Profiling and quantitation of organic acids in dairy products using dual wavelength UV detection

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Introduction

Hi-Plex columns are based on polystyrene divinylbenzene with varying degrees of sulfur cross-linking and different metal counter-ions, and are typically used for the analysis of naturally occurring materials such as carbohydrates, organic acids and alcohols.

An important use for Hi-Plex ligand exchange HPLC columns is in the field of foodstuff analysis, whereby samples are tested to determine various active ingredients in food, or in flavor studies. An example of the superior separation ability of Hi-Plex Hydrogen is demonstrated by the quantitative analysis of organic acids in dairy products, which contain a range of different organic acids and sugars.

Organic acids occur in dairy products as a result of the hydrolysis of butterfat (fatty acids), direct addition as acidulants (e.g. citric, lactic, and propionic acids), normal bovine biochemical metabolic processes (e.g. citric, hippuric, uric, orotic, and ascorbic acids), or bacterial growth (e.g. pyruvic, lactic, acetic, and propionic acids). Quantitative determination of these acids in dairy products is important to flavor studies, for nutritional reasons, and as an indicator of bacterial activity.

Results and Discussion

Nine different dairy products were analysed using a Hi-Plex H, 8 µm, 300 x 7.7 mm, which separates the organic acids by ion exclusion and partition chromatography. Mobile phase used was 0.009M H_2SO_4 at 0.7 mL/min, 65°C.

Uric acid and Formic acid, typically found in dairy, cannot be separated under these mobile phase conditions, however both can be quantified by running dual wavelength UV detection at 220nm and 275nm. At 275nm, only uric acid is visible, which allows quantification of uric and formic acid individually.

Standard Preparation

In order to quantify organic acid content for each dairy product, a standard solution was made up containing the following organic acids in 10 mL of HPLC grade water:

Orotic Acid	0.0307 g	Uric Acid	0.0514 g
Hippuric Acid	0.0514 g	Citric Acid	0.2014 g
Lactic Acid	0.3044 g	Pyruvic Acid	0.0517 g
Propionic Acid	0.5029 g	Formic Acid	0.2056 g
Acetic Acid	0.3124 g	Butyric Acid	0.6051 g

By working out the peak area for each organic content at each wavelength in the standard below, the components of the various dairy samples can now be quantified using ratio's of peak areas. A 5 µL injection of this solution gives the following UV responses:

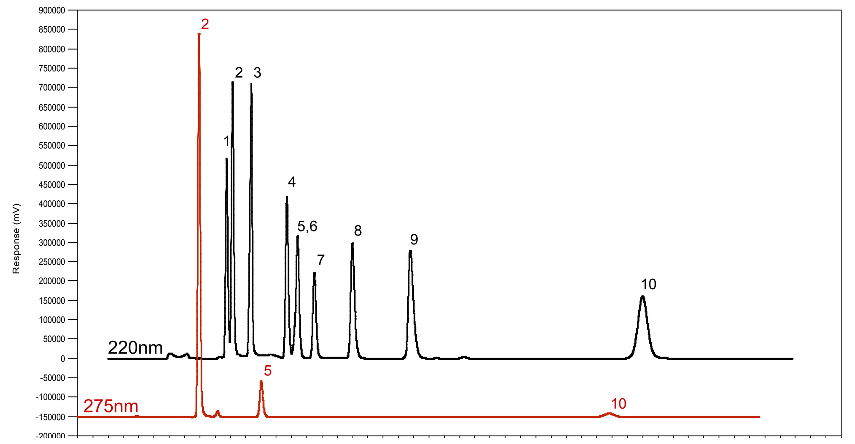

Figure 1: Hi-Plex H, 8 µm, 300 x 7.7 mm with 0.009M H_2SO_4 at 0.7 mL/min, 65 °C
Compounds: 1). Citric Acid, 2). Orotic Acid, 3). Pyruvic Acid, 4). Lactic Acid, 5). Uric Acid, 6). Formic Acid, 7). Acetic Acid, 8). Propionic Acid, 9). Butyric Acid, 10). Hippuric Acid

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 one minute, the sample was centrifuged at 13,000 rpm for two minutes. 25 µl of the resulting supernatant was then injected.

