# Object of Investigation

## Product and Formulation

### Product:

- **Recombinant Horseradish Peroxidase**

  Plant enzyme from horseradish. Produced by bioprocessing of the recombinant yeast strain of *Piccia pastoris* by use of a phosphate buffered minimal medium.

### Formulation:

- **Intermediate Bulk**

  Early intermediate state after biomass separation and buffer exchange

  Serious residuals of nutrient salts and process buffer from the fermentation step

  0.1 M TRIS hydrochloride storage buffer at pH 7.0 for product stabilisation

## Packaging and Freezing

### Packaging:

- **Plastic Bottles of Polycarbonate**

  Cylindrical PC bottles with a total volume of 1 liter and 80 % filling level

### Freezing:

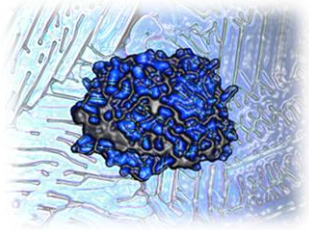
- **$LN_2$ operated Shock-Freezer**

  Transfer to pre-cooled freezer chamber and shock-freezing at -30°C to -70°C

# Product Damage in the Freeze-Thaw-Process

### Cold denaturation:

Direct impact of low temperature. Reduction of intrinsic energy lowers hydrophobic interactions and destabilize protein structure.



***Relevance***
No consideration for modelling: Effects are weak and completely reversible.

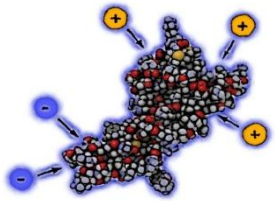
### Protein interaction:

Short scale interaction between protein molecules induced by freeze concentration. Result: Structural damage causes aggregation and precipitation.

***Relevance***
Consideration for modelling only for high product concentrations. No influence for usual protein content.

### Concentration effects:

Short scale interactions between protein and other medium components, induced by freeze concentration. Serious structural damage to the product.



***Relevance***
Serious mechanism of product injury.
Needs modelling!

### pH shifts:

Redistribution of buffer components in the freezing process causes local changes of pH value. pH can influence the hydration state of proteins and increase or decrease the amount of other damaging effects

***Relevance***
Consideration as process variable or impact factor needed for modelling

# Model Assumptions

Distribution of *solute concentrations* and *pH value* in the frozen good are sufficient for quantitative prediction of product injury.

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Product injury by both effects can be detected independently from the freezing process.

*

The pH value can be derived from the solute concentrations in the frozen good.

**Product injury can be predicted based on simulated concentration data only!**

*Concentration profile for $PO_4$ (mol/l)*

| position | center | → | border |
|---|---|---|---|
| top | 0.016 | 0.008 | 0.009 |
| | 0.008 | 0.008 | 0.009 |
| | 0.010 | 0.010 | 0.009 |
| | 0.011 | 0.011 | 0.009 |
| | 0.019 | 0.019 | 0.019 |

*Concentration profile for Cl (mol/l)*

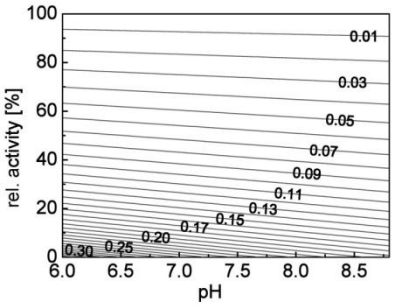
| position | center | → | border |
|---|---|---|---|
| top | 0.057 | 0.037 | 0.042 |
| | 0.031 | 0.031 | 0.038 |
| | 0.040 | 0.040 | 0.042 |
| | 0.043 | 0.043 | 0.036 |
| | 0.082 | 0.082 | 0.067 |

*Concentration profile for TRIS (mol/l)*

| position | center | → | border |
|---|---|---|---|
| top | 0.098 | 0.059 | 0.064 |
| | 0.076 | 0.059 | 0.063 |
| ↓ | 0.139 | 0.069 | 0.067 |
| | 0.184 | 0.077 | 0.068 |
| bottom | 0.216 | 0.114 | 0.110 |

# Choice of Different Model Approaches

## 1) Additive analytical model approach



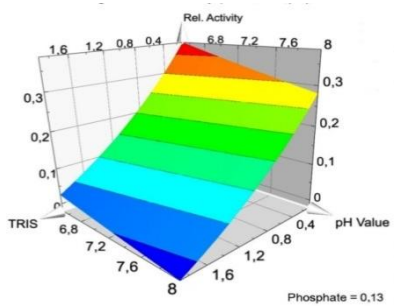

Good reproduction of the spatial distribution of product injury in the frozen good.

Prediction of total product injury is too low.

**Outcome:**

- → Good reproduction of the modelled correlations
- → No consideration of possible interactions of damaging medium components

## 2) DoE-based statistical model approach




Poor reproduction of the spatial distribution of product injury in the frozen good.

Prediction of total product injury seems to fit.

**Outcome:**

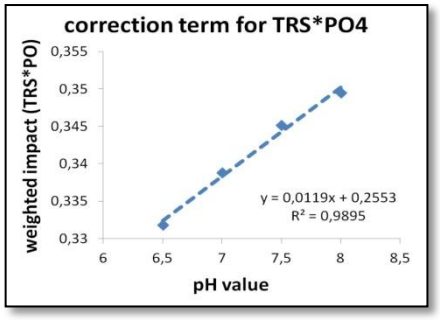
- → Statistical model 'underfits' the natural correlations between cause and effect.
- → Interactions are respected as general part of the model equation

## 3) Mixed model approach

Combination of both model approaches to eliminate their typical weaknesses:

Determination of the interaction impact from the statistical model equation.

Extension of the analytical model equation with the derived interaction term.




**Outcome:**

- → Perfect prediction accuracy in quality and quantity.
- → Correct determination of product loss for any process conditions.

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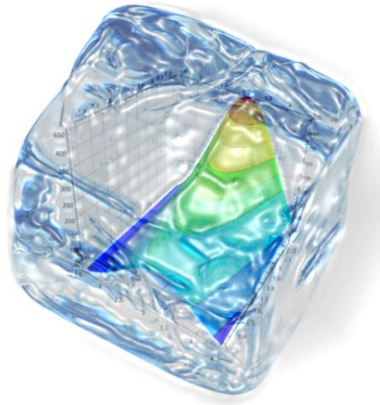
# Prediction of Product Loss in Protein Freezing



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