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Detection of Wine Alterations by Sensory Analysis: Overview of Results obtained from Interlaboratory Tests

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Abstract

Throughout history wine has been appreciated thanks to its distinctive sensory characteristics. Wine quality control is crucial for wine producers, which need to identify any alterations throughout all the winemaking process. Among the techniques used to determine the quality of wines, the most important is sensory evaluation by trained experts, as it is directly related to the organoleptic characteristics of wines. However, wine is a complex matrix and several factors can affect assessors' perception. The same defect can be perceived at different intensities according to experience, training and cultural origins of the panel. This could be problematic for winemakers who need to have an objective analysis. Participation in interlaboratory studies is an interesting tool for a sensory analysis laboratory that needs to demonstrate that its results are the same as those obtained by other laboratories or bodies. Moreover, participation in interlaboratory tests can provide precious information about the performance of assessors.

The purpose of this paper is to critically summarize results obtained from interlaboratory tests for the identification of a main defect in wines which have been artificially contaminated.

Keywords: sensory analysis; wine defects; wine quality control; interlaboratory test; tasting panel; olfactory alterations; taste and tactile alterations

Introduction

A wine can be defined as altered when an undesirable compound is present at a concentration above the detection limit. The latter can be very variable depending on the substance, whose concentration can be measured in grams, milligrams, micrograms or even nanograms per litre.

Molecules responsible for wine alteration may have different origins [1]. Some defects are directly linked to grapes and abnormalities resulting from the maturity of the grapes and/or fungal diseases. Other ones, related to the techniques used, pre-fermentation operations or alcoholic fermentation, can be attributed to the main products of yeast and bacterial metabolism or be directly associated with bacterial metabolism.

Negative attributes of wines can be classified in visual, olfactory, taste and tactile alterations.

The main visual alterations are hazy appearance, browning and pinking effect on white wine, and, for red wines, lack of colour and premature brick-red colour. Causes of these defects are various: hazy appearance is due to the presence of yeasts and bacteria, proteins or tannins, browning and pinking effect are caused respectively by the oxidation of polyphenols or phenolic acids and the presence of polyphenols and an excess of sulphites. Lack of colour of red wines is related to lack of pigments and an excess of sulphites and the cause of premature brick-red colouris an insufficient tannin-anthocyanin combination.

The best-known taste and tactile alterations, acidity, bitterness and astringency are considered defects only when they impact ex-

cessively the wine structure. Acidity is due to the presence of an excessive amount of tartaric or malic acids which give to wines a taste of unripe grape and lemon. Wine tannins or grape tannins are the main elements responsible for the bitter defect (raw chicory, pure cocoa, green tea, quinine taste) and the astringent tactile alteration as feeling of dryness and roughness in the mouth [2]. Other taste alterations can be due to the presence of acetyltetrahydropyridine (odour reminiscent of mouse and acetamide) or excess of ethyl hexanoate or decanoate (taste of soap).

Among olfactory alterations, the attributes vegetable, mouldy, acetic, reduced, oxidised, animal and lactic are the most frequent terms mentioned by tasters.

The main molecules responsible for vegetable attributes are methoxypyrazines, which give the wine herbaceous notes (green pepper, ivy) and 1-hexanol, which is found in the wine as a result of the mechanical crushing of the skins and which also gives distinct notes of cut grass [3].

The mouldy defects (also known as corked taste) are considered among the most harmful because they cannot be eliminated. This detrimental taste effect is generally perceived as a musty earthy aroma (musty-dusty, musty and/or damp cellar) that can mask the natural nuances of the wine and irreversibly affect its quality [4]. Although various compounds have been identified in relation to this spoilage [5, 6], most authors in the literature agree that haloanisoles, particularly chloroanisoles, are the main compounds responsible for this problem [7]. These molecules come from the transformation by moulds of less-odorous halophenols into highly-odorous haloanisoles by methylation in a humid atmosphere and in a confined environment.

Geosmin, 2-isopropyl-3-methoxypyrazine and 2-methyl-isoborneol, responsible for the wet-earth, earthy-mould and mushroom odours and tastes (earthy defect), originate from agro-viticultural factors, such as berries with fragile skins and undesirable mould attacks.

The 1-octen-3-one gives wines a fresh mushroom smell. Although difficult to eliminate, its presence can be ephemeral.

