

Milk safety and heat treatments: the efficiency of the ALP test with CDR FoodLab®

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1. Introduction and biochemical importance of alkaline phosphatase (ALP)

In the dairy industry, **alkaline phosphatase (ALP)** is not simply an endogenous enzyme, but represents the **fundamental analytical pillar** for validating microbiological safety. As an **intrinsic time-temperature indicator**, ALP serves as a biochemical marker whose inactivation signals the success of heat treatment, protecting public health and ensuring legal compliance.

Definition and enzyme kinetics

Alkaline phosphatase is an enzyme naturally present in the raw milk of various species (**bovine, ovine, caprine**). Its biochemical function lies in the cleavage of phosphate groups in an alkaline environment (optimal pH **8-10**). The strategic importance of ALP as a process indicator lies in its **thermoresistance**, which is slightly higher than that of the non-spore-forming pathogens most critical to human health.

In terms of heat resistance, the thermal inactivation curve of alkaline phosphatase exhibits a kinetic characteristic that has been exploited by the dairy industry as a process indicator: its denaturation temperature is in fact just above that of the non-spore-forming pathogenic microorganisms most critical to human health. Among these, *Mycobacterium tuberculosis* stands out, historically used as a benchmark for defining minimum treatment parameters, and especially *Coxiella burnetii* (the causative agent of Q fever), now universally recognized as the most heat-resistant non-spore-forming pathogen present in raw milk. The cardinal principle of the '**safety margin**' is based precisely on this biochemical peculiarity: since ALP can withstand slightly higher time and temperature combinations than these bacteria, its complete inactivation guarantees that the target pathogen load has been effectively eliminated. It is important to emphasize that a negative ALP test is the specific indicator for the destruction of vegetative bacterial forms, but it does not guarantee the absence of spore-forming microorganisms, which require more severe heat treatments (such as UHT). Nonetheless, the test constitutes legal and technical proof that the minimum pasteurization parameters established by regulations (72°C for 15 seconds or 63°C for 30 minutes) have been reached, confirming the microbiological safety of fresh milk placed on the market.

2. Control of heat treatments in the production process

Pasteurization represents the **critical control point (CCP)** par excellence in a plant. Inefficient management of this parameter not only compromises safety but also exposes the company to financial risks resulting from logistical bottlenecks and product recalls.

Risk prevention, interspecific variability and regulatory limits

Rigorous ALP monitoring responds primarily to public health imperatives, acting as a fundamental bulwark in preventing the transmission of pathogens responsible for serious diseases transmissible to humans (zoonosis) and ensuring the safety of the finished product. However, the effectiveness of this control requires a thorough understanding of interspecies variability. Baseline ALP levels vary dramatically depending on the animal species: sheep's milk has an initial enzymatic activity approximately three times higher than cow's milk, while goat's milk shows levels up to five times lower. Consequently, adopting a standardized analytical approach without targeted species calibration risks being misleading and commercially unsafe.

This biological complexity is compounded by strict legal parameters. For cow's milk, the international safety threshold is set at 350 mU /L (milliunits per liter), beyond which the test instantly signals insufficient pasteurization or post-process recontamination with raw milk. The sensitivity of this biochemical marker is so high that it can detect even infinitesimal percentages of untreated milk, between 0.02% and 0.1%, protecting the entire production line from cross-contamination.

The phenomenon of " Reactivation " (Enzyme Reactivation)

A complex biochemical phenomenon that challenges quality control laboratories is ALP reactivation. This anomaly frequently occurs in high-fat products, such as cream, or in milk subjected to UHT treatment and subsequently stored at temperatures above 30°C, especially in the presence of magnesium salts.

A sector operator must be able to distinguish a true pasteurizer failure from a reactivation phenomenon or the presence of microbial ALP, produced by heat-resistant bacteria. In this context, the analysis follows two distinct paths. The re-pasteurization

test (performed in the laboratory at 63°C for 30 minutes) allows the isolation of microbial ALP: being highly heat-stable, its activity will not significantly decrease after the treatment, unlike the native ALP in milk, which will be destroyed. To differentiate residual ALP (from failed pasteurization) from reactivated ALP, the magnesium activation test is used, comparing the enzyme's increased levels under controlled conditions and thus absolutely ruling out a false positive or a real technical failure in the system. It is therefore clear that monitoring Alkaline Phosphatase is crucial for safety and regulatory compliance. Managing numerous variables in real time requires analytical responsiveness that traditional laboratory methods, often time-consuming and complicated, struggle to guarantee. To implement efficient *Positive Release dynamics* and avoid bottlenecks during storage, it is therefore essential to adopt analytical solutions capable of transferring the accuracy of the official laboratory directly to the production site.

3. ALP analysis with the CDR FoodLab® system

The **CDR FoodLab® system** is capable of transferring the accuracy of official fluorimetry directly to the production line through a rapid and intuitive approach, optimized for any type of operator. Overcoming the complexity of traditional methods, this solution for the **determination of alkaline phosphatase** eliminates operational criticalities thanks to the use of pre-vialled, ready-to-use reagents. The analysis is completed in just **25 minutes** and allows for the **processing of multiple samples simultaneously**, thus further reducing analysis time, obtaining results without requiring specialized personnel or laborious calibrations. The instrument operates on a **measurement range from 0.1 to 7 U/L**, with a **fine resolution of 0.01 U/L** and a **repeatability of 0.12 U/L**. This versatility transforms the system into a complete analytical platform: efficient ALP monitoring can be integrated simultaneously with the control of other key chemical parameters, offering the user a 360-degree overview of the quality of the raw material and the integrity of the production process.

Beyond ALP: Integrated screening with CDR FoodLab®

On the same analytics platform, you can monitor:

- **Peroxidase (POD):** Critical for detecting overpasteurization. A **Positive POD** result is the target: it demonstrates that the milk has not been excessively heat-stressed, preserving its nutritional value and proteins.

- **Furosine/ Fructosyl-lysine:** A key indicator of heat treatment and freshness. A low furosine level guarantees the absence of excessive heat treatments or the addition of reconstituted milk powder, thus ensuring the integrity of essential amino acids.
- **Lactic Acid:** A marker of primary hygiene. A low lactic acid level demonstrates the quality of the raw material that will then undergo heat treatment.
- **Urea and Ammonia:** Indicators of bovine health and feed efficiency, essential for monitoring the upstream supply chain.

4. Conclusions

In conclusion, **Alkaline Phosphatase monitoring** remains crucial for validating heat treatments and protecting public health in the dairy industry. The technological evolution interpreted by the **CDR FoodLab® system** demonstrates how it is now possible to combine the scientific rigor of official methods with the speed and flexibility required by modern production lines. Furthermore, the ability to expand screening beyond ALP on the same platform—integrating real-time analysis of **peroxidase, furosine (Fructosyl-lysine), lactic acid, urea, and ammonia**—allows the plant to go beyond simple compliance checks. Quality control allows for the protection of the nutritional value of raw materials, optimization of company processes, and increased consumer safety.

Bibliography / Bibliographic References

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