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# Monitoring Fermentation by Benchtop NMR

Fermentation is the alteration or production of products with the help of microorganisms. It can be used to alter or preserve foods, for example in bread, yoghurt, vinegar or wine. Fermentation can be spontaneous, like milk turning sour in the fridge, or controlled by adding specific microorganisms. Examples of controlled fermentation are the production of cheese or wine. In industrial processes, it is important to monitor the fermentation progress as uncontrolled fermentation through contamination can spoil entire batches of a product.

In this note, we show how the Spinsolve benchtop NMR spectrometer can be used to monitor the conversion of sugar into ethanol through fermentation without any sample processing. The different outcomes of controlled and wild fermentation are demonstrated.

# Fermentation Process

The fermentation happens in several stages. In a first step, the dimer sucrose ($C_6H_{12}OH$) is broken into two glucose molecules:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ C_6H_{12}O_6$$

The next step is the conversion of glucose into ethanol and carbon dioxide:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

The production of carbon dioxide creates the characteristic bubbles observed during fermentation. Under the presence of oxygen, acetic acid bacteria, *acetobacter*, convert ethanol into acetic acid:

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O$$

## Sample preparation and measurement

Two samples from a batch of fresh, untreated apple juice were transferred into three litre plastic bottles. One of these samples was inoculated with a brewer's yeast for the controlled fermentation. The other sample was left without any further treatment to undergo wild fermentation. Both bottles were kept at room temperature.

An example spectrum of the wild fermentation sample is shown in Figure 1.

Figure 1

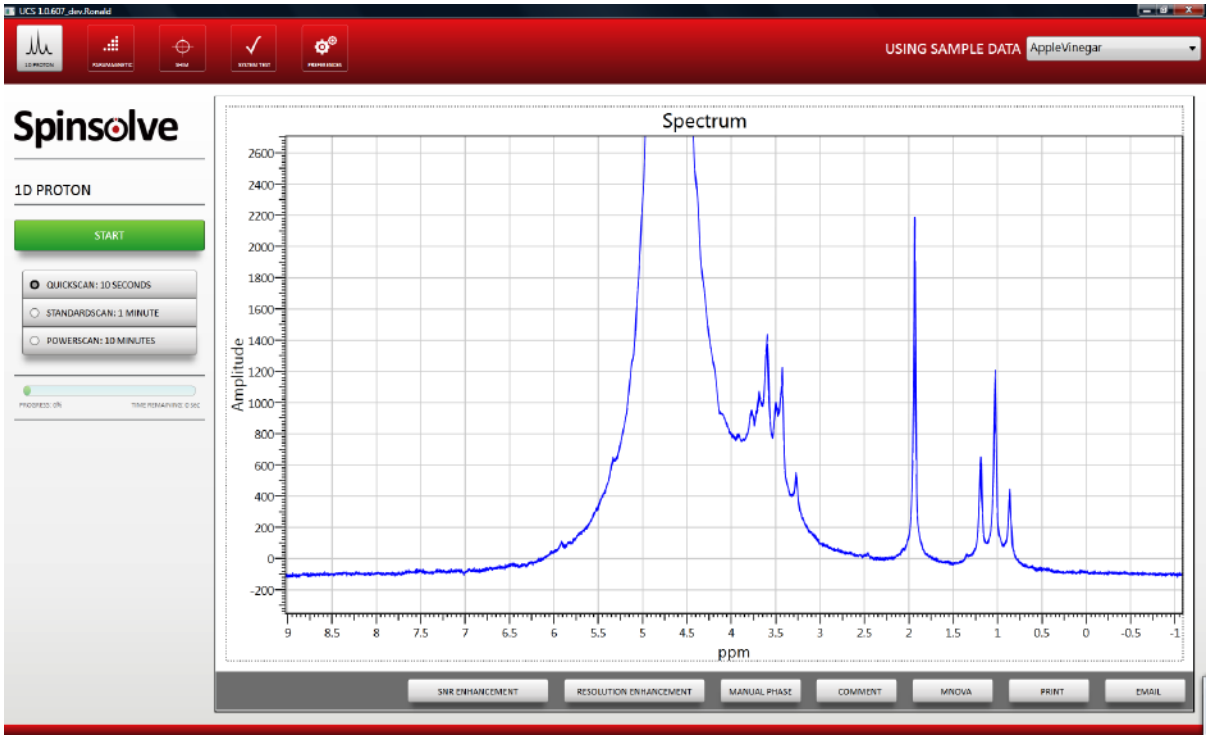


*Figure 1: NMR spectrum apple juice after undergoing wild fermentation.*

# NMR Spectra of Fresh and Fermented Juice Samples

The NMR spectra of the completely fermented samples (the juice now converted to cider) are shown in Figure 2 along with the spectrum of the fresh juice. All sample spectra show a large peak at 4.7 ppm, which is due to the water.

A vertical expansion reveals a number of smaller peaks between 1 and 4 ppm. These peaks are assigned in Figure 2.

