



REVIEW ARTICLE

Chitosan and its applications in oenology

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ABSTRACT

This paper reviews the main applications of the biopolymer chitosan, the main derivative of chitin, a material usually obtained from natural sources accessible at low cost, i.e., industrial wastes from fisheries. Due to its natural origin, which confers biodegradability and biocompatibility properties, in addition to its low toxicity, chitosan has been gaining attention in numerous sectors, such as agriculture, food, medicine, pharmaceuticals, etc., including also important oenological applications due to its potential as a green alternative to the use of sulphite. Among the many applications that can be generated from these materials in the wine-making area, their use has been reported for the clarification of must; in the preparation of films for the removal of contaminants, whether organics such as ochratoxin A or inorganics such as some metal ions and their salts; the control of turbidity caused by protein precipitation; the encapsulation of yeasts of oenological interest and enzymes for the control of adverse microorganisms such as *Brettanomyces*; the manufacture of sensors and nanosensors for the quantification of contaminants, the quality control of starting materials and final products, the optimisation of fermentation processes, the monitoring of storage conditions, etc. As a result of this review, significant development of the applications of this material in the oenological area can be expected, especially due to the possibilities of preparing new derivatives, including the great variety of these that have been recently proposed through click reactions, as well as the growing incursion of chitosan in nanobiotechnology.

KEYWORDS: sulphite alternative, contaminant removal, nanosensors, ochratoxin A, clarification

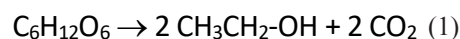
INTRODUCTION

Wine is an old and very dear friend of man. It has also been considered since antiquity as one of the criteria marking the social evolution of humankind, as Herodotus states when referring to the advice given by the Lydian Sandamis to King Croesus in his struggles against the Persians. Throughout history, wine has also provided sublime examples of its use, such as the high regard given to it in Christianity as the blood of Christ, as well as biblical examples of the nefarious side of its abuse, as can be seen in the book of Genesis, chapter 9, verses 20–27, where it is narrated that one of Noah's first activities after the flood was to plant a vineyard, getting drunk afterwards with the wine produced and remaining naked in the sight of his youngest son Ham, who told his brothers Shem and Japheth, who covered him with their clothes but avoided seeing his nakedness. After waking up and realising what had happened, Noah cursed Canaan, son of Ham, condemning him to be a slave of Shem and Japheth.

Throughout history, many renowned scientists have made important contributions that have led to today's knowledge of winemaking processes. A brief summary to mention some of them includes the Dutchman Antoni van Leeuwenhoek, who developed high quality lenses for the time and was able to observe with his microscope, for the first time, some microorganisms he called "*animalcula*" (a word that has been translated as very small animals) in 1639 (van der Leeuwenhoek, 1939); the Frenchman Antoine Lavoisier, creator of the law of conservation of matter, who estimated in 1789 the proportions of sugars and water at the beginning of the fermentation reaction, adding yeast (calling it ferment) to continue the alcoholic reaction, and compared them with the proportions of alcohol and carbon dioxide obtained at the end, coming to the conclusion that sugars decompose into alcohol and carbon dioxide (Lavoisier, 1789), thus, providing a clear view of the basic principles of the chemical reactions necessary to produce alcohol; the French chemist Louis Joseph Gay-Lussac, in whose honour the degrees of alcohol in a wine are known as degrees GL, and who in 1810 carried out experiments with grape juice packed in closed bottles and heated for a time in boiling water, which were kept for a year without fermentation being observed, but after exposure to air were able to ferment, thus, concluding that heat inactivates yeast (Gay-Lussac, 1810); the German physiologist Wilhelm Friedrich Kuhne, who in 1878, to avoid the confusion caused by the double meaning of the word ferment, proposed to use the term "enzyme" for soluble substances that cause fermentation (even giving it a more general connotation and not restricted only to the fermentation process), and to leave the word ferment only to designate yeasts (Kuhne, 1878).

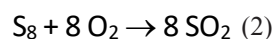
It is also important to mention that Gay-Lussac made calculations with the quantities of reactants and products in the fermentation processes, and his work in 1815 (Gay-Lussac, 1815) has been credited with the development of the chemical equation (1) describing the transformation of

glucose into ethyl alcohol and carbon dioxide, although some researchers do not share this opinion (Barnett, 1998).



However, the real beginning of the systematic approach to the chemical and biological study of alcoholic fermentation can be dated back to 1857, in the work of the French chemist Louis Pasteur (Pasteur, 1857; Pasteur, 1860), who later became one of the most renowned bacteriologists of the modern world. Pasteur demonstrated, for the first time, that fermentation occurs only by the action of "live" yeasts, which transform glucose into ethanol, and that the process occurs in the absence of oxygen. From his experiments, he concluded that fermentation is a vital process that he called "airless respiration".

