Lactose Analysis: Evolution of Enzymatic Methods and Optimization of Analytical Performance in Complex and Delactosed Matrices

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P2621

Abstract

The aim of this paper is to demonstrate how CDR FoodLab® represents a practical evolution of traditional UV enzymatic methods for lactose analysis, overcoming some of their operational limitations related to sample preparation, reagent management, response times, and susceptibility to interference. The method is not presented as an absolute replacement for HPLC in official validation contexts, but as a rapid system with reliability comparable to the chromatographic reference for operational control of the lactose -free process.

1. Introduction: The Analytical Scenario and the Strategic Relevance of Lactose Control

Determining lactose in " lactose -free" products represents one of the most arduous analytical challenges for the dairy industry today. In a market where product purity is a prerequisite, quality integrity management must address a crucial technical issue: eliminating the risk of false negatives in the certification of residual lactose.
Lactose intolerance, linked to a deficiency of the lactase enzyme, requires manufacturers to guarantee extremely low concentrations, making analytical uncertainty a factor in economic and legal risk.
When **monitoring residual lactose**, the analytical method adopted determines not only the laboratory result, but also the manufacturer's ability to monitor the process with reliable data that can be used for operational decisions. Rapid availability of reliable data is essential for validating delactosation processes , optimizing the use of industrial enzymes, and avoiding batch holdups.
Understanding the chemical principle underlying the measurement therefore becomes a fundamental prerequisite for correctly interpreting the data and ensuring food safety.

2. Chemical Principles Compared: Signal Reactivity and Specificity

The enzymatic determination of lactose can be conducted using different approaches, which differ in the reaction principle, the detection system and the management of possible matrix interferences.

- **Traditional UV Method:** Uses a two-step reaction. Lactose is hydrolyzed by β -galactosidase into D-glucose and D-galactose. The latter is oxidized to D- galactonic acid by β-galactose dehydrogenase (β -Gal-DH) in the presence of NAD^+. The signal is generated by the production of NADH, measured at 340 nm. This approach often requires a differential calculation to determine the actual lactose.
- **CDR FoodLab® System**: While maintaining the initial hydrolysis step, the analytical signal is generated by residual glucose. Through a peroxidase-mediated reaction, glucose reacts with a phenolic compound to form a pink quinonimine complex. Absorbance is measured at 505 nm.

Parameter	Traditional Method (UV)	CDR FoodLab® System
Reaction principle	D-galactose oxidation (NADH)	Chromogenic reaction of glucose
Wavelength	340 nm (UV)	505 nm (Visible)
Sample Preparation	Clarification (Carrez) or Deproteinization	Simple dilution
Dosing points	4 separate reagents + redistilled water	Pre-filled reagent + addition of R1a and R2
Analysis time	> 60 minutes (30+30 min incubation)	10 minutes

Table 1: Comparison of traditional enzymatic method vs. CDR FoodLab®

3. Beyond the enzymatic method: The role of HPLC

To complete the picture of analytical techniques used in lactose control, it is also appropriate to consider HPLC, High Performance Liquid Chromatography , a method described by the ISO 22662 standard for the determination of lactose by chromatographic separation.
HPLC allows the separation and quantification of individual sugars, making it a go-to approach when in-depth analytical characterization of the carbohydrate profile is required. However, its use requires more structured operating conditions than routine enzymatic methods.
In particular, chromatographic analysis involves:

- **Specific technical skills** required for instrument management, preparation of mobile phases, column control and interpretation of chromatograms.
- **Longer analytical times** , linked to system preparation, column conditioning and the chromatographic sequence.
- **Higher operating costs** , associated with the use of HPLC-purity solvents, chromatographic columns, instrument maintenance and waste management.

In this context, while HPLC maintains a central role in final compliance and validation analyses, a rapid enzymatic system can operate in perfect complementarity for daily production monitoring.

4. Optimization for the "Lactose-Free" Range and Robustness of the Method

In the determination of residual lactose, particularly in the critical range of lactose-free products (0.01 – 0.1 g/100 g), accuracy is closely linked to the management of matrix effects.

Classic UV enzymatic methods claim a suitable theoretical limit of detection (LOD) (approximately 7 mg/L), but this often requires the introduction of a large volume of sample into the cuvette (up to 0.50 mL). This requirement amplifies turbidity problems due to suspended proteins and fats, making the reading unstable in the ultraviolet range (340 nm) and necessitating complex pre-treatments (such as clarification).

The **CDR FoodLab® system** addresses this limitation by transferring readings to the visible range at **505 nm** . This configuration significantly reduces the optical impact of protein and fat residues, even in matrices subjected to ultra-high heat treatments (UHT). The system allows the entire delactosation cycle to be monitored directly in the plant, promptly identifying the moment when lactose drops below the desired threshold (0.1 g/100 g).

