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Review

CHITOSAN APPLICATION TOWARDS THE IMPROVEMENT OF GRAPEVINE PERFORMANCE AND WINE QUALITY

APLICAÇÃO DE QUITOSANO PARA MELHORAR A PERFORMANCE DA VIDEIRA E DA QUALIDADE DO VINHO

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SUMMARY

Intensification of agrochemicals application in vineyards has raised several concerns in Viticulture and Oenology value chain. Efforts have been developed to optimize grapevine health and productivity, assuring that viticulture is sustainable and competitive in today's wine market. Viticulture practices have constantly been improved for a more sustainable and environment-friendly production, reducing the application of agrochemicals, replacing them by natural compounds that can have a double effect: protect grapevine against pathogens and improve compounds related to grape organoleptic quality. In this context, the development and optimization of alternative strategies to improve and enhance plant defences and grape/wine quality is becoming a necessity. Since the 1980s, chitosan has become a compound of special interest due to its double effect as elicitor and grapevine biostimulant, representing a complement to soil fertilisation, and reducing the negative effects nutrients leaching into the groundwater. The present review aims to present the wide possibilities of chitosan applications on grapevines to prevent and combat the main diseases and to improve wine quality. In this way, relevant studies about chitosan application will be presented as well as some concerns and limitations in order to cover the knowledge gaps inherent to its application in vineyard and wine as well.

RESUMO

A intensificação da utilização de agroquímicos na vinha tem aumentado as preocupações na cadeia de valor da Viticultura e Enologia. Têm sido desenvolvidos esforços no sentido de otimizar a sanidade e produtividade da vinha, assegurando a sustentabilidade e competitividade da viticultura no mercado vitivinícola atual. As práticas culturais têm sido constantemente melhoradas para uma produção mais sustentável e amiga do ambiente, reduzindo a aplicação de agroquímicos, substituindo-os por compostos naturais que podem ter um efeito duplo: proteger a videira contra agentes patogénicos e melhorar os compostos relacionados com a qualidade organolética da uva. Neste contexto, o desenvolvimento e otimização de estratégias alternativas para melhorar cada vez mais as defesas das videiras e a qualidade da uva/vinho tem-se tornado uma necessidade. Desde a década de 1980, o quitosano tornou-se um composto de especial interesse devido ao seu duplo efeito, como elicitador e bioestimulante, representando um complemento à fertilização do solo, diminuindo alguns efeitos negativos devido à lixiviação de nutrientes para as águas subterrâneas. O presente artigo revisão pretende apresentar as possibilidades de aplicação do quitosano na videira, para prevenir e combater as principais doenças bem como para melhorar a qualidade do vinho. Desta forma, serão apresentados estudos relevantes sobre a aplicação de quitosano bem como algumas preocupações e limitações sobre a sua utilização, a fim de colmatar as lacunas no conhecimento inerentes à aplicação deste composto na vinha e no vinho.

Keywords: Chitosan, grapevine biostimulant, grape and wine quality, grapevine phytosanitary status, sustainable viticulture.
Palavras-chave: Quitosano, bioestimulante da videira, qualidade da uva e do vinho, estado fitossanitário da videira, viticultura sustentável.

INTRODUCTION

Viticulture is one of the most economically relevant agricultural activities in the world. In 2019, 7.4 million ha of cultivated vineyard were registered worldwide, similar to the cultivation area of 2018, despite different regions varying heterogeneously (OIV, 2020). Slightly more than 50% of grape production in 2020 (OIV, 2021) was centred in Spain, China, France, Italy and Turkey, including table and dried grapes, and wine (Table I). European vineyard area seems to have stablished at 3.3 million ha, however, an heterogenous behaviour has been reported between countries (OIV, 2021). In recent years, chitosan has gained special attention by the

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Table I

Five grape producing countries with more than 50% of vineyard cultivation in the world, including table and dried grapes, and wine (OIV, 2021)

	2019	2020
Spain	13.1	13.1
China (mainland)	11.5	10.9
France	10.7	10.7
Italy	9.6	9.8
Turkey	5.9	5.9
% of vineyard area	50.8	50.4

scientific community since it has demonstrated efficacy as plant biostimulant in vegetables, ornamentals, and fruit crops, promoting fruit quality and plant protection (Pichyangkura and Chadchawan, 2015; Pandey *et al.*, 2018). Several authors have reported the activity of chitosan as a phytosanitary product, especially in organic viticulture (Heloir *et al.* 2019). Recently, the elicitation mechanism of secondary metabolites on fruit crops has risen the interest of application to improve berry and wine quality (Vitalini *et al.*, 2011; Tessarin *et al.*, 2016; Singh *et al.* 2019; Silva *et al.*, 2020). The application of plant biostimulants has been considered a sustainable strategy (Zheng *et al.*, 2020) for crop growth and development enhancement, quality improvement, resistance to pathogenic organisms and abiotic stresses, and also suited for food industry due to its low-allergenicity, biocompatibility and biodegradability (Hamed *et al.*, 2016; Cheba 2020). Moreover, chemical properties of chitosan have allowed its extended role in vineyards and wine cellars (du Jardin, 2015).

Chitosan is a biopolymer, which can be obtained from animal sources, extracted from the exoskeleton of insects and crustaceans, or from fungi, such as *Mucor rouxii* Calmette, *Absidia glauca* Hagem, *Aspergillus niger* Tiegh., *Gongronella butleri* (Lendn.) Peyronel & Dal Vesco, *Pleurotus sajor-caju* (Fr.) Fr., *Rhizopus oryzae* Went & H.C. Prinsen Geerligs, *Lentinus edodes* (Berk.) Pegler, and *Trichoderma reesei* Simmons (Philibert *et al.*, 2017; reviewed by Huq *et al.*, 2022).

The interest in chitosan arises from its several applications in different fields such as medicine and agri-food chains (Kim *et al.*, 2008; Kabanov and Novinyuk, 2020; Zheng *et al.*, 2020). Its versatility and interest are also related to its biocompatibility, safety, biodegradability, sorption performance of radionuclides and heavy metals (Alves and Mano, 2008; Kabanov and Novinyuk, 2020). Chitosan possesses multiple bioactivities such as antioxidant, antiviral and antitumor along with antimicrobial, which has become of major interest for food and agriculture among other industries (Qin and Li, 2020).

When applied to crops, chitosan has been noticed to affect cell growth and slightly inducing *trans*-resveratrol synthesis in grapevine (Laura *et al.*, 2007) as well as in other crops (Franco and Iriti, 2007; Harding and Sashiwa, 2015). Several works reported the application of chitosan in vineyards as elicitor, promoting plant resistance against several pathogenic related microorganisms, and improving grape and wine quality, thus with extreme importance in viticulture and oenology (Barka *et al.*, 2004; Nge *et al.*, 2006; Vitalini *et al.*, 2011; Tessarin *et al.*, 2016; Singh 2019; Silva *et al.*, 2020).

The present review aims to focus on research works concerning the application of chitosan in vineyards and/or grapes as well as its effect on different grapevine cultivars as growth promotor, grape and wine quality enhancer, and as a phytosanitary agent, fitting important aspects to Viticulture and Oenology sector.

Chitosan classification and regulation

Extreme meteorological events have been more frequent due to climate change, leading to a demand for research in new mitigation strategies for crops, which is one of the top priorities for 21st century Europe's agricultural sector (Costa *et al.*, 2019). Furthermore, the need to improve crop productivity and efficiency as well as to reduce environmental impact on ecosystems and human health is of high priority (Rouphael and Colla, 2020). The application of pesticides and fertilisers has been a common practice to overcome these unfavourable conditions to agriculture, however it brings serious concerns to agriculture sustainability as well as for public and environmental health (Rouphael and Colla, 2020). Plant biostimulants arise as a possibility to overcome adverse abiotic factors, stimulating plant growth and development, crop production and nutrient use efficiency (Calvo *et al.*, 2014), thus improving the organic farming practices (du Jardin, 2015).

