The Analysis of Lactose in Milk and Cheese Products by HPLC with RI Detection

Introduction
Carbohydrates are one of the more important energy sources in foods. They account for about 40-80% of total energy consumption. Key foods containing carbohydrates include cereals, fruits, vegetables, sugar crops and dairy products. In particular, lactose, also referred to as milk sugar, is the primary carbohydrate present in dairy products. Globally, the analysis of lactose is especially important due to the many individuals suffering from lactose intolerance. Such intolerance is typically due to the shortage of the enzyme lactase. As a result of this deficiency, lactose is not completely digested in the body, leading to a number of symptoms having a negative health impact.

The work presented herein provides a method for the routine sample preparation and quantitative determination of lactose in mozzarella and ricotta cheeses and milk. As the monosaccharaides glucose and galactose can be hydrolytically or enzymatically produced from lactose, these two sugars were also chromatographically separated, though not quantitated. The method uses a PerkinElmer HPLC system with refractive index (RI) detector, which is especially effective for analyzing analytes with little or no UV absorption, such as many dietary sugars.
Experimental

Hardware/Software

For all chromatographic separations, a PerkinElmer HPLC system was used, including a solvent and sample module, integrated vacuum degasser/column oven and RI detector. All instrument control, analysis and data processing was performed using a chromatography data system platform.

Method Parameters

The HPLC method parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade and filtered via 0.45-µm filters. Water was used for all dilutions.

Standard solutions of lactose, glucose and galactose were prepared. For all three sugars, a concentration range between 0.005% and 0.25% was prepared.

The samples included mozzarella cheese, ricotta cheese and an easily-digestible low-lactose milk. For each of these, 5 g of sample was weighed out in a 50-mL volumetric flask. Subsequently, each flask was filled to mark with 7% perchloric acid (HClO₄). The acid was used to precipitate the proteins, with the sugars remaining in solution. Each sample flask was then agitated for 10 minutes. The resulting mixtures were brought to pH 7 with 0.1 N NaOH (so as to avoid possible hydrolysis of the lactose), transferred to centrifuge tubes and centrifuged at 6000 rpm. 1 mL of supernatant from each flask was collected and filtered into individual 2-mL vials, using 0.22-µm PTFE filters. Overall, there was a 10-fold sample dilution during sample preparation. 10 µL of each prepared sample was then injected onto the HPLC.

Prior to injection, all calibrants and samples were filtered through 0.45-µm filters to remove any small particles.

Results and Discussion

For lactose, the resulting calibration had a linear fit of $R^2 = 0.997$ and, for both glucose and galactose, the fit was 0.999 (curves not shown).

Figure 1 shows the chromatogram of the 0.005% (50 ppm) standard solution, with the three analytes separated in under eight minutes. The additional 12 minutes were used to assure that all residual matrix components eluted off of the column between injections.
Figures 2 and 3 show the chromatograms of the prepared mozzarella and ricotta cheese, respectively. Only lactose was detected in each of these cheeses. Adjusting for the 10-fold sample dilution during sample preparation, both cheeses showed significant amounts of lactose. Though the diluted concentration of 0.275% for ricotta cheese was slightly above the 0.25% high calibration level, this wasn’t considered significant. Subsequently, this could easily be accommodated by using an additional calibration level.
Figure 4 shows the chromatogram of the prepared low-lactose milk sample. All three sugar analytes were detected. Lactose was found at 0.074%. This concentration level was not surprising, considering that this particular milk was of the easily-digestible variety, expected to contain <0.1% lactose. Though significant amounts of glucose and galactose were detected, as they were not the focus of this work, they were not quantitated.

**Conclusion**

The results demonstrated the effective use of this HPLC method for the isocratic separation of lactose, glucose and galactose and the quantitative analysis of lactose in milk and cheeses, using RI detection. The method allowed for lactose quantitation down to 0.005% (50 ppm) and the calibration range encompassed the highest analyte concentration typically found in such samples. If required, additional sensitivity may be obtained by increasing the injection volume, though it is recommended not to go beyond 50 μl, taking care not to overload the column.