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Review

The Management of Compounds that Influence Human Health in Modern Winemaking from an HACCP Point of View

Santiago Benito

Departamento de Química y Tecnología de Alimentos, Universidad Politécnica de Madrid, Ciudad Universitaria S/N, 28040 Madrid, Spain; santiago.benito@upm.es; Tel.: +34-913363984

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Abstract: The undesirable effects of some hazardous compounds involved in the different steps of the winemaking process may pose health risks to consumers; hence, the importance of compliance with recent international food safety standards, including the Hazard Analysis and Critical Control Point (HACCP) standards. In recent years, there has been a rise in the development of new technologies in response to the hazardous effects of chemical compounds detected during the winemaking process, whether naturally produced or added during different winemaking processes. The main purpose was to reduce the levels of some compounds, such as biogenic amines, ethyl carbamate, ochratoxin A, and sulfur dioxide. These technological advances are currently considered a necessity, because they produce wines free of health-hazardous compounds and, most importantly, help in the management and prevention of health risks. This review shows how to prevent and control the most common potential health risks of wine using a HACCP methodology.

Keywords: biogenic amines; ethyl carbamate; ochratoxin A; sulfur dioxide; phthalates; HACCP

1. Introduction

During the last few decades, grape fermentation products have shown positive health effects when consumed responsibly. Wine is common in the diet of many countries whose populations have high life expectancies, such as Spain. However, there are several health risks related to alcoholic beverages and specifically to wine. Those risks are usually related to specific groups of consumers, such as people suffering from allergies, pregnant women, or alcoholics. In this work, we focus on those health risks that can be avoided by a responsible consumer.

The Hazard Analysis and Critical Control Point (HACCP) theories emerged during the 1970s. Implementation of HACCP is now compulsory for food industries in most countries in order to protect consumers [1]. This article discusses the hazards associated with wine consumption, following the principles of the HACCP, in order to make it easy to understand and applicable for those who work in the wine industry. The HACCP theory is a preventive measure rather than a reactive policy. For this reason, this work shows that most of the known ways to prevent the appearance of human health hazards in wine begin with vineyard management. The first goal of HACCP is to control micro-organisms that could potentially harm regular consumers. From this perspective, wine is a simple food product to control, as no dangerous pathogens (such as *Clostridium botulinum*, *Salmonella enteritidis*, *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Campylobacter jejuni*, or *Aeromonas hydrophila*) can develop in a medium that contains an ethanol level of approximately 10–14%, high acidity, phenols, and sulfide. Indeed, in big cities (before chloride made water safer to drink) alcoholic beverages were consumed instead of water in order to avoid water pathogens that develop under unhygienic conditions. Nowadays, all developed countries and most developing

countries have high-quality public water from a food safety point of view. This fact makes the situation completely different, and although no pathogenic micro-organisms can easily develop in wine, new food safety problems (unknown until recent years) have begun to appear.

The old approaches of HACCP were based on the belief that no pathogenic micro-organisms could reach the consumer through wine and were focused on other food safety hazards, such as chemical or physical risks [2,3]. However, recent research has shown that some potentially indirect pathogenic micro-organisms that are not able to colonize a human body, such as lactic bacteria or grape fungi, can generate dangerous metabolites under specific circumstances. These compounds, in fixed concentrations, can be put into danger-specific groups of the population, or even regular consumers. Some of these compounds are biogenic amines, ethyl carbamate, or ochratoxin A (OTA).

Food safety controls were originally based on testing analyses of final products. The main problem of this approach was the impossibility of analyzing entire productions. In the case of winemaking, it would mean analyzing each bottle. Another specific problem of the enology industry is the price of specific analyses related to food safety, which can easily reach 100 € per unit and analysis, depending on the studied hazard. For these reasons, HACCP theories are based on preventive principles, such as routine control measures during manufacturing, in order to keep production under controlled conditions. In the past, the HACCP focused on pathogenic micro-organisms; however, today it also seeks to control physical and chemical hazards [4]. Such hazards are of great importance in the wine industry. For that reason, we discuss chemical hazards, such as pesticides, commonly used in vineyards or common additives, such as sulfites or fining agents. Physical hazards common in wine industries, such as glass, are also studied. These problems are generally easier to avoid than microbiological hazards, as they are more predictable than micro-organisms.

Because the HACCP is considered to be the most international system for preventing food hazards, we will discuss in detail how to follow a structure based on the seven principles that constitute this theory. This methodology easily allows the reader to identify where potential hazards appear in the winemaking process, their dangerous levels, their origins, and how to prevent them through systematic controls. It also shows how to verify from time to time that the whole system is under control by using more complex and expensive methodologies.

The Codex Alimentarius Guidelines [5] show seven principles to guide the implementation of a HACCP system, as follows.