Accordingly to European Union definition, published in 2019, plant "biostimulants shall be an EU fertilising product the function of which is to stimulate plant nutrition processes independently of the products nutrient content with the sole aim of improving one or more of the following characteristics of the plant and plant rhizosphere: i) nutrient use efficiency, ii) tolerance to abiotic stress, iii) quality traits, or iv) availability of confined nutrients in the soil or rhizospfere" (Rouphael and Colla, 2020).

The classification of a biostimulant has been discussed, and constantly redefined by diverse authors over the years. In a recent work, Rouphael and Colla (2020) classified plant biostimulants based on agricultural function as (i) humic and fulvic acids, (ii) animal and vegetal protein hydrolysates, (iii) microalgae seaweed extracts and (iv) silicon. In this context, microorganisms are also included as plant biostimulants, namely (i) arbuscular mycorrhizal fungi and (ii) N-fixing bacteria (e.g., *Rhizobium* spp., *Azotobacter* spp. and *Azospirillium* spp.).

The application of chitosan hydrochloride has been approved by EU (Commission Implementing Regulation EU No 563/2014) in accordance with Regulation (EU) No. 1107/2009 as a plant protection product to enhance plant resistance against pathogenic fungi and bacteria in fruits, berries and small fruit, vegetables, cereals, spices, crops for animal feed, seed treatment (cereals, potatoes, sugar beet), ornamental bulbous plants and beet crops. Moreover, chitosan treatment in vineyards is restricted to foliar spraying application, from leaf to fruit development stages, between 50-200 g/hL and 200-400 L/ha, and a maximum of eight treatments each campaign, with two weeks interval.

Chemistry of chitosan

Chitosan is a linear biopolymer composed of repeated monomeric units of D-glucosamine and N-acetyl glucosamine. It is a chitin derivative, obtained by deacetylation of N-acetyl group of D-glucosamine, converting acetamide into an amine group (Aranaz *et al.*, 2021). In comparison with chitin, the interaction between deacetylated groups and hydrophilic compounds allow chitosan to be more soluble in acidic solutions than chitin (Hamed *et al.*, 2016; Kabanov and Novinyuk, 2020). Chitosan can be obtained from animal sources through chitin deacetylation at high temperature and pH, extracted from the exoskeleton of insects and crustaceans, or from fungal sources (Kaur and Dhillon, 2014; Kabanov and Novinyuk, 2020). Molecular weight, acetylation and polymerization degrees influence its chemical properties, such as solubility in water and biological eliciting capacity (Kauss *et al.*, 1989; Harding and Sashiwa, 2015). Supporting this, applications of chitosan monomers (D-glucosamine and N-acetyl-glucosamine) did not affect cell growth and have slightly induced *trans*-resveratrol synthesis, in grapevine (Laura *et al.*, 2007) as well as in other crops (Franco and Iriti, 2007; Harding and Sashiwa, 2015).

Chitosan applications

Foliar application of chitosan has become an effective strategy to prevent plant diseases and to enhance grape quality. Moreover, the application of plant biostimulants can have a positive secondary effect on soil fertility. Nevertheless, the effect of biostimulants in vineyards depends on the compounds and their bioactive potential, concentration, and frequency of applications as well as grapevine cultivars (Gutiérrez-Gamboa *et al.*, 2017).

Chitosan forms a semi-permeable film around plant tissues, acting as a physical barrier, that gives protection against the growth of several pathogenic fungi, and also induce host-defence responses, reducing conidial germination and mycelial growth (Romanazzi *et al.*, 2006). Chitosan also has potential for long-lasting protection of harvested grapes (El Ghaouth *et al.*, 1994; Romanazzi *et al.*, 2002), even though it may reduce the bioavailability of nutrients, causing nutrient deprivation to the pathogens deposed on grapevine leaves (Barka *et al.*, 2004).

The following sub-sections present the possibilities of chitosan applications in vineyards and/or grapes as well as its effect on different grapevine cultivars as a growth promoter, grape, and wine quality enhancer, and as a phytosanitary agent to prevent and combat the main grapevine diseases.

Effects on grapevine phytosanitary status

Plant diseases are of main concern for all crops, responsible for several economic and nutritional crises, affecting growth, development, yield, and fruit quality (Burketova *et al.*, 2015). The main diseases of grapevine are caused by viruses (e.g., grapevine leafroll-associated viruses, grapevine fanleaf virus), fungi (*Botrytis cinerea*, *Erisyphe necator*, and complex of fungi associated to grapevine trunk diseases), oomycetes (*Plasmopara viticola*) and phytoplasmas ('Bois noir' and 'Flavescence dorée'). Chemical control of grapevine diseases depends on the use of synthetic agrochemicals such as succinate-dehydrogenase inhibitors (SDHI), quinone outside inhibitors (QoI), quinone inside inhibitors (QiI), strobilurin and carbamates, with a severe negative impact on vineyard ecosystem (such as soil and beneficial insects) and on human health (farmers and consumers) as well (Burketova *et al.*, 2015). Due to these negative impacts, alternative and sustainable approaches are needed. Alternatives include organic and integrated viticultural practices, biological control, use of tolerant cultivars and application of natural compounds, generically called as elicitors. Elicitors are organic biostimulants that trigger plant defence reactions, by mimicking pathogen's attacks, through activation of defence mechanisms that stimulate plant immune system, by the synthesis of secondary metabolites mainly phenolic and volatile compounds (Gutiérrez-Gamboa *et al.*, 2017; Gutiérrez-Gamboa and Moreno-Simunovic, 2021).

Since the 1980s, chitosan has been used for crop farming as biopesticide, biofertilizer, for seed coating formulation, and agricultural film, demonstrating a positive effect against bacteria, fungi, and nematodes (Héloir *et al.*, 2019). It is worthy of special attention because of its use as an elicitor by inducing systemic acquired resistance (Iriti and Varoni, 2015). Chitosan effect is caused by a defence response triggered by chitin receptors recognition (Chen and Xu, 2005; Kaku *et al.*, 2006; Petutschnig *et al.*, 2010). Since plants lack an immune system that surveys the entire organism, as in animals, each cell possesses its own defence mechanisms, releasing chemical signals upon ligand recognition. Thus, plant biostimulants consist of two types of receptors: pathogen associated molecular patterns (PAMP's), including chitosan, and damage-associated molecular patterns (DAMP's). The recognition of specific molecular patterns triggers a defence response by chitin elicitor binding protein (CEBiP) and chitin elicitor receptor kinase 1 (CERK1) receptors that changes cellular metabolism, which is dependent on the chitosan biopolymer molecular weight and deacetylation degree (Zheng *et al.*, 2020). Defence signal leads to intracellular changes in cytosolic Ca^{2+}, increasing enzymatic activity, phytohormones (such as abscisic, jasmonic and salicylic acids) synthesis and pathogen-related proteins accumulation (Iriti *et al.*, 2009). After application, chitosan is deposited in the surface of leaves surface, triggering a hypersensitive response through the binding to a specific receptor in the plant cell surface, activating genetic, biochemical, and morphological/anatomical responses. After recognition, chitosan activates transcriptional factors, leading to the octadecanoic pathway activation, production of pathogenesis-related (PR) proteins, phytoalexins and salicylic acid (SA), the accumulation of nitric oxide (NO) and hydrogen peroxide (H_2O_2), and activation of several oxidative stress enzymes, such as polyphenol oxidase (POX), glucanases and chitinases (Walters *et al.*, 2007; Burketova *et al.*, 2015). All these genetic and biochemical responses change normal plant physiology by cell wall reorganization, callose deposition and wavy thylakoids (Walters *et al.*, 2007).