Milk – Effect of Bacterial Spoilage

The chromatograms below show the effects of bacterial spoilage on the content of fresh milk. A sample of fresh milk was injected onto the Hi-Plex H column then allowed to sour in the fridge for one week before re-injecting. See Figure 2 below:

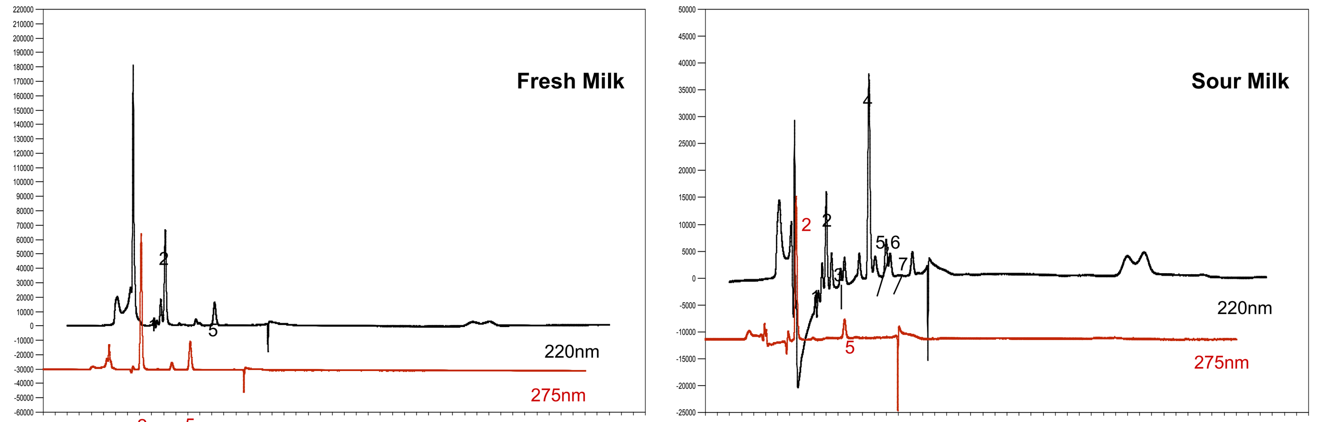

Figure 2: Hi-Plex H, 8 µm, 300 x 7.7 mm with 0.009M H_2SO_4 at 0.7 mL/min, 65 °C. Compounds: 1). Citric Acid, 2). Orotic Acid, 3). Pyruvic Acid, 4). Lactic Acid, 5). Uric Acid, 6). Formic Acid, 7). Acetic Acid, 8). Propionic Acid, 9). Butyric Acid, 10). Hippuric Acid

The main constituents of *fresh* milk include citric acid, orotic acid, uric acid and hippuric acid. However, once the fresh milk had been allowed to go sour it also contained a large amount of lactic acid (a bi-product of bacterial action) and other unknown compounds (which are likely to be some form of ammonia products that give this sample its distinctive smell).

Dairy Product Comparison

Each of the samples tested in this investigation contain a different amount of the expected organic acids. Figure 3 below shows comparative organic acid content data for all samples tested:

	Organic Acid Content (mg/mL*)							
	Fresh Milk	Sour Milk	Mild Cheese	Strong Cheese	Blue Cheese	"Live" Yoghurt	Cottage Cheese	Single Cream
Citric Acid	0.146	0.040	0.050	0.041	0.042	0.073	0.093	0.173
Orotic Acid	0.056	0.014	0.011	0.008	0.009	0.049	0.050	0.059
Pyruvic Acid	0	0.003	0.008	0.005	0.004	0.019	0.002	0.005
Lactic Acid	0	0.509	1.084	1.126	0.479	1.735	1.091	0.221
Uric Acid ***(µg/mL)**	0.061	0.011	0.051	0.073	0.041	0.104	0.081	0.079
Formic Acid	0.049	0.012	0.030	0.021	0.040	0.101	0.092	0.090
Acetic Acid	0	0.058	0.026	0.035	0	0.064	0.016	0.021
Propionic Acid	0	0	0.200	0.188	0.273	0.041	0	0.043
Butyric Acid	0	0	0	0	0	0	0	0
Hippuric Acid	0	0.004	0	0	0	0	0	0

Figure 3: Organic Acid content of different dairy products.

