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Ozone as a Sanitation Method in Winemaking: Improving Fermentation Control in the Context of Climate Change

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Abstract

Climate change presents a challenge for global viticulture due to rising temperatures and water stress, which accelerate grape ripening, increase sugar levels, and reduce acidity. This compromises wine quality and microbial stability, resulting in higher reliance on sulfur dioxide (SO_2). However, SO_2 can inhibit desirable fermentations, including those carried out by non-*Saccharomyces* yeasts, which are key biotechnological tools for climate adaptation due to their ability to modulate acidity, aroma, and ethanol. Therefore, alternative disinfection methods are needed to control wild microbiota without hindering inoculated yeasts. This review critically analyzes ozone (O_3) as a non-thermal disinfection technology for winemaking. It examines the antimicrobial mechanism of ozone, its efficacy against wine-related microorganisms, its impact on the physicochemical and aromatic parameters of grapes, and its practical viability. Ozone effectively reduces spoilage-causing microbiota while preserving crucial grape compounds and providing a favorable environment for novel fermentation biotechnologies. Compared to other emerging technologies and SO_2, ozone offers a balanced profile: effective disinfection, minimal residues, cost-effectiveness, and compatibility with sustainable winemaking. Ozone is emerging as a promising alternative to facilitate controlled fermentations and improve wine quality among the current climatic and oenological challenges.

Keywords: ozone; sulfur dioxide alternatives; non-*Saccharomyces* yeasts; emerging sanitation technologies; winemaking

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1. Introduction

1.1. The Impact of Climate Change on Viticulture

Climate change has become an increasingly significant problem for global viticulture, requiring sustainable and cost-effective adaptation responses [1]. Data from the International Organisation of Vine and Wine (OIV) for 2024 highlights the significant and heterogeneous impact of this climate change on vineyards in the European Union, where episodes of severe water stress coexist with torrential rains and unprecedented destructive storms. This extreme variability affects the world's leading producers: Italy (44.1 mhl), France (36.1 mhl), and Spain (31.0 mhl) [2]. In addition, the Mediterranean basin, the epicentre of global wine production as just noted, is particularly vulnerable to increasing aridity, characterized by lower rainfall, higher temperatures, less cloud cover, and greater solar radiation, which has a major influence on the soil conditions where the grapes for winemaking will grow and their subsequent growth and development [1].

Physiologically, the projected temperature increase (between 0.3 °C and 1.7 °C over the next 20 years) accelerates and shortens the phenological stages, reducing the time for biomass accumulation and compromising the final yield. These changes have a direct effect on the composition of the berry, causing modifications in both primary metabolites (such as sugars or organic acids) and secondary metabolites (phenolic and aromatic compounds), which will be reflected in the resulting wine [3,4].

As a result, the following trend has already been observed in recent years: an increase in alcohol potential of more than 2% vol., a decrease in total acidity of approximately 1 g/L of tartaric acid, and an increase in pH of 0.2 units [5]. Studies on varieties such as Malbec, Syrah, and Bonarda confirm earlier phenological phases, lower yields, higher Brix degrees, and lower acidity [6].

The oenological implications for wine quality are becoming increasingly notorious. Musts with a pH above 4, now common even in traditionally cold regions, result in wines with high alcohol content (often above 14% vol., a level considered excessive for optimal quality), lower freshness, and compromised microbiological stability [5,7]. A high pH reduces the molecular fraction of sulfur dioxide, decreasing its antimicrobial efficacy and forcing an increase in doses to control contaminating yeasts such as *Brettanomyces bruxellensis*, which generates a vicious cycle of increased sulfite addition [5].

1.2. Non-Saccharomyces Yeasts as a Biotechnological Tool for Adaptation and Improvement

In this scenario, the re-evaluation of the role of non-*Saccharomyces* yeasts has led to their use in controlled fermentations (mixed or sequential) as a central strategy for modern winemaking [8,9]. Their value relies on their ability to specifically modulate the composition of wine, offering solutions to the climatic challenges presented above.

Some commercialized species, like *Torulaspora delbrueckii*, *Schizosaccharomyces pombe*, *Metschnikowia pulcherrima*, *Lachancea thermotolerans*, and *Pichia kluyveri*, have metabolic characteristics that make them especially interesting for winemaking [9]. It is worth pointing out *L. thermotolerans* for its ability to act as a natural biological acidifier which has already been documented precisely for its use in warm climates [10]. It produces L-lactic acid from sugars, increasing acidity (up to 6.83 g/L), reducing pH (up to 3.02), and improving freshness and microbial stability, providing an alternative to the addition of water and tartaric acid in overripe musts [11–13]. *M. pulcherrima*, on the other hand, promotes greater aromatic complexity through the significant production of esters (such as isoamyl acetate) and other volatile compounds (2-phenylethanol), while also contributing to the reduction of ethanol content [14,15]. Sequential fermentations combining both species have produced wines with greater overall aromatic complexity and better sensory performance [13].

