Ede, The Netherlands
09 June 2026

Subject: *Endorsement industrial statement*

To whom it may concern,

Through this letter, NIZO endorses the industrial statement issued by Ripple Foods, Royal FrieslandCampina, Tetra Pak, SPX Flow and HP Hood on “Bacterial Spores in Plant-Based Ingredients Intended for Beverage Applications”.

The industrial statement is based on pre-competitive research performed by NIZO food research B.V., HAS Green Academy and Wageningen University in a public-private consortium project with 11 industrial partners.

Within the food, beverage and ingredients industry, the results of this project have greatly increased awareness on microbial risks associated with plant-based ingredients, supported by increased confidence that we are able to reliably predict and interpret those risks to minimize negative consequences. When it comes to food safety, the whole market and society benefit from sharing knowledge and experiences and joining forces to reach a common goal: ensuring a safe and sustainable food supply. This consortium project has offered a very successful platform for knowledge exchange and collaboration between the partners and beyond, with the industrial statement on “Bacterial Spores in Plant-Based Ingredients Intended for Beverage Applications” as a result.

At the start of this joint project, critical knowledge gaps existed regarding risk and control of spore-forming bacteria in plant-based foods. This related to the occurrence of spores in plant-based ingredients from different sources and on the inactivation characteristics and growth boundaries of species that can emerge in different food product applications. With financial support from the industry and the Dutch Topsector Agri & Food, such critical information has now been generated through the activities and results of this consortium project. More information about the results of this project is provided in the Annex to this endorsement letter.

Peer reviewed publications on results and insights generated in the project form crucial independent reference documents for ingredient suppliers and ingredient buyers. They supports the dialogue between the two to reach agreement on reasonable specifications on spore-formers in plant-based ingredients for different applications, to help minimize risks of product spoilage and ensure safety.

Yours truly,
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About NIZO

NIZO operates one of the most advanced R&D centres and laboratories in the world and develops the latest food technologies in its food grade pilot plant.

NIZO is a leading contract research organisation supporting dairy, food, and health companies with accelerating innovation, developing new applications, and securing the quality and safety of their products, thus improving the time to market. NIZO has strong expertise in proteins, bacteria, and processing, which it leverages to help food companies in the transition from animal to plant as most important source of proteins and in the development of safe and healthy products.

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ANNEX

The Plant Protein Contaminants consortium project

In 2022, NIZO initiated and coordinated a public-private consortium research project involving 11 industrial and three knowledge partners to carry out a multi-year research project aimed at investigating the potential food safety and quality risks of microbial contaminants in plant-based food products, such as dairy alternatives. The consortium included, amongst others, Ripple Foods, FrieslandCampina, HP Hood, SPX Flow and Tetra Pak.

In the project, the partners delivered insight into microbes (mainly spore-formers) that are common in plant-based ingredients, their ability to survive processing, and the risk of growth and toxin production in foods and beverages containing such ingredients. The project was finalized in Dec 2024.

The consortium aimed to help reduce food waste and ensure food safety, through filling of knowledge gaps and generating better predictive models for assessing microbial risk and identifying critical control points.

Industrial impact of the research

Plant-based (dairy) alternatives add a level of complexity when it comes to safety and shelf life compared with conventional dairy. This is due to the large variation in ingredient sources, each with their own variation in microbiota and physical properties that may impact food production and processing. Overall, the consortium project led by NIZO demonstrated that the types of microbial contaminants (species) in plant-based ingredients are not new to the food industry, but that their levels may be highly variable. Furthermore, before the consortium project, little knowledge was available within the industry on how to test this and interpret analytical results to agree on reasonable specifications and solutions for control.

Of the species that were further investigated, spore heat resistance and (out)growth profiles were in line with expectations, which is reassuring as no ‘super-bugs’ were associated with the ingredients included in the study. At the same time, it is clear that some of the encountered species can pose a risk of spoilage or even safety, depending on processing conditions, product characteristics and storage conditions in the chain. Thus, adequate measures need to be taken at the start of the R&D stage of plant-based product developments to properly predict and mitigate potential risks. This requires access to detailed data and insights on levels of (potentially problematic) spores in the sourced ingredients.