Another important point in the evolution of wine is related to the use of additives to prevent its decomposition, especially with the use of sulphur dioxide (SO₂), also known as sulphurous anhydride, sulphite, sulphur oxide, etc. Although the preservative and antiseptic effect of burning sulphur inside houses were known since ancient times, as mentioned by Homer in *The Odyssey*, the first recorded mention of SO₂ and its effects on wine seems to have also been made by Pasteur in 1866, demonstrating its antiseptic and antioxidant effects and recommended burning sulphur (Equation 2) inside wine barrels to make them more stable (Pasteur, 1866). At the time, the aim was to control the deterioration suffered by French wines during transport and storage for export (Nous les Vignerons de Buzet, 2017).



Since then, SO₂ has remained an unrivalled additive in wine production, although, in more recent times, legal aspects have been regulated to control, and even try to eliminate in some wines, the use of sulphites. The main reasons that have led to these new practices include the need to improve the image of the naturalness of some wines and the prevention of some health damage associated with SO₂ consumption, such as allergies observed in sensitive consumers. In this regard, the acceptable daily intake established by the World Health Organization (WHO) for sulphites is 0.7 mg/kg body weight (World Health Organization, 2009), which means that the acceptable amount for an average person (~75 kg body weight) would be around 53 mg per day, a value that is reached with a daily intake of half a bottle (375 ml) of a red wine complying with the European Unión standard (up to 150 mg SO₂/l for red wines (European Union, 2019)) and even more easily in other countries such as the United States (up to 350 mg SO₂/L for red wines), where only one glass (150 ml) per day would suffice.

The search for alternatives to the use of SO₂ has led to the testing of various methods and substances for the protection and improvement of wines at different stages of their processing, whether these involve the application of physical processes (microfiltration, ultrasound, ultraviolet radiation, electrical pulses, microwaves, etc.), the use of chemical substances (sorbic acid, dimethyl carbonate, lysozyme,

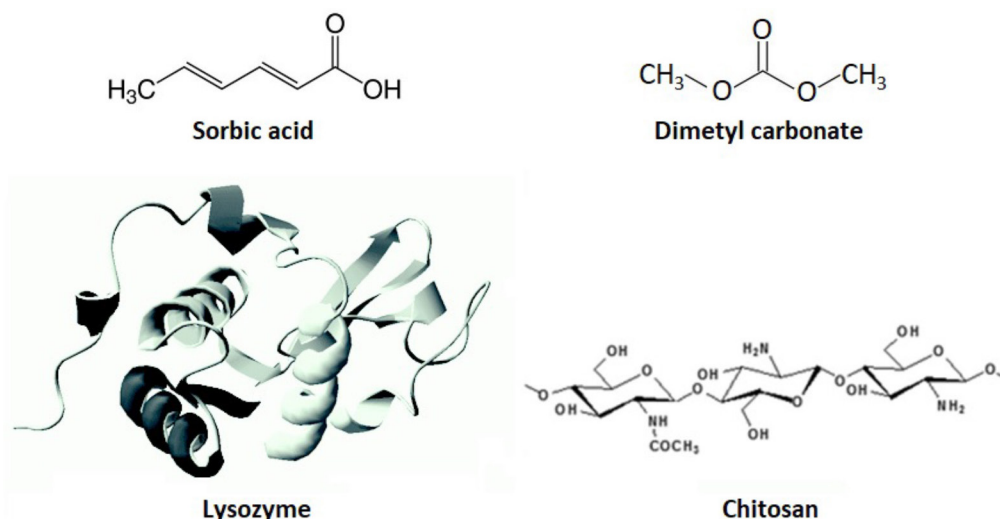


FIGURE 1. Structures of some additives currently used in oenological processes as green alternatives to SO₂.

chitosan, etc., see Figure 1) or the development of biological strategies such as the use of yeast strains with low capacity to produce SO₂ (Rauhut and Cottureau, 2009).

Despite the numerous studies carried out to date, with some of them showing satisfactory results, it has not yet been possible to completely replace the excellent duality of performance that this additive has for the preservation of wine properties, as an antimicrobial agent and as an antioxidant agent (Lisanti *et al.*, 2019). Thus, over a long time, SO₂ has demonstrated a high efficacy in preventing the biological deterioration of wine and in the stabilisation of some of its most appreciated properties, such as fragrances and colour. Therefore, the current oenological task seems to be more focused on obtaining complementary methods to lower the sulphite content than on its total elimination.

Among the most prominent materials that have been investigated as an alternative to the use of SO₂ in oenological applications is chitosan. Its use has also been considered at various stages of the oenological process, including must clarification with approval of the Organization International of the Vine and Wine (OIV) (OIV, 2009a), during the fermentation process (Scansani *et al.*, 2020), before packaging (Mármol *et al.*, 2012), during storage (Nunes *et al.*, 2016), etc. Additionally, chitosan could also be used as a matrix for the encapsulation of yeasts of oenological interest, like what has been tested in bioethanol production (Namthabad and Chinta, 2012), as well as for the preparation of membrane-based membranes for the nanofiltration of effluents in winemaking (Miao *et al.*, 2008).