Beyond these species, the use of other non-*Saccharomyces* yeasts also offers a wide range of diverse benefits: increased glycerol and polysaccharides (improving smoothness and body), color stabilization, reduction of undesirable compounds (ochratoxin A, ethyl carbamate, biogenic amines), and a decrease in the necessary dose of SO_2 by acting as a biological control agent [9,16,17].

However, its effective implementation faces two main limitations: its low fermentative power compared to *Saccharomyces cerevisiae*, and its lower overall tolerance to SO_2 [14,18]. This implies that the high concentrations of SO_2 used to control the indigenous microbiota of grapes can inhibit the desirable metabolic activities of the non-Saccharomyces yeasts themselves [19]. In this regard, to achieve optimal implementation of these new fermentation biotechnologies, it is necessary to explore sanitization methods that control wild microbiota without relying on high doses of sulfites.

1.3. Non-Thermal Sanitization Technologies as an Alternative for Sulfites

The search for alternatives to chemical sanitizers and thermal treatments, which can have negative effects on several properties of fresh grapes, has been focused on non-thermal physical technologies [20]. These technologies reduce the wild microbiota present in grapes, preserving their sensory quality and creating an environment that facilitates the implantation of non-Saccharomyces yeasts. Some of the most notable methodologies that have already been researched for potential use in grapes for winemaking are: Pulsed Electric Field (PEF) [18], Pulsed Light (PL) [21,22], High Hydrostatic Pressure (HHP) [23,24], Ultrasound (US) [25,26]; and, in particular, the use of ozone, which is emerging as a disinfection method with a highly interesting profile and on which various advances in its use have been published over the years [27–30] and which has been evaluated in literature for its characteristics as an alternative method of grape sanitation alongside the other methods mentioned [31].

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1.4. Objective

This review presents a critical analysis of the use of ozone as a sanitization technique for grapes in winemaking illustrating how it also allows controlled fermentation and the development of new technologies in this field. The following will be evaluated in depth: Mechanism of action and antimicrobial efficacy against microorganisms relevant to the winemaking process, viability in winemaking practice, impact on the physical-chemical and aromatic parameters of grapes, along with a comparison with other emerging techniques in this field, mentioned above.

2. Ozone as a Sanitizing Agent

2.1. General Information

Ozone (O_3) is a bluish gas with a pungent odor, predominantly generated in the food industry by exposing oxygen (O_2) to a high-voltage electrical discharge (corona method) or ultraviolet radiation [32–34]. Recognized as an eco-friendly technology and a powerful sanitizer, its main appeal lies in its high oxidizing capacity and the fact that it leaves no residue, as it decomposes rapidly in the environment after application [33–35]. Its antimicrobial mechanism of action, in both the gaseous and aqueous phases, is based on the induction of oxidative stress that leads to the disintegration of microbial cell walls [36]. In terms of direct application, antimicrobial efficacy is modulated by extrinsic factors such as ozone concentration, exposure time, and application method (gaseous, aqueous, or combined). Generally, an increase in concentration and time improves activity, although this effect may reach a plateau, possibly due to the protective interference of non-viable microbial cells or the food matrix. However, its practical efficacy is linked to a complex interaction of physicochemical and operational parameters. In the gas phase, relative humidity and air temperature emerge as essential environmental parameters for disinfection [37]. In aqueous application, ozone is more stable at acidic pH (<7), where it persists in molecular form; in contrast, at alkaline pH (>7.5) it undergoes accelerated spontaneous decomposition, generating even more reactive hydroxyl radical [32]. The presence of organic matter in water is a critical factor that consumes active ozone, drastically reducing its concentration and half-life. For example, a Chemical Oxygen Demand (COD) equal to or greater than 9 mg/L can degrade ozone to biologically insignificant levels in just 5 minutes, while in higher purity waters its concentration and antimicrobial activity can be maintained for longer periods [35]. Temperature has a dual influence: although higher temperatures reduce the solubility of the gas in water [32], they also linearly increase its degradation rate [35]. However, it has been observed that the increase in ozone reactivity at higher temperatures can compensate for its lower stability, resulting in comparable biocidal efficacy. Furthermore, operating parameters such as bubble size, contact time, gas flow, and contact equipment design are essential for maximizing mass transfer and effective solubilization of ozone in water [33]. Overall, ozone represents a versatile and powerful technology, whose successful deployment requires a comprehensive understanding and control of the variables that define its stability, reactivity, and transfer to the target.

