Developing smart control strategies for freeze-drying of lactic acid bacteria

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The CAFE European Project

Computer-Aided Food processes for control Engineering (CAFÉ)

Objective:
Provide new paradigms for the smart control of food processes by combining reliable and novel sensing systems and advanced simulation tools.

- Bioconversion (wine fermentation)
- Separation (beer filtration)
- Struturing (ice cream)
- Preservation (bacteria FD)

- Reconstruct of unmeasured state of the process and product parameters
- Provide in real time predictions of future scenarios and robust control strategy
**Background**

Production of freeze-dried concentrates of lactic acid = a complex process

- **Fermentation**
- **Cooling**
- **Concentration**
- **Formulation**
- **Freeze-drying**
  - **Freezing**
  - **Sublimation**
  - **Desorption**
- **Storage**

**Functionality:**
acidification activity (CinAc®)
Bacteria Freeze-Drying: The approach

LAB concentrate
Protective medium: (200 g/L sucrose)

Freezing (tray)
Primary drying
Secondary drying

Product

3 Process variables
✓ Shelf temperature
✓ Chamber pressure
✓ Time

Critical Process Parameters
✓ Product temperature
✓ Water activity, moisture content
✓ End point of drying steps

Quality attributes
✓ Viability
✓ Acidification activity
✓ Structure
✓ Storage stability

Productivity

Raw material
✓ Tcoll, Tg

Modelling & advanced optimization tools

Sensing method
Predict in real time the CPP
Reconstruct product history

Optimal product history

Quantitative relationships
Relationships between CPP and bacteria activity

- Study the whole process
- Quantify the biological degradation after each step of the freeze-drying process
- Impact of the sublimation conditions
- Impact of the residual moisture content

Functionality: acidification activity (CinAc®)
Relationships between CPP and bacteria activity

Tcoll: the minimum temperature at which the ice sublimation is accompanied with the collapse of the dried structure

Freeze-drying microscopy: (FDCS 196, Linkam Sci. Inst.)

Direct observation of the structure during primary drying (sublimation)

Maltodextrin DE 5-8: 10% solution

\[ T_{coll} = -7^\circ C \approx T_g' \]

\[ \text{BUT for bacteria suspension } T_{coll} = -27^\circ C \gg T_g' = -36^\circ C \]
Formulations with **low value of Tg’** and **Tcoll >> Tg’**

⇒ Performing primary drying at product temperature higher than Tg’

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**Differences in biological activity recovery after freeze-drying?**

**Probably Yes**
Samples analysis

1- Viability $\rightarrow$ Plate counts

2- Technological property: Acidification activity $\rightarrow$ CINAC

The higher the $tm$ value, the lower the acidification activity

3- Residual moisture content: Karl Fisher titration, water activity, Tg

Corrieu et al. 1988 (Patent FR 2629612)
Some experimental results

Primary drying (DI)

Loss of biological activity during FD

<table>
<thead>
<tr>
<th></th>
<th>-20°C/20 Pa</th>
<th>0°C/20 Pa</th>
</tr>
</thead>
<tbody>
<tr>
<td>tm1 before FD</td>
<td>261 min</td>
<td>240 min</td>
</tr>
<tr>
<td>tm2 after FD</td>
<td>475 min</td>
<td>366 min</td>
</tr>
<tr>
<td>dtm=tm2-tm1</td>
<td>214 min</td>
<td>126 min</td>
</tr>
</tbody>
</table>

Close to Tg’ Close to Tcoll

Loss of biological activity during storage

k=2.8

k=2.1

FD: freeze-drying
Relationships between CPP and bacteria activity

Can we integrate in the model the degradation of “biological activity”?

Hypothesis: Degradation rate $K = f (\text{Product } T^\circ - T_g)$

- Freeze-dried samples of LAB
- Equilibration at various water activity values
- Storage at 25°C under vacuum
- Measurement of biological activity at 7, 10 and 29 days

$d_{tm} = k$  

$K = k_0 e^{k_1(T_{storage} - T_g)}$  

$k_0 = 3.89$  

$k_1 = 0.032$
Relationships between CPP and bacteria activity

Integration of the equations describing the degradation rate in the lyo-model

Simulation of the evolution of tm during the process

DI: Primary drying

DI = -20° C, 20 Pa

Close to Tg'

Close to Tcoll

DI = 0° C, 20 Pa

19 min

Close to Tg'

Close to Tcoll
Relationships between CPP and bacteria activity

Freeze-drying protocol

- Fermentation
- Cooling
- Concentration
- Formulation
- Freeze-drying
  - Freezing
  - Sublimation
  - Desorption
- Storage

Conservative

Aggressive

Pilot plant equipped with a sample thief and a hygrometer

(Pilot plant) (Usifroid, SMH 15)
Effect of water content on the acidification activity

Analysis of samples removed at various times of the desorption step

Whey medium

Linear evolution of tm during storage

⇒ Slope k = loss rate of acidification activity
Effect of water content on storage stability

Comparison with data obtained from freeze-dried samples equilibrated at various values of relative humidity

$T_g = 25 \, ^\circ\text{C}$
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Modelling & advanced optimization tools

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Quantitative relationships
Modelling and optimization tool

A one-dimensional model of heat and mass transfer

Mass transfer

Heat transfer

Sensor
Modelling and optimization tools

Parameter estimation using a specific tool developed by IIM-CSIC

Advanced Model Identification using Global Optimization
Modelling and optimization tools

Estimation of parameters of the freeze-drying model

Experimental description

- Six experiments (Primary drying)
- Product temperature, vapor pressure
- No replica: experimental errors were fixed at 10%

<table>
<thead>
<tr>
<th></th>
<th>$k_D$</th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$h_{L,1}$</th>
<th>$h_{L,2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PE</td>
<td>0.0435</td>
<td>$10^5$</td>
<td>$10^7$</td>
<td>10.1</td>
<td>0.4</td>
</tr>
<tr>
<td>After PE</td>
<td>0.252</td>
<td>$7 \times 10^4$</td>
<td>$1.93 \times 10^7$</td>
<td>9.32</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Bound active

Mass transfer

Heat transfer

Experiment 1

Experiment 2
Modelling and optimization tools

Robust Identification

Cloud $h_{L,2}$ vs. $h_{L,1}$

Histogram $h_{L,1}$

Cloud $k_D$ vs. $k_1$

Histogram $h_{L,2}$

Heat transfer

Mass transfer

Optimal experimental design
Bacteria freeze-drying

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Quantitative relationships
Development of sensing methods

To measure or predict in real time the critical process parameters or product quality

**Common sensors**
- Product temperature probe
- Vapor pressure
- Pressure rise test

**New sensors**
- Electronic noise
- Acoustic impedance probe

- Electronic noise
- Water activity
- Acidification activity
Development of sensing methods

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Development of sensing methods

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Acoustic impedance probe

Automatic measurements – Direct presentation of characteristics parameters

Detection – control of glass transition
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Demonstration: Data & Knowledge Management

M3
8 sensors, 2 actuators

M2
1 high frequency pressure measurement

M1
8 thermocouples

S3
Pm_CAFE Local Database

S1
Pm_CAFE Local Database

Optimizer

Other plants involved in LAB production

Embedded board

Scientific database
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CAFÉ Project: Computer-Aided Food processes for control Engineering