United States Department of Agriculture
Agricultural Marketing Service | National Organic Program

Document Cover Sheet

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

www.ams.usda.gov/rules-regulations/organic/national-list

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (NOSB) [7 CFR 205.607(a)].

www.ams.usda.gov/rules-regulations/organic/nosb

National List Petition Guidelines for submitting a petition are available on the AMS website at How to File a Petition.

www.ams.usda.gov/rules-regulations/organic/national-list/filing-petition

AMS posts completed petitions online for public access on the NOP Petitioned Substances Index

www.ams.usda.gov/rules-regulations/organic/petitioned-substances

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List. Technical reports are completed by third-party contractors and AMS posts completed technical reports online for public access on the NOP Petitioned Substances Index. Contractor names and dates completed are available in the report.

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Read more about the technical report process in section **H. SUBSTANCE/MATERIALS REVIEW PROCESS** in the NOSB Policy & Procedures Manual posted on the AMS website.

www.ams.usda.gov/sites/default/files/media/NOSB-PolicyManual.pdf

www.ams.usda.gov/rules-regulations/organic/nosb

Chitosan
Handling/Processing

Identification

Chemical Names:
poly-D-glucosamine; poly(beta-(1,4)-2-amino-2-deoxy-D-glucose); poly(beta-(1,4)-D-glucosamine); poly-*N*-acetylglucosamine

Other Name:
deacetylated chitin; poliglusam/polyglusam; water-soluble chitin (some forms)

Trade Names:
Bactiless; Chitocell®; EnartisStab Micro; No[OX]; NO BRETT INSIDE™; Oneobrett®; QI'UP XC

CAS Numbers:
9012-76-4

FDA Unique Ingredient Identifiers (UNII):
23R93M6Y64 (chitosan oligosaccharide)
5GV09YMO52 [chitosan medium molecular weight 200-400 mPa.s)]
7SRJ3W89J8 [chitosan high molecular weight (400-800 mPa.s)]
82LKS4QV2Y [polyglusam; chitosan medium-high molecular weight (200-800 mPa.s)]
SBD1A2I75N [chitosan low molecular weight (20-200 mPa.s)]

Summary

This full scope technical report provides information to the National Organic Standards Board (NOSB) to support the initial review of fungal chitosan, used as a processing aid in winemaking. The petitioner is requesting that chitosan be added to the National List of Allowed and Prohibited Substances (hereafter known as the "National List") under 7 CFR 205.605(b) as a nonagricultural (nonorganic) substance permitted in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s)" (Enartis USA Inc., 2024). The petition focuses on use of chitosan, derived from fungi, as a processing aid in winemaking, to improve clarification and filterability, prevent and treat volatile aromas, and boost microbial stability.

There were two previous petitions to include chitosan on the National List. The first petitioner requested that chitosan be added as an adhesive adjuvant, used in conjunction with fungicides (Hadwiger, 2004; NOSB, 2004). The NOSB recommended the addition of chitosan to § 205.601 for use in organic crop production with the annotation "as an adjuvant only" (NOSB, 2005). In consultation with the Environmental Protection Agency (EPA), the National Organic Program (NOP) determined that chitosan, when used as an "adjuvant" (not having pesticidal activity), is already allowed under the existing inert ingredient provisions of § 205.601(m) of the USDA organic regulations (72 FR 69569, December 10, 2007).

The second petitioner in 2019 requested that chitosan be added to § 205.601(j)(4) for plant disease control (Aleman et al., 2019). However, the NOSB did not recommend its addition to the National List for this use (NOSB, 2021). These sources of chitosan are typically derived from chitin, found in crab or shrimp exoskeletons as well as insects and fungi. The chitin is then synthetically converted to chitosan. The previous petitions referred to crustacean chitosan sources. The 2020 technical report *Chitosan (Crops)*, written in support of the NOSB's 2021 review, is still relevant (NOP, 2020). While the following technical report confirms many of the same conclusions included in the 2020 report, it also provides more details on fungal chitosan properties and use as a fining agent in winemaking.

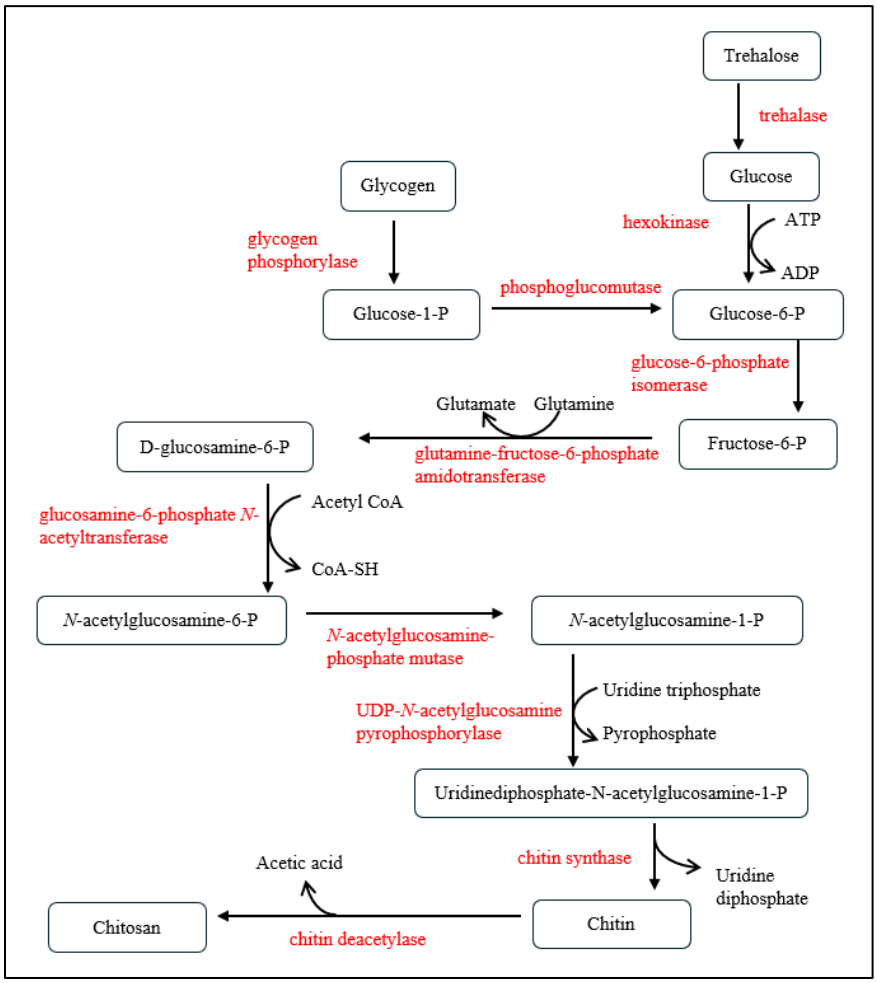
In this report, we compare chitosan and other wine fining agents currently allowed by the NOP. Where possible, we illustrate a few comparisons between chitosan and other materials used as fining agents. However, chitosan effects in wine can extend beyond clarification, such as stabilization and antimicrobial effects that are not necessarily achieved by standard fining agents (*e.g.,* bentonite). Hence, our comparisons with alternatives might not cover all possible effects or alterative substances/practices.

Characterization

Composition of the Substance

Chitin, the precursor for chitosan, is more formally recognized as poly[β-(1→4)-2-acetamido-2-deoxy-D-glucopyranose]. Chitin is structurally similar in many ways to cellulose. However, unlike cellulose, chitin also contains both amino ($–NH_2$) and acetamido ($CH_3CONH–$) chemical groups (Roberts, 1992). Chitin is a linear polysaccharide biosynthesized from trehalose or glycogen and can be further converted into chitosan through a deacetylation step (see Figure 1). This process removes an acetyl group ($CH_3CO–$) from the acetamide chemical group, leaving behind an amino group (Crognale et al., 2022).

Figure 1: Biosynthetic pathway of chitin and chitosan from glycogen and trehalose in arthropods and fungi. Enzymes are depicted in red font. Adapted from Crognale et al. (2022)

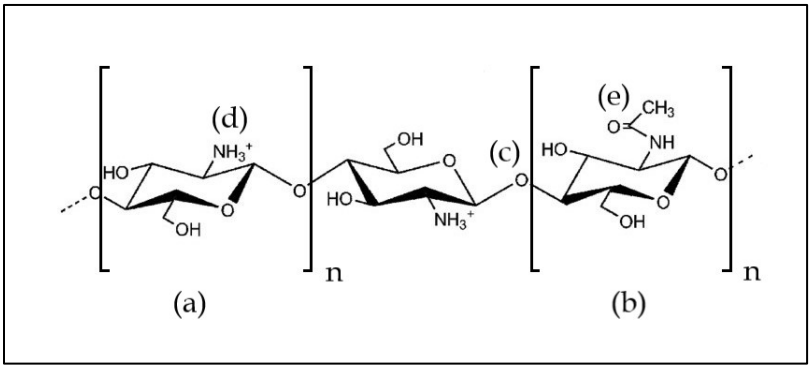


Chitosan refers to a variety of similar molecules that are represented by the chemical formula $(C_6H_{11}NO_4)n$ (Zakharov et al., 2017). While similar, chitosan molecules can differ in (Aranaz et al., 2021):

- composition
- size
- distribution of monomers

Chitosan is a copolymer of *D*-glucosamine and *N*-acetyl glucosamine (see Figure 2) that repeat in no particular order (Pillai et al., 2009; Rocha et al., 2017). Since the *N*-deacetylation of chitin is almost never complete, chitosan compounds have different degrees of deacetylation (Islam et al., 2017). There is no agreement among researchers or regulators on the minimum degree of deacetylation required for a product to qualify as chitosan. Most authors report medium thresholds of deacetylation [*e.g.,* 50% by Kozma et al. (2022), 60% by Dhillon et al. (2013) and Islam et al. (2017), and 65% by Ravi Kumar (2000)] although values as low 33.9% have been reported in literature (Mathaba & Daramola, 2020). According to Brasselet et al. (2019), chitosan products usually have 70% – 80% deacetylation rate. The 14th edition of the Food Chemicals Codex defines chitosan as having 70% – 95% deacetylation.

Figure 2: Structure of chitosan under acidic conditions. Glucosamine monomers (a) are bonded to additional glucosamine monomers or N-acetylglucosamine monomers (b) via 1-4 glycosidic linkages (c). Glucosamine monomers possess an amine group (d), ionized due to low pH. N-acetylglucosamine monomers possess the acetamide (amino-acetyl) group (e). Drawing adapted from Nilsen-Nygaard et al. (2015)



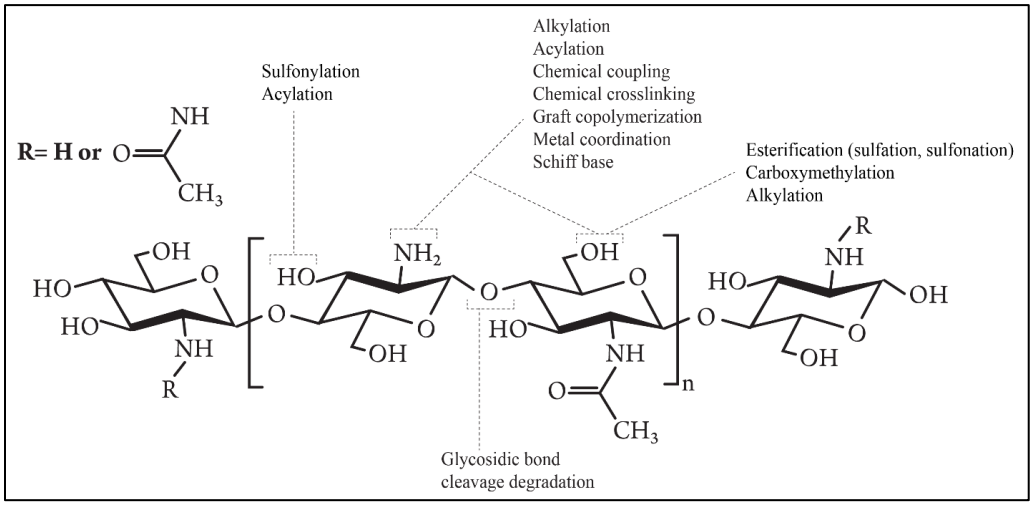
Due to the acetamido and amino groups, chitin and chitosan contain 5–8% nitrogen (Islam et al., 2017), which is proportional to the degree of deacetylation (Ravi Kumar, 2000). These nitrogen containing groups enable distinctive biological functions, and facilitate modification reactions (see Figure 3) to produce derivatives such as *N*-acyl chitosans, *N*-carboxyalkyl (aryl) chitosans, *N*-carboxyacyl chitosans, *o*-carboxyalkyl chitosans and semisynthetic resins of chitosan (Ravi Kumar, 2000).

The properties of chitosan also differ based on its origin (see Table 1). Vendramin et al. (2021) compared ten different chitosans, including:

- four from *Aspergillus niger*
- five from shrimp
- one from crab shells

The molecular weight of chitosan from fungal sources (30-84 kDa[1], mean 49 kDa) is much smaller than the weight of chitosan from shrimp (51-282 kDa, mean 195.6 kDa) or crab (478 kDa). In addition, the wine fining properties of chitosan from these sources differ significantly. For example, chitosan from shrimp and crab show a faster and greater sedimentation compared to those from fungal sources (Vendramin et al., 2021).

Figure 3: Functional groups in chitosan's structure that can be chemically modified. Drawing adapted from Aranaz et al. (2021) and Brasselet et al. (2019)



Source or Origin of the Substance

Chitin, from which chitosan derives, is the second most abundant polysaccharide biosynthesized on earth, after cellulose (Roberts, 1992). Chitin is an important constituent of the exoskeleton in many shelled aquatic (*e.g.,* crustacea) and land (*e.g.,* molluscs, insects) organisms (Roberts, 1992). In addition, it is the principal fibrillar polymer in the cell wall of certain fungi (see Figure 4) (Roberts, 1992). Both chitin and chitosan occur naturally as structural components in the cell walls of fungal classes Basidiomycota, Ascomycota, Mucoromycota (formerly

[1] Kilodalton (kDa): a unit of mass equal to 1,000 daltons. It is the equivalent of 1/12 of the mass of an atom of carbon-12, or 1.66033×10^{-27} kg. It is used to measure the molecular mass of large molecules like proteins and polysaccharides (Oxford Reference, 2025).

Zygomycota), and Deuteromycota (Abo Elsoud & El Kady, 2019; Dhillon, Kaur, Brar, et al., 2013; Gow et al., 2017).

In filamentous fungi, the main cell wall components are polysaccharides such as glucans, glycoproteins, and chitin representing, respectively, 50–60%, 20–30%, and 10–20% of the cell wall dry weight (Garcia-Rubio et al., 2020). Some *Aspergillus* species, including *Aspergillus niger*, possess chitin deacetylase enzymes which catalyze the conversion of chitin to chitosan (Zhang et al., 2024). Chitosan polymer chains, resulting from the deacetylation of chitin, form microfibrils in fungal cell walls. They are embedded in an amorphous matrix consisting of (Dzurendova et al., 2022):

- proteins
- mannoproteins
- lipids
- glucans that appear to cross-link the chitosan fibers

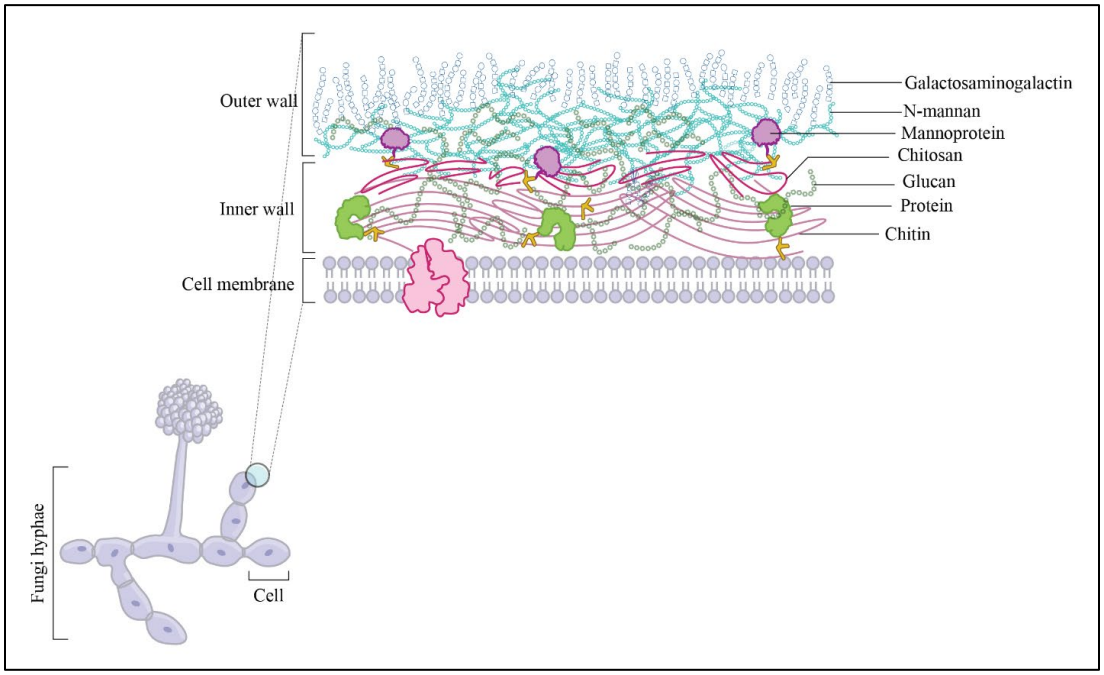
Krake et al. (2024) reported the following concentrations in *Aspergillus niger*:

- glucans (48.6%)
- proteins (22.2%)
- chitin and chitosan (16.0%)

Table 1: Selected properties of chitosan from different sources (Pochanavanich & Suntornsuk, 2002)

Chitosan source	Degree of deacetylation ± SD (n = 3) (%)	Molecular weight (Da)	Viscosity (cP)
Crab shell (Sigma)	97.9 ± 0.9	9.4×10^5	372.7
Apergillus niger TISTR3245	90.0 ± 2.1	1.4×10^5	6.2
Rhizopus oryzae TISTR3189	87.9 ± 2.1	6.9×10^4	3.5
Lentinus edodes no. 1	86.5 ± 2.2	1.9×10^5	5.8
Pleurotus sajo-caju no. 2	83.8 ± 0.1	1.1×10^5	5.6
Zygosaccharomyces rouxii TISTR5058	85.1 ± 1.1	2.7×10^4	3.3
Candida albicans TISTR5239	83.8 ± 0.8	1.1×10^5	3.1

Figure 4: Cell and cell wall composition in the fungus *Aspergillus niger*. Drawing adapted from Gow & Lenardon (2023) and Rocha et al. (2017)



Researchers have identified several fungal taxonomic groups that produce chitosan (see Table 2). Ascomycetes (*e.g., Aspergillus*) are the primary industrial source of fungal chitosan due to their (Krake et al., 2024):

- higher chitin concentrations
- scalability
- regulatory approval

Chitosan yields vary based on strain, mycelial age, cultivation medium, and extraction methods (Abdel-Gawad et al., 2017; Kumaresapillai et al., 2011; Pochanavanich & Suntornsuk, 2002).