2.2. Antimicrobial Mechanism of Action

Ozone is recognized as a broad-spectrum biocide, effective against bacteria (both Gram-positive and Gram-negative), viruses, fungi (yeasts and molds), and spores, mainly due to its high oxidation-reduction potential (2.08 eV) and its non-specific mechanism of action[33,35,37,38].

The powerful biocidal activity of ozone is attributed to a double mechanism of action involving both molecular ozone and radical species derived from its spontaneous decomposition (Figure 1) due to its inherent instability, generating a cascade of this highly reactive radical species, including the hydroxyl radical ($\bullet OH$) and superoxide ($\bullet O_2^-$) radicals [32]. Ozone acts as a dipolar molecule with electrophilic and nucleophilic properties, reacting directly and selectively with organic compounds that have unsaturated bonds or electron-rich functional groups [33].

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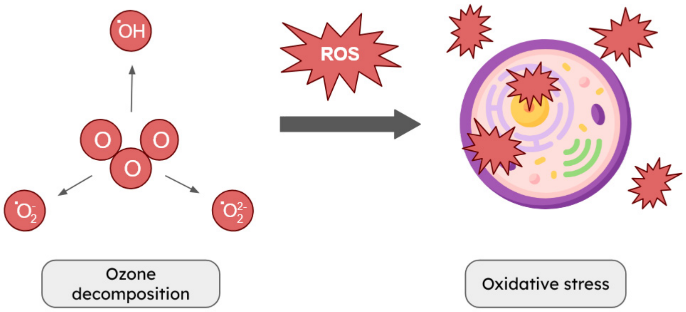

Figure 1. Decomposition of molecular ozone into various reactive oxygen species (ROS) with the ability to damage cell integrity.

Its antimicrobial action is not the result of a single event, but rather a complex and sequential process involving a simultaneous attack on multiple structural and functional targets within the cell (Figure 2). Inactivation typically begins in the cell wall and membrane. Ozone rapidly oxidizes the polyunsaturated fatty acids, glycoproteins, and glycolipids that compose the cytoplasmic membrane and, in the case of bacteria, the cell wall [32,35].

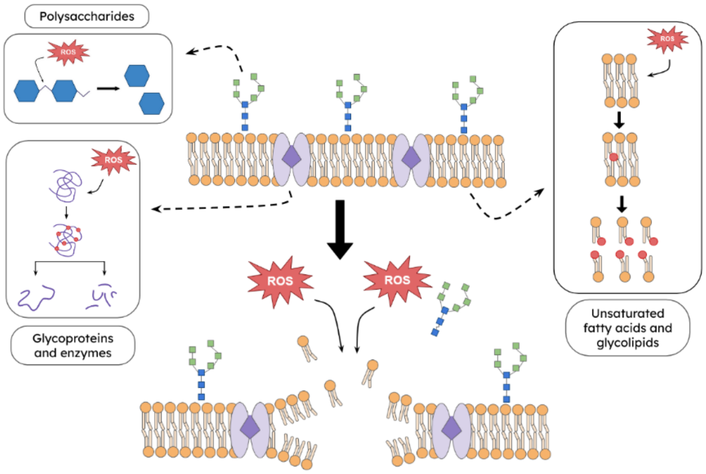

Figure 2. Mechanism of action of ozone, resulting in cell lysis and involving a cascade of reactions with each of the components of the cell wall and membrane.

This oxidative attack leads to lipid peroxidation, a process that profoundly alters the fluidity, integrity, and functionality of the membrane. The products of this peroxidation, such as hydroperoxides, change the physical properties of the lipid bilayer, causing depolarization, inhibition of transport proteins and membrane enzymes, and culminating in a massive loss of selective permeability [32]. The resulting leakage of cellular constituents is a critical event that precedes cell lysis. At the same time, ozone has a powerful effect on protein components. The oxidation of sulfhydryl (-SH) groups and amino acid residues (especially tyrosine, tryptophan, histidine, and cysteine) in essential peptides, proteins, and enzymes leads to their inactivation and denaturation, disrupting vital metabolic processes such as cellular respiration [32,33]. Once the integrity of the membrane barrier is compromised, ozone and the reactive oxygen species it generates, can penetrate the cytoplasm. Similar reactions occur inside the cell, affecting organelles and the organism's genetic material. The oxidation of nitrogenous bases in DNA, with a particular affinity for thymine, guanine, and uracil, as well as the breaking of bonds in the sugar-phosphate chain, cause irreparable genotoxic damage that prevents replication and transcription, leading to cell death [37]. This sequential model of action (damage to the envelope, followed by cytoplasmic oxidative stress and finally DNA damage) explains the high efficacy of ozone as an antimicrobial agent.

