Object of Investigation

Product and Formulation

Product:
- Recombinant Horseradish Peroxidase
  Plant enzyme from horseradish. Produced by bioprocessing of the recombinant yeast strain of *P. 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:
- LN2 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.

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!
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:

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

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:

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

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