The acetic attribute refers principally to the presence of acetic acid or ethyl acetate. Acetic acid, which produces a pungent, acrid smell in the nose and a bitter taste in the mouth, is one of the best-known defects. This molecule can develop through the presence of acid rot on the grapes or contamination by indigenous yeasts. It can also be produced during alcoholic fermentation, by yeasts or lactic bacteria or, in the refining phase, by acetic bacteria. Ethyl acetate, which is responsible for the odours of glue, nail polish and/or solvent, is mainly attributable to the indigenous yeast micro flora present on the grapes. It is produced by yeasts and acetic bacteria during alcoholic fermentation or by acetic bacteria during the ageing and storage phase of the wine.

The reduced defect is due to the presence of volatile sulphonated compounds, responsible for rotten-egg, gas, garlic and cabbage odours, which are mainly generated by yeasts during alcoholic fermentation. These molecules include ethanethiol, mercaptans and hydrogen sulphide.

Acetaldehyde gives the wine an odour of stale apple and/or *rancio*, whichare descriptors of the oxidised attribute. This aldehyde is the result of the metabolism of yeasts or bacteria and the oxidising process of the wine in contact with oxygen. If in red wines its presence in small quantities is positive, as it favours the stabilisation of the colour and the polymerisation of phenolic compounds, in excess or in white wines it can be considered a defect.

Diacetyl is responsible forlactic, buttery, butyric odours in wines (the lactic attribute). This molecule is produced by lactic bacteria during malolactic fermentation. Similar fermentative aromas are also given by other compounds such as ethyl lactate, acetoin, butanediol and pentanedione.

The animal attribute is used to describe phenolic, leather, stable, horse-sweat or foxy odours in wine. These defects have different origins. The o-aminoacetophenone is responsible for the foxy odour. The biochemical mechanisms that cause its formation have not

yet been fully elucidated. During the bottle-ageing phase, some dry white wines show signs of premature ageing, such as the change of colour to orange tones and the loss of the fruity aromas typical of young wines. Phenolic, stable, horse-sweat odour can be associated with the presence of 4-ethylphenol and 4-ethylguaiacol [8]. The4-ethylguaiacol is an aromatic phenol compound found in red wines contaminated by *Brettanomyces spp*. It is not a particularly unpleasant substance in itself, as it is smoky and spicy, but its presence is often associated with that of 4-ethylphenol. The latter mainly affects the fruit note and the wine is more astringent when tasted. 4-ethylguaiacol is derived from phenolic acids, constituents of the grape, which are transformed into 4-ethylphenol by the double action of decarboxylation and reduction.

Lastly, wines can be affected by other olfactory alterations due to specific compounds such as benzaldehyde (bitter-almond, bug, specific unpleasant odour), 4-vinylphenol and 4-vinylguaiacol (pharmaceutical, medicinal odour), cresols (camphorated and iodinated odours), biogenic amines (mousy taste, rotten meat and putrefaction), dimethyl disulphide (cabbage odour) 1,6-trimethyl-1,2-dihydronaphthalene (hydrocarbon odour).

Any wine alteration must be identified as soon as possible throughout all the winemaking process. Nowadays, sophisticated chemical analytical methods are used to characterize wine and to investigate any presence of unwanted compounds. To identify organoleptic defects, chemical analysis of wine is generally accompanied by sensory evaluation by trained experts. In the last few years, the number of laboratories who wish to incorporate a sensory analysis process into their quality system is increasing. However, authorized accreditation bodies only accredit objective sensory tests which are properly documented and validated. In this framework, participation in interlaboratory tests can provide precious information about the performance of assessors. Thanks to this tool, a sensory analysis laboratory has the possibility to demonstrate that its results are the same as those obtained by other panels. Nevertheless, wine is a complex alcoholic beverage and several factors can affect assessors' perception. The same defect can be perceived at different intensities according to the panel experience, training and cultural origins.

This work describes results obtained in interlaboratory tests on wines artificially altered with a specific compound. Over three years, 14 different wines were submitted to laboratories. Each wine proposed was altered with a molecule that corresponds to a main olfactory, taste or tactile alteration. The perception of the defects mouldy-earthy, acetic, reduced, oxidized, animal, acid, bitter, and astringency was tested.

These tests were not implemented for sensory analyses of wines, but their principal objective was to test laboratories in finding a main defect artificially added to matrices.