The susceptibility of microorganisms to this multi-attack is not uniform and is influenced by their structure and physiology. Gram-positive bacteria, which have a thick layer of peptidoglycan in their cell wall, generally show greater resistance than Gram-negative bacteria. This can be explained by the fact that the structural polymer of peptidoglycan, whose basic unit contains N-acetylglucosamine, is relatively more resistant to rapid oxidation by ozone compared to other components [33]. Among fungi, a sensitivity gradient is observed: yeasts, such as *S. cerevisiae*, are very susceptible, achieving reductions of 3-4 log CFU in minutes with low concentrations of ozone (0.1-0.6 ppm) in water [39], while filamentous molds, protected by their mycelial structures and spores, may be more tolerant [32]. Studies applied to viticulture have corroborated these differences; ozone treatment of grapes drastically reduced the population of acetic bacteria (from 32.6% to 3.7% incidence) and *Botryotinia* mold (from 61.6% to less than 5%), while lactic acid bacteria, which are anaerobic in nature, were particularly sensitive, which is technologically beneficial for preventing unwanted malolactic fermentation [40]. Additionally, as a result of the mechanism of action of ozone, which destroys microorganisms through cell lysis, no development of resistance to ozone disinfection has been observed [36].

However, the effectiveness of ozone can be considerably attenuated by environmental and microbial factors, as mentioned above. For this reason, certain factors must be considered, such as, high cell concentration (e.g., ≥10^6 CFU/mL) and, importantly, the ability to form biofilms since this confers significant protection. The exopolysaccharide matrix of biofilms not only acts as a physical barrier that hinders ozone diffusion, but its components also react with ozone, decreasing its concentration. A direct correlation has been observed between increased biofilm-forming activity and increased ozone resistance, requiring higher concentrations and longer exposure times to achieve inactivation [35,37]. Therefore, the biocidal capacity of ozone must always be contextualized within the specific conditions of application, where the organic composition of the medium and the organization of the microbial community are determining variables for the effectiveness of disinfection.

2.3. Applications in the Wine Industry

Initially, ozonation treatment was limited to water purification processes, but this technology has gradually become established globally in the agricultural, food, and wine sectors [34]. The implementation of equipment that allows the use of ozone in any industry is quite simple, since the requirements for setting it up can be summarized as follows: a gas source (air or pure oxygen), an ozone generator, a source of electrical power, a diffuser in the case of aqueous treatments, a gas excess removal unit, and an ozone analyzer to record the concentration of this gas during the process [32]. Currently, high-quality, reliable ozone generation equipment is widely available [41].

Its technical simplicity, combined with the studies published in recent years on ozone treatment of grapes, demonstrates that this technique is viable and relevant in this sector. A selection of examples is presented below (Table 1) that demonstrate the versatility of ozone application and the diversity of results obtained during research.

Table 1. Selection of studies involving the use of different ozone application methodologies for table grapes and wine production. The grape variety used is included, as well as the conditions of each experiment and a brief summary of the results obtained in each of them.

Grape variety	Ozone treatment	Conditions	Results	Reference
Moscato bianco	Two different ozone concentrations of	20 ± 2 °C and 60 ± 5% relative humidity (RH).	Changes in volatile organic compounds concentrations	[42]

	gaseous ozone for 24 and 48 hours		present in grapes after ozone treatment	
Moscato giallo, Sauvignon blanc, Gewürztraminer and Riesling	After a preliminary step of saturation of the chamber (30 min), continuous gaseous ozone for 6 h every 6 days during 6 weeks	20 °C	Control the microorganisms in grapes that cause deterioration without altering the volatile profile of the grapes.	[40]
Moscato bianco	24 days under air ozone-enriched atmosphere	20 ± 2 °C and 60 ± 5% RH.	Increase in the total content of volatile organic compounds.	[43]
Nebbiolo and Barbera	Continuous 30 μL/L ozone concentration for 24 and 72 h	20 °C and 70% RH.	The extraction capacity of phenolic compounds is not affected or even improves in grapes treated with ozone, depending on the variety and exposure time.	[44]
Sangiovese	Continuous gaseous ozone at the maximum flow rate for 12 hours	in a cold room at 4 ± 0.5 °C and 70% RH	Increase in total polyphenol and flavonoid content and upregulation of specific genes involved in polyphenol biosynthesis in grapes.	[45]
Thompson Seedless', 'Autumn Seedless', 'Redglobe', 'Black Seedless', and 'Ruby Seedless'	Fumigation with ozone gas at various concentrations and for different periods of time.	5 ± 2 °C	High concentrations of ozone significantly reduced the number of *Botrytis cinerea* infections.	[46]
Muscat Hamburg	Three different concentrations of ozone for 30 min every day during 80 days	0 ± 1 °C	Changes in the fungal community on the surface of the grape and reduction in fungal diversity, which reduces the occurrence of diseases.	[47]
Petit Verdot	Ozone fumigation overnight (12 h)	in a cold room at 4 °C and 70% RH	Reduction in the microbial count of the initial population in the grapes and increase	[27]