1.1. Principle 1: To Conduct a Hazard Analysis

All hazards relating to a food product that can negatively influence the health of any consumer must be identified at their source. Possible preventive measures should also be described. Hazards must be divided into three groups: microbiological, chemical, and physical. As we explained before, from a microbiological point of view, no human pathogenic bacteria, fungi, or virus can successfully develop in wine due to its ethanol content. However, some micro-organisms that commonly appear in wine or grapes, such as lactic bacteria or fungi, are able to produce some potentially dangerous compounds, such as biogenic amines, ochratoxin A, or ethyl carbamate. There is generally low awareness of these problems of microbial origin in the wine industry, and there is some controversy about which preventive measures are most effective. These compounds constitute the main health hazards of microbiological origin in the wine industry. The main chemical hazards are the pesticides used in the vineyard to protect the plant and grapes from diseases produced by fungi. Migrations emanating from the packaging or containers where the wine is stored or manipulated are also chemical problems. Some fining agents that, on occasion, can be potential allergen compounds for specific groups of people are used to fine the wine in order to reduce the initial turbidity. Additives that can stabilize wine against micro-organism spoilage or against spoilage processes, such as oxidation, in over dosage can also produce health risks. The main physical hazards in the winemaking process are remains of machinery particles that can end up in the wine and glass particles from deteriorated bottles in which the final wine is stored.

1.2. Principle 2: To Determine the Critical Control Points

After conducting a study of all the possible hazards and their potential detriment to health and the probability of occurrence, we must establish how to control these risks. Critical control points (CCPs) are phases in the food process where it is essential to control some parameter that can prevent or eliminate the potential food safety hazard or reduce it to an acceptable level. For example, if a hazard comes from the grapes row material, the best moment to control it is before processing so as to make it easier to isolate the source. Therefore, it would not make any sense to control it at the last stage of the process. Thus, efforts must be made to identify the problem as soon as possible.

1.3. Principle 3: To Establish Critical Limits

Once it has been established where a hazard is going to be controlled, we must establish a criterion that allows for differentiating between what is acceptable and what is not. That criterion is defined according to a critical limit. Most of the time, critical limits are established according to the legal limits defined by legislation, such as that pertaining to histamine, ochratoxin A, ethyl carbamate, or legalized additives.

1.4. Principle 4: To Establish a Monitoring System

Once the stage where we have to control a hazard and its critical limit have been established, we must establish the kind of control to use, its frequency, and the qualified responsible person to use it. These controls are usually analyses that are fast and economical but allow for very quick decision-making. It is very common to use semi-quantitative methodologies that are not the official methods and are usually expensive and require specific equipment not commonly available from every winery. The official methods are commonly used in HACCP Principle 6.

1.5. Principle 5: To Establish Corrective Actions

When a deviation from the established critical limits occurs, a corrective action must be performed in order to restore the control and avoid potentially dangerous wine reaching the consumer. The most drastically corrective action is to eliminate the product. Nevertheless, several other options permit removing the hazard or procuring a secondary product less valuable but with a residual economical value. The principle also proposes to review the cause of the mistake or the imprecise action that generated the deviation in order to correct the procedure.

1.6. Principle 6: To Establish Verification Procedures

Hazard Analysis and Critical Control Point (HACCP) verification is defined as those activities, other than monitoring, that establish the validity of the HACCP plan and ensure that the HACCP system is operating according to the plan. Verification is done to determine whether the HACCP plan is being implemented properly, whether practices used are consistent with the HACCP plan, whether the HACCP system is working to control significant hazards, and whether modifications of the HACCP plan are required to reduce the risk of recurrence of deviations [6]. In winemaking, to verify the success and correct implementation of control measures, which are in most cases based on fast and semi-quantitative analyses, the most common procedure is to perform periodic checks using the official methodology. For that reason, it is very common to perform the verification analyses in accredited laboratories that possess advanced equipment, such as HPLC or GC/MS, and qualified professionals to run them.

1.7. Principle 7: To Establish Documentation Concerning All Procedures and Records That Are Appropriate to These Principles and Their Applications

A HACCP manual must be written. It describes the methodologies to follow in the HACCP system and how to apply them to this specific industry. It also describes potential hazards and their

effect on human health, critical control points, critical limits, corrective actions, control measures, and verification measures. The manual also keeps records of all performed operations in order to help produce safe products.

The main purpose of this review is to show wine manufacturers the main hazards in the wine industry and how to manage them according to HACCP theories (Table 1).

2. Ochratoxin A

2.1. Toxicity

Mycotoxins are toxic compounds of fungal origin that, when ingested, absorbed, or inhaled, can cause illness or death in humans. Ochratoxin A is a common compound in wines. It is considered hazardous to human health because of its nephrotoxic, neurotoxic, immunotoxic, mutagenic, and teratogenic properties [6–8]. Recently, the International Agency for Research of Cancer classified OTA as a carcinogenic compound [9]. The tolerable daily intake of OTA ranges from 0.3 to 0.89 μg/day for a person weighing 60 kg. It can cause instant poisoning in doses between 12 and 3000 mg for a person of that weight [10]. The Food and Agricultural Organization (FAO) and the World Health Organization set the daily upper limit intake to 14 ng/kg and the weekly intake to 100 ng/kg of body weight [10].

2.2. Origin

The origin of mycotoxins in enology are several fungi species in rotten grapes that are able to produce them. The OTA formation is related to the raw grapes, and it is not possible for OTA-producing fungi to develop in liquid juice or wine, as all fungi responsible for its formation are strictly aerobic, such as *Aspergillus carbonarius* [11,12]. The main species able to produce OTA in grapes, must and wine are *A. carbonarius* [13], *Aspergillus fumigatus* [14], *Aspergillus niger* [15], *Aspergillus tubingensis* [16], *Aspergillus japonicus*, and *Penicillium tubingensis* [10].

