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Grape (Vitis vinifera) Pomace as Natural Antimicrobial Agent

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Abstract

Grape (Vitis vinifera) is one of the most produced crops worldwide used mainly for wine production. Grape pomace is the solid waste produced after the alcoholic fermentation of wine. Grape and grape pomace content compounds with beneficial biological activities, such as phytosterols and phenolic compounds. Their concentration and profile of these compounds present great differences in different grape pomace, and this fact could be due to the use of different cultivars and varieties of the plants, differences in the edaphoclimatic conditions and differences in the extraction procedures used, for example. Terpenoid and phenolic compounds from vegetables and grape pomace have antibacterial and antifungal activities against several microorganisms. The predominant phytosterols in vegetables were β-sitosterol, campesterol, and stigmasterol. So, it is possible to reuse grape pomace as a natural antimicrobial agent. Even though, that extracting phenolic compounds or phytosterols will not solve totally the grape pomace problem, could be a partially solve by transform a waste to raw material, giving an environment friendly solution. 

Keywords: winery residues, grape pomace, valorization, polyphenols, phytosterols, antimicrobial activity.

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1. INTRODUCTION

Grape (Vitis vinifera) is one of the main cultivated crops worldwide. In 2017, at global level, the grape production was higher than 77,000 tones [1]. However, the grape is not used in its total for wine making, producing a great quantity of by-products (seeds, skin and pulp). Grape pomace is the most abundant by-product of wine-making. 

Approximately, half of the global production is used for wine-making, but this percentage varies among countries. Actually, approximately 20-30% (w/w) of the total grapes production destined to wine-making ends up as this organic residue, which represents about 9 million tons generated per year in the world [2].
The extracts from grape pomace by-products can present great differences on their content in active compounds. These differences can be explained due to the use of different cultivars and varieties of the plants, differences in the edaphoclimatic conditions in which the plants are grown and in the maturation state of the fruits and differences in the extraction procedures used [3].

In the case of a grape skin extract obtained by the same extraction process, from the same species Vitis vinifera but from a different cultivar, the catechin content is very different. For the grape cultivar Muscat Alexandria, the catechin content is 628 µg/g, but for the extract of the grape cultivar Gharra Shani, the catechin content is 945 µg/g [3, 4].

Phenolic compounds are secondary metabolites of plants, which are synthesized by plants and play important structural roles in the cell wall, act as a defense against UV light, protect against pathogen ingress and are involved in repair of injury. Many foods and beverages contain high levels of phenolic compounds, which often provide colour, taste, astringency and other sensory characteristics [5].

Chemically, phenolics can be defined as substances possessing an aromatic ring bearing one or more hydroxyl groups, including their functional derivatives [6]. Polyphenols are compounds that have more than one phenolic hydroxyl group attached to one or more benzene rings [7].

Phenolic compounds may affect growth and metabolism of bacteria [8, 9].

Peixoto et al., [10] tentatively identified twenty eight phenolic compounds (non-anthocyanin and anthocyanin) in the methanol/water (80:20, v/v) extracts prepared from grape pomace mixture, seeds and skins. The authors identified eleven flavon-3-ols (catechin and epicatechin derivatives and proanthocyanins), seven anthocyanins (malvidin, delphinidin, petunidin and peonidin derivatives), six flavonols (quercetin, laricitrin and syringetin).

Grape skins constitute the major component of grape pomace, containing the highest amounts of anthocyanins and tannins with a high polymerization degree [11, 12]. Grape skins are rich in hydroxycinnamic acids and especially in tartaric esters of these acids, mainly caftaric acid and coumaric acid [13, 14]. Phenolic content of seeds represent about 70% of total extractable polyphenolic compounds from grape pomace. Polyphenolics in grape seeds are mainly flavonoids, including flavan-3-ols and proanthocyanidin oligomers and polymers [15, 16]. The high phenolic content in grape seeds is due to the minimal proportion extracted during pressing in winemaking process.

Rodríguez-Vaquero et al., [18] reported the phenolic compounds concentration in Malbec, Merlot and Cabernet sauvignon Argentinean wines. The authors also demonstrated that polyphenols present in wines have antibacterial activity against Proteus mirabilis, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, S. aureus, listeria monocytogenes and E. coli [17, 18, 19].

Triterpenoids are compounds found in abundance in various plant tissues. Phytosterols comprise a complex class of natural compounds within the triterpene group [19], and are fundamental structural components of all plant membranes [20]. Phytosterols are both structurally and functionally resembling cholesterol, except for a distinctive structural methyl or ethyl group at the C24 carbon atom of their side chain [21]. All phytosterols present

Figure 1. Basical chemical structure of phenolic compounds.
one double bond at the C5 position and the saturation of this double bond can succeed either via enzyme reaction in vivo or by hydrogenation [19]. More than 100 types of phytosterols have been reported in plant species, but the most abundant are sitosterol (C-29), stigmasterol (C-29) and campesterol (C-28) [22, 23, 24, 25].

Ruggiero et al., [26] determined the presence and concentrations of \( \beta \)-sitosterol, stigmasterol and campesterol in grapes from Groppello cultivar. The presence of phytosterols in grape pomace from Malbec cultivars was reported by Sosa-Mármol et al. [27].

Phytosterol are well-known for cholesterol-lowering effects, and the structures and forms of phytosterols affect their bioactivity.

Some authors reported several biological activities such as antiinflammatory, antibacterial, antiviral, hepatoprotective, immunomodulatory, hypolipidemic, among others [28, 29, 30, 31, 32]. Sosa-Mármol et al., [27] determined the antibacterial effect of phytosterols from grape pomace against Xanthomonas citri sp. citri.