Materials and Methods

Aninterlaboratory test entails the analysis by different laboratories of the same analytical parameters on identical samples. The setting up of these tests can be schematized by 3 main steps: preparation of homogenous samples, analyses by the laboratories and the statistical treatment of the data.

Sample production and shipment

The most crucial aspect for the implementation of an interlaboratory testprogramme is the production of homogeneous and stable samples. For this study, all samples of wine proposed were spiked to obtain a main defect. By basing these interlaboratory tests on the notion of a flagrant defect, the spiking concentrations were above the detection limits of the molecules. The wines' characteristics, and their spiking levels by matrix and by attribute are summarized in Table 1. After spiking, matrices were homogenized in a dedicated glass vessel with a floating cover. Sufficient contact time for mixing (defect + wine) was guaranteed (between 1 and 3 days, according to the spiking molecule). Samples were packaged in glass vials with no headspace and stored in a thermostatic chamber at 12-20°C.

Homogeneity and stability check of the batches were performed according to the requirements of ANNEX B of the ISO 13528 standard [9]. For both studies, chemical analyses to confirm the same concentration of the spiked molecule in tested samples were

performed according to the Compendium of the OIV (International Organization of Vine and Wine) [10].

Analyses and statistical treatments

Prepared samples were sent to laboratories, without any indication about the spiking molecules (blind test). Given the nature of the product, participants must organize the sensory sessions as soon as possible after the reception of the samples. Laboratories were invited to follow the analysis method prescribed by OIV [11]. The tasting panel must be composed of a minimum of 10 assessors in order to be taken into account in the statistical treatment. Reply forms indicate the main visual, olfactory, taste and tactile alterations:

- Visual alterations:
  - Hazy appearance
  - Browning
  - Pinking effect (white wine)
  - Lack of colour (red wine)
  - Premature brick-red colour (red wine)
- Olfactory alterations:
  - Vegetable
  - Mouldy-earthy
  - Acetic
  - Reduced
  - Oxidized
  - Animal
  - Lactic
  - Other notes (to specify).
- Tactile and taste alterations:
  - Acid
  - Bitter
  - Astringency.

Assessors were invited to evaluate the alterations according to an intensity scale from zero (if the attribute is not found as a wine adulteration) to 5. A value higher than zero is an indication of the defect identified. If the wine presents more than one defect, attributes must be evaluated individually.

The intensity of the negative attributes predominantly perceived must also be indicated, as well as acceptance or refusal of the tested wine. An Excel file was sent to panels for individual data. Median, inter-quartile range, robust standard deviation, relative coefficient of variation, higher and lower confidence intervals were calculated for each tasting panel. Medians of intensity of each defect were represented graphically. As an example, the graph of results of the panel of a laboratory participant to the test on red wine spiked with 4-ethylphenol is shown in Figure 1.

The data of each tasting panel were collected and treated statistically: for a given wine and for each defect, the median of the intensity level of a perceived defect was estimated from data indicated by panels. The results were presented graphically in the final statistical report for each attribute. An example of graphical representation of medians of the intensities returned by each panel for a specific alteration is shown in Figure 2. The median of medians of the intensity of perceived defect of laboratories for which the panel was composed by at least 10 assessors is indicated as a line in these graphs.

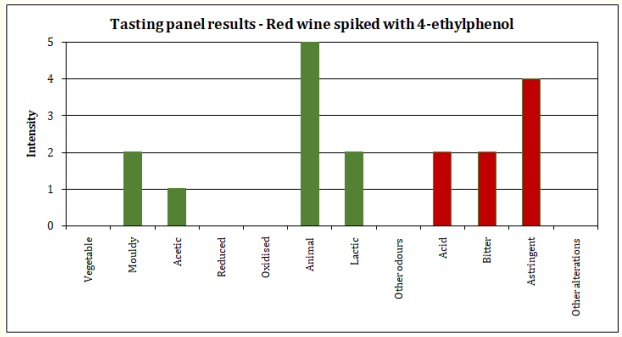

Figure 1: Graphical representation of results obtained by the tastingpanel of a laboratory participating in the test on red wine spiked with 4-ethylphenol. The x-axis lists the different defects that could be found in wine. The y-axis represents the medians of the intensity of perception of each defect detected by tasters. Olfactory alterations are indicated in green, tactile and taste ones in red. The tasting panel of this laboratory detected the main defect caused by the presence of 4-ethylphenol (animal olfactory alteration), with an intensity of 5 (high level).

