# Utilization of grape pomace for the production of microbial protein-A review

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**ABSTRACT.**

Grapes and their obtained products (wine, grape juice and raisins) constitute an economically important factor [19]. Grape production in Greece is estimated in 1.045.990 tones [13]. About 80% is used in wine making and 20% of the weight of grapes processed remains as pomace [19]. A large volume (200.000 tones) of winery wastes are produced with most of them remaining unexploited every year on an international level [13].

Grape pomace (GP) is the main waste in the wine industry and it consists of skin residues, broken cells with pulp remains, stalks, and seeds [33]. According to various analyses, grape pomace could be competitive with other typical agroindustrial wastes used as substrates in solid state fermentation processes [23, 25].

In this respect, the major aim of this research work is to explore possible ways for the use of winery wastes and more precisely for grape pomace. The objective is to develop value-added products through innovative technologies and more precisely through solid state fermentation, a promising microbial technology for primary and secondary metabolite production [31], in order to reduce the winery waste generation and disposal, provide further alternatives to diminish the environmental impact of winery activity and introduce additional sources of income.

More particularly, grape pomace of different grape varieties collected from wineries of Attica region were used as substrates. The substrates were inoculated with commercial strains of the mushroom *Pleurotus ostreatus*. The fermentation process was held in a solid state fermentation bioreactor, designed specifically for the needs of our research where conditions like temperature and pH were measured on a regular basis.

This research focuses on the utilization of grape pomace and on the production of microbial protein. The results of the study reveal a potential use in the food and animal feed industry.

## Introduction

During the wine making process, there are produced several by-products that are rich in biodegradable organic matter that can cause, if not treated correctly, potential environmental hazards. More precisely, inappropriate grape pomace disposal can attract flies and pests and can easily create unwanted hazards [8].

Moreover, utilization of wine by-products in combination with the production of sustainable wine can provide enough advantages in order to differentiate.

Vineyard and winemaking by-products contain valuable chemical compounds that are not extracted during wine making process.

The nutritional concentrations vary on the different type of each by-product (marc, pomace, grape seeds) and also on the different wine making process (white or red vinification). The starting point of the research is that all the by-products are rich in organic and they are able to provide the fermentation's necessary nutrient substrate.

Single Cell Protein (SCP)/microbial protein is one of the alternative protein sources that can supplement the conventional protein sources [27]. SCP refers to dead, dry microbial cells or total proteins that are extracted from pure microbial cell culture and is produced using a number of different microorganisms (fungus, algae and bacterium). SCP contains high protein, fats, carbohydrates, nucleic acids, vitamins and minerals [2, 18] and also essential amino acids like lysine and methionine that are not found in most plants and animals.

## Grape by-products

According to FAO (2014), 67.1 million tons of grapes were utilized in wine production in 2013. 20% of the weight of processed grapes is not found in the final product [24]. There are three main by-products derived from the wine making process, which are the stalks, grape pomace/marc and wine lees [8]. Stalks and grape pomace are the left-over from pressing grapes and wine lees (dead yeasts, yeast residue and other particles) is a material derived throughout the whole wine making process.

Grape by-products contain a respectful amount of organic components (sugars, phenolics, lipids, pectins) and are recognized as environmental pollutant [21].

In the recent years, there are various attempts to use those by-products not only as a way to protect the environment by minimizing their unwanted effects arising from their disposal but also as a way to provide an extra income to the wine industry. Grape by-products can be used as nutraceuticals, antioxidants, colorants, antimicrobials, animal feed, fertilizers, in combustion process, biomass for biofuels, medical remedies, cosmetics and natural ingredients to improve nutritional value of food [6].

## Grape pomace

Grape pomace is a major by-product of wine making, as it is equivalent to about 20% of the grapes used. It consists of pressed skins and pulp (10-12% of grapes), grape seeds (3-6%) and stems (2,5-7,5%) [40] and contributes to approximately 62% of the organic waste [28].

GP is a rich source of high value products including ethanol, tartates, malates, citric acid, grape seed oil, hydrocolloids and dietary fiber [40, 17, 7, 14].

Furthermore, GP has been found to be an excellent source of low-ash carbon whose extensive porosity [42]. The overall properties of GP has widen their utilization and supported the sustainable agricultural production [19] as long as the existence of phenolic phytochemicals, that are found in abundance in grapes, wines and wine by-products [6]. GP provides phenolic compounds as they share characteristics with anthocyanins in terms of solubility in waster and stability to temperature and oxygen [6].

GP is a good source of soluble fiber, with high antioxidant activity due to the natural presence of polyphenols and other bioactive compounds [37, 23, 12].Thus, GP and grape seeds extracts can be used in healthy food products such as yoghurt and salad dressing [39].