Regarding the effect on viruses, it is described that chitosan activates resistance against several local and systemic viral infections in host plant species, such as pea (*Pisum sativum*), tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*) (Iriti and Varoni, 2015). Although some studies report chitosan application against viral diseases, so far there are no studies that have proved its effect on grapevines infected with viruses.

Fungi and oomycetes are some of the most important grapevine pathogens. Among them, *B. cinerea*, *E. necator*, the oomycete *P. viticola* and the complex of fungi associated to grapevine trunk diseases are responsible for the most significant damages in grapevines (Ferreira *et al.*, 2004). Chitosan activity against filamentous fungi, yeasts and Gram-positive and Gram-negative bacteria is well-documented. Chitosan antifungal activity mainly affects mycelial growth, sporulation, germination and morphology of spores and hyphae (Cárdenas-Triviño *et al.*, 2018). Xu and colleagues (2007) have reported that cationic characteristic of oligochitosan may have the capacity to disrupt endomembrane of several fungi, with more efficacy on *Phytophtora capsici*.

Grey mould (Botrytis cinerea)

Grey mould, also known by botrytis bunch rot, is an important grapevine disease worldwide caused by the necrotrophic anamorphic fungus *B. cinerea* (Pers.: Fr), which affects grapevine and grapes, leading to high yield losses and berry quality decreases (Elad, 1994; Jacometti *et al.*, 2010). Canopy management and prophylactic use of fungicides are the most common control methods. However, the application of fungicides has only been partially successful due to emergence and establishment of resistant *Botrytis* spp. strains, impacts on human and environmental health, and their residues in wine (Barka *et al.*, 2004; Jacometti *et al.*, 2010).

To study preventive and curative potential of chitosan preparations, these compounds and their different derivatives have been used in both *in vitro* and *in vivo* research studies. Barka *et al.* (2004) have used *in vitro* cultured plantlets of cv. 'Chardonnay' grown on chitogel-supplemented medium. Chitogel-supplemented medium or chitogel-free medium (as foliar treatment) demonstrated to be efficient to promote plant growth and, to a certain extent, to increase tolerance against grey mould (Barka *et al.*, 2004). Using the same plant culture system, Trotel-Aziz *et al.* (2006) showed that low molecular weight oligochitosan induced protection in grapevine leaves against *B. cinerea*. This study has shown that necrotic lesions caused by post-infection were significantly reduced when leaves were pre-treated with a solution of chitosan 75 mg/L and suppressed by a concentration of 150 mg/L. In laboratorial conditions, chitosan inhibited germination and growth of *B. cinerea* in liquid culture at concentrations higher than 125 mg/L and suppressed grey mould on detached grapevine cv. 'Chardonnay' leaves and bunch rot in commercial wine grapes (Reglinski *et al.*, 2010). De Bona *et al.* (2021) confirmed the fungistatic and filmogenic properties of chitosan in potted plants. During the first days after treatment, chitosan acted as a physical barrier to fungal attack and inhibited its growth. That study showed that grapevines cv. 'Merlot' treated with chitosan, with a molecular weight of 173 kDa and a degree of acetylation of 17%, before *B. cinerea* infection protected grapevines' leaves against the pathogen. Moreover, chitosan induced grapevine defence mechanism, triggering gene expression, and leading to the induction of jasmonic acid and ethylene-mediated response and the accumulation of phytoalexins such as *trans*-resveratrol.

In vineyard, crab-shell chitosan 1% was applied once (21 days before harvest) or twice (21 and 5 days before harvest) to grapevines and bunches of table grapes cv. 'Italia' (Romanazzi *et al.*, 2002). In this study, chitosan had the same ability to protect the grapes from postharvest grey mould as the strategy based on the application of synthetic fungicides, with storage in the presence of sulphur-dioxide-releasing pads. Similar trials were conducted in California (USA) in cv. 'Thompson Seedless', comparing different chitosan formulations, highlighted the elicitor activity on the berry tissues and effects on the properties of the bunches after storage (Feliziani *et al.*, 2013). Another formulation of chitosan – chitosan ethylcarbamate 2% – proved to be efficient against *in vitro* cultures of *B. cinerea* isolated from table grapes, presenting a minimum inhibitory concentration (MIC) of 1250 mg/L (Cárdenas-Triviño *et al.*, 2018). Reglinsk *et al.* (2010) demonstrated that chitosan spray treatments from bunch closure until 2 weeks preharvest decreased 7.4% botrytis bunch rot in cv. 'Chardonnay' comparing with decreases of 15.5% and 5.9% in untreated and conventional fungicide treatment, respectively. In cv. 'Sauvignon blanc' chitosan and conventional treatments reduced botrytis bunch rot severity to 4%, which was significantly lower from the untreated control (11.5%) (Reglinski *et al.*, 2010).

Due to its harmless activity on human health and environment, chitosan was studied as an alternative to the application of conventional chemical preservatives (such as sulphur dioxide, SO_2) during table grapes' long-distance transport. Applications of 0.1, 0.5 and 1.0% crab-shell chitosan (w/v) demonstrated beneficial effects in controlling pre- and post-harvest decay, inducing significant reductions on grey mould storage rot in a two-year experiment. On the other hand, preharvest application of chitosan did not show significant differences to procymidone and SO_2 treatments and it did not also affect naturally occurring microflora of yeast and yeast-like fungi (Romanazzi *et al.*, 2002). Romanazzi *et al.* (2006) reported that preharvest application of 1% chitosan significantly reduced grey mould incidence and severity on cvs. 'Thompson Seedless', 'Autumn Black', and 'Emperor' up to 88%, despite its effect decreased with time after chitosan application increased. Nevertheless, combined application of chitosan and UV-C (254 nm) on cv. 'Autumn Black' and 'selection B36-55' presented a synergistic effect reducing grey mould incidence and severity up to 90% and blue mould (*Penicillium* sp.) incidence as well (Romanazzi *et al.*, 2006). Meng and Tian (2009) demonstrated that a combined treatment of 1 g/L chitosan and *Cryptococcus laurentii* applied on preharvest table grapes of cv. 'Jingxiu' stored at 0 ºC significantly reduced grey mould decay with a concomitant improvement of fruit quality. A recent study revealed that preharvest treatment of chitosan conjugated with salicylic acid (CTS-*g*-SA) is an excellent tool to improve quality and postharvest life of table grapes cv. 'Youngyou' (Shen and Yang, 2017), decreasing grey mould severity by diminishing lesion diameter in coated berries, when compared with chitosan applied alone or combined with SA. Moreover, combined effect of chitosan with ethanol, organic and inorganic acids, and antagonistic yeasts to control postharvest grey mould on table grapes have been already explored (Romanazzi *et al.*, 2007; Meng and Tian, 2009; Romanazzi *et al.*, 2009a). In fact, the combination of 0.5% chitosan with 10 or 20% ethanol improved decay control compared to their single treatments, on single berries stored 7 days (at 15 ± 1 ºC) and on small clusters stored 60 days (0.5 ± 1 ºC) up to 97% and 94%, respectively (Romanazzi *et al.*, 2007). Particularly, on small clusters, combined treatment of chitosan with 10 or 20% ethanol reduced grey mould incidence by 47% and 60% in cv. 'Thompson Seedless', and 70% and 94% in cv. 'Autumn Seedless', respectively, compared to untreated controls.

