Agilent Hi-Plex Columns for the Analysis of Organic Acids in Dairy Products

Application Note

Food

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Introduction

Quantitative determination of organic acids in dairy products is important in flavor studies, for nutritional reasons, and as an indicator of bacterial activity.

Here, we used an Agilent Hi-Plex H column to analyze the organic acid content of various dairy products.

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Sample Preparation

For each dairy product, 0.125 g of sample was added to 125 µL of distilled water in a centrifuge tube, followed by 0.5 mL of HPLC-grade acetonitrile (to precipitate out proteins in the sample). After shaking for 1 minute, the sample was centrifuged at 13,000 rpm for 2 minutes. Ten µL of the resulting supernatant was then injected.

Method

Column	Agilent Hi-Plex H (hydrogen) (8% crosslinked), 7.7 x 300 mm, 8 µm (p/n PL1170-6830)
Mobile phase	0.009 M H_2SO_4
Flow rate	0.7 mL/min
Temperature	65 °C
Detector	Dual wavelength UV at 220 nm and 275 nm (for quantification of uric acid and formic acid)

Results

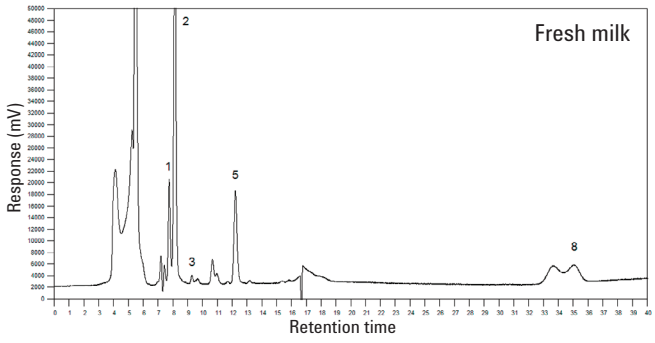
When analyzed, a different number of organic acids were present in each milk sample. The fresh milk sample contained only a few acids, while the sour milk contained each acid noted earlier, in addition to various unknown compounds. The initial solvent peaks for all chromatograms occur between 4 and 6 minutes and result from water, phosphates, and other unretained compounds. The negative peak occurring in all sample chromatograms at approximately 17 minutes is due to the acetonitrile denaturant/solvent.

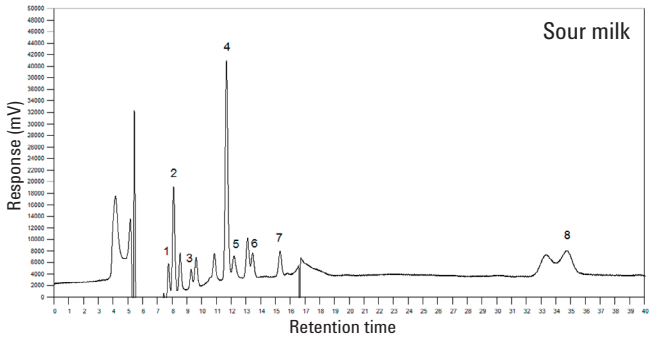
The main constituents of fresh milk include citric acid, orotic acid, uric acid, and hippuric acid, which would be expected as these are produced by the bovine metabolism. Cheese is manufactured by heating milk to a temperature that promotes the growth of lactic acid bacteria, which in turn leads to fermentation of lactose to lactic acid. As a result of this manufacturing process, the UV chromatograms for all three of the cheese samples show a distinct lactic acid peak. In addition, the cheese samples also give responses for pyruvic acid and propionic acid, which further proves that some form of bacterial action has taken place. It is worth noting, however, that blue cheese, despite containing a large amount of mold, contains the least amount of lactic acid.

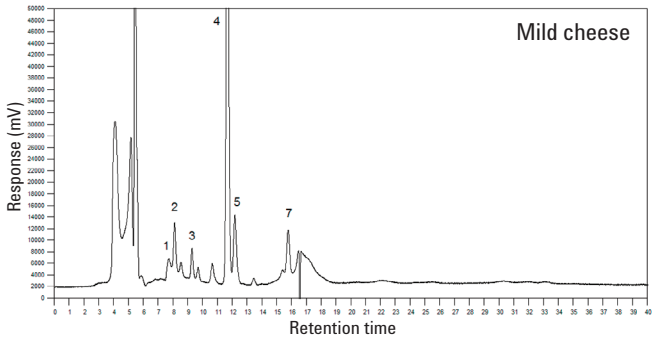
Yogurt is made in a similar way to cheese, as fermentation of the milk sugar (lactose) produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic flavor. This is also reflected in the UV chromatograms for the two yogurt samples. Both samples contain live bacteria, which may account for the slightly higher levels of lactic and acetic acids.