From the point of view of legal regulations for its use in oenology, chitosan obtained from safe and abundant food or biotechnological fungal sources, such as *Agaricus bisporus* or *Aspergillus niger*, was accepted in 2009 by the OIV for the improvement of some oenological processes (OIV, 2009a; OIV, 2009b; OIV, 2009c; OIV, 2009d). Similarly, the proposal of the Belgian company KitoZyme SA to consider

chitosan, specifically that obtained from *Aspergillus niger*, as a “generally recognised as safe” (GRAS) material for use in the production of alcoholic beverages was accepted without objection by the US Food and Drug Administration (US FDA) in 2011 (US Food and Drug Administration, 2011), while in 2022, the same agency accepted without objection the proposal of the Canadian company Chinova Bioworks Inc. to consider chitosan, specifically that obtained from white mushrooms (*Agaricus bisporus*), as a GRAS material for use as an antimicrobial in food and alcoholic beverages (US Food and Drug Administration, 2022).

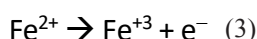
This paper presents an overview of the use of chitinous materials in oenological applications, with special emphasis on those works that promise possibilities to reduce the use of SO₂ without detriment to the preservation of the most appreciated properties of wines.

CHITOSAN APPLICATIONS IN OENOLOGICAL PROCESSES

1. Inhibition of chemical browning

Browning is one of the oldest known problems in winemaking. It derives from the set of chemical reactions that occur during winemaking, ageing, and storage. Due to these reactions, the colour of the wine can change, thus, affecting the quality of the final product. Many of the constituents of wine, such as phenolic compounds, certain metals, sugars, lipids, and amino acids such as tyrosine, aldehydes, etc., are susceptible to oxidation reactions during the manufacturing processes, which can also influence other sensory properties such as loss of flavour, aroma and nutritional value, increased astringency, etc., in addition to the colour changes. The main oxidation reactions can be caused by so-called Reactive Oxygen Species (ROS), which can originate due to some transition metal ions in their reduced form, which are usually present in them, e.g., divalent iron and copper ions (Oliveira *et al.*, 2011).

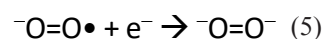
For ferrous ions, the reaction would be initiated by its oxidation to give up an electron:



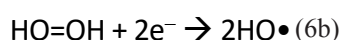
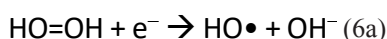
which is transferred to the oxygen present in the system (triplet O_2 , $\text{O}=\text{O}$), generating the superoxide radical anion:



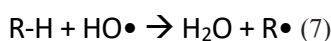
at the usual pH values in wines, it is in its protonated form ($\text{HO}=\text{O}^{\bullet}$, hydroperoxyl radical); the transfer of a second electron leads to the formation of the peroxide anion:



which exists in its protonated form ($\text{HO}=\text{OH}$, hydrogen peroxide). The subsequent transfer of one or two electrons generates a more reactive specie, the peroxide radical:



which is capable of abstracting hydrogen atoms from various organic compounds present to form water, one of the end products of oxygen reduction, and an organic radical:



The many different R-radicals that can form in wines lead to a variety of products that are ultimately responsible for the changes that occur in wines, including those that cause spoilage.

On the other hand, due to the antioxidant properties attributed to chitosan, some applications have been proposed in food preservation processes (Schreiber *et al.*, 2013; Friedman and Juneja, 2010) and various oenological treatments (Castro-Marín *et al.*, 2019; Chinnici *et al.*, 2014). For the latter, only the use of chitosan of fungal origin, specifically those extracted from *Agaricus bisporus* or *Aspergillus niger*, has been considered, either in the treatment of musts, where it is used as a clarifying agent for the settling process (additionally preventing protein breakdown) (OIV, 2009a), or in wine, where it is used to reduce the content of heavy metals (iron, lead, cadmium and copper, preventing ferric or cupric cracking), and to reduce the presence of undesirable microorganisms such as *Brettanomyces* (OIV, 2009c). In this sense, it has been assumed that chitosan can act by complexing Fe^{2+} and Cu^{2+} ions, thus, decreasing Fenton reactions in the presence of tartaric acid (Rocha *et al.*, 2020). Similarly, chitosan has been observed to reduce

the content of some phenolic compounds, such as ellagic acid, which are actively involved in co-pigmentation with anthocyanins (Castro-Marín and Chinnici, 2020). In both cases, a decrease in wine colouring should be expected, although in the second case, the observed effect was marginal. Other mechanisms that have been considered for the inhibition of wine browning by chitosan include its reaction with HOO^{\bullet} and HO^{\bullet} radicals (Friedman and Juneja, 2010) which can abstract hydrogen from chitosan leading to its depolymerisation (Lárez-Velásquez and Zambrano Díaz, 2011), and the displacement of the tartrate anion from the tartrate/Fe(III) complex], thus, blocking Fe(II) regeneration (Castro-Marín *et al.*, 2021).