Most commercial production of chitosan relies on artificially deacetylating chitin from crustaceans in order to produce chitosan (Shigemasa & Minami, 1996). Unlike with crustacean sources, chitosan derived from fungi avoids the need for the demineralization and decolorization steps (see Chemical extraction methods for details). The 2020 *Chitosan (Crops)* technical report provides more information on the steps involved in making chitosan from crustaceans/invertebrates (NOP, 2020).

Manufacturers of fungal chitosan products extract both chitin and chitosan from fungal cell walls, and convert the chitin fraction into chitosan using chemical deacetylation to increase yields (Krake et al., 2024). Species in the Mucoromycota taxonomic division (*e.g., Rhizopus*) have a high chitin content but are less commonly used in commercial production of chitosan (Vellozo-Echevarría et al., 2024). Strains of the ascomycete *Aspergillus niger* have been widely used on an industrial scale, since 1919 (Papagianni, 2007), for the production of citric acid and other biotechnological and pharmaceutical products such as antibiotics (Dhillon, Kaur, Brar, et al., 2013; Muzzarelli et al., 1980). The 2023 *Citric acid and salts (Handling/Processing)* technical report provides recent information on the process and strains involved (NOP, 2023). Microbial production is responsible for about 99% of world production of citric acid, and the weight ratio of *Aspergillus niger* waste produced to citric acid is about 10–15 g *Aspergillus niger* dry biomass for every 110–140°g of citric acid (Max et al., 2010). Companies use these mycelial wastes as an abundant and inexpensive feedstock source for fungal chitosan extraction as an alternative to crustacean sources (Tianjin Taikang Biopharmaceutical Co., Ltd, 2011).

177 **Table 2: Examples of major fungi used for chitosan production. The term "N.d." indicates where no data was available**

Fungal classes and major species	Chitosan yield (%)*	Chitosan yield of alkali insoluble materials (%)*	Degree of deacetylation (%)	Method/Comments	Reference
Ascomycota					
Aspergillus niger	11.2	N.d.	N.d.	Alkali and acid extraction.	(Nadarajah et al., 2001)
Aspergillus niger	3.79 – 8.12	N.d.	72.3 – 86.0	Alkali (sodium hydroxide) and acetic acid extraction.	(Abdel-Gawad et al., 2017)
Aspergillus niger	5.5	30.5	89.6	Alkali (sodium hydroxide) and acid (acetic or sulfuric acid) extraction.	(Krake et al., 2024)
Aspergillus niger TISTR3245**	10.7	90	N.d.	Cultured on potato dextrose broth. Alkali and acid extraction.	(Pochanavanich & Suntornsuk, 2002)
Aspergillus niger NRRL 567	6.4	N.d.	78	Citric acid, solid-state fermentation in apple pomace.	(Dhillon, Kaur, Sarma, et al., 2013)
Aspergillus niger MTCC strain 872	N.d.	17 – 23.1	85	The yield depends on the growth media and increases with incubation period (72 – 120 h).	(Kumaresapillai et al., 2011)
Aspergillus niger MTCC strain 1785	N.d.	18.3 – 25.2	85	The yield depends on the growth media and increases with incubation period (72 – 120 h).	(Kumaresapillai et al., 2011)
Aspergillus niger MTCC strain 2208	N.d.	21.2 – 26.1	85	The yield depends on the growth media and increases with incubation period (72 – 120 h).	(Kumaresapillai et al., 2011)
Penicillium chrysogenum	5.7	N.d.	84	Very low cost since it is a by-product of the antibiotic industry. A large amount of *P. chrysogenum* mycelia are disposed of by incineration.	(Tianwei et al., 2002)
Basidiomycota					
Agaricus bisporus SMR 13	1	N.d.	91	Limited due to lower yields.	(Di Mario et al., 2008)
Agaricus bisporus	2.5	N.d.	96	Direct deacetylation.	(Hassainia et al., 2020)
Agaricus bisporus	41	N.d.	82.7	Deacetylation of chitin after deproteinization and acid hydrolysis.	(Hassainia et al., 2020)
Agaricus bisporus	15.4	N.d.	N.d.	One of the highest yields reported in literature.	(Sousa et al., 2025)
Auricaularia sp.	5.81	N.d.	N.d.	Alkali (sodium hydroxide) and acid (acetic acid) extraction.	(Chang et al., 2019)
Lentinus edodes no. 1	3.3	N.d.	86.5	Cultured on potato dextrose broth. Alkali and acid extraction.	(Pochanavanich & Suntornsuk, 2002)
Ganoderma lucidum	8.3	N.d.	80.3	Ultrasound extraction of chitin. Deproteinization with NaOH.	(Mesa Ospina et al., 2015)
Mucoromycota (used to be under Zygomycota)					
Rhizopus oryzae TISTR3189	13.8	87.9	N.d.	Early research, less common today.	(Pochanavanich & Suntornsuk, 2002)
Rhizopus oryzae 0263	4.43	20.7	N.d.	Alkali (sodium borohydride, sodium hydroxide) and acid (acetic acid) extraction.	(Tan et al., 1996)
Rhizopus oryzae		~45 – 61.1	90.2	Solid-state biomass. Maximum biomass achieved after 12 days of incubation. Maximum chitosan concentration after 9 days. Alkali and acid extraction.	(Khalaf, 2004)
Rhizopus sp. KN01	19.0	36	N.d.	Alkali and acid extraction.	(Nadarajah et al., 2001)
Rhizopus sp. KN02	21.3	66.7	N.d.	Alkali and acid (hydrochloric, formic, or acetic acid) extraction.	(Nadarajah et al., 2001)
Absidia caerulea	11.5 – 27	Up to 57.5	N.d.	Highest value obtained with 36 h hours of cell harvest time (vs 24 and 48 h).	(McGahren et al., 1984)
Absidia glauca	5.37	23.59	N.d.	Alkali (sodium borohydride, sodium hydroxide) and acid (acetic acid) extraction.	(Tan et al., 1996)
Mucor sp. KN03	25	37.9	N.d.	Alkali and acid extraction.	(Nadarajah et al., 2001)
Mucor rouxii	4 – 8	10 – 39	89 – 100	Alkali and acid (hydrochloric, formic, or acetic acid) extraction. Highest yield with hydrochloric acid.	(Satari et al., 2016; White et al., 1979)
Mucor hiemalis	5.77	19.99	N.d.	Alkali (sodium borohydride, sodium hydroxide) and acid (acetic acid) extraction.	(Tan et al., 1996)
Gongronella butleri USDB 0489	4.73	31.68	N.d.	Alkali (sodium borohydride, sodium hydroxide) and acid (acetic acid) extraction.	(Tan et al., 1996)
Gongronella butleri USDB 0201	5.74	22.56	N.d.	Alkali (sodium borohydride, sodium hydroxide) and acid (acetic acid) extraction.	(Tan et al., 1996)

178 * Presented as a percentage of dry mycelia cell weight.
179 ** Strain name.
180

Properties of the Substance

The 2020 technical report *Chitosan (Crops)* describes the general properties of chitosan, derived from invertebrate animals such as shrimp and crabs (NOP, 2020). This information is mostly current although fungal chitosan properties (see Table 3) and production methods might be slightly different. In addition, the use of chitosan in this petition is different from the 2019 petition.

Chitosan presents several interesting functional properties such as antimicrobial activity, antioxidant activity, absorptivity, metal chelation ability, film- and coating-forming ability, flocculation, and biocompatibility (Arfin, 2017; Brasselet et al., 2019; S. Kumar et al., 2020; Ravi Kumar, 2000; Rocha et al., 2017). These functional properties are determined by some chemical and physical attributes, such as (Rocha et al., 2017):

- molecular weight
- degree of deacetylation
- pattern of acetylation
- polydispersity[2]
- purity

Most of the chemical and physical properties described above, as well as chitosan's functional properties mentioned above, strongly depend on the extraction method, source (see Table 2), and polymer structure (Abdel-Gawad et al., 2017; Aranaz et al., 2021; El Knidri et al., 2018; Rocha et al., 2017; Vendramin et al., 2021). Deacetylation (see Chemical extraction methods) decreases the molecular weight, drops the C/N ratio (Krake et al., 2024), and produces free, reactive amino groups ($–NH_2$) that give chitosan a positive charge on multiple sites in acidic media such as wine (Castro Marín et al., 2021; Ravi Kumar, 2000). These amino groups are also responsible for antimicrobial activity, metal chelation, and ability to create derivative compounds (see Action of the Substance) (Ravi Kumar, 2000). In addition to one amino group, a chitosan polymer has two hydroxyl (–OH) groups, with the primary one (at the C6 position) being more reactive than the secondary one (at the C3 site), giving chitosan more reactive sites (see Figure 3) (Ravi Kumar, 2000). The acetamide groups on individual monomers can also be considered functional groups (Aranaz et al., 2021).

The three types of functional groups (amino, hydroxyl, and acetamide) allow for a great number of modifications and the generation of various novel biofunctional macromolecular products that can have the original organization or new organizations (Aranaz et al., 2021; Ravi Kumar, 2000). The amino group is available for reactions with aldehydes and ketones (Schiff's base), chelation of metals, alkylation, sulfonation, carboxymethylation, grafting acetylation, quaternization (Brasselet et al., 2019). The hydroxyl groups enable hydrogen bonding, solubility (to a degree, see Table 3), and chemical modification (*e.g.,* etherification, esterification). The polysaccharides are available for chemical modifications, such as sulfonation, carboxymethylation, phosphorylation, or hydroxyethylation (Aranaz et al., 2021; Ravi Kumar, 2000).

Table 3: Properties of chitosan derived from *Aspergillus niger*

Property	Value/Description	Reference
Physical state and appearance	Solid; powder or flakes; fibrous or granular	(Rinaudo, 2006)
Odor	Odorless or faint shellfish smell (depends on purity)	(Ravi Kumar, 2000)
Taste	Bitter	(No et al., 2007)
Color	Variable, mostly white to off-white, sometimes brown to orange	(Krake et al., 2024; Shahidi & Abuzaytoun, 2005)
Molecular weight	Variable, 6.6 - 560 kDa	(Hussain et al., 2025)
Specific gravity	~1.35–1.40	(Khor, 2001)
pH	Weak base; pH of 1% solution ~6–7	(Rinaudo, 2006)
Solubility	Insoluble in neutral water; soluble in dilute acids (acetic, formic, lactic); soluble in hot sulfuric acid, not cold	(Rinaudo, 2006)
pKa	~6.3 – 6.5 (primary amine)	(Roberts, 1992)
Boiling point	Not applicable; thermal degradation at high temperatures; decomposes before boiling	(Khor, 2001; Rinaudo, 2006)
Melting point	Decomposes ~220 – 260 °C	(Rinaudo, 2006)
Critical temperature	Not applicable for a polymeric solid	
Vapor pressure	Negligible	
Stability	Stable under dry, cool conditions; hygroscopic	(Rinaudo, 2006)
Reactivity	Reacts with acids to form salts; amino groups enable derivatization	(Roberts, 1992)
Nitrogen content	5-8%	(Ravi Kumar, 2000)

In neutral pH solutions (*e.g.,* deionized water), the hydroxyl and amino groups in chitosan form strong inter- and intramolecular hydrogen bonds that give it a stable semicrystalline structure (Sogias et al., 2010). This structure prevents water molecules from entering and dissolving the chains above pH 6, and chitosan typically precipitates

[2] Polydispersity refers to the variation in size, shape, or molecular weight within a population of particles or macromolecules.

from the solution. In acidic conditions below pH 6, such as wine (pH 3-4), chitosan's amine groups can be partially protonated, corresponding to the material's pKa value, causing repulsion between positively charged macrochains, and allowing water molecules to diffuse and macromolecules to dissolve (Hussain et al., 2025; Sogias et al., 2010). Removing acetyl groups (deacetylation) increases solubility by breaking the hydrogen bonding causing the crystalline lattice structure (Mourya et al., 2011; Novikov et al., 2023; Roberts, 1992).

Specific Uses of the Substance

Petitioned Use

Chitosan from *Aspergillus niger* is petitioned for use as "a nonagricultural (nonorganic) substance permitted in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))." The petitioner requested its addition to the National List § 205.605(b) so that chitosan may be used as a processing aid in organic wine production (Enartis USA Inc., 2024). Winemakers use chitosan for:

- clarification
- filtration
- stabilization and preservation
- enzyme and flavor enhancement

The petitioner bases their request on chitosan's ability to achieve the following objectives (Enartis USA Inc., 2024):

- preventing microbial contamination and oxidation, thereby maintaining lower levels of sulfur dioxide
- eliminating spoilage microorganisms such as *Brettanomyces bruxellensis*, known for causing off-flavors
- removing oxidative precursors like catechins and inhibiting enzymes such as laccase that contribute to wine spoilage
- chelating pro-oxidant metals like copper and iron, thus reducing the potential for oxidation

The uses and action mechanisms of chitosan are detailed in Action of the Substance. In brief, chitosan achieves the objectives above by controlling microbial growth and spoilage microorganisms, removing contaminants (ochratoxins) and preventing oxidative browning caused by enzymes and metals (Lárez Velásquez, 2023).

Other Uses

Other uses for chitosan have been discussed in the 2020 *Chitosan (Crops)* technical report (NOP, 2020). Chitosan and chitosan-based substances are used in a wide range of food processing and other applications. The beverage industry utilizes chitosan and its derivatives for (Castro Marín et al., 2021; Rocha et al., 2017; Sharkawy et al., 2020):

- clarification
- preservation (including microbial control)
- encapsulation
- product packaging (*e.g.,* wine, beer, fruit juice, tea, etc.)

Due to its antioxidant and antiradical functions and biological origin, researchers have developed other uses of chitosan in food processing (Castro Marín et al., 2021). Examples include chitosan coatings and films for processed foods to enhance food preservation (S. Kumar et al., 2020). Chitosan is also used in pickering stabilizers due to its biodegradable, biocompatible, and nontoxic properties (Sharkawy et al., 2020).[3] In addition, chitosan is inexpensive and easily converted into products of different forms and sizes (Rocha et al., 2017). Product forms include aqueous acid solutions, powders, hydrogels, and films or membranes, beads, and nano/micro particles (No et al., 2007; Rocha et al., 2017).

Besides those uses in the food industry, chitosan and its derivatives have a wide variety of uses and applications in the biomedical, industrial, environmental, and agricultural fields. For example:

- Agriculture: antimicrobial agent (fungicide); promotion of plant growth; fruit and vegetable coating. When applied to produce, chitosan forms a semipermeable film coating on the surface, thereby reducing respiration rate, decreasing weight loss, maintaining the overall produce quality, and prolonging its shelf life (Romanazzi et al., 2017). Chitosan is also used as an inert ingredient in pesticides, as described in the 2004 *Poly-D-Glucosamine (Chitosan, Crops)* (NOSB, 2004) and 2020 *Chitosan (Crops)* technical reports (NOP, 2020).
- Water treatment: flocculant/coagulant in water purification; antimicrobial agent (Ahmed & Ikram, 2017); wastewater treatment (Kim, 2010).

[3] Pickering emulsions are a type of emulsion stabilized by solid particles adsorbed at the interface between two immiscible liquid phases, rather than by traditional surfactants. These emulsions can be water-in-oil or oil-in-water, and the particles' ability to stabilize the emulsion depends on their wettability in both phases (Polotti, 2020).

- Industrial applications: separation membranes; cosmeceuticals; biocomposites and thickeners imparting increased viscosity and gel consistence to liquid systems (Ahmed & Ikram, 2017).
- Biomedical applications: drug formulations (anticancer, antidiabetic, cholesterol lowering, veterinary medicine) (Kim, 2010); drug and vaccine delivery (Hussain et al., 2025; Kantak & Bharate, 2022); nano-particle gene therapy; DNA and siRNA carriers; antibacterial in oral hygiene (Kim, 2010); wound healing and tissue (bone, cartilage, cardiac, corneal, periodontal) regeneration (Desai et al., 2023).