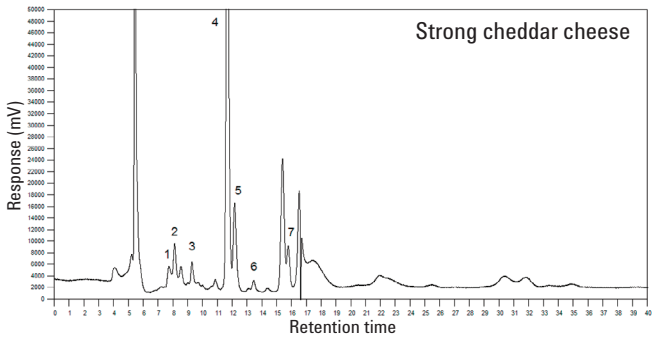
Key

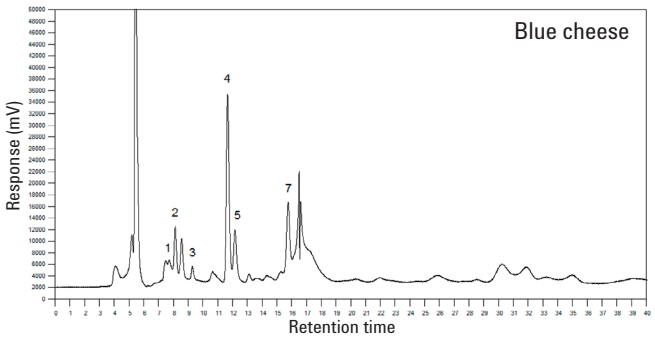
1). Citric acid, 2). Crotic acid, 3). Pyruvic acid, 4). Lactic acid, 5). Uric acid + formic acid, 6). Acetic acid, 7). Propionic acid, 8). Hippuric acid

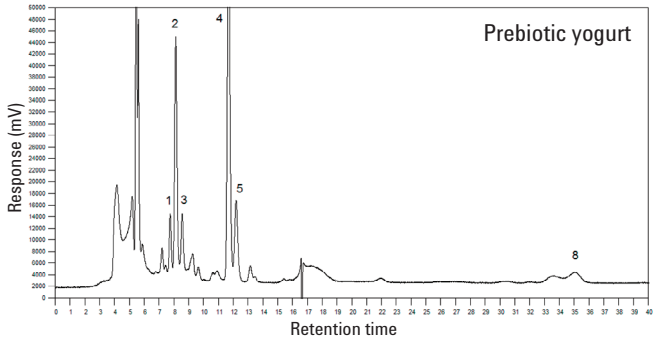


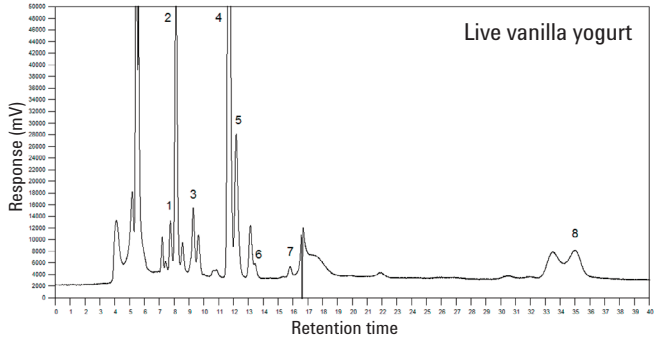


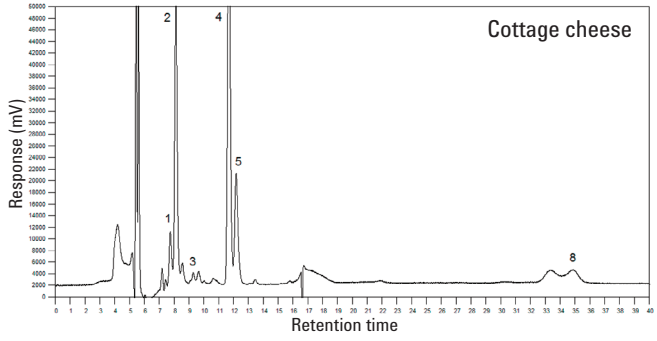












Cottage cheese is a mild white cheese made from the curds of soured skimmed milk and therefore contains a relatively high concentration of lactic acid. Fresh milk that has been allowed to go sour contains a large number of unknown compounds in addition to those expected from bacterial growth. These are likely to be some form of ammonia products that give this sample its distinctive smell.

Conclusion

The Agilent Hi-Plex H column can be used to quantify the concentrations of a variety of organic acids in aqueous samples from dairy products. A potentially useful application of this HPLC procedure is to supply support data in microbiological studies by quantitating bacterial metabolites.

Reference

1. "High Performance Liquid Chromatographic Determination of Organic Acids in Dairy Products." *Journal of Food Science*. Volume 46, issue 1, pages 52-57 (January 1981).

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