Since fruit by-products are considered food waste, their economic value is low. This, combined with their high biological activities, makes them a potential source of bioactive compounds to be included in nutraceutical and pharmaceutical compounds [33].

2. EXPERIMENTAL SETUP, RESULTS AND DISCUSSION.

2.1 Antibacterial and antifungal activities of phenolic compounds and phytosterols from grape pomace

Depending on the concentration, the effect of different phenolic compounds could be beneficial to growth of lactic acid bacteria [34] or inhibitory [35].

The effect of pure phenolic compounds against lactic acid bacteria was reported by several authors. Landete et al., [36], demonstrated that none of the nine phenolic compounds analyzed (cinnamic acid, p-hydroxybenzoic acid, hydroxytyrosol, oleuropein, protocatechuic acid, sinapic acid, syringic acid, tyrosol and vanillic acid) inhibit the growth of \( L. \) plantarum.

It was reported that the growth and metabolism of \( Lactobacillus \) hilgardii 5w a wine spoilage lactic acid bacteria, at concentrations normally present in wine of catechin and gallic acid, not only stimulated the growth rate but also resulted in greater cell densities during the stationary growth phase. But higher concentrations of phenolic compounds inhibited bacterial growth [37].

On pathogenic microorganisms the antibacterial activity depends on the phenolic compounds and of the strains tested [17, 38, 39]. Quercetin, rutin and caffeic acid was the most effective
compounds as antibacterial agent against *Serratia marcescens* *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Flavobacterium sp* and *Listeria monocytoes* [17, 18, 19]. The authors reported a decrease of two, one and half log cycles of *Listeria monocytoes* in presence of 500 mg/l caffeic acid, rutin and quercetin, respectively.

Some authors reported the antimicrobial activity of grape seed extracts, against several pathogenic and spoilage bacteria, such as *Bacillus cereus*, *Enterobacter aerogenes*, *Aeromonas hydrophila*, *Enterococcus faecalis*, *Escherichia coli*, including *E. coli O157:H7*, *Klebsiella pneumoniae*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Salmonella Enteritidis*, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica* [40, 41, 42].

The antifungal and antibacterial activities of olive leaf aqueous extracts against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* bacteria, *Candida Albicans* and *Candida neoformans* were reported by Pereira et al., [43]. Some studies showed the potential of grape pomace phenolic compounds to be used as preservatives, by preventing lipid oxidation and suppressing the growth of some bacterial strains, such as *Staphylococcus aureus*, *Streptococcus mutans* and *Escherichia coli* [44].

The antibacterial activity of methanol/water (80:20, v/v) extracts prepared from grape pomace mixture, seeds and skins was reported against Gram negative (*E. coli*, *Klebsiella pneumonia*, *Morganella morganii* and *Pseudomonas aeruginosa*) and Gram positive bacteria (*Enterococcus faecalis*, *Listeria monocytoes*, *methicillin-resistant Staphylococcus aureus* and *methicillin-susceptible Staphylococcus aureus*) [10]. The authors reported that the lowest minimum inhibitory concentrations (MIC) values were observed in seeds and against Gram-positive bacteria, *Enterococcus faecalis*, MRSA and MSSA (2.5, 5 and 5 mg/ml, respectively). For Gram-negative bacteria, seeds also exhibited the lowest MIC values against *K. pneumoniae* (10 mg/ml). The skins showed the poorest antibacterial activity, revealing MIC values > 20 mg/ml for almost all bacteria strains. They demonstrated that the antibacterial activity have a strong correlation with the presence of phenolic compounds.

Anastasiadi et al., [45] tested extracts of *Vitis vinifera* grape pomace (var. Mandilaria, Voidomato, Asyrtiko and Aidani) against *Listeria monocytoes*, and described that the seed extracts were significantly more antimicrobial than stems.

Antibacterial effect depends on the grape variety. White grape skin extracts presented lower MICs against both Gram-positive (*S. aureus* and *B. cereus*) and Gram-negative bacteria (*E. coli, Salmonella Infantis, Campylobacter coli*) than red ones [46].

Some authors had demonstrated the effect of terpenoids compounds from vegetable extracts as antimicrobial agent against several microorganisms. Burčová et al., [47] reported the antimicrobial activities of *β*-sitosterol against *Pseudomonas aeruginosa* and *Alternaria alternate*; Kumari et al., [48] demonstrated that *β*-sitosterol is present in stem of *Nyctanthes arbor-tristis* (Harsingar) and that the chloroform and ethyl acetate fractions possess antibacterial activity against *Xanthomonas citri*, *Pseudovorax sp.* and *Dickeya zeae*.

The presence of phytosterols, such as *B*-sitosterol and stigmasterol in grape pomace from *Malbec* cultivars was reported by Sosa-Mármol et al.,
Later, the authors reported the antibacterial activity against the etiologic agent of lemon canker. Candidiasis is an infection caused by yeast of the genus Candida. These eukaryotic microorganisms are part of the saprophyte microbiota of humans in the skin, mouth and mucous membranes of the female genitalia. The antifungal agents used for their treatment are imidazole derivatives, however at present a decrease in their effectiveness is observed, mainly due to the emergence of resistant yeasts. Perez-Merello et al., [51] demonstrated the antifungal activity of phytosterols in grape pomace from Malbec cultivars, against Candida albicans, and the possibility to reutilized natural compounds in this residue as antifungal agents in the treatment of candidiasis.

3. CONCLUSIONS

Phenolic fraction and fatty fraction of grape pomace have antibacterial and antifungal activities. So, it is possible to reuse a winery waste as a natural antimicrobial agent. Even though, that extracting phenolic compounds or phytosterols will not solve totally the grape pomace problem, could be a partially solve by transform a waste to raw material, giving an environment friendly solution.

4. REFERENCES