Approved Legal Uses of the Substance

Wine makers use chitosan derived from *Aspergillus niger* as a clarifying agent. The U.S. Food and Drug Administration (FDA) and the Alcohol and Tobacco Tax and Trade Bureau (TTB) regulate the relevant legal uses of this substance (US FDA, 2023a).

Standard of identity for chitosan under FDA

The FDA does not include a standard of identity for chitosan in regulations.

GRAS affirmation for fungal chitosan under FDA

The FDA does not list chitosan as a GRAS material in regulations. However, in 2011, the FDA released GRAS Notice No. GRN 397 pertaining to the use of chitosan from the fungus *Aspergillus niger* (US FDA, 2011). This notice was in response to a query by KitoZyme SA, Herstal, Belgium related to their product "KitoZyme." The FDA noted the intended use "as a secondary direct food ingredient in alcoholic beverage production at levels between 10 and 500 grams per hectoliter (100 liters)" (equal to 100 – 5000 ppm). The FDA had "no questions" regarding the stated use of chitosan from *Aspergillus niger*, meaning the agency did not object to the company's claim that this chitosan product was Generally Recognized as Safe (GRAS) under the specified conditions of use. The petition rereferred to this material as a "food additive" (Enartis USA Inc., 2024). According to the FDA, a "secondary direct food additive" is a substance "that has a technical effect in food during processing but not in the finished food (*e.g.,* processing aid)" (21 CFR part 173).

In July 2021, the FDA issued GRAS Notice No. GRN 997 in response to a letter from Chinova Bioworks Inc. (US FDA, 2021). Again, the FDA did not have any questions about the assertion made by Chinova Bioworks that chitosan from white button mushroom (*Agaricus bisporus*) is GRAS. In this case, Chinova Bioworks specifically noted that chitosan was GRAS for use as a food ingredient for addition to a number of food and beverage products across multiple categories, including alcoholic drinks. They proposed a maximum level of 0.04 of g/100 g for alcoholic drinks.

In February 2022, the FDA issued an amendment to GRAS Notice No. GRN 997, which restricted the amount of chitosan for alcoholic cocktails to 0.02 g/100 g (*i.e.,* 200 ppm) (US FDA, 2022). In December 2023, the FDA published a follow-up letter to their GRAS Notice No. GRN 997 in which the FDA did not oppose the use of chitosan in wine and alcoholic drinks at a maximum level of 0.04 g/100 g (*i.e.,* 400 ppm), which was the level mentioned in the original July 2021 notice and the company letter (US FDA, 2023b).

Specifications for chitosan by the Alcohol and Tobacco Tax and Trade Bureau

The Alcohol and Tobacco Tax and Trade Bureau (TTB) allowed chitosan derived from *Aspergillus niger* as a "wine treating material" as "acceptable in good commercial practice" (TTB, 2025). The material is approved "to remove spoilage organisms such as Brettanomyces from wine, and for clarification, fining, and removing off flavors from wine and juice." The amount used must not exceed 500 grams per 100 liters (5000 ppm) of wine per GRAS Notice No. GRN 397. A final rule published in August 2022 reiterated this rate of 500 grams per 100 liters. This decision was made after reviewing the FDA GRAS Notice No GRN 397, and consulting with stakeholders such as the Wine Institute and European Union (for trade purposes) (TTB-185, Wine Treating Materials and Related Regulations, 2022).

Specifications for chitosan in the Food Chemicals Codex

The 14th edition of the Food Chemicals Codex defines the following specifications for chitosan:

> **Description:** Chitosan occurs as a white to light yellow powder. It is an unbranched binary polysaccharide consisting of *N*-acetyl-d-glucosamine and d-glucosamine units linked in a β(1→4) manner. Chitosan is obtained by partial deacetylation of chitin, which is extracted from the shells of edible shrimps and crabs suitable for human use.
>
> **Function:** Antimicrobial; stabilizer; acidity regulator
>
> **Identification:** The degree of deacetylation is 70.0%–95.0%

Apparent average molecular weight and molecular weight distribution: The values of apparent weight-average molecular weight and polydispersity are 85%–115% of the respective values claimed by the manufacturer.
Arsenic (as As): Not more than 0.5 mg/kg.
Cadmium (as Cd): Not more than 0.2 mg/kg.
Chromium (as Cr): Not more than 1 mg/kg.
Lead (as Pb): Not more than 0.5 mg/kg.
Mercury (as Hg): Not more than 0.2 mg/kg.
Nickel (as Ni): Not more than 1 mg/kg.
Iron (as Fe): Not more than 10 mg/kg.

Specifications for chitosan under the Flavor and Extract Manufacturers Association of the United States (FEMA)
As of 2022, the Flavor and Extract Manufacturers Association of the United States (FEMA) lists chitosan as GRAS flavoring substance under number 4946 (Cohen et al., 2022).

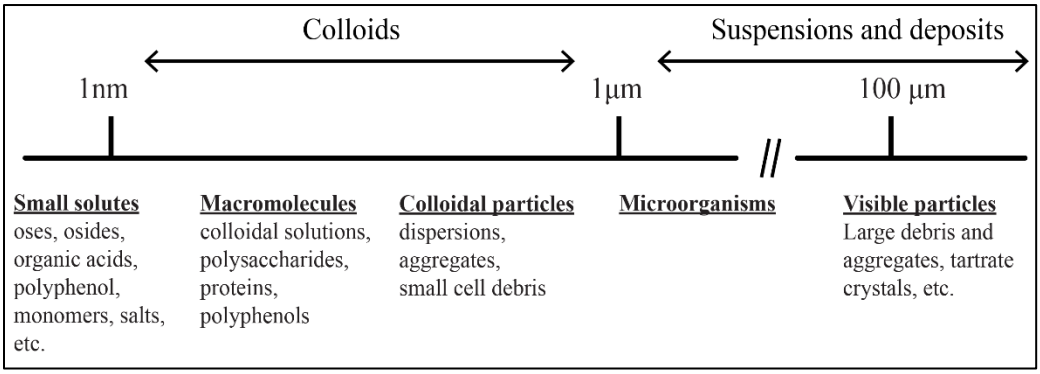
Action of the Substance

Chitosan is involved in four types of winemaking processes: clarification (fining), preservation, enzyme enhancement and contaminant removal. After use, chitosan and its floccules, resulting from the processes detailed below, are removed from the wine by simple racking or filtration, leaving no unwanted residue (Anonymous, 2025; Paulin et al., 2024).

Wine clarification
Clarification is a crucial process in winemaking that improves the wine's visual appeal and prevents unwanted changes to flavor and aroma. Fining agents are added to wine to selectively remove unwanted components that can negatively impact visual clarity, stability, or sensory attributes (Y. Kumar & Suhag, 2024). Clarification involves removing unstable proteins or other insoluble, suspended, and colloidal particles and macromolecules (see Figure 5). These compounds create turbidity and can denature or aggregate over time, causing haziness in wine after bottling (Cosme et al., 2020; Mierczynska-Vasilev & Smith, 2015). Haze formation can be caused by:

- Pathogenesis related proteins: They include chitinases and thaumatin-like proteins associated with grape ripening and berry softening, and constitute a defense mechanism against fungal attack. The constitutive levels of these proteins are typically low in healthy plants, but the proteins are induced in response to wounds or pathogen attack (van Loon, 1985). These proteins can precipitate, especially in white wines and at higher pH levels. These proteins are positively charged at wine pH (3.0 – 3.6), leading to their aggregation. These proteins survive the winemaking process, and can aggregate when wine is exposed to unfavorable environmental conditions, such as heat (Dawes et al., 1994) during transportation and storage, resulting in the appearance of a haze (Waters et al., 2005).
- High levels of sulfate. Excessive sulfate concentrations decrease the solubility of proteins in water and promote their denaturation and aggregation (Pocock et al., 2007).
- High concentrations of phenolic compounds: These contribute to the color, flavor, and mouthfeel of wine. However, phenolic compounds can undergo oxidative reactions leading to browning, bitterness, and astringency, especially in red wines. These include tannins, phenolic acids/esters, and small flavonoids (Y. Kumar & Suhag, 2024).

Figure 5: Wine constituents before clarification and their size range. Adapted from Vernhet (2019).



Chitosan is polycationic, carrying multiple positive charges. This enables it to be an effective clarifying agent in acidic media since its positive charges interact with negatively charged compounds (like sulfate) through electrostatic interactions (Rocha et al., 2017). Chitosan can also remove other haze-active molecules (*e.g.,* polyphenols, proteins rich in proline, polysaccharides, and metal ions) (Rocha et al., 2017). This occurs

through hydrogen bonding and van der Walls forces with chitosan's functional groups below, as further explained in *Properties of the Substance* (Castro Marín & Chinnici, 2020):

- hydroxyl (-OH)
- amino ($-NH_2$)
- acetamido ($-CONH_2$)

Gassara et al. (2015) evaluated the flocculation effects of chitosan and a traditional mixture of gum arabic/bentonite in beer production. They found that chitosan (0.005 g/L) was more effective in flocculating colloidal particles in the beer than gum arabic/bentonite at (0.8 g/L of gum arabic + 0.14 g/L of bentonite). They attributed the result to chitosan's high positive charge density.

Castro Marín and Chinnici (2020) used *Aspergillus niger* chitosan (80–90% deacetylated, 10–30 kDa molecular weight) at 0.5 g/L (in powder or solution forms) to evaluate its effects on physicochemical features of 'Sangiovese' wine. They found slightly lower concentrations of phenolics such as hydroxycinnamic (caftaric, caffeic, and coumaric) acids following chitosan treatment. They also noted a 40% drop in ellagic acid concentration. The authors also observed a decrease in anthocyanins and flavonols responsible for the wine's reddish and bluish pigmentation after the addition of chitosan powder or solution of (Castro Marín & Chinnici, 2020). Chitosan attaches to these compounds by cooperative adsorption allowing hydroxycinnamates to attract molecules already adsorbed on chitosan surface. They concluded that chitosan does not substantially impair the overall physical-chemical features and quality of wines.

Chitosan from both invertebrate animal and fungal sources can be an effective clarifying agent. For example, Rungsardthong et al. (2006) compared shrimp and *Absidia glauca* var. *paradoaxa* (fungal) chitosan effects on apple juice clarification. They observed that clarity and color changes of the apple juice were closely correlated for both fungal and shrimp chitosan sources. The fungal chitosan was more effective in reducing the apple juice turbidity than the shrimp chitosan.

Wine stabilization and preservation

Wine preservation refers to the prevention of oxidative browning due to chemical or enzymatic reactions and microbial degradation. Oxidative reactions that can occur during the fermentation processes alter many wine components such as (Lárez Velásquez, 2023):

- phenolic compounds
- certain metals
- sugars
- lipids
- amino acids
- aldehydes

These changes can deteriorate sensory properties such as flavor and aroma, increase astringency, alter the color, and decrease the nutritional value (Lárez Velásquez, 2023).

Chitosan helps preserve wine (and juice) by acting as an antioxidant (Petriccione et al., 2018) and antimicrobial compound (Kong et al., 2010) (see Figure 6). Its antimicrobial effects have been observed on a wide spectrum of microorganisms, including the following:

- Brettanomyces (major wine spoilage yeast) (Taillandier et al., 2015). Fungal chitosan (0.8 g/L) effectively inhibits *Brettanomyces bruxellensis*, a major cause of off-flavors (*e.g.,* "barnyard" or "band-aid" aromas) in wine.
- Zygosaccharomyces (spoilage yeast in beverages) (Roller & Covill, 1999). This group of yeasts is known for causing refermentation and gas production in sweet wines and juices. Chitosan can help control their growth, particularly in low-alcohol or high-sugar environments.
- Acetobacter (vinegar-producing bacteria) (Valera et al., 2017). Chitosan suppresses *Acetobacter* spp., which can oxidize ethanol into acetic acid, leading to wine spoilage. Chitosan is at least as effective as sulfur dioxide, the standard clarification/fining agent material.
- Lactic acid bacteria (Castro Marín et al., 2021). Chitosan helps manage the following lactic acid bacteria populations, especially in certain wines (*e.g.,* sweet, sparkling base wines, crisp aromatic whites) where malolactic fermentation is undesired:
    - *Oenococcus oeni* (used in malolactic fermentation but can spoil wine if uncontrolled)
    - *Pediococcus* (can produce biogenic amines and viscous spoilage)
    - *Lactobacillus* (can cause off-flavors and turbidity)

Three mechanisms have been proposed to explain the antimicrobial (bacteria, yeast, molds) activity by chitosan and its derivatives (Kong et al., 2010; Rocha et al., 2017):

- Cations from chitosan interact with the anionic groups on the microbial cell walls (Li et al., 2022). These cations may block the transport of essential substances to the interior of cells and may damage the cell wall or cell membrane (see Figure 6). In addition, chitosan oligosaccharides with a high molecular weight exhibit antifungal activity. The microbial cell wall perceives chitosan as an analog of its own chitin/chitosan cell wall fragments and incorporates it into the fungal cell wall organization, which impairs the cell integrity (Li et al., 2022).
- Chitosan may inhibit RNA synthesis and disrupt protein synthesis. As a result, the cell membrane becomes more permeable, causing further disruptions to cellular functions that may lead to cell death.
- Chitosan forms complexes with nutrients and metals, decreasing their bioavailability. This deprives microorganisms of elements required for toxin production.

However, depending on concentration, not all chitosan sources are equally effective antimicrobial compounds. Tedesco et al. (2025) conducted a study to compare three chitosan products from different sources (shrimp, fungi, and insects) at four concentrations (12.5, 25, 50 and 100 mg/L) for effectiveness against five strains of *Brettanomyces bruxellensis*. This wine spoilage yeast is responsible for producing volatile phenols (4-ethylphenol and 4-ethylguaiacol) that lead to undesirable sensory defects (*i.e.,* barnyard, horsey, band-aid, or medicinal flavors) commonly referred to as "Brett" character. Shrimp chitosan and insect-based chitosan samples induced rapid and severe membrane damage in *Brettanomyces bruxellensis*, whereas fungal chitosan presented a slower effect. At the highest concentration, all chitosan products were equally effective in reducing the yeast population. Fungal chitosan, however, began to lose effectiveness at 50 mg/L and it had no effect on cell growth when the concentration was further reduced to 25 mg/L, with cell numbers, measured as OD_{600} being similar as the untreated control, and more than double those treated with shrimp chitosan.[4] In addition, fungal chitosan effect on the strains was variable with some strains being more sensitive than others.

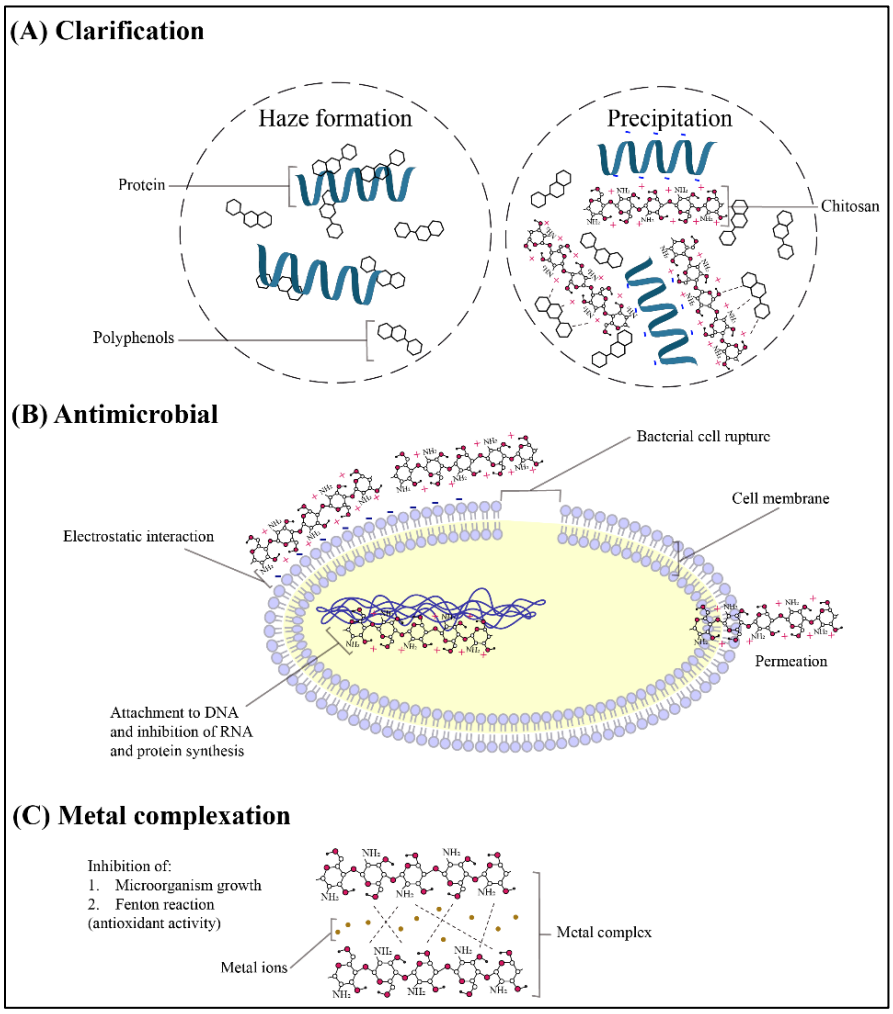
In another study, Filipe-Ribeiro et al. (2018) tested four chitosans (one fungal and three crustacean) and two crustacean chitins, applied to wine with high "Brett" character at 100 mg/L. They found that the addition of chitosan of crustacean or fungal origin decreased the negative sensory phenolic attributes and bitterness in red wines and increased the wines' positive sensory fruity and floral attributes. The authors attributed these results to the chitosan reducing the volatilization of phenolic compounds. Of all chitosans, fungal chitosan was the least effective in reducing 4-ethylphenol (decreased by 14.2%) and 4-ethylguaiacol (decreased by 10.8%), compared to the untreated control. The corresponding reductions caused by crustacean chitosans were 18.5-22.6% for 4-ethylphenol and 23.1-26.1% for 4-ethylguaiacol. They found that the removal of volatile phenols was positively correlated to the degree of deacetylation, with chitins being the least effective. Fungal chitosan, however, was an outlier to that correlation, possibly due to its higher content of glucans (Filipe-Ribeiro et al., 2018).

