Enzymatic Decomposition of Dextran in Sucrose Solutions
Outline

• Motivation
• Current Situation
• Research Approach
• Analytical Results
• Preliminary Results Application
• Conclusions
Origin of Dextrans

Presence in beet and cane caused by action of Leuconostoc mesenteroides

- Dextrans are co-extracted in the mill
- Dextranucrase hydrolyses sucrose into fructose and glucose
- Glucose polymerization into polysaccharides
- Structure of dextrans
  - Main glycosidic linkage is $\alpha-(1\rightarrow6)$
  - Irregular branching via $\alpha-(1\rightarrow2)$-, $\alpha-(1\rightarrow3)$-, $\alpha-(1\rightarrow4)$ link
Dextran Related Processing Problems

- Effect of dextran is size dependent
  - Lower molecular weight – change of crystal morphology (elongation)
  - High molecular weight – increased viscosity causes problems in
    - Settlement, filtration, evaporation, and crystallization rate

![Diagram showing preferred effects according to molecular weight and competitive adsorption model](image-url)

Stokes' settling velocity:

\[ v_p = \frac{(\rho_p - \rho)gr^2}{18\cdot \eta_F} \]

Viscosity increase (\( \eta \))

Crystal morphology

Settling rate, filterability, crystallization rate, evaporation rate

Hagen-Poiseulle: flow velocity in a pore:

\[ v = \frac{\Delta p d^2}{92\eta l} \]

Diffusion: diffusion coefficient

\[ \frac{dm}{dt} = \frac{kT}{6\pi\eta l} \frac{dc}{dx} \]

Heat Transfer:

\[ Q = \alpha A\Delta T \] [W]

Heat transfer coefficient:

\[ \alpha = f(\text{Fluid(Viscosity ...), Flow, Fouling}) \]
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Established Boundaries and Mitigation

- Dextran contamination of syrups is a recurring problem in both cane and beet processing
- High molecular weight dextrans at levels above 500 mg/kg sucrose are considered critical
- Smaller size dextrans considered less problematic

- Established and acknowledged method of mitigation
  Treatment with dextranse to reduce the levels of dextran
  - Common analysis of dextran levels gives no indication of molecular weight
  - Dextran decomposition process and its products not well understood
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Research Goals

Elucidation of the decomposition process of dextran on dextranase action
- Evaluate the analytical methods established
- Perform enzyme treatments at most relevant conditions
- Characterization of resulting regarding poly- and oligosaccharides

Study the effect of different dextran fractions on sucrose crystallization
- Crystallization at most relevant conditions
- Synthetic juices with
  1. No dextran present
  2. Controlled concentrations of different dextran fractions
  3. Dextranase treated dextran contaminated juices
Experimental Design

Dextranase treatment of synthetic juices
• Sucrose levels 15% and 65% (w/w)
• Dextran levels 2000 and 5000 mg/kg sucrose
  • High, medium and low mol. weight dextrans (2000, 500, 40 kDa)
• Various enzyme dosages
• Incubation at 65°C for 10 and 20 minutes
• Deactivation by high temperature
Analytical Methods

Haze Method (ICUMSA GS1/2/9-15, 2011)
- Pre-treatment with subsequent spectrophotometric determination
- Limited to high molecular weight dextrans, >40kDa

Roberts’ Copper Method
- Pre-treatment with subsequent spectrophotometric determination
- Determines practically all sizes down to bigger than trisaccharides

Gel Permeation Chromatography
- Chromatography with detailed separation of molecular sizes
- Relevant juice concentration far below detection limit

HPLC
- Only mono- to trisaccharides

(Adapted from Eggleston et al, 2009)
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Analytical Results – Dextranase Treatment

Thin juice (15% Sucrose) 2000 mg high molceular weight dextran/kg sucrose
- Haze method: indicates all treatments yield dextran levels below 500 ppm
- Roberts’ method: indicates only higher enzyme dosage yield low levels

- Higher sucrose levels (65%) slow the decomposition process down (see far right with high enzyme levels)

Sample Index:
Enzyme concentration in mg/kg juice
Treatment time

Initial Dextran Level = 2000 mg/kg Sucrose

Roberts’ Copper
Haze

<table>
<thead>
<tr>
<th>Sample Index</th>
<th>Enzyme Concentration (mg/kg)</th>
<th>Treatment Time (min)</th>
<th>Dextran (mg/kg sucrose)</th>
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<tr>
<td>2 mg/kg</td>
<td>10 minutes</td>
<td>1558</td>
<td></td>
</tr>
<tr>
<td>4 mg/kg</td>
<td>10 minutes</td>
<td>1625</td>
<td></td>
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<tr>
<td>4 mg/kg</td>
<td>20 minutes</td>
<td>1003</td>
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<td>10 minutes</td>
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<td>10 minutes</td>
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<td>10 minutes</td>
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<tr>
<td>80 mg/kg</td>
<td>10 minutes</td>
<td>540</td>
<td></td>
</tr>
</tbody>
</table>

Sostenibilidad de la agroindustria azucarera
Analytical Results – Dextranase Treatment

Thin juice (15% Sucrose) 5000 mg high molecular weight dextran/kg sucrose
- Haze method: indicates practically all dextran decomposed for enzyme dosage of 4 mg/kg juice and higher
- Roberts’ method: indicates only higher enzyme dosage yield low levels
- Higher sucrose levels (65%) slow the decomposition process down

Sample Index:
Enzyme concentration in mg/kg juice
Treatment time

Initial Dextran Level = 5000 mg Dextran/kg Saccharose
Characterization of dextrans in 15% sucrose solution