This glucose-based approach also mitigates **the risk** of cross-reactivity with L- arabinose , a limitation present in UV systems that use β -galactose dehydrogenase. Arabinose , absent in pure milk, can be found in formulations such as yogurt with fruit (due to pectin degradation), plant-based drinks, or matrices containing thickeners and fibers (such as gum arabic). Since classic β -Gal-DH also shows affinity for this pentose, traditional methods run the risk of overestimating residual lactose. By modifying the analytical target, the CDR FoodLab® system minimizes the potential impact of arabinose , promoting greater data selectivity even in multi-ingredient formulations.

6. Operational Efficiency and Analytical Risk Reduction

In process quality control, analytical efficiency is closely linked to workflow standardization and the minimization of manual variables, factors that directly impact data repeatability.

- **Critical Issues with the Traditional Method:** Sample preparation requires the use of Carrez reagents or perchloric acid for clarification, laborious procedures that introduce variability. Reagent management is complex: the NAD/Citrate solution is stable for only **3 months** after preparation, with the risk of using partially degraded reagents. Furthermore, manually pipetting four different reagents plus redistilled water significantly increases the coefficient of variation (CV%).
- **® System :**
  - **Simplified Workflow:** Milk requires a simple 1:10 dilution, while solids (cheese/butter) are handled with a quick 3-minute extraction in water via a Stomacher and subsequent filtration.
  - **Ready-to-use reagents and stability** : Unlike traditional methods that require the extemporaneous preparation of solutions with a rather short shelf-life, CDR reagents are pre-vialized and ready to use, with an expiry date of 12 months.
  - **Real-Time Response:** With an analysis time of just 10 minutes (versus 60+ minutes with the UV method), the CDR FoodLab® allows for line adjustments during production, transforming quality control from a bottleneck to a factor in production agility.

7. Experimental validation: correlation with HPLC and applicability to lactose -free control

The analytical robustness of the CDR FoodLab® system is supported by experimental evidence showing a high correlation with chromatographic methods (HPLC), historically considered the reference standard for the quantification of sugars. The reliability of the method was confirmed through two different levels of evaluation:

- **Validation of ACTALIA Cecalait** : conducted on lactose-free milk, showed excellent precision compared to HPLC ($R^2 = 0.9882$) with a low standard deviation of repeatability (0.017 g/100 g)
- **Verification under routine conditions (IZS Laboratory)** : a comparative study on commercial samples, managed by different operators with different instruments, confirmed the robustness of the system in the field, recording a correlation equal to $R2 = 0.9903$ compared to the official HPLC method.

These data confirm the positioning of CDR FoodLab® : an instrument designed not to replace HPLC in fully compliant laboratories, but to directly transfer into process control a rapid, repeatable determination that is perfectly consistent with the operating thresholds of the lactose -free market.

Evidence	Source/ Context	Key data	Meaning operating
Repeatability	ACTALIA	S_r = 0.017 g/100 g	Suitable for control operating

Evidence	Source/ Context	Key data	Meaning operating
Correlation with HPLC	ACTALIA	R^2 = 0.9882	High alignment with chromatographic reference
Standard error	ACTALIA	±0.09 g/100 g	Compatible with lactose - free threshold 0.1 g/100 g
IZS Correlation	Commercial samples	R^2 = 0.9903	Confirmation on real samples and external laboratory

Conclusions

The evolution of the *lactose -free market* requires dynamic quality control, capable of integrating into the production flow without slowing it down. While HPLC remains the benchmark for legal compliance and the traditional UV method suffers from operational complexity and the risk of overestimation (as in the case of L- arabinose), the **CDR FoodLab® system** offers an ideal combination of scientific rigor and plant operations:

- **Specificity and interference control:** The 505 nm readout and glucose-focused kinetics protect the data from turbidity, protein residues and false positives caused by extraneous sugars in complex matrices.
- **Process Speed:** Analysis in just 10 minutes, combined with minimal sample preparation, allows you to monitor delactosation progress in real time for rapid in-line decisions.
- **Validated robustness:** The high correlation with the HPLC standard ($R^2 > 0.9882$), certified by independent bodies such as ACTALIA and IZS, guarantees maximum data reliability even in routine multi-operator conditions.

In summary, the integration of CDR FoodLab® optimizes laboratory flows and eliminates waiting times, transforming the control of residual lactose from a potential bottleneck into a strategic factor for efficiency and food safety.

Useful links

- Validation of Enzytec ™ *Liquid* Combi Lactose/D-Galactose for Enzymatic Determination of Lactose and D-Galactose in Selected Foods: Official Method 2024.10 First Action
- Analytical validation of an advanced U-HPLC–MS/MS method for lactose detection in food supplements and pharmaceuticals
- Determination of lactose in milk and dairy products
- CDR FoodLab® milk analysis evaluation report - ACTALIA
- Correlation study of lactose analysis with reference method