Assessment of the use of grape stem from wineries as a second-generation feedstuff to produce a new feed ingredient for ruminants (dairy sheep and cattle).

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Introduction

The EU is the leading wine producer globally, with 167 million hectolitres annually. Significant winery by-products include grape stems, pomace, and lees. Approximately 3-7% of the total amount of grapes resulted in grape stems, which are often discarded or composted, incurring economic and environmental costs. Grape stems contain unseparated grapes with high sugar content valuable for biotechnology applications (46 g sugar/Kg). They are also rich in fibre (cellulose, hemicellulose, lignin) and polyphenols, beneficial for animal nutrition. However, they require drying for stabilization. While in-vitro assays have shown benefits of using these by-products as feed, further in-vivo tests on livestock (e.g., dairy cattle, sheep) are needed to assess potential improvements in milk quality and production. However, the use of grape stem as new ingredient in animal feed is limited by their high lignin content which limit their level of inclusion.

Current study aims to analyse the effect of applying different hydrolysis strategies in the digestibility of grape stems for ruminants, through in vitro and in vivo assays.

Methodology

The grape stem (GS) samples were collected from Baigorri S.A. winery from Samaniego Spain. In order to improve GS digestibility two consecutive experimental designs were conducted at laboratory scale:

- A full factorial design including, a washing step and comparison between alkali hydrolysis (NaOH, 1 % w/v, at a sample:solvent ratio of 1:1.25 w/w) and enzymatic hydrolysis, which were selected as the most appropriates for fibre hydrolysis based on bibliography and previous research (Ping et al, 2011, Filippi et al, 2021) (Ultimase® and Viscozyme® (Novozymes A/S, Bagsvaerd, Denmark).
- A Box Behnken design to optimize alkali hydrolysis conditions of GS to maximize both digestibility and nutritional benefits. To achieve this, temperature, time, and solid concentration, along with their interactions, were analyzed.

Both, basic and enzymatic hydrolysis were performed at laboratory-scale using a Sell Symphony 7100 Bathless Dissolution Distek equipment (Distek Inc., North Brunswick, USA), controlling and monitoring temperature, time and stir speed. Optimal processing conditions were selected based on the nutritional value of the hydrolysates that was measured by applying the Association of Official Analytical Chemists (AOAC) Official Methods, ruminal in vitro organic matter digestibility (IVOMD), and ruminal fermentation patterns (SCFA, short chain Fatty acids).

Subsequent to the optimizing process, technical processing was validated at higher TRL (6-7) and in vivo animal feeding trials were conducted. The technical process consisted in a preliminary grinding (Comitrol® Processor Model 1700 (Urschel, Chesterton, Indiana, USA) and a washing process developed by adapting a pulp washing technology. This process adds 1 litre of water per kilogram of grape stem, and the liquor with the soluble sugars is separated from the solid part using centrifugal force. The liquid part is removed for human food applications.

The hydrolysis was performed with the selected conditions in the small-scale tests. For this process a large bulk container was adapted with a stirrer, 4 heating elements and a pH meter for pH control. The stabilization and adaptation of the solid part (grape stems) to the needs of the animal feed sector was performed using RINA-JET turbo-dryer. This technology is suitable for thermosensitive products.

Finally, grape stem-based ingredients were validated in two ruminant production systems: dairy cattle and sheep. Firstly, isonitrogenous and isoenergetic diets for both ruminant production systems were formulated by substituting conventional feed ingredients and by meeting the nutrient requirements. Percentage of inclusion was decided based on the nutritional value and digestibility. Diets were fed ad libitum to about 5 to 10% orts. 36 six multiparous dairy ewes (150 days) and 16 cows (120 days) were used to validate hydrolyses and non-hydrolyses ingredients.

Results and discussion

Grape stems ar a by-product of wine production that can carry a greater or lesser number of grapes that have not been harvested in the grape harvest. This grape contains a high sugar content, which can enrich the final product in easily assimilated sugars compared to the high amount of fibre (approximately 65 %) contained in the stem. Even so, these sugars can be a problem in the drying process, so a preliminary washing may help in the subsequent drying process and can be used as a carbon source for biotechnology processes.

The washing process releases around 50-60 g of sugar/L, which significantly improves the drying efficiency, however, also reduces the digestibility of the GS (from 37.6 to 48.6%). Enzymatic hydrolysis involves a centrifugation step prior to drying process which implies a loss of nutrients compared to the control and led to a decrease in polyphenols and sugars and IVOMD values. Alkali hydrolysis, although involving a centrifugation and an apparently an increase of fibre fractions also led to an increase of IVOMD of GS compared to the washed control sample, therefore, it was selected for further optimization. Using Box Behnken design, the best alkali hydrolysis conditions were selected for IVOMD improvement: Temperature 90 ºC, time 2.3 h and solids 33%. The composition of both hydrolysed and non-hydrolysed sample is shown in the next table:

	Hydrolysed sample	**Non-hydrolysed samples**
Reducing sugars (mg/g)	192.7	234
Polyphenols (mg/g)	29.6	38.0
NDF (%)	47.3	45.0
ADF (%)	47.7	40.6
Lignin (%)	29.9	24.6
Digestibility (%)	41.9	30.3

In dairy sheep including 10% GS or HGS in the concentrate compared to CTR did not affect dry matter intake (DMI). In addition, including 10% GS or HGS in the concentrate did not impair milk yields or milk fat, protein, or lactose proportions. As for methane emissions formulating 10% GS or HGS did not significantly reduce daily methane emissions or methane intensity compared to CTR. Compared to CTR, including GS in the concentrate resulted in an increase in the individual proportions of C18:2 cis9 cis12 n6, C18:3 n3 and C20:3 n3, C22:2 n6, which resulted in an increase in total milk polyunsaturated acid (PUFA), n6 and n3, not altering the n6:n3 ratio. This higher PUFA content in milk resulted in a higher PUFA:SFA ratio. Finally, regarding sensory properties of the final products, consumers were not able to distinguish between those curds produced with milk obtained from GS-fed-ewe or CTR-fed-ewes.

In dairy cows feeding GS did not affect daily yields of milk, lactose, protein. Similarly, including GS in the concentrate did not affect milk proportions of lactose or fat, but tended to decrease those of protein crude. Regarding methane emissions, including GS in the concentrate did not significantly affect CH4 production or intensity. Including GS in the concentrate increased total milk PUFA, the PUFA:SFA ratio, total n6, but it did not affect total CLA, atherogenic index or n3 proportions. Finally, milk consumers were not able to distinguish between the milk obtained from a GS-fed-cow or CTR-fed-cow.

Conclusions

The grinding process has favoured the subsequent processing of the raw material and has been carried out without any technical difficulty.
The washing process has made it possible to reduce the sugar content in order to improve drying efficiency and solve the fermentation processes associated with the high sugar content in the raw material. Although alkali hydrolysis also reduced TRS content and increased fibre components, digestibility was improved compared to control. It could be hypothesized that in the present study the alkali hydrolysis succeeded in acting into the

lignocellulosic bounds, leading to a more degradable fibre fraction. Therefore, although a loss of soluble sugars occurred in the hydrolysis process, this fact was offset by a more digestible fibre fraction.

Regarding animal feeding trials, including 10% GS or HGS in the concentrate in dairy sheep or GS in dairy cows did not impair productive performance or alter curds or milk consumer acceptance, and therefore can be used as raw material in ruminant feeding. However, little advantage can be gained from their inclusion because it did not reduce enteric methane emissions. Including 10% GS over GSH offers the advantage of improving the milk fatty acid composition towards a healthier one.

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