Sousa et al. [36] reported that the microbiological quality of GP can be consumed by humans due to its unfavorable conditions of low moisture and pH lower than 4.

According to Avantaggiato et al.[3], GP could remove in vitro a number of mycotoxins from liquid media, with the biosorbent efficacy in the order aflatoxin B1>zearalenone, ochratoxin A and fumonisin B1>>deoxynivalenol.

**Fermentation process**

Grape and wine by-products are a good source of carbon and have been used to generate various high-value products like as citric acid, lactic acid, gluconic acid, ethanol through submerged and solid state fermentation [6]. *Trichoderma harzianum, Aspergillus niger, Penicillium chrysogenum* and *P.citrinum* have been used in order to degrade winery biomass [20].

Fungi belonging to the division Ascomycota, such as *Trichoderma* spp., *Aspergillus* spp. and *Penicillium* spp., are known for their biomass degrading ability and should prove useful in grape biomass degradation. The fungi have been studied also for their ability to produce high levels of cellulose and hemicellulase degrading enzymes [20].

Protein rich products can be used as feedstock for animals. The protein content of grape marc has been managed to be increased from 7% to up to 27% in five days using solid state fermentation process, certain fungal strains and specific conditions like temperature and moisture content [43].

Submerged fermentation of grape waste using *Monascus purpureus* produced a red pigment to be used in food industry and the usage of *Lactococcus lactus* and *Lactobacillus pentosus* led to the production of lactic acid and *Trametes pubescens* to produce laccase [36].

Pleurotus ostreatus-complex is the third most important edible mushroom cultivated worldwide [32]. It can easily decompose lignocellulose without chemical or biological pretreatment as it possesses an enzymatic complex system that includes phenol oxidases and peroxidases [22]. Therefore, they can be utilized and recycled by solid state fermentation (SSF) using various strains of mushroom [16].

Solid-state fermentation (SSF) is defined as the fermentation involving solids in absence of free water. However, substrate must possess enough moisture to support growth and metabolism of micro-organism [31]. All the fermentation processes used in ancient time were based on the principles of SSF. Nowadays, SSF offers numerous opportunities in processing of agro-industrial residues.

It stimulates the growth of micro-organisms in nature on moist solids, it has lower energy requirements, it produces lesser wastewater and it is environmental-friendly as the problem of solid wastes disposal is solved.

The bioconversion of vineyard prunings and grape pomace by Pleurotus spp. with SSF was evaluated by measuring the fruiting body as alternative attempt for recycling of winery agroindustrial wastes [34].

**Microbial protein**

Grape pomace can be useful substrate for production of microbial proteins. The utilization of grape by-products in the production of Single Cell Protein will help in controlling pollution and also in solving waste disposable problem to some extent in addition to satisfy the world shortage of protein rich food [5].

SCP has high protein content (about 60-82% of dry cell weight), fats, carbohydrates, nucleic acids, vitamins and minerals. It is rich in certain essential amino acids like lysine, methionine which are limiting in most plant and animal foods [1]. This protein can be used as additive added to the main diet instead of sources known very expensive such as soya bean and fish [15].

Grape pomace waste with good nutrient content can be converted into food enriched with protein and feed and by properly utilizing them, will bring an end to the protein deficiency around the world. Furthermore, good nutritious food can be supplied with least expenditure of cost.

**Animal feed and health**

Some industrial uses currently under investigation for GP waste include use as animal feed, as possible nutrient ingredient for value added products, as natural pigment using the anthocyanins from grape skins [3, 26] and as a material for the production of alcoholic (distilled) beverages [11].

Pomace can be used as animal feed, especially in the dry season when pastures are scarce. High costs of handling and transportation usually limit the direct benefit of feeding animals with many agricultural by-products.

**Remarks and future research**

One of the most significant facts that needs research is in the field of physiological activities of phenolics. There are many studies that justify positive health effects, basically associated with the known antioxidative properties of GP. The sustainable exploitation of GP will be a useful strategy for wineries with the aim of reducing environmental contamination and as an alternative to reduce the carbon footprint in the whole production process. In this sense, simplified processes will be the choice with the aim of an easier scale-up as well as achieving a cheaper production.

A series of experiments are planned to be performed to obtain an optimized protocol for degrading winery biomass waste. Both submerged fermentation and SSF processes will be assessed using the fungi *Pleurotus ostreatus* .The fermentation process will be held in a solid state fermentation bioreactor, designed specifically for the needs of this research where conditions like temperature and pH were measured on a regular basis. Various analyses (temperature, pH, ash, total nitrogen) will be performed on both, the substrate and the fermented product.

With further research elements, the present study aims to prove that grape pomace have compounds with beneficial effects when correctly used as a substrate  allowing the valorization of winery by- products and produce value-added products.

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