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Prospects for the use of sensors/biosensors for detecting food safety parameters in wine

Perspectivas de utilización de sensores/biosensores para detección de parámetros de seguridad alimentaria en vino

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Abstract. The implementation of food safety assurance systems in wineries is a mandatory requirement and prevents wines from containing chemical hazards that may affect the health of consumers. Traditional analytical techniques require the transfer of the sample to specific laboratories, with highly sophisticated instrumentation requiring high maintenance costs and highly qualified personnel, which makes the rapid determination of these undesirable substances in grapes or wine unfeasible. The use of selective, sensitive and portable sensor/biosensor devices represents an advantage for the real-time control of harmful substances in the wineries. This work has investigated the use of sensors/biosensors for the monitoring of chemical hazards such as cyanide, PAHs, allergens and sulphites, as well as for the verification of pesticide residues, mycotoxins, heavy metals and other harmful compounds that might be present in wine. The bibliography consulted has shown that there are studies in which sensors/biosensors applicable to the food industry in general and the wine industry in particular have been developed and, although there are not too many devices commercially available, these studies provide a solid basis for the future development of devices useful in the wine industry.

Resumen. La implantación de sistemas de garantía de inocuidad alimentaria en bodegas de vino es un requisito obligatorio, y evita que los vinos contengan peligros químicos que puedan afectar la salud de los consumidores. Las técnicas de análisis tradicionales requieren el traslado de la muestra a laboratorios específicos, con instrumentación altamente sofisticada que necesita un elevado coste de mantenimiento y un personal altamente cualificado, lo que hace inviable la determinación rápida de estas sustancias indeseables en la uva o el vino. El empleo de dispositivos sensores/biosensores selectivos, sensibles y portátiles representa una ventaja para el control en tiempo real de sustancias perjudiciales en la industria vitivinícola. En este trabajo se ha investigado el uso de sensores/biosensores para la vigilancia de peligros químicos como el cianuro, HAPs, alérgenos y sulfitos, así como para la verificación de residuos de plaguicidas, micotoxinas, metales pesados y otros compuestos perjudiciales que podrían estar presentes en el vino. Se ha comprobado que existen estudios en los que se han desarrollado sensores/biosensores aplicables a la industria alimentaria en general y a la del vino en particular y, aunque no hay demasiados dispositivos disponibles comercialmente, estos estudios proporcionan una base sólida para el futuro desarrollo de dispositivos útiles en la industria vitivinícola.

1. Introduction

The implementation of food safety assurance systems in wineries involves ensuring that the wines produced do not pose a risk to consumer health and, therefore, substances that, such as allergens, could constitute a danger to some consumers are identified on the labelling and, furthermore, are free or have a limited content of harmful substances, such as those that could be incorporated during the production process (pesticides, mycotoxins, additives, etc.).

In this respect, the OIV's Hazard Analysis and Critical Control Point (HACCP) guide identifies the food safety parameters that must be considered as significant risks and, therefore, kept under control during wine production.

The official analysis techniques require the transfer of the sample to specific laboratories equipped with sophisticated instrumentation that requires high maintenance costs and the need for highly qualified personnel. This makes on-site monitoring difficult, which would be desirable for the rapid detection of possible wine contamination problems at any stage of wine production. Sensor/biosensor devices are selective, sensitive, portable and easy-to-use analysis techniques that provide reliable results in a short time and are very useful for in situ and real-time control and monitoring of substances harmful to health (pesticides, additives, mycotoxins, etc.) in the wineries.

Therefore, the design and manufacture of new and robust sensors/biosensors is emerging as an effective solution to carry out, in situ and quickly, the analysis of compounds of interest for quality control and safety assurance in wineries.

2. Objective

This work has two objectives: i) To identify the analytical parameters of interest to ensure food safety in wineries. ii) To assess the feasibility of using sensors/biosensors to carry out analyses in the winery, either during the monitoring of the Critical Control Points or during the verification of the effectiveness of the implementation of the HACCP system.

3. Material and methods

A study has been carried out to identify the need for the development of sensor/biosensor devices that could be used in wineries, during the winemaking process, to analyse the presence of substances of interest in ensuring the safety of wine.

