EN

FINAL DRAFT RESOLUTIONS

to be submitted

to the 20th General Assembly

of the

International Organisation of Vine and Wine

Ensenada (Mexico) – 2022

N°	Ref. Resolution	Step	Title
1	**VITI-PROTEC 21-705**	8	OIV recommendations on alternatives to herbicides use in vineyards
2	**VITI-GENETPROTEC 14-565**	8	OIV guidelines for the harmonization of requirements- for exchange of viticultural plant material: phytosanitary and genetic aspects
3	**VITI-ENVIRO 20-677**	8	OIV Definition and recommendations about functional biodiversity in the vineyards
4	**VITI-SCRAISIN 20-678 A**	8	OIV Definition of grape nectar and carbonated grape nectar
5	**CST 12-503H**	8	OIV general principles for the communication related to the carbon footprint for enterprises/organisations and products in the grape vine and wine sector
6	**OENO-TECHNO 14-567B2**	8	Distinction between additives and processing aids – Carbon dioxide
7	**OENO-TECHNO 14-567B4**	8	Distinction between additives and processing aids - Dimethyl dicarbonate
8	**OENO-TECHNO 14-567C1**	8	Distinction between additives and processing aids - Skimmed milk
9	**OENO-TECHNO 20-684A**	8	Use of selective plant fibres in wine – update to Resolution OIV-OENO 582-2017
10	**OENO-TECHNO 20-684B**	8	Use of selective plant fibres in mustp

N°	Ref. Resolution	Step	Title
11	**OENO-TECHNO 21-689**	8	OIV Maximum limit for gum arabic – update
12	**OENO-TECHNO 21-707**	8	Wines – treatment with silver chloride
13	**OENO-TECHNO 21-708**	8	Update to file 2.1.14 - flotation
14	**OENO-MICRO 20-686**	8	Review of the code of good vitivinicultural practices in order to avoid or limit contamination by brettanomyces
15	**OENO-SPECIF 16-602**	8	General principles to take into account in the assessment of oenological practices/substances
16	**OENO-SPECIF 17-624**	8	Update to the monograph on oenological tannins
17	**OENO-SPECIF 20-674**	8	Update to the monograph on yeast mannoproteins (E-COEI-1-MANPRO)
18	**OENO-SPECIF 20-675A**	8	Specific monographs for procyanidins/prodelphinidins
19	**OENO-SPECIF 20-675B**	8	Specific monographs for ellagitannins
20	**OENO-SPECIF 20-675C**	8	Specific monographs for gallotannins

N°	Ref. Resolution	Step	Title
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22	**OENO-SPECIF 20-681**	8	Food cellulose
23	**OENO-SCMA 19-662A**	8	Measuring ochratoxin a in grape juice, reconstituted grape juice, concentrated grape juice and nectar, after going through an immunoaffinity column and high liquid performance chromatography with fluorescence detection
24	**OENO-SCMA 19-662C**	8	Determination of volatile acidity in grape juice, reconstituted grape juice, concentrated grape juice and nectar
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29	**CST-SCMA 20-668**	8	Opinion of the OIV on total dry extract (total dry extract, total dry extract without sugars, residual extract)
30	**ECO-DROCON 20-685**	8	Update of the international standard for wine labelling: medals and distinctions

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32	**SECSAN-SECUAL 21-709**	8	Update to the resolution OIV-OENO 427-2010 on criteria for the quantification of allergens
33	**SECSAN-SECUAL 21-710**	8	Update to the resolution OIV-OENO-SECSAN 520-2014 on the code of good fining practices for wine to be applied in the use of proteinaceous wine fining agents with allergenic potential
34	**SECSAN-CONUSA 20-679**	8	Recommendations on the dissemination of information on wine consumption
35	**SECSAN-CONUSA 21-711**	8	Recommendation on the evaluation/assessment and the communication of epidemiological studies concerning the consumption of grapes, wine and other vitivinicultural products

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DATE		10/2021	10/2021			05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
VITI-PROTEC 21-705 Et8

OIV RECOMMENDATIONS ON ALTERNATIVES TO HERBICIDES USE IN VINEYARDS

THE GENERAL ASSEMBLY

ON THE PROPOSAL of Commission I Viticulture and the group of experts "Vine Protection and Viticultural Techniques",

IN VIEW OF the Article 2, paragraph 2 b) i and c) iii of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine, and under points 1.a.iii, 1.b.1, 1.b.4, 1.b.6, 1.b.7, 1.c.iii, and 1.f. of the OIV Strategic Plan 2020-2024 which relates the promotion of environmentally friendly vitiviniculture, and the consideration and responses to the challenge of climate change, and the improvement of environmental performance.

CONSIDERING Resolution CST 1~~/~~ 2004 on Development of sustainable vitiviniculture

CONSIDERING Resolution OIV VITI 333 2010 establishing the concept of terroir,

CONSIDERING the OIV resolution OIV-VITI 592-2018 OIV good practices for minimizing the impacts associated with the application of plant protection products in vineyards,

CONSIDERING the OIV resolution OIV-VITI 569-2018 OIV protocol for the sustainable use of water in viticulture, in particular for the point concerning "Water consumption in the vineyard",

CONSIDERING the OIV resolution OIV CST 518-2016 on general principles of sustainable vitiviniculture, and especially the principle 2: Sustainable vitiviniculture respects the environment and the parts concerning to preserve the biodiversity,

CONSIDERING the OIV resolution OIV-VITI 641-2020 guide for the implementation of principles of sustainable vitiviniculture,

CONSIDERING the adoption of the Strategic Plan for Biodiversity 2011-2020 under the Convention on Biological Diversity (CBD; 2010) and the 2030 Agenda for Sustainable Development of the United Nations committed the international community to a set of ambitious goals on 'living in harmony with nature' and 'leaving no one behind, which requires immediate and ambitious action to protect life both below water and on land, by reducing pressures on biodiversity and ecosystems.

CONSIDERING the strengthening at the international level of public policies and societal expectations on the protection of biodiversity and natural resources (in particular water and soil resources)

CONSIDERING the growing demand for agricultural and food products (products from organic farming, integrated production or other system aimed at meeting sustainability objectives) resulting from production methods that minimize the use of inputs

CONSIDERING the innovative technics, agricultural equipment and technical means~~materials available~~, that allow alternative interventions~~=~~ that facilitate the reduction or replacement of the use of herbicides,

CONSIDERING the increasing professionalization of winegrowers facilitating the transition to new cultivation practices, with innovative tools, allowing the use of less inputs,

CONSIDERING the progress of scientific knowledge and the numerous experimental results relating to the maintenance of vineyard soils without the use of herbicides,

CONSIDERING the potential for increasing the carbon storage in grapevine soils through the development of adequately selected and managed cover crops and inherent organic matter, aware of its beneficial effect on soil health and as potential carbon sink in the face of climate change

CONSIDERING the political and socio-economic importance of the OIV's approach to sustainability for national and regional legislators, scientific institutions, professional organisations and the winegrowers themselves,

RECOGNIZES:

- ✓ Various chemical formulations and substances with herbicidal qction are allowed for use in viticulture.
- ✓ The use of herbicides of synthesis may have negative effects on the environment as they can impact the biological activity of the soil ~~because they can~~ through ~~persist~~persistence in the soil and ~~migrate away from cultivated plots~~ percolation in the deep soil and contaminat~~e~~inge soil surface or groundwater resources in a persistent manner.
- ✓ However, given the current state of knowledge and available alternative methods, herbicides are still indispensable for soil maintenance in vineyards in certain specific situations (where mechanisation is not ~~im~~possible or is very difficult or the less sustainable option).
- ✓ The alternative to the elimination of spontaneous vegetation by active substances is based on the implementation of the following ~~four~~ current methods, operated alone or in combination:
  - i) elimination of spontaneous vegetation by mechanical (or physical) means without deep plowing,
  - ii) establishment and adequate maintenance of a plant cover (spontaneous vegetation, seeded or selectively planted)
  - iii) application of compostable materials to soil to avoid or limit the growth of spontaneous and competing herbaceous vegetation.
  - iv) se of bio-based substances with herbicidal effect (bio-herbicides)
- ✓ Strategies based on the combination of one or more of these agronomical methods with the application of herbicidal substances are quite common in many vineyards.
- ✓ The choice to reduce and/or abandon the use of herbicides implies significant systemic changes for the grape growers, and the transition to other practices for the maintenance of the floor surface requires specific hardware equipment, longer and more frequent intervention times and an adapted qualification of the operators.
- ✓ Despite significant constraints, ~~reducing the use of herbicides~~ the reduction and total elimination of the use of herbicides for maintaining vineyard soils must be encouraged in all situations where this is technically feasible with available~~conventional~~ means. However, it is important to ensure that the transition to these new methods does not undermine the economic balance of farms.
- ✓ In some cases, this change of viticultural practices may~~can~~ also result in undesirable ~~an~~ increase in greenhouse gas emissions or soil erosion, which should be weighed against the procured benefits. In addition, in situations of sloping or terraced vineyards, available soil maintenance

techniques without herbicides may prove to be unsuitable because they are economically unsustainable.

- ✓ Additionally, under wet conditions mechanical weed control of the grapevine row (inter-row operations) might not be efficient and must be repeated several times during the year, leading to detrimental consequences (higher fuel consumption, increased soil compaction).
- ✓ Application of compostable materials might lead to ~~phyto~~sanitary problems like ~~bud pests or~~ root rots, nutritional disorders, etc.
- ✓ The techniques and devices available for mechanical maintenance of the soil surface are many and varied, and new tools are appearing regularly on the market. In the case of mechanical and physical interventions, it is advisable to research and choose, for each pedoclimatic~~ and soil~~ situation and type of vineyard, the techniques or combinations of techniques that are best suited.
- ✓ The strategy based on controlled soil cover with spontaneous (where is no alternative) or seeded herbaceous vegetation has multiple advantages. Nevertheless, it requires very good technical mastery and adaptability to the constraints of the plot management. It is applied temporarily or permanently, depending on the case, and concerns the entire surface of the vineyard or only a part of it (e.g. the strip between the grapevine rows).
- ✓ The implementation of controlled soil cover strategy is very dependent on pedoclimatic water balance and impacts the vigour of lower vine and its production. However, when competition for water between grapevine and cover crop is balanced and greening is carried out in an appropriate situation, the plant cover of the soil is effective in controlling erosion, improving the bearing capacity for machines, limit compaction and sequestering carbon in the soil by increasing its richness in organic matter.

RECOMENDS TO MEMBER STATES

- ✓ To initiate or develop actions to study the feasibility of the transition towards alternative practices to weed control in the vineyard under regional conditions.
- ✓ To initiate research focu~~s~~sing on alternative practices to the chemical weed control in viticulture, under regional conditions ~~regarding~~with regard to adequate sustainability indicators~~ the objectives of sustainability~~ (energy consumption, toxicity risk, soil compaction, effect on production~~ wine quality~~).
- ✓ To develop technical training (particularly in the proper use of the innovative and adapted tools, and on the knowledge and management of spontaneous (where is possible) and seeded vegetation), and actions with the aim of educating, communicating and raising awareness on this topic.~~,~~
- ✓ To develop and encourage research and experiments to study the feasibility and impacts of soil surface management without herbicides, by getting producers involved (participatory networks), i.~~ I~~n ~~particular~~particular about ~~with regard to~~ erosion and the sustainable use of water.
- ✓ To promote innovations for soil maintenance via different methods (physical or others).~~ I~~n particular, research into innovative mechanical tools for working the soil correctly on the grapevine row should be encouraged in order to limit damage to the vine (injuries) and at the same time reduce work times, including in vineyards with high planting density, when possible used unmanned ground vehicles to weed physical control.

- ✓ To encourage the international exchange of the results of experiments and feedback on experiences of soil maintenance without weed control.
- ✓ To support alternative products ~~s~~ and/or viticultural practices to promote ~~in order to include~~ the sector's sustainability objectives, especially those in direct relation to sustainable soil management (balanced water budget, low toxicity risks, ~~CO_2 footprint,~~ carbon emission and long-term sequestration and organic matter in soils, microbial biodiversity, soil health, etc.).

STEP	1	2	3	4	5	6	7	8
DATE		03/2014	10/2014	04/2016	06/2016	04/2017		
		04/2015	10/2015		10/2017	04/2018		
					10/2018	04/2019	07/2019	
						10/2019		
					06/2020	10/2020		
					10/2021		11/2022	11/2022

FINAL DRAFT RESOLUTION
VITI-GENETPROTEC 14-565 Et8

OIV GUIDELINES FOR THE HARMONIZATION OF REQUIREMENTS FOR EXCHANGE OF VITICULTURAL PLANT MATERIAL: PHYTOSANITARY AND GENETIC ASPECTS

THE GENERAL ASSEMBLY,

ON THE PROPOSAL of Commission I "Viticulture",

IN VIEW OF Article 2, paragraph 2 b) i and c) iii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine, and under points 2.b.ii and 2.d.iii of the OIV Strategic Plan 2015-2019, which foresaw the definition of different categories of grapevine products, including that regarding the grapevine germplasm material, and to harmonize the diagnosis and the identification of grapevine diseases and pathogens with technical methods, as well as the point 3.b.ii which relates to plant material exchanges, and promotes the identification and the sanitary and phytosanitary control tools,

CONSIDERING the many works presented during the meetings of the expert groups, particularly the "Genetic Resources and Vine Selection" (GENET) and "Vine Protection" (PROTEC) Expert Groups, and following a proposal made by both Groups,

CONSIDERING Resolution OIV/-VITI 424/-2010, "Conservation of Vine's Genetic Resources", concerning the conservation of grapevine genetic resources, and especially the part that refers to facilitating its implementation in order to catalogue plant material, promote its conservation and actively support research in the interest of improving the different conservation systems,

CONSIDERING Resolution ~~OIV-~~VITI 01/-2002, "Conservation of Diversity", concerning the maintenance of the widest possible genetic diversity, the technological value of this diversity and the encouragement of the development of local varieties,

CONSIDERING Resolution OIV-VITI 539-2017, "OIV guidelines for recognising grapevine collections", concerning the establishment the requirements that an ampelographic collection must have to be included in the specific list of the OIV,

CONSIDERING Resolution OIV-VITI 609-2019, "OIV protocol for identification of varieties", concerning the procedures to be followed to identify vine varieties and to harmonize these criteria,

CONSIDERING the demands expressed by the grapevine's propagators related to the definition of harmonized criteria for enhancing the exchanges of grapevine propagation material at an international level,

CONSIDERING the existence of laws that are currently applicable at an international level, in addition the availability of various diagnostic methods for genetic and phytosanitary identification, and the need for standardised criteria for safe exchange of plant material among different countries,

DECIDES to adopt the following OIV guidelines for the harmonization of methods and criteria for the exchange of viticultural plant material: phytosanitary and genetic aspects

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1. Introduction

The OIV Member States have their own regulations in place through National Plant Protection Organisations (NPPOs)[1], or have established the necessary agreements among themselves that apply to the production, introduction, and safe exchange of grapevine propagation material. However, considering there may be discrepancies between producing and importing countries, the OIV considers it important to harmonise said regulations and agreements, and establish standards with a global reach to be used by all Member States.

2. Objectives

The main objective of this resolution is to define principles and practices that make it possible to guarantee and preserve the genetic quality and phytosanitary initial level of plants during all phases of vegetative propagation. In addition, these indications furnished by the OIV can help the international agreements between producers and importers of grapevine plant material.

OIV guidelines shall consider and recommend minimum requirements for the vegetal material exchange, for each type of grapevine variety (rootstocks and/or wine table and raisin grape varieties from the subgenus *Vitis*, ~~(ex~~~~formerly~~ *Euvitis*).

The plant propagation material can be exchanged if it has been successfully submitted to the control and/or certification procedures, if the case, envisaged by the relevant legislation of each State have been successfully performed.

The principles and practices defined in this document may~~do~~ not concern the exchange of propagating material of grapevine varieties for experimental or demonstrative aims.

3. Glossary

For the purposes of globally standardising the terms used for the processes of exchange, production and commercialisation of viticultural plant material, the following glossary is proposed[2]:

- Propagation (vegetative) material: grapevine plants, cuttings, scions, and canes taken from the mother plants.
- Mother plant: a grapevine plant grown destined for the production of scions, cuttings and canes intended for vegetative propagation.
- Mother grapevine block: cultivation, in an identifiable location, of vine mother plants destined for the production of cuttings, grafts and vine shoots.
- Foundation block: planting of grapevines propagated and maintained that are intended as a source of foundation material.
- Varietal Collection: collection of varieties or clones that meets the criteria indicated by Resolution OIV-VITI 539-2017.

[1] The list of NPPOs of IPPC Contracting parties is available via the IPPC website: https://www.ippc.int/en/countries/nppos/list-countries/. The list of Regional Plant Protection Organizations (RPPOs) is also available via the IPPC website: https://www.ippc.int/en/external-cooperation/regional-plant-protection-organizations/

[2] For more details see the phytosanitary terms used by NPPOs relating to the exchange and production of plant material provided by FAO/IPPC International Standard for Phytosanitary Measures No. 5 Glossary of phytosanitary terms.

- Grapevine plant
  - ✓ Rooted cuttings: young plant or ungrafted shoots of rooted grapevine obtained from lignified shoots, canes or herbaceous shoots, intended for planting ungrafted or for use as rootstock, whose lower part is rooted.
  - ✓ Rooted grafts (grafted plant): young plant obtained from grapevine lignified shoot, cane or herbaceous shoot ~~of which the underground part is rooted~~, and joined by grafting with rootstock cuttings, whose lower part is rooted.
  - ✓ Potted plant: young plant rooted or grafted in vegetative or dormant phase raised and preserved in pot structure.
- Vine plant parts:
  - ✓ Cane: one-year-old and lignified branches.
  - ✓ Herbaceous Shoot: fraction of herbaceous not lignified shoots of the grapevine with vegetative tip, leaves, lateral shoots, buds and inflorescence sketches.
  - ✓ Nursery cuttings: pieces of grapevine lignified shoot, sprouts, cane or herbaceous shoot intended for the production of rooted cuttings
  - ✓ ~~Hardwood r~~Rootstock cutting: piece of cane /~~or~~ shoot of ~~obtained from different~~ rootstocks intended to form roots system of cuttings after grafting.
  - ✓ Scion: piece of cane /~~or~~ shoot ~~(from a fruit variety)~~ with at least one bud intended to form the epigeal part of the grafted vine or to form rooted cuttings to be planted in the field ~~to be grafted to form a grapevine above ground after grafting and grafts planting in situ after planting roots~~.

- Nursery: site ~~(land)~~ destined for the production of ~~rooted~~ grapevine plant.
- Lot: a set of grafts, cuttings, plants frank or grafted of the same variety and/or a same varietal clone of grafts and/or rootstock, from the same field of mother plants or from the same nursery, produced in the same vintage
- Certification: a public procedure established by a recognised~~n official~~ body, based on specific national or international standards which establish authorisations and certifications of genetic and phytosanitary conformity of the propagation material.
- Certified material: reproductive material from mother plant or nurseries that meets the certification requirements and subjected to favourable genetic and phytosanitary control.
- Categories ~~(level)~~ of propagation material: initial, base, certified, standardised or equivalent categories according to national regulations.
- Productive use of grapevine plants: vine varieties for the use of rootstock, wine grapes, table grapes, dried grapes, nectar, grape-juice, canned and frozen fruits, biomass.
- Accession: isolated or selected genotype of one variety of a selected clone or germplasm mother plant still grown in ampelographic collections or under study and evaluation.
- Selected clone: A clone is the vegetative progeny of a single vine plant. For selection purpose this single plant is chosen for its varietal identity, its phenotypic traits and its phytosanitary state (OIV-VITI 564A-2017).
- Polyclonal selection~~material~~: the selection of a group of 7 to 20 genotypes from an experimental ~~of an ancient variety in a~~ field trial containing a representative sample of the intra-varietal variability of ancient variety using ~~diversity, based on~~ quantitative genetic tools to enable high,

stable, and predictable genetic gains. ~~Material to be distributed and planted as a balanced and indivisible mixture~~ (OIV-VITI 564B-2019)

4. Crop monitoring

Regular monitoring and inspections of mother blocks and nurseries are necessary in order to detect any possible varietal impurities harmful to vine propagation to be found and also, for diseases of mother plants, nurseries and ampelographic collections as recommended by resolutions OIV-VITI 424-2010 and OIV-VITI 539-2017.

The visual inspections must be done during a period favourable to the expression of the vine phenotypic features and symptoms of the diseases being monitored. It must be conducted by qualified personnel.

For correct monitoring a mother-plant vineyard and each plot in a collection must be identified by at least one sign post indicating their variety and clone or an identity code.

5. Varietal authenticity: verification of the identity of varieties

The OIV recommends using the adopted and updated (by the OIV) varietal identification protocols: molecular analyses, DNA tests (SNPs or SSRs), and ampelographic exams according to the resolution OIV-VITI 609-2019 and to the OIV descriptors list for grape varieties and *vitis* species and its updates, which could be used in order to evaluate the authenticity of each plot or plant batch with respect to its variety.

Other standardised methods of validation and evaluation for trade purposes can be established between the interested parties, prior to their use within the framework of protocols for variety identification.

Identification and denomination of a variety should be in accordance with a common list of existing varieties (names and synonyms) and a database containing descriptive characters both morphological and molecular). The application and its acceptance as an international reference are highly recommended.

In addition, the OIV recommends mentioning the use of the following international lists of vine varieties and their synonyms:

- ✓ The OIV International list of vine varieties and their synonyms,
- ✓ The UPOV[3] PLUTO database.

6. Phytosanitary criteria

It is up to the importing Countries of plant material of the grapevine to fix phytosanitary prophylaxis measures prior the introduction of the material in its own territory and exercise phytosanitary control to avoid the introduction of harmful organisms in their country. The OIV recommend that phytosanitary control should be subject to consultation between accordance between producer and importer, in accordance with the regulations in force.

6.1. Basic measures

The exchange plant materials must be free of quarantine organisms (harmful) established by the States involved in the exchange.

