Cereulide in Dairy Matrices

Last Updated February 2024

<u>Question:</u> Please summarize studies conducted on cereulide in dairy matrices, focusing on the toxin itself or any chemistry of the toxin and with less emphasis on B*acillus cereus* growth and toxin production.

<u>FRI response:</u>

What is cereulide?

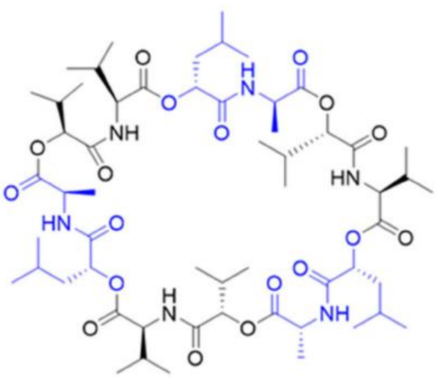

Figure 1. Chemical structure of cereulide with D-*O*-Leu-D-Ala in blue and L-*O*-Val-L-Val in black.

- Cereulide is a toxin produced by some strains of *Bacillus cereus* that is responsible for emetic intoxication when the preformed toxin is consumed in foods (Bamnia and Kaul, 2015).
- Additionally, some strains of *B. cereus* may produce enterotoxins (hemolysin BL, non-hemolytic enterotoxin, and cytotoxin K) (Sarkar et al., 2023).
- Cereulide is a cyclic dodecadepsipeptide of approximately 1.2 kDa.
    - Depsipeptides are compounds that contain both ester and amide bonds (Alonzo and Schmeing, 2020).
    - Other cyclic depsipeptides include ionophores, quorum sensing modulators, toxins, and antiobiotics, including the anticancer agent valinomycin and the pesticide antimycin (Alonzo et al., 2015; Alonzo and Schmeing, 2020).
    - The cereulide peptide is made of 12 alternating aminoacyl and hydroxyacyl residues moieties (Alonzo et al., 2015) and contains three tetradepsipeptide repeat units (Alonzo et al., 2015; Naka et al., 2020; Walser et al., 2022).

- Its rigid, cyclical structure increases its resistance to agents such as proteases or thermal treatments (Walser et al., 2022).
- The side chains of the amino acids and hydroxy acids in the cereulide structure contain only hydrocarbons (Naka et al., 2020). As a result, the toxin is highly lipophilic, facilitating its absorption from the gut into the bloodstream and making it poorly soluble in water (Paananen et al., 2002).
- In culture supernatants, cereulide degrades by hydrolysis (probably enzymatic) into smaller depsipeptides. The biological activities of these hydrosylate depsipeptides are unknown (Naka et al., 2020).
- Variants of cereulide are found in different *B. cereus strains* that have different (in some cases, increased) toxicity (Marxen et al., 2015; Ducrest et al., 2019; Walser et al., 2022).

How is it generated?

- Cereulide is not synthesized by ribosomes; it is synthesized by a special two-protein nonribosomal peptide synthase found in *B. cereus* (Arnesen et al., 2008).
- The cereulide variants are believed to be made as a result of a relaxed proof-reading function of the nonribosomal peptide synthase (Marxen et al., 2015).
- Different levels of toxin are produced in different types of foods, with lower levels found in egg, meat, and milk than in boiled rice and farinaceous foods (Arnesen et al., 2008).
- Higher levels of cereulide are made during the stationary phase of growth and in food compared to under aerated incubation in growth media (Arnesen et al., 2008).
- Cereulide production occurs over a narrower range of temperatures than does growth, with cereulide production low or absent at 37°C but usually optimal (and plateauing) at ~30°C (Ellouze et al., 2021).
- Growth of *B. cereus* is usually optimal around 40°C. Dairy matrices tend to support intermediate cereulide formation rates, but dairy matrices also result in higher *B. cereus* growth rates when compared to cereal, meat and vegetable matrices (Ellouze et al., 2021).
- The presence of lactose inhibits cereulide production in milk (Zheng et al., 2024).

Mechanism of toxicity?

- Cereulide has a "high affinity for complex formation with alkali metal ions (Li+, Na+, and K+) and ammonium ions" (Walser et al., 2022).
- Cereulide's toxicity is a result of this affinity for potassium: cereulide is a potassium ionophore and transporter that appears to inhibit mitochondria by damaging the cell membrane potential (Bamnia and Kaul, 2015) (Naka et al., 2020; Walser et al., 2022).

How is it detected in foods?

- The small size and unusual shape of cereulide makes it relatively non-immunogenic, so few antibody-based tests for the toxin exist (Ducrest et al., 2019).
- Bioassays or mass spectrometry assays have typically been used for cereulide detection, although MALDI-TOF has more recently shown promise for detection of the toxin (Ducrest et al., 2019).

Stability of cereulide to heat and acid?

- Due to its structure, cereulide is extremely heat stable.
    - It can withstand 126°C for 90 minutes according to one review (Bamnia and Kaul, 2015).
    - The toxin appears less stable to heat at higher pHs and lower concentrations, but is more stable in the presence of oil (Rajkovic et al., 2008):
        - At pH 7, cereulide is stable for more than 2 hours at 121°C.
        - At pH values between 8.7 and 10.6, no loss in cereulide toxicity was observed after 150 min at 100°C.
        - At concentrations of 5 µg/mL, toxin was inactivated after 180 min at 100°C and pH>9.25. At a concentration of 0.5 µg/mL and a pH of 8.6, cereulide was inactivated after 90 min at 100°C.
        - In buffers containing olive oil, no loss of toxic activity was observed after 180 min at 100°C. Oil was protective to a smaller extent at higher temperatures.
        - In some cases, the inactivation by heat appears reversible.
- Cereulide is resistant to a wide range of pH values (2 to 11) and towards proteases (Rajkovic et al., 2008).

How does cereulide behave in dairy matrices?

- Within cow milk, cereulide showed a clear affinity towards the hydrophobic, lipid phase, in line with the toxin's hydrophobicity (Walser et al., 2021).
    - As milk fat content of milk increased, (0.5 to 50%), the percentage of cereulide located in the lipid phase increased from 13.3 to 78.6% (Walser et al., 2021).
    - Interestingly, increasing the fat content of milk with sunflower oil up to 50% only resulted in a 47.8% of cereulide to be found in the lipid phase (Walser et al., 2021). The authors of the study hypothesized that this difference arose from the better homogenization of the milk with milk fat vs. sunflower oil or from the different chemical composition of the lipid sources.

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Other papers on *B. cereus* in dairy environments

- Other references which look at *B. cereus* growth in dairy environemnts that might be interest include (da Silva et al., 2022; Jovanovic et al., 2022; Meng et al., 2022; Tirloni et al., 2022; Torii and Ohkubo, 2023)

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