Downy mildew (Plasmopara viticola)

Besides its filmogenic capacity, chitosan also presents antifungal activity due to its capacity to interact with phospholipids of fungus membrane, destroying them (Romanazzi *et al.*, 2009a). Therefore, chitosan has become a promising tool to combat biotrophic pseudo-fungi and fungi, like downy mildew and powdery mildew, respectively.

Downy mildew is caused by the obligative biotrophic oomycete *P. viticola*, which is a great concern worldwide, especially where grapevines suffer with high humidity and abundant rainfall in the spring. It is characterised by the presence of oil spots on the surface of leaves and white sporulation that can be seen on the bottom page of the leaves, canes, and bunches in periods of high humidity, causing a significant impact on yield if control measures are not accurately implemented (Gessler *et al.*, 2011).

Crab-shell chitosan oligomers at 200 µg/mL have promoted plant defence reactions and significantly reduced infection severity in *in vitro* leaves of explants of grapevine cv. 'Chardonnay' clone 7535, triggering the accumulation of secondary metabolites, such as phytoalexins, *trans*- and *cis*-resveratrol and their derivatives, ε-viniferin and piceid (Aziz *et al.*, 2006). Romanazzi *et al.* (2021)

demonstrated that chitosan could be a good alternative product to copper to control downy mildew on cv. 'Montepulciano', especially in organic farming. This study also shown that, under low disease pressure, solutions of chitosan 0.5 and 0.8% reduced significantly downy mildew incidence compared with untreated control plants.

Powdery mildew (Erisyphe necator)

Powdery mildew, caused by the biotrophic ascomycete *E. necator*, is another fungal disease, which is of great concern in viticulture worldwide. Usually, phytosanitary control of this grapevine disease consists on the application of several synthetic agrochemicals and sulphur, which have a great impact on environmental and human health (Iriti *et al.*, 2011; Héloir *et al.*, 2019).

Recently, it has been shown that a mixture of chitosan and oligogalacturonides (COS-OGA) protects grapevines cv. 'Carignan' against powdery mildew (van D'Abruzzo *et al.*, 2014). Moreover, Iriti *et al.* (2011) demonstrated that foliar application of a commercial formulation of chitosan not only has protected grapevines cv. 'Montepulciano' against powdery mildew but also improved total polyphenol content and free radical scavenging potential in both grapes and wine. Despite the slightly lower activity as fungicide (of about 2.39%), compared to a synthetic product (0.92%), chitosan formulation showed to be a promising anti-fungal agent to be applied with synthetic compounds (Iriti *et al.*, 2011). Although the application of commercial formulation of chitosan has been effective in the treatment of powdery mildew infection comparatively to penconazole and methyldinocap (Iriti *et al.*, 2011), further studies are required to demonstrate its capacity to prevent fungus sporulation and efficiency to prevent powdery mildew regardless of disease pressure.

Grapevine trunk diseases

Grapevine trunk diseases (GTD) are also of great concern for viticulturists and nurserymen worldwide and effective control strategies are not yet available for many of them. GTD have many associated fungi, such as *Botryosphaeriaceae fungi* (dieback and cane blight), *Phomopsis* sp. (Phomopsis cane and leaf spot), *Eutypa lata* (eutypa dieback), black foot disease associated fungi, such as *Campylocarpon* sp., *Cylindrocladiella* sp., *Dactylonectria* sp., *Ilyonectria* sp., and *Thelonectria* sp., *Phaeomoniella chlamydospore* (Petri disease and esca), *Phaeoacremonium minimum* (Tul & C. Tul) and *Fomitiporia* sp. (esca decline) (Nascimento *et al.*, 2007; Matei *et al.*, 2009; Bertsch *et al.*, 2013; Gramaje *et al.*, 2015; Gramaje *et al.*, 2018).

Some studies have been carried out in order to better understand the role of chitosan in GTD's management. Two studies highlighted that application of 0.5 % chitosan revealed an effective inhibition of mycelial growth of grapevine wood fungi (Nascimento *et al.*, 2007; Matei *et al.*, 2009). Nascimento *et al.* (2007) demonstrated that chitosan applied on potted grapevines cv. 'Castelão' significantly reduced *Ne. liriodendri* and *Pa. chlamydospora* up to 32.05% and 3.30%, respectively, compared to commercial fungicides as carbendazim+flusilazole, cyprodinil+fludioxonil and tebuconazole. Moreover, chitosan showed an inhibitory effect on mycelial growth of the main GTD fungi, as a significant reduction was observed in *Botryosphaeria* sp. (EC_{50} 1.56 mg/L), *E. lata* (EC_{50} 3.26 mg/L), *Pa. chlamydospora* (EC_{50} 1.17 mg/L) and *Fomitiporia* sp. (EC_{50} 1.53 mg/L) growth (Nascimento *et al.*, 2007). This significant reduction of *Ne. liriodendri* and *Pa. chlamydospora* was accompanied by the enhancement of grapevine growth. However, no statistically significant differences were observed between fungicides and chitosan (Nascimento *et al.*, 2007). Cobos *et al.* (2015) reported that low molecular weight chitosan was more effective than medium- and high-molecular weight ones to control GTDs pathogens. Chitosan combined with garlic extract and vanillin reduced the infections of *D. seriata* and *Pa. chlamydospora* artificially inoculated onto treated pruning wounds. Moreover, that mixture revealed itself to be effective under field conditions, leading to lower mortality rate of vines and low percentages of re-isolation of the pathogens (Cobos *et al.*, 2015). This study contributed to the development of new tools and strategies to manage and combat GTDs in organic viticulture. A recent study conducted by Buzón-Durán *et al.* (2021) tested the efficacy of chitosan oligomers-amino acid conjugate (viz. cysteine, glycine, proline, or tyrosine) complexes against three fungal species belonging to the *Botryosphaeriaceae* family, both *in vitro* and *in planta*, revealing that *in vitro* assays led to EC_{50} and EC_{90} between 254.6-1498.5 µg/mL, depending on the amino acid involved in the conjugate complex and on the pathogen assayed. It was also observed a synergistic effect between chitosan oligomers and the amino acids against *D. seriata* and *Botryosphaeria dothidea*. The bioassay performed in potted plants have proven that the chosen formulations induced a significant decrease in disease severity against *Neofusicoccum parvum* and *B. dothidea* (Buzón-Durán *et al.*, 2021).

Despite the new approaches that have been developed using chitosan (free or in conjugated with other natural compounds), more studies are needed to validate and strengthen this new promising treatment.

Phytoplasmas

Phytoplasmas are phloem-limited prokaryotic organisms, evolved from Gram-positive bacteria, without cell wall, and which cannot be cultivated in axenic culture (Weisburg *et al.*, 1989). 'Bois noir' and 'flavescence dorée' are the most important grapevine phytoplasma diseases in Europe, leading to severe yield losses (Dermastia *et al.*, 2017). Plants infected with phytoplasmas develop leaf rolling, leaf yellowing or reddening (depending on berry colour), stunted growth, unripe cane wood and shrivelled berries, impairing plant yield (Ahmed *et al.*, 2016). The severity of these symptoms depends on the grapevine cultivar, plant vigour, presence of other pathogens and degree of infection (Zahavi *et al.*, 2013). Up to now there are no efficient methods to eradicate these pathogens; the strategies to contain infection dissemination are the uprooting of infected plants, preventive hot water treatment of plant propagation material, insecticide treatments against vectors, and the use of phytoplasma-free propagating material (Oliveira *et al.*, 2020). However, some studies using elicitors to trigger grapevine defences in order to fight against phytoplasmas and eradicate them inside the plant were carried (Oliveira *et al.*, 2019a,b). So far, there is only two studies using chitosan against "boir noir" in cv. 'Chardonnay' (Romanazzi *et al.*, 2009b; Romanazzi *et al.*, 2013). However, since chitosan is a biopolymer that produces a protection film against pathogens, its application on phytoplasma-infected grapevines had no significant effects compared with most powerful compounds such as benzothiadiazole, glutathione supplemented with oligosaccharines, methyl jasmonate or salicylic acid (Oliveira *et al.*, 2019a,b). Nevertheless, more studies should be performed to better understand the role of chitosan on triggering defence mechanisms activation in grapevines infected with phytoplasmas or even the protection of grapevines against phloem-feeding insects that can be vectors of these diseases.