[3] International Union for the Protection of New Varieties of Plants

Each State, in relation to the phytosanitary situation of its own territory and with reference to the international agreements, would adopt and take the necessary measures to protect their own territory from harmful organisms, indicated in tables 1a and 2 of annex A in the case exchange of propagation material.

~~Exchange plant material must provide health guarantees as indicated in Annex A, tables 1a and 2.~~

Material coming from countries that adopt process or product certification procedures for vine propagating material, which foresee the control of the harmful organisms listed in table 1a and 2 of annex A, can be exchanged without further analytical investigations.

In other case the exchange ~~shall~~ may take place following the performance of the analyses foreseen in table 1a and 2 of annex A on a representative sample of the lot and according to the methods defined by the countries concerned in accordance with the provisions of point 6.4.2 below.

Derogations on the phytosanitary status of plant material exchanged for non-commercial reasons from that provided for in Table 1 may be established in advance by the States involved in such exchange.

6.2. Quarantine disease and pest lists

In order to facilitate the exchange of vine plant material, the OIV has decided to publish and update a general list of links relating to regulated and unregulated grapevine pests on its website (see tables 1a~~,~~ ~~1b~~ and 2 of ~~A~~annex A).

For the control of quarantine organisms, the official methodologies regulated by each state should be adopting, where applicable, analytical standards recommended by EPPO.

6.3. Appropriate protection in field

- ✓ Good protection practices should be observed in collections and mother blocks according to OIV and National regulation, to make sure that there is a high physiological quality of cuttings ~~(maturity and stock of the wood)~~. The same level of protection must be applied in nursery fields.

6.4. Phytosanitary prophylaxis methods

6.4.1. Principles

Fields in which the plant material for propagation is grown must ensure the appropriate, effective guarantees of the absence of the pathogen agents and the vectors of viruses, bacteria and phytoplasma that could reduce the quality of the propagation material.

On the designated vineyards of mother plants chosen for the production of plant material propagation in nurseries, mother plants must be regularly monitored for the presence of primarily viruses, ~~bacteria and~~ phytoplasma and bacteria, or any other infection-causing agent considered dangerous.

It is recommended that the mother plants should be free of the main grapevine trunk diseases (GTD) such as esca disease, eutipiosis and Botryosphaeria decay. ~~All mother plants showing symptoms (leaf discolouration, wood discolouration, etc.) should be removed from the vineyard immediately and burnt or buried.~~

Foundation blocks must be protected from pathogen vectors (nematodes, mites, leafhoppers, scales etc.),

To achieve acceptable levels of phytosanitary standards, it is envisaged that the following measures shall be applied: visual phytosanitary control to monitor the appearance of transmissible pathogens and, where appropriate, sampling and laboratory tests of vegetable material for checking the phytosanitary status. Phytosanitary interventions (e.g. chemical and mechanical treatment) may be

required to avoid and control the appearance of vectors of viruses, phytoplasmas and bacterium *Xylella fastidiosa*.

6.4.2. Sampling of plant material for phytosanitary analyses of international exchange batches

In absence of normative and international treaties (subscribed by the Member States), specific agreements between the countries are settled to establish control methods, pathogenic organisms to be excluded, the entity of the sample, the type of tissue and the period of the year in which the test is conducted and admissible health and tolerance levels expressed in percentages of the lot.

The sample number depends on the batch size, the required confidence level and the particular level of detection. Appropriate sample sizes are provided by FAO/IPPC Intern. Standard for phytosanitary measures No. 31.

Batches must be clearly identified in terms of lot in accordance with the provisions of the glossary.

6.4.3. Methods of analysis of harmful organisms affecting the quality of the propagating material

For virus analysis, the OIV recommends that phytosanitary control should be carried out to ensure the absence of these organisms, in accordance with the methods described in the table 1a of Annex. And additionally, in accordance with the agreements established between the parties for dangerous organisms recognized by the international scientific community. Only lots~~plants~~ that have been subjected to analysis according to the indications shown in tables 1a of the Annex A or in Annex III of resolution OIV-VITI 564A / 2017 give the best levels of phytosanitary guarantee in terms of viral agents.

In the case of propagation material produced according to certification protocols, reference must be done to the legislation in force at the moment in the country of origin as for the exemption of viruses and viral diseases.

In ~~the~~ case of exchange of propagation material coming from collections, it is necessary to declare its sanitary status. ~~the health status must be declared, and the material may be affected by one or more of the viruses listed in Appendix table 1.~~

For the analysis of diseases caused by phytoplasma (table 2 of annex A), molecular techniques (PCR, real-time PCR) with extraction of DNA from herbaceous tissues and cane material (leaves, petioles, etc.) are recommended.

For the analysis of diseases caused by bacteria, molecular techniques are recommended. The FAO/IPPC diagnostic protocol (FAO/IPPC International Standard for Phytosanitary Measures No. 27) provides the appropriate sample sizes and molecular techniques such as for the case of *Xylella fastidiosa* (Pierce's disease causal agent).

~~Finally, for the purposes of phytosanitary prophylaxis, nucleous and foundation plants must be free of main grapevine trunk diseases (GTD) such as Esca, Eutypa and Botryosphaeria dieback. All symptomatic mother plants (tigers leaves etc.) must be removed immediately from the vineyard and destroyed in coherence with the regional/territorial legislations.~~

6.4.4. Prophylaxis on exchange material

To promote better phytosanitary prophylaxis against phytoplasmas, bacteria, nematodes, and phylloxera diseases (EPPO, ANSES) and fungi associated with the main wood diseases is recommended interventions with hot water treatments and external disinfection of the propagation material. These interventions may be mandatory in accordance with the only legislation in force and provided by each Member State.

6.4.4.1. Hot water treatment (HWT)

Hot water treatments (applied to woody plant propagation material) are considered preventative measures of phytosanitary prevention to avoid the spread of phytoplasmas and bacteria (to a lesser degree).

In addition, the OIV recommends respecting the national legislation, exemptions and regulations in force in the countries of origin and destination and reviewing these before applying any treatment. Considering the possible risks on the ability of vegetative growth of this plant material, treated with hot water and destined for long distances (export and import), they must have decided the treatments' conditions, to be carried out preferably in the country of arrival.

Several differences were found among the national schemes and standards in force (Table 3 in Annex A). These differences depend on both the purpose of the treatment and the type of plant material that needs to be treated.

In any case, the hot water treatment of vine propagation material is not an absolute guarantee of a good phytosanitary state or of disinfection for all types of pathogens or pests.

Its application requires particular measures to be taken in order to avoid damage to cells, to tissues and to reduce risks with regard to the growth capacity of the plant material.

To avoid the appearance of potential~~whatever vegetal~~ problems related to ~~involving~~ the plant material propagation, the OIV proposes the following guidelines regarding the use of hot water treatments for the specific pathogens. The following criteria are decisive for success on the pathogen or vector treatments and also, the survival of plant material:

- ✓ High physiological quality standard and good dormancy status of the material to be treated.
- ✓ Storage of the plant material to be treated under optimal conditions
- ✓ Hot Water Treatment should be applied during the dormancy phase of graft wood or plants.
- ✓ Temperature and time: Appropriate combination of treatment time and temperature for the specific pathogen. The evaluation of specific protocols is necessary, in accordance with Table 3 of Annex A.
- ✓ Vegetal Material cleaning: phytosanitary products residue, presence of (paraffin) waxes, soil residue or peat on the roots should be avoided for plants with roots.
- ✓ HWT Method: complete immersion of packaged plant material treated in batches in tanks to assure rapid attainment of homogeneous temperature in the whole tank.
- ✓ Storage post treatment: progressive acclimatisation to avoid thermal shock, in a humid and well-ventilated atmosphere.
- ✓ Checking: analysis of the vegetative growth of the heat-treated material.

7. Storage and conservation of plant material

Before and during exchange of propagation material (scions, rootstocks, cuttings, rooted plants and grafts, potted plants…), all plant material must be stored in optimal conditions to ensure its subsequent vegetative growth.

The propagation material:

- ✓ Should have a visual technical purity of 100%. Absence of wounds (E.g.: hail, insects, other), necrosis, symptoms of external fungi (E.g.: mildew, dead arm, other).
- ✓ Should be protected against physical and physiological damage.

- ✓ Should be kept separate from unidentified propagation material and from material of other lots.

7.1. Environmental conditions of buildings

After harvesting and during the processing, preparation and disposal, storage and transport phases the material is conserved in optimal (standard) conditions to ensure its long-term viability and use possibility. To this end, it is proposed to conserve them in a cold storage from 2-5 °C, and relative humidity around 85%.

In case of transport over a long distance, exchange material must be preserved under temperature and moisture conditions suitable to the final destination.

The vegetative material used for nursery production should be harvested during the same campaign (harvest) that the mother plants in question, with the object of avoiding the use of cuttings, scions and canes having stayed a year or more in a cold storage. Plant material from these plots must be clearly identified, in terms of lot~~the variety and/or the clone or polyclonal selection~~.

7.2. Packaging

Cardboards, boxes, bags of propagation material must be sealed in such a manner that they cannot be opened without damaging the seal, if any.

Each container, box or batch of propagation material should carry a label that can be clearly visible and easily accessed.

8. Labelling and management of plant material

The guarantee of traceability should be based on the documentation and compliance with existing or future protocols.

Possible additional measures can be adopted, as required by the recipient of the materials of propagation.

There are protocols linked to other quality labels (such as ISO 9001 or ISO 17025) or certified through an external or auditing company (e.g. SGS and the New Zealand system), while other protocols refer to national or international regulations with regard to traceability or recommendations for use.

8.1. Labelling standards

Letters and figures used to indicate the relevant characteristics of the material on such labels should:

- ✓ be of a font that is easy to read, or, as in the case of international trade, in a language commonly known to the countries concerned, also using the national code of origin country or another international code recognized among the parts,
- ✓ be of a colour that is in clear contrast with the colour of the labels on which they appear,
- ✓ be indelibly printed and clearly legible.

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10. ANNEXES

ANNEX A: Phytosanitary Requirements and Recommendations

Table 1. The main grapevine viral diseases with the associated viruses and the diagnostic methods for their control in viticultural propagation material for the purposes of international exchange.

This table has been established taking into account that:

i) Exchange of grapevine propagation material infected by harmful organisms is the primary means of dissemination of viral agents and the associated graft transmissible diseases;

ii) Infectious degeneration and leafroll complex ~~(table 1a)~~ are the most damaging and widespread virus diseases of grapevine and contemplated in the processes of clonal selection (table 1a);

1a. Main diseases: serious **virus diseases** in all viticultural areas, and test required.

Diseases to be controlled and excluded/eradicated from propagation material	**Associated agents**	**Evident symptoms or on appropriate *Vitis* indicator[2]**	**Laboratory diagnosis**
Infectious degeneration and decline[1], induced by *Nepoviruse*	- Grapevine Fanleaf virus, GFLV - Arabis Mosaic Virus, ArMV	Indicator: on *Vitis rupestris*	Serologicaly and/or, Molecular
Leafroll Disease[1]	Grapevine Leafroll associated Virus, GLRaV 1, 2, 3~~, 4~~	Visible in varieties rich in anthocyanins or in *Vitis* indicators	Serologicaly and/or, Molecular

1: Appropriate indicators should be chosen from the plants according to relevant technical standards only on selection phase (e.g. EPPO PM 4/8 (2); Pathogen-tested material of grapevine varieties and rootstocks http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2338.2008.01258.x/full).

Information on the~~all~~ known grapevine viruses is provided by:

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Table 2. List of phytoplasma diseases of grapevine

Molecular diagnostic tests are available with PCR test, real time PCR for identification and detection of grapevine phytoplasmas from the mother plant material present in the nursery, in ampelographic collections. Tests carried out on woody material are not safe in order to exclude phytoplasmas in propagation material.

Diseases	Acronym	Pathogen	Phylogenetic group / sub-group	Main vectors to the grapevine	Categoria
Flavescence dorée	FD	Grapevine flavescence dorée phytoplasma	16SrV-C, 16SrV-D and genetic variants	*Scaphoideus titanus*	Quarantine organism in Europe
Bois noir	BN	*Ca. Phytoplasma solani*	16SrXII-A, F, G, J, K	*Hyalesthes obsoletus, Reptalus panzeri and other local leafhoppers*	~~Quarantine organism in Europe~~
Palatinate Grapevine Yellows	PGY	Alder Yellows Phytoplasma	16SrV-C	*Oncopsis alni*	
Australian Grapevine Yellows	AGY	*Ca. P. australiense* *Ca. P. australasia* -	16SrXII-B 16SrII-D	*unknown*	
North American Grapevine Yellows	NAGY	Ca. P. asteris Ca. P. pruni	16SrI-A 16SrIII-A	*unknown*	
Other Grapevine Yellows	GY	*Ca. P. asteris*	16SrI-B	*unknown*	
Buckland valley Grapevine Yellows	BVGY	*Buckland valley grapevine yellows P.*	16SrXXIII	*unknown*	
Aster yellows of grapevine	AY	*Ca. P.asteris*	16SrI	*Mgenia fuscovaria* *Aconurella prolixa*	
Chilean Grapevine Yellows	ChGY	*Aster yellows P.* *Western X-disease P.* *Elm yellows P.* *Ash yellows P.* *Ca. P. solani*	16SrI-B, 16SrI-C 16SrIII-J 16SrV-A 16SrVII-A 16SrXII-A	*unknown*	

Table 3. Excerpt of ~~some~~ phytosanitary hot water treatment techniques for some vine diseases on material propagation.

Disease or treatment	Temperature of water	Type of material	Duration	Country	References
Phytoplasmas (FD and BN)	50°C	Cuttings and dormant top graft cuttings (scions) or dormant grafted vines	45 min	EPPO (Italy, France, etc)	✓ Method by Caudwell et al. 1991 ✓ Manini *et al*., 2007 and 2009 ✓ EPPO Standard 10/18(1), DOI: 10.1111/epp.2594
Industry and Interstate conditions within Australia	50°C	Cuttings and dormant top graft cuttings	30 min	Australia	AS588 Australia
Xylella fastidiosa	50°C	Cuttings and dormant top graft cuttings	45 min	Europe	✓ EFSA scientific opinion, 2. Sept. 2015, doi:10.2903/j.efsa.2015.4225
Partially *Agrobacterium vitis* and several other pests	50°C	Dormant canes	45 min	EPPO	EPPO Standard 10/18(1), DOI: 10.1111/epp.2594
General treatment	50°C	Not detailed	45 min	FAO/IBGRI	FAO/IBPGR Technical Guidelines for the Safe Movement of Grapevine Germplasm
	45°C	Not detailed	3h	FAO/IBGRI	

ANNEX B: Quarantine pest lists by country

In order to comply with national and international laws, OIV strongly recommends consulting each quarantine pest lists by concerned countries or these provided by the International Plant Protection Convention (IPPC: https://www.ippc.int/). However, OIV can provide some useful links in the Quarantine pests list of vine plants and grapes for OIV (http://www.oiv.int/public/medias/3310/quarantine-pests-list-vine-plants-and-grapes-oivvf.pdf).

STEP	1	2	3	4	5	6	7	8
DATE	06/2020	06/2020	10/2020	10/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
VITI-ENVIRO 20-677 Et8

OIV DEFINITION AND RECOMMENDATIONS ABOUT FUNCTIONAL BIODIVERSITY IN THE VINEYARDS

THE GENERAL ASSEMBLY

AT THE PROPOSAL of Commission I « Viticulture » and the group of experts “Sustainable development and Climate Change”,

IN VIEW of article 2, paragraph 2 iv of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine, and under the axe 1 of the OIV Strategic Plan 2020-2024, which foresees to “Promote an environmentally-friendly vitiviniculture”,

CONSIDERING Resolution ~~OIV~~ VITI ~~0~~1/~~-~~2002 on preservation of the diversity,

CONSIDERING Resolution OIV/~~-~~VITI 333/~~-~~2010 establishing the concept of terroir,

CONSIDERING the FAO definition of Regenerative Agriculture and its 10 elements of agroecology,

CONSIDERING the OIV resolution OIV CST 518-2016 on general principles of sustainable vitiviniculture, and especially the principle 2: Sustainable vitiviniculture respects the environment and the parts concerning to preserve the biodiversity,

CONSIDERING the OIV resolution OIV-VITI 641-2020 guide for the implementation of principles of sustainable vitiviniculture,

CONSIDERING the OIV collective expertise document «Functional biodiversity in the vineyard» published by the OIV in 2018,

CONSIDERING the OIV Resolution OIV-VITI 655-2021 on OIV recommendations about valuation and importance of microbial biodiversity in a sustainable vitiviniculture context.

CONSIDERING the works of the International Organisation for Biological and Integrated Control (IOBC) on integrated control, integrated protection and pest management, and landscape management for functional biodiversity,

CONSIDERING the adoption of the Strategic Plan for Biodiversity 2011-2020 under the Convention on Biological Diversity (CBD; 2010) and the 2030 Agenda for Sustainable Development of the United Nations (UN) through which the international community committed to a set of 17 ambitious goals on ‘living in harmony with nature’ and ‘leaving no one behind’, which requires immediate and ambitious action to protect life both below water and on land, by reducing pressures on biodiversity and ecosystems. In particular the goal 15 meant to protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.

RECOGNIZED THAT:

- ✓ Biodiversity across trophic levels is necessary for the balance of ecosystems and life on our planet; vitiviniculture and its production systems can contribute to preserving and strengthening it.
- ✓ Biodiversity is an important regulator of agroecosystem functions, not only in the strictly sense of its impact on production, but also in satisfying a variety of needs of the farmer and society at large.
- ✓ Agroecosystem managers, including farmers, can build upon, enhance, manage, steer and maintain multitrophic biodiversity, thus contributing to support the essential ecosystem services that are needed to work towards sustainable and regenerative viticultural production.
- ✓ Development of innovative, sustainable and regenerative viticultural practices, including the use of ancient and/or traditional viticultural practices is essential for resilience of viticultural systems. These agroecological approaches and guidelines must emphasize the conservation and sustainable management of biodiversity, soil, water, and other resources thereby improving efficiency waste management to meet the growing array of socioeconomic and environmental challenges.
- ✓ Biodiversity can provide multiple benefits through application of better farming practices following ecosystem-based approaches designed to improve the sustainability and resilience of production systems. These approaches meet consumer expectations for products produced in an environmentally and socially responsible way.
- ✓ Reaping benefits from biodiversity in agroecosystems is a key ecological strategy to bring sustainability to production.
- ✓ A wide array of complementary ecological infrastructures fostering multitrophic biodiversity should be integrated and managed in and around vineyards and respected by management.

DECIDE to adopt the following definition of "functional biodiversity" in the vineyards

"Functional Biodiversity (FB) is defined as a set of organisms, microorganisms and their species that contribute to ecosystem functions in an agroecosystem which promotes sustainability and resilience of production systems. It refers to the utilitarian part of biodiversity that can be of direct or indirect use to the farmer (e.g., conservation biological control of pests).

Functional Biodiversity seeks to integrate ecological infrastructures (hedgerows, woodlands, dry-stone walls, ground covers, insect hotels, etc.) and management practices (e.g. cover crops, livestock, microbial inoculation, etc.), in order to support and enhance ecosystem services and biodiversity in and around the vineyard and to facilitate their management by increasing the quality of the production, while recovering and maintaining the quality and functionality of landscapes."

RECOMMENDS

A) TO THE MEMBER STATES:

- ✓ To promote the FB approach which takes into account the benefits that can be derived from its establishment in the management of the vineyard,
- ✓ To support the development and promotion of the FB approach and its application in vineyards agroecosystems, as an important tool to support sustainability in the vitivinicultural sector.
- ✓ To promote the establishment of public policy measures to promote FB in vineyards agroecosystems and evaluate its outcomes.

B) TO THE SCIENTIFIC COMMUNITY

- ✓ To promote targeted actions for surveying, inventorying, and conserving existing biodiversity in and around the vineyards, discriminating through analysis of the current situation of existing ecosystems (functions of state or status) and identification of ongoing evolutions in response to disturbances caused by human, climatic or other stressors (functions of tendency),
- ✓ Carry out biodiversity evaluations of organisms, including those in the rhizosphere, and an identification of useful microorganisms living in beneficial interaction with the grapevine that may contribute to its well-being as well as the function of stimulation of self-defence mechanisms,
- ✓ To identify the effects of FB on the landscape and terroir in and around vineyards with social, environmental and economic implications, including those related to tourism,
- ✓ To continually elaborate a critical review about the ongoing study and development of tools to assess and evaluate about the role and importance of FB for the vitivinicultural sector, to publish a summary of the state of the art, at least every three years.

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FINAL DRAFT RESOLUTION
VITI-SCRAISIN 20-678A Et8

OIV DEFINITION OF GRAPE NECTAR AND CARBONATED GRAPE NECTAR

THE GENERAL ASSEMBLY,

AT THE PROPOSAL of Commission I « Viticulture » and the Sub-Commission "Table Grapes, Raisins and Unfermented Vine Products",

IN VIEW of article 2, paragraph 2 iii of the Agreement of 3 April 2001, establishing the International Organisation of Vine and Wine, and under the axe 1 of the OIV Strategic Plan 2020-2024, which foresees to "Promote an environmentally-friendly vitiviniculture",

IN VIEW of the OIV Strategic Plan for the period 2020-2024, in particular on its AXIS IV: Pursue the development of a harmonized regulatory environment and its subsections: A,1: Develop recommendations on the definition of the different categories of vitivinicultural products, A,2: Develop recommendations for labelling and for the presentation of vitivinicultural products,

CONSIDERING the works presented during the meetings of Sub-Commission 'Table grapes, raisins and unfermented vine products',

CONSIDERING Resolution AG~~OIV~~ 18~~/~~73-OEN, establishing among others the definition of grape juice and concentrated grape juice,

CONSIDERING Codex STAN 247-2005 "General standard for fruit juices and nectars",

CONSIDERING the need to establish the appropriate definitions of the vitivinicultural products in order to contribute to international harmonization, for regulatory and legal purposes and to improve the development and marketing of vitivinicultural products,

DECIDES to include in Part I chapter 6 of the OIV Code of Oenological Practices the following definitions bellow:

6.12. Grape Nectar

Grape nectar is an unfermented beverage (clarified or with pulp) for direct consumption[1]obtained by adding potable water and/or sugars*, and/or honey**, and/or syrups***, and/or sweeteners**** (up to 20 % of the total weight of the finished products), and/or vitamins and minerals to the following grapevine products: grape juice[2], concentrated grape juice[2], dehydrated grape juice[3], water extracted grape juice[3], grape puree[3], concentrated grape puree[3] or a mixture of these products. Aromatic substances, exclusively recovered from grape and obtained by suitable physical means can be added.

