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

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WATER COUNCIL
CORFU2022

NEWFEED:

Turn food industry by-products into secondary feedstuffs via circular-economy schemes

Project Case Studies

Three value chains at the Mediterranean area will be validated to create new business opportunities based on a multi-actor approach in their conception, configuration and its sustainability assessment:

1st — Grape stem from wineries as a second-generation feedstuff to produce a new feed ingredient for ruminants (dairy sheep and cattle). AZTI / Spain.

2nd — Orange peel from orange juice industry to produce an improved feed ingredient for ruminants (dairy sheep). NTUA / Greece.

3rd — Olive cake from olive oil industry to produce an improved feed ingredient for poultry (broiler chicken). HUSD / Egypt.

Project Partnership

AZTI, NEIKER, Riera Nadeu, UAGA, BAI GORRI, cesfac, Heliopolis University for Sustainable Development, SEKEM Development Foundation, iSiS, ΣΕΒΤ, ELGO-DIMITRA, University of Western Macedonia, Orta Doğu Teknik Üniversitesi / Middle East Technical University

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

newfeed — FEED FROM FOOD INDUSTRY BY-PRODUCTS

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

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).

1.5-7%

- Fibres

cellulose, hemicellulose, lignin

- Polyphenols

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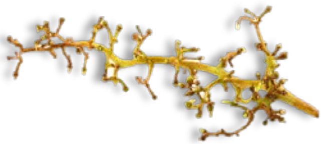


1.5-7% → **Flash dryer** → **Grape stem ingredient** → **Feed formulation** → **Feeding test**

- Fibres

cellulose, hemicellulose, lignin

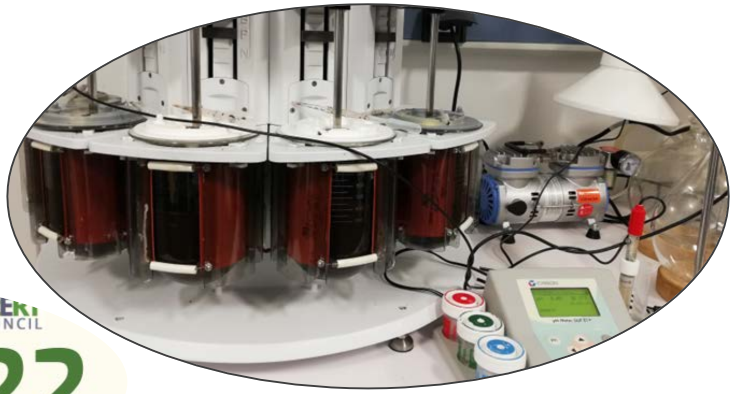
- Polyphenols

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

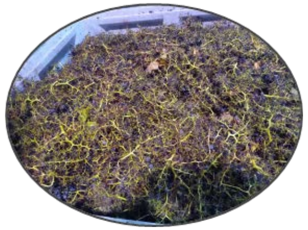
↑ % of inclusion

Methodology

Optimization of the hydrolysis process

Factorial experimental design

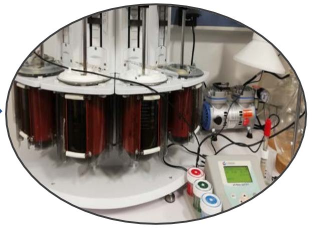
Washing	Hydrolysis	Conditions
Yes	1. NaOH	1% NaOH, ratio 1:1.25 w/w 90 ^{o}C 3 h 250 rpm
No	2. Cellulolytic Enzymes	Ultimase-Viscozyme 2 % 55 ^{o}C 20h
	3. NaOH + Cellulolytic enzymes	Consecutive processes



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)