Figure 2

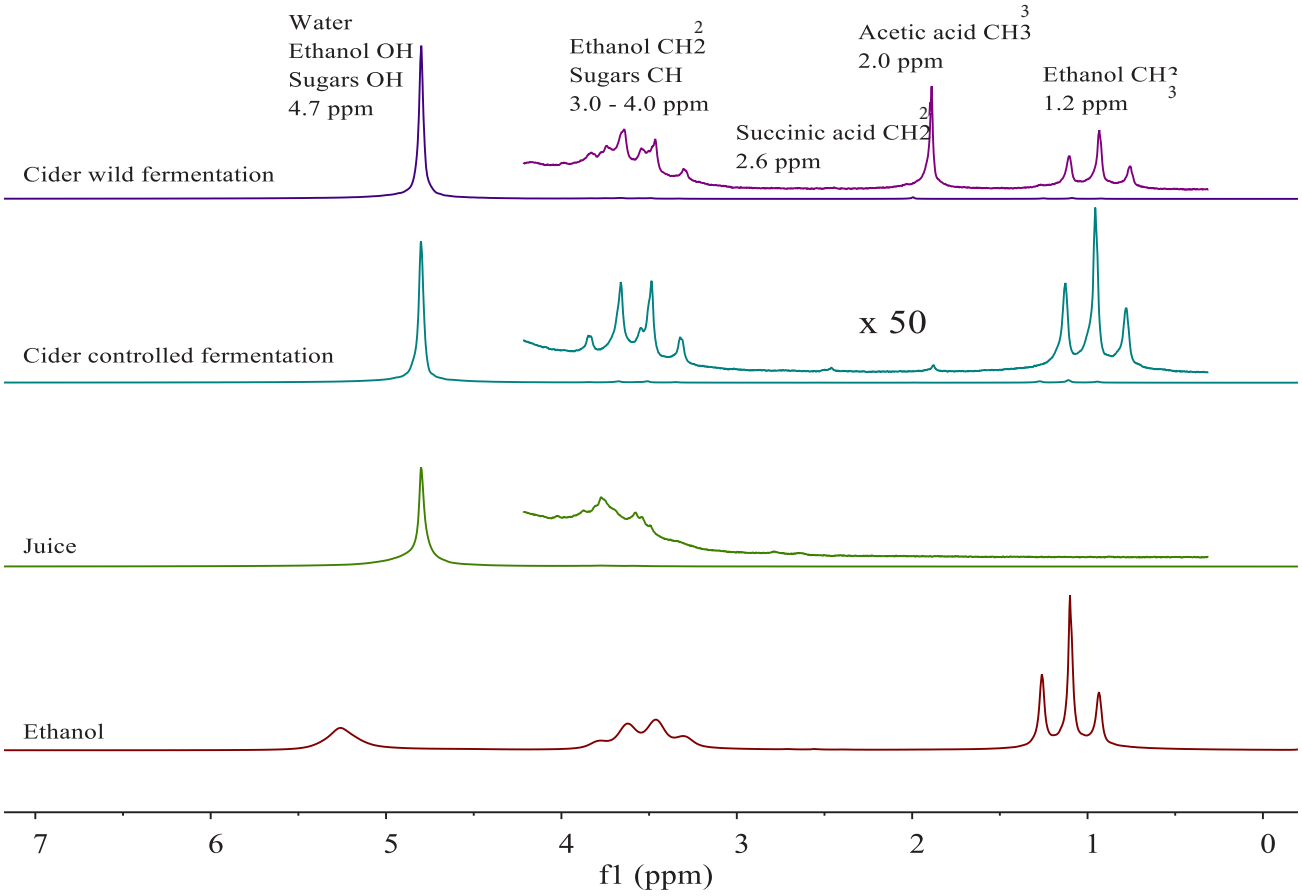


*Figure 2: NMR spectra of fresh apple juice and apple cider from wild and controlled fermentation after 20 days. The juice and cider spectra are dominated by the prominent water peak, but the vertical expansion by a factor 50 reveals the presence of smaller peaks, which can all be assigned. In order to aid peak assignment, the spectrum of a 99% ethanol/ water mixture is also shown.*

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## Measurement of Alcohol Content and Conversion Rate

The $CH_3$ peaks at 1.2 and 2.0 ppm are well resolved and can be integrated for a quantitative measurement of ethanol and acetic acid content. A plot of the measured volume concentrations is shown in Figure 3. Characteristic for the controlled fermentation is the higher conversion rate and the absence of secondary fermentation.

Figure 3

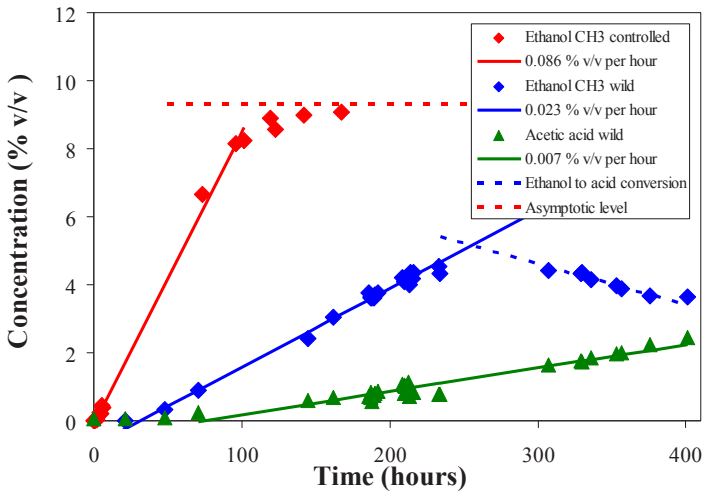


*Figure 3: Volume concentrations of ethanol and acetic acid obtained from the $CH_3$ peak integral of the NMR spectra. The lines show the regression of the data points in the linear region.*

## Conclusion

The Spinsolve benchtop NMR spectrometer has been used to monitor the fermentation process of untreated apple juice. Clear differences between controlled and wild fermentation were detected. The samples were taken straight out of the fermentation vessel without any further processing or purification. This shows that the Spinsolve benchtop NMR spectrometer can be readily used as an on-line process monitoring tool.

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