Effect of Raw Milk Temperatures on the Growth of Enterotoxin Producing Pathogens: Use of ComBase Predictive Models for Deviation Variance

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Abstract: The Pasteurized Milk Ordinance (PMO) requires that raw milk for Grade A plants be shipped and stored at temperatures below 45 °F to ensure that microbial counts are kept at a minimum prior to pasteurization. Chilled storage is especially important to control growth of bacterial pathogens that are capable of producing heat-stable enterotoxin that are not inactivated by pasteurization that is suitable to kill vegetative cells. However, there are deviations where milk is measured at a temperature between 46°F to 50 °F upon arrival at the facility. To address this issue, the effect of elevated storage temperature on the growth of toxigenic microbes were evaluated using ComBase Predictive Model. The model showed <1 log increase of *Staphylococcus aureus* and *Bacillus cereus* in a sterile matrix with pH 6.8 when held at temperatures as high as 51°F for up to 72 hours; this growth limit is considered to be below the threshold level needed for development of heat-stable enterotoxin. Therefore, in the rare event that milk temperature upon receipt exceeds the regulatory limit (>45°F), the output from the model can be used as documentation to support safe use of the milk for pasteurization and further processing provided that the milk was stored at ≤50°F for ≤72 hours. In addition to regulatory compliance, milk temperature limits of no greater than 45°F is still recommended to avoid quality issues.

Objective: to provide safety evaluation of milk delivered at temperatures that exceed the limit of 45°F defined by the PMO.

Introduction: Raw milk is known to contain pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus* and others. Grade A products are regulated by the Pasteurized Milk Ordinance issued by the FDA. This regulation requires that raw milk be shipped and stored at temperatures below 45 °F for Grade A plants to ensure that microbial counts are kept at a minimum of growing prior to pasteurization, especially pathogens capable of producing heat-stable enterotoxin. There are some instances where milk is measured at a temperature between 46 °F to 50 °F upon arrival at the facility. If the temperature deviates from required maximum limit, the load must be rejected unless the deviation is evaluated for safety and temperature history is sufficiently conservative to prevent enterotoxin production. Surveys of populations of *Bacillus cereus* and *Staphylococcus aureus* in raw milk are typically $<10^3$ CFU/g

(1, 4), > 3-log increase would be needed to produce sufficient enterotoxin to cause illness *(5, 6)*. Furthermore, staphylococcal enterotoxin is produced at temperatures of 59°F (15°C) or above *(3)*. This evaluation is intended to serve as scientific evidence for disposition of milk when received at temperatures that exceed the temperature of 45°F.

Methods: ComBase Predictor (https://www.combase.cc/index.php/en/) for *Staphylococcus aureus* and *Bacillus cereus* was used to predict growth at temperatures ranging from 45 to 55°F (7.2 to 12.8°F). The predictive model is based on growth characteristics in sterile laboratory (nutritive) media (worst case scenario without competitive microbes)*(2)*. Therefore, growth is typically "over predicted" (i.e., considered to be fail-safe compared to behavior of microbes in foods). For this study, the model was run at pH 6.8 and water activity 0.99 to mimic characteristics of fluid milk, and at temperatures ranging from 45 to 55°F (7.6 to 12.8°C, lower limits of the model) for *S. aureus*, and from 5.6 to 12.8°C for *B. cereus*. Although there are strains of psychrotrophic *B. cereus* that are capable of growth temperatures between 48-51°F, these typically are spoilage rather than pathogenic, or growth occurs extremely slow (such over weeks) at those temperatures and there is no clear evidence that enterotoxin production will be produced at the lower temperatures. As noted in the introduction, production of enterotoxin by *S. aureus* requires temperatures of 59°F or greater. Pass/fail limit is designated at 10^6 CFU/ml, populations associated with sufficient enterotoxin production to cause illness.

Results: Output of the models are reported in Figures 1 and 2. The model showed that growth of either pathogen is limited to less than a 1-log increase when temperatures are less than 51°F (10.6°C) for 72 hours in a sterile media (without competitive microbiota, such as lactic acid bacteria). Using a worst-case initial population of 10^3 CFU/ml of either pathogen, populations at the end of the 72-h hold would be 10^4. Using the pass/fail limit of 10^6 CFU/ml, these storage conditions provide a 100-fold margin of safety. The presence of indigenous microbes in the raw milk, specifically lactic acid bacteria, are expected to be competitive to pathogens, particularly at slightly elevated temperatures.