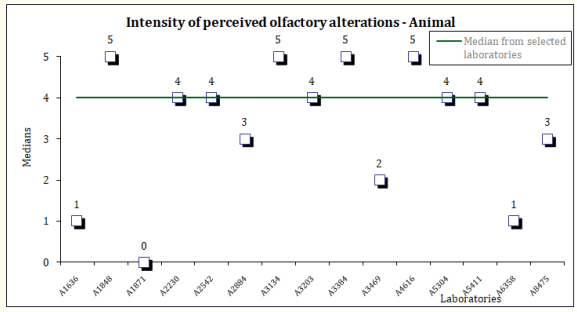

Figure 2: Graphical representation of medians obtained by laboratories on the animal defect perception for a red wine spiked with 4-ethylphenol.
All laboratories participating to this test are represented in the x-axis using a confidential code. The y-axis represents the medians of the intensity of perception of the animal olfactory alteration detected by tasting panels of each laboratory. The green line indicates the median of the medians of laboratories which panel consists of at least 10 assessors (selected laboratories).

Results and Discussions

The number of participating laboratories, mainly from Europe and America, varies from 15 to 31, depending on the trial. An overview of the results of the tests is summarized in Table 1. Results are satisfactory: the main laboratories found the defect linked to the presence of the spiking molecule in almost all the proposed samples. Even if wines with a neutral sensory profile were chosen for these tests, adding some molecules can alter the sensory equilibrium and highlight also defects which were not the target. Radar charts representing the intensity of each perceived defect on each proposed wine are shown in Figure 3. In these graphs, dotted lines represent the medians of intensity of defects perceived by laboratory and red continuous lines indicate the medians of these medians.

Four different samples were proposed for detection of mouldy-earthy alteration in red wines. Two wines were spiked with trichloroanisole (TCA) and geosmin was added to the other two samples.

Trichloroanisole spiking concentrations were 38 ng/L for the first trial and 29 ng/L for the second one. Mouldy-earthy olfactory alteration was indicated by 61% of participants for the first tests and 83% for the second one. The medians of the intensity of perceived alteration of selected laboratories were 3 and 4 respectively. It can be noticed that results of the second test were more satisfactory, the same defect was detected by a larger population of laboratories and the median obtained was higher even if the spike concentration was lower. The olfactory identification of trichloroanisole can be difficult due to the influence of the matrix, the difference in sensitization of the panel, the training of the panel and finally the rapid saturation of sensory receptors.

Spiking concentrations of geosmin were 702 ng/L and 451 ng/L respectively. 88% out of laboratories participating in the first test detected the mouldy-earthy alteration. All of the laboratories participating to the second one detected this defect. The median of the intensity of perceived this defect was 4 for the two tests, despite the difference in spiking concentration.

Two different tests were organized for the acetic olfactory alteration: white wine samples were spiked with ethyl acetate at 160 mg/L for the first test and 226 mg/L for the second one. Acetic defect was not indicated as a glaring defect by the laboratories participating to the first test, even if the amount added to the wine normally allows detection of this defect. Only 20% out of participating laboratories found this alteration. The predominant negative attributes were acidic for 30% of the laboratories and bitter for 20% of participants (taste alterations). In the second test, 60% of the laboratories detected the acetic defect due to the presence of ethyl acetate in the wine. The higher concentration of this molecule in wine allowed more consistent results compared to the first test, the medians of the intensity of perceived alteration of these two tests were 1 and 3 respectively. Moreover, a wine with different characteristics was chosen for the second test and this may have played a role in the degree of perception of acetic defect.

Alteration molecule / Defect	*WineCharacteristics[1] and test*	*Spiking Concentration*	*Number of participants*	*Laboratories identifying the main defect (%)*	*Intensity of perceived main defect[2]*	*Laboratories refusing wine (%)*
Trichloro anisole Mouldy-earthy	Red wine (Merlot, Languedoc Roussillon) T1 ME	38 ng/L	31	61%	3	90
	Red wine (Syrah, LanguedocRoussillon) T2 ME	29 ng/L	15	83%	4	100
Geosmin Mouldy-earthy	Red wine (Merlot, LanguedocRoussillon) T3 ME	702 ng/L	15	83%	4	100
	Red wine (Shiraz, Syrah, Grenache, Carignan, Cotes du Rhone) T4 ME	451 ng/L	18	100%	4	100
Ethyl acetate Acetic	White wine (Chardonnay, LanguedocRoussillon) T1 Acetic	160 mg/L	15	20%[3]	1	25
	White wine (Sauvignon, Grenache, Vaucluse) T2 Acetic	226 mg/L	18	60%	3	100