[1] Not used for the winemaking process.
[2] According to OIV resolution 18/73
[3] According to the definitions of Codex STAN 247-2005

Grape nectar must contain a minimum amount (% volume of finished product) of 50% of grape juice and/or grape puree.

For grape nectar the potable water used for reconstitution must, as a minimum, comply with the latest edition of the Guidelines for drinking water quality of the World Health Organization.

* Sugars: sugars must comply with the ones listed in the CODEX STAN 247-2005: sucrose, dextrose anhydrous, glucose, fructose.

**Honey: according to CODEX STAN 12-1981.

*** Syrups: syrups, as defined in the Codex Standard for Sugars, must comply with ones listed at the CODEX STAN 247-2005 : liquid sucrose, invert sugar solution, invert sugar syrup, fructose syrup, liquid cane sugar, isoglucose, high fructose syrup.

**** Sweeteners: as listed in the General Standard for Food Additives. Includes products that are preparations of high-intensity sweeteners (e.g. acesulfame potassium) and/or of polyols (e.g. sorbitol) which may contain other additives and/or nutritive ingredients, such as carbohydrates. These products, which are sold to the final consumer, may be in powder, solid (e.g. tablets or cubes), or liquid form.

6.13. Carbonated Grape Nectar

Carbonated Grape nectar is an unfermented beverage for direct consumption produced according to the definition of Grape nectar in which carbon dioxide is added.

STEP	1	2	3	4	5	6	7	8
DATE	03/2012	03/2013	10/2013	03/2014				
			10/2014	03/2015				
			10/2015	04/2016	10/2016	04/2017		
					10/2017	04/2018		
					10/2018	04/2019		
					10/2019	06/2020		
			10/2020	04/2021				
			10/2021			05/2022	11/2022	11/2022

OIV GENERAL PRINCIPLES FOR THE COMMUNICATION RELATED TO THE CARBON FOOTPRINT FOR ENTERPRISES/ORGANISATIONS AND PRODUCTS IN THE GRAPE VINE AND WINE SECTOR

THE OIV GENERAL ASSEMBLY,

CONSIDERING the resolution OIV-CST 431-2011, and in particular its decision to specify the modalities of communication of the results of calculation of the greenhouse gases emission balance for the vitiviniculturalgrapevine and wine sector in the later documents:

CONSIDERING the resolution OIV-CST 503AB-2015 which provides specific information on the items to be considered when accounting for greenhouse gases balance of an enterprise or of a specific product as well as detailed information on the greenhouse gases to be considered,

CONSIDERING the resolution OIV-VITI 640-2020 - "Multi-criteria assessment of environmental impact in vitivinicultural sector – life-cycle assessment (LCA): general principles for conduction and communication of results",

CONSIDERING the standard ISO 14020:2000 "Environmental labels and declarations – General Principles",

CONSIDERING the standard ISO 14025:2006 "Environmental labels and declarations – Type III environmental declarations – Principles and procedures",

CONSIDERING the standard ISO /TS 14027 "Environmental labels and declarations — Development of product category rules",

CONSIDERING the standard ISO/TS 14067:2018 "Greenhouse gases - Carbon Footprint of Products – Requirements and guidelines for quantification,

CONSIDERING the standard ISO 14064-1:2006 "Specification with guidance at the organization level and reporting of greenhouse gas emissions and removals",

CONSIDERING the standard ISO 14026:2017 "Environmental labels and declarations -- Principles, requirements and guidelines for communication of footprint information",

CONSIDERING the standard ISO 14044 "Environmental management – life cycle assessment – requirements and guidelines",

DECIDES to adopt the following ***"OIV General principles for the communication related to the carbon footprint for products in the vitiviniculturalgrapevine and wine sector***".

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Scope

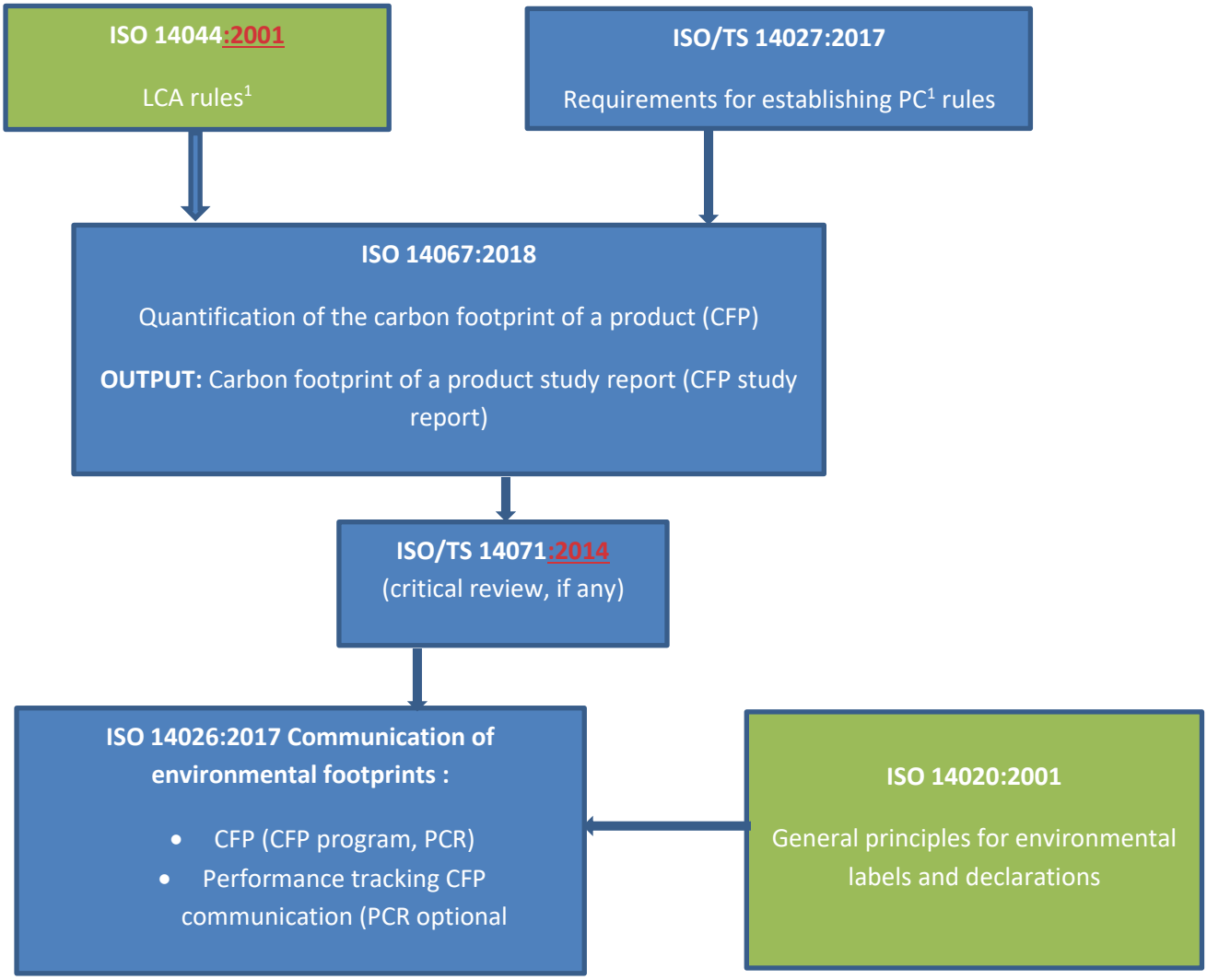
The objective of this document is to give guidance towards~~as far as the~~ performing, evaluating, and reviewing the communication of the results of the greenhouse gases accounting in the framework of the OIV protocol in the vitiviniculturalgrapevine and wine sector.

A general framework on communication requirements and options for carbon footprint of products are given in this document.

I. Relevant standards of ISO applicable to the vitivinicultural products

Communication rules and requirements provided here below for carbon footprint communication of products in the vitivinicultural sector are compatible with those defined by ISO standards 14020:2001, ISO/TS 14027, ISO 14026:2017, 14067:2018.

The articulation of pertinent ISO standards can be schematized as following:



Here below you will find a short outline of requirements set up for communication of carbon footprint for vitivinicultural products as set up by ISO standards.

II. General considerations

Standardised criteria for carbon footprint communication are necessary to ensure the precision and reliability of information communicated to consumers, as well as to minimize the number of misleading declarations and labels.

Finally, common criteria for carbon footprint communication will facilitate international trade.

III. Terms and definitions

OIV GHGAP: OIV Greenhouse Gases Accounting Protocol (defined by the resolutions OIV-CST 431-2011 and OIV-CST 503AB-2015)

Carbon footprint of a product (CFP)[1]: balance of greenhouse gas emissions and sequestrations in a product system, expressed as CO_2 equivalents and based on a life cycle assessment (LCA) (scope 1, scope 2 and scope 3) using the single impact category of climate change. The results of the quantification of the CFP are documented in a CFP study report.

Carbon footprint of a product study report / CFP study report[2]: report that documents the CFP study, presents the CFP or partial CFP, and shows the decisions taken within the study.

Product Category Rules (PCR)[3]: set of specific rules, requirements and guidelines for developing environmental declarations for a product category

Product category[4]: group of products that can fulfill the same functions

CFP communication program[5]: program for the development of CFP communication based on a set of operating rules. The footprint communication program can be voluntary or mandatory, international, regional, national, local and/or sectoral.

QR Code: - Quick Response Code is a two-dimensional (2-D) matrix code that can store more data than previous 1-D and 2-D codes. In addition, an advanced error-correction method and other unique characteristics allow the QR Code to be read more reliably and at higher speeds than other codes.

Functional Unit:

- The functional unit describes the functionality of a product as a quantified unit facilitating comparison across similar studies of carbon footprint. For the specific purposes of this resolution, the OIV recommends to follow the prescription established in resolution OIV-CST 503AB-2015 «Greenhouse gases accounting in the grapevine and wine sector – recognised gases and inventory of emissions and sequestrations».

- The declared unit is the unit used in communication of specific carbon footprints. For the sake of consistency and comparability, the OIV recommends the declared unit to be the same as the functional unit.

[1] ISO 14067: 2018
[2] ISO 14067:2018
[3] ISO 14027 :2017
[4] ISO 14027 :2017
[5] ISO 14026:2017

IV. General principles [6]

The following general principles are applicable to all carbon footprint labels and climate declarations of vitivinicultural products.

1. The labels and declarations shall be accurate, verifiable, relevant
2. The labels and declarations must in no way imply obstacles to international trade
3. The information provided must be based on scientific methodology as described in the OIV GHG accounting protocol for grapevine and wine sector (resolutions OIV-CST 431-2011 and OIV-CST 503AB-2015).
4. The information used by labels and declarations must be publicly available and transparent
5. The labels and declarations must take into consideration all relevant aspects of the life cycle of the product
6. The labels and declarations must ensure the issue of comparability. Footprint communications are intended to enable comparison, based on the area of concern, between products in the same product category and having the same functional or declared unit.
7. The labels and declarations must take into consideration the different local and regional contexts for the calculation of the carbon footprint.
8. The labels and declarations must ensure the fairness of Greenhouse Gas Accounting Plan (GHGAP) through a single category assessment of climate change impact and not overall environmental performance.
9. Any administrative requirements or information demands related with labels and declarations must be limited to necessary administrative requirements or information demands
10. The development of labels and declarations must include a participatory consultation with interested parties
11. The relevant labeling and declaration information must be available along the supply chain

V. Requirements for CFP communication

1. General requirements

A CFP communication of a vitivinicultural product shall be based on a footprint study which is based on methodology as described in the OIV GHG accounting protocol for grapevine and wine sector (resolutions OIV-CST 431-2011, OIV-CST 503AB-2015 and ISO 14026).

2. Intended audience: business-to-business or business-to-consumer

A CFP communication of a vitivinicultural product may be intended for business-to-business or business-to-consumer communication.

3. Information to be provided

The CFP communication shall include the following information:

- the functional unit or declared unit to which the CFP communication refers;

[6] For further information, please see the general principles of communication for products defined by ISO 14020

- identification of the life cycle stages that are covered by the footprint communication;
- an unambiguous indication (e.g. a link to a website or a QR code) on how to access the supporting information which may be on a website, at the point of sale or any other publicly available communication means.

4. Critical review

In compiling the CFP study, a critical review enhances the credibility of CFP. It is mandatory in the case of comparative LCA results. A critical review of CFP studies, if any, shall be performed in accordance with ISO/TS 14071.

STEP	1	2	3	4	5	6	7	8
DATE		03/2014	10/2014	03/2015	10/2015	04/2016		
					10/2016	04/2017	06/2017	
					10/2017	04/2018	11/2018	
					11/2018	04/2019	07/2019	
							10/2019	
					10/2019			
					10/2020			
					10/2021	10/2021	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 14-567B2 Et8

DISTINCTION BETWEEN ADDITIVES AND PROCESSING AIDS – Carbon dioxide

THE GENERAL ASSEMBLY,

IN VIEW OF article 2, paragraph 2 b) ii of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING that in the framework of its competence, the objectives of the OIV are to contribute to international harmonisation of existing practices and standards and, as necessary, to the preparation of new international standards in order to improve the conditions for producing and marketing vine and wine products, and to help ensure that the interests of consumers are taken into account,

CONSIDERING the definitions of additives and processing aids issued from OIV resolution OIV-OENO 567A-2016,

CONSIDERING the different oenological substances adopted by the OIV and published in the *International Code of Oenological Practices* and *International Oenological Codex*,

CONSIDERING the works of the "Technology" Expert Group, and the OIV Task Force on additives in wines on the evaluation of the status as additives or processing aids of the substances adopted by the OIV,

CONSIDERING that this distinction will help further harmonisation between intergovernmental organisations and will facilitate international trade in wine,

CONSIDERING that the table below is not an exhaustive list of the additives and processing aids and that the OIV continues to review and consider the additives and processing aids proposed for use in winemaking,

CONSIDERING the works of the electronic working group created during the meeting of the TECHNO group of experts in June 2020,

DECIDES, following a proposal made by Commission II "Oenology", to adopt the following distinction between additives and processing aids for the substances already permitted by the OIV and listed below,

DECIDES to classify carbon dioxide as an additive or a processing aid according to each oenological function,

DECIDES to incorporate this distinction into the corresponding files of the *International Code of Oenological Practices* and also present it in the form of a summary table to be inserted into said Code:

Substance	**SIN or CAS No.**	**Code of Oenological Practices ref.**	**Codex file ref.**	**Additive**	**Processing aid**
Gaz					
Carbon dioxide	SIN 290		COEI-1-DIOCAR		
		1.7 Carbonic maceration			X
		2.2.3 Preservation by addition of carbon dioxide to must or carbonation of must		X[i]	
		2.2.5 Protection under inert atmosphere			X
		2.3.9 Warm post fermentation maceration of red grapes called warm final maceration			X
		3.2.3 Racking			X
		3.5.17 Management of dissolved gas in wine using membrane contactors		X	
		4.1.10 Decanting			X
		4.5 Carbonated Wines		x	
		6.15 Carbon dioxide use		X	

[i] Carbon dioxide is considered an additive when it is added to the must. However, to continue with the production phases, after storage, it is eliminated from the must, and therefore it can be considered a processing aid

STEP	1	2	3	4	5	6	7	8
DATE		03/2014	10/2014	03/2015	10/2015	04/2016		
					10/2016	04/2017	06/2017	
					10/2017	04/2018		
					10/2018	04/2019		
					10/2019	06/2020		
					10/2020	03/2021		
					10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 14-567B4 Et8

DISTINCTION BETWEEN ADDITIVES AND PROCESSING AIDS - Dimethyl dicarbonate

THE GENERAL ASSEMBLY,

IN VIEW OF ARTICLE 2, paragraph 2 b) ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING that In the framework of its competence, the objectives of the OIV is to contribute to international harmonisation of existing practices and standards and, as necessary, to the preparation of new international standards in order to improve the conditions for producing and marketing vine and wine products, and to help ensure that the interests of consumers are taken into account,

CONSIDERING the definitions of additives and processing aids issued from OIV resolution OIV-OENO 567A-2016,

CONSIDERING the different oenological substances adopted by the OIV and published in the *International Code of Oenological Practices* and *International Oenological Codex*,

Considering the works of the “Technology” Expert Group, and the OIV Task Force on additives in wines on the evaluation of the status as additives or processing aids of the substances adopted by the OIV,

CONSIDERING that this distinction will help further harmonisation between intergovernmental organisations and will facilitate international trade in wine,

CONSIDERING that the list below is not an exhaustive list of the additives and processing aids and that the OIV continues to review and consider the additives and processing aids proposed for use in winemaking,

CONSIDERING that the use of DMDC in beverages including wine as a microbial control agent is permitted in many economies. In wine, DMDC is typically added to the beverage during the processing stage before it is filled into packages. DMDC breaks down in the presence of water to form primarily methanol and carbon dioxide. This breakdown occurs within hours in typical applications. There is no ongoing antimicrobial activity from DMDC, or the breakdown products, in a product sold through a normal commercial beverage distribution system. No residue of DMDC remains in the treated beverages ready for consumption however methanol and traces of other DMDC breakdown products such as dimethyl carbonate (DMC), methyl ethyl carbonate (MEC) and methylcarbamate (MC) may be found in the finished product.

CONSIDERING that some countries, due to the presence in the treated wines of some breakdown products of DMDC, have legislated in their relevant laws that DMDC is an additive, whereas other countries, considering the absence del DMDC in the treated wines, have legislated that it is a processing aid,

DECIDES, on the proposal of Commission II "Oenology", to add, in the table of classification of additives or processing aids of the International Code of Oenological Practices, a line related to DMDC as follows:

Substance	**SIN or CAS No.**	**Code of Oenological Practices ref.**	**Codex file ref.**	**Additive**	**Processing aid**
Stabilising agents					
Dimethyl dicarbonate	SIN 242	Fiche 3.4.13	COEI-1-DMDC	See footnote[1]	See footnote[1]

1 According to the national laws of different countries, DMDC is considered as an additive in some countries whereas in in other countries it is considered as a processing aid.

STEP	1	2	3	4	5	6	7	8
DATE		03/2014	10/2014	03/2015	10/2015	04/2016		
					10/2016	04/2017	06/2017	
					10/2017	04/2018		
					10/2018	04/2019		
					10/2019	06/2020		
					10/2020	10/2021	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 14-567C1 Et8[1]

DISTINCTION BETWEEN ADDITIVES AND PROCESSING AIDS - Skimmed milk

THE GENERAL ASSEMBLY,

IN VIEW OF ARTICLE 2, paragraph 2 ii of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING that In the framework of its competence, the objectives of the OIV is to contribute to international harmonisation of existing practices and standards and, as necessary, to the preparation of new international standards in order to improve the conditions for producing and marketing vine and wine products, and to help ensure that the interests of consumers are taken into account,

CONSIDERING the definitions of additives and processing aids issued from OIV resolution OIV-OENO 567A-2016,

CONSIDERING the different oenological substances adopted by the OIV and published in the *International Code of Oenological Practices* and *International Oenological Codex*,

Considering the works of the "Technology" Expert Group, and the OIV Task Force on additives in wines on the evaluation of the status as additives or processing aids of the substances adopted by the OIV,

CONSIDERING that this distinction will help further harmonisation between intergovernmental organisations and will facilitate international trade in wine,

CONSIDERING that the list below is not an exhaustive list of the additives and processing aids and that the OIV continues to review and consider the additives and processing aids proposed for use in winemaking,

DECIDES, following a proposal made by Commission II "Oenology", to adopt the following distinction between additives and processing aids for the substances already permitted by the OIV and listed below,

DECIDES to incorporate this distinction into the corresponding files of the *International Code of Oenological Practices* and also present it in the form of a summary table to be inserted into said Code:

[1] Pending further information, the draft of resolution, which remained at this stage, has not been modified compared to the previous version of 2020.

Substance	SIN or CAS No.	Code of Oenological Practices ref.	Codex file ref.	Additive	Processing aid
Clarifying agents					
Skim milk[2]		fiche 3.2.1	COEI-1-LAIECR		X

[2] Skimmed milk is a foodstuff used in enology as a processing aid.

STEP	1	2	3	4	5	6	7	8
DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 20-684A Et8

IMPORTANT: This draft resolution modifies the following resolution:
- OIV-OENO 582-2017

USE OF SELECTIVE PLANT FIBRES IN WINE – update to Resolution OIV-OENO 582-2017

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 b) ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Technology" Expert Group,

CONSIDERING Resolution OIV-OENO 582-2017 adopted in 2017,

DECIDES, at the proposal of Commission II "Oenology", to modify file 3.4.20 in part II, chapter 3, "Wines", of the *International Code of Oenological Practices* of the OIV as follows:

PART II

3. Wines

TITLE:

USE OF SELECTIVE PLANT FIBRES IN WINE

Definition:
Use in wines of a selective adsorbent composed of plant fibres.

Objectives:
a) To reduce the ochratoxin A levels in wines;
b) to reduce the number and levels of phytosanitary product residues in wines.

Prescriptions:

a) Selective plant fibres are to be used as processing aids and incorporated either into wine or during filtration with continuous deposition, during cross-flow filtration, or as a constituent of a filter sheet;
b) the recommended dosage is to be determined according to the filtration technique used, without exceeding 1.5 kg/m^2 of the filter sheet surface area or 200 g/hL in wine;
c) selective plant fibres used in wines are to be removed by racking after sedimentation, centrifugation, or filtration;

d) selective plant fibres are to be used in wines in compliance with the regulatory requirements, particularly the permitted limits for phytosanitary product residues;
e) selective plant fibres should comply with the prescriptions of the *International Oenological Codex*.