Conclusion: In the event of a deviation for milk temperature at receipt (PMO requirement of no greater than 45°F), ComBase predictive model and other known characteristics for growth and enterotoxin production for *S. aureus* and *B. cereus* show that raw milk that is stored below the temperature of 50°F for less than 72 hours is considered to be safe to pasteurize and will not have a significant food safety risk. Temperature limits for 45°F is still recommended to avoid quality issues.

References:

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Figure 1. Growth of *Staphylococcus aureus* in model media representing milk composition (pH 6.8, a_w 0.99), ComBase Predictor, no competitive microbiota

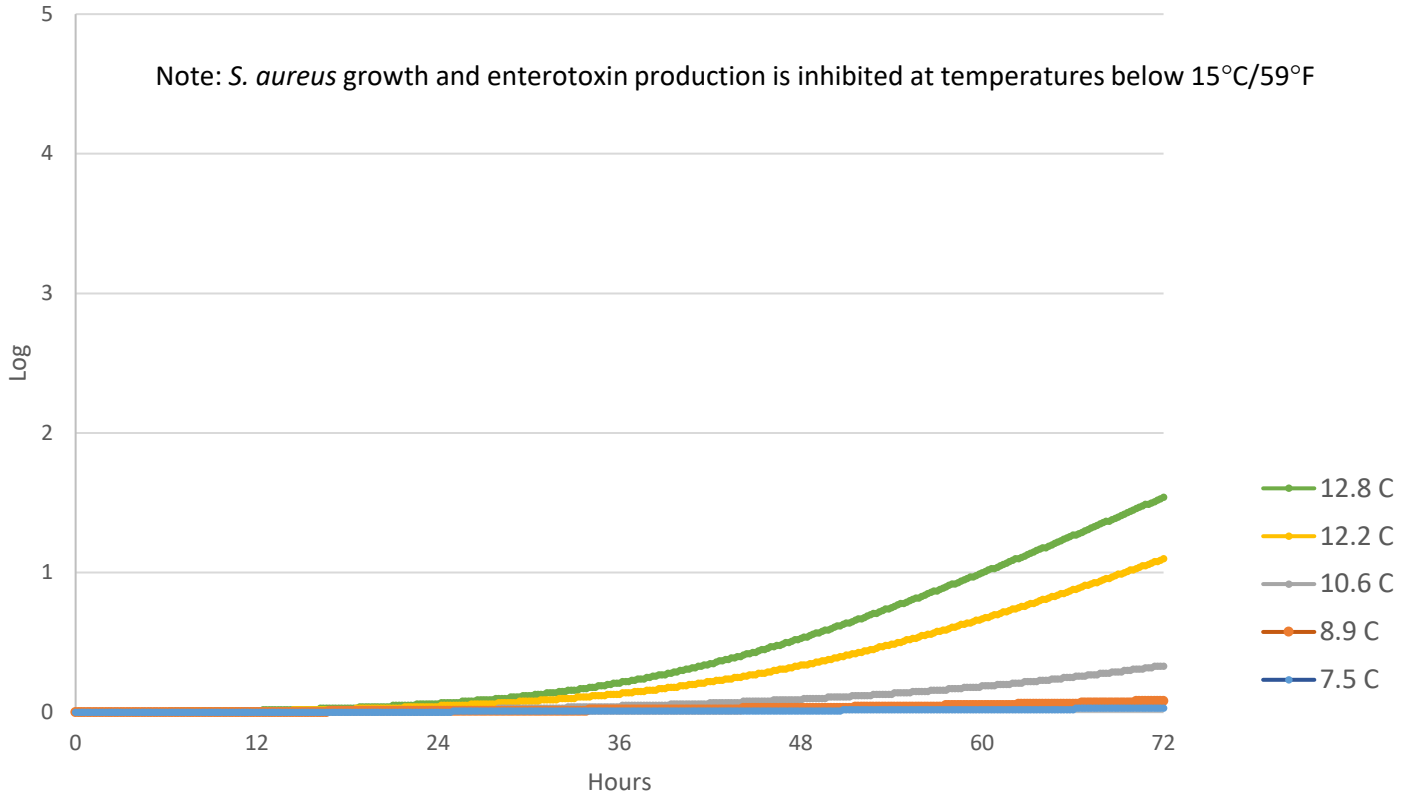

Figure 2. Growth of *Bacillus cereus* in model media representing milk composition (pH 6.8, a_w 0.99), ComBase Predictor, no competitive microbiota