4. Results

4.1. Use of sensors/biosensors in the implementation of HACCP systems.

The food industry is primarily responsible for taking the lead in developing and elaborating national guides to good practice. However, in the case of the European Union, the Member States have the power to study the national guides developed by each sector to ensure that they comply with the requirements established by Regulation 852/2004 on the hygiene of foodstuffs.

In Spain, in order to facilitate these procedures, in 2010 the Institutional Commission of the Spanish Agency for Food Safety and Nutrition (AESAN) approved the ‘Procedure for a guide on good hygiene practices and/or the application of HACCP principles to be considered as a national guide’. This procedure includes the possibility for the Regional Governments to draw up their own guides in collaboration with the central administration. In the case of the wine sector, the guide to good hygiene practices for the wine sector in Catalonia has been published.

The HACCP plans implemented in wineries must include a procedure for monitoring the Critical Control Points (CCPs) (Principle 4 of the Codex Alimentarius) and a procedure for verifying the plan (Principle 6).

According to the guide of good hygiene practices for the wine sector of the Generalitat de Catalunya [1] and the HACCP guide of the OIV [2], it is recommended that the following compounds related to wine safety be controlled in the CCP monitoring procedure: Presence of cyanide derivatives, Polycyclic Aromatic Hydrocarbons (PAHs), SO2, and allergens related to clarification and stabilisation (egg proteins or milk proteines).

On the other hand, for the verification of the correct implementation of the HACCP plan, the analysis of other parameters related to the safety of the final product is recommended, among them: Ochratoxin A, pesticide residues, heavy metals, residues of cleaning products, Bisphenol A and derivatives, ethylene glycol and diethylene glycol, ethyl carbamate, biogenic amines, phthalates and nonylphenolic compounds, dioxins, furans and polychlorinated biphenyls [2].

The use of sensors/biosensors for the rapid analysis of these compounds would be of great interest to the wine industry.

4.2. General characteristics of sensor / biosensor devices.

Sensors/biosensors are integrated devices that combine a recognition element, chemical in the case of sensors and biological in the case of biosensors, with a transduction element, and are capable of providing specific quantitative or semi-quantitative analytical information. These devices are highly selective due to the possibility of adapting the specific interaction of the analyte with a recognition element in the sensor substrate that has specific binding affinity to the molecule of interest.

A sensor/biosensor consists of several fundamental components:

– Analyte: The substance to be analysed or quantified.
– Receptors/bioreceptors: These are materials that have a high selectivity for the analyte. In the case of biosensors, these are biological materials such as enzymes or antibodies.
– Transducer: converts the chemical or biochemical signal into an electrical signal.
– Amplifier: Amplifies and processes the electrical signal obtained from the transducer.

- Display: Output system that shows the results in the form of curves, graphs, images or tables.

Figure 1 shows a diagram of a generic biosensor with its parts.

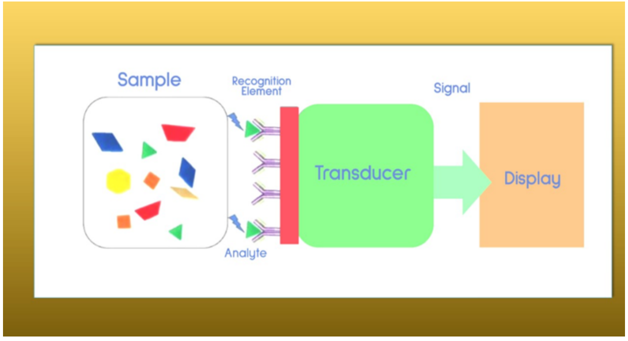

Figure 1. Diagram of a biosensor.

Depending on the type of transducer element, they can be classified as follows:

- Electrochemical: They measure the current, voltage or impedance resulting from the biochemical reaction.
- Optical: Detect changes in optical properties such as absorbance, fluorescence, etc.
- Thermal: measure changes in temperature produced by the biochemical reaction.
- Piezoelectric: Detect changes in mechanical properties such as mass or elasticity [3].

4.3. Sensors/biosensors of potential use in the monitoring and verification of HACCP plans in wineries.

4.3.1. Monitoring of CCPs in the wine industry: Control of chemical hazards recommended by the OIV, using Sensors / Biosensors.

The OIV recommends monitoring of cyanide, PAHs, allergens used in wine clarification and residual levels of SO_2

Hydrogen cyanide is a very toxic compound that could be released by combination with potassium ferrocyanide, which is added to some wines to stabilise them and avoid turbidities or metallic precipitations. The wine must be free of cyanide [1].