2.3. Critical Limit

Nowadays, OTA concentration in wine is regulated in certain European Union countries. We propose a critical limit that corresponds with the European legal limit of 2 μg/kg (available online: http://europa.eu/rapid/press-release_IP-04-1215_en.htm). The average value of OTA in European wines is about 0.19 μg/L [10]. According to some research, Spanish wines show an incidence of 1% of being over the legal limit [17].

2.4. Preventive Measures

Preventive measures mainly involve vineyard management being used to avoid the development of undesirable fungi capable of rotting the grapes. Some of those species are powdery mildew [18], *Rhizopus stolonifera*, or *Botrytis cinerea* [19] that favor berry colonization by the *Aspergillus* genus. Those vineyard diseases are well-known by viticulturists and in most cases are easily treated through phytosanitary controls. The insect known as *Lobesia botrana* usually produces small injuries in grapes that favor the latter's colonization by the former fungi. The insect plays an important role in OTA formation as fungi, such as *A. carbonarius*, are not able to attack the grape skin and invade the pulp by themselves [20]. Thus, previous skin damage is needed for colonization [12]. This insect management is also well-known by viticulturists. Nowadays, there is a trend to use a methodology based on sexual confusion through hormones in order to avoid the use of dangerous chemical compounds. Some alternative options for avoiding undesirable fungal developments and the use of pesticides are the biocontrol agents, such as *Aureobasidium pullulans* [21], *Kluyveromyces thermotolerans* [22], and *Lanchacea thermotolerans* [23,24]. The biocontrol strategy consists of colonizing plant surfaces or wounds for long periods under dry conditions before fungal attacks take place under wet conditions. Another trend is to use vineyard management that exposes the grapes to the sun and allows for higher air-stream circulation. In such microclimates, the development of fungi is more limited.

Table 1. The main wine-industry hazards and their management from a Hazard Analysis and Critical Control Point (HACCP) point of view.

Hazard	Toxicity	Origin	Critical Limit	Preventive Measures	Control Measures	Corrective Measures	Verification
Ochratoxin A	Nephrotoxic, neurotoxic, immunotoxic, mutagenic, teratogenic, carcinogenic	Fungus *Aspergillus Penicillium*	2 µg/kg	Vineyard management, phytosanitary controls, yeast biocontrol agents	Fungi visual control, gluconic acid, immunoaffinity	Maceration, Finning agents, selected yeast, amicrobic filtration	HPLC with fluorescent detector, 80 €
Biogenic amines	Several allergenic disorders	*Lactic bacteria*, *Pediococcus*, *Oenococcus*, *Lactobacilluas*, *Leuconostoc*	2 mg/L	Antibacterial agents, sulfur dioxide, lysozyme, chitosan, yeast inoculation	Selected lactic bacteria, *Schizosaccharomyces pombe/Lachancea thermotolerans*, semi-quantitative control	Unknown	fluorescence HPLC, 40 €
Ethyl carbamate	Carcinogenic and genotoxic	Urea evolution and lactic bacteria metabolism	15 µg/L	Nitrogen management, alternatives to malolactic fermentation	Urease enzyme, selected yeasts or bacteria.	Unknown	GC/MS, 40 €
Sulfur Dioxide	Irritation, bronchospasm, pulmonary edema, pneumonitis, and acute airway obstruction	Common additive in wine (antimicrobial and antioxidant)	150 mg/L	Sulfur dioxide alternatives: sorbic acid, lysozyme, chitosan, ascorbic acid, thermovinification, high hygiene	Calculation, sulfur dioxide stock control, Ripper method	To oxygenate, dilution	Paul method, 5 €
Wine Food Allergens	Allergic reactions	Present in fining agents (egg white, caseinates, or fish gelatin)	Traces	Fining agent dose evaluation before adding. Stabilization tests. No animal origin fining agents.	Label control	Unknown	ELISA tests, 50 €
Pesticides	Dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine negative effects	Vineyard protection against fungi and insects	Ditianon 5 mg/kg	Vineyard management	Field practice notebook registration (residual period control)	Unknown	ECD gas chromatography, 21 €
Genetically Modified Organisms (GMOs)	Precaution measures until total harmlessness is proved	Better performance of GMO yeasts	Residual presence, ML01 and ECMo01 0.005% (mass/mass)	Spontaneous alcoholic and malolactic fermentations	Yeast labeling control before use (free GMO product)	Unknown	Polymerase Chain Reaction, 100 €
Physical hazard	Cuts, bleeding, infection, and choking	Installations, raw materials, bad manipulation	2 mm	Raw material inspection, preventative equipment maintenance, good practice guidelines	Filtration	To refiltration	Random control
Phthalates	Endocrine disrupting, estrogenic, carcinogenic, and mutagenic	Equipment, pipes, plastic boxes, or epoxy resin surfaces	DBP 0.3 mg/kg, DEHP 1.5 mg/kg and DINP 9 mg/kg	Food quality material free of phthalates	Food quality material inspection	Unknown	ECD gas chromatography, 80 €

2.5. Possible Control Measures

The main strategy to avoid possible contaminations by OTA is to control the sanitary status of the grapes by visual control prior to processing them. This methodology allows the producers not to accept any spoiled grapes or to remove the affected grape bunches in a selective process before fermentation. Some authors report a success of about 98% using this methodology [25], when they establish a critical limit of tolerating just 1% of infected grapes.

