IMPACTING BETTER FOOD™

Mitigating Age Gelation Caused by AprX Enzyme Activity in UHT Milk

JOHA® Functional Phosphates Inhibit AprX Proteolytic Activity and Protect Casein Micelle Stability

Ultra-high temperature (UHT) milk is widely adopted in the dairy industry due to its extended shelf life, microbiological safety, and convenience for ambient storage and global distribution. UHT processing typically involves heating milk to temperatures above 135 °C for a few seconds, effectively inactivating vegetative microorganisms and most spoilage enzymes while retaining the majority of the milk's nutritional value. As a result, UHT milk can be stored for several months without refrigeration, offering significant logistical and economic advantages, particularly in regions with limited cold-chain infrastructure and for applications such as export, food service, and shelf-stable beverage formulations.

Despite these benefits, UHT milk is not exempt from quality deterioration during storage. One of the most critical defects affecting UHT milk quality is age gelation, a phenomenon characterized by the gradual transition of milk from a fluid to a gel-like state during storage. This defect leads to unacceptable changes in viscosity, texture, and sensory perception, ultimately resulting in consumer rejection, even when products are processed and packaged under aseptic conditions.

Age gelation in UHT milk is strongly associated with the activity of heat-stable proteases, including both microbial enzymes such as AprX and endogenous enzymes like plasmin. AprX is an extracellular metalloprotease produced by psychrotrophic bacteria such as *Pseudomonas fluorescens* during raw milk storage. This enzyme exhibits remarkable thermostability, surviving UHT processing and maintaining proteolytic activity throughout shelf life. Its primary action involves hydrolysis of κ-casein, releasing caseinomacropeptides and disrupting micellar integrity, which promotes protein aggregation and ultimately leads to gel formation.[1-3]

In parallel, plasmin—the major endogenous protease in milk—also contributes to age gelation. Derived from plasminogen activation, plasmin preferentially hydrolyzes β-casein and αs2-casein, targeting regions critical for micellar stability. Although less heat stable than AprX, plasmin partially survives thermal treatment and remains active during storage, promoting proteolysis that destabilizes the colloidal matrix and facilitates protein network formation.[4]

The combined action of these proteases poses significant challenges for UHT product stability during shelf life, requiring stringent control strategies from raw milk handling to processing.

The economic implications of age gelation are substantial, encompassing:

- Product recalls
- Increased waste
- Diminished consumer confidence

Consequently, strategies to mitigate this phenomenon and stabilize casein micelles are of paramount importance for manufacturers seeking to ensure product reliability and profitability.

To address this challenge, ICL collaborated with the Federal University of Viçosa (Brazil) to investigate the efficacy of JOHA® functional phosphate blends in mitigating AprX-induced age gelation in UHT milk.

By examining enzyme activity and micellar stability under controlled conditions, we aimed to provide a scientifically validated, cost-effective approach to improving UHT milk shelf life and maintaining consistent product quality.

Materials and Methods

Sample Preparation

The sample was prepared by reconstituting skimmed milk powder at 10% (w/w) with distilled water. The mixture was mixed until complete hydration of the powder. Subsequently, the milk was divided into equal portions in 250 mL Erlenmeyer flasks for phosphate addition, except for the control sample. Each sample was then supplemented with AprX at a concentration of 116 U/mL. The mixtures were mixed again to ensure complete incorporation of the enzyme.

Phosphate Treatment

JOHA® functional phosphate blends were evaluated at level of 0.05% relative to control formulations without added phosphates. All treatments were prepared in triplicate to ensure experimental reproducibility and statistical robustness.

