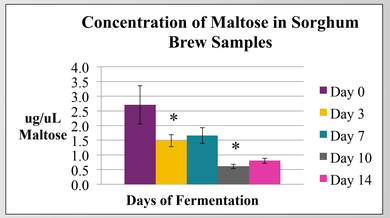
Results



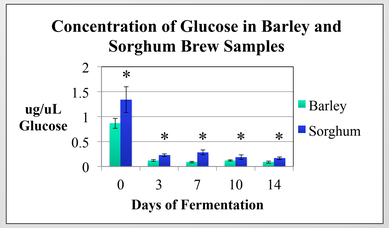


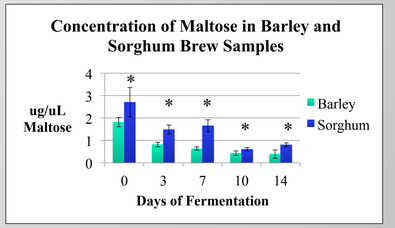
N values for Day 0, 3, 7, 10, and 14 are 18, 40, 40, 48, and 48, respectively.
(*P < 0.05 represent significant differences compared to data collected on the previous day of fermentation)





N values for Day 0, 3, 7, 10, and 14 are 18, 48, 48, 40, and 40, respectively.
(*P < 0.05 represent significant differences compared to data collected on the previous day of fermentation)





*P < 0.05 represent significant differences compared to data collected on the previous day of fermentation.

Conclusions

- Standard curves for glucose and maltose were linear over a concentration range of .0200-3.00 ug/uL and prepared each time brew samples were run.
- The intrarun of glucose and maltose standards had a relative standard deviation of 1.92 and 5.75%, respectively.
- The fermentation of glucose and maltose in the barley brew occurs within the first three days of the fermentation process. After day 3, there was no statistically significant difference in the concnetrations of glucose and maltose.
- The fermentation of glucose in the sorghum brew occurs within the first three days of fermentation with no statistically significant difference in the concentration of glucose after day 3.
- Interesting, the concentration of maltose in the sorghum brew decreased significantly by day 3 of the fermentation process. There was another statistically significant difference in the concentration of maltose during the fermentation process between day 7 and day 10.
- The concentrations of glucose and maltose in sorghum brew were significantly greater than in the barley brew during the entire fermentation process

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