Sustainable exploitation of wine lees for winemaking applications: a circular economy approach

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Grape consists a fruit that has been cultivated and consumed in numerous ways since ancient times. It is grown all over the world, with an annual (2021) production of grapes of 69.5 million tons (49% of which were wine grapes), while the wine production for that year was estimated at 26 billion liters (OIV, 2021). It is well known that the global wine sector is a market that is continuously expanding and is expected to reach a market value of 343.6 billion USD by the end of 2027 (Santos *et al*, 2022). As it can be deduced, extended cultivation of grapes and their further transformation into wine possesses a significant share of the agro-industrial economic pie.

The process of winemaking produces significant amounts of by-products, such as vine shoots, grape pomace and wine lees. Wine lees is a sludge material that can be described as the muddy residue that accumulates in vessels after fermentation or during storage of wine (EEC regulation No. 337/79). It contains mostly dead yeast cells, ethanol and organic acids, along with other valuable compounds, such as polyphenols (Jara-Palacios, 2019). It has been estimated that wine lees correspond to 2-6% of wine produced and 14-25% of total winery by-products (Dimou *et al*, 2016), whereas approximately 60 kg of wine lees are produced per ton of grapes crushed. In this context, 2.66 million tons of wine lees were accumulated during 2018 (Oliveira and Duarte, 2016). Traditionally, wine lees have been exploited for the extraction of their main components, while more novel applications include their use as a fermentation substrate for metabolites harvesting and supplement for cell growth (Kokkinomagoulos and Kandylis, 2020). Exploitation of such materials is an issue of vital importance to global good environmental governance, directly linked with environmental, economic and social impacts of life.

Yeast extract is a powder material that is widely used in the food industry, either directly as a flavoring agent in a variety of food products, for example, snacks, sauces and soups, or indirectly as part of culture media for the growth of useful microorganisms. It consists primarily of amino acids, peptides, nucleotides and other soluble components of yeast cells. For the manufacturing of yeast extract, cells are broken down, either by autolysis (through chemical or enzymatic hydrolysis of yeast cells) or cellular disruption, followed by concentration and drying protocols. Main source for the production of yeast extract in the industry is thought to be high-protein yeast species of the genus *Saccharomyces*, which are grown specifically for this purpose.

Due to its high concentration in yeast cells, wine lees appear as a promising alternative substrate for the preparation of yeast extract-type powders. In this frame, aim of the present study consists the valorization of wine lees, an agro-industrial by-product, through the production of a novel, low-cost, commercial yeast extract substitute. Specifically, the process of wine lees autolysis was optimized regarding temperature, pH and solid concentration. Moreover, the amino acid profile of autolysates was also investigated. In order to assess the efficiency of the autolysis process, free α-amino nitrogen (FAN) was determined in autolysates. In fermentation procedures, FAN is utilized as a nitrogen source for yeast growth, thus adequate concentrations are required for a successful fermentation (Duodu and Dowell, 2019). Chemically, FAN includes individual amino acids and short peptides that are produced by the hydrolysis of proteins (Taylor and Duodu, 2017), and therefore an increase in FAN can be utilized as a means of comparison for hydrolysis processes, e.g., autolysis. The produced powder will be used as a growth medium for lactic acid bacteria and yeasts, microorganisms that are utilized in winemaking, and the functionality of the novel yeast extract-type powder will be determined and compared to commercially available yeast extract.

Wine lees originating from white winemaking of Assyrtiko grapes were kindly provided by Ktima Gerovassiliou (Epanomi, Greece). Experimental process regarding the autolysis of wine lees is presented in Figure 1 (Dimou *et al*, 2015, with modifications). A Central Composite Design was applied using the statistical software Minitab (v. 15.1.20). The effect of temperature, pH and solid concentration on the efficiency of autolysis was investigated. Temperature ranged between 40 and 65 ºC, pH between 4.0 and 9.0 and solid concentration between 50 and 400 g/L. Efficiency of autolysis was calculated based on the increase of FAN found in the extract, which was determined through the ninhydrin spectrophotometric method. Analysis of amino acid profiles was performed on autolysates by HPLC. Yeast extract-type powder was obtained after autolysis under optimum conditions, concentration and freeze-drying of autolysates, and is expected to be used as a yeast extract substitute in culture media for the growth of lactic acid bacteria and yeasts.

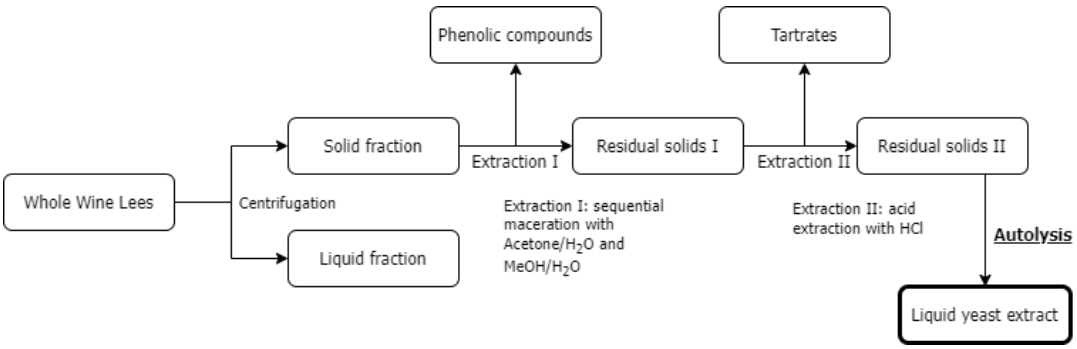

Figure 1. Autolysis experimental process.

Regarding autolysis experiments, a significant increase of FAN was observed in all cases, from 50.13 (before autolysis) to a maximum of 236.21 mg α-amino N/kg (after autolysis). Increase of pH and solid concentration appeared to negatively affect the efficiency of the process, with maximum efficiency being observed at pH 5 and solid concentration 120.94 g/L. The amino acid profile of autolysates was significantly affected by autolysis temperature, with higher temperatures promoting the release/formation of asparagine, alanine, proline, leucine and isoleucine, while also increasing total amino acid concentration (mg/kg wine lees). This finding can probably be attributed to the different proteolysis profiles during autolysis at higher temperatures.

The results of the present study appear promising towards the holistic revalorization of wine lees for their potential use as a growth medium for microorganisms, such as wine yeast. Therefore, this by-product can be utilized in the same industrial process from which they originate, namely winemaking, promoting the concept of circular economy and sustainable development for wineries. Additionally, the application of approaches based on the exploitation of industrial wastes is considered advantageous, as it is in agreement with the public consensus on the important benefits associated with environmental protection. In such manner, the ultimate goal should be the enhancement of the Greek winemaking industry's competitiveness by the development of feasible, marketable and environmentally friendly processes.

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