Plant-Based Meat Analogues; How far of an Analogue in Microflora?

Moderator: Joyjit Saha, Kerry

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Plant-Based Meat Analogues; How far of an Analogue in Microflora?

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10am Eastern Time
Alejandra Ramirez is a Senior Scientist at Impossible Foods, working as the Team Lead for R&D Food Safety. In her role, she supports validating the microbiological shelf life of plant-based meat products to ensure safety and investigates microbial population dynamics throughout the production process.
Nicolette Hall is a Senior Technologist and Plant-Based R&D lead at Kerry Ingredients in the Food Protection and Preservation team. In her role, she supports the validation of preservation tools for food safety and shelf life of plant-based meat products.
Agenda

1. Microbial profiling in plant-based meat
2. Potential shelf life and food safety solutions
3. Understanding spoilage and sustainability

Alejandra Ramirez
Sr. Scientist
Impossible Foods

Nicolette Hall
Sr. Technologist
Kerry

Joyjit Saha
R&D Manager
Kerry
Microbial Profiling in Plant-Based Meat

Alejandra Ramirez
Senior Scientist- Impossible Foods
Plant-based meat
Market Trends

Increase demand and rapid market growth of plant-based meat worldwide.

Global Plant-Based Meat Industry Sales

CAGR: +31%

2019 2020 2021 2022 2023 2024 2025

Latin America  Asia Pacific  Western Europe  United States

UBS (Retail + Food Service)  Future of Food I: Is Plant-Based Meat Poised to Rebalance Global Protein Consumption?
Plant-based meat
Industry challenges

- Diversity of ingredient sources and types, including in the processing of plant-based meats (flours, concentrates, pastes, syrups etc.)
  - Soy and pea proteins are the most widely used ingredients for the manufacturing of major alternative meats on the market.

- No food microbiological guidelines or specifications for microbial quality and safety of plant-based meat products.
  - Unknown microbial populations on plant-based ingredients
  - New processing technologies
  - Lack of standardized approaches to assess microbiological safety and quality
Plant-based meat
Factors affecting microbial growth

- Intrinsic factors
  - Ingredients
  - pH
  - Water activity
  - Moisture content
  - Antimicrobial agents

- Extrinsic factors
  - Thermal treatment
  - Cooling
  - Storage temperature
  - Packaging

- Other factors
  - Processing conditions
  - Environmental contaminants unique from manufacturing sites
Microbial profiling of plant-based meat

Samples:
- Ingredients
- Intermediates
- Finished goods

Process samples
Homogenization (1:10 in buffer media)

Dilution and plating on agar media
TSA or MRS

Isolation and purification of bacterial colonies

DNA extraction

16S PCR

Sanger DNA Sequencing

Microbial Identification

Microbial Population

Metagenomics Sequencing

16S rRNA PCR and Library prep

DNA extraction
Microbial profiling of plant-based ingredients

Identify potential microbial targets & hazards
- Lactic acid bacteria
- Enterobacteriaceae
- *Listeria monocytogenes*
- *Bacillus cereus* group (toxin-producing strains)

Define microbiological limits and specifications from the ingredient providers
- APC levels < 10,000 CFU/g
- *B. cereus* group < 1000 CFU/g
- Absence of *L. monocytogenes* and *Salmonella enterica*

Integrate mitigation measures
- Heat treatment
- Antimicrobial agents:
  - Cultured dextrose
  - Vinegar
  - Sodium and Potassium lactates
88 different plant-based ingredients including pea, faba bean, oat, almond and coconut.

Microbial loads vary widely not only between but also within ingredient groups, and can reach up to $5.3 \text{ Log}_{10} \text{ CFU/g}$.

*Bacillus licheniformis* and *B. cereus* group strains were most frequently detected among *Bacillus* isolates, and these species originated primarily from pea and oat samples.

Among the *B. cereus* isolates, 9% (13/147) contained the *ces* gene, which encode the heat-stable cereullide toxin.

*B. cereus* spores represent a food safety risk when they can survive the processing conditions, germinate and grow and produce toxins.
Microbial profiling of plant-based meat
Shelf life & Micro ID

Microbial profiles of raw plant-based meat under refrigerated conditions. Grey shade represents the SD of the mean values (n = 3). Detection limit 10 CFU/g.