Sensors based on noble metal nanomaterials (Au and Ag) with an optical transducer have been described for the determination of cyanide content. The advantages of these sensors are their portability, selectivity, speed and low detection limit. One of these sensors uses gold nanoparticles functionalised with β cyclodextrin that gives a red colour to the negative sample, decreasing the intensity of the red colour in the presence of cyanide. In this way, very low cyanide concentrations (93 nM) have been measured in drinking water [5]. Its use could be studied in white wines.

The use of sensors made of cerium/gold nanoclusters (NCs) stabilised with bovine serum albumin (BSA-Ce/Au NCs). When excited at 325 nm, BSA-Ce/Au NCs exhibit two fluorescence bands centred at 410 and 658 nm, which are assigned to BSA-Ce/Au complexes and Au NCs, respectively. The fluorescence at 658 nm is quenched by etching the Au core of the BSA-Ce/Au NCs with CN-, while the fluorescence at 410 nm increases during complex formation between BSA, Ce4+ and [Au(CN)2]-. A detection limit of 200 nM is achieved with this technique. [6].

Finally, the use of an amperometric biosensor using cyanide inhibition of the activity of a peroxidase enzyme (horseradish peroxidase) that catalyses the oxidation of caffeic acid in the presence of hydrogen peroxide has been described. The enzyme is immobilised on a surface of carbon sonogel and gold nanoparticles. This biosensor has been found to have a detection limit of 0.03 µM. [7]. The application of this biosensor in wine could be studied, as the colour would not influence the results.

PAHs have been classified by the International Agency for Research on Cancer as probable human carcinogens, therefore, maximum levels of these compounds in the final product are being limited. Wine could be contaminated with PAHs by exposure to toasted wood barrels during ageing, so it is important to measure these compounds in barrel-aged wines.

Although no literature has been found on sensors/biosensors to determine PAHs in wine, they have been developed to determine PAHs in water. This methodology could be used as a basis for sensors that can be used in wine.

Sensors have been used by modifying a gold electrode screen-printed with 11-mercaptodecanoic acid (11-MUA) for the determination of benzopyrene in water at 300 mV, achieving a detection limit of 0.01 ppm [8].

On the other hand, a sensor that measures benzopyrene by differential pulse voltammetry, with a detection limit of 0.67 nM, has been used. It uses a glassy carbon electrode in a binary medium of acetonitrile and water. This sensor uses the electrochemical oxidation of benzopyrene to determine its concentration in water samples [8].

In the wine fining stages, proteins that are considered as **allergens** in the European Union regulations (egg and milk derivatives) may be used. Depending on the procedure used by the winery to clarify the wine, traces of these allergens could remain in the final product and, although the OIV admits the presence of casein and ovalbumin with a detection limit of 0.25 mg/L, according to the regulations in force in the EU, it would be necessary to specify this on the labelling. If it is not desired to include this information on the label, it is necessary to guarantee the absence of traces of these allergens in the wine.

ELISA tests are currently commercially available to quantify the presence of allergens in wine. These tests take 2 to 3 hours to perform. However, the use of techniques based on sensors or biosensors that could do this more quickly could be of interest for the control of allergens in wine.

A voltammetric biosensor using an electrode with gold nanoparticles, on which anti-casein immunoglobulins are adsorbed, has been used for the quantification of caseins in solid foods. The presence of casein in the sample decreases the peak current of the redox couple and the casein present in the sample can be quantified with a detection limit of 0.05 ppm [9]. The application of this biosensor to liquid foods such as wine could be considered.

The concentration of egg proteins in white and rosé wines can be determined using a surface plasmon resonance (SPR) biosensor. This technology allows the detection and quantification of proteins through changes in the refractive index on the surface of a sensor when egg molecules present in the wine sample bind to specific antibodies immobilised on that surface. To quantify the presence of protein, this sensor would have the disadvantage of requiring a rather complex preparation of the sample to be measured, since it is necessary to purify it beforehand by Size Exclusion Chromatography (SEC). The biosensor would have a detection limit of 0.03µg/ml [10]. However, since the only presence of traces of egg protein would require its identification in the labelling, its use without prior purification of the sample could be considered.