However, in some cases, early contaminations by fungi cannot be detected by human eyes. Therefore, the control of fungi chemical indicator parameters, such as gluconic acid in grape reception, allows one to eliminate possible human subjectivities. A fast enzyme test able to analyze that compound is commonly used as a HACCP control measure, as it is relatively cheap: about 1 €/sample [26]. There are also commercial kits based on immunoaffinity [27] that offer good accuracy and a rapid solution. The official detection method of OTA is commonly used as a verification measure in accredited laboratories due to its price, which is around 30–40 € [28,29] currently. Early detection allows for the removal of traceability lots and allows one to apply corrective measures that are quite effective in this specific case.

2.6. Corrective Measures

Once the presence of OTA concentration over the critical limit is detected, several corrective measures can be applied before eliminating the lot. Some methodologies, such as reducing maceration in the case of contaminated grapes, fining activated carbon [30], or fermenting with selected yeast, can reduce OTA concentration in final wine from 70 to 32% [31]. Non-*Saccharomyces*, such as *Schizosacchromyces*, look to be very promising in reducing the content by about 70% during fermentation [31,32]. A regular amicrobic filtration before bottling about 0.45 µm of wine can easily reduce the final concentration in OTA by about 80% [33].

All these options make it easy to manage OTA when it is detected. For that reason, affected lots are usually not disqualified due to the high number of possibilities of corrective measures.

2.7. Verification

The official methodology in Europe is HPLC with a fluorescent detector. The detection price in an accredited laboratory varies from 30 to 40 € currently [28,29].

3. Biogenic Amines

3.1. Toxicity

Biogenic amines are over-specific concentrations able to produce undesirable effects, such as headaches, respiratory distress, blushing, heart palpitation, hyper or hypotension, tachycardia, itching, skin irritation, vomiting, and several allergenic disorders [34,35]. The levels found in wines are far from being able to produce such harmful effects in regular consumers. There are some specific groups of people, such as those who are allergic to histamine, for whom the effects could be especially dangerous. The most toxic biogenic amine that can appear in wine is histamine [36,37]. Human metabolism possesses several enzymes, such as monoamine oxidase and diamine oxidase, that degrade the toxic compound histamine for regular cases. However, specific groups of people have gradually inhibited those enzymatic activities. Another specific parameter of wine is the presence of ethanol, which can also inhibit those enzymes or alternative medication [36].

3.2. Origin

Biogenic amines production is mainly related to bacteria metabolism [38–40]. The main bacteria genera involved in the process are *Pediococcus*, *Oenococcus*, *Lactobacillus*, and *Leuconostoc*. Histamine formation depends on the genes of histamine decarboxylase activity. Lactic acid bacteria produce

biogenic amines during the malolactic fermentation that takes place in almost every red wine after alcoholic fermentation [41], although other micro-organisms, such as yeasts, are able to produce biogenic amines in smaller amounts [42].

3.3. Critical Limit

Although there are no specific laws, several countries have established rules for the specific biogenic amine histamine, whereas other biogenic amines remain free of control. Some recommended limits are 10 mg/L in Australia and Switzerland, 8 mg L in France, 3.5 mg L in the Netherlands, 6 mg L in Belgium, and 2 mg/L in Germany [37,43]. These recommended levels could become compulsory in the near future. According to these data, we can establish an industry critical limit of 2 mg/L, which is the most restrictive reported concentration.

3.4. Preventive Measures

All the preventive measures are designed to avoid uncontrolled bacteria developments in the grapes or during alcoholic fermentation. The use of sulfur dioxide is the traditional way of inhibiting lactic bacteria during alcoholic fermentation. The conventional enology sulfur dioxide doses allow yeasts to develop and ferment while bacteria are inhibited. An alternative is the inoculation of a high number of commercial yeasts that makes the development of other competitor micro-organisms impossible. Nevertheless, other modern products, such as lysozyme or chitosan, also effectively inhibit lactic acid bacteria development, consequently reducing the incidence of biogenic amines in wine. These products can also be used if an undesirable lactic bacteria development takes place during alcoholic fermentation, in order to stop it as soon as it is detected. Nevertheless, there are other types of management than additive provision, such as high levels of hygiene, that reduce the initial population of undesirable micro-organisms, such as wild lactic bacteria, in any installation that is in contact with wine [44]. Biofilm techniques can considerably reduce the risk of bacteria able to produce biogenic amines. Biofilm techniques consist of directly colonizing the wine and preventing the development of spoilage micro-organisms. For that purpose, species such as *Torulaspora delbrueckii* are used to minimize the use of additives, such as sulfites [45].

3.5. Possible Control Measures

The management of these risky compounds at the industry level is commonly based on the use of selected lactic bacteria that do not possess histamine decarboxylase enzymatic activity [40]. Approximately 20% of bacteria do not possess that undesirable enzymatic activity [40]. Nowadays, it is relatively easy to detect which bacteria are able to decarboxylase amino acids precursors to the unhealthy biogenic amine forms [46]. All lactic bacteria available in the market underwent selection processes in order not to develop such enzymatic activities, a part of classic selection parameters, such as malic acid degradation, performance at low temperatures, and sulfur dioxide tolerance. Therefore, the inoculation of those strains, instead of performing a spontaneous process, and the control of a proper devolving of malolactic fermentation through the monitoring of bacteria implantation through microscopic observation or more advanced techniques or malic acid degradation and evolution after bacteria inoculation are some of the most common ways to control enzymatic activity.