**Quality defects:**
- Bloated packages
- Sour smells
- Rancid and mushy

**Microbial profiles:**
- **Lactobacillus sakei**
- **Lactobacillus curvatus**
- **Leuconostoc mesenteroides**
- **Carnobacterium divergens**
- **Bacillus licheniformis**
Microbial profiling of plant-based meat
16S Metagenomics

- *Lactobacillus acidophilus* and *Lactococcus lactis* were the dominant bacteria species up to 19d-RSL.
- *Lactobacillus sakei* take over the microbial population being the dominant species at day 26 and 36.

- *Lactobacillus sakei* appeared earlier at d12-RSL and was the dominant species until the end of study.
Challenges in the Plant-Based Meats

*Lactobacillus sakei*

**L. sakei**
- Often isolated from food of vegetable sources like various flours, sourdoughts, or fermented cabbage, but it is systematically associated with **refrigerated meat products** under vacuum packaging (Zagorec et al., 2017).
- *L. sakei* has the ability to adapt to various habitats, thanks to its metabolic activities and phenotypic traits that are well adapted to its growth and survival under the conditions present during meat storage and processing.
- **Bacteriocin production** against foodborne pathogens, like *L. monocytogenes* and *Escherichia coli*.
  - Ongoing investigation to determine the presence of bacteriocin-producing *L. sakei* strain isolated from plant-based meat products.
**Microbial profiling of plant-based meat**

**Pathogen challenge test**

Pathogen growth profiles in artificially inoculated vacuum-packed raw plant-based meat samples under refrigerated conditions. Grey shade represents the SD of the mean values (n = 3). Detection limit 10 CFU/g.

**Assessing the risk:**

*Salmonella enterica* and *L. monocytogenes* were **unable to grow** in raw plant-based meat samples at refrigerated at 4°C for 28 days.

**Criteria:**

- *Salmonella*: < 1 log outgrowth for 1.25X RSL
- *L. mono*: < 2 log outgrowth for 1.25X RSL
Microbial profiling of plant-based meat
How to utilize the data of microbial profiling data?

- Measure changes in the microbial population diversity over time and in response to ingredients/process/sanitation changes.
  - Reformulation, new antimicrobials supplier
- Identify emerging or previously known quality and safety risks
  - Troubleshooting spoilage failure
  - Relevant and prevalent pathogens of concern
- Incident investigation & resolution (out of specifications)
- Support extension of shelf life
- Define food safety risk assessment programs

Limitations: knowledge gaps, cost, time, data analysis and interpretation.
How different are bacteria in plant-based vs animal meat?

**Plant-based meat**

**Spoilage**
- Lactic acid bacteria (LAB)
  - *Lactobacillus* spp.,
  - *Pediococcus* spp.,
  - *Lactococcus* spp.,
  - *Leuconostoc* spp.,
  - *Carnobacterium* spp.
- *Bacillus* spp.
  - *Bacillus subtilis*,
  - *Bacillus licheniformis*
- *Paenibacillus* spp.

**Safety**
- *Bacillus cereus* group
  - Spore heat resistance
- *Listeria monocytogenes*

**Animal meat**

**Spoilage**
- *Pseudomonas* spp.,
- *Enterobacteriaceae*
- Lactic acid bacteria (LAB)
  - *Lactobacillus* spp.,
  - *Pediococcus* spp.,
  - *Leuconostoc* spp.,
  - *Carnobacterium* spp.
- *Flavobacterium*
- *Brochothrix thermosphacta*

**Safety**
- *Salmonella* spp.,
- Shiga toxin-producing *E. coli* O157:H7
- *Listeria monocytogenes*
Native microflora in ground beef and plant-based meat analogues

- Ground beef had the highest initial native microflora among the three meats, followed by pea-based meat and soy-based meat.
- Although the APC counts were significant at day 0 for all three samples, the final population of 10d-RSL was > 7.0 Log CFU/g.
- The growth of LAB was more significant in soy-based meat and pea-based meat at the end of the RSL.
- By the end of 10d, pea-based meat has the highest coliform levels (~ 6.0 Log CFU/g).

Changes in native microflora in ground beef and plant-based meats during the 10-day storage at 4C (n = 6).
Artificially inoculated spoilage microorganisms in ground beef and plant-based meat analogous

- No significant changes in *B. thermosphacta* for the first 5d-RSL for all three meat samples.
- Pea-based meat had the highest increase of *B. thermosphacta* at 10d-RSL, followed by Soy-based meat and ground beef.
- The growth of *P. fluorescens* was rapid in ground beef and pea-based meat while the levels in soy-based meat were not changed.