Alteration molecule / Defect	*WineCharacteristics[1] and test*	*Spiking Concentration*	*Number of participants*	*Laboratories identifying the main defect (%)*	*Intensity of perceived main defect[2]*	*Laboratories refusing wine (%)*
Ethanethiol Reduced	White wine (Sauvignon, Grenache, Cotes du Rhone) T1 Reduced	59 ng/L	15	45%[4]	2	93
	Red wine (Syrah, LanguedocRoussillon) T2 Reduced	81 µg/L	18	60%	3	94
Acetaldehyde Oxidized	White wine (Colombard,Ugniblanc, Listan, Gascogne) T1 Oxidized	135.6 mg/L	18	89%	4	100
4-Ethylphenol Animal	Red wine (Merlot, LanguedocRoussillon) T1 Animal	441 µg/L	15	100%	4	87
Tartaric acid Acid	White wine (Sauvignon, LanguedocRoussillon) T1 Acid	2.2 g/L	31	16%[5]	4	97
	White wine (Sauvignon, LanguedocRoussillon) T2 Acid	1.1 g/L	15	50%	3	60
Quinine Sulphate Bitter	White wine (Chardonnay, LanguedocRoussillon) T1 Bitter	28.3 mg/L	15	63%	3	50
Grape tannins Astringency	Red wine (Merlot, LanguedocRoussillon) T1 Astringency	1087 mg/L	15	56%	3	71

1. Grape variety and origin
2. Median of selected panels
3. 30% of laboratories detected the "Acid" defect, median: 2
4. 33% of laboratories detected the "oxidized" defect, median: 2
5. 68% of laboratories detected the "Mouldy-earthy" defect, median: 4

Table 1: Summary of results obtained in interlaboratory tests for identification of wine defects.

For the detection of reduced defect, a white and a red wine were spiked with ethanethiol at a concentration of 58 ng/L and 81 mg/L respectively. 44% of the laboratories detected the reduced defect in white wine, even if the median of the intensity of this perceived alteration was low (2). In this same sample, 33% of laboratories indicated the oxidized defect. 60% of the laboratories detected the reduced defect in the red wine (3 was the median of the intensity). The higher concentration and the different wine characteristics likely contributed to the improved results of this second test.

Concerning the oxidized defect, white wine samples were spiked with acetaldehyde at 135.6 mg/L; 89% of the laboratories detected the presence of this alteration in wine. The median of the intensity of perceived defect of selected laboratories was 4.

For the detection of the animal defect, a test on red wine samples spiked with 4-ethylphenol at 441 mg/L was organized. 100% of the laboratories detected this olfactory alteration. The median of the intensity of perceived defect of selected laboratories was 4.

Two different tests were proposed for the acid taste alteration: white wine samples were spiked with tartaric acid at 2.2 g/L for the first test and 1.1 g/L for the second one. Only 16% of laboratories participating to the first test found the acid defect as the major one. Two different tests were proposed for the acid taste alteration: white wine samples were spiked with tartaric acid at 2.2 g/L for the first test and 1.1 g/L for the second one. Only 16% of laboratories participating to the first test found the acid defect as the major one. The median of the intensity of perceived taste alterations of selected laboratories was 4. The analysis of laboratories' results showed also a mouldy-earthy olfactory alteration, with a median of the intensity equal to 4. This negative attribute, detected by 68% of the participants, mainly derives from the matrix. Concerning the second test, 50% of the laboratories detected the acid alteration.

Quinine sulphate was added to white wine samples at a concentration of 28.3 mg/L to highlight the bitter defect. 63% of the laboratories detected this alteration, with a median of the intensity of the perceived defect of 3.

Finally, red wine samples were spiked with grape tannins: 56% of the laboratories detected the astringency defect due to the presence of excess tannins in wine. The median of the intensity of the perceived defect of selected laboratories was 3.

Almost all wines were refused by the majority of panels, except the first white wine spiked with ethyl acetate, which was refused only by 25% of participants. However, it is important to remember that wine is a very complex matrix and a defect perception may be influenced by the characteristics of the spiked product.

