Optimization of bioethanol production from a sugar-rich liquor and a functional animal feed ingredient obtained in the biorefinery scheme of grape stem

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Introduction

The EU is the world's leading wine producer, with an average annual production of 167 million hectolitres (Eurostat 2019). Among the winery by-products, grape stems, pomace and lees are the most significant, with grape stems representing between 1.4% and 7.0% of the raw material initially processed.

Grape stems are rich in fibre, such as cellulose, hemicellulose and lignin, and in polyphenols, of great interest in animal nutrition. However, at present, grape stems are not valorized as highly profitable by-products and are sent for composting or disposed of in open spaces, causing a negative environmental impact.
In addition, at the destemming stage, part of the grape goes with the stems, resulting in a significant sugar content that is lost if not recovered. To avoid the loss of these sugars that could be used as a source for biotechnological applications and to facilitate the processing and drying of the grape stems within the biorefinery project, a cutting and washing process is proposed prior to the drying process of the solid part.

Within this framework, the present study proposes the following diagram to valorise grape stems to obtain a sugar-rich liquor for bioethanol production and an ingredient for animal feed.

Figure 1. Proposed diagram for valorising grape stems from wineries.

Material and methods

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

A preliminary washing process was 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 was characterized, and the production of bioethanol was optimized using fermentation processes (0,2 g/L *Saccharomyces cerevisiae* BO213, Laffort) at pH 5 and 28 ºC. Two optimization trials were carried out during 96 h at 50 mL volume combining different sugar and nitrogen concentrations to determine the better conditions for maximizing the bioethanol production. A final trial of bioethanol production under the optimized conditions was carried out in a 5 L stirred tank reactor (MINIFORS 2, Infors HT). The liquor (4.5 L) was inoculated with the yeast at pH 5, maintaining the temperature at 28 °C and under stirring at 150 rpm for 72 h.

In addition, having seen that the liquor is a source rich in sugars and with potential for bioethanol production, a test was defined to monitor the degradation of sugars present in the stems, throughout their collection in the wineries. The objective of this test was to assess the degradation and the production of the compounds present in the grape stems over time (10 days of storage) in order to define the optimal collection of

the by-product. For this purpose, grape stems were collected in 2 wineries in the Rioja Alavesa region, were stored in bags (8 kg of stems per bag) and incubated in temperature-controlled chambers simulating the night and day temperatures of La Rioja Alavesa between 1999 and 2019. Each day, samples were grinded and washed as previously defined in the grape stem washing processes (1:1 ratio of stem to water). Sugar analysis of the washing liquids obtained each day gave us information about the degradation suffered by the stems during storage in the winery.

The content of sugars of the obtained liquor was monitored during all the process by measuring the Brix° content and the reducing sugars using Dinitrosalicylic acid (DNS) assay. On the other hand, the nutritional value of the prototypes was measured by applying the Association of Official Analytical Chemists (AOAC) Official Methods. Ethanol production was determined using the Ethanol Assay Kit (Liquid Ready), based on the AOAC method 2017.07 (Megazyme).

Results and discussion

The liquor obtained after the washing of the grape stems showed a high initial sugar content (between 54 and 64 g/L). The data also showed a rapid consumption of the sugars, decreasing to 25 g/L (winey 1) and 13 g/L (winery 2) at 72 h of incubation. In addition, spontaneous ethanol was also produced during the incubation at controlled temperature, reaching 17 g ethanol/L after 168 h of storage at the winery. Bioethanol production optimization trials showed that the highest production was achieved using the liquor samples produced after the harvesting of the stems (highest sugar concentration), without any day of storage. Bioethanol production results showed a production yield close to 100%, obtaining bioethanol values greater than 30 g bioethanol/L.
At 40 g initial sugar/L the production values were close to 20 g bioethanol/L and in the case of the samples with 20 g initial sugars/L the production was of 12 g bioethanol/L. In the bioreactor, production peaks were again reached at 72 h with up to 32 g of bioethanol/L and yields close to 100%.

Regarding the functional ingredient from grape stems (Table 1), after washing and drying process, it has a residual sugar content (12 mg/g dry ingredient) and a content of interest in polyphenols (3%) with its associated antioxidant capacity (35 mg Trolox equivalents/g). Polyphenols can have a clear benefit in animal nutrition and therefore, confer a bioactivity to these ingredients. In terms of fibre concentration, the prototype has a high content of neutral detergent fibre (cellulose + hemicellulose + lignin). This means that its inclusion percentage in a concentrated feed cannot exceed 10% to avoid affecting its nutritional value.

Table 1. Chemical composition of dried grape stem-based ingredient.

Parameters	
Sugars (mg/g)	12.36
Polyphenols (mg/g)	30.55
Antioxidant activity (mg/g)	35.34
Protein (%)	5.66
Ash (%)	7.42
ADF (%)	50.95
Lignin (%)	22.36
NDF (%)	53.18
VFA (mmol/100 mL)	5.45

ADF: Acid detergent fibre; NDF: Neutral detergent fibre; VFA: Volatile Fatty Acids.

Conclusions

Grape stems are an available and cheap raw material with high potential to be valorised to obtain a sugar rich liquor and an ingredient for animal feeding.

They have a high sugar content due to the presence of a significant number of grapes and the defined washing process has been validated as an effective pre-treatment to obtain a sugar rich liquor with a great value in the food industry for bioethanol production.

The optimization of bioethanol production led to a final production of 30 g bioethanol/L, production yield close to 100%. Depending on the starting sugars, the final bioethanol production will be higher or lower and this can help to better define the collection periodicity and the associated business model.
The high polyphenols content of washed grape stems gives them a high potential to be used an alternative and functional ingredient for animal feeding. However, t its inclusion percentage in a concentrated feed cannot exceed 10% to avoid affecting its nutritional value due to the high fibre content. Even so, this 10% will reduce

dependency on other cereals while providing an ingredient rich in polyphenols with possible positive effects on animal health.

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