Rapid SUGAR Series: Fermentation Monitoring and Sourdough Cultures

Introduction

Traditional fermentation techniques, including sourdough cultures, are created from communities of microbes. At home, sourdough cultures can be created from the yeast and bacteria populations naturally present in the air, flour, and on surfaces[1]. The yeast and bacteria convert fermentable sugars into CO_2 and compounds like lactic acid, creating sourdough's characteristic rise and sour flavor. In this application note, a "homemade" sourdough starter culture was created and analyzed using a new rapid ligand exchange method. Key fermentation products were analyzed at different time points over the course of 24 h with the new Rapid SUGAR SH1011 8C column.

The Shodex™ Rapid SUGAR SH1011 8C is a styrene divinylbenzene based Hydrogen ligand exchange column and is designed for rapid fermentation monitoring, allowing for the analysis of saccharides, organic acids, and ethanol in less than 5 min. The method uses simple aqueous conditions and RID detection, ideal for large sample workflows and QC environments.

Experimental

Seven common fermentation compounds (maltotriose, maltose, glucose, lactic acid, acetic acid, glycerol, and ethanol) were used as standards. The sourdough starter culture was adapted from King Arthur[2] with the procedure repeated once daily for two weeks.

Sample Preparation

Samples were taken starting on Day 15 at three time points with the mixture well stirred

Time 0: Sampled after completed addition of flour and water

Time 2: 12 hours after mixing

Time 3: 24 hours after mixing

Approximately 10 g of starter was homogenized with 90 mL distilled water (maximal speed, 30 s). Five milliliters of 1 mol/L $HClO_4$ solution was added to a 10 mL aliquot of the homogenate. The mixture was centrifuged for 15 min at 4000 g at 15C, the supernatant was neutralized (pH 7.0) with 2 mol/L KOH and the volume was adjusted to 25 mL with distilled water. After 30 min precipitation on ice, the solution was filtered on 0.45 mm cellulose filter (adapted reference 3).

Picture 1: Sourdough Bread (pre-oven)

Conditions

Column: Shodex SUGAR SH1011 8C (8.0 mm I.D. × 100 mm, 6 µm)

Detector: Shodex RI-501 **Eluent :** 1 mM H_2SO_4 **Temp:** 75 °C **Flow rate:**1.0 mL/min

