Safety of Plant-Based Meat Alternatives

In a Reverse Engineering Approach

5 May 2022, Masja Nierop Groot, Hasmik Hayrapetyan
The global plant-based meat market is growing

In 2020: 0.3% of total meat sales

In 2030: possibly up to 5% of total meat & fish market

Growth is largely resulting from consumption by flexitarians

Research focus on plant-based meat alternatives

- Improvement of texture & juiciness
- Flavour improvement
  - Lack of the appreciated meat flavour
  - Beany volatiles and unpleasant flavours due to oxidation*
- Diversification of plant protein sources
- Less refined ingredients
- Impact of further processing
- Nutritional value
  - Low intake of micronutrients such as iron, zinc, and vitamin B12**

- Food safety & spoilage aspects have received less attention

* Kaneko et al (2011)  
** Pereira & Vicente (2013)
Survey among Dutch flexitarians

Replies to the question “It is important to me that the meat substitute (e.g., vegetarian burger) I eat on a typical day...”.

Multi-Criteria Assessment Platform for informed and weighed decisions for plant based products

WUR multi-disciplinary project KB Reverse Engineering

Case study:
Plant-based burger

Scenario ranking
Recipe 1
Recipe 2
Recipe 3
Recipe 4
Recipe 5
Multi-Criteria Assessment Platform for informed and weighed decisions for plant based products

Case study:
Plant-based burger

This presentation

Scenario ranking
Recipe 1
Recipe 2
Recipe 3
Recipe 4
Recipe 5
What is reverse-engineering in food?

- Pre-existing product
- Alternative product

**Product**
- Stakeholder & Consumer wishes
- Accountable Key Performance Indicators (KPIs) / Criteria
- Use of models & data
- Evaluation
- (Re)design

Optimization of new products and recipes
Factors affecting microbial contamination of plant-based burger

**Protein source**
- Soy
- Wheat
- Potato
- Pea
- Lentil
- Faba bean
- ...

**Protein ingredient**
- Protein isolate
- Protein concentrate
- Level of refinement
- ...

**Processing steps**
- Extrusion
- Mixing
- Shaping
- Cooking
- Deep frying
- ...

**Product properties**
- pH
- Salt (Aw)
- Organic acids
- ...

**Storage conditions**
- Time
- Temperature
- Packaging atmosphere
- ...
Microbiological safety: Performance Objective approach

\[ N_0 - \sum R + \sum I \leq PO \]

- Initial contamination level of the product
- Reduction (processing)
- Increase (growth and/or re-contamination)
- Performance Objective (maximum acceptable level)

*ICMSF* defined approach for microbial risk management

*International Commission on Microbiological Specifications for Foods*
Factors included in the approach

- **Storage (product properties)**
  - pH
  - Gas composition
  - Aw
  - Temp

- **Storage (product temp)**
  - Maximum acceptable level

- **Processing (thermal)**
  - Thermal resistance (D- and z-values)
  - Growth rates

**Databases:**
- Scientific literature review
- Expert opinion

- Hazards per ingredient category
- Initial contamination levels
- Thermal resistance
- Growth rates
Inclusion of factors affecting growth rate: based on gamma concept

\[ \mu = \mu_{\text{optimum}} \times \gamma \]

\[ \gamma = \gamma(T) \times \gamma(pH) \times \gamma(a_w) \times \gamma(O_2) \]

- Factors are considered independent.
- The effect on growth rate is multiplied.
- Growth model: Modified Gompertz model.
- Cardinal values retrieved from literature

Reference parameters used for plant-based meat alternatives

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>pH</th>
<th>Storage time (days)</th>
<th>Water activity</th>
<th>Oxygen availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>6</td>
<td>10</td>
<td>0.9991</td>
<td>With O2</td>
</tr>
</tbody>
</table>

Hazards per ingredient category

Databases: hazards linked to ingredients

Based on: systematic literature reviews, reports on outbreaks, RASFF, reports by ICMFS, EFSA, WHO

Dairy chain
Egg chain
Potato chain
Nuts and seeds
Fruit chain
Fruiting vegetable chain
Mushroom chain
Leafy vegetables
Root/stem vegetables
Legumes
Sprouts
Seasonings
Pipeline selection most relevant hazards for a plant-based burger (chilled storage)

Long list of pathogens in the database, relevant for plant and dairy-based ingredients

- Bacillus cereus
- Campylobacter spp.
- Clostridium botulinum (proteolytic)
- Clostridium botulinum (non-proteolytic)
- Clostridium perfringens
- Cronobacter spp.
- Escherichia coli, pathogenic
- Listeria monocytogenes
- Vibrio spp.
- Salmonella
- Shigella spp.
- Staphylococcus aureus
- Yersinia spp.
- Hepatitis A virus
- Hepatitis E virus
- Norovirus
- Cryptosporidium spp.
- Cyclospora cayetanensis
- Giardia duodenalis
- Toxoplasma gondii

Short list of relevant pathogens for burger ingredients

- Bacillus cereus
- Clostridium botulinum (proteolytic)
- Clostridium botulinum (non-proteolytic)
- Clostridium perfringens
- E. coli (pathogenic)
- Listeria monocytogenes
- Salmonella spp.
- Staphylococcus aureus

