Hydrolysis strategies for the valorisation of Grape stems to improve their value in ruminant feeds

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NEWFEED: Turn food industry by-products into secondary feedstuffs via circular-economy schemes
Case study 1: grape stem-based ingredients for dairy sheep and cattle
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Assess the use of grape stem from wineries as a second-generation feedstuff to produce a new feed ingredient for ruminants (dairy sheep and cattle).

- Fibres: cellulose, hemicellulose, lignin
- Polyphenols: 1.5-7%
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Fibres
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- Polyphenols

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- Polyphenols

Introduction

1.5-7%

Flash dryer

Grape stem ingredient

Feed formulation

Feeding test
Case study 1: grape stem-based ingredients for dairy sheep and cattle

Optimization of the Valorization and feeding strategies

1. Definition of the Feedstock supply and logistics strategy and characterisation of grape stem
2. Optimization of the Non-hydrolysed grape stem drying process
3. Optimization of the hydrolysis process

Fibre fraction
% of inclusion
Optimization of the hydrolysis process

Methodology

Factorial experimental design

<table>
<thead>
<tr>
<th>Washing</th>
<th>Hydrolysis</th>
<th>Conditions</th>
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<tbody>
<tr>
<td>Yes</td>
<td>1. NaOH</td>
<td>1% NaOH, ratio 1:1.25 w/w 90 °C 3 h 250 rpm</td>
</tr>
<tr>
<td>No</td>
<td>2. Cellulolytic Enzymes</td>
<td>Ultimase-Viscozyme 2 % 55 °C 20h</td>
</tr>
<tr>
<td></td>
<td>3. NaOH + Cellulolytic enzymes</td>
<td>Consecutive processes</td>
</tr>
</tbody>
</table>

Collect in winery

Grinding

Washing

Hydrolysis

Characterisation of non hydrolyzed and hydrolyzed prototypes

1. Nutritional characteristics
   Dry matter, ash, crude protein, ether extract, NDF, ADF, LAD, NDICP and ADICP, sugars, polyphenols

2- In vitro digestibility value
   In vitro organic matter digestibility (IVOMD) and short chain fatty acid production determination (acetic, propionic, butyric, isobutyric, valeric and isovaleric).

3- Ruminal fermentation kinetics
   Rate and extent of gas production (mL/g DM)
Optimization of the hydrolysis process

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Objective

With the aim of increasing their inclusion level in ruminant diets

Characterisation of non hydrolyzed and hydrolyzed prototypes
Results

Optimization of the hydrolysis process

Washing

**Washing step** → reducing free sugars and facilitate the drying process

Ratio grape stem:water = 1:1.5 (w/w)
Time = 60 minutes

Solid fraction
Continue with the process
- Freeze-dry
- Hydrolysis processes

Liquid fraction
Sugar extraction
22 g/L
Optimization of the hydrolysis process

Significant interactions
Washed:
- Alkali-H increases antioxidant capacity of prototypes
- E-H decreases TE in prototypes

No Washed:
- Only E hydrolysis decreases TE/g sample
Optimization of the hydrolysis process

Results

Significant interactions
Washed:
- Alkali-H increases polyphenol content of prototypes
- E-H decreases polyphenols

No Washed:
- E and Alkali-E hydrolysis decrease polyphenol content of samples
Optimization of the hydrolysis process

Results

Significant interactions

Washed:
- Alkali-H increases sugar content of prototypes compared to E and Alkali-E hydrolysis

No Washed:
- All treatments decrease sugar content compared to control
Optimization of the hydrolysis process

- There are no significant interactions
- Significant differences are only seen due to hydrolysing
- E hydrolysis decreases cumulative gas production compared to control
Results

Optimization of the hydrolysis process

- Time required for half of the potential gas production to be reached ($B$, h)

- There are no significant interactions

- Significant differences are only seen due to washing

- Washing increases the time needed to reach half of the potential gas production

\[ P = 0.013 \]
Optimization of the hydrolysis process

Results

- In vitro digestibility (%)

**Washed:**
- Alkali-H improves digestibility compared to control
- E-H decreases the digestibility compared to control, no differences with Alkali-E

**Not Washed:**
- Alkali-E and E-H decrease digestibility compared to Alkali-H and control

**Washing**

**Hydrolysis**

- There are significant interactions

![Graph showing in vitro digestibility (%) for different conditions: Basic, Basic-enzymatic, Enzymatic, No.](chart.png)

- Washed conditions:
  - Basic: 60% digestibility
  - Basic-enzymatic: 50% digestibility
  - Enzymatic: 40% digestibility
  - No: 30% digestibility

- Not Washed conditions:
  - Basic: 70% digestibility
  - Basic-enzymatic: 60% digestibility
  - Enzymatic: 50% digestibility
  - No: 40% digestibility

* Significant differences indicated by asterisks (*)
Conclusions

- Enzymatic hydrolysis processes involve subsequent mechanical drying (centrifugation) which implies a loss of nutrients compared to the control.

- Therefore, the enzymatic hydrolysis processes lead to a decrease in the content of Trolox equivalents, polyphenols and sugars in the final prototypes.

- Alkali hydrolysis, although involving a mechanical drying, fibre degradation increases the content of Trolox equivalents and polyphenols compared to the control, only when a previous washing step has been carried out.

- The washing process itself leads to a loss of sugars in the final samples.

- E hydrolysis decreases cumulative gas production mainly due to nutrient release in the mechanical drying.

- Washing step releases sugars → increasing the time needed to reach half of the gas production.
Conclusions

- Digestibility: Alkali-H improves digestibility compared to all treatments only when samples are washed.

- When there is no washing, the samples without hydrolysis do not improve with any of the processes proposed. Instead, washing releases many nutrients that are readily available, causing the ingredient to decrease in value. In this case, there is a margin for improvement that can be obtained after the degradation of the fibre by applying the alkali hydrolysis.

- Alkali hydrolysis is selected for further optimization. As alkali hydrolysis already includes a wash itself, this factor is removed from the study.
On going-Next steps

✓ Second experimental design
  • Alkali hydrolysis (pH 9)
  • Not washed sample
  • Response surface methodology

✓ Selection of the best condition

✓ Scale-up and Validation of the non-hydrolyzed and hydrolyzed conditions

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<th>Time (h)</th>
<th>T° (°C)</th>
<th>S/L ratio (% solids)</th>
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<tr>
<td>1</td>
<td>60</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>75</td>
<td>36.5</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>40</td>
</tr>
</tbody>
</table>
Thank you for your attention!

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Project Partnership