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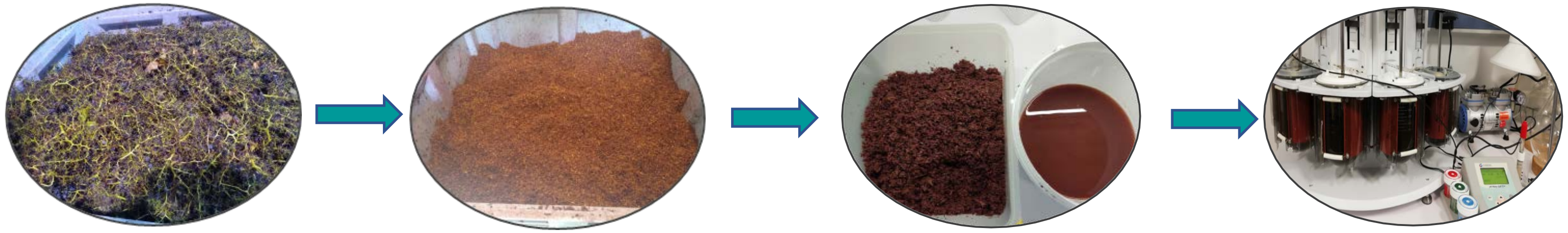


Collect in winery → **Grinding** → **Washing** → **Hydrolysis**

↓

Characterisation of non hydrolyzed and hydrolyzed prototypes

Objective

With the aim of increasing their inclusion level in ruminant diets

Optimization of the hydrolysis process

Washing

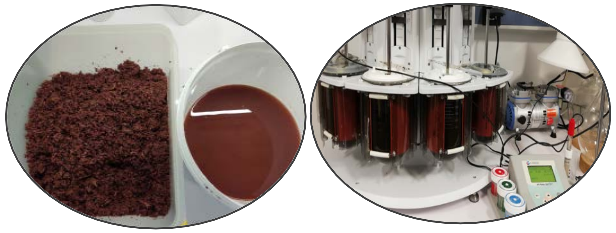
Washing step → reducing free sugars and facilitate the drying process