Main conclusion of the study:

- Plant-based meat analogous, regardless of the type of plant protein contained lower indigenous microbial loads compared with ground beef.
- Plant-based meat have a more complex manufacturing process and the involvement of heat treatment during the processing could explain why their microbial levels are lower.
- Growth of indigenous microflora was observed in all tree meat sample types. LAB was able to grow in all three meat sample.
Key Takeaways

- The market for plant-based meat requires a solid foundation of knowledge in microbial quality and safety to support this industry’s continuous sustainable growth.
- Microbial profiling of plant-based ingredients and finished goods provides crucial information to understand microbial spoilage and food safety risks.
  - Unique profile based on the ingredients (type and source), processing, packaging and storage conditions
  - Risk-based assessment for food safety and quality programs
- Plant-based meat products are susceptible to microbial spoilage throughout the refrigerated shelf life due to their neutral pH, high water and protein content.
- Finally, the risk of pathogen contamination in plant-based meat is different compared to animal meat due to the nature of its ingredients, processing, and the addition of antimicrobial agents.
THANK YOU!
Potential Shelf Life and Food Safety Solutions
Plant-based products are top of mind for food safety

<table>
<thead>
<tr>
<th>Category</th>
<th>Concerned About (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Meat</td>
<td>66%</td>
</tr>
<tr>
<td>Processed Meat</td>
<td>51%</td>
</tr>
<tr>
<td>Other Plant-based Dairy</td>
<td>51%</td>
</tr>
<tr>
<td>Meat Alternatives</td>
<td>49%</td>
</tr>
<tr>
<td>Fresh Milk</td>
<td>43%</td>
</tr>
<tr>
<td>Frozen Chicken</td>
<td>43%</td>
</tr>
<tr>
<td>Snack Dips</td>
<td>43%</td>
</tr>
<tr>
<td>Spoonable Yogurt</td>
<td>42%</td>
</tr>
<tr>
<td>Sweet Baked Goods</td>
<td>42%</td>
</tr>
<tr>
<td>Non-Dairy Milk</td>
<td>41%</td>
</tr>
</tbody>
</table>

Source: Kerry 2021 Proprietary Research “Food Safety Fundamentals”
Considerations for Food Protection and Preservation in Plant-based Meat Alternatives

Positive impact on shelf-life extension and reduction in food waste

Improved food safety efficacy

Impact on taste and texture of the preservation used.

Ingredient impact on sodium content and label

Better freshness and flavor delivered throughout shelf life

Impact on cost
Customer problem: unknown microorganism limiting shelf life

- Isolate and determine the spoilage micro-organisms
- Advanced broth screening to evaluate the sensitivity of the isolated micro-organisms
- Run shelf life and challenge studies in application
- Use model food systems and accelerated shelf-life testing

Result: unlocking next generation shelf life for your product

Accommodating various conditions:
- temperatures
- atmospheric

Microorganisms in scope:
- Spoilage
- Pathogenic
- Demanding bacteria

Applications:
- Meat
- Bakery
- Beverage
- Dairy
- Plant-based
- Meals
- Pet
The Same Rules Do Not Apply!
Typically, higher limits of bacteria per gram of product are allowed in plant-based products in comparison to meat. If you normally work with meat, don’t assume the same limits apply.

### Primary Sources of Microbial Risk in Meat and Plant-based Meat Alternatives

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Animal Protein</th>
<th>Plant Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spore formers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td><strong>Vegetative/Pathogen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Enterohemorrhagic E. coli</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>Spoilage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid bacteria</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Yeast &amp; Mold</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Plant-based Meat Alternative Roadmap: Analysis of Products on the Market

In collaboration with University College Cork
Why Market Screen Was Conducted

Product / Market Knowledge & Market Demand
- Market is still relatively new, but evolving rapidly
- Limited shelf-life and food safety data
- Processors not using shelf-life extension ingredient or using similar ingredient to their meat equivalents (e.g., CL acetate, Sulphites)

Regional Regulatory Challenges
- In regions such as EU and Canada, buffered vinegar is not considered ‘natural’ due to the use of chemical neutralisers
- New food protection solutions required for the market - challenge test model required for testing and validation
Objectives

1. Determine food quality (spoilage) and safety features of plant-based meat alternatives.

2. Determine main spoilage organisms.

3. Acquire market intelligence and identify opportunities for solutions.

4. Establish a product relevant model system to test and validate new food protection and preservation solutions.
Population Analysis of Plant-based Meat Alternatives using 16S Metagenomics
What is 16S Metagenomics?