Sulphites (SO_2 and derivatives) have an inhibitory function in wine, inhibiting the growth of bacteria, moulds and yeasts. In addition to being an allergen capable of causing respiratory crises, their content in the final product is limited by regulations. Therefore, in addition to identifying on the labelling that the wine contains sulphites, it is necessary to check that the quantity present does not exceed the legal limits.

Optical colorimetric sensors have been developed using sensor membranes that are prepared by dissolving indicator ion pairs in solvents together with a silicone or ormosil prepolymer. The ion pairs are prepared by reacting the indicators used: bromothymol blue (BTB), bromocresol purple (BCP) or bromophenol blue (BPB) with quaternary ammonium salts. When SO_2 reacts with the indicators, a colour change from blue to yellow occurs, which allows the SO_2 present in the sample to be quantified. The detection limit of the sensor depends on the indicator used: 1 µM for BTB, 4 µM for BCP and 5 mM for BPB [11].

Similarly, an amperometric sensor has been developed using an electrode on which an osmium redox polymer, which acts as a mediator for electron transfer, and the enzyme sulphite oxidase, which catalyses the oxidation of sulphite to sulphate, are immobilised together. The current generated by oxidation is measured at a working potential of - 0.1 V. This biosensor has a detection limit of 1 µM of sulphite (0.08 ppm). Although it is applicable to both white and red wines, its accuracy in red wines is lower than that obtained in white wines, because the higher polyphenol content of the wines inhibits the detection of sulphite [12].

Table 1 summarises the sensors/biosensors that could be used to monitor the CCPs recommended by the OIV. Either directly or after adaptation to the wine matrix.

Table 1. Sensors/Biosensors for CCP monitoring.

Parameter	Sensor/Biosensor type	Advantages / Disadvantages	LOD	Reference
Cyanide	Optical made of Ay nanoparticles functionalised with β ciclodextrin	Interference with the red colour of rosé wines	93 nM	[5].
	BSA-Ce/Au NCs	Interferences from different polyphenols, tannins and anthocyanins should be studied before being applied to wine	200 nM	[6].
	Enzyme receptor with peroxidase and amperometric transducer	This is a very fast technique, but would need to be validated in wine	0.03 µM	[7].
PHAs.	Electrochemical electrode with Au electrode screen-printed with 11 MUA	It would be necessary to adapt it to measure in wine.	0.01 ppm.	[8].
	Glassy carbon electrode in a binary medium of acetonitrile and water	It would need to be adapted for measurement in wine	0.67 nM	[8].
Allergens (casein).	Voltammetric with immunoglobulins adsorbed on Au NPs.	It would need to be adapted for measurement in wine	0.05 ppm	[9].
Allergens (egg protein).	SPR Biosensor	Complex sample preparation is necessary to achieve low LODs	0.03µg/ml	[10].
SO_2	Optical colourimetric using ion-pairing sensor membranes	Colour interference in red wines.	1 µM – 5 mM depending on the indicator	[11]
	Amperometric with sulphite oxidase enzyme	The polyphenol content of red wines makes the accuracy lowe	0.08 ppm	[12]

4.3.2. The verification of the HACCP plan in wine industries: Control of chemical hazards recommended by the OIV using Sensors / Biosensors.

In the verification of the effectiveness of the implementation of HACCP plans in the wine industry, the absence of the following chemical hazards in the final product must be verified: Pesticide residues, mycotoxins such as ochratoxin A, heavy metals, bisphenol A, ethylene glycol, biogenic amines, phthalates, dioxins and furans.

The **residues of pesticides** with which grapes can be treated have harmful effects on the health of consumers if unauthorised pesticides are used or, even when authorised, the residual concentration of pesticides in grapes exceeds the Maximum Residue Limits (MRLs) established in the regulations.

Despite the difficulty of having sensors/biosensors to quantify residues of the huge variety of authorised pesticides in wine grapes, biosensors have been developed to determine some types of pesticides.

Carbendazim can be quantified with a detection limit of 51.6 nM using modified glassy carbon electrodes. Its detection is based on the electrochemical oxidation of the pesticide which is detected as an oxidation peak at + 0.2 V in a pH 7 phosphate buffer solution [13].

On the other hand, a biosensor has been developed that uses an electrode modified with palladium nanoparticles to detect Rutin. When Rutin is oxidised, a signal is generated with a peak at +0.4 V in a phosphate buffer at pH 5. It is a very sensitive biosensor, with a detection limit of 0.006 nM [13].