			in the extraction of phenolic substances and the aroma of the final wine.	
Pignola	Ozone fumigation for 18 h	10 °C	Reduction of microbial count in grapes without affecting their polyphenol and carotenoid content.	[28]
Grechetto	Constant ozone fumigation in air and in nitrogen atmosphere for 12 h	10 ± 1 °C and 90 ± 2% RH	Preserves fruit quality and have a great impact on secondary metabolites like flavonols.	[48]
Barbera	Recirculation of ozone-enriched air for 12 and 24 h + Spray with aqueous ozone for 6 and 12 min	20 ± 1 °C and 57 ± 3% RH and water temperature of 25 °C for aqueous ozone	Reduction in the initial population of microorganisms on grape skin	[29]
Tempranillo	Spray ozonated water on a sorting table	Water temperature of 15ºC	Reduction in the initial population of microorganisms on the grape skin, allowing the implantation of non-*Saccharomyces* yeast.	[49]
Red Globe	After a preliminary step of atmosphere saturation, continuous fumigation with gaseous ozone for 30 min and immersion on ozonated water for 30 min	20ºC	Reduction in the initial population of microorganisms on the grape skin with both treatments, allowing the implantation of non-*Saccharomyces* yeast.	[50]

Based on the scientific literature cited above, numerous studies have shown that ozone's action as an antimicrobial agent eliminates or inhibits the growth of a wide variety of fungi, yeasts, and bacteria that can cause defects in grapes:

Ozone prevented the growth of microflora present in grapes by reducing the microbial population by up to 3 Log CFU/mL. From a qualitative point of view, ozone particularly reduced the incidence of microorganisms that cause deterioration, such as *Botrytis cinerea*. The elimination of lactic acid bacteria reduces the risk of unwanted malolactic fermentation, which is particularly noticeable in wines made from aromatic grapes. In addition, acetic bacteria, which are primarily responsible for sour rot, are effectively limited by ozone [40].

[46] also describes that the high concentrations of ozone applied resulted in a significant reduction in the number of *B. cinerea* infections, most likely by reducing the number of viable conidia on the surface of the berry. In addition to the microbiological analysis, this study also revealed some

very interesting findings related to fungicides. Residues of four fungicides commonly used in vineyards (fenhexamid, cyprodinil, pyrimethanil, and pyraclostrobin) applied to control *B. cinerea* were significantly reduced by ozone fumigation. In contrast, residues of iprodione and boscalid were not degraded by ozone. The degradation of fungicide residues in grapes by ozone may have interesting applications in industry, but for this approach to become a viable option, the degradation products of fungicides and their safety must be studied in depth.

Other interesting data related to this subject were obtained by [29]. The plate count revealed a significant difference between ozonated and untreated grapes. Both gaseous and aqueous ozone reduced yeast populations from 5.0 Log CFU/mL in untreated grapes to between 4.5 and 4.0 Log CFU/mL after treatment. It should be noted that the decrease in yeast populations in the treated samples (both with gaseous and aqueous ozone) proved to be differential between the different yeasts involved, with this decrease being more severe in apiculated yeasts. This result is interesting due to the negative impact of this species on the composition of wine because of its ability to produce high levels of acetic acid.

Further evidence of the powerful sanitizing effects of ozone was provided by [27]. In the samples treated with ozone, a significant reduction was observed in acetic acid bacteria, various lactic acid bacteria, yeasts such as *Hanseniaspora uvarum* and *Pichia membranifaciens*, for which reductions of between 1-3 orders of magnitude were recorded, but especially in *S. cerevisiae*, for which the population was reduced from 5.0 Log CFU/mL to less than 1.0 Log CFU/mL.

In addition, due to its mechanism of action, it also creates an environment suitable for the implantation of yeasts that are inoculated later. It has been reported that, compared to control fermentations in which sulfites were used to treat the grapes, samples treated with ozone showed a significantly faster start to fermentation and more regular development [27]. This is particularly relevant for non-*Saccharomyces* yeasts, due to their lower fermentative power and competition with other yeasts combined with their sensitivity to sulfites, its implementation can be challenging. However, studies have been published showing that ozonation creates a suitable environment for the implementation and development of *L. thermotolerans* during fermentation [49,50].

