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

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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
- Liquid processing
- Centrifugation
- PC

**Feed**

- Dehydrated alfalfa (99 %)
  - France : 800 000 t/year

- Proteins concentrate (1 %) +

- Low added value
- Disadvantageous energy balance
I. Introduction

Biomass

Alfalfa

33 million ha (700 000 in France)

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

Biorefinery

Products

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

Diversification

Added value

Energy input

Pollution

Dehydrated alfalfa

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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
### 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

<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>

S1: Buffer solution
S2: Alfalfa juice
### Operating conditions

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$C_0$ (g.L$^{-1}$)</th>
<th>Dilution</th>
<th>Superficial velocity (cm.min$^{-1}$)</th>
<th>Concentration of NaCl (mol.L$^{-1}$)</th>
<th>Superficial velocity (cm.min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>1.13</td>
<td>1:10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>1.55</td>
<td>1:8</td>
<td></td>
<td></td>
<td>5.3</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>2.20</td>
<td>1:6</td>
<td></td>
<td>0.5</td>
<td>0.65 (fixed bed mode)</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>13.35</td>
<td>1:1</td>
<td></td>
<td>0.40 (fixed bed mode)</td>
<td>0.40 (fixed bed mode)</td>
</tr>
</tbody>
</table>

Experiment 4 (Centrifuged raw juice)

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

Breakthrough curves

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

**Determination of dynamic capacity**:

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

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

- **Q_{10\%} = 37 \text{ gBSA/L}_{\text{Q Hyper Z}}** (Vergnault, 2004)

- **Q_{10\%} = 35.9 \text{ mgBSA/ml}_{\text{Q Hyper Z}}** (Xia et al., 2007)
VI. Results

Elution curves

- Elution velocity \( U \), 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

Qualitative analysis

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

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