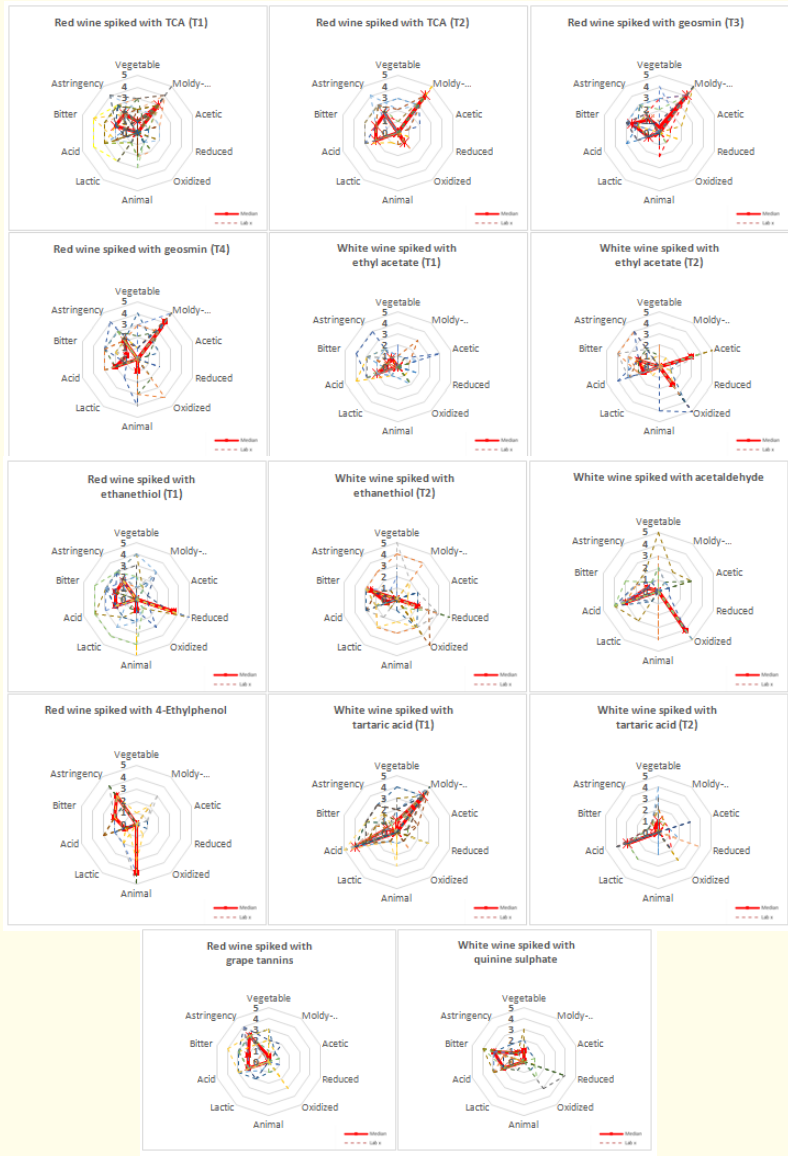

Figure 3: Radar charts of panel results for identification of alterations in wines. Studied wines and spiking molecules are indicated at the top of each graph. The red continuous line indicates the median of the medians of intensity of defects perceived by laboratories (dotted lines).

Conclusion

Results obtained in overall tests highlight that some alterations are well-known and easier identified by panels (i.e., mouldy-earthy and animal). In general, laboratories better detect the artificially added defect if the molecule responsible for the alteration has already been proposed in previous tests. Data concerning the intensity of the main perceived defect consolidate this observation as low dispersions of panellists' answers is noticed when laboratories are faced with an alteration for the second time. These results show that interlaboratory tests support laboratories in their process of continuous improvement and can be useful as sensory training to improve tasters' sensibility, providing precious information about the performance of assessors.

In conclusion, sensory analysis is an excellent tool for wine quality control, as the product is analysed in its entirety. As any scientific tool, this discipline requires a lot of rigour to guarantee reliable and quality results. Authorized accreditation bodies only accredit objective sensory tests which are properly documented and validated: participation in interlaboratory tests is an interesting tool for laboratories for satisfying the conditions required by the ISO/IEC 17025 standard [12] and gaining the trust of accreditation bodies and customers.

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