Besides microbiological parameters, it’s worth noting the research done on the possible changes that ozone can cause in grapes, which could affect important compounds during the fermentation process or the wine’s aromatic profile.

Regarding the integrity of the grapes after exposure to ozone, there is no evidence that ozone causes damage to the berries themselves [51]. In terms of the physical-chemical matrix of the grapes, ozone treatments can modulate the concentration of certain metabolites. [40] demonstrated that ozone-treated grapes retain parameters crucial for optimal fermentation, such as readily assimilable nitrogen. Moreover, [42] reported significant effects on some compounds. Exposure to ozone caused a significant decrease in the content of tartaric and citric acids, with the most marked reduction in samples subjected to the longest treatment. No significant differences were found for malic acid, glucose, or fructose between the treated samples and the control. On the other hand, the glycerol content decreased significantly in the samples exposed to ozone.

About the aromatic profile, the results obtained in different studies do not show a consistent pattern for certain specific compounds. On the one hand, in the analysis of numerous volatile compounds, both in their free and glycosylated forms, in treated and untreated grapes, no alterations attributable to ozone were detected [40]. On the other hand, [42] observed that short-term ozone treatment caused a significant decrease in the total content of free volatile organic compounds (VOCs) in fresh grapes, mainly attributable to the degradation of terpenes. Within this group, linalool, geraniol, and nerol, considered key aromatic markers in varieties such as Moscato bianco, showed differential behaviors. While the contents of geraniol and nerol did not show significant changes, only free linalool decreased significantly and only in this grape variety. In contrast, the longer and more intense treatment induced the synthesis of this compound, probably as a physiological response to greater abiotic stress. This could indicate that the impact of ozone on VOCs would be dependent on the dose and exposure time. It is important to note that volatile compounds in their glycosylated form, which are aroma precursors, did show consistent results in all treatments, with no significant variations between controls and treated samples.

A more recent study indicates that ozone treatment can improve the oenological properties of the final wine, including anthocyanin concentration, oxidative stability, color, and the concentration of desirable volatile compounds and esters [34].

Furthermore, regarding the adaptability and versatility of ozone treatments, as well as being applied directly to grapes, they can also be applied to equipment in the winery that is relevant to the winemaking process. Studies have been carried out on the viability of ozonated water as a disinfectant for winery machinery. On the one hand, there is a study that looked to determine its effectiveness in hoses for possible application in a CIP cleaning system, with the result that the use of ozonated water is more effective than the isolated use of peracetic acid and the combined use of soda and peracetic acid [52]. Another study aimed to define the application and possible risks associated with the use of ozone as a disinfectant in barrels, considering that some serious microbial contamination can occur during the aging of wine in barrels. The study focused on the effectiveness of ozone in combating microorganisms that cause wine deterioration and that have adapted well to the barrel environment, such as the impact of ozone on simple phenols, which represent a significant fraction of the extractable components that are transferred to wine through barrels or oak pieces. The results obtained demonstrated its effectiveness against microorganisms that may be present under these conditions and showed no significant alteration in terms of simple phenols in wine [35].

Overall, these findings support the controlled application of ozone as a viable tool to ensure the sanitization of grapes and even improve the chemical and sensory quality of the treated grapes, with possible positive effects on the characteristics of the wine obtained from these grapes.

2.4. Regulatory Framework

The approval by the US Food and Drug Administration (FDA) of ozone as a direct food additive, following a petition submitted in August 2000, allowed its safe use in the treatment, storage, and processing of food in gaseous and aqueous phases [33]. This substance, classified as GRAS (Generally Recognized as Safe) since 2001, is regulated for use in food under code 21 CFR 173.368 [53]. In addition, ozone is recognized as compatible with organic production by the USDA National Organic Program due to its residue-free application [51].

3. Comparison with Other Methods of Grape Sanitization

This section presents a comparative analysis of several emerging technologies: ozone, pulsed light, high hydrostatic pressure, ultrasound, and pulsed electric fields, alongside SO_2 as the conventional reference. The objective of this systematic comparison, grounded in scientific literature, is to evaluate not only their antimicrobial efficacy but also the impact on product quality, operational safety, cost, and sustainability.