Variability in antimicrobial effectiveness has also been reported within different fungal chitosans, mostly due to differences in molecular weight. Miot-Sertier et al. (2022) conducted a large study focusing on the antimicrobial effects of two fungal chitosans, varying in molecular weight and viscosity. They experimented on 206 microbial strains from 27 species present in musts and wines. The researchers found that during alcoholic fermentation, chitosan treatment does not seem to be appropriate to limit the musts microbial pressure and does not control acetic acid bacteria. After alcoholic fermentation, however, chitosan was effective against most microbial populations present in both red and white wines, even when used at a low dose of 0.04 g/L. The effects persisted for at least three months. An exception to this were acetic acid bacteria, which were tolerant to the chitosan treatment (Miot-Sertier et al., 2022). Strains of the same species can differ in their susceptibility to chitosan (Taillandier et al., 2015). This may be due to the presence or absence of cell surface charges that allow chitosan to interact (Taillandier et al., 2015).

Chitosan can also improve wine stability by removing acids, such as hydroxycinnamic acids, that cause off aromas (Castro Marín & Chinnici, 2020). Colangelo et al. (2018) reported that a dose of 1 g/L of fungal chitosan was sufficient to remove chitinases and reduced tartaric and malic acids, potassium, iron, and free terpenols from an aromatic wine. In addition, the heat stability of the wine samples fined with chitosan was highly improved compared with a control without added chitosan. The desirable glycosylated terpenols and the fermentative aroma compounds were not affected by chitosan treatment (Colangelo et al., 2018).

[4] OD_{600} is a method used in microbiology to estimate the cell density of a microbial culture by measuring how much light at a 600 nm wavelength is scattered by the cells. It is a quick and convenient way to monitor bacterial or yeast growth in real-time by using a spectrophotometer to measure the turbidity of the culture.

Figure 6: Three modes of action for chitosan in wine: (a) clarifying agent, (b) antimicrobial preservation and (c) metal complexation. Adapted from Rocha et al. (2017).



Enzyme and flavor enhancement

Grape enzymes and added yeast assist in the biotransformation of juice to wine (Hüfner & Haßelbeck, 2017). Chitosan is sometimes added to wine during fermentation to extend enzyme action. Chitosan can bind with anionic regions in enzymes β-D-glucosidase and α-L-arabinofuranosidase, thus entrapping and immobilizing the enzymes to extend their activity. Tavernini et al. (2020) showed that enzymes can retain their full activity after 91 days of incubation under winemaking conditions, which enhanced the wine aroma.

Due to its positively charged NH_3^+ groups, chitosan can entrap enzymes by binding with anionic regions within the enzyme (Tavernini et al., 2020). These researchers added *Aspergillus niger* chitosan beads to entrap cross-linked enzyme aggregates of β-D-glucosidase and α-L-arabinofuranosidase. This entrapment temporarily immobilized the enzymes retaining their full activity after 91 days of incubation under winemaking conditions, which enhanced the wine aroma.

Wine filtration

Winemakers have successfully used chitosan to remove organic and inorganic contaminants (*e.g.,* metals and their salts) from wine (see Figure 5) (Lárez Velásquez, 2023). One contaminant of particular interest is the mycotoxin ochratoxin A (Lárez Velásquez, 2023), due to its carcinogenic potential (National Toxicology Program, 2021). Bornet and Teissedre (2008) found that chitosan, and to a lesser extent, chitin-based treatments, of fungal origin can reduce the levels of iron, heavy metals (lead, cadmium), and mycotoxins (*e.g.,* ochratoxin A) in wine, thereby improving wine safety and quality. Chitosan's ability to remove contaminants increases with the dose, degree of deacetylation, concentration of ferric ions in solution, and with lower pH of the medium (Burke et al., 2000). When added at 2 g/L, chitosan reduced iron in red wine from 23 mg/L to 2 mg/L, while lower concentrations of 0.5 and 0.1 g/L chitosan resulted in 3 and 5 mg/L iron, respectively (Bornet & Teissedre, 2008). The distribution of acetyl and amino groups along the polymer chain plays a role in the effectiveness of chitosan in filtration (Bornet & Teissedre, 2008).

Chitosan can remove metals from solutions by three main mechanisms, depending on the metal, pH and the matrix of the solution (Guibal, 2004):

- Chelation (see below)
- electrostatic attraction (see below)
- physical adsorption (see below)

Chelation/Complexation

Chitosan has amino and hydroxyl groups that can chelate metal ions depending on pH of the solution. The free amines in some of the chitosan repeat units get protonated in dilute acidic solutions and form multiple bonding sites that can chelate metals like copper and zinc (Guibal, 2004). In addition, hydroxyl groups located in position C-3 of chitosan can chelate lead and cadmium (Bornet & Teissedre, 2008). Chitosan's chelation ability depends on its physical state (powder, gel, fiber, film) and its degree of deacetylation (Rinaudo, 2006). Chitin with a higher degree of acetylation is more effective at chelation (Bornet & Teissedre, 2008; Rinaudo, 2006).

Ion exchange/Electrostatic attraction

The presence of ligands in chitosan and the pH of the solution strongly control chitosan's sorption performance (sorption isotherm) and the uptake mechanism. The binding mechanism can change based on the species of metal. Depending on pH, chitosan may stop chelating metals, and instead ionically bond with the metal (Guibal, 2004). The amine groups, which are easily protonated in acidic solutions, may electrostatically attract anionic compounds, including metal anions, anionic ligands, and anionic dyes (Spagna et al., 1996). This force is also largely responsible for the removal of ochratoxins from wine. The protonation of chitosan's amino groups at lower pH causes an ionic binding with ochratoxin's negatively charged ions (Quintela et al., 2012).

Physical adsorption

Chitosan's ability to adsorb heavy metal ions could also be attributed to the deposition of metal hydroxide aggregates in the pores of chitosan particles (Park et al., 1984). Metals can be trapped in chitosan's porous structure via hydrogen bonding or van der Waals forces (Spagna et al., 1996). This mechanism is less effective at removing unwanted compounds from wine than the chelation/ion exchange mechanism but remains significant in some applications. Chitosan efficiently removes iron from solutions, which may occur due to nitrogen and oxygen atoms in the repeat unit of chitosan. These can cause a surface complex to form on insoluble chitosan particles that adsorbs Fe^{+3} ions (Bornet & Teissedre, 2008).

Variation in pH can influence the ability of chitosan to remove iron, lead, and cadmium through physical adsorption. A lower pH (approximately 3.1) helps chitosan be more effective in removing iron in different types of wines. An increase of 1 pH unit to around pH 4.1 reduces the efficiency of chitosan's iron removal about two-fold for white and sweet wines (Bornet & Teissedre, 2008).

The physical adsorption mechanism can also remove organic compounds. Spagna et al. (1996) attributed a drop in phenolic compounds to this mechanism. Gruppi et al. (2024) reported that chitosan adsorption was responsible for a linear decrease in tartaric acid concentration in wine. However, adsorption was less effective on malic acid, due to its higher acid dissociation constant (pKa).

Combinations of the Substance

Chitosan is not a precursor to, or component of, substances on the National List—with the exception that it is an allowed inert in pesticide formulations [see 2020 *Chitosan (Crops)* technical report]. Chitosan is present in the cell walls of many fungi (see Source or Origin of the Substance) and might therefore be present in allowed nonsynthetic substances incorporating fungal ingredients.

The petitioned substance contains ascorbic acid (E300 L), lactic acid (E270), and citric acid (E330) (Enartis USA Inc., 2024). Other chitosan products have added substances. For example, Oenobrett® is a specific combination of a chitosan and a pectinase/glucanase enzymatic preparation that facilitates the lysis and elimination of the *Brettanomyces* spoilage yeast in red wines (Laffort, 2025). Some products contain a combination of different fining agents, such as Super Kleer K.C., a clarifying product for wine and beer containing a mixture of colloidal silica (kieselsol) and shellfish chitosan (Winemakeri, 2025). Another group of commercial chitosan products such as No Brett Inside™ (Lallemand Oenology, 2024) and Chitocel (AEB, 2025) have no added components besides chitosan.

There are many chitosan derivatives (*e.g.,* carboxyalkyl chitosan, propyl chitosan, etc.) prepared for various industrial applications (Pestov & Bratskaya, 2016), although we found no evidence that they are used in winemaking.

Status

Historic Use

There were two previous petitions to include chitosan on the National List, under the Crops scope (see Summary, above).

There is no history of using chitosan in USDA certified organic handling/processing. However, chitosan of fungal origin is approved in organic winemaking in the European Union and the UK as a clarifying agent since 2009 (International Organisation of Vine and Wine, 2009). It is approved for enological use (yeast/bacteria control) to achieve the following objectives with the maximum doses in parentheses (International Organisation of Vine and Wine, 2009):

1. Reduce heavy metal content, notably iron, lead, cadmium, copper (1 g/L).
2. Prevent iron haze, copper haze (1 g/L).
3. Reduce possible contaminants, especially ochratoxin A (5 g/L).
4. Reduce undesirable micro-organisms, notably *Brettanomyces* (0.1 g/L).

In addition to the maximum doses above, the organization sets the following conditions of use for chitosan:

1. Sediments are eliminated by physical procedures.
2. Chitosan of fungal origin may be used alone or together with other admitted products.
3. Chitosan must comply with the requirements of the International Oenological Codex.

The current OIV standards are very similar to the European organic program rules, allowing the addition of chitosan of fungal origin as a processing aid for the purpose of fining musts. The specific objectives are to facilitate settling and clarification and to carry out a treatment to prevent protein haze (International Organisation of Vine and Wine, 2025a).

In the US, fungal chitosan (from *Aspergillus niger*) is allowed for use in winemaking by the Alcohol and Tobacco Tax and Trade Bureau (TTB, 2025). Although the level of adoption and use in winemaking is not clear, chitosan is regularly referred to in extension publications, winemaker meetings (Eastern Winery Exposition, 2023; Rieger, 2019), winemaker magazines (Smith, 2025), winemaker blogs (Crowe, 2024) and winemaking protocols (Ting, 2018), indicating it might be commonly used. Winemakers also use chitosan as a post-harvest disease control agent on grapes (Belcher & Crolley, 2025).

Organic Foods Production Act, USDA Final Rule

The Organic Foods Production Act (OFPA, 1990) does not include any reference to chitosan.

The USDA organic regulations do not currently include chitosan as a non-organic substance allowed for use in organic processing or handling. However, for crop production purposes, USDA organic regulations allow chitosan for use as an inert ingredient in combination with permitted active pesticidal ingredients (OMRI, 2025). Such pesticide formulations may only be used if the requirements of § 205.206(e) are met, which require the use of preventive, mechanical, physical, and other pest, weed, and disease management practices.

International

As with the USDA organic regulations, other international organic standards and guidelines have rules that are specific to the phases of organic production. For each of the standards below, we focus on the allowance of chitosan during the handling or processing phase of production. These materials may have different allowances during other phases of production.

Chitosan is not described by many of the international standards that we reviewed. However, it is described and allowed in some parts of Europe (see below) and Great Britain (see below).

International Organic Food Standards: CODEX Alimentarius Commission—Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999)

Chitosan is not included in CODEX guidelines.

International Organic Agriculture Standards: IFOAM – Organics International (International Federation of Organic Agriculture Movements)

Chitosan is not mentioned by IFOAM guidelines.

Canada: Organic production systems-General principles and management standards (CAN/CGSB-32.310), Organic production systems-Permitted substances list (CAN/CGSB-32.311)
Chitosan is not listed on the permitted substances lists of the Canada organic program (Organic Production Systems Permitted Substances Lists, 2020).

Europe and United Kingdom (Norther Ireland): European Economic Community (EEC) Council Regulation (EC No. 2018/848 and 2021/1165)
Chitosan is approved for use in organic wine in the European Union since 2018 (Implementing Regulation - 2018/1584 - EN - EUR-Lex, 2018). A recommendation was made in 2015 based on a petition to include chitosan to Annex VIIIa to Regulation (EC) No 889/2008 (Expert Group for Technical Advice on Organic Production, 2015). The following text is excerpted from the EU report (page 36):

> "Chitosan seems to have a better ability to eliminate contamination with Brettanomyces yeast than chitin-glucan, whose objective is more anti-oxidant activity… The Group considers now that the use of Chitosan is in line with the objectives, principles and criteria of the organic regulation." (Expert Group for Technical Advice on Organic Production, 2015).

Prior to that, chitosan was approved in European non-organic winemaking in 2011, when the European Union published Regulation (EU) No 53/2011 amending Regulation No 606/2009 to approve the use of "chitosan of fungoid origin" (E1109), among other materials, as a fining agent and antimicrobial treatment in wine, with a maximum dose limited to 1 g/L [Commission Regulation (EU) No 53/2011, 2011]. The EU regulation 53/2011 specifies in Appendix 13 the following areas of application and maximum relevant concentrations and requirements:

a) reduction in the heavy metal content, particularly iron, lead, cadmium and copper, (1 g/L)
b) prevention of ferric casse and copper casse; (1 g/L)
c) reduction of possible contaminants, especially ochratoxin A; (5 g/L)
d) reduction in the populations of undesirable micro-organisms, in particular *Brettanomyces*, solely by means of treatment with chitosan (0.1 g/L)

The dose levels to be used are determined after a qualification test. Sediments are removed using physical processes [Commission Regulation (EU) No 53/2011, 2011].

In 2021, the EU published an amendment allowing the use of "Chitosan derived from *Aspergillus niger*." Also, "Chitosan hydrochloride" was allowed with the specific condition "obtained from *Aspergillus* or organic aquaculture or from sustainable fisheries, as defined in Article 2 of Regulation (EU) No 1380/2013 of the European Parliament and of the Council" (Authorised Products and Substances for the Production and Conservation of Organic Grapevine Products of the Wine Sector, 2021).[5]

Japan: Japan Agricultural Standard (JAS) for Organic Production
Chitosan (from any source) is not included in Annex B [Additives (for Alcohol Beverages)] of the Japanese Agricultural Standard for Organic Processed Foods (Japanese Agricultural Standard, 2024).

Switzerland: Federal Office for Agriculture (FOAG), Switzerland Organic Ordinances, Organic Farming Ordinance (SR 910.18), EAER Ordinance on Organic Farming (SR 910.181), FOAG Ordinance on Organic Farming (SR 910.184)
As of January 1, 2019, chitosan derived from *Aspergillus niger* is listed under Annex 3b, *Products and substances allowed for winemaking* of the Swiss Organic Farming Ordinance for use in organic wine processing without further annotation (Ordonnance du DEFR sur l'agriculture biologique, 2018).

Taiwan: Organic Agriculture Regulations
Chitosan is not listed in the Taiwan organic regulation (Certification Standard for Organic Agricultural Products and In-Conversion Agricultural Products and Allowable Substances in Their Production, Processing, Packaging, Distribution, and Sale, 2019).

United Kingdom (Great Britain): Organic Products Regulations (2009), Retained Council Regulations (EC) (834/2007, 889/2008, and 1235/2008)
As of June 2025, chitosan derived from *Aspergillus niger* is listed under GB 6.9.3 *Additives and processing aids* for use in organic wine clarification without further annotation (Organic Standards for Great Britain, 2025).

[5] Chitosan hydrochloride (chitosan HCl) is a water-soluble derivative of chitosan with many applications, including in pest management.

Evaluation Questions

Classification of the Substance

Evaluation Question #1**(A)** Describe if this substance is extracted from naturally occurring plant, animal, or mineral sources.

Chitosan is chemically extracted from naturally occurring organisms that contain chitin (Abdel-Gawad et al., 2017; Dhillon, Kaur, Brar, et al., 2013). In some cases, these organisms contain both chitin and chitosan. For example, *Aspergillus niger* is one fungal species that naturally produces chitosan in its cell walls (see Figure 4).

Drake et al. (2024) established methods to determine the total composition of *Aspergillus niger* mycelia. Using the acid detergent fiber method to quantify chitin and acid extraction to determine chitosan content, they observed chitin and chitosan yields of 14.4% and 5.5% of the fungal mycelia dry weight, respectively.

Commercial production of fungal chitosan is entirely done using the alkali-acid method (Hussain et al., 2025) and is discussed in detail in Evaluation Question #1(B) below. Briefly, the production process includes at least one of the following three steps, noting that the last two steps can be done in a variable order (Krake et al., 2024):

- a deproteination step for the removal of protein (using NaOH)
- an acid extraction of freely accessible chitosan (using sulfuric or acetic acid)
- a deacetylation process to convert the remaining purified chitin to chitosan

The chitosan is afterward potentially purified in a solubilization-precipitation process. The steps mentioned above for chitosan production from fungal sources are performed using chemicals such as:

- sodium hydroxide (NaOH)
- disodium phosphate (Na_2HPO_4)
- acetic acid (CH_3COOH)
- sulfuric acid (H_2SO_4)
- hydrochloric acid (HCl)
- formic acid (HCOOH)

Enzymes are also used in some of the production processes.