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



Washing **Hydrolysis**

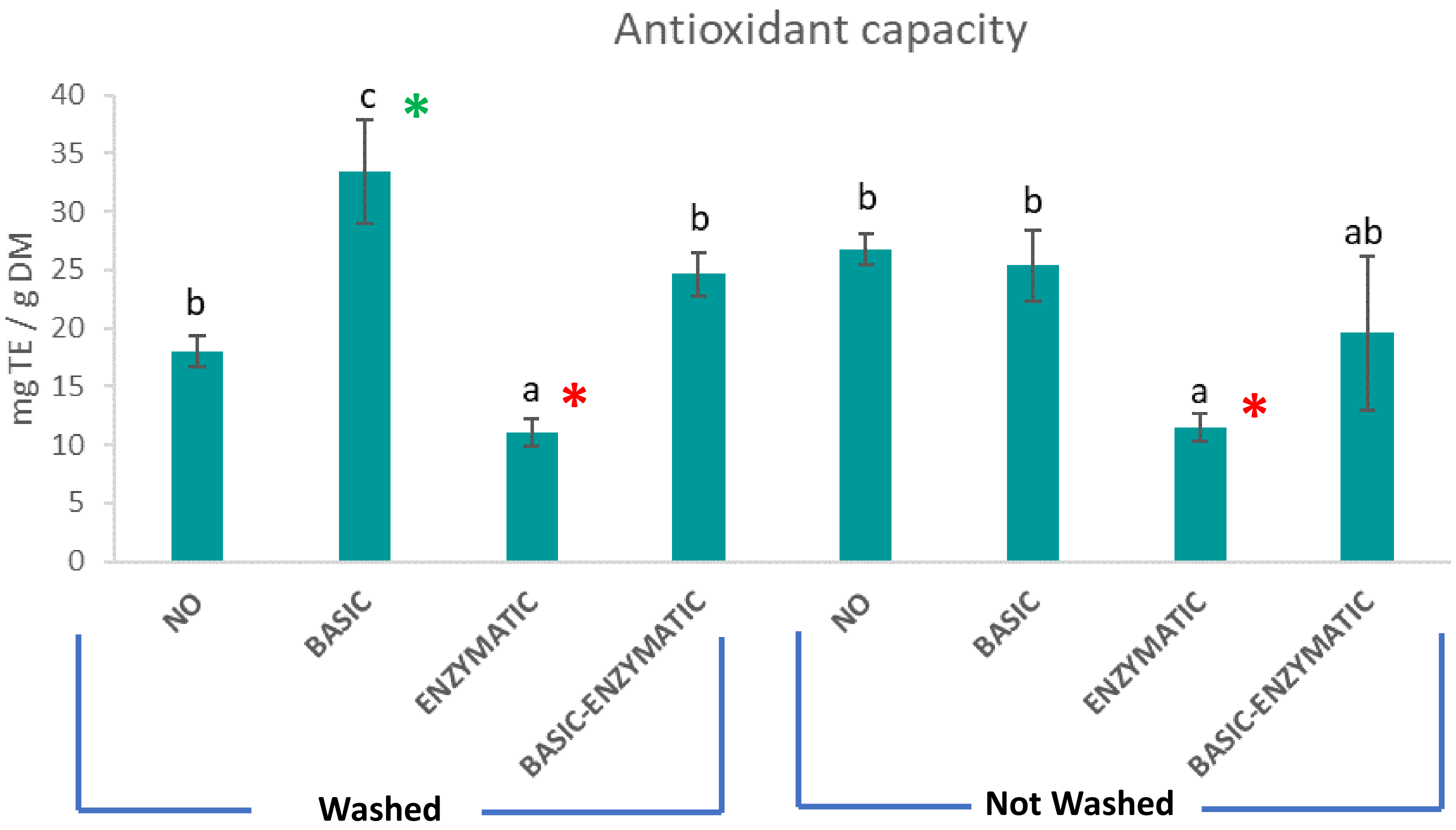
Significant interactions

Washed:

- Alkali-H increases antioxidant capacity of prototypes
- E-H decreases TE in prototypes

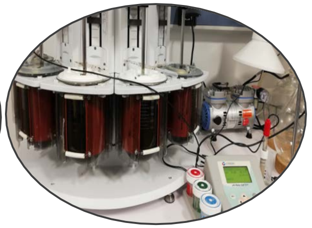
No Washed:

- Only E hydrolysis decreases TE/g sample



Optimization of the hydrolysis process

Washing



Hydrolysis

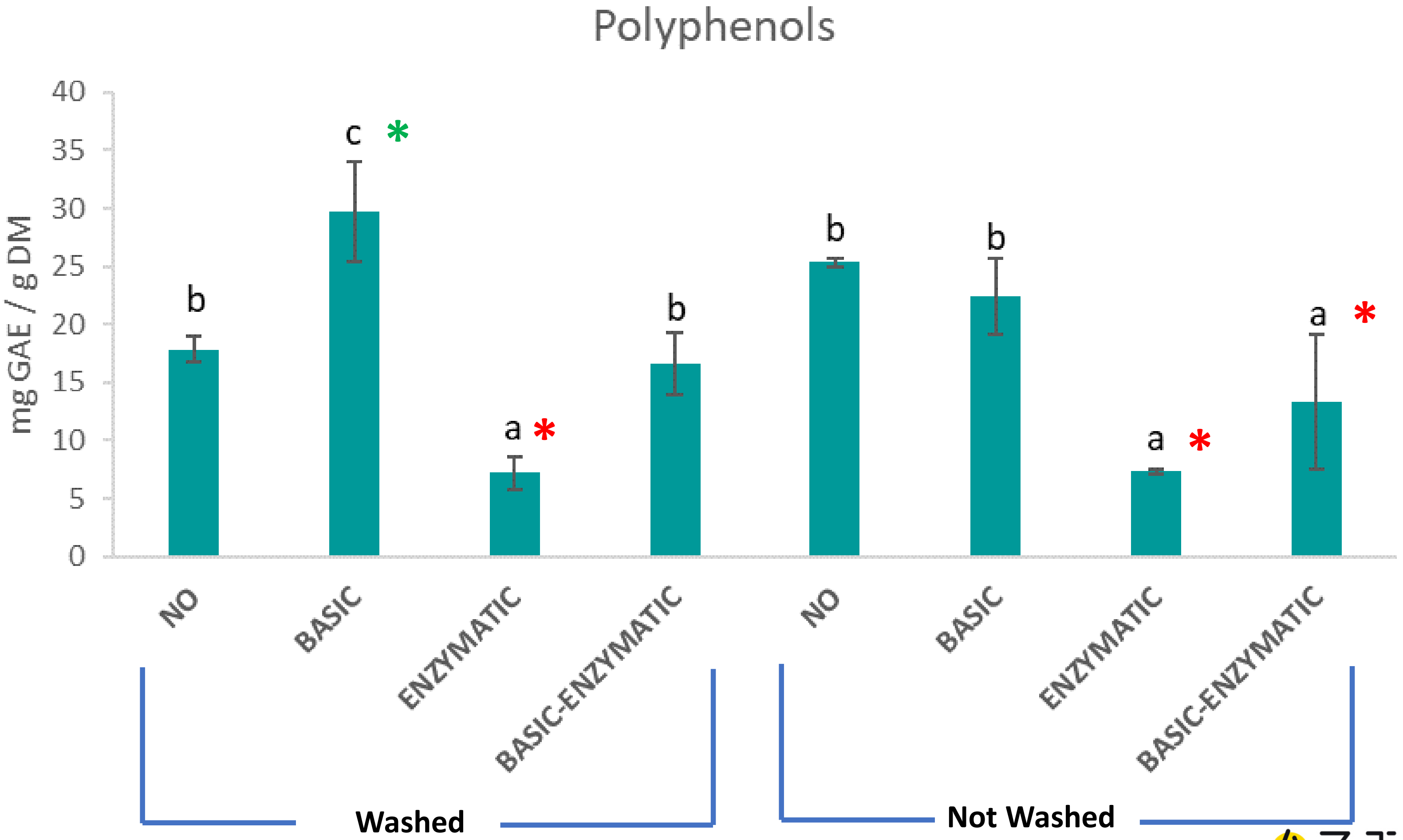
Significant interactions

Washed:

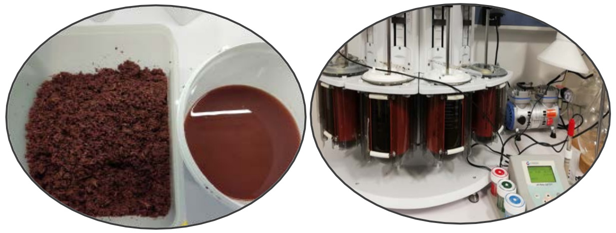
- Alkali-H increases polyphenol content of prototypes
- E-H decreases polyphenols

No Washed:

- E and Alkali-E hydrolysis decrease polyphenol content of samples



Optimization of the hydrolysis process



Washing **Hydrolysis**

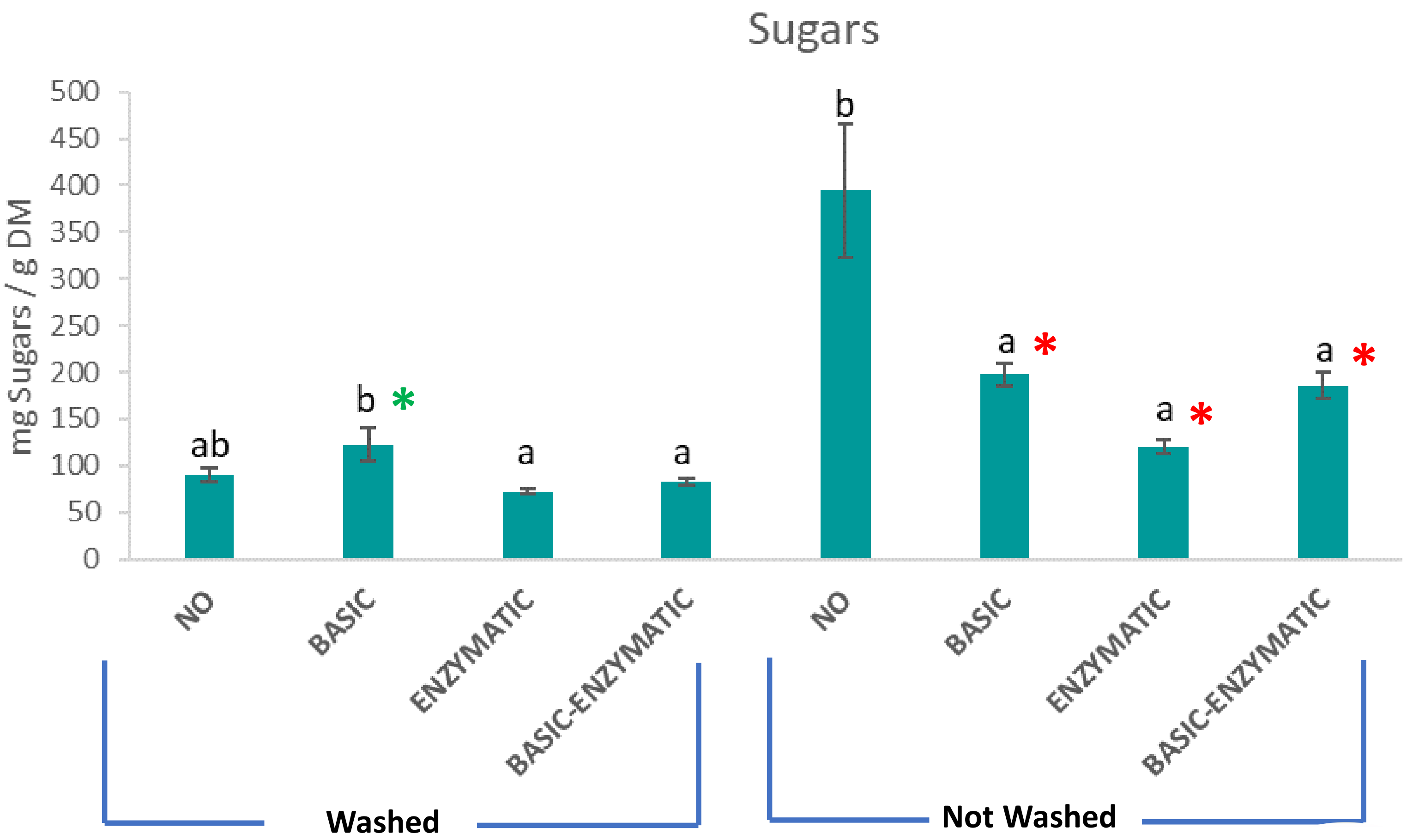
Significant interactions

Washed:

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

No Washed:

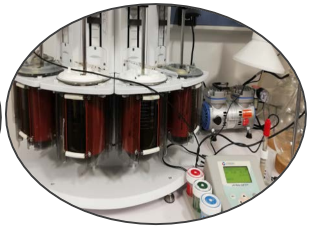
- All treatments decrease sugar content compared to control