Similarly, a sensor is used to determine Morin. In this case with a carbon paste electrode with nickel phthalocyanine. Its oxidation is detected at + 0.1 V in a phosphate buffer solution pH 7 [13].

An enzymatic biosensor using laccase (oxidoreductase) that catalyses the oxidation of formetanate can be mentioned. The enzyme is immobilised on an electrode modified with gold nanoparticles, and allows its quantification with a detection limit of 1 ppm [14].

In addition to the biosensors developed specifically for wine, there are more than 100 different sensors/biosensors described that have been designed for other foods and beverages, and that could be considered for modification for application in grapes or wine [15].].

Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus* and *Penicillium* moulds with the ability to cause chronic disease and is classified as a possible human carcinogen (International Agency for Research on Cancer Group 2B).

A biosensor based on white light reflectance spectroscopy (WLRS) has been developed. This device uses polyclonal anti-OTA antibodies on the surface of a silicon chip. When OTA is present in the sample, it interacts with the antibodies, causing an increase in the thickness of the biomolecular layer on the chip, which shifts the reflectance spectrum towards higher wavelengths and allows the concentration of OTA to be determined as a function of the thickness of the biomolecular layer. This biosensor can quantify concentrations as low as 0.03 ng/ml in wine in approximately 25 minutes [16].

Heavy metals have a toxic effect on human health, which is enhanced by their capacity for bioaccumulation. This fact has led the OIV to establish maximum limits for the content of metals such as lead and cadmium in wine.

In addition to traditional methods for the detection of these heavy metals, such as atomic absorption spectroscopy, some studies propose the use of voltammetric and potentiometric sensors to measure the content of Pb, Cd, Zn, Fe, Cu and Hg in wine [17] [18].

An electronic tongue has been developed that uses an array of potentiometric chemical sensors to detect traces of Pb, Cd and Cu in wine, with a detection limit of 0.1 µg/L for Pb and Cd and 0.02 mg/L for Cu. This is a very sensitive method, which allows the simultaneous detection of several metals, but requires prior digestion of the wine sample [17].

Similarly, modified graphite thin films containing electrodes have been used to quantify Pb and Cd in wines without the need for pre-processing of the samples. It is a more sensitive method than using Hg electrodes, and without the drawbacks of generating toxic residues, detecting concentration levels of 1 mg /mL [18].

Finally, the device for the determination of heavy metals in wine by differential pulse anodic stripping voltammetry (DPASV) can be mentioned. This technique is based on the electrochemical reduction of metals at an electrode and subsequent oxidation during a potential sweep, generating current peaks that are proportional to the concentrations of the metals present. It has been used to determine, in addition to Pb and Cd, Zn and Cu. This technique does not require prior digestion of the sample, although given the complexity of the wines, some of their components could interfere with the electrochemical measurements. With this sensor, detection limits of 4.57 µg/L for Pb, 1.74 µg/L for Cd, 4.64 µg/L for Zn and 1.0 µg/L for Cu have been achieved. [19].

Some wineries use tanks coated with epoxy resins that could lead to migration of **bisphenol A** and derivatives into the wine. These compounds are harmful to health and maximum limits have been set for them.

Biosensors are available to quantify bisphenol A in food and wine. There has been developed an electrochemical biosensor that uses a microelectrode with Au nanoparticles to increase its 3D surface area. This biosensor uses aptamers, which are single-chain oligonucleotide molecules that have the ability to bind specifically with the molecule to be determined, BPA in this case, this binding induces changes in the shape of the aptamers that can be used to detect the analyte. In this biosensor, when the BPA present in the sample binds to the specific aptamers, messenger DNA (mDNA) strands that were bound to magnetic particles are released. The mDNA strands bind to capture DNA (cDNA) strands on the electrode. The action of the enzyme Exonuclease III (Exo III) cleaves the cDNA strands releasing more mDNA. The more BPA in the sample, the more mDNA is released and the more cDNA is cut. The cDNA molecules remaining on the electrode bind to DNA labelled with methylene blue, which generates an electrical current that can be measured (the measured signal is inversely proportional to the amount of BPA present in the sample). It is a very sensitive biosensor, capable of measuring concentrations as low as 10 pg/mL [20].