Recommendation of the OIV:

Accepted.

STEP	1	2	3	4	5	6	7	8
DATE		6/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 20-684B Et8

USE OF SELECTIVE PLANT FIBRES IN MUST

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 b) ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Technology" Expert Group,

CONSIDERING Resolution OIV-OENO 582-2017 adopted in 2017 on the use of plant fibres in wines,

DECIDES to widen the scope of application to musts and, at the proposal of Commission II "Oenology", to introduce the following oenological practice into part II, Chapter 2, "Musts", of the *International Code of Oenological Practices*:

PART II

2. Musts

TITLE:

USE OF SELECTIVE PLANT FIBRES IN MUST

Definition:
Use in musts of a selective absorbent composed of plant fibres.

Objectives:
a) To reduce the ochratoxin A levels in musts;
b) to reduce the number and levels of phytosanitary product residues in musts.

Prescriptions:
a) Selective plant fibres are to be incorporated into must, or during fermentation;
b) the dose to be used is to be determined depending on the must and levels of molecules to be adsorbed, and should not exceed 200 g/hL;
c) selective plant fibres are to be removed by racking after sedimentation, by centrifugation or by filtration;
d) selective plant fibres are to be used in musts in compliance with the applicable authorisations and limits for phytosanitary product residues;
e) selective plant fibres should comply with the prescriptions of the International Oenological Codex*.*

OIV recommendation:

Accepted.

PHASE 1	1	2	3	4	5	6	7	8
DATE		03/2021	10/2021	05/2022			11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 21-689 Et8

WARNING: this resolution amends the following resolution:
- AG 12/72-OEN

OIV MAXIMUM LIMIT FOR GUM ARABIC – Update

THE GENERAL ASSEMBLY,

CONSIDERING Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work carried out by the working group to update or confirm the use limits for specified additives or processing aids,

CONSIDERING the work done by the "Technology" Expert Group during its June 2020 session,

CONSIDERING file 3.3.6 of the *International Code of Oenological Practices* relating to treatment with gum arabic, limited to a maximum use level of 0.3 g/L,

CONSIDERING the work undertaken by the working group that specifies the following:
- some liqueur wines with very high colouring matter require a greater amount than 0.3 g/L to stabilise the colloidal colouring matter,
- the addition of a 0.8 g/L dose to very unstable red liqueur wines is required to achieve stability of the colloidal colouring matter,
- this 0.8 g/L dose does not change the sensory characteristics of these wines,

CONSIDERING that even though an acceptable daily intake has not been specified and there is no proven health risk, numerical gum arabic use levels are necessary to ensure the authenticity and identity of these vitivinicultural products,

DECIDES, at the proposal of Commission II "Oenology", to modify Resolution AG 12/72 OEN and, consequently, file 3.3.6, which appears in part II, chapter 3 of the *International Code of Oenological Practices*, in relation to the current limit permitted for the treatment of wines with gum arabic:

- Prescription b) is modified as follows:

B) The dose used shall not exceed 0.8 g/L for red liqueur wines and 0.3 g/L for other wines.

STEP	1	2	3	4	5	6	7	8
DATE		10/2021	10/2021			05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 21-707 Et8

IMPORTANT: The present resolution revokes the following resolutions:
- OIV/OENO 145/2009
- OIV-OENO 505-2014

WINES – TREATMENT WITH SILVER CHLORIDE

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the "Technology" Expert Group,

CONSIDERING the results of the questionnaire indicating that the oenological practice is no longer used,

DECIDES, at the proposal of Commission II "Oenology", to withdraw the resolution OIV-OENO 505-2014 and consequently delete the monograph COEI-1-CHLARG from the *International Oenological Codex*,

DECIDES, at the proposal of Commission II "Oenology", to withdraw the resolution OIV-OENO 145-2009 and consequently delete the following oenological practice from part II of the *International Code of Oenological Practices*:

WINES – TREATMENT WITH SILVER CHLORIDE

PART III

3. WINES

3.5.15. TREATMENT WITH SILVER CHLORIDE

STEP	1	2	3	4	5	6	7	8
DATE		03/2021	10/2021			05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-TECHNO 21-708 Et8

IMPORTANT: This resolution modifies the following resolution:
- OIV-OENO 2/99

UPDATE TO FILE 2.1.14 - Flotation

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Technology" Expert Group,

DECIDES, at the proposal of Commission II "Oenology", to modify specifically point "a" of the prescriptions of Resolution OENO 2-1999 and consequently file 2.1.14 "Flotation" in part II, chapter 2 of the *International Code of Oenological Practices* as follows (strikethrough text is to be deleted and text in *italics* is to be added):

Part II

Chapter 2: MUSTS

UPDATE TO SHEET 2.1.14. - Flotation

Prescriptions:

Clarification can be done:

a) Protected from air by using nitrogen or ~~carbon dioxide~~ *argon*, or with compressed air to encourage the oxidation of oxidable compounds and render the color of the wine more stable;

STEP	1	2	3	4	5	6	7	8
DATE		10/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-MICRO 20-686 Et8

IMPORTANT: This draft resolution supersedes the following resolution:
- OIV-OENO 462-2014

REVIEW OF THE CODE OF GOOD VITIVINICULTURAL PRACTICES IN ORDER TO AVOID OR LIMIT CONTAMINATION BY *BRETTANOMYCES*

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Microbiology" Expert Group,

CONSIDERING that this code determines the measures to be set up in vineyards and in wineries in order to contribute to reducing the risks linked to the presence of *Brettanomyces*,

DECIDES to replace Resolution OIV-OENO 462-2014, 'Code of Good Vitivinicultural Practices in order to Avoid or Limit Contamination by *Brettanomyces',* by the following text:

CODE OF GOOD VITIVINICULTURAL PRACTICES IN ORDER TO AVOID OR LIMIT SPOILAGE OF WINES BY *BRETTANOMYCES BRUXELLENSIS*

PREAMBLE

- Among processes that deteriorate wine quality, the production of volatile phenols by *Brettanomyces bruxellensis* is widespread and increasingly problematic. These compounds are characterised in particular by aromas of ink or glue, and horse sweat, leather or stable taints, which can mask wines' fruity characteristics.
- Volatile phenols, mainly 4-ethylphenol and 4-ethylguaiacol, are produced from p-coumaric acid and ferulic acid respectively after enzymatic decarboxylation (cinnamate decarboxylase, PAD) and reduction (vinylphenol reductase, VPR). These precursors are naturally present in grape musts. The decarboxylation step caused by cinnamate decarboxylase activity has been described for many bacteria, yeast and fungi species, while the reduction step caused by vinylphenol reductase activity, or VPR, is more specific to the *Brettanomyces* species.
- *Brettanomyces* are present on grapes and on winemaking equipment, meaning that these yeasts may proliferate in wines during or after alcoholic and/or malolactic fermentations, during wine maturing or after packaging.

INTERVENTIONS IN THE VINEYARD

Not applicable (to our knowledge, no study available). However, *Brettanomyces* yeasts have been detected on grape skins after enrichment steps, from the first stages of berry development. The microbial ecology of grape surfaces has shown great diversity, with a small population for each species. An initial preventive approach, consisting of rigorous selection of healthy grapes, can play a role in reducing the risk of *Brettanomyces*, which is generally found in greater numbers on spoilt grapes.

INTERVENTIONS DURING THE GRAPE HARVEST

Grape management:
Brettanomyces is present on grapes (small population) but is not the major species of yeast. However, the removal of damaged or botrytised grapes could limit *Brettanomyces* spoilage.
The harvest of overripe berries is more and more common, and in this case particular precautions could be considered. These interesting impacts at the organoleptic level may increase the risk of volatile-phenol production because overripe grapes contain more volatile-phenol precursors. Working in these conditions does not necessarily increase the presence of *Brettanomyces*, but increases the risk of activity (a lower total acidity and higher pH directly impacts on molecular SO_2 levels and consequently on the growth of *Brettanomyces*).

INTERVENTIONS IN THE CELLAR

Due to multiple factors, including the increase in alcohol content, a reduction in microbial diversity is observed during alcoholic fermentation. However, as *Brettanomyces* has good resistance to ethanol

and its presence does not decrease. Therefore, perfect hygiene is essential during winemaking (sound grapes, winemaking and storage equipment, etc.).

Pre-fermentative operations and treatments

- Ensuring that suitable hygiene practices are applied in the cellar is recommended.

- The most important factors are sulphiting and temperature:
  - sulphiting is the most effective preventative action at the pre-fermentative stage for limiting the development of *Brettanomyces* populations, however it is recommended that excessive sulphiting (>8 g/hL), which could delay malolactic fermentation, is avoided,
  - high-temperature, pre-fermentative maceration (above 65 °C) results in the inactivation of *Brettanomyces*, but also of other microorganisms in winemaking, and cold maceration at a temperature lower than or around 10 °C prevents their proliferation, but does not kill them.

- The use of some enzymes containing cinnamoyl esterase activities may increase the risk of volatile phenol production. In all circumstances, subsequent contamination is possible.

Fermentation operations

Alcoholic fermentation (AF):

- During AF, microbial diversity decreases and *Saccharomyces cerevisiae* becomes the main species. However, due to its ethanol resistance and lower nutrient demand, *Brettanomyces* can grow as AF slows down or stops. The oenological practices commonly recommended for management of alcoholic fermentation should be implemented.
- Inoculation of musts with selected yeasts helps to achieve a more reliable AF.
- The environment becomes more favourable to the multiplication of *Brettanomyces* if AF slows down or stops. In the case of the latter, using a process to restart AF as soon as possible is recommended.
- Residual sugars (mainly glucose and fructose) are substrates for *Brettanomyces* growth. Wines are generally considered as dry when the glucose + fructose level is below 4 g/L. A concentration of 0.3 g/L of glucose + fructose is sufficient for the development of *Brettanomyces* biomass capable of producing over 1000 µg/L of volatile phenols.
- Yeast nutrients (that may also benefit *Brettanomyces*) should only be added if really necessary to avoid stuck fermentations.

Lag period before malolactic fermentation (MLF):

- Once AF is completed, conditions favour not only lactic bacteria but also *Brettanomyces*, although proliferation remains slow.

- It is important to monitor the *Brettanomyces* population since the environment is relatively low in microorganisms.
- Factors favourable to the growth of *Brettanomyces* during this phase are as follows: final high-temperature macerations (40-45 °C), micro-oxygenation, the release of sugars in the case of uncrushed harvests, and partially raisined harvests.
- Co-inoculation of selected yeasts and lactic bacteria may help to reduce the lag phase between AF and MLF, and consequently the development of *Brettanomyces*.

Malolactic fermentation (MLF):

- Physicochemical parameters (pH, temperature, total SO_2) affect the progress of MLF. If MLF is delayed, the risk of production of volatile phenols increases because *Brettanomyces* can take advantage of this time to multiply.
- The use of malolactic starters is a good way to limit *Brettanomyces* development.
- After malolactic fermentation, it is recommended to eliminate all microorganisms, particularly by adding SO_2. These quantities must be adjusted according to the pH of the wine. The use of physical techniques (HHP or ~~,~~ UHPH ~~or PEF~~) is also possible.

Maturation and clarification operations

The first necessary precaution is to carry out regular tasting and a complete microbiological analysis, which includes specific counting of *Brettanomyces* and/or analysis of volatile phenols. This analysis must be repeated throughout the maturation period.

- SO_2 management is crucial to limit the development of *Brettanomyces*. The recommended concentration is about 0.5 to 0.8 mg/L of molecular SO_2[1]. In case of *Brettanomyces* strains tolerant/resistant ~~–~~to ethanol or SO_2, ~~strains,~~ alternative methods are recommended (chitosan, filtration or heat treatment).
- Ageing on lees is an additional risk factor because *Brettanomyces* are quite able to survive and to proliferate in lees (which release nutrients into wine).
- Clarification by racking, fining and filtration are essential to reduce the viable and viable but non-culturable *Brettanomyces* populations that can multiply by metabolising residual sugars.
- Some fining agents are more efficient than others. Treatments with fining proteins can reduce populations by a factor of 40-2000. Fining using casein or potassium caseinate may reduce the ethylphenol levels if these are not too high.
- The addition of chitosan is one alternative for controlling the growth of undesirable microorganisms, particularly *Brettanomyces* and when dealing tolerant/resistant strains to ethanol or SO_2.
- Some winemaking operations (racking, topping up, filtration, bottling, etc.) may result in oxygen dissolution in wine, favouring the multiplication of *Brettanomyces*.
- If micro-oxygenation is practised, the absence of *Brettanomyces* should be checked using appropriate analyses.

[1] The final product should comply with the regulations in force regarding total SO_2 limits.

– During wine aging, the cellar temperature should be carefully controlled especially during the summer period, and long periods at over 14 °C should be avoided to prevent *Brettanomyces* growth.

NB:

1- Upon addition of SO_2, the *Brettanomyces* population can switch (fully or partially) from a viable state to a viable but non-culturable (VBNC) state. These changes lead to a reduction in the size of the yeasts, so it is necessary to adapt filtration.
2- It is also important to note here that enumeration of VBNC microorganisms cannot be performed by routine analysis, for example enumeration on a Petri dish, but rather by qPCR or flow cytometry, which enumerates VBNC and viable forms of *Brettanomyces*.
3- A PCR test is available to predict *Brettanomyces* sulphite tolerance/resistance.

Barrel maturation

Barrel maturation is considered to be the period most sensitive to spoilage by *Brettanomyces*, particularly with new barrels.
During sampling, cross contaminations have to be avoided.

As for any microbial spoilage, the wine used for topping up must not be contaminated.
Wood is favourable to growth of *Brettanomyces*, which is capable of growing pseudohyphally, of colonising wood micropores, and of using cellobiose as a carbon source. Barrels are difficult to clean and disinfect.
Old, poorly-cleaned barrels are known sources of contamination by *Brettanomyces.* However, new barrels also favour yeast multiplication and the production of volatile phenols, since they release more nutrients. Moreover, new barrels are more permeable to O_2, which favours a relatively high redox potential and decreases the (active or molecular) SO_2 concentration – two parameters favourable to *Brettanomyces* growth.
Different approaches have been investigated for the sanitisation of barrels, but none of them have enabled the complete removal of *Brettanomyces* on the internal stave surface or bunghole. Indeed, the natural microporosity of the wood makes its complete disinfection difficult because microorganisms stay alive in the cavities of the deep layers of the wood. A deeply-acting treatment is essential for long-lasting efficiency and results over time.

Nevertheless, some techniques for the disinfection of barrels significantly reduce *Brettanomyces* populations and may be used where permitted by the applicable regulations in question, for example:

- steam treatment: deep disinfection requires a sufficiently long treatment time (cold water rinse, hot water rinse at 70 °C and low pressure steam for 10 min). A treatment by immersion in hot water at 60°C for an exposure time of 19 min allowed to eliminate the yeast populations up to a log count reduction of 8,~~.~~
- ozone sanitisation: performed either with gaseous ozone combined with a hot water treatment at 82 °C for 20 min or with ozonated water, by reacting with materials with a high organic load the ozone does not deeply penetrate into the wood,

- SO_2 sanitisation: a minimum of 5 g per barrel of gaseous SO_2 should be used to disinfect empty and dry barrels, with SO_2 being very efficient both on the surface and also in deeply penetrating the first millimetres of the wood,
- barrel shaving and re-heating: this treatment does not disinfect the wood but enables the most contaminated part to be removed, enabling an 80% reduction of volatile phenols compared to a non-treated barrel,
- ultrasound: this technique removes more than 90% of viable *Brettanomyces* (up to 2-4 mm below the internal stave surface).

Pre-packaging operations

The risk of volatile-phenol production should be evaluated before the packaging operations using analytical checks (both chemical and microbiological). When the risk has been evaluated, suitable operations should be planned in order to prevent post-packaging development of *Brettanomyces*:

- sterilisation by membrane filtration (0.45 to 0.65 µm) or cross-flow filtration, for efficient removal of *Brettanomyces* yeasts, followed by sterile packaging,
- use of DMDC for non-lasting protection,
- use of antimicrobials with lasting protection (sorbic acid, only if lactic bacteria have been removed completely; management of SO_2, taking the pH, acquired alcoholic strength by volume, and temperature into account). An online calculator taking into account different parameters (ph, alcohol and temperature) can be used.~~,~~
- heat treatment,
- use of physical techniques (HHP or UHPH ~~or PEF~~) is also possible.

Storage conditions

In order to prevent *Brettanomyces* proliferation in bottles during storage (and the production of volatile phenols), keeping bottles below 12 °C is recommended, especially for lightly-filtered wines or wines containing low levels of SO_2.

CONCLUSIONS

- Frequent analyses are highly recommended in order to detect any contamination by *Brettanomyces* at an early stage. During sampling, particular attention should be paid to avoid cross-contaminations.
- The maintenance of the best possible hygiene conditions in the cellar is highly recommended.
- Sulphiting and molecular SO_2 management are recommended.
- Temperature management is recommended.
- Preventive actions are preferable to curative processes.

The present recommendations are based on current knowledge and are liable to be updated according to ongoing research.

Brettanomyces management should be a global preventative approach throughout the winemaking process.

STEP	1	2	3	4	5	6	7	8
DATE		4/2016	10/2016	04/2017				
			10/2017	04/2018				
			10/2018	04/2019				
			10/2019	10/2020				
			10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SPECIF 16-602 Et8

GENERAL PRINCIPLES TO TAKE INTO ACCOUNT IN THE ASSESSMENT OF OENOLOGICAL PRACTICES AND SUBSTANCES COMPOSING OENOLOGICAL PRODUCTS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work accomplished by the "Specifications of Oenological Products" Expert Group during its March 2016 session,

CONSIDERING the definitions of wine and other viticultural products,

CONSIDERING that the oenological practices and oenological substances recommended by the OIV are intended for the production, preservation and development of wine-sector products,

CONSIDERING that the *International Oenological Codex* contains descriptions of the main products used in the making and storage of wine, and the conditions, method and limits for their use are set by the *International Code of Oenological Practices*,

CONSIDERING the above and given the scope of Resolutions CST 1/2004 'Development of sustainable vitiviniculture', OIV-OENO 567A-2016 'Distinction between additives and processing aids – Part 1', OENO-TECHNO 14-567B 'Distinction between additives and processing aids – Part 2',

DECIDES to adopt the following as general principles that will appear in the preamble of the *International Code of Oenological Practices* and *International Oenological Codex*,

GENERAL PRINCIPLES TO TAKE INTO ACCOUNT IN THE ASSESSMENT OF OENOLOGICAL PRACTICES AND SUBSTANCES COMPOSING OENOLOGICAL PRODUCTS

Oenological practices:

- are safe biological, physical and chemical practices intended for the production and preservation of vitivinicultural products,
- preserve the natural and essential characteristics and composition of wine,
- should not cause consumer confusion or facilitate this.

(Wherever possible and reasonable, preference should be given to substances that are extracted from grapes/substances of vitivinicultural origin).

Oenological practices and the use of oenological substances should not mask the effects of the use of defective raw materials, faulty raw materials or unhygienic practices[1].

Taking this into consideration the OIV establishes a specific description of each oenological practice and any use of oenological substances, including its objectives, purposes and conditions of use. Oenological practices and the use of oenological substances recommended and published by OIV shall only be used for the purposes described and in the manner described to provide a benchmark for good manufacturing practice.

Oenological practices and oenological substances are used:

- to prevent or eliminate wine defects (oxidation, bacterial contamination, tartaric precipitation, etc.),
- to improve the process of winemaking (control the fermentations, improve the filtrability, improve clarification,…),
- to improve the storage capacity or stability of the vitivinicultural product and maintenance of its organoleptic qualities,
- as additives or processing aids, while the status of the substance depends on their use.

There is a sufficient technological need to justify the use of the oenological substance, or the treatment gives evident advantages, economically or technically, compared to the present practices and consumers are not misled through the use of the oenological treatment agent; the oenological substance provides advantages to consumers and consequently fulfils one or several objectives such as:

- preservation of the nutritive quality of the vitivinicultural products,
- improvement of the storage capacity or stability of the vitivinicultural product and maintenance of its organoleptic qualities.

The OIV establishes a specification for each oenological substance concerning the origin, purity and other necessary indications.
The OIV evaluates and develops new oenological practices or revises those that already exist, also taking into account environmental impact/sustainability according to technological innovations.
The OIV recommends that:

- the quantity of an oenological treatment agent added in the elaboration of a product shall be limited to the lowest possible level necessary to accomplish its desired effect,

[1] "hygiene", means the measures and conditions necessary to control hazards and to ensure fitness for human consumption of wine, wine-based beverages, table grapes, raisins and other vine-based products taking into account its intended use

- the quantity of the oenological treatment agent that as a result of its usetransforms to another product not intended to accomplish any physical or other technical effect in the vitivinicultural product itself, is reduced to the extent reasonably possible.

The OIV recommends maximum numerical use levels for oenological substances with an acceptable daily intake (ADI) set by JECFA or other Food Safety Authorities, also taking account of the opinion of the OIV's "Food Safety" Group. The OIV also recommends numerical limits for best use of the technological function and/or for quality purposes on the basis of experimental data analysed by its own experts. In the absence of fixed numerical limits from the OIV, the OIV recommends oenological substances shall be used at levels established for GMP, which are the lowest levels possible to provide a specific technological function.

STEP	1	2	3	4	5	6	7	8
DATE		4/2017	10/2017	04/2018				
			10/2018	04/2019				
			10/2019	06/2020	10/2020			
					10/2021			
						05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SPECIF 17-624 Et8

WARNING: this resolution revokes the following resolutions:
OENO 12/2002, OENO 5/2008, OENO 6/2008, OIV/OENO 352/2009, OIV-OENO 574-2017 and OIV-OENO 554-2015

UPDATE TO THE MONOGRAPH ON OENOLOGICAL TANNINS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

DECIDES, at the proposal of Commission II "Oenology", to replace monograph COEI-1-TANINS in the *International Oenological Codex* by the following:

OENOLOGICAL TANNINS

1. GENERAL STATEMENTS

Oenological tannins contain polyphenols. Their complex structure is generally made up of a great variety of monomer units that are covalently bound to each other.
These are derived from different parts of plant species and extracted using solvents permitted under current regulations. They are classified into two classes according to the nature of the monomeric units that characterise them: hydrolysable tannins and condensed tannins *or proanthocyanic tannins*. Besides the two classes of tannins, each monomer unit itself (e.g. flavan-3-ol monomers, gallic acid, ellagic acid) can also occur in tannin preparations.