Effects on grapevine physiology, grape, and wine quality

The application of chitosan as an elicitor of plant systemic defence mechanism, by mimicking pathogens attack, was firstly thought as a substitute to environmental harmful agrochemicals, to promote a sustainable agriculture. Elicitation mechanisms are not fully understood and can be triggered by physical (e.g., low or high temperatures) or chemical (e.g., chitosan, benzothiadiazole, methyl jasmonate) stimuli, that prompt plant defence responses with synthesis of secondary metabolites such as polyphenolic compounds (Ruiz-García and Gómez-Plaza, 2013). This effect has raised the interest of the food industry that needs high quality and sustainable products for consumer demands. Wine industry faces prominent commercial challenges, such as the demand for eco-friendly products from biological and organic viticulture, keeping the high quality of wine organoleptic features.

In the following sub-sections, the biostimulant activity of chitosan in grapevine physiology and grape/wine quality will be discussed.

Enhancer of grapevine physiology

Chitosan application has shown activity as a growth biostimulator in plants (Barka *et al.*, 2004; Nge *et al.*, 2006). In potted grapevines, cv. 'Castelão' infected with *Ne. liriodendri*, a microorganism responsible for black foot disease, chitosan oligossacarin (Gofar Agro) had efficacy of a synthetic fungicide, and enhanced plant height and number of shoots (Nascimento *et al.*, 2007). In cv. 'Chardonnay' plantlets, 1.75% (v/v) chitosan improved *in vitro* vegetative growth, increased root and shoot biomass, stem length and number of nodes (Barka *et al.*, 2004), which might be related to the improvement of photosynthetic parameters, increasing oxygen production and carbon dioxide fixation. Romanazzi *et al.* (2006) determined that net photosynthesis was reduced after plant treatment with a solution of 0.8% of chitosan, caused by reduction of stomatal conductance, leaf area and leaf dry weight without impact in the production and juice quality (Romanazzi *et al.*, 2006).

Most studies report the positive effect of chitosan in grapevine vigour and growth rate (that is, root and shoots), which may be related photosynthesis (oxygen production and carbon dioxide fixation) (Barka *et al.*, 2004; Nge *et al.*, 2006; Nascimento *et al.*, 2007). In addition, the fact that chitosan showed potential as anti-transpiring agent in beans (Iriti *et al.*, 2009) may be of interest for agricultural sector, especially as a mitigation strategy for climate change. However, it is important to understand if this effect is also present in grapevine.

Enhancer of grape and wine quality

The relationship between wine and society dates back thousands of years. OIV reported that global wine trade reached 29.6 billion EUR in 2020 (OIV, 2021) highlighting the weight of wine business in the world economy. Wine quality and its acceptance by consumers is closely related to the organoleptic features. Phenolic and volatile composition of grapes are the most important players in red wine expression through aroma, colour, bitterness, astringency, and flavour (Garrido and Borges, 2013).

Climate change and associated climate instability (e.g., hot waves, extreme raining, high thermic fluctuations) can compromise grape production, as viticultural sector is extremely vulnerable to weather conditions. In this context, it is of utmost importance to define new strategies to face the consequences of adverse climatic events, maintaining or even improving product quality while being environmentally friendly. Elicitors/biostimulants application may be a promising strategy by acting as phytochemicals and improving fruit quality, contributing to wine quality while being more sustainable than the agrochemical in use. Herein, the findings of chitosan treatment on grapevine cultivars, namely in phenolic, volatile and nitrogenated compounds, according to European Union mission and directives to achieve a more sustainable and competitive agri-food industry, will be reported.

Oenological Parameters

Wine quality control protocol at harvest time measures the pH, ºBrix, probable alcohol, total acidity (g/L tartaric acid) and malic acid concentration. Quality parameters are measured before, during and after fermentation as a general quality control and characterisation in the winemaking process. Treatment with chitosan demonstrated to not interfere with grapevine productivity and berry weight (Garde-Cérdan *et al.*, 2017), having a slight effect, however not significant, on chemical oenological parameters such as probable alcohol (Portu *et al.*, 2016, Tessarin *et al.*, 2016, Gutierrez-Gamboa *et al.*, 2017), pH (Portu *et al.*, 2016, Gutierrez-Gamboa *et al.*, 2017), total acidity (Portu *et al.*, 2016; Gutierrez-Gamboa *et al.*, 2017), tartaric acid (Portu *et al.*, 2016, Gutierrez-Gamboa *et al.*, 2017), malic acid (Portu *et al.*, 2016, Gutierrez-Gamboa *et al.*, 2017) lactic acid (Portu *et al.*, 2016) and color intensity (Portu *et al.*, 2016). Despite these variations in the wine parameters, sensorial analysis of chitosan treated wines were generally more accepted by consumers (Vitalini *et al.*, 2014), which may suggest that grape metabolism is slightly altered comparatively to untreated grapevines.

Phenolic composition

Phenolic compounds belong to an heterogenous group of secondary metabolites produced in plants. It is estimated that 20% of the fixed carbon by photosynthesis leads to the synthesis of flavonoids and stilbenes (Pereira *et al.*, 2009). Phenolic compounds include phenolic acids, flavonoid and non-flavonoid molecules, tannins, stilbenes and coumarins, which are biosynthesized via phenylpropanoid metabolic pathway (Ferreira and Antunes, 2021), may be found in vegetables, fruits as raw or processed products such as wine, fruit juices and other plant derived industrial products (de la Rosa *et al.*, 2018). Elicitation by chitosan was reported to activate phenylalanine-ammonia liase (PAL) enzyme, therefore, contributing to increased phenolic composition of grapes (Boss *et al.*, 1996, Kobayashi *et al.*, 2002, Chen *et al.*, 2006, Reglinsky *et al.*, 2010, Zhao *et al.*, 2016) in cvs. 'Cabernet Sauvignon' (Duxbury *et al.*, 2004; Tessarin *et al.*, 2016), 'Montepulciano' (Iriti *et al.*, 2011), 'Gropello', 'Merlot' (Vitalini *et al.*, 2011), 'Tempranillo' (Portu *et al.*, 2016), 'Tinto Cão', 'Touriga Franca' (Singh *et al.*, 2019; Singh *et al.*, 2020), and 'Sousão' (Silva *et al.*, 2020). Altogether, these studies suggested that chitosan treatment, alone or in combination with other phytochemicals, induced a differentiated response in grape phenolic composition of the abovementioned cultivars, as discussed below. Moreover, phenolics play a wide range of functions in plant defence mechanisms, signalling and gene induction (Dixon *et al.*, 2002).

Weekly foliar application of chitosan solution in cv. 'Montepulciano' resulted in the accumulation of total phenolics by 19% in epidermal tissues and 22.5% in seeds. Phenolics were accumulated in grape structures after chitosan treatment, with a slight decrease in total phenolic composition in wines from chitosan-treated grapes, while the conventional fungicides caused a higher decrease of those compounds (Iriti *et al.*, 2011).