Regarding antimicrobial effectiveness, ozone, as has already been previously established in the above section, although some heterogeneity depending on its application may be observed, is always highly effective in eliminating the microbiota of the grape, allowing controlled fermentation to subsequently take place. PL also achieves significant reductions in yeast and bacteria on the surface of grapes; however, its main limitation is the “shadow effect,” which protects microbes lodged in cracks or irregular surfaces, reducing the effective radiation dose [22,54]. Microbial reduction levels between 2 and 1.7 log10 have been observed in various fresh fruits inoculated with *S. cerevisiae* treated with PL. Nevertheless, due to the irregular nature of the fruit skin, the inactivation curve of microorganisms is not linear, and the results obtained can be highly variable [55]. Meanwhile, HHP has a selective inactivation profile: it effectively eliminates wild yeast populations less than Log 1 CFU/mL, but bacteria may tend to persist with reduced counts [23]. US offers fairly effective inactivation against fungi and bacteria, although its use has been studied more for food processes, such as the extraction of certain compounds [25,56]. However, ultrasound alone has often proven insufficient for substantially reducing microbial populations. Consequently, it should be applied in conjunction with other sanitizing methods to increase the effectiveness of the treatment [57]. Finally, PEF act through electroporation, creating pores in cell membranes that significantly inactivate all kind microorganisms very successfully [56,58]. The antiseptic properties of sulfur dioxide have been clearly demonstrated and the concentrations used today for wine production are strictly defined by the OIV [59]. SO_2 inhibits the development of microorganisms such as yeasts, lactic acid bacteria and, to a lesser extent, acetic acid bacteria. Its action prevents yeast haze formation, undesirable secondary fermentation, *Brettanomyces* growth, the development of mycodermic yeasts, and various types of bacterial spoilage [60]. Despite its well documented effectiveness, it should be considered that, due to its mechanism of action, microorganisms present in grapes may develop resistance to sulfites. As more studies are published, they reveal a greater complexity and strain dependence in sulfite detoxification mechanisms [59].

The impact of these technologies on product quality is also a decisive factor. Ozone does not negatively affect the visual quality of the grapes, nor does it significantly affect compounds of interest for fermentation. PL, although considered mostly non-thermal, can induce surface heat damage if high fluences are used or without an adequate cooling system, which limits the treatment duration and intensity [54,61]. In some studies, the temperatures recorded on the fruit surface immediately after exposure to treatments that reduced the microbiota increased considerably compared to the initial temperatures. This could lead to partial dehydration and alterations in nutritional and sensory properties [55,62]. Conversely, HHP, PEF, and US are recognized for their potential to improve the extraction of compounds of interest (phenolics, aromas) without severe negative thermal effects [19,63]. A comparative study showed that both HHP and PEF allowed a higher extraction of polyphenols and total anthocyanins in grapes [64]. In contrast, the traditional method with sulfur dioxide, despite its broad effectiveness, can impart unpleasant odors and flavors in young wines and, more critically, inhibit desirable fermentations such as malolactic fermentation or those carried out by non-*Saccharomyces* yeasts [19]. High doses of sulfites can neutralize the aroma of wine and even lead to characteristic aromatic defects, such as a smell of wet wool that quickly becomes suffocating and irritating, along with a sensation of burning in the aftertaste. Moreover, in red wine production, sulfation promotes the dissolution of phenolic compounds (anthocyanins and tannins), which are responsible for color [60].

Operational safety is particularly relevant. For ozone, at low concentrations, it can cause mucosal irritation, and prolonged exposure to higher concentrations has toxic effects, mainly on the respiratory system [33]. Although its toxicity is low, it must be considered when taking measures to ensure minimal exposure to the agent to avoid health risks. The other technologies present risks mainly associated with the handling of high-pressure equipment (HHP), electricity (PEF), or radiation (PL).

Cost analysis reveals different scenarios. Although ozone requires an initial investment in generators, it is considered a cost-effective and environmentally friendly technology in the long term, as it is produced on site, eliminating the costs of storage and transport of chemicals [32,33,41]. PL is very efficient in terms of sterilization doses, but the energy consumption required to generate the pulses and the potential need for cooling systems can increase operational expenses [61]. Technologies such as PEF and HHP require a significant initial financial investment due to the complexity of the machinery, which can be a barrier when considering the implementation of these techniques [56,65]. For ultrasound, industries can be provided with practical and reliable ultrasound equipment easily without a big investment [25].

Finally, regarding environmental and health impact, some of these techniques have considerable advantages. Ozone decomposes rapidly into oxygen, leaving no persistent chemical residues [33]. It could also be used as a disinfectant in winery machinery instead of the chemicals that are usually used, allowing for the reduction or elimination of waste generated during equipment cleaning [35,52]. Similarly, pulsed light neither uses nor generates residual chemical compounds, making it a clean process from a waste perspective [54]. Ultrasound, considered as green novel technology, has also attracted the attention to its role in environment sustainability [25]. This contrasts with the use of SO_2, whose residue in wine and potential allergenic impact are points of growing concern. It should be noted that the use of SO_2 as a food preservative is regulated and is the method discussed here with limits strictly imposed by the European authorities. This is because excessive sulfite consumption has been shown to cause symptoms such as headaches, nausea, stomach irritation, and breathing difficulties, especially in people with asthma. This is very relevant for consumers, as chemical-free products are becoming increasingly important in the market [66,67]. Additionally, there is significant demand for increased investment and acceptance due to consumer interest in less processed and organic foods [56].