Hydrolysable tannins form a heterogeneous class that is divided into two sub-classes:

- gallotannins or gallic tannins consist of a glucose or quinic acid unit that is esterified with one or more molecules gallic acid and/or its depsides.
- ellagitannins or ellagic tannins consist of glucose unit(s) that is/are esterified with one or more molecules of ellagic acid and related molecules. In addition, the glycosidic unit(s) may be esterified with gallic acid and/or its/their depsides.

The structure of hydrolysable tannins varies according to the degree of esterification and polymerization (1)

Condensed or proanthocyanic tannins (of the procyanidin and prodelphinidin sub-classes and the profisetinidin and prorobinetidin sub-classes) and consist of polymers of flavan-3-ols. The flavan-3-ol units may differ stereochemically as well as in the degree of hydroxylation and may be present as gallic acid esters. The large variety of monomeric units, different interflavan bonds and the degree of polymerization result in a large number of possible structures of proanthocyanidins. Depending on the molecular structure, the reactivity of proanthocyanidins can vary greatly.

Oenological tannins come exclusively in powder, granule or flake form, from light beige or beige and brick red to dark brown in colour.
They can be dissolved in a portion of must or wine for incorporation into the total amount of must or wine.

2. LABELLING

The labelling of oenological tannins must specify the following:
- their botanical origin (e.g. oak, quebracho, etc.),
- the classes to which they belong (hydrolysable or condensed),
- the sub-classes (e.g. gallotannins, prodelphinidins, etc.),
- the batch number and the expiry date,
- the minimum content of total polyphenols according to the method in Annex 1,
- the technological functions,
- when tannin preparations are a mixture of several tannin classes or tannin sub-classes the different tannin classes or sub-classes and associated technological functions,
- the recommended dosage and conditions of use,
- the storage conditions to guarantee their stability,
- the possible presence of potentially allergenic residues,
- where applicable, the indication that the oenological tannins were obtained from genetically-modified plants.

3. PERMITTED OENOLOGICAL TANNINS

The oenological tannins that are described in Chapter 1, that have demonstrable and measurable properties as well as a technological interest duly proven in practice, and that completely fulfil the conditions and criteria mentioned below, are permitted in compliance with the files of the Code of Oenological Practices.
The oenological tannins used should not:

- liberate substances in concentrations that could lead to potential health risks,
- cause fraud resulting from the addition of aromas or coloring,
- be harmful to the quality of the products made,
- result in the modification of the well-known organoleptic profile of wine (flavouring).

4. REACTIVITY PROPERTIES AND FUNCTIONS OF OENOLOGICAL TANNINS

4.1 Reactivity

Tannins are likely to be involved in numerous reaction pathways in wine. The reactivity of oenological tannins is directly related to the specific characteristics of their chemical structures; it also depends on the technical operations of production (techniques of extraction, concentration, fractionation, etc.), which influence the polyphenol content (thus the degree of purity) and the proportion of free

functional groups. The nature of the plant determines the tannin class(es) or sub-class(es) to which they belong.

4.2 Properties and functions of tannins

The functions of oenological tannins are directly linked to their properties. The methods of analysis used to determine the functions of tannins should correspond to the state of the art and, if possible, be validated according to the appropriate international standards.
The following table lists properties as well as oenological applications, some of which are recognised and some of which remain to be demonstrated.

Theoretical properties	**Possible oenological applications**
Reactivity with proteins	Clarification aid
Reactivity with oxygen	Antioxidant
Iron chelation	Reduction of iron content
Polymerisation	Colour stabilisation
Formation of complexes	Colour stabilisation
Anti-l~~l~~accase activity	Inhibition of laccase-activity
Microbiological action	
Antibacterial effect	Microbiological stability, reduction in SO_2 use

The properties and resulting oenological applications will be the object of specific monographs by classes of tannins and/or sub-classes, in which the methods of measurement shall be specified.

4.3 Estimation of the total polyphenol content

The estimation of the total polyphenol content of preparations of oenological tannins is measured by the method described in Annex 1.
The total polyphenol content should be greater than or equal to 65% and a maximum concentration is not required~~.~~

5. PHYSICAL PROPERTIES

5.1 Insoluble materials

Place 5 g tannin in a solution of 100 mL double-distilled water at room temperature and stir for 15 minutes. After that filter this solution over a 0.8 µm membrane previously weighed. Evaporate and dry the membrane at 100-105 °C. Weight the membrane. The insoluble matter content should not be greater than 5% w/w.
The procedure described later in the total polyphenol content method (Annex 1, point 4.3) could be used as an alternative.

5.2 Loss on drying

Determined up to constant weight, for a 2 g sample, the weight loss in an oven at 100-105 °C for 2 hours should be less than 10%.

6. MAXIMUM CONTAMINANTS LEVELS

Oenological tannins should be produced in accordance with good manufacturing practices. According to the origin of the plant used, some maximum contaminant levels – such as those for heavy metals – may be different (e.g. the iron content of chestnut-derived tannins).

All of the limits set below relate to dry products.

6.1 Pentachlorophenol

Proceed with determination according to the method described in the OIV Compendium of the International methods of musts and wines. Content should be less than 1 µg/kg.

6.2 Total polycyclic aromatic hydrocarbons (PAH)

Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoroanthene and chrysene
Proceed with determination according to the method described in Chapter II of the International Oenological Codex. Content should be less than 30 µg/kg.

6.3 Benzo(a)pyrene

Proceed with determination according to the method described in Chapter II of the International Oenological Codex. Content should be less than 5 µg/kg.

6.4 Ashes

Without going above 550 °C, progressively incinerate the residue left by the determination of the loss on drying. The weight of the ashes should be less than 5%. A Higher concentration could mean that the extraction was done with non-authorized solvents.

6.5 Preparation of the test solution

Dissolve the ashes obtained from 2 g tannin into 1 mL diluted hydrochloric acid (R) and a drop of concentrated nitric acid (R). Heat in a water bath at 100 °C for a short time to ensure dissolution is complete. Transfer to a 50-mL calibrated flask, rinsing the capsule with distilled water, and make up to the mark. The test solution is ready to dose following elements.

6.6 Arsenic

For 0.25 g tannin, detect arsenic using the atomic-absorption-spectrophotometry method described in Chapter II of the International Oenological Codex or using ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis, after destruction of the organic matter using the wet method. The arsenic content should be less than 3 mg/kg.

6.7 Iron

On the basis of 10 mL of test solution prepared according to paragraph 6.5, determine the iron using atomic-absorption-spectrometry according to the method in Chapter II of the International Oenological Codex or using the ICP/MS method described in the Compendium of International Methods of Wine and Must Analysis.
The iron content should be less than 50 mg/kg, with the exception of chestnut-derived tannins, whose iron content should be less than or equal to 200 mg/kg .

6.8 Copper

Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis. Content should be less than 5 mg/kg.

6.9 Lead
Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis. Content should be less than 5 mg/kg.

6.10 Mercury
Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis. Content should be less than 0.5 mg/kg.

6.11 Cadmium
Proceed with determination according to the method described in Chapter II of the International Oenological Codex or by ICP/MS according to the method described in the Compendium of International Methods of Wine and Must Analysis. Content should be less than 0.5 mg/kg.

6.12 Salmonella
Proceed with counting according to the method described in Chapter II of the International Oenological Codex. Absence should be checked on a 25 g sample of dry matter.

6.13 Total coliforms
Proceed with counting according to the method described in Chapter II of the International Oenological Codex. Content should be less than 30 CFU/g of dry matter.

6.14 Escherichia coli
Proceed with counting according to the method described in Chapter II of the International Oenological Codex. Absence should be checked on a 25 g sample of dry matter.

6.15 Moulds
Proceed with counting according to the method described in Chapter II of the International Oenological Codex. Content should be less than 100 CFU/g of dry matter.

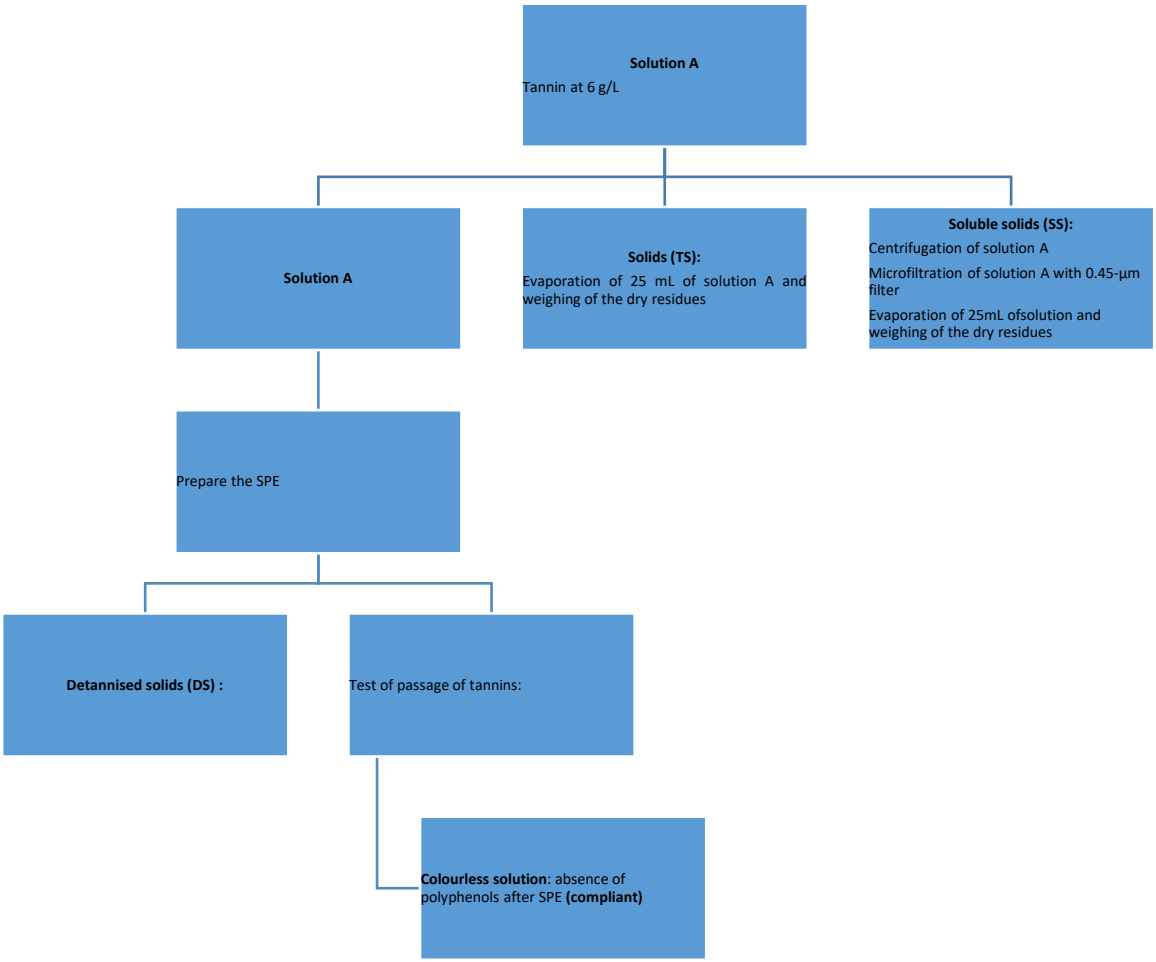
(1) Encyclopeadia health food Pub off 00683

Annex 1

METHOD FOR THE ESTIMATION OF THE TOTAL POLYPHENOL CONTENT

1. PRINCIPLE

This method is designed to measure the concentration in polyphenols of preparations of oenological tannins and is based on gravimetric analysis using solid-phase extraction (SPE). In an SPE column, tannins in aqueous solution are adsorbed onto a polymer (in this case polyvinylpolypyrrolidone) capable of retaining polyphenols. The substances not retained by the PVPP correspond to non-phenolic compounds that were present in the original sample.
The full diagram of the method is presented below:


Solution A
Tannin at 6 g/L
Solution A
Solids (TS):
Evaporation of 25 mL of solution A and weighing of the dry residues
Soluble solids (SS):
Centrifugation of solution A
Microfiltration of solution A with 0.45-µm filter
Evaporation of 25mL ofsolution and weighing of the dry residues
Prepare the SPE
Detannised solids (DS) :
Test of passage of tannins:
Colourless solution: absence of polyphenols after SPE (compliant)

2. REAGENTS, MATERIALS AND APPARATUS

2.1 Reagents

2.1.1 PVPP (approx. 100 µm polyvinylpolypyrrolidone, CAS No. 9003-39-8)
2.1.2 Aqueous solution of $FeCl_3$ (1 g/L)
2.1.3 Double-distilled water
2.1.4 Ethanol (20 % v/v)

2.2 Materials

2.2.1 Aluminium dishes (70 mL)
2.2.2 Disposable tubes with a conical base and stoppers (50 mL)
2.2.3 SPE columns (70 mL reservoir, 150 x 29,75 mm)
2.2.4. Frits for SPE column (diameter 27 mm, 20 µm PE)
2.2.5 1 000-mL Pyrex flask
2.2.6 50-mL Class-A test tubes
2.2.7 0.45 µm Cellulose acetate membrane filter, Ø 47 mm
2.2.8 Plastic 50-mL syringe
2.2.9 25-mL Class-A graduated glass pipettes (2 marks)

2.3 Apparatus

2.3.1 Thermostatic bath at 20 °C
2.3.2 Technical balance with precision of 0.01 g
2.3.3 Analytical balance with precision of 0.1 mg
2.3.4 Thermostatic oven at 105 °C
2.3.5 Thermostatic oven at 80 °C or, failing this, a thermostatic bath
2.3.6 Centrifuge
2.3.7 Vacuum manifold
2.3.8 Class-A volumetric glassware
2.3.9 Desiccator

3. PREPARATION OF SAMPLES

The solution (referred to below as solution A) is used to measure the total solids (TS), soluble solids (SS) and detannised solids (DS).
Weigh approximately 6 g tannins on the analytical balance and make a note of the weight. Dissolve the tannins in around 950 mL hot double-distilled water (60-70 °C) in a 1-L borosilicate-glass flask and mix well. Leave the flask to rest at room temperature for 30 minutes. Cool the solution at 20-22 °C in a thermostatic bath, make up to volume with double-distilled water and mix well.

4. PROCEDURE

4.1 Measurement of total solids (TS)

- Take a sample and transfer 25 mL of solution A to an aluminium dish (2.2.1),
- evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residue (cool the dish in the desiccator before weighing).

The formula to be applied for the calculation of the total solids (TS) is as follows:

$$\%\mathrm{TS} = \frac{\mathrm{TS_dry_residues\ (g)}}{\mathrm{tannins_weight\ (g)}} \cdot \frac{1\,000}{\mathrm{solA\ (mL)}} \cdot 100$$

4.2 Measurement of soluble solids (SS)

- Centrifuge solution A at -10 000 g during 5 minutes, ~~3 000 g,~~
- microfilter solution A centrifuged through a membrane filter in order to obtain a clear solution, then leave 25 mL of solution to evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residue (cool the dish in the desiccator before weighing).

The formula to be applied for the calculation of soluble solids (SS) is as follows:

$$\%\mathrm{SS} = \frac{\mathrm{SS_dry_residues\ (g)}}{\mathrm{tannins_weight\ (g)}} \cdot \frac{1\,000}{\mathrm{solA\ (mL)}} \cdot 100$$

4.3 Measurement of insoluble solids (IS)

Calculate the difference between the total solids and soluble solids:

$$\%\mathrm{IS} = \%\mathrm{TS} - \%\mathrm{SS}$$

4.4 Measurement of detannised solids (DS)

- Prepare the SPE columns: introduce the first frit, 7.0 g ± 0.1 g PVPP rehydrated beforehand with a hydro-alcoholic solution at 20% for 15 minutes, as well as the second frit, then pack the stationary phase in well,
- place the SPE column in a vacuum manifold (example in Figure 1),
- pack the column with three rinses (do not dry out the PVPP and apply a vacuum of approx. 0.2 bar in order to avoid compacting of the polymer): a first rinse with 50 mL ethanol (20 % v/v), a second rinse with 50 mL double-distilled water, and a third rinse with 20 mL solution A in order to eliminate the water residues of the PVPP,
- add 30 mL of solution A to the column and collect 30 mL eluate (DS, detannised solids) in a 50-mL Falcon tube with a conical base, interrupting the elution when the liquid reaches the level of the upper frit,
- take a sample of 25 mL eluate and transfer this into an aluminium dish,
- leave to evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residue (cool the dish in the desiccator before weighing).

The formula to be applied for the calculation of detannised solids (DS) is as follows:

$$\%\mathrm{DS} = \frac{\mathrm{DS_dry_residues\ (g)} - \mathrm{BC\ (g)}}{\mathrm{tannins_weight\ (g)}} \cdot \frac{1\,000}{\mathrm{solA\ (mL)}} \cdot 100$$

where BC is the value of the blank measured after SPE (see 4.5).

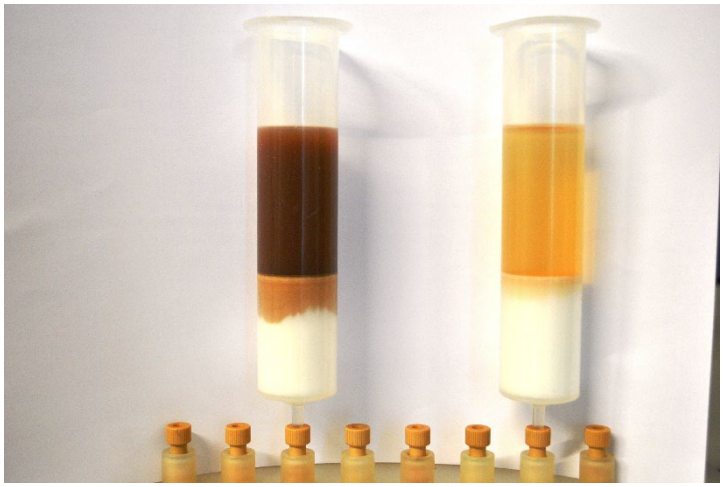


Figure 1 - Example SPE extraction

In order to guarantee the absence of polyphenols in the eluate after passing through the column, add 3 drops of an aqueous solution of $FeCl_3$ to 3 mL detannised solids (DS) in solution. If the solution develops a blue-black hue, this means that the polyphenols have passed through the polymer; the analysis should then be repeated by reducing the quantity of initial product. If the solution remains colourless after this treatment, proceed with gravimetric analysis.

4.5 Measurement of the blank (BC)

A blank test should be carried out before conducting the SPE elution, in order to evaluate any interference caused by the analytical process. Proceed as follows:

- prepare the SPE columns: introduce the first frit, 7.0 g ± 0.1 g PVPP rehydrated beforehand with a hydro-alcoholic solution at 20% for 15 minutes, as well as the second frit, then pack in well,
- place the SPE column in a vacuum manifold (example in Figure 1),
- pack the column with two rinses (do not dry out the PVPP and apply a vacuum of approx. 0.2 bar in order to avoid compacting of the polymer): a first rinse with 50 mL ethanol (20 % v/v) and a second rinse with 70 mL double-distilled water,
- add 30 mL double-distilled water to the column and collect 30 mL eluate (blank for detannised solids) in a 50-mL Falcon tube with a conical base, interrupting the elution when the liquid reaches the level of the upper frit,
- take a sample of 25 mL eluate and transfer this into an aluminium dish, then leave to evaporate to dryness in a thermostatic oven at 80 °C,
- transfer to a thermostatic oven at 105 °C in order to proceed with drying until a constant weight is obtained, then weigh the residues (cool the dishes in the desiccator before weighing).

5. EXPRESSION OF RESULTS

Measurement of the percentage of total polyphenols (%polyphenols):

The formula to be applied for the calculation of the percentage is as follows:

$$\%\text{polyphenols} = \frac{\%\text{SS} - \%\text{DS}}{\%\text{TS}} \cdot 100$$

- Determination of the suitability of the PVPP: refer to OENO 11-2002 - COEI-1-PVPP: 2007, § 6.

STEP	1	2	3	4	5	6	7	8
DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SPECIF 20-674 Et8~~7~~

IMPORTANT: This resolution modifies the following monograph and resolution:
E-COEI-1-MANPRO
OENO 26/2004

UPDATE TO THE MONOGRAPH ON YEAST EXTRACTS CONTAINING MANNOPROTEINS (E-COEI-1-MANPRO)

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

DECIDES, at the proposal of Commission II "Oenology", to update Resolution OENO 26-2004 and, accordingly, Monograph E-COEI-1-MANPRO in Chapter I of the *International Oenological Codex* as follows:

UPDATE TO THE MONOGRAPH ON YEAST EXTRACTS CONTAINING MANNOPROTEINS (E-COEI-1-MANPRO)

The title of the monograph is changed to: "Yeast extracts containing mannoproteins".

The following sentence is to be added to point 3.2:

Their water solubility is determined by comparing the total dry matter (DM) with the insoluble DM that remains after a hot wash, according to the method described in Annex 2. The percentage of insoluble DM should be less than or equal to 1% ± 0.5.

Point 3.3. is to be deleted.

Point 5 is modified as follows (italic part added):

Solid mannoproteins ~~have a two years shelf life~~ *must be* stored away from humidity in a sealed pack in a temperate room.

The following Annex 2 is to be added to the monograph (Annex 2):

Annex 2

DETERMINATION OF THE PERCENTAGE OF INSOLUBLE DRY MATTER

1 – PRINCIPLE

The analysis consists of comparing the total dry matter (DM) of the yeast mannoprotein preparation with the insoluble DM that remains after a hot wash.