Higher concentration of (+)-catechin, (-)-epicatechin and procyanidin B2 (epicatechin dimer) were registered in grapes of organically managed cv. 'Cabernet Sauvignon' after treatment with Kaitosol® in three development stages – pre-v*eraison*, post-*veraison* and preharvest (Tessarin *et al.*, 2016). Flavanols (or flavan-3-ols) family of flavonoids includes catechin and its enantiomer epicatechin as well as gallocatechin, epigallocatechin, and their 3-*O*-gallates and polymers (de la Rosa *et al.*, 2018). Catechins are of special interest due to its role in flavour, contributing to bitterness. When polymerized, they originate tannins, contributing to wine structure, texture, and ageing potential (Garrido and Borges, 2013).

Singh *et al.*, (2020) also reported the increase of catechin content in grapes of cv. 'Tinto Cão' after chitosan treatment. In addition, quercetin-2-*O*-galactoside, rutin and several monomeric anthocyanins were found in higher concentration as response to the upregulation of PAL genes, induced by chitosan treatment in grapes and leaves.

Anthocyanins belong to another flavonoid family and are considered the main components for wine colour. They may also form adducts with tannins and co-polymerize, resulting in brownish pigments associated with wine ageing (Garrido and Borges, 2013). Singh *et al.* (2020) also reported distinct responses in grape cultivars after the treatment with chitosan: 'Touriga Franca' cultivar showed higher levels of total phenolic composition and total tannins, while the effect of chitosan in cv. 'Tinto Cão' was extended to anthocyanins synthesis, as well.

Polyphenols in leaves have a distinct effect of chitosan in red grapevine cv. 'Touriga Franca' registered a decrease when reaching maturation while the opposite response was observed for cv. 'Tinto Cão' (Singh *et al.*, 2019). Although in general chitosan promoted total polyphenol accumulation in berries, grapes of cv. 'Tinto Cão' had higher antioxidant activity, while no differences were observed in cv. 'Touriga Franca', reinforcing the varietal effect. Moreover, tannin composition increased in seeds and general phenolics accumulated in green structures such as stems and leaves (Singh *et al.*, 2019). This study also showed that chitosan application triggered genes that encode enzymes of the reactive oxygen species (ROS) pathway, such as iron-superoxide dismutase, copper-zinc-superoxide dismutase, catalase, glutathione reductase, glutaredoxin, respiratory burst oxidase, amine oxidase, peroxidase, and polyphenol oxidase (Singh *et al.*, 2019). In addition, Vitalini *et al.* (2011) reported the eliciting effect of chitosan and chitosan-copper treatment on biosynthesis of phenolics compounds in cvs. 'Gropello' and 'Merlot', respectively.

Tessarin *et al.* (2016) reported no effects on grape and wine phenolic composition in organically managed cv. 'Sangiovese' after chitosan treatment, unlike cv. 'Cabernet Sauvignon'.

In addition, Duxbury *et al.,* (2004) reported no significant changes in total phenolics and anthocyanins in grape skins, total phenolics and catechins and proanthocyanins in grape seed extracts, and total phenolic composition of wine after foliar spraying of chitosan since fruit set phenological stage to harvest in cv. 'Cabernet Sauvignon'. Noteworthy, the formulations used in both studies were not the same; Duxbury *et al.* (2004) sprayed a 150 mg/L chitosan solution in leaves with 3 weeks interval while Tessarin *et al.* (2016) studied the effect of a 125 g/L of chitosan (Kaitosol®), which could be indicative that the formulation (concentration and coadjutants) in chitosan solutions might also influence the elicitation mechanism.

The fact that different formulations (Vitalini *et al.*, 2011) caused different effects in phenolic synthesis and accumulation on distinct cultivars, together with the different effects reported in cv. 'Sangiovese' and 'Cabernet Sauvignon' (Tessarin *et al.*, 2016) strongly suggest that chitosan biostimulant activity is cultivar dependent.

Other than cultivar specific responses, chitosan solution formulation seems to influence chitosan elicitation mechanism. In fact, foliar spraying of chitosan nanoparticles and chitosan solution in cv. 'Sousão' resulted in grape extracts (skin, seeds, and stems) with different polyphenol contents. Although chitosan nanoparticles did not show any significant effect on polyphenol synthesis, a decrease in total phenolics and total tannins was found in all bunch structures with no effect on anthocyanins synthesis. Therefore, it is important to consider a possible effect of degree of polymerization of chitosan and the acetylation pattern on the elicitation mechanism (Das *et al.*, 2015). Chitosan alone was noted to increase phenolics in bunch structures, but only with significance in the stem. Although chitosan did have a small eliciting effect, the results suggest a differentiated effect dependent on the chemical formulation of the compound (Silva *et al.*, 2020).

In addition, no major phenolics (anthocyanins and flavonols) were elicited by chitosan in cv. 'Tempranillo' except for epicatechin-3-*O*-gallate in grapes, which was significantly reduced, while no changes were reported in wines. However, wines showed lower content in vitisins A and B (Portu *et al.*, 2016). Table II summarizes the abovementioned studies, detailing the effect of chitosan treatments in different grapevine cultivars, the aims of studies, chitosan composition and number of applications.

In order to contribute to wine competitiveness in international markets and, at the same time, to a more sustainable viticulture and resilient sector to climate change in the upcoming years, chitosan-based products are likely to be a promising strategy as a substitute of synthetic agrochemicals. In addition, by eliciting secondary metabolism and accumulation of phenolics in some red grape cultivars, chitosan may be used as a grape quality enhancer. However, there is still the need to better understand which cultivars may be prone to chitosan effect, product formulations, the number of treatments and coadjutants and method and timing of application that better conduct to a desired response.

Nutraceutical effect

Grape and wine are rich in antioxidant phenolic compounds and are considered nutraceutical food products by some researchers (Iriti and Faoro, 2009). Phenolic compounds have different biological activity in living organisms, which rose interest in their study by scientists of the food and health industries (Pereira *et al.*, 2009; de la Rosa *et al.*, 2018). Bioactivity of phenolic compounds in living organisms includes antioxidant, anticancer, antimicrobial activities, among other positive health benefits, therefore nutraceutical value is of interest to health and food industries, including wine sector. Vitalini *et al.* (2011) demonstrated that treatment of cvs. 'Gropello' and 'Merlot' with chitosan and chitosan-copper increased antioxidant activity in wines. Another chitosan-copper treatment tested by Iriti *et al.* (2011) increased free radical scavenging activity in cv. 'Montepulciano' grapes. Antioxidants inhibited ROS production during cellular metabolism, preventing damage of biochemical structures, and providing a protective role of cell viability.