Nevertheless, during the last several years, new biotechnologies based on the use of yeasts able to remove the malic acid from wine while avoiding any possible bacteria activity are becoming popular, especially in those regions where performing malolactic fermentation can mean a drop in quality [41]. The use of *Schizosaccharomyces pombe* is the best option, although in grape juices that are not very acidic it must be combined with *L. thermotolerans* to avoid excessive deacidification [41]. These new biotechnologies are usually combined with other technologies, such as lysozyme or chitosan, to avoid any undesirable bacteria development that could generate detrimental biogenic amines production. Therefore, in those cases, the production of biogenic amines is not possible, and the final concentration is null.

Another control measure is direct analysis of biogenic amines, such as histamine. This control is highly recommended in wineries that perform spontaneous malolactic fermentations. Some affordable options are the use of rapid techniques, such as enzymatic analysis [26], which is fast and relatively cheap: about 1 € per sample. The official methodology is usually performed for verification purposes, as it is much more expensive and requires specific instrumental equipment.

3.6. Corrective Measures

Even though some yeasts can remove small amounts of biogenic amines during alcoholic fermentation or during lees contact processes, there is no effective way of removing biogenic amines when they appear in finished wine. For that reason, all efforts must be focused in order to avoid their undesirable formation, as to date it has not had any effective corrective solution.

3.7. Verification

After using protocols that reduce the incidence of biogenic amines, in most cases the verification measure is performed by fluorescence HPLC chromatography in accredited laboratories [29]. The price in the market varies from 40 to 70 € [29].

4. Ethyl Carbamate

4.1. Toxicity

Ethyl carbamate (EC) is a known carcinogen compound present in a variety of fermented foods [47]. Since the 1940s, the literature has considered it a toxic compound. In 1943, it was proven to be carcinogenic [48,49]. A common use of ethyl carbamate was as a sedative and anesthetic for animals. Ethyl carbamate is carcinogenic and genotoxic for several species, including hamsters, rats, mice, and monkeys, which suggests a high potential carcinogenic risk for humans [50,51]. Ethyl-carbamate absorption implicates three pathways: N-hydroxylation or C-hydroxylation, hydrolysis, and side-chain oxidation. The main pathway is Ethyl carbamate (EC) hydrolysis through liver microsomal esterases to carbon dioxide, ammonia, and ethanol. The International Agency for Research on Cancer (IARC) classifies ethyl carbamate as a group 2A carcinogen (i.e., probably carcinogenic to humans) [52].

4.2. Origin

Ethyl carbamate is mainly produced in wines due to the evolution of urea. Urea is a regular metabolite produced by most yeasts and bacteria during their regular metabolisms. Urea is slowly combined with ethanol, producing ethyl carbamate. This is why incidence is higher in old aged wines. Other secondary production pathways can be created by the action of lactic bacteria and specific amino acids metabolism. Citrulline is an intermediate of arginine degradation by wine lactic acid bacteria during malolactic fermentation. Citrulline is the second precursor in the formation of ethyl carbamate after urea [53]. A high percentage of heterofermentative wine lactic bacteria, such as *Oenococus oeni*, are able to degrade arginine. The enzyme arginine deiminase produces that phenomenon.

4.3. Critical Limit

European legislation does not specify any legal limit regarding ethyl carbamate. Nevertheless, some specific countries possess a legal limit or a recommended level. Some examples are Canada (30 μg/L), Czech Republic (30 μg/L), South Korea (30 μg/L), and the United States (15 μg/L) [54]. We propose the lowest referenced level of 15 μg/L as the critical limit to be considered, especially for companies from countries where ethyl carbamate is not legislated but with possibilities of exporting to countries with legal limits.

4.4. Preventive Measures

Possible preventive measures are to reduce nitrogen fertilization in vineyards, especially the direct use of urea. Another measure is to use only the necessary nutrient supplementation before and during fermentation, as increases in nitrogen composition will increase the final production of urea [55,56]. Any alternative to malolactic fermentation is an effective way to avoid ethyl-carbamate formation from that bacteria metabolism or their urea formation [45].

4.5. Control Management

Current strategies are based on the use of urease enzyme [57], which can reduce levels of urea down to 0 mg/L or non-detectable levels. Some companies commercialize the enzyme, and its use is common in companies that export to countries with legal limits. Another more recent alternative is the use of yeast species that naturally possess urease activity. Some of them are able to complete an alcoholic fermentation by themselves, such as *S. pombe*, whereas others can be used in combined fermentations with a more powerful fermenter, such as *Saccharomyces cerevisiae* [41]. Some experiences demonstrate that the final urea values in these cases are close to 0 mg/L. Another option is the use of selected malolactic bacteria that cannot excrete citrulline from arginine degradation [53].

4.6. Corrective Measures

Once it is produced, there is no corrective methodology that can effectively reduce the final concentration to regular levels.

4.7. Verification

Accredited laboratories offer GC/MS as a detection technique. The price in the market varies from 40 to 100 € [29].

5. Sulfur Dioxide

5.1. Toxicity

According to the Agency for Toxic Substances and Disease Registry (ATSDR) [58], sulfur dioxide may cause irritation, and is especially dangerous when exposed to the eyes, mucous membranes, skin, and respiratory tract. Direct exposure can cause such problems as bronchospasm, pulmonary edema, pneumonitis, and acute airway obstruction.

Nevertheless, for the regular levels that can appear in wine, the main issue is people who suffer from chronic pulmonary diseases, such as asthma [59], that can easily evolve to bronchospasm. For that reason, in some markets, it must be indicated in the labeling that the wine contains sulfites in order to protect that specific high-risk group, as they can easily identify any risks by reading the label before consumption.