2 – MATERIAL AND REAGENTS

2-1 4200 rpm (6000g) Centrifuge and accessories
2-2 Scales with 0.1 mg precision
2-3 Oven at 105 °C +/- 1 °C

3 – PROCEDURE

Obtaining the insoluble part of the yeast mannoprotein preparation
- Place approx. 10 g of yeast mannoprotein preparation, previously placed in an oven at 105 °C until constant weight, in a tared centrifuge bucket. Note the exact weight, which will be referred to as M1.
- Suspend in hot water (70-80 °C).
- Stir.
- Centrifuge for 10 min at 4200 rpm (6000g).
- Discard the supernatant, stir again into hot water and centrifuge for 10 min at 4200 rpm.
- Carry out the procedure a third time.
- Place the tared centrifuge bucket containing the centrifugation pellet in an oven at 105 °C until constant weight. Note the weight. M2 is the weight of the residue of yeast mannoproteins preparation that make up the insoluble DM.

4 – CALCULATIONS

Insoluble dry matter percentage (Insoluble DM %) = (M2/M1) x 100

STEP	1	2	3	4	5	6	7	8
DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SPECIF 20-675A Et8

SPECIFIC MONOGRAPHS FOR PROCYANIDINS/PRODELPHINIDINS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

CONSIDERING the draft resolution OENO-SPECIF 17-624 “Update to the monograph on oenological tannins” which describes a general monograph,

CONSIDERING the need to set up specific monographs for each family of tannins,

DECIDES, at the proposal of Commission II "Oenology", to add the monograph COEI-1-PROCYA to the *International Oenological Codex*:

OENOLOGICAL TANNINS

Specific monograph for oenological tannins containing procyanidins/prodelphinidins

Procyanidins/prodelphinidins are a sub-class of condensed (or proanthocyanic) tannins. Tannins from *Vitis vinifera* grapes, grape skins and grape seeds are included in this sub-class.

1. Method for the determination of sub-class affiliations
Characterisation by high-performance liquid chromatography (HPLC)

1.1. Principle

This method is designed to verify the presence of characteristic components of condensed tannins of the procyanidin/prodelphinidin sub-class and measure their total concentration. It is applicable to preparations of oenological tannins that are declared as pure and thus as not containing tannins with small masses from different families or sub-classes.

1.1.2. Reagents, material and apparatus

1.1.2.1. Reagents

(+)-catechin, CAS No.: 154-23-4
Ultrafiltered water (resistivity: 18.3 MΩ·cm)
Water (HPLC quality)
Methanol (HPLC quality)
Formic acid (HPLC quality)

1.1.2.2. Materials

100-mL borosilicate-glass flask
Cellulose filters with 0.45 µm pore size diameter

Plastic 1-mL syringe

1.1.2.3. Apparatus

Analytical balance with precision of 0.01 g
Analytical balance with precision of 0.1 mg
Class-A volumetric glassware
Mass chromatographic system with spectrometry detection composed of:

- Gradient pump for binary or quaternary mix
- Injector with a loop of 10 µL
- Spectrophotometric detector at 280 nm fixe wavelength
- Column Eclipse Plus C-18 (for example): 2.1 x 100 mm, 1.8 µm particle size
- ESI-SIM (Selected Ion Monitoring mode via Electro Spray Ionisation) ionisation source
- Mass spectrometer detector: quadrupole time of flight (Q-TOF)

1.1.3. Preparation of samples and standards

Samples: weigh approximately 0.5 g of oenological tannins on the analytical balance and make a note of the weight. Dissolve the oenological tannins in 100 mL of ultrafiltered water in a 100-mL borosilicate-glass flask and mix well.

Preparation of standard solutions: put 10 mg of (+)-catechin in solution into 50 mL of ultrafiltered water, corresponding to a 200 mg/L concentration. Then carry out dilutions in ultrafiltered water to obtain 5, 10, 20, 40, 60, 80 and 100 mg/L concentrations.

Solvent A: HPLC-quality water containing 0.1% of formic acid.

Solvent B: methanol containing 0.1% of formic acid.

1.1.4. Procedure

The sample solution and standard solutions are filtered on 0.45 µm (pore size diameter) filters and analyse by chromatography under the following conditions:

Injected volume: 10 µL of sample solution or standard solution of (+)-catechin
Detection at 280 nm
Composition of elution gradient: (time, % of solvent A)
0 min, 99.0%; 0.5 min, 94.0%; 20 min, 50.0%; 25 min, 0.0%; 32 min, 94.0% and 10 min for equilibrium.
Flow rate: 0.3 mL/min

Quantification and detection of the characteristic components of condensed tannins of the procyanidin/prodelphinidin sub-group (sub-class) ((+)-catechin, (-)-epicatechin, (+)gallocatechin, epigallocatechin, (-)-epicatechin-3-*O*-gallate, (-)-epigallocatechin-3-*O*-gallate, dimers B1, B2, B3 and B4, B2-3-*O*-gallate, trimers) according to the ESI-SIM scan and Q-ToF detection, for example.

Table 1: Example chemical formulas and masses of the different procyanidins/prodelphinidins

Compound	Chemical formulas	m/z
(+)-catechin	$C_{15}H_{14}O_6$	290.1
(-)-epicatechin	$C_{15}H_{14}O_6$	290.1

(-)-epicatechin-3-O-gallate	$C_{22}H_{18}O_{10}$	442.1
Dimer B1	$C_{50}H_{26}O_{12}$	578.1
Dimer B2	$C_{50}H_{26}O_{12}$	578.1
Dimer B3	$C_{50}H_{26}O_{12}$	578.1
Dimer B4	$C_{50}H_{26}O_{12}$	578.1
B2-3-O-gallate	$C_{37}H_{30}O_{16}$	730.1
Trimers	$C_{45}H_{38}O_{18}$	866.2

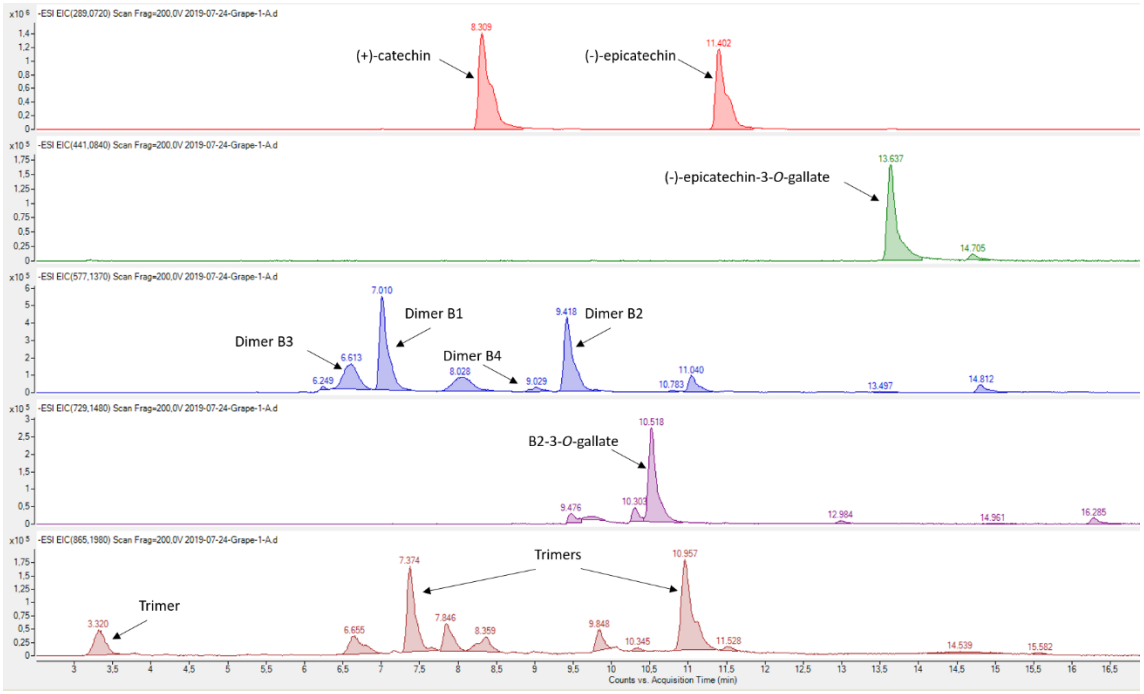


Figure 1: Example of ESI-SIM scan of procyanidins/prodelphinidins

1.2. Conclusion

An oenological tannin is recognised as a procyanidin/prodelphinidin when:

- **its total polyphenol content is higher than 65% (gravimetric method in Annex 1 of the general monograph 17-624),**
- **its procyanidin/prodelphinidin content as characterised by the HPLC method is higher than 120 mg equivalent of (+)-catechin per gram of oenological tannins.**

2. Methods of measurement of properties and functionalities

The following compliance methods and criteria are only applicable when the property/functionality is claimed on the preparation of tannins.

2.1. Antioxidant ability

2.1.1. Principle

Determination of procyanidins/prodelphinidins' ability to contribute to the protection of must and wine from oxidation.

2.1.2. Products

2.1.2.1. Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl): MM = 394.32
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid): MM = 250.29
Methanol at 99.9% volume
96-well microplate reader (FLUOstar Omega - BMG Labtech, for example)

2.1.2.2. Direct oxygen consumption (OCR)

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Iron (III) chloride hexahydrate: MM = 270.30, CAS No. 7705-08-0
Copper (II) sulfate pentahydrate: MM = 249.68, CAS No. 7758-98-7
Clear glass bottles with inserted pills of 0.75-cL capacity
NomaSens oximeter, for example

2.1.3. Protocols

2.1.3.1. Antioxidant capacity (DPPH assay)

0.15 g/L oenological tannin solution: dissolve 37.5 mg of oenological tannins in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5). Dilution of oenological tannins solution could be needed if the measurement absorbance is higher than 1 unit (in this case the dilution should be included in the calculation).

1mM Trolox solution: dissolve 125 mg of Trolox in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Calibration curve: dissolve in 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mL of 1mM Trolox solution into 0, 0.2, 0.4, 0.6, 0.8 and 0.9 mL of model wine solution. These quantities correspond to 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mM final concentration of Trolox respectively.

6.10^{-5} M DPPH solution: dissolve 2.36 mg of DPPH in 100 mL of methanol. The solution should be freshly prepared.

2.1.3.2. Direct oxygen consumption (OCR)

1 g/L oenological tannin solution: dissolve 0.75 g of oenological tannins in 750 mL of model wine solution.

Model wine solution: dissolve 4 g of tartaric acid, 2.25 mg of Iron (III) chloride hexahydrate and 0.225 mg of Copper (II) sulfate pentahydrate in 90 mL of ethanol and 660 mL of distilled water. The pH should be adjusted at 3.5.

2.1.4. Tests

2.1.4.1. Antioxidant capacity

First a blank containing solely the DPPH reagent (RB) is measured at 515 nm by placing 190 µL of DPPH solution (1.3.1) in all the wells of the plate. Then, add 10 µL of oenological tannin solution (samples), distilled water (blank) or Trolox curve solution (standards) into the wells and measure (MS) at 515 nm after 30 min.

See Figure 2 for an example of how to fill the plate.

The formula to be applied for the calculation of the antioxidant capacity is as follows:

1) $RB - MS = x$
2) $antioxidant\ capacity\ (mg\ eq.Trolox\ per\ g\ of\ tannins) = \frac{250.29\ (mg)}{0.15\ (g)}\ x\ \frac{x-b}{a}$

where "a" and "b" correspond respectively to the slope and the constant of the Trolox calibration curve: Absorbance = f ([Trolox]) → Absorbance = ax + b

In all cases, procyanidins/prodelphinidins should demonstrate antioxidant capacity, and more specifically they should have more than 500 ± 50 mg equivalent Trolox per gram of tannins (commercial extract).

	1	2	3	4	5	6	7	8	9	10	11	12
A	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
B	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
C	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
D	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
E	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
F	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
G	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
H	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11

T = Trolox OT = Oenological Tannins

Figure 2: example 96-well plate

2.1.4.2. Direct oxygen consumption (OCR)

First the model wine solution is saturated with oxygen at 8 mg/L by bubbling with air for 10 min at 20-25 °C. Then, add the oenological tannins to the model wine solution in the bottles filled to 0.75 cL. Seal the bottles hermetically and shake to fully homogenise.

1. Measure the oxygen consumed every two days starting 1h after the filled of the bottles.
2. To determine the oxygen consumption rate, follow the pathway as shown in **Figure 2**:
    - represent the oxygen consumption versus the time,
    - then represent the inverse of the oxygen consumed versus the inverse of the time,
    - the oxygen consumption rate corresponds to the inverse of the slope coefficient:
    - OCR t_0 mg of O_2 consumed per day and per g of tannins = 1/A, A being the slope coefficient

In all cases, procyanidins/prodelphinidins should demonstrate an ability to consume the oxygen directly, and more specifically they should be able to consume at least 0.10 ± 0.05 mg of O_2 per litre, per day and per gram of tannins (commercial extract).

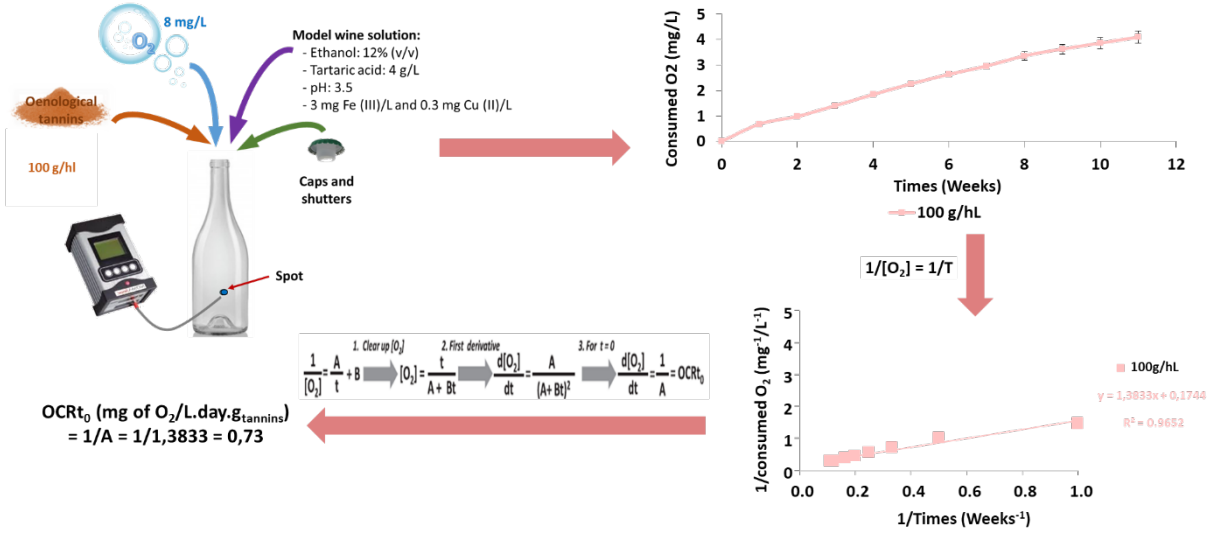


Figure 3: Pathway to determine oxygen consumption rate

2.1.4.3. Antioxidasic ability

2.1.4.3.1. Principle

Determination of procyanidins/prodelphinidins' antioxidasic ability to contribute to antioxidasic protection in terms of the laccase activity of compounds in must and wine.

2.1.4.3.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Sodium acetate: MM = 82.03, CAS No. 6131-90-4
Syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine): MM = 360.36, CAS No. 14414-32-5
Polyvinylpolypyrrolidone (PVPP): CAS No. 25249-54-1
Must botrytised with laccase activity
Distilled water (HPLC quality)

2.1.4.3.3. Protocols

2 g/L oenological tannin solution: dissolve 200 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Buffer solution (8.2 g/L): dissolve 410 mg of sodium acetate in 50 mL of distilled water.

Syringaldazine solution (0.06 g/L): dissolve 30 mg of syringaldazine in 500 mL of ethanol.

2.1.4.4. Tests

1) Add 4 mL of botrytised must to 1 mL of oenological tannin solution in one tube, which will correspond to sample modality.
2) Add 4 mL of botrytised must to 1 mL of model wine solution in one tube, which will correspond to control modality.
3) After 4 minutes (precisely), add 0.8 g of PVPP to both tubes (sample and control modalities), stir and centrifuge for 10 minutes at 8,500 rpm.
4) Recover 1 mL of the supernatant (for both sample and control modalities), into 1.4 mL of buffer solution and 0.6 mL of syringaldazine solution. Put the mixture into a plastic spectrophotometer cuvette (10 mm path length).

5) Measure the absorbance at 530 nm every minute for 5 minutes (including time measurements at 0 minutes).
6) Then determine the laccase activity and the residual laccase activity by using the following equations and **Figure 3**:

$Laccase\ activity$ = 46.15 $x\ \Delta A$ µ$mol.\ L^{-1}.\ min^{-1}$ = 46.15 $x\ \Delta A\ UL$

$\%\ of\ residual\ activity$ = ($laccase\ activity_{sample}$ / $laccase\ activity_{control}$) x 100

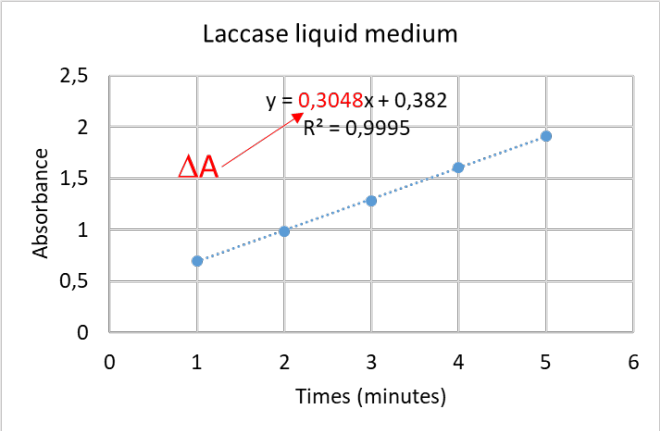


Figure 4: Example determination of ΔA.

In all cases, procyanidins/prodelphinidins should demonstrate an antioxidasic ability, and more specifically they should be able to reduce the residual laccase activity by at least 50%. This value is valuable for must and wine containing less than 5 UL (units of laccases).

2.1.5. Colour stabilisation

2.1.5.1. Principle

Determination of procyanidin/prodelphinidin colour stabilisation properties to promote the expression, stabilisation and preservation of colour in red must and wine.

2.1.5.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Malvidin-3-*O*-glucoside: MM = 528.87, CAS No. 18470-06-9

2.1.5.3. Protocols

0.8 g/L oenological tannin solution: dissolve 80 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

0.1 g/L malvidin-3-*O*-glucoside solution: dissolve 10 mg of malvidin-3-*O*-glucoside in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

2.1.5.4. Tests

1) Place 0.75 mL of oenological tannin solution and 0.75 mL of model wine solution in one 2-mL stoppered conical tube – hereinafter a “tube” – and keep it in the dark at room temperature. This tube will be called “T_0”.
2) Place 0.75 mL of malvidin-3-*O*-glucoside solution and 0.75 mL of model wine solution in one tube and keep it in the dark at room temperature. This tube will be called “M”.

3) Place 0.75 mL of oenological tannin solution and 0.75 mL of malvidin solution in one tube and keep it in the dark at room temperature. This tube will be called "T_M".
4) After 7 days, measure the absorbance at 450, 520, 570 and 630 nm of the three tubes (T_M, T_0 and M).
5) Subtract the absorbance values of T_0 to T_M to obtain the absorbance, taking into account the absorbances of the oenological tannins used.
   $A(T_M) – A(T_0) = A(T)$
6) Then, determine the CIELAB coordinates (L*, a* and b*) corresponding to the tannin solution with malvidin-3-*O*-glucoside (T) and malvidin-3-*O*-glucoside solution (M) with the free MSCV software (https://www.unirioja.es/color/descargas.shtml) or equivalent.

   The formulas to be applied for the calculation of the copigmentation index are as follows:

$$1)\ \Delta E_{ab}.TS = \sqrt{(L^*_T - L^*_W) + (a^*_T - a^*_W) + (b^*_T - b^*_W)}$$

$$2)\ \Delta E_{ab}.CS = \sqrt{(L^*_M - L^*_W) + (a^*_M - a^*_W) + (b^*_M - b^*_W)}$$

$$3)\ \text{Copigmentation Index (\%)} = 100 \times \frac{\Delta E_{ab}.TS - \Delta E_{ab}.CS}{\Delta E_{ab}.CS}$$

   ΔE_{ab}.TS: total colour difference between the solution of malvidin-3-*O*-glucoside containing commercial tannins (T) and a pure white colour solution (W).

   ΔE_{ab}.CS: total colour difference between the solution of malvidin-3-*O*-glucoside (M) and a pure white colour solution (W).

   The CIELAB coordinates of a pure white colour solution are L* = 100.00, a* = 0.00 and b* = 0.00.

   In all cases, procyanidins/prodelphinidins should demonstrate an ability to stabilise the colour, and more specifically their copigmentation index should read as higher than 7.0 ± 2.0% after 7 days.

Note: Alternative methods of determination can be used in place of any of the methods described, on the condition that these have been internally validated.

3. Bibliography

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STEP	1	2	3	4	5	6	7	8
DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SPECIF 20-675B Et8

SPECIFIC MONOGRAPHS FOR ELLAGITANNINS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

CONSIDERING the draft resolution OENO-SPECIF 17-624 “Update to the monograph on oenological tannins” which describes a general monograph,

CONSIDERING the need to set up specific monographs for each family of tannins,

DECIDES, at the proposal of Commission II "Oenology", to add the monograph COEI-1-ELLAGI to the *International Oenological Codex*:

OENOLOGICAL TANNINS

Specific monograph for oenological tannins containing ellagitannins

Ellagitannins are a sub-class of hydrolysable tannins. Chestnut and oak tree tannins are included in this sub-class.

1. Method for the determination of sub-class affiliations

1.1. Characterisation by high-performance liquid chromatography (HPLC)

1.1.1. Principle

This method is designed to verify the presence of ellagitannins in oenological tannins and measure their total concentration.