A DNA-based technique that simultaneously detects all bacteria in a given sample based on the sequence of the conserved, 16S ribosomal RNA gene (rDNA), at genus (sometimes species) level.

Why did we use 16S Metagenomics?

To understand the detailed make-up of the microbial populations in different products and different manufacturers.

How was the analysis done?

- Samples were taken at expiry and post-expiry (7 days post expiry)
- DNA extracted from samples and 16S (V3-V4 region) sequencing libraries prepared
- 16S library samples sent for sequencing
- Bioinformatic analysis carried out at APC UCC
16S Metagenomic Results - All Plant-Based

- C1-C8 – Company1-8
- B – Burger
- M – Mince
- S – Sausage
- C – Chicken Bucket
- C10 – Chicken Bucket (Expiry + 10 days)
- Dk – Duck
- PP – Pulled Pork
- PP10 – Pulled Pork (Expiry + 10 days)
- Exp – Expiry Date
- PExp – Post Expiry (Expiry +7 Days)
One of the main objectives of the study was to isolate relevant spoilage related strains from plant-based meat alternative products, to establish a realistic inoculum for a spoilage Challenge Test model system.

13 of the 53 isolates chosen for full 16S gene sequencing.

5 strains chosen for inclusion in the Spoilage Challenge Test pool.

1. Lactobacillus sakei
2. Leuconostoc mesenteroides
3. Leuconostoc carnosum
4. Carnobacterium divergens
5. Brochothrix thermosphacta

Protocol developed and validated in collaboration with UCC.

<table>
<thead>
<tr>
<th>Plant based isolates-colony PCR 16S v3-v4</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuconostoc mesenteroides</td>
<td>17</td>
</tr>
<tr>
<td>Leuconostoc carnosum</td>
<td>6</td>
</tr>
<tr>
<td>Lactobacillus sakei</td>
<td>6</td>
</tr>
<tr>
<td>Leuconostoc kimchi</td>
<td>5</td>
</tr>
<tr>
<td>Carnobacterium divergens</td>
<td>4</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>3</td>
</tr>
<tr>
<td>Brochothrix thermosphacta</td>
<td>3</td>
</tr>
<tr>
<td>Leuconostoc lactis</td>
<td>2</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>2</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>1</td>
</tr>
<tr>
<td>Leuconostoc pseudomesenteroides</td>
<td>1</td>
</tr>
<tr>
<td>Leuconostoc citreum</td>
<td>1</td>
</tr>
<tr>
<td>Lactobacillus graminis</td>
<td>1</td>
</tr>
<tr>
<td>Carnobacterium gallinarum</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>53</strong></td>
</tr>
</tbody>
</table>
How to Approach
Where can Food Protection and Preservation Solutions Be Built into Plant-based Meat Alternatives?

**Built into Base Plant Protein Formula**
- Built into ready-to-use specialty protein ingredients like textured vegetable protein (TVP) application formula

**Integrated into Masking or Seasoning Blends**
- Built into natural flavours - so one less step for operations
- Food safety built into flavour - additional step

**Multi Ingredient Systems**
- In combination, these Protection and Preservation systems can tackle several issues at once, making them cost effective
- The combination of different ingredients work together to address different problems from Spoilage to Colour and Food safety to Shelf Life
- Can be Liquid or Dry Solutions

**Applied as Single Ingredients**
- Single functional components
- Liquid and dry solutions possible
- Spectrum of inhibition
Preservation Solutions

Conventional and clean label solutions to meet evolving needs

<table>
<thead>
<tr>
<th>Conventional</th>
<th>Clean Label / Consumer-Friendly</th>
<th>+ Taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>Fermentation</td>
<td>Vinegar</td>
</tr>
<tr>
<td>• Acetates</td>
<td>• Organic acids</td>
<td>• Dry buffered vinegar</td>
</tr>
<tr>
<td>• Propionates</td>
<td>• Peptides</td>
<td>• Liquid buffered vinegar</td>
</tr>
<tr>
<td></td>
<td>• Natural cure</td>
<td></td>
</tr>
</tbody>
</table>

Multiple pillars come together as

**Multifunctional Systems**

Combined technologies / products to meet specific goals.