Main project results

Levels and types of microbial contaminants in plant-based ingredients

In total, 45 plant-based ingredient samples (8x almond, 11x pea, 12x oat, 10x faba bean and 4x coconut) were collected by the consortium. These were analyzed for levels of different groups of

microbial contaminations (with a focus on spore-formers) by using seven different (agar plating) assays, followed by identification of bacterial isolates. The data showed large variation in levels of bacterial (heat resistant) aerobic and anaerobic spores, not only between the samples of the same ingredient source, but also between the individual samples of the same source and type. Variation in spore levels between samples of the same source and type (e.g. pea isolates) can range between <1 log CFU/g up to 4 or 5 log CFU/g.

Identification results of spore-forming species revealed representatives of 32 different bacterial genera in the ingredient samples, with prevalence of *Bacillus* and *Clostridium* species (±70% and ±9% of all identifications, respectively). The spores of *Bacillus* and *Clostridium* species can have a wide range of heat resistance, which can vary between but also within species. Bacilli and the strictly anaerobic Clostridia can be associated with food spoilage or even food poisoning when the organisms grow out in the product and such foods are consumed. For bacterial isolates identified as *Bacillus cereus* group species, genes encoding enterotoxins or emetic toxin synthetase were detected in the isolates, indicating a potential food safety issue if these spores survive food processing and germinate and grow out in the finished product.

In addition to Bacilli and Clostridia, *Geobacillus stearothermophilus* was the main species identified as part of Heat Resistant Thermophilic Spores, mainly encountered in ingredient samples from pulses. High concentrations of HRTS in food ingredients should be avoided when manufacturing Ultra High Temperature (UHT) products that are stored at ambient conditions. This is because part of the spores can withstand even UHT heating and commercial non-sterility issues can arise when the final product is exposed to high temperatures in the distribution chain, allowing for these thermophiles to grow.

The results on levels and types of microbial contaminants in plant-based ingredients, included a detailed recommendation for spore enumeration methods, was published in the International Journal of Food Microbiology (Kyrylenko *et al*., Int J Food Microbiol. 2023 Dec 16:407:110392).

Spore heat resistance and heat inactivation kinetics

After identifications, spores from 96 bacterial strains (isolated from five different sources of plant-based ingredients) were subjected to spore heat resistance screening. Results revealed three highly heat resistance strains of *Bacillus subtilis* and *Bacillus amyloliquefaciens*. Also strains of *Geobacillus subterraneus* and one strain of *Paenibacillus* spp. showed a high level of spore heat resistance (<1log reduction). Detailed spore heat inactivation kinetics (D-values and estimated z-values) were further investigated for a sub selection of 15 bacterial isolates, followed by in-product validation of the findings in plant-based pH neutral beverages. Results provide insights on required processing settings needed to inactivate spores and prevent spoilage in plant-based products and were published in the International Journal of Food Microbiology (Karamcheti *et al*., Int J Food Microbiol. 2025 Dec 2:443:111426). It is important to note that sterilization and UHT treatments effectively inactivate spores of most of the plant-based isolates, but not all. Presence of highly heat resistant spores in

ingredients that are able to survive such product processing conditions is highly undesirable, since most plant-based beverage formulations support germination and outgrowth of such spores (see below), leading to product spoilage and potential food safety risks. More stringent thermal inactivation treatments to increase inactivation of highly heat resistant spores could lead to overprocessing and deterioration in product quality.

Risk of spore outgrowth and product spoilage in plant-based dairy alternatives

Follow-up investigations on growth cardinal values of these isolates in plant-based dairy alternative food products, such as drinks, yogurts and cheeses, showed that many bacterial isolates were thermotolerant and less were psychrotolerant. The majority of isolates are capable of growth at pH 5 at 42°C and no growth was observed at pH 5 and 10°C. Verification studies in pH neutral plant-based beverages showed growth of all selected spore-formers in products stored at ambient temperatures.

Importantly, cereulide measurements after fermentation of plant-based yogurts spiked with a *ces*-positive strain of *B. cereus* revealed that the cereulide toxin is produced during slow fermentation conditions, but not during fast fermentation conditions.

The results of this work will be submitted for publication in a peer-reviewed journal in Q3 2026.