Based on all the points discussed in this section, Table 2 below provides a summary using a color code to indicate the advantages and disadvantages of each method. Consequently, green would indicate advantages or strengths in that field; yellow would indicate the absence of advantages compared to other methodologies or slight disadvantages to be taken into account; finally, red would indicate clear weaknesses or disadvantages in that specific field.

Table 2. Comparative summary of the different grape sanitization methodologies studied.

Methodology	Antimicrobial effectiveness	Implications for product quality	Safety concerns	Cost analysis	Environmental and health impact
Ozone (O_3)	🟢	🟢	🟡	🟡	🟢
Pulsed Light (PL)	🟡	🟡	🟡	🟡	🟢
High Hydrostatic Pressure (HHP)	🟢	🟢	🟡	🔴	🟡
Ultrasound (US)	🟡	🟢	🟡	🟡	🟢
Pulsed Electric Field (PEF)	🟢	🟢	🟡	🔴	🟢
Sulfur Dioxide (SO_2)	🟢	🔴	🟢	🟢	🔴

Furthermore, for a more detailed and comprehensive view of these variances between the different methodologies, a radar chart (Figure 3) has been created, providing approximate numerical scores for each characteristic analyzed. This shows how each method has a different profile, with its strengths and weaknesses depending on what priorities are considered most important. Although there are certain similarities between them, ozone stands out because, despite not having the best score in all parameters, it is the most stable of all the methods. By contrast, the use of sulfites is at the complete opposite side of the spectrum: while it is the most effective in terms of antimicrobial activity and cost, it comes with serious problems when the product quality or health impact are considered.

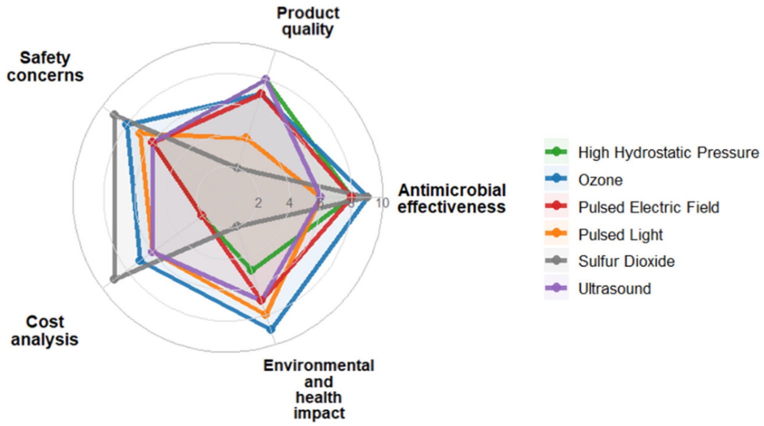

Figure 3. Radar chart showing the strengths and weaknesses of the different methodologies studied, based on a numerical score.

4. Conclusions

In summary, ozone is emerging as a viable, sustainable, and effective disinfection technology for grapes intended for winemaking. It is a valuable tool for modern winemaking, as it meets both quality requirements and the need to adapt to climate change. Its broad-spectrum biocidal action, mediated by oxidative damage to cell walls, membranes, and genetic material, significantly reduces populations of microorganisms that cause spoilage, including fungi, yeasts, and bacteria. By reducing the initial microbial load without relying on sulfites, ozone creates favorable conditions for fermentation and facilitates innovation in this process. Furthermore, the impact of ozone on grape quality parameters is generally neutral or positive, so it is not expected to cause any defects during processing. In addition, when evaluated based on various criteria that may affect its industrial incorporation, such as antimicrobial efficacy, impact on product quality, safety, cost, and environmental footprint, ozone demonstrates balanced and stable performance providing certain advantages when compared to other emerging non-thermal methodologies.

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Abbreviations

The following abbreviations are used in this manuscript:

SO_2	Sulfur dioxide
O_3	Ozone
OIV	International Organisation of Vine and Wine
PEF	Pulsed Electric Field
PL	Pulsed Light
HHP	High Hydrostatic Pressure

US	Ultrasound
COD	Chemical Oxygen Demand
ROS	Reactive Oxygen Species
CFU	Colony Forming Unit
VOCs	Volatile Organic Compounds
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe

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