Results

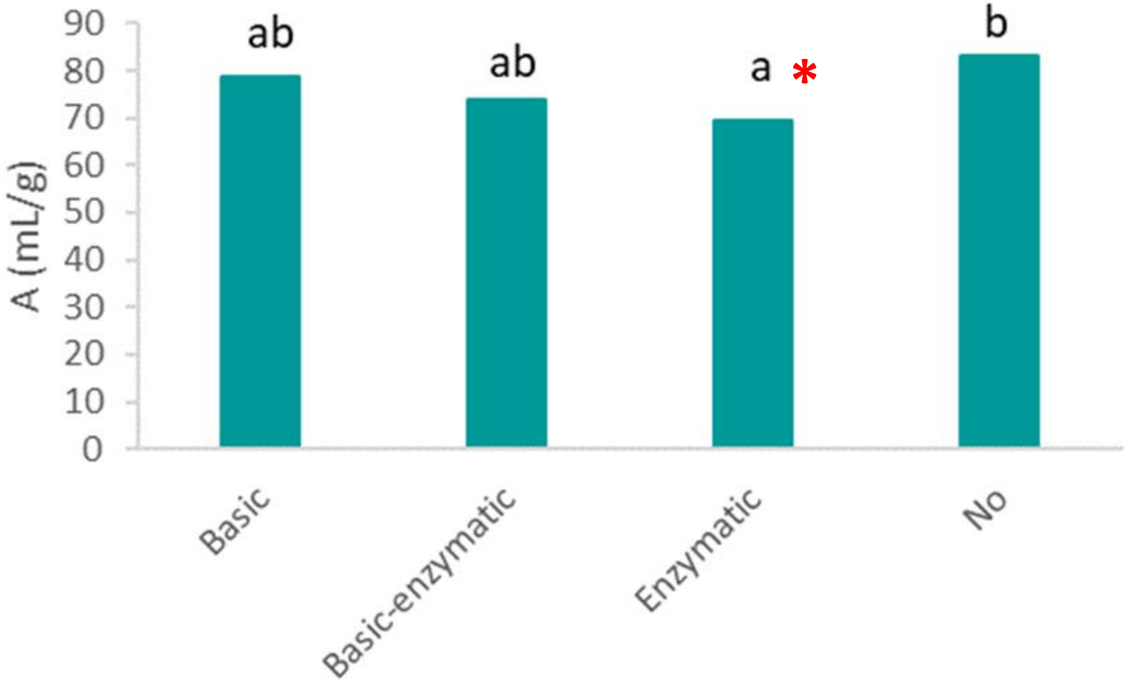
Optimization of the hydrolysis process

Washing



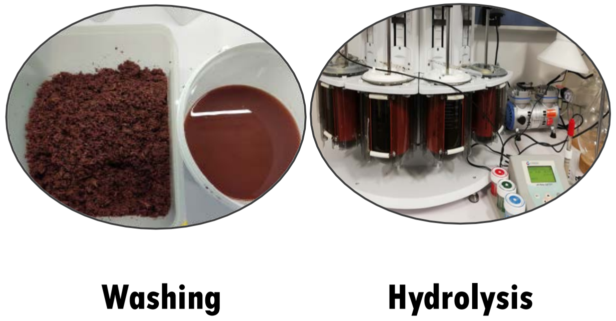
Hydrolysis

- Cumulative gas production (A, mL/ g)

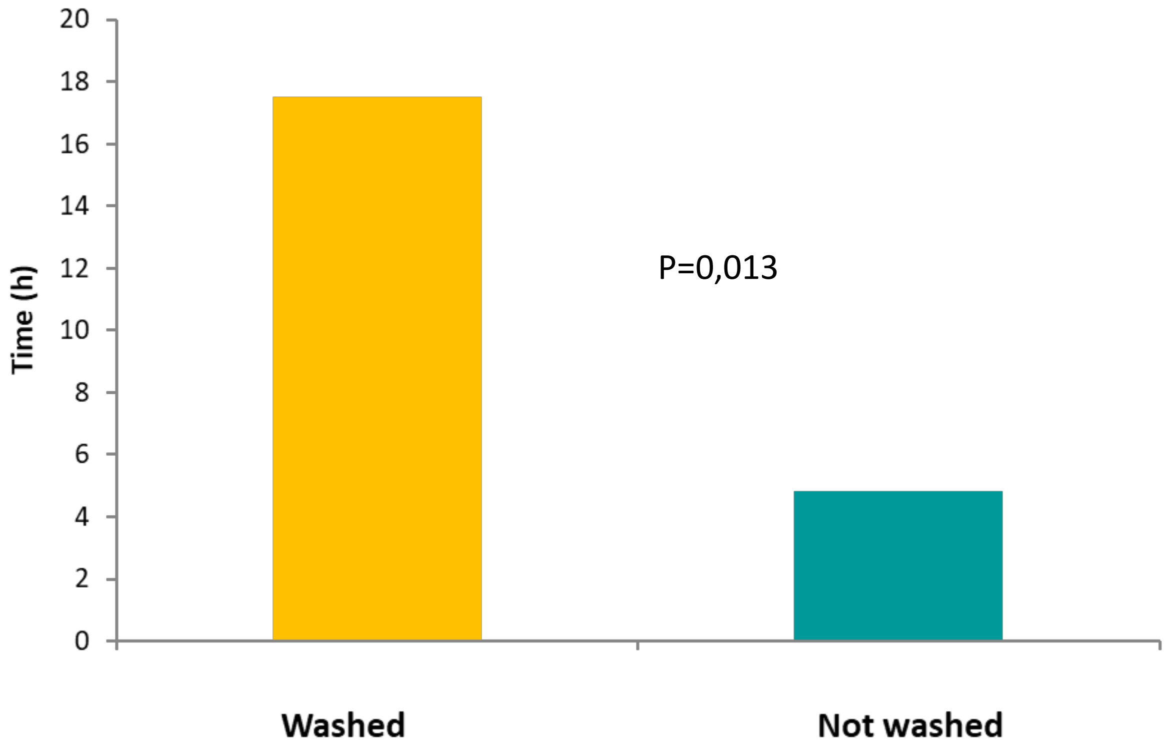


- There are no significant interactions
- Significant differences are only seen due to hydrolysing
- E hydrolysis decreases cumulative gas production compared to control