1.1.2. Reagents, materials and apparatus

1.1.2.1. Reagents

Vescalagin (purity > 96%), CAS No. 36001-47-5
Ultrafiltered water (resistivity: 18.3 MΩ·cm)
Water (HPLC quality)
Methanol (HPLC quality)
Formic acid (HPLC quality)

1.1.2.2. Materials

100-mL borosilicate-glass flask
Cellulose filters with 0.45 µm pore size diameter
Plastic 1-mL syringe

1.1.2.3. Apparatus

Technical balance with precision of 0.01 g

Analytical balance with precision of 0.1 mg

Class-A volumetric glassware

Mass chromatographic system with spectrometry detection composed of:

- Gradient pump for binary or quaternary mix
- Injector with a loop of 10 µL
- Spectrophotometric detector at 280 nm fixe wavelength
- Column Phenomenex Kinetex (for example): 150 x 3.0 mm, 2.6 µm particle size
- ESI-SIM (Single Ion Monitoring mode via Electro Spray Ionisation) ionisation source
- Mass spectrometer detector: triple quadrupole time of flight (Q-Tof)

1.1.3. Preparation of samples and standards

Samples: weigh approximately 0.5 g of oenological tannin on the analytical balance and make a note of the weight. Dissolve the oenological tannin in 100 mL of ultrafiltered water in a 100-mL borosilicate-glass flask and mix well.

Preparation of standard solutions: put 10 mg of vescalagin in solution into 50 mL of ultrafiltered water, corresponding to a 200 mg/L concentration. Then carry out dilutions in ultrafiltered water to obtain 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 40, 50 and 100 mg/L concentrations for the calibration curve.

Solvent A: water (HPLC quality) containing 0.1% of formic acid (HPLC quality).

Solvent B: methanol containing 0.1% of formic acid (HPLC quality).

1.1.4. Procedure

Oenological tannin solution and standard solutions are filtered on 0.45 µm (pore size diameter) filters and analyse by chromatography under the following conditions given by way of example:

Injected volume: 10 µL of oenological tannin solution or standard solution of vescalagin

Detection at 280 nm

Composition of elution gradient: (time, % of solvent A)

0min, 99.0%; 2 min, 98.0%; 5 min, 97.0%; 6 min, 96.5%; 7 min, 96.0%; 8 min, 95.5%; 10 min, 95.0%; 14 min, 90.0%; 17 min, 85.0%; 23 min, 80%; 35 min, 1.0% and 10 min for equilibrium.

Flow rate: 0.4 mL/min

Quantification and detection of the eight principal ellagitannins (vescalagin, castalagin, roburin A, B, C, D, E and grandinin) according to DAD (UV 280 nm) or the ESI-SIM scan with Q-ToF detection, which allows for more precise detection and quantification.

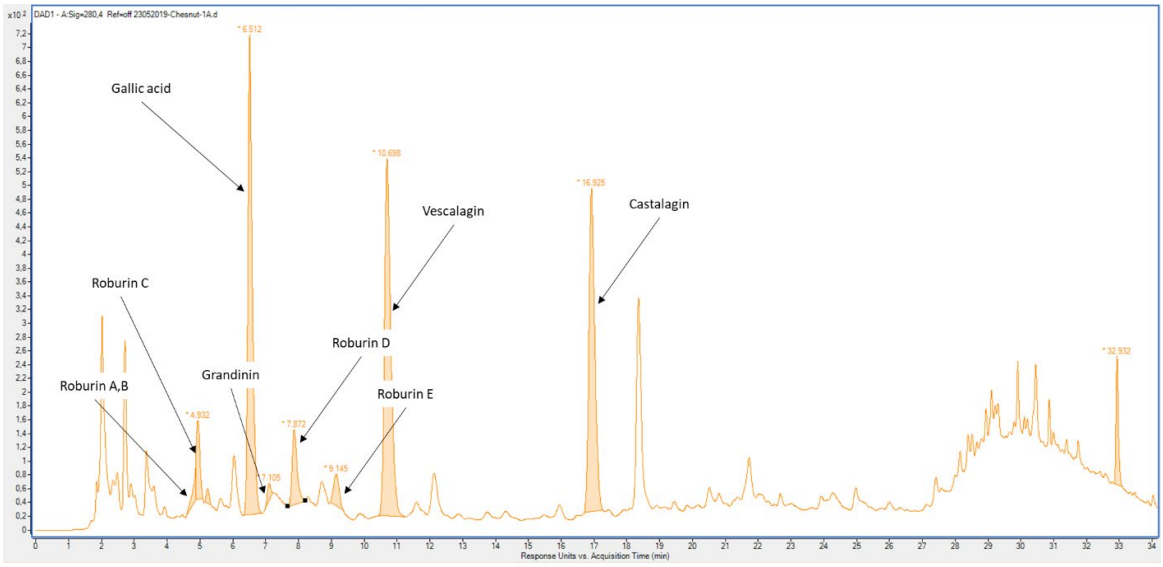

Figure 1: Example ellagitannin chromatogram at 280 nm

1.2. Conclusion

An oenological tannin is recognised as an ellagitannin (or ellagic tannin) when:

- **its total polyphenol content is higher than 65% (gravimetric method in Annex 1 of the general monograph 17-624),**
- **its ellagitannin content is higher than 200 mg equivalent gallic acid per gram of oenological tannins (characterised by the HPLC method).**

2. Methods of measurement of properties and functionalities

The following compliance methods and criteria are only applicable when the property/functionality is claimed on the preparation of tannins.

2.1. Antioxidant ability

2.1.1. Principle

Determination of ellagitannins' antioxidant ability to contribute to the protection of must and wine from oxidation.

2.1.2. Products

2.1.2.1. Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl): MM = 394.32
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid): MM = 250.29
Methanol at 99.9% volume
96-well microplate reader (FLUOstar Omega - BMG Labtech, for example)

2.1.2.2. Direct oxygen consumption (OCR)

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Iron (III) chloride hexahydrate: MM = 270.30, CAS No. 7705-08-0
Copper (II) sulfate pentahydrate: MM = 249.68, CAS No. 7758-98-7
Clear glass bottles with inserted pills of 0.75-L capacity
NomaSens oximeter (for example)

2.1.3. Protocols

2.1.3.1. Antioxidant capacity (DPPH assay)

0.15 g/L oenological tannin solution: dissolve 37.5 mg of oenological tannins in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5). Dilution of oenological tannins solution could be needed if the measurement absorbance is higher than 1 unit (in this case the dilution should be included in the calculation).

1mM Trolox solution: dissolve 125 mg of Trolox in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Calibration curve: dissolve in 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mL of 1mM Trolox solution into 0, 0.2, 0.4, 0.6, 0.8 and 0.9 mL of model wine solution. These quantities correspond to 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mM final concentration of Trolox respectively.

6.10^{-5} M DPPH solution: dissolve 2.36 mg of DPPH in 100 mL of methanol. The solution should be freshly prepared.

2.1.3.2. Direct oxygen consumption (OCR)

1 g/L oenological tannin solution: dissolve 0.75 g of oenological tannins in 750 mL of model wine solution.

Model wine solution: dissolve 4 g of tartaric acid, 2.25 mg of Iron (III) chloride hexahydrate and 0.225 mg of Copper (II) sulfate pentahydrate in 90 mL of ethanol and 660 mL of distilled water. The pH should be adjusted at 3.5.

2.1.4. Tests

2.1.4.1. Antioxidant capacity

First a blank containing solely the DPPH reagent (RB) is measured at 515 nm by placing 190 µL of DPPH solution (1.3.1) in all the wells of the plate. Then, add 10 µL of oenological tannin solution (samples), distilled water (blank) or Trolox curve solution (standards) into the wells and measure (MS) at 515 nm after 30 min.

See Figure 2 for an example of how to fill the plate.

The formula to be applied for the calculation of the antioxidant capacity is as follows:

1) $RB - MS = x$
2) $antioxidant\ capacity\ (mg\ eq.Trolox\ per\ g\ of\ tannins) = \frac{250.29\ (mg)}{0.15\ (g)}\ x\ \frac{x-b}{a}$

where “a” and “b” correspond respectively to the slope and the constant of the Trolox calibration curve: Absorbance = f ([Trolox]) → Absorbance = ax + b

In all cases, ellagitannins (or ellagic tannins) should demonstrate an antioxidant capacity, and more specifically they should have more than 600 ± 50 mg equivalent Trolox per gram of tannins (commercial extract).

	1	2	3	4	5	6	7	8	9	10	11	12
A	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
B	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
C	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
D	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
E	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
F	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
G	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
H	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11

T = Trolox OT = Oenological Tannins

Figure 2: example 96-well plate

2.1.4.2. Direct oxygen consumption (OCR)

First the model wine solution is saturated with oxygen at 8 mg/L by bubbling with air for 10 min at 20-25 °C. Then, add the oenological tannins to the model wine solution in the bottles filled to 0.75 cL. Sealed the bottles hermetically and shake to fully homogenise.

1) Measure the oxygen consumed every two days starting 1h after the filled of the bottles.
2) To determine the oxygen consumption rate, follow the pathway as shown in **Figure 2**:
   - represent the oxygen consumption versus the time,
   - then represent the inverse of the oxygen consumed versus the inverse of the time,
   - the oxygen consumption rate corresponds to the inverse of the slope coefficient:

     OCR t_0 mg of O_2 per L consumed per day and per g of tannins = 1/A, A being the slope coefficient

In all cases, ellagitannins (or ellagic tannins) should demonstrate an ability to consume the oxygen directly, and more specifically they should be able to consume at least 0.50 ± 0.05 mg of O_2 per litre, per day and per gram of tannins (commercial extract).

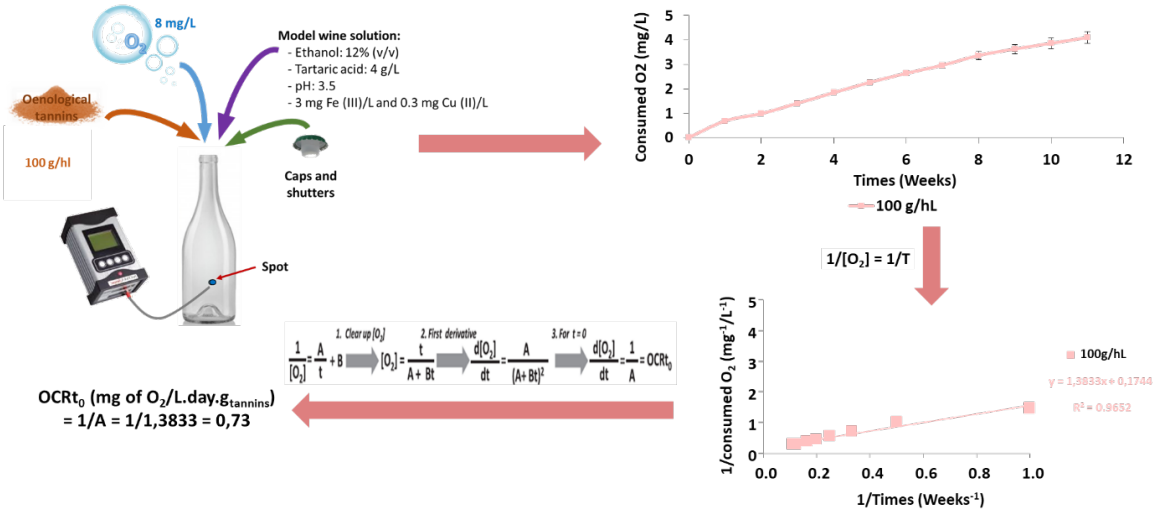

Figure 3: Pathway to determine oxygen consumption rate

2.2. Antioxidasic ability

2.2.1. Principle

Determination of ellagitannins' antioxidasic ability to contribute to antioxidasic protection in terms of the laccase activity of compounds in must and wine.

2.2.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Sodium acetate: MM = 82.03, CAS No. 6131-90-4
Syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine): MM = 360.36, CAS No. 14414-32-5
Polyvinylpolypyrrolidone: PVPP, CAS No. 25249-54-1
Must botrytised with laccase activity
Distilled water (HPLC quality)

2.2.3. Protocols

2 g/L oenological tannin solution: dissolve 200 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Buffer solution (8.2 g/L): dissolve 410 mg of sodium acetate in 50 mL of distilled water.

Syringaldazine solution (0.06 g/L): dissolve 30 mg of syringaldazine in 500 mL of ethanol.

2.2.4. Tests

1) Add 4 mL of botrytized must to 1 mL of oenological tannin solution in tube, which will correspond to sample modality.
2) Add 4 mL of botrytized must to 1 mL of model wine solution in tube, which will correspond to control modality.
3) After 4 minutes (precisely), add 0.8 g of PVPP in both tube (sample and control modalities), stirred and centrifuged for 10 minutes at 8,500 rpm.
4) Recover 1 mL of the supernatant (for both sample and control modalities), into 1.4 mL of buffer solution and 0.6 mL of syringaldazine solution. Put the mixture into a plastic spectrophotometer cuvette (10 mm path length).
5) Measure the absorbance at 530 nm every minute for 5 minutes (including time measurements at 0 minutes).
6) Then determine the laccase activity and the residual laccase activity by using the following equations and **Figure 3**:

$$Laccase\ activity = 46.15\ x\ \Delta A\ \mu mol.\ L^{-1}.\ min^{-1} = 46.15\ x\ \Delta A\ UL$$

$$\%\ of\ residual\ activity = (laccase\ activity_{sample} / laccase\ activity_{control})\ x\ 100$$

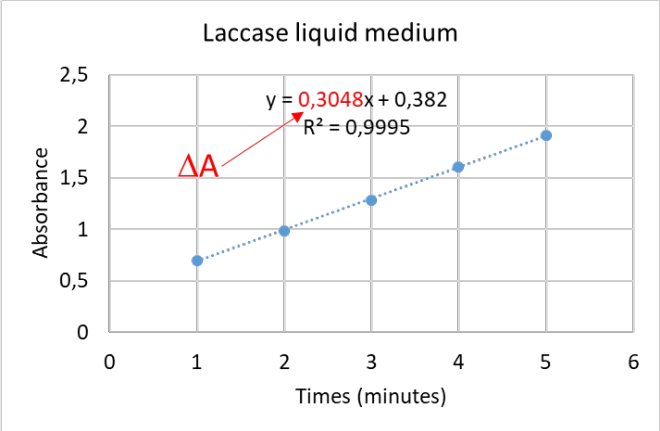

Figure 4: Example determination of ΔA.

In all cases, ellagitannins (or ellagic tannins) should demonstrate an antioxidasic ability, and more specifically they should be able to reduce the residual laccase activity by at least 40%. This value is valuable for must and wine containing less than 5 UL (units of laccases).

2.3. Colour stabilization

2.3.1. Principle

Determination of ellagitannins' colour stabilisation properties to promote the expression, stabilisation and preservation of colour in red must and wine.

2.3.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Malvidin-3-*O*-glucoside: MM = 528.87, CAS No. 18470-06-9

2.3.3. Protocols

0.8 g/L oenological tannin solution: dissolve 80 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

0.1 g/L malvidin-3-*O*-glucoside solution: dissolve 10 mg of malvidin-3-*O*-glucoside in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

2.3.4. Tests

1) Place 0.75 mL of oenological tannin solution and 0.75 mL of model wine solution in one 2-mL stoppered conical tube – hereinafter a "tube" – and keep it in the dark at room temperature. This tube will be called "T_0".
2) Place 0.75 mL of malvidin-3-*O*-glucoside solution and 0.75 mL of model wine solution in one tube and keep it in the dark at room temperature. This tube will be called "M".
3) Place 0.75 mL of oenological tannin solution and 0.75 mL of malvidin solution in one tube and keep it in the dark at room temperature. This tube will be called "T_M".
4) After 7 days, measure the absorbance at 450, 520, 570 and 630 nm of the three tubes (T_M, T_0 and M).
5) Subtract the absorbance values of T_0 to T_M to obtain the absorbance avoiding the interferences due to the "natural" colour of the oenological tannin.

$A(T_M) - A(T_0) = A(T)$

6) Then, determine the CIELAB coordinates (L*, a* and b*) corresponding to the tannin solution with malvidin-3-*O*-glucoside (T) and malvidin-3-*O*-glucoside solution (M) with the free MSCV software (https://www.unirioja.es/color/descargas.shtml) or equivalent.

The formulas to be applied for the calculation of the copigmentation index are as follows:

$$1)\ \Delta E_{ab}.TS = \sqrt{(L^*_T - L^*_W) + (a^*_T - a^*_W) + (b^*_T - b^*_W)}$$

$$2)\ \Delta E_{ab}.CS = \sqrt{(L^*_M - L^*_W) + (a^*_M - a^*_W) + (b^*_M - b^*_W)}$$

$$3)\ \text{Copigmentation Index (\%)} = 100 \times \frac{\Delta E_{ab}.TS - \Delta E_{ab}.CS}{\Delta E_{ab}.CS}$$

ΔE_{ab}.TS: total colour difference between the solution of malvidin-3-*O*-glucoside containing commercial tannins (T) and a pure white colour solution (W).

ΔE_{ab}.CS: total colour difference between the solution of malvidin-3-*O*-glucoside (M) and a pure white colour solution (W).

The CIELAB coordinates of a pure white colour solution are L* = 100.00, a* = 0.00 and b* = 0.00.

In all cases, ellagitannins (or ellagic tannins) should demonstrate an ability to stabilise the colour, and more specifically their copigmentation index should read as higher than 10.0 ± 2.0% after 7 days.

Note: Alternative methods of determination can be used in place of any of the methods described, on the condition that these have been internally validated.

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STEP	1	2	3	4	5	6	7	8
DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINALDRAFT RESOLUTION
OENO-SPECIF 20-675C Et8

SPECIFIC MONOGRAPHS FOR GALLOTANNINS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

CONSIDERING the draft resolution OENO-SPECIF 17-624 “Update to the monograph on oenological tannins” which describes a general monograph,

CONSIDERING the need to set up specific monographs for each family of tannins,

DECIDES, at the proposal of Commission II "Oenology", to add the monograph COEI-1-GALLOT to the *International Oenological Codex*:

OENOLOGICAL TANNINS

Specific monograph~~s~~ on preparations containing gallic tannins

Gallotannins, or gallic tannins, are a ~~sub-group (or~~ sub-class~~)~~ of hydrolysable tannins. ~~Nut gall, oak in particular~~Oak (and chestnut)~~,~~ galls and tara (*Caesalpinia spinosa*) pod tannins are included in this sub-~~group~~class.

1. Method for the determination of sub-~~group~~ class affiliations

1.1. Characterisation by high-performance liquid chromatography (HPLC)

1.1.1. Principle

This method is designed to verify the presence of gallotannins and measure their total concentration.

1.1.2. Reagents, materials and apparatus

1.1.2.1. Reagents

Gallic acid (purity > 96%), CAS No. 149-91-7
Ultrafiltered water (resistivity: 18.3 MΩ·cm)
Water (HPLC grade)
Methanol (HPLC grade)
Formic acid (HPLC grade)

1.1.2.2. Materials

100-mL borosilicate-glass flask
~~Whatman f~~Filters with 0.45 µm pore size diameter
Plastic 1-mL syringe

1.1.2.3. Apparatus

Technical balance~~s~~ with precision of ± 0.01 g
Analytical balance with precision of ± 0.1 mg
Class-A volumetric glassware
Mass chromatographic system with detection by spectrometry composed of:

- Gradient pump for binary or quaternary mix
- Injector fitted with a loop of 10 µL
- Spectrophotometric detector at 280 nm fixe wavelength
- Column Phenomenex Kinetex (for example): 150 x 3.0 mm, 2.6 µm particle size~~, for example~~
- ESI-SIM (Single Ion Monitoring mode via Electro Spray Ionisation) ionisation source
- Mass spectrometer detector: triple quadrupole time of flight (Q-ToF)

1.1.3. Preparation of samples and standards

Samples: weigh approximately 0.5 g of oenological tannins on the analytical balance and make a note of the weight. Dissolve the ~~gallotannin~~ oenological tannins in 100 mL of ultrafiltered water in a 100-mL borosilicate-glass flask and mix well.

Preparation of standard solutions: put 10 mg of gallic acid in solution into 50 mL of ultrafiltered water, corresponding to a 200 mg/L concentration. Then carry out dilutions in ultrafiltered water to obtain 1, 5, 10, 25, 50, 75 and 100 mg/L concentrations.

Solvent A: HPLC-quality water containing 0.1% of formic acid.

Solvent B: methanol containing 0.1% of formic acid.

1.1.4. Procedure

Sample solution and standard solutions are filtered on 0.45 µm (pore size diameter) ~~Whatman~~ cellulose filters and analyse by chromatography under the following conditions given by way of example:

Injected volume: 10 µL of sample solution or standard solution of gallic acid
Detection at 280 nm
Composition of elution gradient: (time, % of solvent A)

0min, 99.0%; 2 min, 98.0%; 5 min, 97.0%; 6 min, 96.5%; 7 min, 96.0%; 8 min, 95.5%; 10 min, 95.0%; 14 min, 90.0%; 17 min, 85.0%; 23 min, 00%; 25 min, 8.0%; 29 min, 5.0%; 34 min, 1.0%; 45 min, 99.0% and 10 min for equilibrium.

Flow rate: 0.4 mL/min

Quantification and detection of the components of the following gallic tannins: gallic, digallic and quinic acid; 3-, 4- and 5-galloylquinic acid; tri-, tetra-, penta-, hexa-, hepta-, octa-, nona- and decagalloyl-glucose according to the ESI-SIM scan and Q-ToF detection~~,~~ (for example).