Evaluation Question #1**(B)** Describe the most prevalent processes used to manufacture or formulate this substance. Include any chemical changes that may occur during manufacture or formulation of this substance.

Fungal chitosan is usually produced by fermenting *Aspergillus niger* on standard culture media such as:

- potato dextrose agar (Tayel et al., 2011)
- Sabouraud dextrose broth (Raphaël & Meimandipour, 2017)
- agricultural byproducts such as apple pomace (Dhillon, Kaur, Sarma, et al., 2013) or pineapple waste (Davis et al., 2024)

The fermentation can be performed using liquid (submerged) or solid media (Dhillon, Kaur, Sarma, et al., 2013). The mycelium material is harvested via filtration or centrifugation and washed to remove residual medium components before extraction of chitin. Chitin is then deacetylated to produce chitosan.

Prior to extraction, processors may disintegrate the fungal mycelium by milling, freeze-drying (Krake et al., 2024), or by applying sodium phosphates mixed with enzymes (Cai et al., 2006). Krake et al. (2024) found that milling can significantly improve the yields of chitosan compared to deproteination alone.

Following disintegration, processors then extract naturally occurring chitosan or chitin from the mycelium using chemical or enzymatic methods (Kozma et al., 2022; Younes & Rinaudo, 2015). Only the chemical methods are used by manufacturers for the production of chitosan (Hussain et al., 2025).

Chemical extraction methods

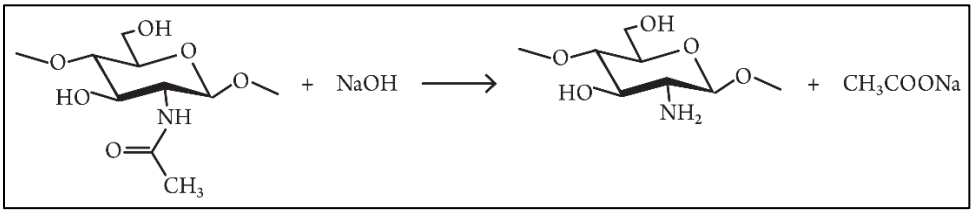
Manufacturers produce chitosan by first breaking up fungal mycelium cells. Then, they remove proteins using sodium hydroxide in a heated solution. Next, they extract chitin, and sometimes a mixture of chitin and chitosan from fungal mycelium using acids. Lastly, they deacetylate chitin to create chitosan. Researchers have used slightly different methods with different concentrations, temperatures, and/or durations (Crognale et al., 2022). Some of the extraction and deacetylation steps have also been reported in different orders (Krake et al., 2024).

Abdel-Gawad et al. (2017) described the following extraction method from *Aspergillus niger*:

1. Cell disruption: Milling of *Aspergillus niger* biomass to make cell constituents more accessible.
2. Deproteination: The milled biomass is mixed with NaOH solution (1:40 w/v) at different concentrations from 1 to 4 M. The different extraction temperatures ranged from 80 to 100 °C, for 1–3 h. The alkali-insoluble material is separated by centrifugation (6000 rpm, 15 min) then washed with distilled water and recentrifuged until reaching neutral pH.
3. Extraction: Isolation of chitin from the alkali-insoluble material by further extraction using 10% v/v acetic acid (1:40 w/v) at room temperature for 6 h on a rotary shaker (200 rpm), after which the acid insoluble residue is discarded by vacuum filtration. Tayel et al. (2011) used 1% acetic acid in their extraction. Other researchers have used sulfuric acid for this step (Dhillon, Kaur, Sarma, et al., 2013; Zamani et al., 2010). Zamani et al. (2010) used a two-step sulfuric acid extraction, with the first one conducted at room temperature to remove phosphates, while another step was conducted at 120° C to extract the chitosan, since they found chitin solubility to be greater in hot sulfuric acid than cold.
4. Deacetylation (see Figure 7): pH is adjusted to 9.0 with 4 *M* NaOH solution. The deacetylation step proceeds rapidly until the polymer is about 75-85% deacetylated, after which further treatment has only a very limited effect on the extent of deacetylation (Roberts, 1992).
5. Purification and drying: The solution is recentrifuged and the precipitated chitosan is washed with distilled water. The material is then dried at 60 °C until reaching constant weight.

The method described in the petition parallels this method, although it does not give any specific details on materials used or concentrations (Enartis USA Inc., 2024).

Figure 7: Deacetylation of chitin to chitosan. Adapted from Novikov et al. (2023)



Enzymatic extraction methods

In 1997, a lab-scale enzymatic method to extract chitosan from *Mucor rouxi* was published by Synowiecki and al-Khateeb (1997). The method uses enzymes lysozyme, snailase, neutral protease, and chitin deacetylase, as well as several chemicals. Another team used glucanase, chitinase, and amylase (Nwe et al., 2010). Enzymatic methods exploit the specificity of enzymes and mild reaction conditions (commonly 25–59°C) to remove proteins with minimal deacetylation and damage to the chitin chain (Kozma et al., 2022). As of 2025, enzymatic methods were still at the laboratory scale and were not being implemented at commercial levels, due to the sensitivity of the processing method (Hussain et al., 2025). Giraldo et al. (2024) reported that protein and mineral removal by enzymatic methods is still insufficient for industrial-scale applications. In addition, enzymatic methods need to increase chitin/chitosan production yield and reduce environmental impacts and enzyme costs for them to become more competitive with chemical methods (Lopes et al., 2018).

Cai et al. (2006) used Synowiecki and al-Khateeb's method for extraction of chitosan from *Aspergillus niger* mycelia waste resulting from citric acid production. This method involves four distinct steps: cell disruption, deproteination, deacetylation and extraction, as follows:

1. Cell disruption: The fresh mycelia (400 g) are suspended in a solution of 0.2 M sodium phosphate buffer (Na_2HPO_4–NaH_2PO_4) at pH 6.4 (120 ml). The solution also contains lysozyme (1 g) and snailase (1 g). The mixture is continuously stirred for 5 h at 50 °C to disrupt the cell wall, and the solid is collected with a centrifuge at 12,000 rpm for 20 min. The solid is washed with demineralized water.
2. Deproteination: The solids resulting from the cell disruption are suspended again in a sodium phosphate buffer at pH 7.0 (1000 ml). Neutral protease (5100 U per 100g of mycelia) is added. The mixture is stirred continuously at 55 °C for 3 h and then the solid is collected with a centrifuge at 12,000 rpm for 20 min. The solid is washed with demineralized water. The objective of this step is to remove glycoproteins, which represent 20-30% of the cell wall components (Garcia-Rubio et al., 2020). This step breaks down disulfide and peptide covalent bonds in proteins, making chitosan more accessible and allowing its direct extraction with acid (Krake et al., 2024).
3. Deacetylation: The solid derived from deproteination is suspended in 50 mM Tris–HCl buffer at pH 7.5 (800 ml). Chitin deacetylase is added, and the solution is stirred continuously at 55 °C for 8 h. This step converts chitin to chitosan. The solid is then collected with a centrifuge at 12,000 rpm for 20 min. The solid is washed with demineralized water.

4. Extraction: To purify the deacetylated chitosan, the solid is dissolved in 2% acetic acid at 30 °C for 16 h. then the mixture is centrifuged at 12,000 rpm for 20 min to remove the remaining solids. The chitosan is precipitated from the acetic acid solution by adding 40% NaOH until pH 9.0 and separated with centrifuge at 12,000 rpm for 20 min.
5. Drying: The precipitated chitosan is washed with demineralized water and 95% ethanol three times and freeze-dried.

The 2020 technical report *Chitosan (Crops)* provides more information on chitosan extraction from crustacean sources (NOP, 2020). Compared with fungal chitosan, extraction of chitosan from crustaceans generally involves two additional steps: demineralization and decolorization. These steps were described by Younes and Rinaudo (2015):

- Demineralization: This step is conducted before the deproteination step to remove the $CaCO_3$ in the shells by reacting them with strong acids such as HCl solutions (concentration ranges 0.6–11.0 M) at room temperatures for 2–3 h, with agitation.
- Decolorization: This step is usually performed using acetone to remove pigments such as *β*-carotene and astaxanthin.

Alternate chemical extraction of chitin without deacetylation

A method to isolate chitin and glucosamine from different mushroom species was described by Grifoll et al. (2024):

1. Deproteination: Researchers generated freeze-dried mushroom flour from fresh, whole mushrooms. They cleaned fresh mushroom samples and subsequently freeze-dried them at −54 °C for 72 h to avoid undesired enzymatic degradation. After grinding using a Retsch ZM200 blender, 4 g of the powdered mushrooms were treated in 1 M NaOH at 80 °C for 120 min at a ratio 1:30 (w/v) under continuous stirring for deproteination.
2. Separation and washing: The solution was centrifuged at 5000 rpm during 10 min, and the supernatant was discarded. The alkali insoluble residue was subjected to washing/centrifugation cycles with demineralized water several times until neutral pH.
3. Chitin extraction: The alkali insoluble residue was freeze-dried at −54 °C for 72 h and stirred in acetic acid (2% v/v) at a ratio of (1:100 w/v) at 95 °C for 6 h. The insoluble material was recovered by centrifugation at 5000 rpm for 10 min, washed several times with demineralized water until pH became neutral, and finally freeze-dried to obtain isolated chitin.

Chitin obtained from such a manufacturing process could vary in its degree of deacetylation. Given that some mushroom species contain naturally occurring chitosan, it could be possible that some "vegetal chitin" products could also contain chitosan. However, we did not find detailed manufacturing processes of commercial vegetal chitin, nor specific information on the degree of deacetylation for such products.

Promising production techniques at an experimental scale

Recent research has been focusing on greener approaches and improving efficiencies to allow commercial scalability. Emerging deproteinization techniques that might become industrially feasible include the use of ionic liquids, deep eutectic solvents, microwave, ultrasound, and pulsed electric field technologies (Pellis et al., 2022).

Evaluation Question #1**(C)** Discuss whether this substance is agricultural or nonagricultural. If the substance is nonagricultural, is it synthetic or nonsynthetic (natural) [7 U.S.C. 6502(22); NOP 5033-1 (Decision Tree for Classification of Materials as Synthetic or Nonsynthetic); NOP 5033-2 (Decision Tree for Classification of Agricultural and Nonagricultural Materials for Organic Livestock Production of Handling)].

Agricultural or nonagricultural classification

Evaluation of chitosan (derived from fungi) against Guidance NOP 5033-2 *Decision Tree for Classification of Agricultural and Nonagricultural Materials for Organic Livestock Production or Handling* (NOP, 2016b) is discussed below.

1. *Is the substance a mineral or bacterial culture as included in the definition of nonagricultural substance at section 205.2 of the USDA organic regulations?*

No, chitin extracted from any fungal source (*e.g., Aspergillus niger* or the white button mushroom *Agaricus bisporus*) is not a mineral or bacterial culture. Chitin is extracted and then chemically or enzymatically deacetylated to form chitosan. Some natural chitosan may also exist in the extracted material prior to the deacetylation process.

2. *Is the substance a microorganism (e.g., yeast, bacteria, fungi) or enzyme?*

No. Chitosan (from all fungal sources) is a polysaccharide (a carbohydrate polymer), derived from chitin, which is extracted from fungi such as *Aspergillus niger* or white button mushrooms.

3. Is the substance a crop or livestock product or derived from crops or livestock?

It depends. When chitosan is extracted from non-crop fungi such as *Aspergillus niger*, no. This fungus is not produced as an agricultural crop. Currently, commercially produced chitosan derived from non-crop fungi such as *Aspergillus niger* should be classified as a nonagricultural substance according to step 3 of the decision tree.

However, another commercial source of chitin and chitosan is common white-button mushroom (*Agaricus bisporus*), which is considered a crop. When chitin is extracted from fungi grown as crops such as the white-button mushroom and subsequently converted to chitosan, yes, the product is derived from a crop. Mushroom-based chitosan is commercially available (Chinova Bioworks Inc., 2025; Huq et al., 2022). However, it still undergoes a chemical deacetylation process.

4. Has the substance been processed to the extent that its chemical structure has been changed?

Yes, for chitosan extracted from any fungal sources. Chitin is naturally present in the cell walls but is usually bound to glucans and proteins. It is chemically extracted to separate it from glucans, proteins and other compounds, then deacetylated to form chitosan using chemical or enzymatic methods.

5. Is the chemical change a result of naturally occurring biological processes such as fermentation or use of enzymes; or a result of mechanical/physical/biological processes described under section 205.270(a)?

No, the deacetylation step is not known to happen naturally in fungi in significant amounts. While chitosan extracted from non-crop fungi should be classified as a nonagricultural substance according to step 3 of the decision tree, chitosan extracted from fungi grown as crops should be classified as a nonagricultural substance according to step 5.

Synthetic or nonsynthetic classification

Evaluation of chitosan against Guidance NOP 5033-1 *Decision Tree for Classification of Materials as Synthetic or Nonsynthetic* (NOP, 2016a) is discussed below.

1. Is the substance manufactured, produced, or extracted from a natural source?

Yes. Chitin (or a mixture of chitin and chitosan) is extracted from fungi such as *Aspergillus niger* and *Agaricus bisporus* (white button mushroom). It is then chemically converted into chitosan through a deacetylation process. Experimentally, researchers have used enzymes to perform the chemical deacetylation process as well.

2. Has the substance undergone a chemical change so that it is chemically or structurally different than how it naturally occurs in the source material?

Yes, while some chitosan occurs naturally in fungi such as *Aspergillus niger* and can be acid extracted, commercial methods use chemicals to convert chitin into chitosan. In white button mushroom, there is very little or no natural chitosan, so all the chitosan produced is achieved through chitin deacetylation.

The degree of deacetylation of the petitioned commercial product (EnartisStab Micro) is not disclosed in the petition (Enartis USA Inc., 2024) or in the technical datasheet (Enartis, 2023). The degree of deacetylation of white button mushroom chitosan produced by Chibio is >90% (Chibio, 2024a).

2b. At the end of the extraction process, does the substance meet all the criteria described at 4.6 of NOP 5033?

Yes. During the chemical extraction process, manufacturers extract chitin or a mixture of chitin and chitosan from fungi. During this part of the process, chitin (or a mixture of chitin and chitosan) is not transformed, and eventually, the extractants are removed. At the end of the process, the synthetic materials used in the alkali deproteination and acid extraction are removed from the final product and have no technical or functional effect in the final product.

However, after the extraction, chitin undergoes further chemical processing to become chitosan.

Experimentally, researchers have used enzymes *prior* to extraction to convert chitin into chitosan.

3. Is the chemical change created by a naturally occurring biological process, such as composting, fermentation, or enzymatic digestion; or, by heating or burning biological matter?

No. While fungi naturally contain some chitosan, commercial chitosan products still rely on chemical deacetylation of chitin. Thus, the material, as commercially produced, is synthetic according to the decision tree.

In experimental chitosan production, deacetylation can be carried out by the enzyme chitin deacetylase. The use of enzymes to produce chitosan in this way is a naturally occurring biological process.

Evaluation Question #1**(D)** Does this substance in its raw or formulated forms contain nanoparticles?

Chitosan does not contain nanoparticles in its raw form as the extraction of chitosan results in a powder with particle size in the micron range (Rinaudo, 2006). However, chitosan nanoparticle compounds have been engineered in

different fields. For example, chitosan nanoparticles are actively researched and intentionally produced in the field of nano-drug delivery and encapsulation using various methods such as ionic gelation, covalent cross-linking, reverse micellar, precipitation/coacervation, and emulsion-droplet coalescence (Garg et al., 2019). Researchers have used chitosan to form metallic nanoparticles with precious metals such as palladium, platinum, gold, and silver (Aranaz et al., 2021).

Evaluation Question #1**(E)** Does this substance in its raw or formulated forms contain ancillary substances?
Chitosan extracted from chitin is in its pure chemical form and does not contain ancillary ingredients. However, the petition mentions that their product contains inactivated yeast, ascorbic acid (E 300) and lactic acid (E 270), all of which are on the National List at § 205.605(a) or (b). Other commercial chitosan products used in wine processing might contain other ingredients (*e.g.,* enzymes, colloidal silica) that also function in wine clarification, as described in *Combinations of the Substance*.

Evaluation Question #1**(F)** Is this substance created using excluded methods?
This substance is derived from *Aspergillus niger*, which is a naturally occurring fungus. The raw material used to extract the petitioned substance consists of *Aspergillus niger* mycelia, a byproduct of the citric acid production process. Both genetically engineered (by radiation) (Show et al., 2015) and non-genetically engineered strains of the fungus are commercially available and used to produce citric acid (Cai et al., 2006). The mycelia of *Aspergillus niger* used in the citric acid production process are a byproduct that manufacturers harvest as feedstock for chitosan extraction (Cai et al., 2006; NOP, 2023). The 2023 technical report on *Citric acid and salts (Handling/Processing)* concluded that the majority of citric acid manufacturers use wild type fungal strains, as well as those produced through classical induced mutagenesis (*i.e.,* mutagenesis caused by exposure to UV light, chemicals, irradiation, or other stress-causing activities). It also noted that the use of organisms developed using excluded methods (*i.e.,* genetic engineering) appears to remain in an experimental phase, and unraveling the microbial origin of each one of the citric acid and citric acid salts in the market requires information that is often not publicly available (NOP, 2023).