2. Enzymatic browning

Wine browning can also occur through chemical reactions catalysed by enzymes generically known as phenol oxidases, which include catechol oxidase (also known by other names such as diphenol oxidase, phenol oxidase, polyphenol oxidase, phenolase, and tyrosinase, is also confused with other types of enzymes such as monophenol monooxygenase), laccase and o-aminophenol oxidase (Oliveira *et al.*, 2011). These reactions occur mostly in the must because the activity of these enzymes is inhibited in wine by the alcohol present (Waliszewski *et al.*, 2009). The oxidation of phenolic compounds involves (i) the hydroxylation at an ortho position to a hydroxyl group present in the phenolic substrate by an enzyme with cresolase activity, (ii) the subsequent oxidation of the product (ortho-dihydroxy-benzene) to ortho-benzoquinones by an enzyme with catecholase activity and (iii) the subsequent reactions of the generated quinones with other species present in the medium, such as other phenols, amino acids, proteins, etc., to generate condensation and polymerisation products (Oliveira *et al.*, 2011), to generate condensation products and usually coloured polymers (see Figure 2).

Although studies on the effects of chitosan on enzymatic browning in wines are scarce, it has been reported that the use of chitin, added as an adsorbent that is removed by filtration before fermentation and packaging, resulting in a significant decrease in colour, catechins, and total polyphenols over storage time (Mármol *et al.*, 2012; Mármol *et al.*, 2009). Therefore, it can be assumed that these materials act by previously decreasing, by adsorption, the concentration of phenolic compounds in the must. Similar reasoning has been used to explain the decrease in browning of pear and apple

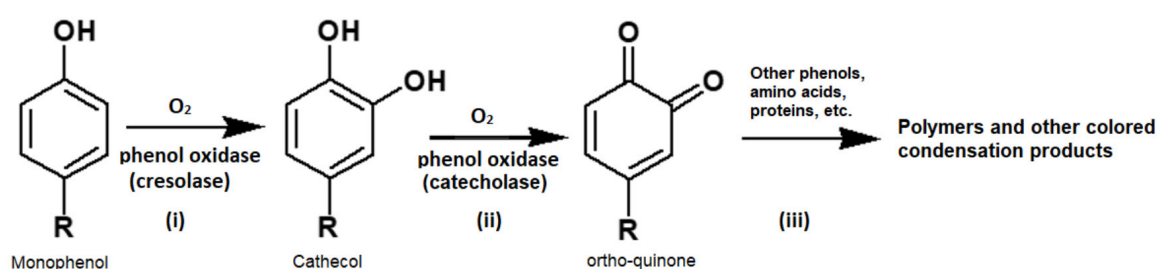


Figure 2. Summary of enzymatic oxidation processes leading to browning in the must.

juices when pre-treatments with chitosan solutions, followed by filtration, were applied before packaging and storage (Sapers, 1992); however, in the latter case, it was proposed that the decrease in browning is since the coagulation processes caused by chitosan allow a more efficient filtration of the smaller insoluble particles, to which the polyphenol oxidases are bound.

3. Control of harmful microorganisms

Due to their origin and taking advantage of the sugar content of fruits, oenological processes are affected by many harmful microorganisms. Among the most harmful microorganisms reported for wine are yeasts of the genus *Brettanomyces*, some of which can lead to the accumulation of volatile phenols in red wines, i.e., *Brettanomyces bruxellensis*, conferring them with strange fragrances that can even permanently damage them commercially (Paulin *et al.*, 2020). For the control of these microorganisms, SO₂ has usually been used as a preservative agent, despite being considered a highly polluting and harmful compound for human health; additionally, in some cases, this compound can generate other complications in wines, such as unpleasant odours, allergic reactions, and headaches in consumers, etc., besides the difficulties arising from the emergence of resistant strains to this chemical may cause (Avramova *et al.*, 2018).

Regarding the use of chitosan as an environmentally friendlier alternative to the use of SO₂ in the control of this type of harmful microorganisms, some studies have also been reported (Tika and Puspaningrat, 2022; Picariello *et al.*, 2020). The antimicrobial properties of chitosan have been known for a long time, although the mechanisms of action have not yet been fully elucidated. Usually, its biocidal effect has been associated with its cationic nature (generated by the protonation of its amino groups), which can cause morphological changes, alteration of the cell membrane, and loss of intracellular material in several pathogenic microorganisms. In addition, other mechanisms have been proposed, such as the chelation of metals necessary for the development of pathogens (through their amino and hydroxyl groups), the alteration of their gene expression, the inhibition of protein synthesis, the blocking of sodium channels, etc. (Li and Zhuang, 2020; Lárez-Velásquez and Rojas-Avelizapa, 2020).