Optimization of the hydrolysis process



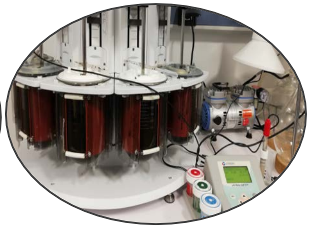
- Time required for half of the potential gas production to be reached (B, h)



- 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

Optimization of the hydrolysis process

Washing



Hydrolysis

- There are significant interactions

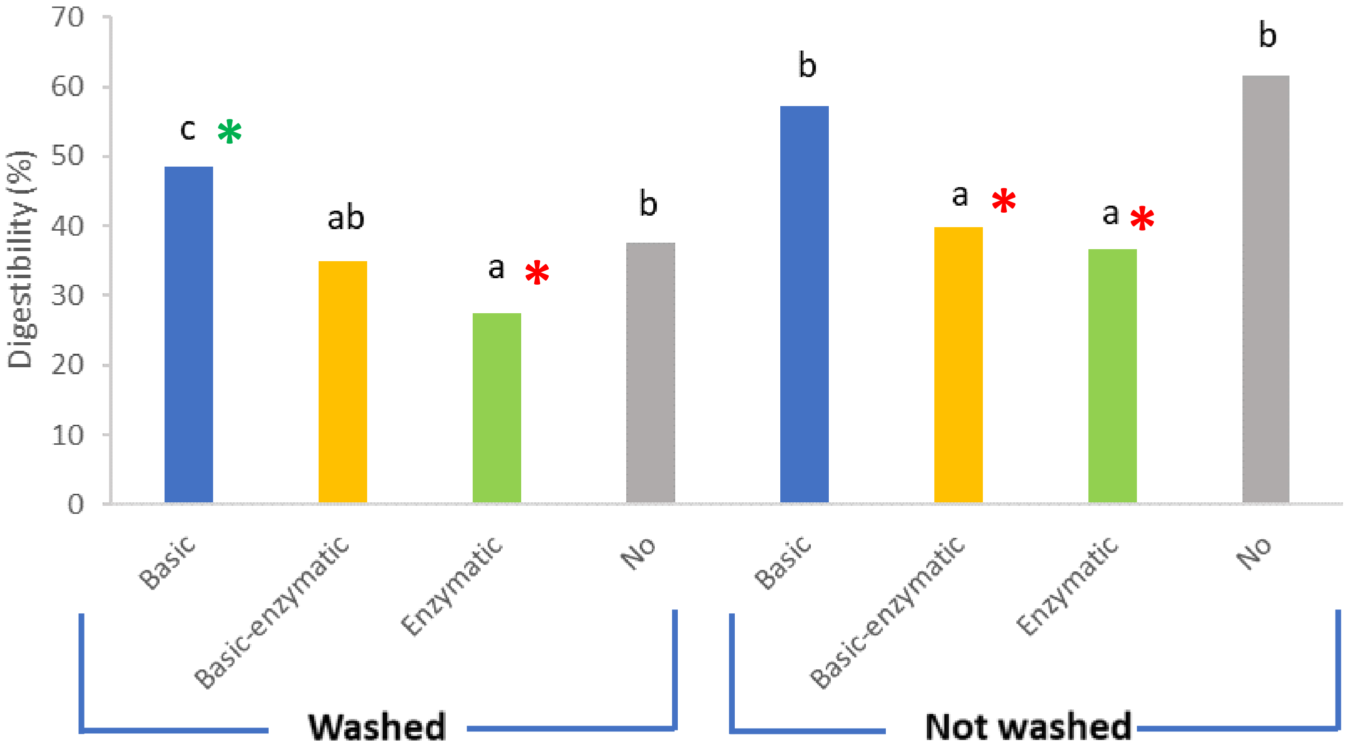
Washed:

- 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

- In vitro digestibility (%)



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

Time (h)	Tª (ºC)	S/L ratio (% solids)
1	60	33
2	75	36.5
3	90	40

✓ Selection of the best condition

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

Thank you for your attention!

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