Results and Discussion

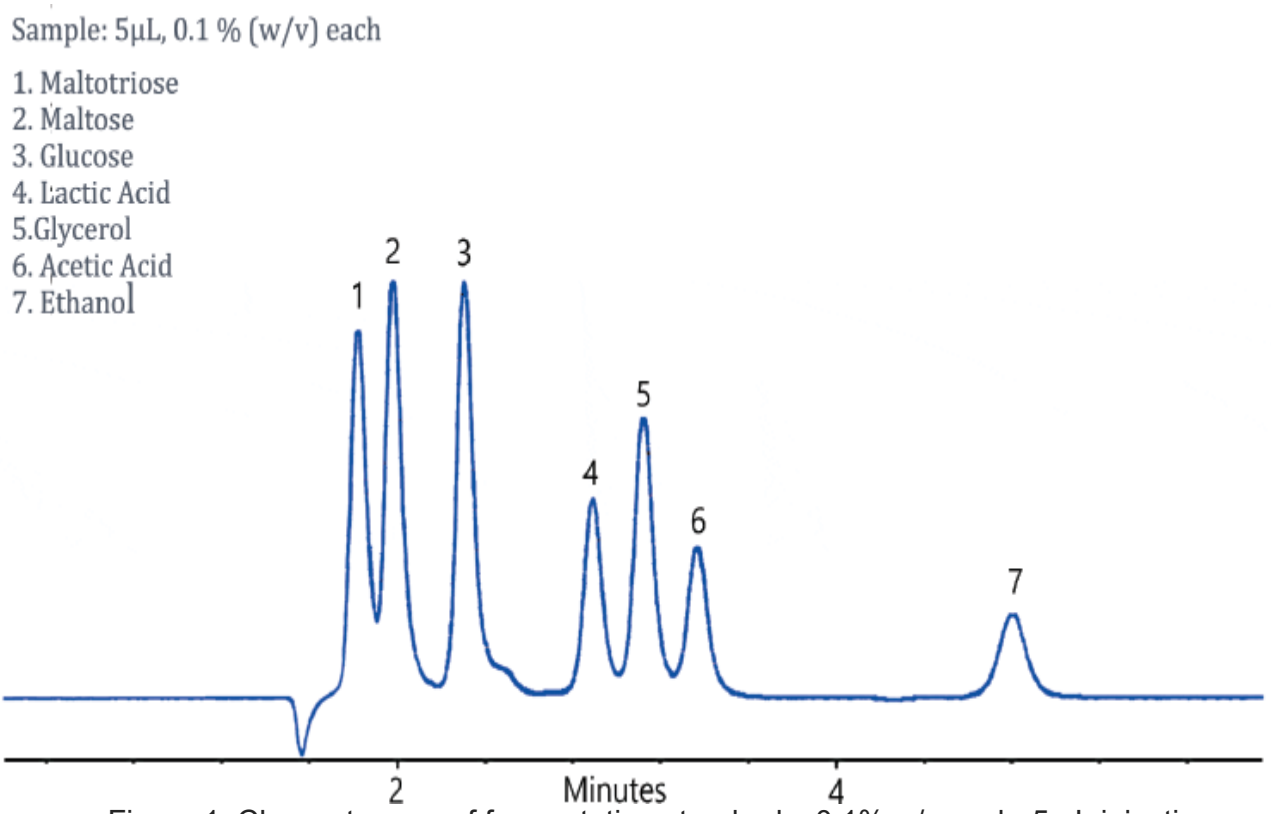

Figure 1: Chromatogram of fermentation standards, 0.1% w/v each, 5 µL injection

Simple eluent preparation of 1 mM sulfuric acid successfully demonstrated analysis of simulated fermentation by-products in less than 5 min, including the column equilibration time.

The SH1011 series features porous beads of styrene divinylbenzene crosslinked to a hydrogen ligand, providing a combination of ligand exchange and size exclusion chromatography. Neutral sugars like maltotriose, maltose, and glucose are separated by their molecular weight. Organic acids and alcohols are retained by a mixture of ligand exchange and reverse phase effects, with the elution of organic acids controlled by the acid concentration of the mobile phase.

For the analysis of the sourdough starter culture a guard column was used: (Shodex™ SUGAR SH-G (6.0 × 50 mm id, 10 µm) (Figure 2).

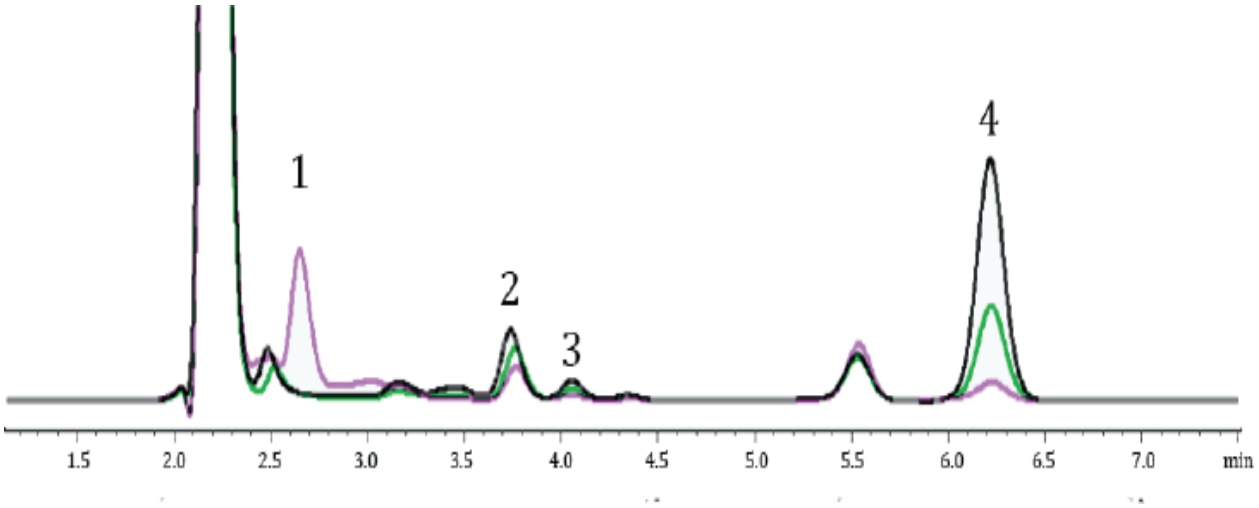

Figure 2: Time 0 - Pink, Time 12H - Green, Time 24H: Black

Peak 1: glucose and sample matrix. Peak 2: lactic acid. Peak 3: glycerol. Peak 4: ethanol

A method for rapid fermentation monitoring was developed using the Shodex SUGAR SH1011 8C column. Fermentation sample measurement completes in 6 min. While not explored in this application, sample preparation included protein and particulate removal, a recommended step to improve robustness of the method.

An increase in the peak response of ethanol and organic acids was observed at each time point. A corresponding decrease in the peak response of glucose was also observed. The results show that the SH1011 8C can be used to monitor fermentation at different timepoints, tracking the conversion of saccharides into metabolites.

Reference

(1) J. Marx, J.H. Litchfield, A Revolution in Biotechnology, *Cambridge University Press*, 71(1989).

(2) The King Arthur Flour Baker's Companion: The All-Purpose Baking Cookbook. *The Countryman Press*, (2012)

(3) D. Lefebvre, V. Gabriel, Y. Vayssier, and C. Fontagné-Faucher, *Food Sci. Tech* **35.** 407–414 (2002).