In this regard, it has been found that, under well-established conditions, chitosan does not impair the important properties of wine but, on the contrary, improves some of them, such as decreasing its browning (due to its antioxidant

effects). However, it can delay the initial lag phase during the fermentation process with *Saccharomyces* strains (Castro-Marín *et al.*, 2018), although it simultaneously acts as an antimicrobial agent, with variable effectiveness, against different strains of *Brettanomyces* (Paulin *et al.*, 2020). Studies have also been reported where chitosan activity is highly selective, retarding the growth of *Brettanomyces* strains in the presence of *Saccharomyces* (Gómez-Rivas *et al.*, 2004). Additionally, chitosan treatment is effective in reducing the activity of acetic acid bacteria during fermentation, with its remarkable effect being observed immediately after application, especially in the most active strains (Valera *et al.*, 2017); similar effects have been observed against lactic acid bacteria (Elmacı *et al.*, 2015).

In parallel to applications based on its recognised antimicrobial properties, chitosan can be used for the transport and release of bioactive substances, even at the nanoscale (Lárez-Velásquez, 2018). In applications related to oenological processes, for example, it has been reported that treatments with low molecular weight chitosan matrices, which act as an antimicrobial agent, loaded with antimicrobial fungal extracts (obtained by spray/drying), showed a notorious synergistic effect of the components for the control of *B. bruxellensis* (Choque *et al.*, 2019).

4. Chitosan for the removal of contaminants from wine

The use of chitosan and chitin–glucan, both of fungal origin, in treatments for the removal of contaminants in wine has been approved in the European Union since 2011 (European Union, 2011). As mentioned above, chitosan has been tested in the removal of contaminants present in various stages of the oenological process, both for contaminants of an organic nature, such as ochratoxin A (Figure 3), perhaps the contaminant that has received the most attention for its removal due to its carcinogenic potential (Abbas *et al.*, 2018), and inorganic ones, such as various metals and their salts. Thus, the treatment of different wine samples (red, white, and sweet) using chitosan as an adsorbent achieves notable reductions in the concentrations of iron, copper, and cadmium while simultaneously reducing the content of ochratoxin A (Bornet and Teissedre, 2008). Likewise, the use of nano adsorbents for the removal of ochratoxins, including those based on chitosan, seems to merit consideration by the oenological sector, as it has started to be tested in the food industry for the removal of mycotoxins (Song and Qin, 2022).

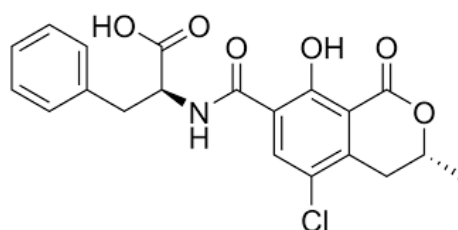


Figure 3. Chemical structure of the ochratoxin A.

On the other hand, Rizzo *et al.* (2010) reported a high efficiency of chitosan in the coagulation processes of wine wastewater, proposing its use as a sustainable alternative to the use of conventional metal-based coagulants, with the additional advantage of producing potentially reusable organic sludge; similarly, it has been reported that the use of chitosan-sepiolite nanocomposites allowed an efficient clarification of effluents from wine-making activities, associating its effect to a process of neutralisation of the charges in the colloids (Rytwo *et al.*, 2013).

5. Chitosan as a support for enzymes and other additives in wine processing

The use of immobilised enzymes in different processes related to oenology (Ottone *et al.*, 2020) is another area where the attractive properties of chitosan can be exploited. Many enzymes face difficulties due to their rapid inactivation, the lack of control over different reaction parameters, their low efficiency, etc. Therefore, their immobilisation, or co-immobilisation in enzyme aggregates, is an alternative whose development has become increasingly interesting. In this sense, some promising results that have been reported are the following: (a) the preparation of chitosan microspheres cross-linked with glutaraldehyde, loaded with β -D-glucosidase (β G) and α -L-arabinofuranosidase (ARA), with retention of enzymatic activity for 91 days of incubation under winemaking conditions, which makes them suitable for application in processes related to wine aroma enhancement (Tavernini *et al.*, 2020), although it might be more advisable to test other less irritant crosslinking agents, such as genipin; b) the encapsulation of the proteolytic enzyme bromelain in clay/chitosan nanocomposites to control the opacification or turbidity caused by protein precipitation in white wines (Benucci *et al.*, 2018), an instability phenomenon of non-microbial origin, leading to the search for mechanisms to control it (e.g., the use of proteolytic enzymes to hydrolyse proteins, including their encapsulation in appropriate and more environmentally friendly matrices); c) covalent

immobilization of the enzyme lysozyme from hen's egg in chitosan spheres to control heterolactic fermentation in sherry wines, showing that the antimicrobial activity of the enzyme is not affected by SO_2 and phenols in white wine (Liburdi *et al.*, 2016); d) immobilization of the urease enzyme on a chitosan-based support (*Chitopearl*) showed no appreciable difference in urea removal speed in white wines (with low tannin concentrations) with respect to soluble urease, however, since soluble commercial preparations usually have limitations for such removal due to their low protein contents (even when using the maximum legally allowed doses), the use of the immobilized enzyme could allow overcoming such limitations since the biocatalyst is insoluble in wine and can be easily removed (Andrich *et al.*, 2010); e) it has been reported that the use of chitosan to encapsulate natural oxidizing agents, obtained as fungal extracts (*Aspergillus tubingensis*), allows to maintain their properties after the encapsulation process and that the retarding effect of chitosan on *Saccharomyces cerevisiae* seems to be diminished (Choque *et al.*, 2019).