For each assay, the initial solvent peaks occurs 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, highlighted above, are 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.

Yoghurt 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 yoghurt its texture and characteristic flavor. This is also reflected in the UV chromatogram for the yoghurt sample. This also contains 'live bacteria' which may account for the slightly higher levels of lactic and acetic acid.

Finally, cottage cheese is a mild white cheese made from the curds of soured skimmed milk therefore also contains a relatively high concentration of lactic acid.

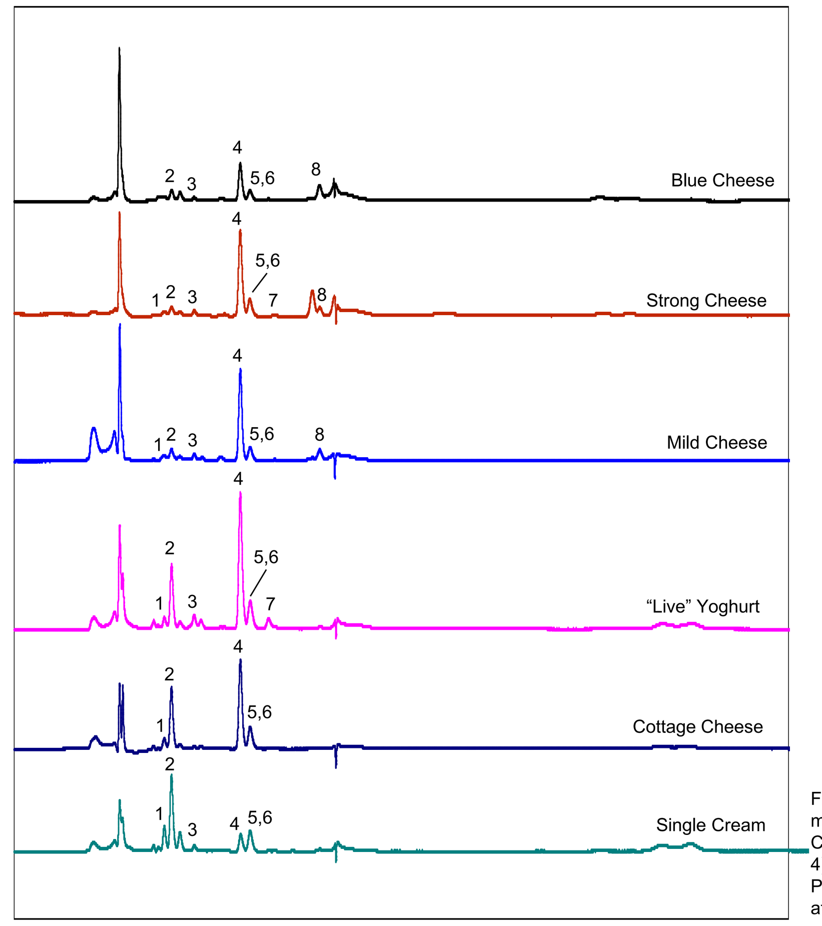

Figure 4 shows a comparison of the UV profiles for a variety of different dairy products analysed in this investigation:

Figure 4: Hi-Plex H, 8 µm, 300 x 7.7 mm with 0.009M H_2SO_4 at 0.7 mL/min, 65 °C.
Compounds: 1). Citric Acid, 2). Orotic Acid, 3). Pyruvic Acid, 4). Lactic Acid, 5). Uric Acid, 6). Formic Acid, 7). Acetic Acid, 8). Propionic Acid, 9). Butyric Acid, 10). Hippuric Acid. Detection: UV at 220nm

Conclusions

This report demonstrates how a Hi-Plex Hydrogen column can be used to quantify the concentrations of a variety of organic acids in aqueous samples from dairy products.

For this application, and where Hydrogen counter-ion ligand exchange columns are used, dilute sulfuric acid is required as eluent, however for most applications on other Hi-Plex phases, only pure HPLC grade water is required for the analysis, and separations can be aided by the manipulation of mobile phase temperature and flow rate.

A potentially useful application of the HPLC procedure described in this poster is to supply support data in microbiological studies by quantitating bacterial metabolites.

References

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