Evaluation Question #2: Specify whether this substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices [7 CFR 205.600(b)(5)]. If not categorized as GRAS, describe the regulatory status.
The FDA does not list chitosan as a GRAS material in regulations. However, in 2011, the FDA released GRAS Notice No. GRN 397 pertaining to the use of chitosan from the fungus *Aspergillus niger* in response to a query by KitoZyme SA, Herstal, Belgium related to their product "KitoZyme." The FDA noted the intended use "as a secondary direct food ingredient in alcoholic beverage production at levels between 10 and 500 grams per hectoliter (100 liters)" (equal to 100 - 5000 ppm) (US FDA, 2011). The FDA's notice in this document indicated that they had "no questions" regarding the stated use of chitosan from *Aspergillus niger* (US FDA, 2011). This essentially means that the FDA did not object to the company's claim that this chitosan product was GRAS under the specified conditions of use.

In December 2023, the FDA published a follow-up letter to their GRAS Notice No. GRN 997 in which the FDA did not oppose the use of chitosan in wine and alcoholic drinks at a maximum level of 0.04 g/100 g (*i.e.,* 400 ppm), which was the level mentioned in the original July 2021 notice and the company letter (US FDA, 2023b).

Purpose and Necessity of the Substance

Evaluation Question #3: Describe whether the primary technical function or purpose of this substance is a preservative [7 CFR 205.600(b)(4)].
The primary technical function of fungal chitosan in food processing is a fining agent.

The U.S. FDA defines a "chemical preservative" as "any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties" at 21 CFR 101.22(a)(1)(5). Chitosan used as a fining agent may contribute to the following qualities that may contribute to shelf life:

- reduction of microbial spoilage (Lárez Velásquez, 2023)
- removal of metal ions that catalyze oxidation (Guibal, 2004)
- clarification and colloidal stability (Castro Marín et al., 2021)

For more information on these properties of Chitosan, refer to the Action of the Substance: section of this technical report.

Many international regulatory agencies (*e.g.,* EU, UK, OIV) consider fungal chitosan a preservative that is allowed for use in winemaking. Specific Uses of the Substance details various types of spoilage microbes and the mechanisms by which chitosan disrupts their action.

Taillandier et al. (2015) found that fungal chitosan induced both physical and biological effects on the cells of the main wine spoilage yeast *Brettanomyces bruxellensis*. Chitosan charges attracted yeast cells removing them from the solution and inactivated them by compromising their plasma membranes.

Evaluation Question #4: Will this substance primarily be used to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law)? If so, describe how [7 CFR 205.600(b)(4)].
The definition of flavor enhancers at 21 CFR 170(o)(11) is "Substances added to supplement, enhance, or modify the original taste and/or aroma of a food without imparting a characteristic taste or aroma of its own."

The primary use of chitosan is a fining agent, but it does improve the flavor, color, and texture of wine. The mechanisms of action are described in Action of the Substance. One of chitosan's functions in wine making is to reduce off aromas that can develop during processing, and to enhance the activity of beneficial enzymes. Adding 0.1 g/L chitosan to red wine did not significantly decrease the content of volatile phenolic compounds in the wine, but did decrease their abundance in the wine headspace (Filipe-Ribeiro et al., 2018). This caused a drop in negative phenolic attributes known as "Brett character" (bitterness and persistency), accompanied by a significant recovery of the fruity and floral attributes (Filipe-Ribeiro et al., 2018). Entrapping enzymes in chitosan before addition to wine can also extend the action of fermentation enzymes responsible for the aroma release in wine (Tavernini et al., 2020).

As for nutritive value, we did not find any studies suggesting that the addition of chitosan would contribute to improving nutritive qualities to any appreciable degree.

Chitosan has a small, but still significant, effect on anthocyanins largely responsible for the wine color (Filipe-Ribeiro et al., 2018); however, the impact on non-phenolic nutrients in wine (such as minerals or residual sugars) is negligible, as these are not the target of the fining process.

Evaluation Question #5: Describe any effect or potential effect on the nutritional quality of the food or feed when this substance is used [7 CFR 205.600(b)(3)].
We found no studies that reported the effects of chitosan treatment on the standard nutritional parameters (*e.g.,* vitamins, minerals, fats and carbohydrates) in wine. However, the nutritional value of wine in terms of vitamins, minerals, fats, and carbohydrates is very low. A serving of red wine (5 oz, ~147 g) contains ~125 calories, 0 g fat, ~3.8 g carbohydrates, ~0.1 g protein, and negligible micronutrients (Frey, 2024). These values are even lower for white wine (Atkinson et al., 2021). Wine's primary nutritional value is linked to polyphenolic compounds (Buljeta et al., 2023). Chitosan fining is intentionally used to remove a portion of phenols to improve sensory properties (reduce bitterness and astringency) (Lárez Velásquez, 2023). However, from a winemaker's perspective, this is a controlled trade-off. The goal is not to strip the wine bare but to achieve a balance by removing undesirable harshness while retaining enough polyphenols to maintain the wine's character, color, and a portion of its potential health benefits. The final nutritional impact on the wine in the bottle is a direct result of the fining decisions made during its production.

Chitosan has a strong affinity for larger, more polymerized tannins, which are the primary contributors to excessive astringency. Phenol removal depends on the dose of fining agent used. Testing three crustacean and one fungal chitosans, Filipe-Ribeiro et al. (2018) found that the fungal chitosan applied at 0.1 g/L did not significantly affect phenols in red wine. However, when the dose increased to 5 g/L, there was a significant decrease in the total phenols and flavonoid phenols. For the non-flavonoid phenols, only one of the commercial crustacean chitosans significantly decreased these compounds. The same chitosan decreased individual phenolic acids such as gallic acid (18% decrease on average), catechin (11% decrease on average), trans-caftaric acid (60% decrease on average), and coutaric acid (52% decrease on average).

Environment and Human Health Effects

Evaluation Question #6: List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in this substance [7 CFR 205.600(b)(5)].
The FDA establishes "action levels" for poisonous or deleterious substances that are unavoidable in human food and animal feed (U.S. FDA, 2000). These include aflatoxin, cadmium, lead, polychlorinated biphenyls (PCBs), and many other substances. The FDA uses different action level tolerances for these substances, depending on the commodity. Commodities are largely food items; however, the FDA also includes tolerances for ceramic and metal

items, such as eating vessels and utensils. Chitosan is not included on the list of commodities with action levels (U.S. FDA, 2000).

The Food Chemicals Codex specifies maximum limits on impurities in chitosan: 0.5 ppm arsenic, 0.2 ppm cadmium, 1.0 ppm chromium, 10 ppm iron, 0.5 ppm lead, 0.2 ppm mercury, and 1.0 ppm nickel (US Pharmacopeia, 2025).

Chitosan's ability to remove heavy metals and ochratoxins from wine reduces the negative health effects from these contaminants (Lárez Velásquez, 2023; Quintela et al., 2012). For example, ochratoxin A is linked to increased cancer risk (Abbas et al., 2018).

Evaluation Question #7: Discuss and summarize findings on whether the manufacture and use of this substance may be harmful to the environment or biodiversity [7 U.S.C. 6517(c)(1)(A)(i) and 7 U.S.C. 6517(c)(2)(A)(i)].
Studies evaluating the environmental impact of fungal chitosan production are rare. In addition to the raw fungal material, chitosan production requires chemicals, energy, water, and growing media. The main environmental concerns with fungal chitosan production are the generation of alkali and acid effluents used in the deproteination and extraction steps (Younes et al., 2014).

The petitioner (Enartis USA Inc., 2024) referred to a study that evaluated the effects of two chitosans (undisclosed origin) on soil water stability and wettability (Adamczuk et al., 2021). They found that the chitosan with lower molecular mass, lower viscosity, and higher degree of deacetylation was more effective in increasing the water stability of the soil. The addition of this chitosan after a single wetting-drying cycle caused the water stability to become one to two hundred times higher than that of the soil. The other chitosan had a much smaller effect on the soil wettability.

The petition (Enartis USA Inc., 2024) referred to a publication on chitosan effects on animals (Boamah et al., 2023). Light molecular weight chitosan is used as an alternative to synthetic antibiotics (Hashem & Gonzalez-Bulnes, 2021), and can improve rumen fermentation in cows (Gandra et al., 2016). Feeding chitosan-ensiled grass, at concentrations up to 1% of the diet dry matter, to beef calves improved their dry matter digestibility, neutral detergent fiber, acid detergent fiber, and live weight (Henry et al., 2015).

We were not able to find life cycle analysis studies specific to chitosan derived from *Aspergillus niger*. However, Grifoll et al. (2024) compared chitin extraction from 22 fungal species not including *Aspergillus niger*. The study estimated global warming potential of fungal chitin between 87.5–589.3 kg·CO_2 eq/kg chitin.

Life cycle analysis studies exist for crustacean chitosan (Muñoz et al., 2018; Riofrio et al., 2021; Vicente et al., 2024). Life cycle analysis numbers for crustacean chitosan vary widely, since production conditions usually have very different environmental profiles, raw materials (shrimp, crab, etc.), production locations (locally produced vs. a global supply chain involving different continents), and applications (general-purpose chitosan vs. chitosan for medical purposes) (Muñoz et al., 2018). Riofrio et al. (2021) estimated the global warming potential of crustacean chitosan production in Ecuador at 59.22 kg CO_2 eq/kg chitosan. Vicente et al. (2024) compared three different pathways for crustacean chitin production and three methods for chitosan extraction from chitin, including innovative research-scale techniques (such as deep eutectic solvents using choline chloride). For commercially used techniques, the most significant contributors to the greenhouse gas (GHG) footprint were acids and electricity. The technology with the lowest GHG footprint (184 g CO_2 eq/g chitosan) was conventional solvents, specifically conventional 50% g/g NaOH. In this technique, electricity contributed 46% to the GHG footprint, followed by NaOH (18%), and other components (6%).

The manufacture of fungal chitosan usually uses fungal waste from the citric acid production process. Fungal chitosan production methods avoid mineral de-ashing, so typically do not need HCl for demineralization. However, crustacean chitosan producers can get GHG credits for producing protein as an animal feed byproduct (Vicente et al., 2024), therefore reducing water needs to grow feed crops and avoiding ammonia emissions from composting or landfills (Muñoz et al., 2018).

Manufacturers can improve the efficiency of the chitosan production process to reduce global warming potential by decreasing the chemical amounts and water used in the different steps. The current mushroom solid to NaOH ratio is 1:30, and chitin to acetic acid is 1:100 (Grifoll et al., 2024).

Replacing chemicals with enzymes during the deproteination step could be another way to improve the environmental footprint of chitosan production. Cai et al. (2006) compared enzymatic deproteination with chemical deproteination (hot and cold 4% w/v NaOH). The deproteination rate was highest using the hot 4% NaOH (62.3% versus 59.9% for the enzymatic method). The authors concluded that enzymatic deproteination could replace

the chemical methods (Cai et al., 2006). Manufacturers could also improve their deproteination processes by replacing NaOH with KOH, which is more environmentally friendly (Shahidi & Synowiecki, 1991).

Evaluation Question #8: Describe and summarize any reported effects upon human health from use of this substance [7 U.S.C. 6517(c)(1)(A)(i), 7 U.S.C. 6517(c)(2)(A)(i), and 7 U.S.C. 6518(m)(4)].
The EPA stated that based on all the information available to the agency, there are low risk concerns for human health or the environment if chitosan is intended for use as a minimum risk pesticide (87 FR 67364, 2022).

Reports of health reactions to chitosan are rare. Researchers in Japan reported an immediate anaphylactic reaction after a person's ingested a health food containing fungal-derived chitosan (Kato et al., 2005), cited in Amaral et al. (2016). The patient was diagnosed with a chitosan allergy. In Portugal, Amaral et al. (2016) carried out a study to evaluate the allergic effects of wine treated with fungal chitosan on 13 high-risk individuals with genetic predisposition to shrimp allergies, and six control individuals with no allergies. The allergic participants had a previous history of anaphylaxis after exposure to shrimp and other seafood. The researchers concluded that wine treated with chitosan derived from fungi is unlikely to trigger allergic reactions in patients with shrimp allergies (Amaral et al., 2016).

While fungal chitosan is generally regarded as safe from an allergic standpoint, there is controversy about the allergenicity of crustacean chitosan. Peng et al. (2022) reported a case of allergic reaction to chitosan after an intra-articular injection in a patient's left knee. However, Muzzarelli (2010) argued that chitosan lacks the allergenic protein tropomyosin because it is a deproteinated product, hence it is generally considered safe for medical use, even when it is derived from crustacean sources.

Alternately, some research has shown that fungal chitosan might be helpful with certain health conditions. In mice, a small (five daily doses of 25 µg) direct intranasal application of water-soluble chitin (the precursor for chitosan) attenuated mucus production and lung inflammation induced by the European house dust mite (*Dermatophagoids pteronyssinus*) (Strong et al., 2002). The authors suggested that the immune system responds to chitin microparticles with a Th1-mediated immune response, which has beneficial effects in reducing the symptoms of an allergy. Muzzarelli (2010) published a critical review on the anti-allergic activity of chitosan and chitin. They enumerated several medical benefits from chitin that are related to boosting immunity and anti-inflammatory reactions in addition to depressing allergic responses.

The petitioner (Enartis USA Inc., 2024) referred to chitosan's potential to reduce the need to use sulfur dioxide in wine treatment, commonly found in higher concentrations in white wines than red wines (Jackson, 2000). Exposure to sulfites in food or drinks has been reported to induce a range of adverse clinical effects in sensitive individuals, including headache, dermatitis, urticaria, flushing, hypotension, abdominal pain and diarrhea to life-threatening anaphylactic and asthmatic reactions (Jackson, 2000). Consequently, legislated maximum concentrations of sulfur dioxide allowed in winemaking have been gradually dropping, triggering efforts to find alternatives (Santos et al., 2012). Several studies have shown that chitosan can be a good alternative to sulfur dioxide for microbial stabilization of wine (Picariello et al., 2020; Valera et al., 2017).

Like other filamentous fungi, *Aspergillus niger* should be treated carefully to avoid the formation of spore dust (Schuster et al., 2002). However, compared with other filamentous fungi, it does not present significant concern as an allergen or with regards to mycopathology. Some researchers reported lung infections due to *Aspergillus niger* exposure, but these were in severely immunocompromised individuals (Schuster et al., 2002). Ear infections (otomycosis) due to *Aspergillus niger* invasion of the outer ear canal can occur in tropical areas when there is mechanical damage to the skin. *Aspergillus niger* can produce ochratoxin A, however, Schuster et al. (2002) report that only 3–10% of strains examined have tested positive for production of this mycotoxin under favorable conditions.

Alternatives

Evaluation Question #9: Are there alternative nonsynthetic (natural) source(s) of the substance [7 CFR 205.600(b)(1)]?
As described in Source or Origin of the Substance, fungal-derived chitosan can be extracted from multiple sources. However, commercial production uses the alkali-acid extraction/deacetylation process to convert chitin into chitosan. Enzymatic deacetylation is not used at the commercial scale. We were unable to definitively find alternative nonsynthetic sources for chitosan.

It is possible that there are products that are partially deacetylated through non-chemical means, but which do not meet the Food Chemicals Codex (FCC) required 70% degree of deacetylation threshold. For example, the product Vegetal Chitin from Chibio specifies that it is sourced from oyster mushroom (*Pleurotus ostreatus*) or *Aspergillus*

niger (Chibio, 2024b). Whether this product is nonsynthetic, or whether it meets *some* definitions for chitosan (*e.g.,* degree of deacetylation greater than 50%) but not the FCCs, we do not know. Similarly, the petition and the product from Enartis (Enartis, 2023) do not disclose the degree of deacetylation of their product.

Evaluation Question #10: Describe all nonagricultural nonsynthetic (natural) substances or products which may be used in place of this substance [7 U.S.C. 6517(c)(1)(A)(ii)]. Identify which of those are currently allowed under the NOP regulations.

The current petition requests the addition of fungal chitosan to the National List under § 205.605(b) as a nonagricultural (nonorganic) substance permitted in or on processed products labeled as "organic" or "made with organic (specified ingredients or food group(s))." The petition specifically mentions its use as a processing aid to improve clarification and filterability, to prevent and treat microbially generated volatile off aromas and to improve wine stability by decreasing the microbial load.

There are many fining agents used in winemaking (see Table 4). Regardless of their legal status in organic production, fining agents can be grouped according to their general nature (Kemp et al., 2022; Y. Kumar & Suhag, 2024):

- nonagricultural
  - nonsynthetic
    - earths: montmorillonite, bentonite, kaolinite/kaolin, perlite
    - biological materials: wood charcoal, carrageenan, enzymes, yeast
  - synthetic
    - silicon dioxide
    - polyvinylpolypyrrolidone (PVPP)
    - ascorbic acid
- agricultural
  - plant proteins: wheat gluten, soybeans, lupine, pea
  - animal proteins: gelatin (pork, cow, fish), isinglass (fish), caseins

In terms of nonagricultural nonsynthetic (natural) substances allowed as fining agents or filter aids for wine, the NOP currently allows bentonite, diatomaceous earth, enzymes, kaolin, and perlite. Synthetic substances allowed for the same purposes include sulfur dioxide, activated carbon, and silicon dioxide.

