Improved Biorefinery Scheme for Winery Grape Pomace By-products

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INTRODUCTION

According to OIV (International Organization of Vine and Wine) the wine making industry produced in the EU around 175 mhl of wine in 2022, being Italy, France and Spain the main producers. Grape processing generates around 20 % of solid by-products, composed mainly of grape pomace (12%) stalks (4%) and lees (4%). In Spain most of the grape pomace and lees generated in red wine production are collected by distilleries to recover the ethanol that still remains in these by-products (about 5% d.w), generally by steam or hot water-drive systems.

This process presents a low yield, and most of the distilleries have to obtain additional products as tartrates or colorants to make the facility profitable. Exhausted grape pomaces are generally composted to obtain an organic amendment or dried to be used as fuel for energy recovery. The development of pre-treatments that could increase the bioethanol production is therefore of great interest. The aim of this study is to investigate the impact of several treatments including enzymatic and heat treatment on the release of reducing sugars that could be further fermented and, maximise ethanol production. In addition, the impact of these treatments on polyphenols extraction is evaluated. Finally, an integrative process for the valorisation of grape pomace is proposed where the main product is bioethanol and the co-products are: (i) polyphenol extracts from grape pomace and seeds, (ii) protein enriched fermented biomass.

METHODS

Grape pomace from red wine production was provided by a winemaking located in Alava (Spain) and a separation of fractions (peels and seeds) was carried out by sieving before storage at -20ºC. *Enzymatic treatments* were developed with a mixture of enzymes provided by Novozymes® and composed of Ultraflo XL, Viscozyme L, Saczyme L and Ultimase BWL 40 at a relative ratio of 1:1:1:2.6 in volume. Incubations were carried out for 24 h at 50 ºC and pH 4.6, at different concentrations. *Polyphenols* were extracted from previously separated grape peels and seeds (after grounding and drying) according to Maidin *et al*. (2018). *Solid state fermentation* tests with grapes pomace were carried out after different treatments through inoculation with *Rhizopus oryzae* (10%) and incubation of 10 gr (d.w) in Petri dishes, per triplicate, during 7 days at 28ºC and humidity between 65- 75%. *Analytical methods.* Total polyphenols by Folin-Ciocalteau method; total protein by Kjeldhal; anthocyanins by the pH differential method; reducing sugars by the DNS method and antioxidant activity by the DPPH method.
Treatments applied to grape marc were: incubation in hot water for 1 h a t 98 ºC (HW), heating for 1 h at 98 ºC without water addition (H), Polyphenols extraction (P), enzymatic treatment (E) and yeast fermentation (YF) for 4 days at 28ºC. Accumulative treatment steps were applied to determine their effect on polyphenols extraction and solid-state fermentation (SSF) processes and propose the most suitable bio-refinery scheme.

RESULTS AND DISCUSION

For **enzymatic treatment** tests, grape peels were first treated with hot water (HW) at 1:4 ratio (wet basis) to remove the remaining ethanolic fraction and further treated with several concentrations of enzymes mixture (from 0.3 to 6 %) also at 1:4 ratio. The release of reducing sugars clearly increased as higher enzyme concentrations were applied. The lack of HW pre-treatment reduced the sugar concentration in a 40%.

Table 1. Effect of enzyme concentration on reducing sugar release.

Enzymatic mixture concentration	Reducing sugars (g/kg d.w)	Expected ethanol production (%)
0	12.12 ±1.22	0.62
0.3	37.42 ± 4.61	1.93
1	78.73 ± 15.81	4.07
3	184.83 ± 5.26	9.56
6	341.64 ± 6.66	17.68
6 (not HW)	202.80 ± 1.05	10.49

The highest result of 176.8 gr/tn (d.w.) corresponds to 83.8 % of the theorical maximum production of ethanol from red wine pomace reported by Corbin *et al* (2015).

The effect of proposed treatments on **polyphenols extraction** was evaluated. As expected, the highest content of polyphenols was found in the fresh grape pomace (2,48% DW) with an anthocyanin concentration of 0.08% DW and 261.4 µmolTE/g DW. Results clearly indicate that all the treatments (HW, HW + E and HW+ E + YF) have a deleterious effect on polyphenol content and specially on anthocyanins (> 99% reduction). Total polyphenol content of separated seeds was 1.84 % DW and 178.3 µmol TE/g DW. In most distilleries, seeds are separated at the end of grape pomace processing for seed oil production. In this case, separation of peels and seeds from the very beginning was proposed to increase extraction yields (Fig 1). This allows to obtain two different extracts for food and/or cosmetics applications: (i) seeds derived (rich in proanthocyanidins) (ii) seedless pomace derived (rich in anthocyanins). The remaining seed biomass could be further valorized by oil extraction or directly become a flour for food/feed applications.

In order to improve the added value of the remaining grape pomace biomass after proposed treatments several **solid-state fermentation** trials were carried out using grape peels inoculated with *Rhizopus oryzae*. In this assay, a new treatment (H) was tested, to avoid sugar removal. Protein contents of initial and final samples were analysed to determine the most suitable treatment to obtain a protein rich ingredient of interest in animal feeding.

Table 2. Effect of several pre-treatments on protein concentration after SSF fermentation with *Rhizopus oryzae* on grape peels

Treatment	Protein increase (% d.w)
Control	25.15 ± 1.63
HW	19.34 ± 7.51
H	31.52 ± 2.37
P +H	39.34 ± 8.50
HW+ E	21.87 ± 7.99
HW+E+F	23.12 ± 6.57

Results indicate that polyphenols extraction followed by removal of ethanol by evaporation (H) seems to produce the highest protein increase on final products (from 10.2 to 14.2%). These results are still lower than those reported by Zepf and Jin (2013) where increments from 7% to 27% were obtained using *Aspergillus oryzae*. Further optimization of the process is needed to obtain standardized and improved results.

According to all the results, the biorefinery scheme proposed for grape pomace is presented in Fig.1.

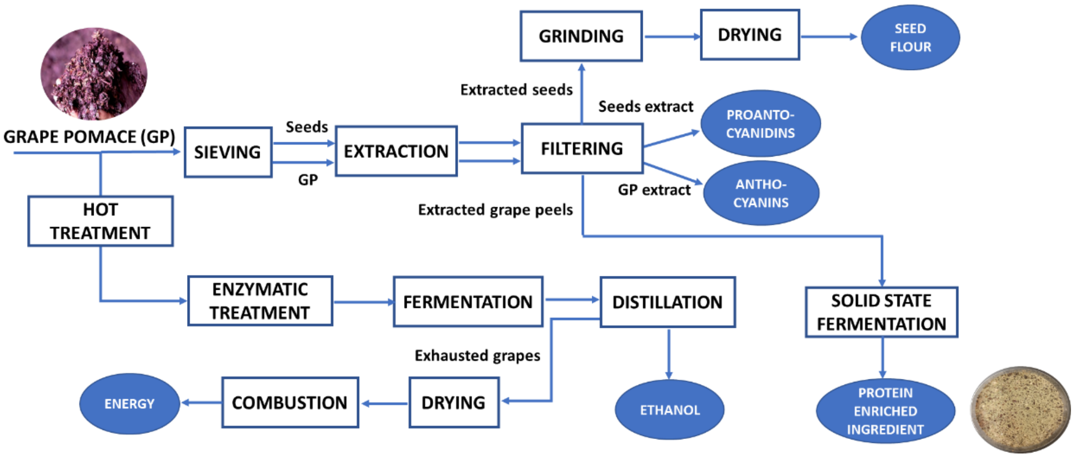

Fig.1. Grape pomace bio-refinery scheme proposal

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