Another interesting point regarding the application of chitosan in the preparation of matrices for enzyme immobilisation is its chemical derivatisation, which can lead to controlled modifications of its chemical structure, allowing to obtain materials better adapted to the specific immobilisation of a particular enzyme, with the consequent improvement of the process to be controlled. In this sense, it has also been reported that, in general terms, enzymes immobilised on chitosan composites show better catalytic activity and operational stability than when they are in their free form (Nunes *et al.*, 2021).

6. Chitosan as an antioxidant agent for thiols

Thiols are organic chemical compounds characterised by the presence in their structure of the functional group -S-H, known as thiol, sulfhydryl, sulfanyl, or mercaptan. These compounds have been identified as key aroma

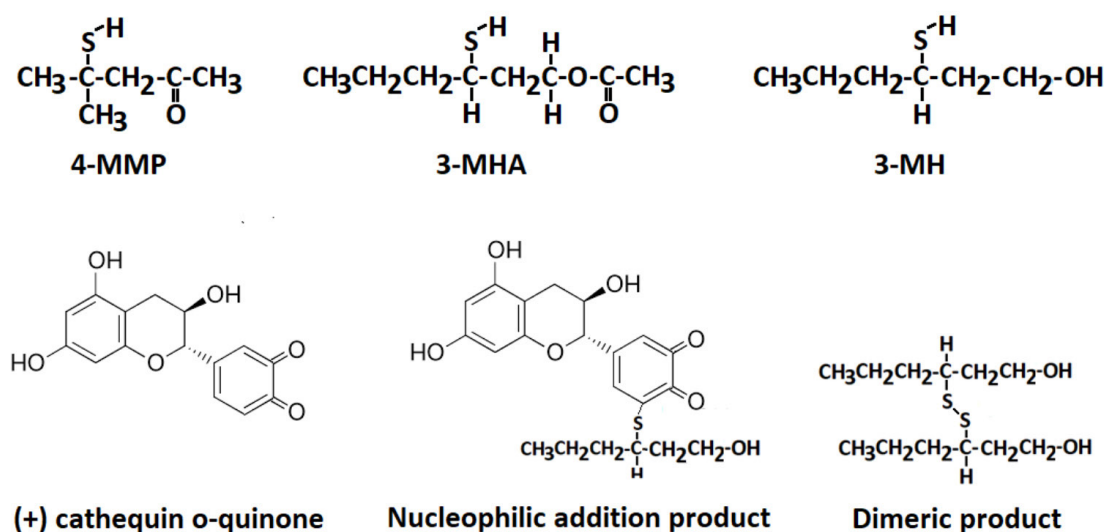


FIGURE 4. Chemical structures of some species of interest in the oxidation of thiols in wines.

components in young wines from different varieties, with 4-mercapto-4-methyl pentane-2-one (4-MMP), 3-mercaptohexyl acetate (3-MHA), and 3-mercaptohexan-1-ol (3-MH) (Figure 4) being among the most common in varietal wines (Roland *et al.*, 2012).

Varietal wines are wines made from a single grape variety or at least 80 % of a single variety, as defined in the European Union (Salvador Insua, 2016), although in some countries such as Argentina, a minimum of 85 % is required (Murgo *et al.*, 2019). In these wines, which constitute complex systems due to the presence of multiple chemical and enzymatic species, thiols are very sensitive to oxidation reactions that could generate a variety of products, among which those resulting from their nucleophilic addition to (+)-catechin-(o-quinone) and dimeric disulphides have been proposed (see Figure 4). The latter would be obtained through autooxidation catalysed by free radicals derived from the redox cycle of some of the metals present, especially the Fe^{2+}/Fe^{3+} couple. In both cases, such free radicals seem to play a major role (Nikolantonaki *et al.*, 2010). On this point, chitosan can significantly lower the oxidation of thiols to maintain the varietal character of wines from aromatic grapes, especially when reduced amounts of sulphite are used in model wine solutions (Chinnici *et al.*, 2014). However, contradictory results also have been reported for the early addition of chitosan in the grape processing stages, which results severely detrimental to the formation of varietal thiols although it was not possible to identify the exact mechanism by which chitosan affected thiol formation, suggesting some impact on enzyme systems (Dias Araujo, 2017).