Most relevant for plant-based burger recipe (chilled)

Relevant hazards depending on processing and storage conditions of the plant-based burger
Model case study: plant-based burger

Protein source

- Soy
- Wheat
- Potato
- Pea
- Lentil
- Faba

Recipe 2,3,4

Recipe 1,5

Ingredients and processes selected based on:
- INNOVA Market Insights database
- Expert knowledge
## Sensitivity analysis: tested parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tested variability (StDev as % of the mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial contamination level</td>
<td>100x (mean±2 logs)</td>
</tr>
<tr>
<td>Heat inactivation rates (D value)</td>
<td>+/- 50%</td>
</tr>
<tr>
<td>Growth rates</td>
<td>+/- 30%</td>
</tr>
<tr>
<td>Storage time</td>
<td>10 days +/- 50%</td>
</tr>
<tr>
<td>Re-contamination</td>
<td>yes/no (recontamination level=0.1 CFU/g)</td>
</tr>
</tbody>
</table>
Recipe testing: assumed initial levels in the plant-based burgers

Sensitivity analysis > robustness of estimates towards uncertainty and variation of data

<table>
<thead>
<tr>
<th>Variable inputs for sensitivity analysis</th>
<th>min=mean*0.01</th>
<th>mean</th>
<th>max=mean*100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial levels in the mixed and shaped burger (CFU/g)</td>
<td>x 0.01</td>
<td>x</td>
<td>x 100</td>
</tr>
<tr>
<td>* Bacillus cereus</td>
<td>5.15</td>
<td>515</td>
<td>51,500</td>
</tr>
<tr>
<td>* Clostridium botulinum (proteolytic)</td>
<td>0.05</td>
<td>5.2</td>
<td>515</td>
</tr>
<tr>
<td>* Clostridium botulinum (non-proteolytic)</td>
<td>0.05</td>
<td>5.2</td>
<td>515</td>
</tr>
<tr>
<td>* Clostridium perfringens</td>
<td>0.70</td>
<td>70</td>
<td>7,000</td>
</tr>
<tr>
<td>* Escherichia coli, pathogenic (STEC, EPEC, ETEC)</td>
<td>0.04</td>
<td>3.9</td>
<td>385</td>
</tr>
<tr>
<td>* Listeria monocytogenes</td>
<td>0.01</td>
<td>1.3</td>
<td>130</td>
</tr>
<tr>
<td>* Salmonella (non typhoidal)</td>
<td>0.05</td>
<td>5.2</td>
<td>515</td>
</tr>
<tr>
<td>* Staphylococcus aureus</td>
<td>0.05</td>
<td>4.8</td>
<td>480</td>
</tr>
</tbody>
</table>

* 5 recipes: assumed the same initial levels (more data for plant-based protein ingredients required!)
Interpretation of figures

Three different values of tested parameter (min/mean/max)

Test PO level

within selected boundary
exceeding selected boundary
Sensitivity of tested parameters on recipes with deep-frying

Without recontamination

**Sensitivity to initial level**

- Recipe 2, 3 & 4

**Sensitivity to shelf life (5, 10 or 15 days)**

- Recipe 2, 3 & 4

**Sensitivity to D-value**

- Recipe 2, 3 & 4

Within the tested range:

Inactivation of all pathogens by >> 12 log

Growth rates, initial levels, storage time or D-values are not sensitive parameters for these recipes.
Sensitivity of tested parameters on recipes with deep-frying

**With recontamination**

**Re-contamination** level tested: 0.1 CFU/g*

*L. monocytogenes*: common re-contaminant and if present can exceed the PO within 10 days for mean growth rate.

> Growth rate and shelf-life are critical parameters

*If re-contamination level is 0.01 CFU/g or 30% reduction of growth rate  > *Listeria* does not reach the PO within 10 days.

(Packaging with oxygen, storage at 7°C)
Sensitivity of tested parameters on recipes with 100°C/30 min

**Without recontamination**

**Sensitivity to initial level**

Within the tested range for these recipes:

- Spore formers survive
- Possibility of outgrowth for spore formers is a critical factor for *B. cereus* and *C. perfringens*

For *C. botulinum*: only based on inactivation levels (turn red if inactivation is less than the desired level (-12 Log or -6 Logs))

**Sensitivity to thermal inactivation**

D values based on upper 95% PI (van Asselt & Zwietering 2006).

If average reported D used: *C. bot* non-proteolytic will be inactivated >6 Log.

Only *C. botulinum* proteolytic will remain as a risk.
Conclusions & outlook

- The platform under development can provide insight in critical parameters for plant-based meat recipes
- Heat treatment conditions used for plant-based meat alternatives are critical (*B. cereus* and *Clostridia* spore survival)
  - Potential outgrowth of spores (storage time, storage conditions important)
- Re-contamination: sensitive (influential) parameter
- More specific data relevant for plant-based ingredients and products needed to further refine the tool
- More scenarios for parameters will be included
Acknowledgement

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KB Reverse Engineering project Team


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