Bentonite (nonagricultural nonsynthetic)

Bentonite clay is a nonsynthetic material allowed for use as a filter aid [7 CFR 205.605(a)(5)]. Bentonites (mainly containing montmorillonite) are the most commonly used fining agent in the wine industry (Kemp et al., 2022) to promote beverage clarity (Redan, 2020). When added to an aqueous solution, bentonite platelets adsorb water and disperse to form a colloidal suspension with a high cation exchange capacity, allowing it to bind to positively charged molecules (Marchal et al., 1995), then gradually separate by gravity (Mierczynska-Vasilev & Smith, 2015). Different types of bentonites exist, mainly based on the type of mineral cation they come with; the main ones being sodium, calcium or magnesium (Marchal et al., 1995). Sodium bentonites more commonly used as fining agents than the other types, as they swell thus presenting greater surface area available to bind to cations (Cosme et al., 2020; Kemp et al., 2022).

One drawback of this material is that it can contain elevated levels of metals and trace elements that can subsequently end up in the processed wine (Catarino et al., 2008; El Youssfi et al., 2023). Nicolini et al. (2004) conducted a study to investigate wine fining with 10 different bentonites (1 g/L) at three pH levels and different timings (before or after fermentation). Bentonite fining resulted in statistically significant increases in concentrations of most elements, but significantly lower levels of copper, potassium, rubidium, and zinc. The addition of bentonite generally increased the arsenic content of wine, regardless of the timing of application (Nicolini et al., 2004). Similar effects were observed by Catrino et al. (2008) who reported significant increases in many toxic elements and heavy metals including arsenic, cadmium, tin, antimony, barium, and thallium.

Another limitation of bentonite is that it will preferentially remove the most positively charged proteins, but is less effective at removing the less positively charged ones (Kemp et al., 2022). Therefore, bentonite alone is not effective at stabilizing certain wines unless used in excessive amounts, resulting in changes to the wine's properties (Kemp et al., 2022).

A few studies provided side by side comparisons of chitosan and bentonite (among other materials) as wine fining agents. One study compared six common fining agents, including shrimp chitosan, and assessed their effects on wine protein haze-forming potential and on the levels of proteins and phenolic compounds in a white wine (Chagas et al., 2012). The authors concluded that bentonite was the most effective fining agent for white wine protein

stabilization. Chitosan was second to bentonite in removing polyphenols from wine and seemed to slightly decrease the potential for haze formation.

Another study, by Quintela et al. (2012), compared several fining agents including bentonite and chitosan) in terms of ability to remove ochratoxin A and effect on wine quality. Bentonite was more effective than chitosan at lower concentrations (0.1 and 0.5 g/L) in ochratoxin A removal than, but chitosan at the higher concentrations (2 and 5 g/L) became more effective than bentonite, reducing ochratoxin A by up to ~67%. However, it strongly affected wine quality parameters (color, polyphenols, etc.) at the higher concentration. Bentonite showed moderate effectiveness at reducing ochratoxin A but came with drawbacks, notably a considerable decrease in color intensity value.

More recently, Arenas et al. (2021) evaluated the effects of sodium bentonite, calcium bentonite, fungal chitosan (among other materials) on chemical attributes (composition of the macromolecular fraction, polysaccharides and proteins, phenolic compounds, chromatic characteristics, and protein stability) of two 'Albariño' white wines. Sodium and calcium bentonite decreased the levels of wine polysaccharides but 16% to 59%, while chitosan decreased them by 60%. Chitosan did not increase the heat stability of both wines, and pathogenesis-related proteins levels remained unaltered.

Carrageenan (nonagricultural nonsynthetic)

Carrageenans are anionic polysaccharides extracted from red seaweed (Glicksman, 1982). Carrageenan is currently classified as a nonsynthetic material allowed for use as a processing ingredient in or on processed products labeled as "organic" or "made with organic [7 CFR 205.605(a)(9)]. Carrageenans are sometimes used as fining agents in the wine industry to promote beverage clarity and heat stability. Ratnayake (2019) conducted a large scale trial to evaluate heat stability of two Chardonnay wines as affected by 11 types of carrageenan and one bentonite added at three different stages (juice, fermentation, wine). Addition of potassium-rich kappa-carrageenan at the wine or fermentation stages was generally more effective than at the juice stage for producing heat-stable wine, with minimal impact on the sensory profile, wine lees, turbidity, and concentration of metal ions compared to that of untreated control wines. Moreover, carrageenans added at the wine stage produce more fruity or floral characters than carrageenans added during fermentation or at the juice stage. Sensory analysis also indicated that wines heat-stabilized with carrageenans had more intense flavor and aroma profiles than wines heat-stabilized with bentonite (Ratnayake et al., 2019).

Arenas et al. (2021) evaluated the effects of fungal chitosan and kappa carrageenan (among other fining agents) on chemical attributes (composition of the macromolecular fraction, polysaccharides and proteins, phenolic compounds, chromatic characteristics, and protein stability) of two 'Albariño' white wines. Overall, kappa carrageenan was more suitable for white wine protein stabilization, having a more desirable impact on the wine macromolecular fraction than the other fining agents. Kappa carrageenan reduced the content of pathogenesis-related proteins and the protein instability in both wines more effectively than chitosan, which did not increase the heat stability of both wines or affect pathogenesis-related proteins levels. Moreover, kappa carrageenan did not affect polysaccharide levels while chitosan decreased their levels by 60%.

Diatomaceous earth (nonagricultural nonsynthetic)

Diatomaceous earth (or diatomite, celite, or kieselgur/kieselguhr) is another material that is frequently used as an inorganic filter aid (Jackson, 2008). Diatomaceous earth is allowed in organic processing as a food filtering aid only at [7 CFR 205.605(a)(10)].

Diatomaceous earth is made from the silica-rich skeletons of diatoms, which are tiny, fossilized, aquatic organisms (US EPA, 1995). Diatoms accumulate over time in the sediment of water bodies. In order to produce diatomaceous earth, sediments containing large quantities of diatoms are mined and often calcined (heated at high temperature) to get rid of any organic matter and agglomerate the diatoms together. Manufacturers also calcine diatomaceous earth to adjust the particle size distribution for specific applications, such as for use as a medium flow rate filter aid (US EPA, 1995).

Diatomaceous earth is used to build a porous filter cake that will trap suspended solids (*e.g.,* yeast, bacteria, proteins, tartrate crystals, colloids). In addition, in wine's acidic conditions there is an abundance of protons that protonate the hydroxyl groups on the silica surface to give it a positive charge that will attracts negatively charged particles (Mierczynska-Vasilev & Smith, 2015). It thus allows the agglomeration of settled colloidal particles. It is effective in removing hazes left from using other fining agents and in removing cations, such as copper. This fining agent has little effect on flavor and color, and is often effective when other fining agents have failed (Mierczynska-Vasilev & Smith, 2015).

We did not find information comparing the effects of chitosan versus diatomaceous earth when used for wine clarification.

Enzymes (nonagricultural nonsynthetic)

The USDA organic regulations include nonsynthetic enzymes derived from edible, nontoxic plants, nonpathogenic fungi, or nonpathogenic bacteria [7 CFR 205.605(a)(11)]. Enzyme products that are allowed in USDA organic production are commercially available on the market (*e.g.,* VinoClear®).

The International Code of Oenological Practices include the following enzymes for wine clarification (International Organisation of Vine and Wine, 2025b):

- arabinanases
- cellulases
- pectinlyases
- pectinemethylesterase
- polygalacturonases
- hemicellulases
- β-glucanases

The use of enzymes, including some of the ones mentioned above and others, in wine clarification is discussed in the 2024 technical report *Enzymes, microorganisms, and yeast* (NOP, 2024b). We were not able to find side by side comparisons of chitosan and enzymes in wine clarification.

Yeast

Yeast is a nonsynthetic material allowed for use as a filter aid with the following limitations: "When used as food or a fermentation agent in products labeled as "organic," yeast must be organic if its end use is for human consumption; nonorganic yeast may be used when organic yeast is not commercially available. Growth on petrochemical substrate and sulfite waste liquor is prohibited. For smoked yeast, nonsynthetic smoke flavoring process must be documented" [7 CFR 205.605(a)(30)]. The 2024 technical report *Enzymes, microorganisms, and yeast* provides information on yeast use in winemaking (NOP, 2024b).

The petitioner indicated the use of yeast as antimicrobial to "inhibit the growth of indigenous flora on grapes" (Enartis USA Inc., 2024). Lochbühler et al. (2015) tested fining effectiveness of a yeast protein extract and a gelatin reference, in red wine. The yeast protein extract, which contains mannoproteins, caused precipitation of proteins and tannins and maintained the initial quality of the wine, with comparable effectiveness to fining with gelatin.

Sommer & Tondini (2021) carried out a comparison of two *Saccharomyces paradoxus* yeast strains with chitosan for reduction of protein instability in several red/rosé and white wines. They showed that yeasts were slightly more effective than chitosan in fining red wines, although both were not acceptable compared to the results of a bentonite material. For white wines, the yeasts removed 50-60% of protein instability while chitosan removed >60%. Bentonite removed ~90% of protein instability.

Activated charcoal (synthetic)

Activated charcoal (also known as activated carbon) (CAS #s 7440-44-0; 64365-11-3) is a synthetic material allowed for use as a filtering aid [7 CFR 205.605(b)(2)]. The 2024 technical report *Activated charcoal* provides more details on this material as a filtering aid for wine and juice (NOP, 2024a).

Activated charcoal acts like a sponge, binding to weakly polar molecules, especially those containing benzene rings, such as proteins and grape phenolics (Kemp et al., 2022). Activated carbon is more effective at removing pesticide residues from wine than most other fining agents (diatomaceous earth, bentonite, polyvinylpolypyrrolidone) (Ruediger et al., 2004). However, this material can decrease the desirable foaming properties of wine (Marchal et al., 2002).

A direct comparison of activated charcoal with chitosan showed that activated charcoal was more effective at reducing 4-ethylphenol and 4-ethylguaiacol (volatile phenols responsible for "Brett" aroma in the wine headspace) than chitosan applied at 0.1 g/L. The percentage of headspace concentration reduction achieved by activated charcoal was 74.1% and 76.9% for the two compounds respectively, versus 36.1% and 16.6% reduction achieved with chitosan, for the same compounds (Milheiro et al., 2017).

Silicon dioxide/synthetic amorphous silica (synthetic)

Silicon dioxide is primarily used in food production in synthetic forms, known as synthetic amorphous silica. It is included on the National List [7 CFR 205.605(b)(29)]. A technical report on silicon dioxide is currently being written.

Colloidal silicon dioxide solutions (also known as kieselsol) consist of negatively charged silica particles (Mierczynska-Vasilev & Smith, 2015). This form of silicon dioxide is used as a clarifying agent to remove haze-forming particles in both musts and wines. When added to wine, the negatively charged silica particles bind to the positively charged proteins. This binding creates larger, heavier complexes that will quickly precipitate, dragging other suspended particles like yeast, bacteria, and unstable phenols with them, resulting in a clear liquid and a compact sediment. The removal of suspended particles makes the wine easier to filter. Mierczynska-Vasilev & Smith (2015) describe combining colloidal silica with other fining agents like gelatin, bentonite, or chitosan for a more effective fining process, even in wines that are otherwise difficult to clear.

Sulfur dioxide (synthetic)
The USDA organic regulations currently include sulfur dioxide (SO_2), for use in wine labeled "made with organic" grapes with total sulfite concentration to not exceed 100 ppm [7 CFR 205.605(b)(35)]. The 2011 technical report *Sulfur dioxide (Handling)* provides details on the use of this material in organic winemaking (NOP, 2011). Sulfur dioxide is the most widely used antimicrobial compound in oenology due to its broad spectrum of action (Ribéreau-Gayon et al., 2006; Tedesco et al., 2025). Even though many of the alternatives to sulfur dioxide are effective antimicrobials in wine, no other physical technique or additive can deliver the efficacy and broad spectrum of action that sulfur dioxide can (both as an antioxidant and antimicrobial). Because of that, Lisanti et al. (2019) argue that the alternative methods should be considered a complement rather than a substitute for sulfur dioxide in low-sulfite winemaking. However, the popularity of sulfur dioxide is decreasing due to growing consumer demand for fewer chemical additives as well as evidence of *Brettanomyces* spp. resistance to its effects (Tedesco et al., 2025).

Chitosan can be advantageous compared to sulfur dioxide at different points in the winemaking process, especially in terms of fining ability. Picariello et al. (2020) compared chitosan with sulfur dioxide in pre- and post-fermentative phases during the production of 'Aglianico' red wine. Two wine batches were prepared: one composed of 100% healthy grapes and one composed of a mixture of 50% healthy grapes and 50% grapes damaged by acid rot. The results indicated that the use of chitosan just after the end of fermentation resulted in a lower occurrence of sulfur dioxide bound to acetaldehyde in finished wines. Furthermore, when chitosan was used in post-fermentative phases, the authors observed a higher content of polymeric pigments and a lower content of tannins that were reactive towards proteins. Overall, chitosan significantly decreased anthocyanins and tannins compared to the untreated control while the addition of sulfur dioxide had no effect (Picariello et al., 2020). In terms of antimicrobial effect, chitosan was slower than sulfur dioxide in controlling non-*Saccharomyces* yeasts (Picariello et al., 2020). However, different results were observed by Valera et al. (2017) who compared chitosan and sulfur dioxide's ability to control two acetic acid bacteria strains, *Acetobacter malorum* and *Acetobacter pasteurianus*. When added to wine, both materials reduced the numbers and metabolic activity of both *Acetobacter* strains. Chitosan was more effective at reducing the bacterial numbers than sulfur dioxide (Valera et al., 2017).

Ascorbic acid (synthetic)
The USDA organic regulations currently include ascorbic acid for use in wine labeled "made with organic" grapes [7 CFR 205.605(b)(6)].

Ascorbic acid can preserve wine longer by reducing oxidation. The combination of ascorbic acid and sulfur dioxide was more effective than a higher concentration of sulfur dioxide alone in preventing negative sensory attributes in white wine exposed to higher oxygen concentration during storage (Morozova et al., 2015).

We could not find published studies that currently offer a direct side-by-side comparison of ascorbic acid and chitosan as fining agents in wine clarification.

Evaluation Question #11: Provide a list of organic agricultural products that could be alternatives for this substance [7 CFR 205.600(b)(1)].
Many materials of proteinaceous origin can be used as fining agents in winemaking. Wine pH (~3–4) protonates amino groups of fining proteins, giving them a net positive charge and making them efficient at binding with negatively charged phenolic compounds and tannins (responsible for astringency and haze in wine), driving electrostatic binding (Marangon et al., 2019). These agents are either plant-based or animal-based, as described below.

Mushroom chitosan
Mushroom-based chitosan is commercially available (Huq et al., 2022). However, it still undergoes a chemical deacetylation process.

Plant proteins

Proteins used as fining agents in wine come from plants such as (Granato et al., 2018; Marangon et al., 2019; Río Segade et al., 2020):

- legumes (*e.g.,* pea, soy and lentil proteins)
- cereals (*e.g.,* wheat gluten, corn zeins)
- potatoes (*e.g.,* patatin)
- seaweeds (*e.g.,* spirulina)
- grape seeds

Marangon et al. (2019) reviewed plant-based proteins used as wine fining agents and found that several have been successfully commercialized and used by winemakers. For example, pea protein is commercially available on the market with several examples of pea-protein fining agents available on the US market (see Table 5, in the Appendix).

A study compared several plant protein fining agents (soy, lentil, wheat gluten, and three pea protein hydrolysates) with gelatin on a red wine at three levels of ageing (young, 12 months old and 24 months old) (Granato et al., 2018). The authors showed that plant proteins were as effective as gelatin in fining, with each plant protein having a different impact on some specific wine traits although they all had similar effects on wine color. There was little effect of fining agents on varietal aroma components. Individual plant proteins and enzymatic hydrolysates differed in their ability to interact with some anthocyanins, with specific proanthocyanidins complexes, and with some aroma components of fermentative origin. Effects of all the fining agents tested in this study on the anthocyanidin components were most noticeable in young red wine, and decreased markedly with older wine (Granato et al., 2018).

Río Segade et al. (2020) evaluated the effects of plant-based (potato and pea origin) and gelatin wine fining agents on four red wines. They concluded that plant-derived fining agents have variable abilities to reduce phenolic compounds depending on their origin, formulation, dose, and the initial phenolic content and profile of the wine. One pea-based fining agent was comparable to gelatin for the removal of polymeric flavanols with a minor loss of anthocyanins and is therefore better at preserving the wine color in terms of intensity and hue.

A side by side comparison of pea protein and chitosan fining ability in white wine found that both fining agents were statistically similar in terms of their effect on total polyphenols and caftaric acid reduction (Rihak et al., 2022).

Gum arabic

Nonorganic, water-extracted gum arabic may be used as an ingredient in or on processed products labeled as "organic," only when the product is not commercially available in organic form [7 CFR 205.606(j)].

Gum arabic has been used in winemaking for years for its ability to prevent cloudiness, tartaric acid and color precipitation, and to improve wine organoleptic properties (Kyzas & Papageorgiou, 2025). Wang et al. (2023) studied the effect of different doses of gum arabic on wine quality. They reported that 0.2 g/L was more effective at reducing astringency than 0.6 and 1.2 g/L. It also reduced phenolic acids mainly by forming soluble ternary complexes with polyphenols and proteins, and preferentially binding proteins/polyphenols to decrease polyphenol-protein reactions. The higher molecular weight and more numerous and longer branches offered more binding sites that inhibited polyphenol self-aggregation protein binding.

Gassara et al. (2015) evaluated two traditional mixtures of gum arabic/bentonite versus chitosan (undisclosed source), or chitin, in terms of their ability to flocculate beer solids. They found the two gum Arabic/bentonite fining agents less effective than chitosan or chitin. The reduction of total solids in the beer was 32% (w/w) and 46% for the two mixtures versus 65% (w/w) for chitosan, and 64% for chitin.