In table grapes cv. 'Thompson Seedless' higher content in quercetin, myricetin and *trans*-resveratrol were found after treatment with ChitoPlant® (Feliziani *et al.*, 2013). Indeed, quercetin and *trans*-resveratrol are two powerful antioxidants in wine with beneficial health effects as cardioprotective, anticancer, anti-diabetic, neuroprotective and anti-ageing properties, which strengthen the 'French paradox' (Carollo and Caimi, 2012; Fernández-Mar *et al.*, 2012). A study carried out in cell suspension culture of *Vitis vinifera* cv. Barbera showed that chitosan treatment elicited *de novo* synthesis or accumulation of stilbene synthases, and thus increased the accumulation of *trans*-resveratrol (Ferri *et al.*, 2009). Romanazzi *et al.* (2006) found that chitosan combined with UV radiation increased catechin and *trans*-resveratrol content in cv. 'Autumn Black' table grapes, while the same treatment led to an accumulation of *trans*-resveratrol in green grape 'selection B36-55' (Romanazzi *et al.*, 2006). Melatonin is another nutraceutical compound

Table II

Summary of biological effect of different chitosan formulations in *Vitis vinifera*

Grapevine cultivar/ Region	Research purpose	Matrix	Biological effect	Mode of application	Formulation	Reference
'Cabernet Sauvignon' (Italy)	Organic composition	Grape and wine	**Grape and wine:** higher concentration of (+)-catechin, (-)-epicatechin and procyanidin B2, coutaric and fertaric acids **Grape:** increase (~50%), in amino acids and amines - Ala, Arg, Cys, Leu, Ile, Ser, GABA, ETA, PUTR, Ammonium ion	3 applications: beginning and end of veraison and preharvest, in shoots and bunches **Quantity:** 600 L/ha	Kaitosol® (12,5 g/L chitosan)	Tessarin *et al.* (2016)
'Cabernet Sauvignon' (New Zealand)	Total phenolic composition	Grape and wine	No effect on total phenolic composition	Since fruit set to harvest (every 3 weeks) **Quantity:** 100 mL/per vine	150 mg/L chitosan in pH 5.5 acetate buffer	Duxbury *et al.* (2004)
'Gropello' (Italy)	Nutraceutical	Wines	Increase of melatonin, total phenolic and antioxidant potential	Since grape susceptibility to fungi infections until veraison (every 10 days), dependent on the meteorological conditions **Quantity:** 800-1000 L/ha	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.03% m/v chitosan	Vitalini *et al.* (2011)
'Gropello Gentile' (Italy)	Aromatic composition	Wines	High concentration of esters and alcohols Volatile aroma of wines varied between years in chitosan treatments	Since grape susceptibility to fungi infections until veraison (every 10 days), dependent on the meteorological conditions **Quantity:** 800-1000 L/ha	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.03% m/v chitosan	Vitalini *et al.* (2014)
'Merlot' (Italy)	Nutraceutical composition of wines	Wines	Increase of total phenolic composition Chitosan-copper lead to higher antioxidant potential	Since grape susceptibility to fungi infections until veraison (every 10 days), dependent on the meteorological conditions **Quantity:** 800-1000 L/ha	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.03% m/v chitosan	Vitalini *et al.* (2011)
'Montepulciano' (Italy)	Protective agent (downy mildew)	Grape must	Oenological parameters of must were not affected Decrease of amino acid content	Since mid-May until the end of July (12 weekly treatments per year). **Quantity:** 1000 L/ha	0,03% m/v chitosan (99.9%) + 0.05% Boron + 0.05% Zinc Chitosan specifications: DA 85%, MW 76 kDa	Garde-Cérdan *et al.* (2017)

Table II (continuation)

Summary of biological effect of different chitosan formulations in *Vitis vinifera*

Grapevine cultivar/Region	**Research purpose**	**Matrix**	**Biological effect**	**Mode of application**	**Formulation**	**Reference**
'Montepulciano' (Italy)	Protective agent (Powdery mildew)	Grape (skin, flesh, and seeds) and wine	Antifungal effect **Grape and wine: i**ncrease of total phenolics and higher antiradical activity comparing with wines made from conventional fungicide treated grapes	Since May to August (every 7 days), dependent on the meteorological conditions **Quantity:** 800-1000 L/ha	Kendal Cops® - 4% chitosan solution with 1,5% Cu^{2+}, 0,5% Mn^{2+} Chitosan specifications: DA 85%, MW 20-30 kDa	Iriti *et al.* (2011)
'Sangiovese' (Italy)	Organic composition	Grape and wine	Increase of PUTR in grapes.	3 applications: beginning and end of veraison and preharvest, in shoots and bunches **Quantity:** 600 L/ha	Kaitosol® (12,5 g/L chitosan)	Tessarin *et al.* (2016)
'Sousão' (Portugal)	Nutraceutical activity	Stems, seeds, and skins	**Grape extracts:** small increase in polyphenols, antimicrobial, and antioxidant activity Chitosan nanoparticles decreased in total phenolic and total tannins and no changes on total anthocyanin in skin extracts	Two treatments spaced by 16 days	Chitosan specifications: DA 85%, MW 76 kDa; fungal chitosan Concentration: - 0.01% (m/v) chitosan in 0.01M acetic acid - 0.001% (m/v) chitosan nanoparticles in 0.01M acetic acid	Silva *et al.* (2020)
'Tempranillo' (Spain)	Elicitor of phenolic content	Grape and wine	**Must:** slight decrease of potassium and epicatequin-3-*O*-gallate; no effect on anthocyanins and flavonols **Wine**: lower content of vitisin A and B	Foliar spraying at veraison and 1 week later **Quantity:** 400 mL/plant	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.01% (m/v) chitosan in 0.01M acetic acid	Portu *et al.* (2016)
'Tempranillo' (Spain)	Effects of chitosan application in musts	Grape must	Decrease of potassium and amino acids: Gln, Glu, Ala, Ser, Thr, Lys, Arg No effect on YAN	Foliar spraying at veraison and 1 week later **Quantity:** 400 mL/plant	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.01% (m/v) chitosan in 0.01M acetic acid	Gutiérrez-Gamboa *et al.* (2017)
'Tinto Cão' (Portugal)	Genes of ROS pathway, antioxidant activity	Grape (seed, skin), leaves, stems, and shoots	**Leaves:** increase of polyphenols **Berries:** increase of total polyphenols, anthocyanins, and tannins **Skins:** increase of antioxidant activity Up-regulation of ROS enzymes genes: *Fe-SOD*, *CAT*, *GR*, *GRx*, *Rboh*, *AO*, *POD*, *PPO* in **all tissues**, excepting for *APX* and *GRx* in **stems**.	2 treatments: at veraison and berry ripening **Quantity:** 200 mL/plant	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.1% (m/v) chitosan in 0.01M acetic acid	Singh *et al.* (2019)

Table II (continuation)

Summary of biological effect of different chitosan formulations in *Vitis vinifera*

Grapevine cultivar/Region	Research purpose	Matrix	Biological effect	Mode of application	Formulation	Reference
'Tinto Cão' (Portugal)	Genes of phenylpropanoid metabolic pathway and phenolic composition	Grape berries and leaves	Increase of monomeric anthocyanins, catechin, rutin, quercetin-3-*O*-glucoside Up-regulation of *PAL*, *CHS*, *F3H*, *ANR*, *UFGT*, *ABCC1*, *GST* and anthocyanin transporter *MATE1*	1 treatment at beginning of veraison	Chitosan specifications: DA 85%, MW 76 kDa; fungal chitosan Concentration: 0.01% (m/v) chitosan in 0.01M acetic acid	Singh *et al.* (2020)
'Touriga Franca' (Portugal)	Genes of ROS pathway, antioxidant activity	Grape (seed, skin), leaves, stems, shoots	**Berries skin**: increase of total polyphenols and tannins with a slight decrease in total anthocyanins at maturation; no effect on antioxidant activity **Leaves:** decrease of total polyphenols at maturation. Up-regulation of ROS enzymes genes: *Fe-SOD*, *CAT*, *GR*, *GRx*, *Rboh*, *AO*, *POD*, *PPO* in **all tissues**, excepting for *APX* and *GRx* in **stems**.	2 treatments: at veraison and berry ripening **Quantity:** 200 mL/plant	Chitosan specifications: DA 85%, MW 76 kDa Concentration: 0.01% (m/v) chitosan in 0.01M acetic acid	Singh *et al.* (2019)