SOME CONCERNS ABOUT THE USE OF CHITOSAN IN WINE TREATMENT

Although the preceding sections have highlighted many of the benefits of these materials for environmentally friendly

applications in the main oenological processes, there are also some concerns regarding their use in some of them. Thus, chitosan for use in wine applications must be of high purity because the material containing proteins, i.e., tropomyosin, may cause intoxication problems in sensitive individuals (Amaral *et al.*, 2016). Although this issue has been practically solved by obtaining chitosan from sources other than crustaceans, a few cases of allergy have also been reported using chitosan from zygomycetes (Kato *et al.*, 2005). A further problem associated with the use of chitosan is the lack of reproducibility in the results usually obtained when using materials from different sources, or even from the same source but obtained by different methods, which implies additional work and costs to carry out tests each time chitosan of different origin is to be used. This is an issue that has generated a lot of attention for a considerable time but has not yet been resolved. Some observations related to the use of chitosan in oenological applications that could be related to these facts are: (a) chitosan has shown a high capacity to reduce the ochratoxin A concentration in highly contaminated red wines, but also appreciably reduces colour intensity (contrary to chitin which reduces ochratoxin A without significantly affecting colour intensity) (Quintela *et al.*, 2012), both reductions being dependent on the doses of chitosan applied (Kurtbay *et al.*, 2008); b) it has been reported that chitosans with low deacetylation rates are not efficient for the removal of volatile phenols that affect the fragrance of red wines, and, therefore, do not help to ameliorate the negative impact of these, in contrast to chitosans with high deacetylation rates (Filipe-Ribeiro *et al.*, 2018); c) it has been reported that chitosan can alter some organoleptic attributes related to astringency and bitterness, due to the removal of compounds such as cinnamic acid and phenols such as procyanidins (Spagna *et al.*, 1996), which can decrease the concentration of vanillin-reactive flavanols (Picariello *et al.*, 2020), although the dose of chitosan added seems to be important to avoid obtaining contrasting results (Colangelo *et al.*, 2018)

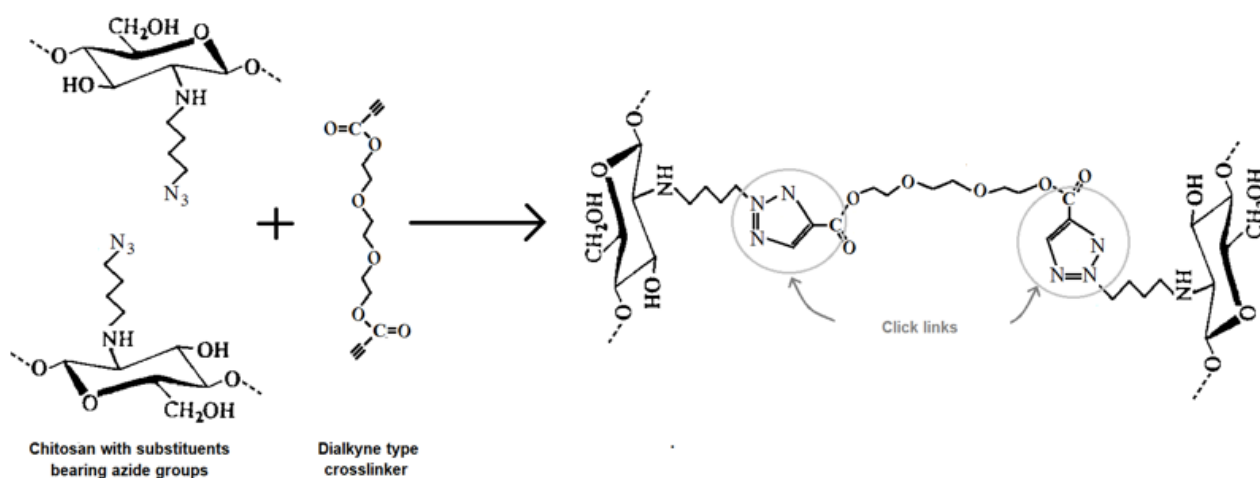


FIGURE 5. A hypothetical cross-linking, via click reaction, for chitosan with substituents bearing azide groups and a dialkyne-type cross-linker.

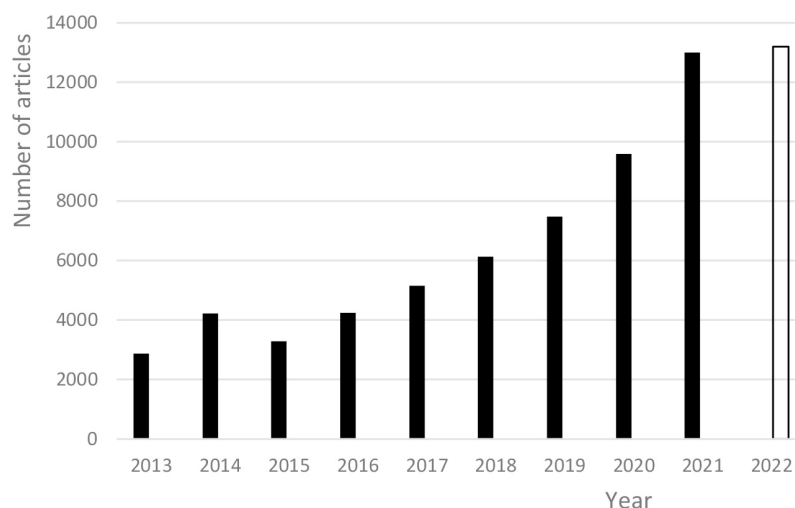


FIGURE 6. Evolution over the last few years of the number of articles found using the academic search engine Google Scholar for the keyword “chitosan-based nanomaterials”. The white column represents the values for the year 2022 until 01/11/2022.