5.2. Origin

Although some toxicological properties are attributed to sulfur dioxide, its use caused a revolution in winemaking, as it is a common additive that possesses several interesting properties from a technological point of view, such as antioxidant, antimicrobial, and inactivator of oxidase enzymes, such as laccase or tyrosinase, properties. Therefore, such properties notably increased the quality of wines once its use became generalized in most wines. The management of rotten grapes is especially difficult without sulfur dioxide if a good-quality wine is the objective of vinification. Nowadays, there is no other single additive that provides a solution to all the former properties.

Sulfur dioxide is commonly used in different phases of the winemaking process, such as reception, grape crushing, alcoholic fermentation, and barrel aging or storage. The main point about using it

in winemaking is to inhibit possible bacteria development during alcoholic fermentation or storage, while protecting against oxidation, which can spoil a wine's aroma and color.

5.3. Critical Limit

The legal limit in Europe varies, depending on the content of sugar and the type of wine, from 150 mg/L to 350 mg/L total sulfur dioxide content [60]. A recent trend is to reduce the legal limit gradually due to its toxicity. Sweet wines and wines produced from rotten grapes are those that have been allowed to reach the highest limits due to their more difficult management from a microbiological and technological point of view. The most consumed wines in Europe—dry red and dry white—have a legal limit of 150 and 200 mg/L, respectively [60,61]. The higher permitted levels for white wines are justified due to their lower protection in antioxidant compounds, such as anthocyanins and tannins, that must be compensated for with higher additions of sulfur dioxide.

5.4. Preventive Measures

Although there are not at this moment any additives that can totally replace sulfur dioxide, many can replace some of its technological properties. The best examples are the ones that possess antimicrobial activity, such as sorbic acid [62], lysozyme [63], and chitosan [64]. Physical methods, such as high-pressure processing, allow one to greatly reduce the need for preservatives due to their capacity for undesirable micro-organism inactivation [65]. Ascorbic acid [66] is effective against oxidations; products that combine sulfur dioxide and ascorbic acid have started to become common in the market. Another option is the removal of oxygen that can react with oxygen before bottling [67]. Therefore, theoretically, it is possible to replace sulfur dioxide with a combination of several additives with different properties. The selection of yeasts with a low production of compounds able to bound to sulfur dioxide, such as acetaldehyde, which decrease the efficiency of sulfur dioxide additions, is an alternative to reducing initial doses [45].

Another alternative is the use of thermovinification, which inhibits most micro-organisms and inactivates such enzymes as tyrosinase or lacassa so that high doses of sulfur dioxide are no longer required. The sanitary initial state of grapes and the hygienic state of winery conditions influence the initial state of microbiota and can contribute to reducing the initial sulfur dioxide doses in winemaking.

5.5. Control Management

Most problems are mistakes in calculations before addition. A regular control measure is to calculate the proper dose and to obtain approval from the enologist before physical addition. Then, the added sulfur dioxide amount is registered and contrasted to the stocked sulfur dioxide in order to detect a possible mistake.

There are several techniques for analyzing sulfur dioxide. It is very common to use, after additions, the cheap and fast analytical method named Ripper, which, though not as accurate as the official method, is accurate enough to detect additions that are excessively high. The official method, which takes 30 min and is named the Paul method, is usually reserved for verification purposes.

5.6. Corrective Measures

One alternative to reducing the concentration is oxygenating [67] the wine through rankings. However, the reduction of high concentrations is very slow and requires large investments of energy to pump. The most common solution is to dilute the wine with another wine whose concentration is below the legal limit. An unrecognized International Organisation of Vine and Wine (OIV) practice is the use of hydrogen peroxide.

5.7. Verification

Verification is performed using the official Paul method. Several companies usually contract the service out to accredited laboratories to verify their internal analyses. The price varies from 2 to 5 € [31].

6. Wine/Food Allergens

6.1. Toxicity

Wines that have been fined using some potentially allergenic products, such as proteins or non-grape tannins, can produce clinical allergic reactions, especially in people who suffer from an allergy to food allergenic proteins [68].

6.2. Origin

During alcoholic and malolactic fermentation, turbidity is an inevitable effect. Potentially allergenic food proteins are used in most wines to achieve specifications related to low turbidity units. Most consumers and wine distributors demand a lack of turbidity in the end product. Although turbidity by itself is not a food safety problem, it is a common reason to refuse lots. Therefore, winemakers commonly use fining agents to produce bottled wines free of any turbidity that could lead to refusal in the market. Several of those fining agents possess allergenic properties, such as egg white, caseinates, or fish gelatin. Residual traces of those compounds could occasion allergenic reactions in allergic individuals [68]. Other modern additives, such as lysozyme (egg allergen), have started to become an interesting option for reducing sulfur dioxide additions in the control of undesirable spoilage bacteria during alcoholic fermentation.

6.3. Critical Limit

Although there is no prohibition of the use of fining agents, there are some that are considered targeted food proteins, and they must be indicated on the labels. European legislation obliges winemakers to label any wine treated with allergenic additives or processing aids if their presence can be detected in the final product [69,70]. We propose as a critical limit to label the product where there is a presence of traces.