YAN - Yeast Assimilable Nitrogen; **DA** - Deacetylation degree; **MW** - Molecular Weight. **Amino acids (AA): Gln** - Glycine, **Glu** - Glutamine, **GABA** - Gamma aminobutyric acid, **Ala** - Alanine, **Ser** - serine, **Thr** - Threonine, **Lys** - Lysine, **Arg** - Arginine, **Cys** - Cysteine, **Ile** Isoleucine. **PUTR** - putrescine (**biogenic amine**). **Phenylpropanoids metabolic pathway genes**: p*henylalanine ammonia lyase* gene - *PAL, chalcone synthase - CHS, flavanone 3-hydroxylase - F3H*, anthocyanidin reductase - *ANR, UDP-glucose: flavonol 3-O-glucosyl transferase – UFGT*. Anthocyanin transporter genes: *ABCC1, MATE1, glutathione S-transferase - GST*. **ROS pathway genes**: iron-superoxide dismutase - *Fe-SOD, copper-zinc-superoxide dismutase - Cu/Zn-SOD, catalase - CAT, glutathione reductase - GR, glutaredoxin - Grx, respiratory burst oxidase - Rboh, amine oxidase - AO, peroxidase – POD, polyphenol oxidase - PPO, ascorbate peroxidase - APX.*

of interest with phytoprotector activity against pathogens in plants and nutraceutical for humans. Vitalini *et al.* (2011) found that chitosan treatments led to an increase of 22% of melatonin in cv. 'Gropello' wines while cv. 'Merlot' wines suffered the highest increase of melatonin (about 15%) in chitosan-copper treatment. In addition, anti-microbial and antioxidant activity were also higher in chitosan treated grapes (Singh *et al.*, 2019; Silva *et al.*, 2020).

The elicitation of secondary metabolism and phenolics accumulation by chitosan is proven to induce accumulation of bioactive compounds, which is of major importance considering the health benefits that moderate wine drinking may have for consumers. This characteristic of wine may be used as an argument in marketing strategies. Nevertheless, the same concerns on the formulation, cultivar dependence, as previously mentioned, on the elicitation mechanism are topics that require further research.

Volatile composition

Wine aroma is of the most important organoleptic features for consumers. Since aroma in wine result from the interaction of volatile molecules between them and wine matrix, the effect of chitosan in aromatic and sensorial profile of wines is of interest for wine industry players. Cv. 'Gropello Gentile' wines, made from chitosan treated grapes, had higher fraction of volatiles, mostly alcohols and acetals, when compared to wines made from conventional fungicides treated grapes. Although treatment with chitosan-copper did not significantly affect wine volatile profile, they were preferred by the tasting panel when compared to wines obtained by conventional fungicide treated grapes (Vitalini *et al.*, 2014). Additionally, grapes of cv. 'Tempranillo' exhibited more volatile compounds belonging to C6 group and less concentration of terpenoids, with exception of limonene and *p*-cymene, benzenoids, except dihydro-β-ionone, and C13 norisoprenoids (Gutiérrez-Gamboa *et al.*, 2019). These compounds play an important role in grape primary aroma, expressing the typicity of a specific *terroir*, which is important in wine typicity and distinctiveness.

Wine aroma results from the sum of many variables, such as grape cultivar, cultural practices, soil, and climate, together with oenological practices and chemical reactions during ageing. In fact, chitosan eliciting effect of secondary metabolism does target aromatic composition. However, the abovementioned observations may suggest that there are several variables involved in the chitosan effect, namely, grapevine cultivar, solution formulation, among others. In addition, elicitation of secondary metabolism may redirect nutrients to phenolics biosynthesis, compromising the synthesis of other aromatic compounds. Considering these hypotheses and evidence, as well as the lack of published studies regarding the volatile fraction in chitosan treated wines, it is of the utmost importance to clarify the effect of chitosan treatment in aromatic and sensory profile of grapes and wines. It should also be noted that none of the reported studies have referred defects related to chitosan odour.

Nitrogen sources composition

Primary sources of nitrogen in musts come from grape both in organic and inorganic forms. Yeast assimilable nitrogen (YAN) comprises the forms of nitrogen that yeast assimilates and involves organic (amino acids with exception of proline) and inorganic (ammonium ion, NH_4^+, and ammonia, NH_3) sources. Therefore, nitrogen composition of must is essential in winemaking to avoid unnecessary prophylactic addition of ammonium sulphate and to prevent sluggish fermentations that may ultimately lead to microbial proliferation and must spoilage (Bell and Henschke, 2005).

Gutiérrez-Gamboa *et al.* (2017) have reported a decrease of 17% in amino acid content of must when chitosan was applied at veraison and one week later, however, no changes were found in YAN. In contrast, the depletion of amino acids in grape must was reported in cv. 'Montepulciano' for both chitosan and chitosan-copper after ten chitosan treatments (Garde-Cérdan *et al.*, 2017). Since chitosan stimulates PAL activity and the reaction cascade that follows, leading to phenolic accumulation, this mechanism response may compromise plant metabolism due to the consecutive synthesis of secondary metabolites (Barbosa *et al.*, 2008). Grapes of cv. 'Cabernet Sauvignon' also showed decreases in several amino acids, while YAN increased. Contrastingly, in cv. 'Sangiovese' no effects were observed in amino acids content, besides accumulation of putrescine (Tessarin *et al.*, 2016).

As mentioned before, amino acids are part of primary metabolism, essential to plants, and play an important role in yeast nutrition during fermentation. Usually, 140 mg/L of nitrogen is considered enough for fermentation to occur (Bell and Henschke, 2005). Some studies suggest that the elicitation mechanism induced by plant biostimulants may have physiological costs for plant metabolism by the continuous stimulation on the absence of a pathogen, which may explain the decrease in amino acids and effect on some aromatic molecules, as previously discussed (Dietrich *et al.*, 2005; Barbosa *et al.*, 2008). Although most studies report no effect on YAN, the qualitative content of amino acids seems to be negatively influenced by chitosan treatments.

Tessarin *et. al.* (2016) reported contradictory evidence concerning the amino acid profile of chitosan treated musts in two different grapevine cultivars. So, there is still the need to understand the extent of secondary metabolism over-activation and the impacts in primary metabolism. Therefore, in order to elucidate the benefits of chitosan for wine industry, more research in this field is necessary to fully understand the extent of biochemical responses of this compound and its real impact on primary metabolism.

CONCLUDING REMARKS

The present review focused on the main advantages, drawbacks, and knowledge gaps of chitosan application to grapevine as elicitor, triggering defence mechanisms, or as biostimulant, improving plant physiology, grape, and wine quality. Chitosan has proven to be a suitable natural compound with special characteristics related to its biocompatibility, safety, biodegradability, sorption performance and multiple bioactivities as well as a promising biocontrol agent.

Chitosan application in vineyards revealed to be a promising approach as phytosanitary treatment against the main causal agents of grapevine diseases, such as botrytis bunch rot and downy mildew. Nevertheless, some studies should be carried out to better demonstrate the role of chitosan on grapevine diseases caused especially by powdery mildew, GTD, viruses and phytoplasmas, and unravel the mechanisms behind chitosan application under vineyard conditions.

Chitosan elicits defence mechanisms that stimulates phenylpropanoid metabolic pathway, but the extent of its effect seems to be cultivar dependent. Disclosure of the mechanisms of chitosan elicitation may be the first step to understand the distinct effects reported on grapevine, especially those related to aromatic profile and primary metabolism Nevertheless, it is already noticed that chitosan impacts volatile composition, reflected in the improvement of organoleptic properties and in a nutraceutical point of view.

In conclusion, chitosan is a promising compound towards a sustainable vitiviniculture, allowing the reduction of the environmental impact of agrochemicals and their economic costs. Additionally, chitosan treatment leads to general improvement of grapevine phytosanitary status, grape quality, and wine acceptance.

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