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Plant-based Burger Challenge Test 1
Plant-based Burger: Shelf-Life Results

Results

- Rapid outgrowth in control sample. Short shelf-life of 2-6 days based on Log 7 cut off.
- Control
  - Log 7 @ day 8
  - Log 8 @ day 13
- Provian NDV and Cloud AC-15
  - Log 7 @ day 14
  - Log 8 @ day 19-20

~50% improvement of shelf-life compared to control
16S Metagenomics Results

- Selected samples analysed to determine the population structure using 16S Metagenomics.
- *Lactobacillus sakei/curvatus* was dominant Genus in both samples.
- *Leuconostoc spp.* detected at very low levels. *Carnobacterium divergens & Enterococcus faecalis* not detected in samples.
- *Alicyclobacillus acidocaldarius, Anoxybacillus flavithermus & Thermoanaerobacterium thermosaccharolyticum* not in the initial inoculum. All Gram positive spore formers, acidophilic and thermophilic. Unsure as to why they are present except as part of an ingredient.
Ensuring control of *L. monocytogenes*

**Results**
- Products were inoculated with a cocktail of 3 *L. mono* strains with starting counts of ~Log 2 CFU/g
- Test failure set at *L. mono* outgrowth ≥2 Log
- ≥2 Log outgrowth was detected in the Control sample at Day 23

All 3 Cloud products fully controlled *L. monocytogenes* outgrowth over the 25-day study
Plant-based Chicken Challenge Test
Aim
Evaluate the efficacy of Kerry’s Food Protection and Preservation solutions in a plant-based chicken application at varying pH. pH was adjusted with a 1:1 combination of malic and citric acid.

Trial Conditions
- Products were prepared with and without the following solutions:
  1. No antimicrobial
  2. 2.25% Buffered Vinegar (BV)/Fermentate
  3. 2.25% BV/Fermentate + 0.10% organic acids
  4. 2.25% BV/Fermentate + 0.20% organic acids
  5. 2.25% BV/Fermentate + 0.40% organic acids
- Products were stored aerobically at 4°C and 7 °C.

Microbiological analysis
- Samples inoculated with ~2 Log LAB cocktail.

Product Characteristics

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>No antimicrobial</td>
<td>6.16</td>
<td>0.9609</td>
</tr>
<tr>
<td>2.25% BV/Fermentate</td>
<td>5.45</td>
<td>0.9559</td>
</tr>
<tr>
<td>2.25% BV/Fermentate + 0.10% OA</td>
<td>5.32</td>
<td>0.9507</td>
</tr>
<tr>
<td>2.25% BV/Fermentate + 0.20% OA</td>
<td>5.12</td>
<td>0.9489</td>
</tr>
<tr>
<td>2.25% BV/Fermentate + 0.40% OA</td>
<td>4.89</td>
<td>0.9524</td>
</tr>
</tbody>
</table>
Results

- At both storage temperatures outgrowth slowed as product pH decreased.
- At 4°C, plant-based chicken remained below the spoilage threshold for up to 32 days with when pH was reduced.
- At 7°C, plant-based chicken with the lowest pH (4.89) stayed below the spoilage limit for 32 days.

2.25% BV/Fermentate provided an additional 14 days (100% increase) in shelf-life vs control.
Science-Backed Meat Alternative Solutions

Partnering together to solve complex challenges in:

- **Food Safety**
  Ensure products are safe with proven efficacy and research-backed solutions

- **Shelf-Life Extension**
  Utilize expertise and solutions to extend shelf life with no compromise on quality

- **Taste, Nutrition, Appeal**
  Elevate visual and sensory appeal while locking in great taste
What happens when we eliminate sources of food waste?
Whether you’re a food manufacturer or a concerned citizen – or both – access our Food Waste Estimator.

With food and energy prices at an all-time high, and climate change at critical levels, now is the time.

Disclaimer: The results of the food waste estimator are indicative and not designed to represent a quantitative measure of a customer's sustainability impact. The outputs should not inform front of pack claims or sustainability claims.

See the impact you could have on the planet’s resources here.
Thank you for attending!

Questions?

Contact Information
Reach Out: nicolette.hall@kerry.com
Questions?
Contact Information

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Nicolette Hall     nicolette.hall@kerry.com
Joyjit Saha        joyjit.saha@kerry.com
Upcoming Webinars

November 17, 2023  1:00 PM  Matrix Additions Part 2: Alternative Approaches for Rapid Pathogen Detection Methods

December 14, 2023  9:00 AM  Impact of Water Use and Reuse in Food Production and Processing on Food Safety at the Consumer Phase: Focus on the Fresh Fruit and Vegetable Products Sector

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