6.4. Preventive Measures

The most common preventive measure is performing stabilization tests to determine whether a fining process is needed and which minimum proper dose is possible to achieve the desired effect. Currently, there are several alternatives to fining processes, e.g., cold stabilization and subsequent filtration. Nevertheless, those processes require specific installations, energy, and more time to be performed. A more recent alternative is the use of fining agents whose proteins are from plants, such as wheat or lupine [71–73]. However, we must take into account that, although it is possible to reduce the turbidity in a similar way to those of an animal nature, some of them can also generate risks for specific groups of people, such as those with celiac disease. Nevertheless, peas and potatoes are nowadays not included in the list of main allergens, and they do not need to be included in labeling [72,73].

6.5. Control Management

One control-management measure is to use alternative fine agents, being always aware of their nature in order to avoid other allergenic reactions. Nevertheless, the most common measure is to label the products according to the specific legislation [74] so as to make it easy for allergic people to identify potentially risky products and avoid accidents. It is common for the quality control manager to check the label before proceeding to bottle any lot. Another option is chemical control [74–76], although it is more commonly used for verification purposes.

6.6. Corrective Measures

If those agents are used, it is difficult to remove them completely from the wine as traces will be detected. Nevertheless, some techniques, such as filtration, or secondary finning treatments can achieve final concentrations that make it impossible to detect their presence in the final product.

6.7. Verification

The official detection methods are based on ELISA tests [29], and the price offered by accredited laboratories is about 50 €. Some recent alternative methodologies are based on mass spectrometry [74,75].

7. Pesticides

7.1. Toxicity

Although there are numerous families of pesticides with different negative health effects, the most common harmful health effects are associated with dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine negative effects [77]. Furthermore, high occupational, accidental, or intentional exposure to pesticides can result in hospitalization and death [78].

7.2. Origin

Several fungi or insects can attack the grapes in the vineyards. On occasion, the fungi attacks can seriously decrease the quality of wine and its final value. Therefore, it is normal to protect the vineyard against some common plagues that appear quite often depending on the year and climatic conditions. In some cases, high residual values can help avoid the proper development of yeast that is also fungi. Some of the most common plagues are mildew, *Lobesia botrana*, and *Botrytis cinerea*. These are much-studied, and most agriculturalists possess some notions about how to treat them in a successful way.

7.3. Legal Limit

Most pesticides can cause serious food poisoning. Thus, the pesticides that can be used in viticulture are legislated and need authorization. Those that are authorized possess legal residual limits in final wine of about μg/L [79,80]. The legal limits vary between countries. Nevertheless, the Codex Alimentarius establishes international standards for grapes [81] of 99 pesticides and their limits, and a limit of 5 mg/kg for dithianon for wine grapes [82].

7.4. Preventive Measures

The main preventive measures are based on the use of vineyard management that avoids propitious microclimates for fungi development. Some examples are the removal of leaves and exposure of grape clusters to sunlight and air currents that avoid high moisture conditions. The development of resistant plants is also of great interest [83,84]. Cultivation in dry areas also significantly reduces the risk of fungal attack and makes it easy to produce organic wines free of pesticides.

7.5. Control Management

Although some pesticides are authorized, their use must be justified and registered in the field practice notebook. The aim of registers is to respect the authorized periods of residuality in order to avoid possible residual values over the limit in final wines. When wineries do not control vineyard management, they sell the grapes to viticulturists. They usually request the field practice notebook in order to register performed treatments before accepting the grapes.

7.6. Corrective Measures

Once contamination takes place in wine, it is not possible to remove it.

7.7. Verification

The most common way to verify whether the system is properly controlling the risk of pesticides in our industry is by contracting for the analysis of the official method in an accredited laboratory. The official methodology includes the analyses of the legislated pesticides by ECD gas chromatography [29], which costs about 21 €.

8. Genetically Modified Organisms

8.1. Toxicity

Several scientific studies confirm that there is no proof of recombinant proteins contained in foods produced by genetically modified organisms (GMOs) being more harmful than regular proteins [85]. Nevertheless, as there is also no proof of their being totally innocuous, they are considered a potential food safety hazard in some countries.

8.2. Origin

Laboratory experimentation with genetically modified yeasts shows remarkable improvements in several quality fermentation parameters, e.g., acidity management [86,87], that led to producing better wines or reducing industrial risks under difficult situations, such as high levels of sugar or a lack of nutrients. These scientific findings tempt winemakers to use those GMO yeasts in real industry.

8.3. Critical Limit

Although there is no scientific proof that GMO wine is more dangerous to human health than non-GMO wine, there is very strict preventive legislation in several countries [88]. They will remain valid for several years until new results prove this biotechnology to be totally safe.

8.4. Preventive Measures

The safest preventive measure derived from HACCP to avoid GMO problems would be to perform spontaneous alcoholic and malolactic fermentations. Nevertheless, some controlled fermentations can originate other problems that can influence wine quality or even food safety from other hazard nature food safety problems. For that reason, another preventive measure is to buy products, such as dried commercial yeasts, from registered companies that comply with the legislation, produce safe products, and have a sanitary registration that certifies their conduct.

8.5. Control Measures

Control measures in wineries are commonly based on supplier controls at the moment when the materials are received, when the sanitary registration of the company is identified before product acceptance. The enologist or qualified assistant will check the labeling of the microbiological dried product, identifying the food quality and the GMO-free indication. If the product does not have food quality certification, the company does not have sanitary registration, or the product is not properly labeled, the use will be voided.

