STATEMENT

Bacterial Spores in Plant-Based Ingredients Intended for Beverage Applications

The plant-based (PB) beverage market has grown over 11% in the past five years and is expected to keep growing as PB beverages evolve from niche alternatives to lifestyle stables. As such, there was a need to evaluate microbial risks when formulating low-acid PB beverages that are distributed in a shelf-stable format. Of particular concern is the presence of spore-forming bacteria that are inherently present in PB ingredients such as almond, faba, oat, and pea through contact with soil.

The presence of spore-forming bacteria is highly undesirable in food ingredients, especially those used in the manufacturing of beverages that don't employ hurdle strategies other than thermal process, such as low product pH or storage under refrigerated conditions.

The research consortium organized by NIZO on microbial contaminants in PB foods referenced below uncovered the following key learnings:

Spores in plant-based (PB) ingredients

Most of the spores isolated from PB ingredients belonged to the *Bacillus*, *Geobacillus*, and *Clostridium* spp, with levels ranging from less than 1 to 4 Log_{10} CFU/g. *G. stearothermophilus* was found to be the most common among the heat-resistant thermophilic spore (HRTS) species isolated from PB ingredients. Processing conditions of the ingredient can affect levels of HRTS. Among the samples tested, levels were generally low, around or below the LOD of 1 Log_{10} CFU/g, with a few exceptions exceeding these levels (Kyrylenko et al., 2023).

The majority of the mesophilic spore isolates were identified as species belonging to the *Bacillus* genus. 42% of the isolates were identified as *B. licheniformis*, 21% as *B. subtilis* and 11% as *B. cereus* group species. Among the *B. cereus* strains, 9% contained the *ces* gene which encodes the heat-stable emetic toxin cereulide. Anaerobic sulfite reducing *Clostridium* spore (SRCS) levels were generally low, around or below the LOD of 1.0 Log_{10} CFU/g with most species identified as *C. sporogenes/tepidum* (Kyrylenko et al., 2023).

Limiting spoilage incidents

Ultra-High Temperature (UHT) treatment effectively inactivates spores of most bacterial isolates from PB ingredients, including strains of *B. cereus*, *B. cytotoxicus*, *B. licheniformis*, and *C. sporogenes* (Karamcheri et al, 2025). However, exceptionally high heat-resistant thermophilic spores, such as spores of *G. stearothermophilus,* can withstand even UHT conditions, leading to commercial sterility issues. Once spores survive and favorable environmental conditions return, they can germinate and grow, compromising the quality of the final product.

Therefore, using ingredients that have low levels of HRTS is crucial to limit spoilage incidents. This is especially important for shelf stable beverages that could be exposed to warmer temperatures during distribution and storage, allowing for thermal process survivors to grow.

It is also important to note that proper hydration of powdered ingredients prior to thermal processing is crucial to prevent "dry pockets" and ensure penetration of wet heat to the spores. Since spores are more resistant to dry heat, these "dry pockets" create areas where heat sterilization has lower efficiency on spore inactivation. This increases the risk of thermal process survivors, including survival of species that are not highly heat resistant and are of safety concern such as *B. cereus*.

Recommendation

It is the recommendation of these industrial leaders that PB ingredient suppliers monitor and control bacterial spores, including HRTS and *B. cereus*, to ensure consistent low levels in PB ingredients intended for beverage applications. This will enable more streamlined verification of incoming ingredients at the customer level.

Enumeration of spores in PB powders

To ensure comparable results across sites, we recommend the adoption of the NIZO spore enumeration methods described in the references below. Of most importance, for HRTS, a heat treatment of 100 °C for 30 min is applied, followed by incubation of the plates at 55 ± 2 °C. For *B. cereus*, the use of selective chromogenic agar is highly recommended.

Additional information on these NIZO learnings can be found in the references below.

References

NIZO food research B.V., Industrial statement endorsement letter, 9 June 2026 *(See attachment)*

Alina Kyrylenko, Robyn T. Eijlander, Giovanni Alliney, Elly Lucas-van de Bos, Marjon H.J. Wells-Bennik, Levels and types of microbial contaminants in different plant-based ingredients used in dairy alternatives, International Journal of Food Microbiology, Volume 407, 2023, 110392, ISSN 0168-1605, https://doi.org/10.1016/j.ijfoodmicro.2023.110392.

Soundarya T. Karamcheti, Karin I. Beekmann-Metselaar, Robyn T. Eijlander, Marjon H.J. Wells-Bennik, Marcel H. Zwietering, Heidy M.W. den Besten, Heat resistance of bacterial spores isolated from plant-based matrices: Consequences for food safety and quality, International Journal of Food Microbiology, Volume 443, 2025, 111426, ISSN 0168-1605, https://doi.org/10.1016/j.ijfoodmicro.2025.111426.