Gelatin

Gelatin (CAS # 9000-70-8) is widely used as a wine clarifying agent or fining agent to remove suspended particles and excess tannins, improving clarity and stability. It is on the National List [7 CFR 205.606(h)] as a nonorganically produced agricultural product and may be used from a nonorganic source if an organic source is commercially unavailable. Certified organic gelatin is commercially available (Enartis, 2025). The 2019 technical report *Collagen gel, Gelatin and Casings (Handling/Processing)* provides some information on gelatin uses in the food industry, beginning on line 199 (NOP, 2019).

Gelatin is positively charged and colloidal in nature. It attracts tannins, which are primarily negatively charged (Mierczynska-Vasilev & Smith, 2015), forming protein-tannin complexes that precipitate out of the wine (Braga et al., 2007). Gelatin also acts on proteins; however, since it requires tannins to flocculate, its application is limited primarily to red wines (Mierczynska-Vasilev & Smith, 2015). Winemakers have used less gelatin since the mad cow

disease outbreak in 1999 (Kemp et al., 2022). In addition, because of its animal origin, gelatin is a concern for vegans and those avoiding pork or bovine products (Karim & Bhat, 2008).

A side by said comparison of gelatin and chitosan showed that at the same concentration (10 g/hL) gelatin was able to remove more ochratoxin A than chitosan (Quintela et al., 2012). Chitosan concentrations of 200 g/hL or 500 g/hL were needed to achieve higher removal of ochratoxin A than gelatin at 14 or 16 g/hL, respectively. The gelatin had a lower impact on color parameters and polyphenol concentration compared with chitosan, which strongly affected these wine-quality parameters.

Albumin

Egg whites contain a protein called albumin, which winemakers consider a traditional fining agent, especially for premium red wines. This filter aid is especially effective on highly tannic red wines (Kyzas & Papageorgiou, 2025). Albumin is positively charged and can bind to negatively charged tannins and astringent phenolic compounds, thus clarifying and softening wine (Kyzas & Papageorgiou, 2025). After the albumin and impurities coagulate and settle to the bottom, the clarified wine is gently and carefully racked (siphoned off), removing the sediment (Staff of the Australian Wine Research Institute, 2025).

A direct comparison of chitosan (undisclosed source) with egg albumin showed that chitosan at 0.1 g/L was slightly less effective at reducing 4-ethylphenol and 4-ethylguaiacol (volatile phenols responsible for “Brett” aroma in the wine headspace). The concentration reductions in these two compounds with chitosan application were 36.1% and 16.6%, respectively, versus 37.4% and 22.4%, respectively, with egg albumin (Milheiro et al., 2017).

Casein

Casein is the classic wine treatment material used to remove flavonoid and nonflavonoid compounds involved in white wine oxidation (Cosme et al., 2020). The proteinaceous nature of casein allows it to form complexes with metal salts haze particles and tannins, therefore reducing unwanted phenolic substances (Mierczynska-Vasilev & Smith, 2015). We found two sources of organic micellar casein in the Organic Integrity Database (NOP, 2025). Whether these are used by winemakers as fining agents is unclear. We were not able to identify any caseinate fining agent products certified to the USDA organic standards.

Spagna et al. (1996) evaluated four different chitosan products against caseinate (a type of casein) and polyvinyl polypyrrolidone to compare their adsorption effects on phenolic compounds in five white wines. They found that chitosans were more effective in removing total phenolic compounds (28.8-32.9% mean removal for the five different wines) than caseinate (22.4%) and polyvinyl polypyrrolidone (23.9%). However, chitosans (13.9-22%) were far less effective than polyvinyl polypyrrolidone (59.5%) or caseinate (35.7%) in removing proanthocyanidins.

A direct comparison of chitosan (undisclosed source) with potassium caseinate showed that chitosan at 0.1 g/L was more effective at reducing 4-ethylphenol and 4-ethylguaiacol (volatile phenols responsible for “Brett” aroma in the wine headspace). While chitosan was able to reduce the two compounds by 36.1% and 16.6% respectively, potassium caseinate did not affect their concentrations (Milheiro et al., 2017).

Comparison of chitosan with other fining agents (a polyvinylpolypyrrolidone/plant protein/amorphous silica combination, bentonites, albumin, isinglass, gelatin, chitin, carrageenans)

Researchers compared six common fining agents (casein, egg albumin, isinglass, shrimp chitosan, chitin, and polyvinylpolypyrrolidone) and assessed their effects on wine protein haze-forming potential and on the levels of proteins and phenolic compounds in a white wine (Chagas et al., 2012). The authors concluded that bentonite was the most effective fining agent for white wine protein stabilization. Chitosan was second to bentonite in removing polyphenols from wine and seemed to slightly decrease the potential for haze formation.

Quintela et al. (2012) conducted an experiment to compare several fining agents (bentonite, albumin, gelatin, a polyvinylpolypyrrolidone/plant protein/amorphous silica combination, chitin, chitosan) in terms of ability to remove ochratoxin A and effect on wine quality. Below are the main findings of their study:

- Generally, the higher the dosage of the fining agent, the more ochratoxin A removed. However, higher dosages also tended to produce more negative effects on wine quality.
- Chitin (at 0.50 g/L) removed approximately 18% of ochratoxin A. Importantly, this was achieved without significant effects on wine quality (color, polyphenols, etc.).
- Gelatine and the combination of polyvinylpolypyrrolidone/plant protein/amorphous silica, at the highest dosages tested, removed 39-40% of ochratoxin A. These had less adverse impact on color and polyphenol content compared to chitosan.
- Chitosan was less effective in ochratoxin A removal than most other fining agents at lower concentrations (0.1 and 0.5 g/L), but became more effective than all other fining agents at the higher concentrations

(2 and 5 g/L), achieving up to ~67% reduction. However, it strongly affected wine quality parameters (color, polyphenols, etc.).
- Bentonite showed moderate efficiency but came with drawbacks, notably a considerable decrease in color intensity (color intensity value decreased substantially).
- Egg albumin only removed up to ~16% of ochratoxin A and strongly affected color intensity parameters.

Arenas et al. (2021) evaluated the effects of pre-fermentative skin maceration and four fining agents on chemical attributes (composition of the macromolecular fraction, polysaccharides and proteins, phenolic compounds, chromatic characteristics, and protein stability) of two 'Albariño' white wines. The fining agents were fungal chitosan, sodium bentonite, calcium bentonite, and kappa carrageenan. The most effective treatment was kappa carrageenan, which reduced the content of pathogenesis-related proteins and the protein instability in both wines to a greater degree than sodium and calcium bentonites. Chitosan did not increase the heat stability of both wines, and pathogenesis-related proteins levels remained unaltered. However, chitosan decreased the levels of wine polysaccharides by 60%. Sodium and calcium bentonites also decreased the levels of wine polysaccharides, although to a lesser extent (16% to 59%). Kappa carrageenan did not affect the wine polysaccharide levels. They concluded that kappa carrageenan was more suitable than the other fining agents for white wine protein stabilization. It reduced the levels of wine pathogenesis-related proteins without impacting polysaccharide composition.

Table 4: Summary of dosage and effects of main fining agents (non-agricultural and agricultural) used in winemaking. Adapted from Y. Kumar & Suhag (2024)

Fining agents	Wine types	Dosage range	Characteristics
Bentonite	White	20–100 g/hL	• Average clarification • Treats and prevents protein and copper casse • Facilitates racking with proteins • Avoids over-fining
	Red	20–50 g/hL	• Clarification of young wines • Eliminates colloidal coloring matter • Facilitates sedimentation of protein fining agents
	Rosé	30–100 g/hL	• Remove proteins and improve clarity and stability
Casein	Must and white	10–50 g/hL	• Good clarification • Treats and prevents yellowing (maderization) • No over-fining • Removes undesirable odors and reduces color of white wines
Isinglass	White	1–2.5 g/hL	• Good clarity • Intensifies yellow color • Light flakes, bulky, settles slowly
Siliceous earths	White	20–100 mL/hL	• Acts on protective colloids in wines that are difficult to clarify • Used with protein fining agents, prevents over-fining and facilitates settling of the lees
Gelatin	Red	3–10 g/hL	• Very good fining agent for tannic wines • Affects only the most aggressive tannins and reduces astringency • May make wine softer or thinner
Egg white	Red	5–15 g/hL (powder), 3–8 fresh egg whites per barrel (225 L)	• Very good fining agent for tannic wines with some age • Sensitive to protective colloids
Maize zeins	Red	5–25 g/hL	• No modification to sensorial properties
Grape seed extract	Red	5–20 g/hL	• Could cause allergies if residues remain after the fining treatment • Desirable effects on wine turbidity, color, oxidative stability • Improvement of wine sensory properties
	White		
Polyvinylpolypyrrolidone (PVPP)	Red	10–45 g/hL	• Eliminates odors described as mushroom-like, moldy, camphoric, or earthy in wines • Removes bitter compounds and browning precursors in both red and white wines • Modulates the intensity and hue of the pink color and prevents some organoleptic degradation in rosé wine
	Rosé	-	
	White	10–80 g/hL	
Carbon	Juice, red and white	5–200 g/hL	• Removes off-odors • Effective in color reduction (browning and pinking)
Caseinates	Red	5–25 g/hL	• Prevent oxidative browning in white wines
	White		
Legume, soybean, lupine, peanuts and pea protein	Red	5–30 g/hL	• Could cause allergies (except for pea protein) • Clarification, removing tannins and reduce astringency in red wines • Lower impact on fermentation aroma compounds
	White		

Evaluation Question #12: Describe if there are any alternative practices that would make the use of this substance unnecessary [7 U.S.C. 6518(m)(6)].

Many wines, especially natural wines or minimal intervention styles (*e.g.,* Muscadet, Barolo), are made without any fining or even filtration (Wei et al., 2023). They may appear slightly hazy but are considered stable and acceptable. However, in commercial winemaking, large-scale producers tend to use at least one fining agent (commonly bentonite for white wines) because it ensures stability during storage and distribution (Eisenman, 1998). Although wine can be clarified by physical methods alone (described below), many producers combine approaches for stability and polish.

Clarification by filtration

Filtration is a separation technique used to eliminate solids in suspension from a liquid by passing them through a filter medium. Filtration preserves wine taste and aroma components and reduces the waste created by filter aids, such as diatomaceous earth and filter pads (El Rayess et al., 2011). Depending on the particle size of molecules to be filtered, the removal of particles can be achieved using either macrofiltration, microfiltration, or ultrafiltration (Mierczynska-Vasilev & Smith, 2015).

Clarification by sedimentation/settling

Stoke's law can predict the settling velocity of suspended spherical particles when a fluid is at rest. Among other factors, the settling velocity is proportional to the particle size and fluid height. As such, natural settling works best for the removal of larger particles such as tartrate crystals and the majority of yeasts, but does not work for colloidal size particles or bacteria (Vernhet, 2019).

Clarification by chilling

Cold treatment is another method than can be used to stabilize wines (Vernhet, 2019). Besides fining, cold treatments are usually performed to prevent the crystallization of tartaric salts, but can also prevent precipitation of colloidal coloring matter in bottled wines. Their effectiveness strongly depends on the wine, the applied temperature, and the treatment duration (Vernhet, 2019). In red wines, cold stabilization by storage, either at -4 °C for 1–5 days or at 2 °C for up to 14 days, was found to be a successful procedure to prevent bottle sedimentation in stored wine while preserving wine quality (Z. Peng et al., 1996). The authors compared the efficacy of cold stabilization to several fining agents including egg white, gelatin, and bentonite. Cold stabilization was more effective at reducing sedimentation than any fining agent tested, followed by bentonite, while egg white and gelatin were not effective in this role (Z. Peng et al., 1996).

Clarification by centrifugation

Centrifugation has many beneficial uses in wine processing, including (Costagli et al., 2016; Mierczynska-Vasilev & Smith, 2015):

- disrupting alcoholic fermentation and eliminating yeast at the end of fermentation
- clarifying wine post-fermentation and after tartaric acid stabilization
- for protein and biological stabilization, and quick separation after fining

Recent advances in centrifugation technology have expanded its long-standing utility in wine processing. For example, centrifuging wine pre-clarification can improve the efficacy of subsequent cross-flow filtration, and new centrifuge designs have minimized the introduction of dissolved oxygen and energy use (Costagli et al., 2016). Centrifuging wine is especially useful for musts that do not have a high content of solid particles and thus would otherwise settle very slowly. According to Costagli et al. (2016), centrifugation technologies should be combined with the use of fining agents for successful clarification of wines.

Clarification using a pulsed electric field

Pulsed electric field is a promising non-thermal technology, which applies a high-intensity electric field pulse for a short time (treatment time usually in the microsecond scale) to food or raw material between two electrodes. The electric field causes electroporation (formation of pores in cell membranes) in microbial cells and plant cells present in the wine or must. This method can be used at different stages during the winemaking to achieve different objectives. This method has the following advantages (Feng et al., 2022):

- it can accelerate particle sedimentation reducing the need for fining agents
- inactivate spoilage organisms to induce microbial stabilization (yeasts, lactic acid bacteria)
- Minimal changes to the aroma

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Annex V. Authorised Products and Substances for Use in the Production of Processed Organic Food and of Yeast Used as Food or Feed. Part D: Authorised Products and Substances for the Production and Conservation of Organic Grapevine Products of the Wine Sector Referred to in Point 2.2. of Part VI of Annex II to Regulation (EU) 2018/848 (2021). https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=PI_COM:Ares(2021)2131426&rid=4

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Appendix

The following table provides examples of commercially available, certified organic fining agent products for use in winemaking as alternatives to chitosan. The alternatives include pea protein, gum arabic, gelatin, and albumen products commercially available in the US. The list is not exhaustive.

Table 5: Alternative fining agent products.

Company name	Product name	Notes	Website
Pea protein			
Lamothe-Abiet, BV North America	GreenFine® Must	100% pea protein	https://www.bvnorthamerica.com/finingshop/greenfine-must
Lamothe-Abiet, BV North America	GreenFine® Nature	Pea protein with inactivated yeasts and calcium bentonite	https://www.bvnorthamerica.com/finingshop/greenfine-nature
Perdomini IOC	Clear GT R	Yeast hulls, pea protein and silica gel	https://www.perdomini-ioc.com/wp-content/uploads/2020/10/PERDOMINI_IOC-organic-winemaking-A4-dfm-2021-1.pdf
Perdomini IOC	Inofine V	Pea protein	https://www.perdomini-ioc.com/wp-content/uploads/2020/10/PERDOMINI_IOC-organic-winemaking-A4-dfm-2021-1.pdf
Enartis	PLANTIS L	Pea protein with SO_2 (1%)	https://www.enartis.com
Gum arabic			
Perdomini IOC	Dry Gum L	Protective colloid on iron, copper, color-producing matter and colloidal substances	https://www.perdomini-ioc.com/wp-content/uploads/2020/10/PERDOMINI_IOC-organic-winemaking-A4-dfm-2021-1.pdf
Perdomini IOC	Dry Gum R	Tartaric acid stabilization	https://www.perdomini-ioc.com/wp-content/uploads/2020/10/PERDOMINI_IOC-organic-winemaking-A4-dfm-2021-1.pdf
Enartis	Maxigum® F	-	https://www.enartis.com/en-us/products/wine/stabilizzanti-en-us/gum-arabic-en-us/maxigum-f/
Enartis	Citrogum	-	https://www.enartis.com/en-us/products/wine/stabilizzanti-en-us/gum-arabic-en-us/citrogum/
AEB	Arabinol Bio D	-	https://www.aeb-group.com/us/arabinol-bio-d-21484?_gl=1*1pb3gdq*_up*MQ..*_ga*MTQ5NTUwNDc2MS4xNzU5NTc0MTQ5*_ga_D4PPVQYBQH*czE3NTk1NzQxNDkkbzEkZzAkdDE3NTk1NzQxNDkkajYwJGwwJGgxMTc1MzExOTk2
Lamothe-Abiet, BV North America	GreenFine® Must	100% pea protein	https://www.bvnorthamerica.com/finingshop/greenfine-must
Lamothe-Abiet, BV North America	GreenFine® Nature	Pea protein with inactivated yeasts and calcium bentonite	https://www.bvnorthamerica.com/finingshop/greenfine-nature
Perdomini IOC	Clear GT R	Yeast hulls, pea protein and silica gel	https://www.perdomini-ioc.com/wp-content/uploads/2020/10/PERDOMINI_IOC-organic-winemaking-A4-dfm-2021-1.pdf
Perdomini IOC	Inofine V	Pea protein	https://www.perdomini-ioc.com/wp-content/uploads/2020/10/PERDOMINI_IOC-organic-winemaking-A4-dfm-2021-1.pdf
Enartis	PLANTIS L	Pea protein with SO_2 (1%)	https://www.enartis.com
Gelatin			
Laffort	Gecoll	Powder	https://laffort.com/en/products/gecoll/
Laffort	Clarpress	Liquid	https://laffort.com/en/products/clarpress/
Laffort	Gecoll Flottation	Liquid gelatin with high reactivity for flotation	https://laffort.com/en/products/gecoll-flottation/
Albumen			
Lamothe-Abiet	Lamothe-Abiet Ovaline	Suited to structured red wines to soften their phenolic profile	https://lamothe-abiet.com/en/fining-agents/ovaline/
Agrovin	Ovovin	For red wines	https://agrovin.com/en/products-oenology/fining-agents/ovovin/
Laffort	Albucoll	Liquid	https://laffort.com/en/products/albucoll/