Enzyme Activity Assay

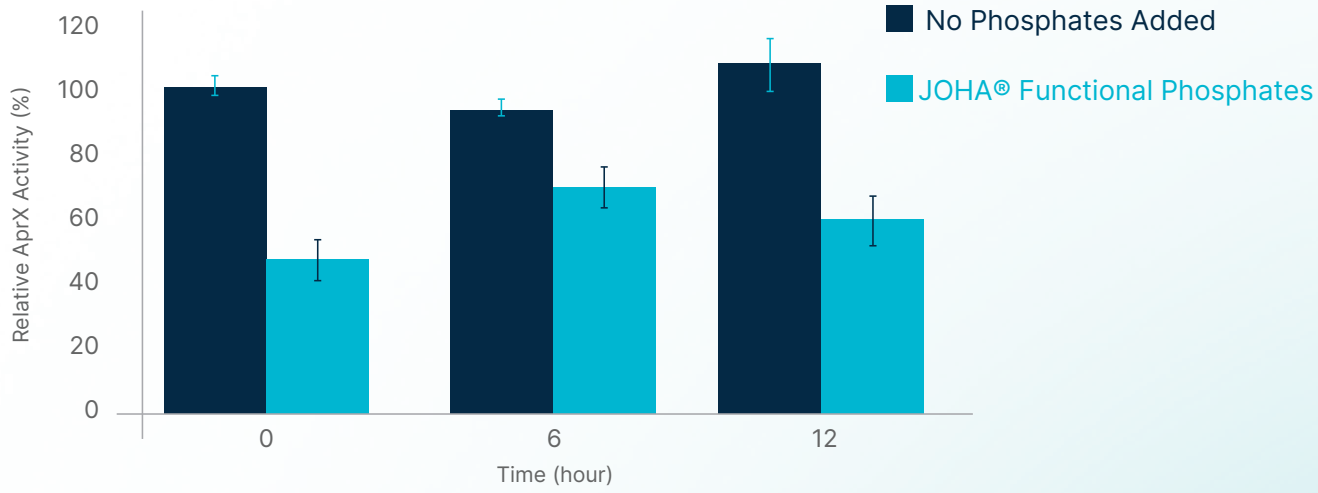
AprX proteolytic activity was quantified using an azocasein-based spectrophotometric assay. The reaction mixture consisted of 125 µL of 2% (w/v) azocasein solution, 50 µL of AprX enzyme extract, and 125 µL of Tris-HCl buffer (240 mM, pH 8.0). Samples were incubated at 37 °C for 1 h to allow proteolysis. The reaction was terminated by adding 600 µL of trichloroacetic acid (TCA, 20% w/v), followed by centrifugation at 10,000 × g for 10 min to remove precipitated proteins. The absorbance of the supernatant was measured at 366 nm using a UV–Vis spectrophotometer. One unit of AprX activity was defined as an increase of 0.01 absorbance units per hour under the assay conditions.

Data Analysis

Results were expressed as relative AprX activity (%) compared to the control at time zero. Statistical analysis was performed using ANOVA followed by Tukey's post hoc test, with significance set at $p < 0.05$.

Results and Discussion

JOHA® functional phosphate blends significantly reduced AprX activity compared to control samples.



In the absence of phosphate intervention, AprX activity remained elevated, underscoring the enzyme's persistent proteolytic potential. This sustained activity is particularly detrimental because AprX targets κ-casein, releasing caseinomacropeptides and disrupting micellar integrity, which accelerates protein aggregation and ultimately leads to gelation.

In contrast, the incorporation of JOHA® functional phosphate blends produced an immediate and substantial inhibitory effect, reducing AprX activity and maintaining significantly lower enzymatic activity throughout the entire testing duration. The mechanism underlying this inhibition is likely multifactorial: phosphate ions modulate calcium equilibrium within the milk system, enhance micellar stability, and may interfere with enzyme-substrate interactions, thereby limiting proteolysis. This reduction in proteolytic activity correlates strongly with improved UHT milk stability, as the preservation of κ-casein structure and micellar integrity mitigates aggregation phenomena and delays gelation, ensuring extended shelf life and superior product quality.

Conclusion

JOHA® functional phosphate blends, applied at exceptionally low inclusion levels of 0.05% (w/w), deliver a statistically proven solution to delay age gelation in UHT milk caused by AprX proteolytic activity.

By optimizing calcium balance and reinforcing casein micelle stability, JOHA® ensures:

- Extended shelf life
- Consistent product quality and product standardization
- Reduced economic risk

This cost-effective approach, backed by ICL Food Specialties' expertise and innovation, provides manufacturers with a reliable, science-driven solution for long-term success.

Why Work with ICL Food Specialties

ICL Food Specialties is an ingredient solutions provider with over 80 years of experience in the food and beverage industry. We combine the power of a global leader with the passion of an agile team of local experts to help you create solutions that impact the future of food.

Contact us at: iclfood.com/contact

References

[1] D'Incecco, P., Rosi, V., Fortina, M. G., Sindaco, M., Ricci, G., & Pellegrino, L. (2022). Biochemical, microbiological, and structural evaluations to early detect age gelation of milk caused by proteolytic activity of Pseudomonas fluorescens. European Food Research and Technology, 248(9), 2097–2107. https://doi.org/10.1007/s00217-022-04033-8

[2] Zhang, C., Bijl, E., Svensson, B., & Hettinga, K. (2019). The extracellular protease AprX from Pseudomonas and its spoilage potential for UHT milk: A review. Comprehensive Reviews in Food Science and Food Safety, 18(4), 834–852. https://doi.org/10.1111/1541-4337.12452

[3] Baglinière, F., Matéos, A., Tanguy-Sai, G., et al. (2013). Proteolysis of ultra high temperature-treated casein micelles by AprX enzyme from Pseudomonas fluorescens F induces their destabilisation. International Dairy Journal, 31, 55–61. https://doi.org/10.1016/j.idairyj.2013.02.011

[4] France, T. C., O'Mahony, J. A., & Kelly, A. L. (2021). The plasmin system in milk and dairy products. In Food Engineering Series (pp. 11–55). Springer. https://doi.org/10.1007/978-3-030-55482-8_2