

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

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## Introduction

Winery industry generate huge amounts of grape stem in the winemaking process. 0.21 kg of grape stems are produced per kg of grapes.

Most of this grape stem finishes in a dump, which involves important economic costs and environmental impacts to the wineries.

Numerous studies have highlighted its phytochemical composition (Gouvinhas et al, 2019), which provides them significant antioxidant properties with beneficial biological and physiological effects if they are included in the diet of animals.

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.

## Material and methods

The grape stem samples were collected from Baigorri S.A. winery from Samaniego Spain.

A full factorial design has been defined including, a washing step, two different crushing pre-treatments, basic 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 appropriate for fibre hydrolysis based on bibliography and previous research (Ping et al, 2011, Filippi et al, 2021). The following commercial enzymes Ultimase® and Viscozyme® (Novozymes A/S, Bagsvaerd, Denmark) were selected as the most appropriate for fibre hydrolysis based on bibliography and previous research. The best hydrolysis conditions for each enzyme were applied. The hydrolysis was inactivated by temperature at 90 °C for 15 minutes. The samples were centrifuged (2,650 x g; 15 min; ambient T<sup>a</sup>) and the solid fraction was separated for animal feed, whereas the liquid sample was removed.

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.

The nutritional value of the hydrolysates was measured by applying the Association of Official Analytical Chemists (AOAC) Official Methods. Then, a digestibility test was carried out with the aim of determining its digestibility.

## Results and discussion

Grape stems are 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. The washing process releases between 15 and 22 g/L of reducing sugars, which could significantly improve the drying efficiency.

The crushing step favours the reduction of particle size and the action of rumen enzymes in the raw material with a high fibre content. This makes the enzymatic action more efficient and improves the digestibility of the products. The basic treatment reduces the lignin content of the grape stems, thus improving the rumen digestibility of the products. On the contrary, the enzymatic treatment degrades part of the grape stalk fibre, but the release of interesting compounds into the liquid fraction, such as easily fermentable sugars, that are not recovered in the solid fraction, makes the hydrolysed products less valuable than the untreated grape stem.

### **Conclusions**

Grape stem is a by-product with a high fibre content which decreases its digestibility, and therefore, reduces its inclusion as a digestible raw material in animal feed. The hydrolysis process could have been a valuable strategy to make the raw material components more accessible to the animals, however, the effect of releasing compounds of interest (sugars, polyphenols...) into the liquid medium during the hydrolysis process, lead to a less valuable ingredient. Furthermore, the effect that enzymes can have on the solid matter is counteracted by the enzymatic action of the ruminal bacteria.

The washing step could be an effective pre-treatment for sugars release, which could significantly improve the drying efficiency, however its effect on final ingredients and the possibilities of using the generated wastewaters should be further analysed.

In addition, combination of basic and crushing processes could lead to the highest digestibility processes, even so, the effect of chemical adding (NaOH) and longer processing of grape stem should be considered from an economic and environmental point of view.

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