- Detection limit for dextran relatively high
  a) 80000 mg high mol weight dextran/kg sucrose
  b) High + middle + low mol weight dextrans each 80000 mg/kg sucrose
- All dextrans show broad mol weight (MW) distribution
- There are practically no small oligosaccharides present
• 15% sucrose solution
• Starting material 80000 mg high mol weight dextran/kg sucrose (40 times 2000 ppm)
• Enzyme dosage per dextran scaled analog to 2 and 4 mg/kg juice (80, 160 ppm)
• Incubation time and temperature as previous
• Mixed dextrans 240000 mg/kg sucrose for reference only

• Significant reduction of dextran molecular size to oligosaccharides (smaller T40)
  • Deposition depending on enzyme dosage
• 15% sucrose solution
• Starting material 200000 mg high mol weight dextran/kg sucrose (40 times 5000 ppm)
• Enzyme dosage kept as before at 80 and 160 ppm
• Incubation time and temperature as previous

• Same reduction of dextran molecular size to oligosaccharides (smaller T40) as at lower (80000 mg/kg dextran) levels
Analytical Methods and Decomposition

Haze method
• Fast reduction of level of high MW dextrans
• Reduction kinetics strongly depending on enzyme dosage

Roberts’ method
• Intermediate decomposition products (dextran, polysaccharide (> tri sac.) )
• Integral level reduces slowly
• Reduction kinetics strongly depending on enzyme dosage

Gel Permeation Chromatography
• Dextran samples have broad molecular weight distribution
• Decomposition at artificially high levels of dextran and enzyme (both 40-fold) indicate decomposition to oligosaccharides
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Crystallisation Experiments

- Batch labscale crystalliser - 5 liter thick juice
- Evaporation crystallization at constant supersaturation
- Process control
  - Refractometer, thermometer, flux of condensate, torque
- Seeding at designed supersaturation
- End point – 50% of solids
Crystallization Experiments – Processing

Process conditions
• Pressure 270 mbar,
• Stirrer speed 60 rpm
• Temperature 75 – 77 °C
• Supersaturation approximately 1.08
• Seed crystals size about 280 µm
• Desired end crystal size 800 µm
• Final crystal content via stirrer torque and off-line

• Centrifugation with z=1970 g
  • 2.5 % of massequite washing water (70 °C)

• Fluidized bed drying
Crystallization – Experimental Design

- Crystallisation Synthetic thick juices with 67% Sucrose
  - Reference – no dextran
  - High molecular weight dextran (T2000) at 2000 and 5000 mg/kg sucrose
  - Low molecular weight dextran (T2000) at 2000 and 5000 mg/kg sucrose

- Enzyme treated thick juice
  - Thin juice with 5000 mg/kg sucrose high MW dextran
  - 20 min. Incubation at 65°C
  - Inactivation at 80°C (20 min)
  - Batch vacuum evaporation to thick juice (67% sucrose)

- Analysis of the particle size distribution by sieving was done according to the ICUMSA-Method GS2/9-37 (2007)
- Microscope and particle sizer

- WORK IS ONGOING
Crystallization preliminary Results

Crystal morphology

Presence of dextrans causes

• Dramatic change in the particle size distribution
• Both high and low MW cause a wider distribution
• Low MW dextrans have a quite profound effect on the crystal elongation along the c-axis

• Further analysis ongoing

No Dextran

T2000 high MW dextran

T40 low MW dextran
Crystallization preliminary Results

Unprocessed data of sieving residue per mesh size
• Mesh sizes according to ICUMSA
  • Fraction distribution skewed due to step size
• Reference sample
Crystallization preliminary Results - T2000

Unprocessed data of sieving residue per mesh size
• Addition of 5000 ppm high MW dextran (T2000)
  • Less large and more small crystals
    ➢ ‘growth rate reduced’

• Further data processing necessary
Crystallization preliminary Results – T40

Unprocessed data of sieving residue per mesh size
- Addition of 5000 ppm high MW dextran (T2000)
- Addition of 5000 ppm low MW dextran (T40)
  - Effect less pronounced for low MW dextran
    ➢ ‘growth rate reduced’

- Further data processing necessary
Crystallization preliminary Results
Enzyme Treatment on 5000ppm T2000

Unprocessed data of sieving residue per mesh size
• Addition of 5000 ppm high MW dextran (T2000) to thin juice
• Enzyme treatment, evaporation and crystallization

• Adequate enzyme treatment practically reverts dextran effect

• Further data processing necessary
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Conclusion and Future Work

Dextran decomposition process
• Combination of analytical methods
  • Fast reduction of high MW dextrans (Haze method)
  • Integral compositin of dextran fragments (>trisaccharides) slowly reduced (Roberts’ method)
  • Analysis at artificially high levels of dextran and enzyme fragments indicates fragment are oligosaccharides - clearly smaller than T40 (Gel permeation) sample framework established

• High sucrose levels (thick juice) slows decomposition process down
• Decomposition of dextrans very sensitive to enzyme concentration

More detailed analysis needed to
• elucidate detailed decomposition process
• kinetic parameters of process
Conclusion and Future Work

Sucrose Crystallisation
• Effect of dextran on crystallisation process
  • Reconfirmation that dextrans disturb crystallisation process
    • Change of crystal morphology and size distribution
    • Effect on growth and process efficiency not yet quantified

• Dextranase treatment (preliminary results)
  • Enzyme treatment can revert effect of high MW dextrans

Future work
• Further improve analytical toolbox
• Elucidate decomposition process and its parameters
• Extend study on crystallisation effects of dextrans to better characterized dextran compositions
• Quantify the effect of dextrans and decomposed dextrans on sugar processing
THANK YOU FOR YOUR ATTENTION