8.6. Corrective Measures

Once the product is identified as GMO wine, there is no known solution. Nevertheless, there are some food industries where the use of modified organisms is not legislated, as the product will not be directly consumed by humans, e.g., bioethanol for cars or for disinfection purposes. In those cases, wine could be sold to those industries, obtaining a residual value.

8.7. Verification

The detection of GMOs in food produce is mostly based on Polymerase Chain Reaction (PCR) methodologies that offer enough sensitivity, accuracy, and precision to use on wine [89]. The price of the analysis is about 100 €. Therefore, this instrumental technique is commonly used only for verification purposes or for inspection reasons for governmental controls.

9. Physical Hazard

9.1. Toxicity

According to the Institute of Agriculture and Natural Resources [90], small physical hazards, such as pieces of glass, can cause potential cuts and bleeding and may require surgery to find and remove. Other foreign objects, such as metal from installations or personal effects from employees, can also generate cuts, infection, and choking.

9.2. Origin

Physical hazards are foreign objects, distinct from the food products, that are able to cause injury or illness to the final consumers. The most common extraneous matters found in food products are bones, metal fragments, pieces of product packaging, stones, wood fragments, insects, or other personal items. The most common physical hazard described in wine is glass, because it is the main material of the final vessel that usually contains the wine when it is sold to the final consumer. The main origin is improper raw materials, such as deteriorated bottles, from the beginning of or during their manipulation. Other origins are improper facilities or equipment or a lack of maintenance that enables strange objects to pass into the wine. Another significant origin is improper performance by employees due to human error, which leads to the dropping of personal effects that can end up in the wine.

9.3. Critical Limit

Several countries' legislation does not indicate quantifiable limits or recommended ones. According to the Division of Compliance Management and Operations (HFC-210) [91], a hard or sharp foreign object larger than 7 mm can produce a serious health hazard for humans. However, other countries consider more restrictive sizes down to 2 mm. Health Canada evaluates injurious extraneous material in food, and it considers 2 mm or greater as the threshold size for consideration as a health risk [92]. Besides size, the risk associated with extraneous material is further evaluated through an assessment of shape, hardness, material, source, target consumer groups, etc.

9.4. Preventive Measures

The main preventive measures in winemaking consist of raw material inspection based on specifications, seller quality certifications, preventative equipment maintenance, and employee training based on good practice guidelines.

9.5. Control Management

The most common way to guarantee that no foreign objects are in a commercial wine is to perform a prior filtration with a proper pore nominal diameter below 2 mm before bottling. The use of filtration materials with a pore diameter down to 0.22 μm is common in some winemaking protocols [93].

9.6. Corrective Measures

In case some deviation takes place during the filtration process, the most common corrective measure is to filter the lot again when the filtration equipment has been optimized.

9.7. Verification

The verification of physical hazard critical control point management is commonly based on the inspection of random bottles of each traceability unit.

10. Phthalates

10.1. Toxicity

Phthalates have been scientifically proven to be endocrine-disrupting [94], estrogenic [95], carcinogenic, and mutagenic [96].

10.2. Origin

Equipment that contains phthalates is commonly used in many industries, as these compounds increase the flexibility of plastic [97]. In the winemaking industry, they usually appear in flexible plastic pipes, plastic boxes to collect grapes, or epoxy resin surfaces. Phthalates can migrate to beverages from plastic equipment or packaging materials [98] because they are not covalently bound to plastics [99].

10.3. Critical Limit

According to the European legislation, some phthalates possess a legal limit. Those phthalates are dibutyl phthalate (DBP) 0.3 mg/kg, diethylexyl phthalate (DEHP) 1.5 mg/kg, and diisononyl phthalate (DINP) 9 mg/kg.

10.4. Preventive Measures

Most common preventive measures consist of using food quality material free of phthalates for contact with grapes and wine. Usually, winemakers have to pay special attention to old pipes that were made before the legislation that regulates phthalates. Another option is the use of non-plastic materials, such as stainless steel for pipes.

10.5. Control Management

It is necessary to check that the materials that are going to be in contact with wine possess the proper standard of food quality and are produced by a certified provider. Clearly, pipes designed to move liquids other than food liquids, such as gasoil or water for refrigeration systems, are not suitable for wine management.

10.6. Corrective Measures

When wine contains phthalates over the legal limit, there is no known solution to remove them. Such wines are usually sold as sub-products to companies that make products not aimed at human consumption, such as bioethanol.

10.7. Verification

The most common way to verify if the system is properly controlling the risk of phthalates in winemaking is contracting for the analysis of the official method out to an accredited laboratory. The official methodology includes the analyses of the legislated phthalates by ECD gas chromatography [29], and the cost is about 80 €.

11. Conclusions

Although wine is a food beverage in which no pathogenic micro-organisms can develop, in a similar way to other food products, such risks as physical hazards must be taken into account; these kinds of hazard are easily controlled with such techniques as modern filtrations. Modern research studies have discovered potential toxicological compounds that must be taken into account in

order to produce healthier wines and protect specific consumers that can be included in risk groups. Some of these hazards are biogenic amines, ethyl carbamate, and OTA. The management of these modern hazards, such as control measures, corrective measures, and verification methods, is not very well-known yet, which makes them difficult to control. Some new winemaking technologies allow one to effectively control those risks in a successful way, which offers solutions to issues that wine industries face today. All these methodologies can be easily implemented using a HACCP system.

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