FUTURE TRENDS ON THE USE OF CHITOSAN IN OENOLOGICAL PROCESSES

Because chitosan is a material with multiple application possibilities in different fields, the intense research that has been carried out on its chemical modification has allowed obtaining increasingly novel derivatives with better control of its properties, including those obtained recently through the so-called click reactions (Truong *et al.*, 2014; Kritchenkov and Skorik, 2017; Rojas-Pirela *et al.*, 2021) (see Figure 5), a new type of chemical reactions whose pioneers have received this year’s Nobel Prize in Chemistry (Nobel Prize Organization, 2022).

In this sense, chitosan click reactions constitute a simple route to generate materials and systems that could quickly find applications related to the encapsulation of yeasts of oenological interest, such as those mentioned above for bioethanol production (Namthabad and Chinta, 2012); for the preparation of new methods for the immobilisation of proteolytic enzymes that help to control the opacification of wines derived from protein precipitation, similar to the systems reported (Benucci *et al.*, 2018); the preparation of systems that promote the controlled release of substances enhancing the qualities of wine (Tavernini *et al.*, 2020); among others.

On the other hand, the development of different chitosan-based nanomaterials has been growing steadily over the last few years, as can be inferred from the number of publications in the last ten years for the Google Scholar search using the words “chitosan-based nanomaterials” (see Figure 6). Some systems based on these nanomaterials could easily be tested for the removal of contaminants in wine, like how some nano-adsorbents have been tested in other media for the removal of organic contaminants such as mycotoxins (Song and Qin, 2022), or inorganic contaminants such as metals and their salts (Haripriyan *et al.*, 2022).

It is estimated that nano-adsorbents can generate better performance in the removal of various substances thanks to the unique properties derived from their nano-dimensions, such as high adsorption capacities, short-time adsorption equilibria, large surface area, and the presence of various active groups for ion binding, etc. (Ali *et al.*, 2020).

Similarly, chitosan offers wide possibilities for the manufacture of molecularly imprinted adsorbents (MIA) (Xu *et al.*, 2015; Karrat *et al.*, 2020), whose preparation consists of using chitosan, or its composites, as a matrix for the prior moulding of an adsorbate of interest (usually molecules or ions but which can also be applied to viruses, bacteria, proteins, etc.) and whose subsequent extraction generates an adsorbent of very high specificity because its active sites have the shape of the adsorbate. Despite this, MIAs have been little exploited as adsorbents, especially in the oenological area, although some interesting studies have been reported in related applications, such as the removal of the toxin patulin in pear juices using a molecularly imprinted adsorbent that additionally possesses magnetic properties (Sun *et al.*, 2020).

The use of ever smaller and more specific sensors is also one of the areas of biotechnology, and nanobiotechnology, that has found a place in different stages of the oenological process, such as quality control of starting materials and final products, optimisation of fermentation processes, monitoring of storage conditions, etc. (Monge and Moreno-Arribas, 2016). Interesting results related to the use of chitosan-based nanomaterials have already been reported in many of these applications. Straightforward examples include the preparation of an amperometric nanosensor based on the immobilisation of the enzyme alcohol dehydrogenase on chitosan/carbon nanotube matrices for the quantification of ethanol in wine, beer, and spirits (Lee and Tsai, 2009) as well as the preparation of voltammetric sensors based on molecularly imprinted chitosan electrodeposited on a

boron-doped diamond electrode for the detection of catechol in wine (Salvo-Comino *et al.*, 2020). Other nanosensors that have been prepared for use in other areas should be of easy application in wine, such as the system for the detection of ochratoxin A prepared using a plasmon resonance biosensor based on chitosan and carboxymethyl-chitosan nanomatrices (Rehmat *et al.*, 2019).

CONCLUDING REMARKS

As can be seen from this brief overview of the multiple uses that chitosan has been experiencing in the oenological area, the possibilities of its utilisation seem to be widening more and more owing to the numerous investigations that are being developed with this material in different fields, in addition to being a material with proven sustainability benefits due to its natural origin and optimisations, in this sense, of the processes to obtain it in an environmentally friendlier way. An important point in favour of chitosan is its versatility to be easily integrated into the preparation of different types of nanomaterials, a previous step for the development of applications in nanotechnology and nanobiotechnology. In the case of oenology, for example, for the manufacture of nanosensors for the detection of contaminants such as ochratoxin A, the nanoencapsulation of strains of interest, the preparation of specific nano-adsorbents, etc.

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