Rubisco recovery from alfalfa juice by ion-exchange chromatography in expanded bed

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PLAN

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II. Objectives
III. Material and methods
IV. Results
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I. Introduction

**Biomass**

Alfalfa

- 33 millions ha (700,000 in France)
- ~ 2500 kg proteins/ha (3x soybean, 4x wheat)

**Fractionation process**

- Mechanical dewatering
- Thermal dewatering
- Grinding
- Dehydrated alfalfa

**Feed**

- Proteins concentrate (1%) +
- Dehydrated alfalfa (99%)

- Low added value
- Disadvantageous energy balance

France: 800,000 t/year
I. Introduction

Biomass

Proteins, amino acids, ethanol, organic acids
Cellulose, enzymes, biofuels …

I. Introduction

Biorefinery

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33 million ha
(700 000 in France)

~ 2500 kg proteins/ha
(3x soybean, 4x wheat)

Products

Proteins, amino acids, ethanol, organic acids
Cellulose, enzymes, biofuels …

Dehydrated alfalfa

Diversification

+ Added value
+ Energy input
+ Pollution

Alfalfa

Diversification

+ Added value
+ Energy input
+ Pollution

Bioenergy

Bio-based Products

Feed

Materials

Chemicals
II. Objectives

Leaf proteins are **abundant** and **renewable**

Proteins in alfalfa juice: up to **20%** of DM:
- 50% **hydrophobic** proteins
- 50% **hydrophilic** proteins

**Rubisco**: Ribulose 1,5 Biphosphate Carboxylase Oxydase
- 70% of **hydrophilic** proteins
- **Valuable** in many fields (human nutrition, pharmaceuticals, environmental ...)

**Aim**
Study of Rubisco recovery from alfalfa juice by ion-exchange chromatography in expanded bed

**Preliminary approach**:
- Centrifuged alfalfa juice
- Different Rubisco content
II. Objectives

- Crude extract
  - Liquid-solid separation:
    - Centrifugation
    - Filtration
  - Concentration
- Primary purification
  - Primary separation:
    - Chromatographic processes
    - Adsorption
    - Membrane filtration
- Purification
- Polishing
- Pure product

Expanded Bed Chromatography (EBC)

Ion exchange
- Good selectivity
- High binding capacity

Expanded Bed
- High treatment capacity

Interesting alternative
III. Material and methods

Vegetable material

Physico-chemical properties of centrifuged alfalfa juice

<table>
<thead>
<tr>
<th></th>
<th>Centrifuged juice</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.8</td>
</tr>
<tr>
<td>Conductivity (mS.cm⁻¹)</td>
<td>17.0</td>
</tr>
<tr>
<td>Viscosity (Pa.s)</td>
<td>2.10⁻³</td>
</tr>
<tr>
<td>Density (kg.m⁻³)</td>
<td>1042</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>8.1</td>
</tr>
<tr>
<td>Total nitrogen content (g.L⁻¹)</td>
<td>19.43</td>
</tr>
<tr>
<td>Hydrophobic proteins content (g.L⁻¹)</td>
<td>1.84</td>
</tr>
<tr>
<td>Hydrophilic proteins content (g.L⁻¹)</td>
<td>16.92</td>
</tr>
<tr>
<td>Rubisco content (g.L⁻¹)</td>
<td>13.3</td>
</tr>
</tbody>
</table>
III. Material and methods

Experimental set-up

![Experimental setup diagram](image)

<table>
<thead>
<tr>
<th>Nature of ionic groups</th>
<th>Q Hyper Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average particle size (μm)</td>
<td>75</td>
</tr>
<tr>
<td>Particle size distribution (μm)</td>
<td>40-105</td>
</tr>
<tr>
<td>Mean particle density (g.mL⁻¹)</td>
<td>3.2</td>
</tr>
<tr>
<td>Binding capacity (mg.mL⁻¹)</td>
<td>80 (BSA)</td>
</tr>
</tbody>
</table>
### Operating conditions

<table>
<thead>
<tr>
<th></th>
<th>Charge</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C₀ (g.L⁻¹)</strong></td>
<td><strong>Dilution</strong></td>
<td><strong>Superficial velocity  (cm.min⁻¹)</strong></td>
</tr>
<tr>
<td>Experiment 1</td>
<td>1.13</td>
<td>1:10</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>1.55</td>
<td>1:8</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>2.20</td>
<td>1:6</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>13.35</td>
<td>1:1</td>
</tr>
<tr>
<td>(Centrifuged raw juice)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ H = 21 \text{ cm} \quad \Phi_{colonne} = 2.5 \text{ cm} \]
VI. Results

Breakthrough curves

Experimental breakthrough curves for Rubisco obtained for a Q Hyper Z column in expanded bed mode.

**Determination of dynamic capacity:**

\[
Q_{10\%} = \frac{C_0 \left( V_{10\%} - V_p \right) - \int_0^{V_{10\%}} C \cdot dv}{V_R}
\]

<table>
<thead>
<tr>
<th>Conductivity (mS.cm(^{-1}))</th>
<th>(Q_{10%}) (g_{Rubisco}.L(^{-1})QHyper Z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>1.22</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>1.43</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>2.11</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>17.00</td>
</tr>
</tbody>
</table>

- \(C_0\) Rubisco \(\uparrow Q_{10\%}\) \(\uparrow\) even if conductivity \(\uparrow\)

- \(Q_{10\%} = 37\ g_{BSA}/L_Q\ Hyper\ Z\) (Vergnault, 2004)
  - \(= 35.9\ mg_{BSA}/ml_Q\ Hyper\ Z\) (Xia et al., 2007)
VI. Results

Elution curves

- Elution velocity ↓, thin elution peak
- High concentration of eluted fraction: up to 60 g/L

Elution curves of Rubisco performed with 0.5 M NaCl at different superficial velocities
VI. Results

Chromatograms obtained by HPLC SEC analysis of eluted fractions (experiment 3).

- High concentration of Rubisco
- Absence « contaminants » peaks in the first eluted fraction
VI. Results

Qualitative analysis

Production of Rubisco fraction at very high concentration and purity when compared with available Rubisco

Comparison between chromatograms obtained by HPLC SEC analysis of commercial purified Rubisco and fraction 5 obtained by EBC process
V. Conclusions and perspectives

• Rubisco can be separated by a simple process with good performances.

• Even the complexity and the high conductivity of the juice, $Q_{10\%}$ obtained are good.

• Dilution of the juice before EBC treatment could be interesting in order to reduce its ionic strength and increase the dynamic capacity of the adsorbent.

• Fixed Rubisco is easily eluted at 0.5 M NaCl.

• Elution in fixed bed mode at low velocity allows the recovery of high concentration Rubisco fractions with high purity.

• Experiments without centrifugation step
• Scale up
• Rubisco binding mechanisms
THANK YOU FOR YOUR ATTENTION