Biogenic amines appear in wine as a result of the decarboxylation of amino acids or the amination of aldehydes and ketones during the fermentation and ageing stages. A high concentration of these substances not only decreases the quality of the wine but can also have toxic effects [21].

There has been developed a biosensor that uses a graphene or platinum electrode on which an enzyme, diamine oxidase (DAO), is immobilised, which catalyses the oxidation of the biogenic amines present in the sample, releasing hydrogen peroxide, this compound is oxidised generating an electric current that is quantified and is proportional to the quantity of biogenic amine in the sample. It allows the determination of histamine, putrescine and cadaverine with a detection limit of 0.1 µM. The only drawback is the possible interference with some compounds in wine [21].

In addition to the above, a glass sensor on which Au or Ag nanoparticles are attached to increase its surface area has been used. Localised Surface Plasmon Resonance (LSPR) is used to measure the amount of biogenic amines. This sensor has a transducer based on surface-amplified Raman spectroscopy. When amines are present in the medium, the Raman signal is significantly amplified and this can be used to quantify the amount of analyte present in the sample. According to the author of the work, this device allows the measurement of amine concentrations in the sample in the order of nM [22].

Ethylene glycol could appear in wine as a result of contamination due to leakage from the cooling system. No literature has been found on the detection of this contaminant using sensors/biosensors applicable to wine.

Toxic substances that could migrate into wine from PVC containers also include **phthalates**. These compounds are harmful to health, as they could act as endocrine disruptors.

Although no specific sensors/biosensors for determining phthalates in wine have been found in the literature, they do exist for other foods. A dual emission fluorescence sensor based on molybdenum disulphide Quantum Dots (MoS_2 QDs) and cadmium telluride (CdTe QDs) has been developed. This sensor uses an aptamer that binds to bis(2-ethylhexyl) phthalate (DEHP), modifying the fluorescence signal of the sensor, has a detection limit of 0.21 µg/L [23] and could be used as a basis for creating new sensors/biosensors applicable to wine.

Finally, **dioxins and furans** could be present in wine by migration from contaminated barrels or from the cork, if it has been treated with chlorinated compounds. Although the scientific community continues to try to develop sensors/biosensors that can determine these compounds in food [24], no conclusive studies have been found in the literature.

Table 2 summarises the sensors/biosensors with potential for use in wineries to verify the effectiveness of the HACCP plan in place.

Table 2. Sensors/Biosensors to verify the effectiveness of the implemented HACCP plan.

Parameter	Sensor/Biosensor type	Advantages / Disadvantages	LOD	Reference
Pesticides	Electrochemical for Carbendazim, Rutin and Morin	Very low LODs.	From 0.006 nM for the Rutin.	[13]
	Enzyme electrode with laccase enzyme with laccase for formetanate detection.	It has been used on fruits and has not been evaluated for use on wine	1 ppm.	[14]
OTA	White light reflectance spectroscopy	The analysis only take 25 minutes.	0.03 ng/mL	[16]
Heavy metals (Pb and Cd)	Electronic tongue with potentiometric sensors	Needs prior digestion of the wine.	0.1 µg/L	[17]
	Electrode-modified graphite films	No pre-digestion required	1 mg/mL	[18]
	DPASV	Possible interference with wine components.	4.57 µg/L for Pb, and 1.74 µg/L for Cd,	[19]
BPA	Electrochemical with a microelectrode with Au nanoparticles and aptamers	To achieve low LODs a complex sample preparation is necessary	10 pg/mL	[20]
Biogenic Amines	Enzymatic electrochemical with DAO	Possible interference with wine components	0.1 µM –	[21]
	LSPR Sensor	Not detailed	1 nM	[22]
Phathalates	Aptameric Emission fluorescence (MoS_2 QDs) (CdTe QDs) sensor	It would need to be adapted for measurement in wine	0.21 µg/L	[23]

5. Conclusion

The determination of compounds of interest in food safety during winemaking could be facilitated by the use of sensors/biosensors due to their ease of use, speed and relatively low cost. For most of the parameters, studies have been published on different types of sensors/biosensors that would allow us to use them as a basis for studying their applicability in wine samples.

However, it has been found that, despite the large number of research works on the development of sensors/biosensors applicable to the food industry, and the great advantages that their use would bring, the number of commercial devices that can be used is comparatively very low. However, this work provides the basis for the development of useful devices in the wine industry.

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