Table 1: Example chemical formulas and m/z of the different gallotannins (or gallic tannins)

Compounds	Chemical formula	m/z
Gallic acid	$C_7H_6O_5$	170.0
Digallic acid	$C_{14}H_{10}O_9$	322.2
Quinic acid	$C_7H_{12}O_6$	192.2
3-galloylquinic acid	$C_{28}H_{24}O_{18}$	648.1
4-galloylquinic acid	$C_{35}H_{28}O_{22}$	800.1

5-galloylquinic acid	$C_{42}H_{32}O_{26}$	952.7
Trigalloyl glucose	$C_{27}H_{24}O_{18}$	636.5
Tetragalloyl glucose	$C_{24}H_{28}O_{22}$	788.6
Pentagalloyl glucose	$C_{41}H_{32}O_{26}$	940.6
Hexagalloyl glucose	$C_{48}H_{36}O_{30}$	1092.8
Heptagalloyl glucose	$C_{55}H_{40}O_{34}$	1244.9
Octagalloyl glucose	$C_{62}H_{44}O_{38}$	1396.9
Nonagalloyl glucose	$C_{69}H_{48}O_{42}$	1548.1
Decagalloyl glucose	$C_{76}H_{52}O_{46}$	1707.2


Decagalloyl-glucose
Nonagalloyl-glucose
Octagalloyl-glucose
Heptagalloyl-glucose
Hexagalloyl-glucose
Pentagalloyl-glucose
Tetragalloyl-glucose
Trigalloyl-glucose
Counts vs. Acquisition Time (min)
Decagalloyl-glucose
Nonagalloyl-glucose
Octagalloyl-glucose
Heptagalloyl-glucose
Hexagalloyl-glucose
Pentagalloyl-glucose
Tetragalloyl-glucose
Trigalloyl-glucose
Counts vs. Acquisition Time (min)



Figure 1: Example ESI-SIM scan of gallotannins (or gallic tannins)

1.2. Conclusion

An oenological tannin is recognised as a gallotannin (or gallic tannin) when:

- **its total phenol content is higher than 65% (gravimetric method in Annex 1 of the general monograph 17-624),**
- **its gallotannin content as characterised by the HPLC method is higher than 190 mg equivalent of gallic acid per gram of oenological tannins.**

2. Properties and functionalities

The following compliance methods and criteria are only applicable when the property/functionality is claimed on the preparation of tannins.

~~2.~~

2.1. Antioxidant ability

2.1.1. Principle

Determination of gallotannins' antioxidant ability to contribute to the protection of must and wine from oxidation.

2.1.2. Products

2.1.2.1. Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyle): MM = 394.32
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid): MM = 250.29
Methanol at 99.9% volume
96-well microplates reader (FLUOstar Omega - BMG Labtech, for example)

2.1.2.2. Direct oxygen consumption (OCR)

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Iron (III) chloride hexahydrate: MM = 270.30, CAS No. 7705-08-0
Copper (II) sulfate pentahydrate: MM = 249.68, CAS No. 7758-98-7
Clear glass bottles with inserted pills of 0.75-cL capacity
NomaSens oximeter, for example

2.1.3. Protocols

2.1.3.1. Antioxidant capacity (DPPH assay)

0.15 g/L oenological tannin solution: dissolve 37.5 mg of oenological tannins in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5). Dilution of oenological tannins solution could be needed if the measurement absorbance is higher than 1 unit (in this case the dilution should be included in the calculation).

1mM Trolox solution: dissolve 125 mg of Trolox in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Calibration curve: dissolve in 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mL of 1 mM Trolox solution into 0, 0.2, 0.4, 0.6, 0.8 and 0.9 mL of model wine solution. These quantities correspond to 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mM final concentration of Trolox respectively.

6.10^{-5} M DPPH solution: dissolve 2.36 mg of DPPH in 100 mL of methanol. The ~~s~~Solution should be freshly prepared.

2.1.3.2. Direct oxygen consumption (OCR)

1 g/L oenological tannin solution: dissolve 0.75 g of oenological tannin in 750 mL of model wine solution.

Model wine solution: dissolve ~~43~~ g of tartaric acid, 2.25 mg of Iron (III) chloride hexahydrate and 0.225 mg of Copper (II) sulfate pentahydrate in 90 mL of ethanol and 660 mL of distilled water. The pH should be adjusted at 3.5.

2.1.4. Tests

2.1.4.1. Antioxidant capacity

First a blank containing solely reagent (RB) is measure at 515 nm by placing 190 µL of DPPH solution (1.3.1) in all the wells of the plate. Then, add 10 µL of oenological tannin solution (samples), distilled water (blank) or Trolox curve solution (standards) into the wells and measure (MS) at 515 nm after 30 min.

See Figure 2 for an example of how to fill the plate.

The formula to be applied for the calculation of the antioxidant capacity is as follows:

1) $RB - MS = x$
2) $antioxidant\ capacity\ (mg\ eq.Trolox\ per\ g\ of\ tannins) = \frac{250.29\ (mg)}{0.15\ (g)}\ x\ \frac{x-b}{a}$

where "a" and "b" correspond respectively to the slope and the constant of the Trolox calibration curve: Absorbance = f ([Trolox]) → Absorbance = ax + b

In all cases, gallotannins (or gallic tannins) should demonstrate antioxidant capacity, and more specifically they should have more than 600 ± 50 ~~milligrams~~ mg equivalent Trolox per gram of tannins (commercial extract).

	1	2	3	4	5	6	7	8	9	10	11	12
A	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
B	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
C	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
D	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
E	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
F	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
G	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
H	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11

T = Trolox OT = Oenological Tannins

Figure 2: Example 96-well plate

2.1.4.2. Direct oxygen consumption (OCR)

First the model wine solution is saturated with oxygen at 8 mg/L by bubbling with air for 10 min at 20-25 °C. Then, add the oenological tannins to the model wine solution in the bottles filled to 0.75 cL. Seal the bottles hermetically and shake to fully homogenise.

1) Measure the oxygen consumed every two days starting 1h after the filled of the bottles.
2) To determine the oxygen consumption rate, follow the pathway as shown in **Figure 2**:
   - represent the oxygen consumption versus the time,
   - then represent the inverse of the oxygen consumed versus the inverse of the time,
   - the oxygen consumption rate corresponds to the inverse of the slope coefficient:

     OCR t_0 mg of O_2 per L consumed per day and per g of tannins = 1/A, A being the slope coefficient

In all cases, gallotannins (or gallic tannins) should demonstrate an ability to consume the oxygen directly, and more specifically they should be able to consume at least 0.10 ± 0.05 ~~milligrams~~ mg of O_2 per litre, per day and per gram of tannins (commercial extract).

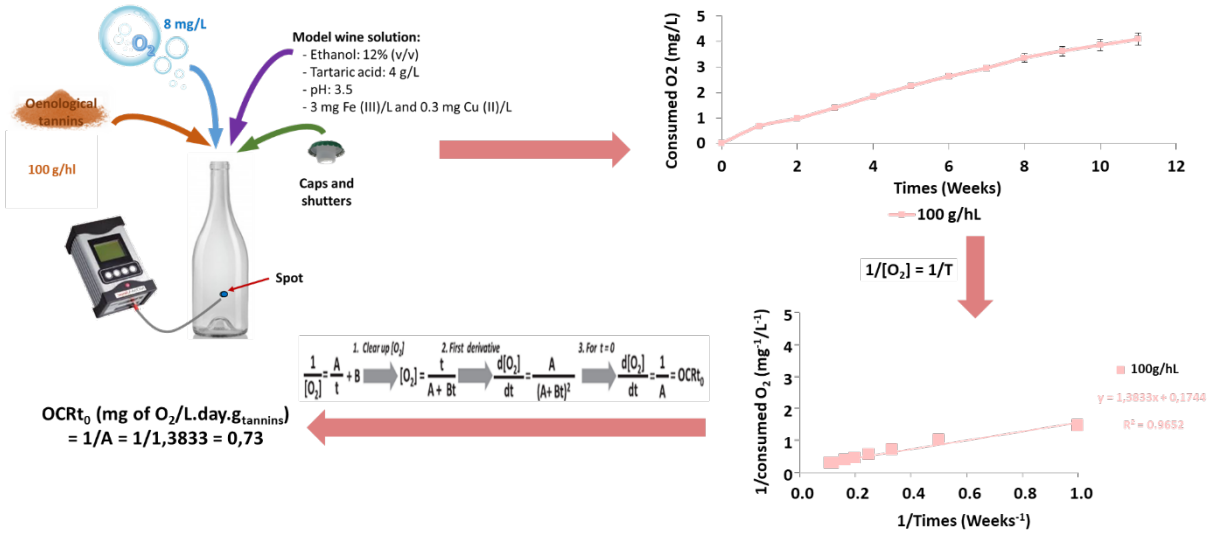

Figure 3: Pathway to determine oxygen consumption rate

2.2. Antioxidasic ability

2.2.1. Principle

Determination of gallotannins' antioxidasic ability to contribute to antioxidasic protection in terms of the laccase activity of compounds in must and wine.

2.2.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Sodium acetate: MM = 82.03, CAS No. 6131-90-4
Syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine): MM = 360.36, CAS No. 14414-32-5
Polyvinylpolypyrrolidone: PVPP, CAS No. 25249-54-1
Must botrytised with laccase activity
Distilled water (HPLC quality)

2.2.3. Protocols

2 g/L oenological tannin solution: dissolve 200 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Buffer solution (8.2 g/L): dissolve 410 mg of sodium acetate in 50 mL of distilled water.

Syringaldazine solution (0.06 g/L): dissolve 30 mg of syringaldazine in 500 mL of ethanol.

2.2.4. Tests

1) Add 4 mL of botrytised must to 1 mL of oenological tannin solution in tube, which will correspond to sample modality.
2) Add 4 mL of botrytised must to 1 mL of model wine solution in tube, which will correspond to control modality.
3) After 4 minutes (precisely), add 0.8 g of PVPP in both tube (sample and control modalities), stirred and centrifuged for 10 minutes at 8,500 rpm.
4) Recover 1 mL of the supernatant (for both sample and control modalities), into 1.4 mL of buffer solution and 0.6 mL of syringaldazine solution. Put the mixture into a plastic spectrophotometer cuvette (10 mm path length).

5) Measure the absorbance at 530 nm every minute ~~during~~ for 5 minutes (including time measurements at 0 minutes).
6) Then determine the laccase activity and the residual laccase activity by using the following equations and **Figure 3**:

$$Laccase\ activity = 46.15\ x\ \Delta A\ \mu mol.\ L^{-1}.\ min^{-1} = 46.15\ x\ \Delta A\ UL$$

$$\%\ of\ residual\ activity = (laccase\ activity_{sample} / laccase\ activity_{control})\ x\ 100$$

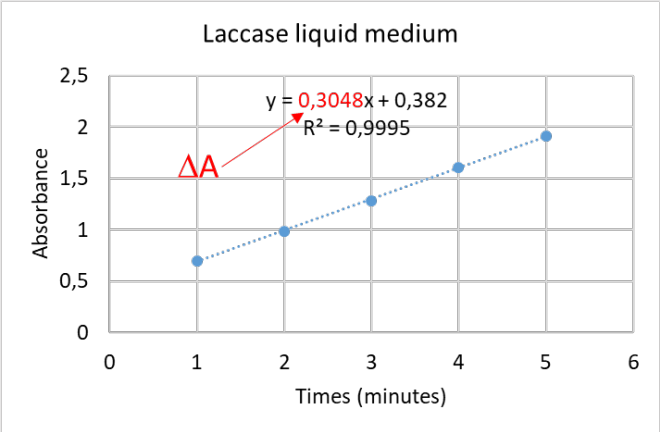

Figure 4: Example determination of ΔA.

In all cases, gallotannins (or gallic tannins) should demonstrate an antioxidasic ability, and more specifically they should be able to reduce the residual laccase activity by at least 50%. This value is valuable for must and wine containing less than 5 UL (units of laccases).

2.3. Colour stabilisation

2.3.1. Principle

Determination of gallotannins colour stabilisation properties to promote the expression, stabilisation and preservation of colour in red must and wine.

2.3.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Malvidin-3-*O*-glucoside: MM = 528.87, CAS No. 18470-06-9

2.3.3. Protocols

0.8 g/L oenological tannin solution: dissolve 80 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

0.1 g/L malvidin-3-*O*-glucoside solution: dissolve 10 mg of malvidin-3-*O*-glucoside in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

2.3.4. Tests

1) Place 0.75 mL of oenological tannin solution and 0.75 mL of model wine solution in one ~~eppendorf~~ 2-mL stoppered conical tube – hereinafter a "tube" – and keep it in the dark at room temperature. This ~~eppendorf~~ tube will be called "T_0".
2) Place 0.75 mL of malvidin-3-*O*-glucoside solution and 0.75 mL of model wine solution in one ~~eppendorf~~ tube and keep it in the dark at room temperature. This ~~eppendorf~~ tube will be called "M".

3) Place 0.75 mL of oenological tannin solution and 0.75 mL of malvidin solution in one ~~eppendorf~~ tube and keep it in the dark at room temperature. This ~~eppendorf~~ tube will be called "T_M".
4) After 7 days, measure the absorbance at 450, 520, 570 and 630 nm of the three ~~eppendorf~~ tubes (T_M, T_0 and M).
5) Subtract the absorbance values of T_0 to T_M to obtain the absorbance, avoiding the interferences due to the "natural" colour of the oenological tannin.
$A(T_M) – A(T_0) = A(T)$
6) Then, determine the CIELAB coordinates (L*, a* and b*) corresponding to tannin solution with malvidin-3-*O*-glucoside (T) and malvidin-3-*O*-glucoside solution (M) with the free MSCV software (https://www.unirioja.es/color/descargas.shtml) or equivalent.

The formulas to be applied for the calculation of the copigmentation index are as follows:

$$1)\ \Delta E_{ab}.TS = \sqrt{(L^*_T - L^*_W) + (a^*_T - a^*_W) + (b^*_T - b^*_W)}$$

$$2)\ \Delta E_{ab}.CS = \sqrt{(L^*_M - L^*_W) + (a^*_M - a^*_W) + (b^*_M - b^*_W)}$$

$$3)\ \text{Copigmentation Index (\%)} = 100 \times \frac{\Delta E_{ab}.TS - \Delta E_{ab}.CS}{\Delta E_{ab}.CS}$$

ΔE_{ab}.TS: total colour difference between the solution of malvidin-3-*O*-glucoside containing commercial tannins (T) and a pure white colour solution (W).

ΔE_{ab}.CS: total colour difference between the solution of malvidin-3-*O*-glucoside (M) and a pure white colour solution (W).

The CIELAB coordinates of a pure white colour solution are L* = 100.00, a* = 0.00 and b* = 0.00.

In all cases, gallotannins (or gallic tannins) should demonstrate an ability to stabilise the colour, and more specifically their a copigmentation index should read as higher than 30.0 ± 2.0% after 7 days.

N~~Footn~~ote: Alternative methods of determination can be used in place of any of the methods described, on the condition that these have been internally validated.

3. Bibliography

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STEP	1	2	3	4	5	6	7	8
DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SPECIF 20-675D Et8

SPECIFIC MONOGRAPHS FOR PROFISETINIDINS/PROROBITENIDINS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

CONSIDERING the draft resolution OENO-SPECIF 17-624 "Update to the monograph on oenological tannins" which describes a general monograph,

CONSIDERING the need to set up specific monographs for each family of tannins,

DECIDES, at the proposal of Commission II "Oenology", to add the monograph COEI-1-PROFIS to the *International Oenological Codex*:

OENOLOGICAL TANNINS

Specific monograph for oenological tannins containing profisetinidins/prorobitenidins

Profisetinidins/prorobitenidins are a sub-class of condensed (or proanthocyanic) tannins. Tannins from the quebracho and acacia *spp*. tree are included in this sub-class.

1. Method for the determination of sub-class affiliations

1.1. Characterisation by high-performance liquid chromatography (HPLC)

1.1.1. Principle

This method is designed to verify the presence of characteristic components of condensed tannins of the profisetinidin/prorobitenidin sub-group and measure their total concentration. It is applicable to preparations of oenological tannins that are declared as pure and thus as not containing tannins with small masses from different families or sub-families (or classes).

1.1.2. Reagents, material and apparatus

1.1.2.1. Reagents

(+)-catechin, CAS No. 154-23-4
Ultrafiltered water (resistivity: 18.3 MΩ·cm)
Water (HPLC quality)
Methanol (HPLC quality)
Formic acid (HPLC quality)

1.1.2.2. Materials

100-mL borosilicate-glass flask

Filters with 0.45 µm pore size diameter
Plastic 1-mL syringe

1.1.2.3. Apparatus

Technical balance with precision of 0.01 g
Analytical balance with precision of 0.1 mg
Class-A volumetric glassware
Mass chromatographic system with spectrometry detection composed of:

- Gradient pump for binary or quaternary mix
- Injector fitted with a loop of 10 µL
- Spectrophotometric detector at 280 nm fixe wavelength
- Column Eclipse Plus C-18 (for example): 2.1 x 100 mm, 1.8 µm particle size
- ESI-SIM (Single Ion Monitoring mode via Electro Spray Ionisation) ionisation source
- Mass spectrometer detector: quadrupole time of flight (Q-TOF)

1.1.3. Preparation of samples and standards

Samples: weigh approximately 0.5 g of oenological tannins on the analytical balance and make a note of the weight. Dissolve the oenological tannins in 100 mL of ultrafiltered water in a 100-mL borosilicate-glass flask and mix well.

Preparation of standard solutions: put 10 mg of (+)-catechin in solution into 50 mL of ultrafiltered water, corresponding to a 200 mg/L concentration. Then carry out dilutions in ultrafiltered water to obtain 5, 10, 20, 40, 60, 80 and 100 mg/L concentrations.

Solvent A: water (HPLC quality) containing 0.1% of formic acid.

Solvent B: methanol containing 0.1% of formic acid.

1.1.4. Procedure

The sample solutions and standard solutions are filtered on 0.45 µm (pore size diameter) filters and analysed by chromatography under the following conditions given by way of example:

Injected volume: 10 µL of sample solution or standard solution of (+)-catechin
Detection at 280 nm
Composition of elution gradient: (time, % of solvent A)
0min, 99.0%; 0.5 min, 94.0%; 20 min, 50.0%; 25 min, 0.0%; 32 min, 94.0% and 10 min for equilibrium.
Flow rate: 0.3 mL/min

Quantification and detection of the characteristic components of condensed tannins from the profisetinidin/prorobitenidin sub-group or sub-class according to the ESI-SIM scan and Q-ToF detection (for example).

Table 1: Example chemical formulas and m/z of the different profisetinidins/prorobitenidins (for the quebracho tree)

Compound	Chemical formula	m/z
(+)-catechin	$C_{15}H_{14}O_6$	290.1
(-)-epicatechin	$C_{15}H_{14}O_6$	290.1
Catechin-fisetinidol dimers	$C_{30}H_{26}O_{11}$	562.1
Catechin-fisetinidol trimers	$C_{45}H_{38}O_{16}$	834.2
Catechin-fisetinidol tetramers	$C_{60}H_{50}O_{21}$	1106.3

Table 2: Example chemical formulas and m/z of the different profisetinidins/prorobitenidins (for the acacia tree)

Compound	Chemical formula	m/z
(+)-catechin	$C_{15}H_{14}O_6$	290.1
(-)-epicatechin	$C_{15}H_{14}O_6$	290.1
(-)-epicatechin-3-O-gallate	$C_{22}H_{18}O_{10}$	442.4
Fisetinidol-gallocatechin dimers	$C_{30}H_{26}O_{12}$	578.1
Robinetinidol-catechin dimers	$C_{30}H_{26}O_{12}$	578.1
Chalcan-flavan dimers (gambiriin)	$C_{30}H_{28}O_{12}$	580.1
Catechine-fisetinidol-robinetinidol or gallocatechin-fisetinidol-fisetinidol trimers	$C_{45}H_{58}O_{17}$	850.2
Robinetinidol-robinetinidol-catechin or gallocatechin-fisetinidol-robinetinidol trimers	$C_{45}H_{38}O_{17}$	866.2

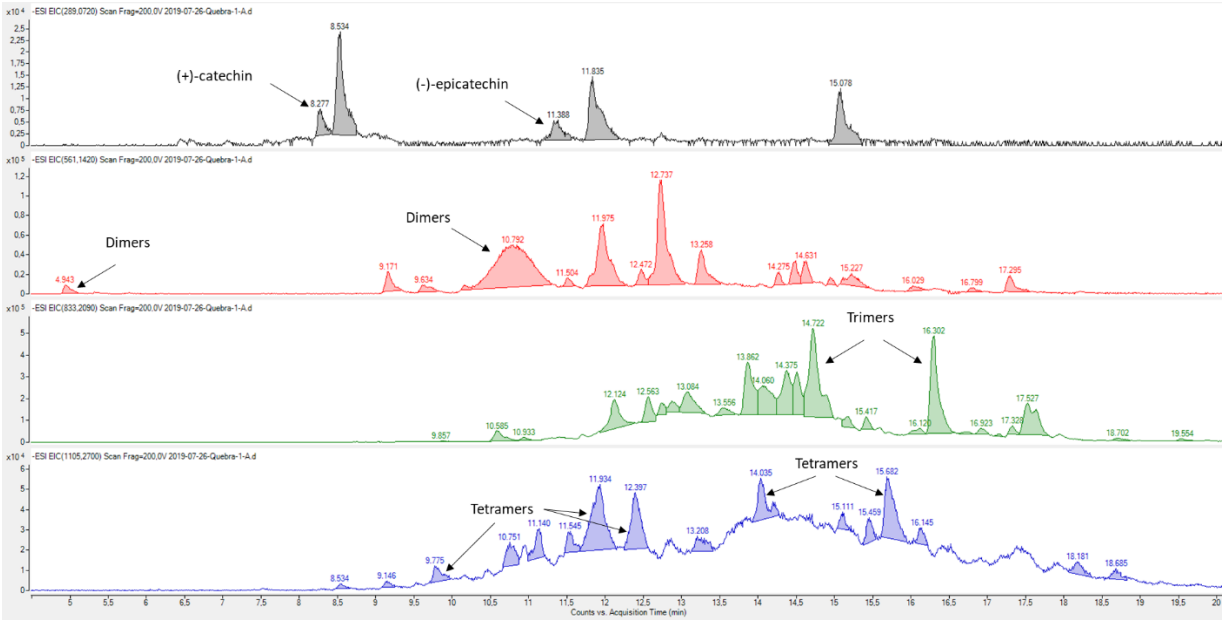

Figure 1: Example ESI-SIM scan of quebracho tree

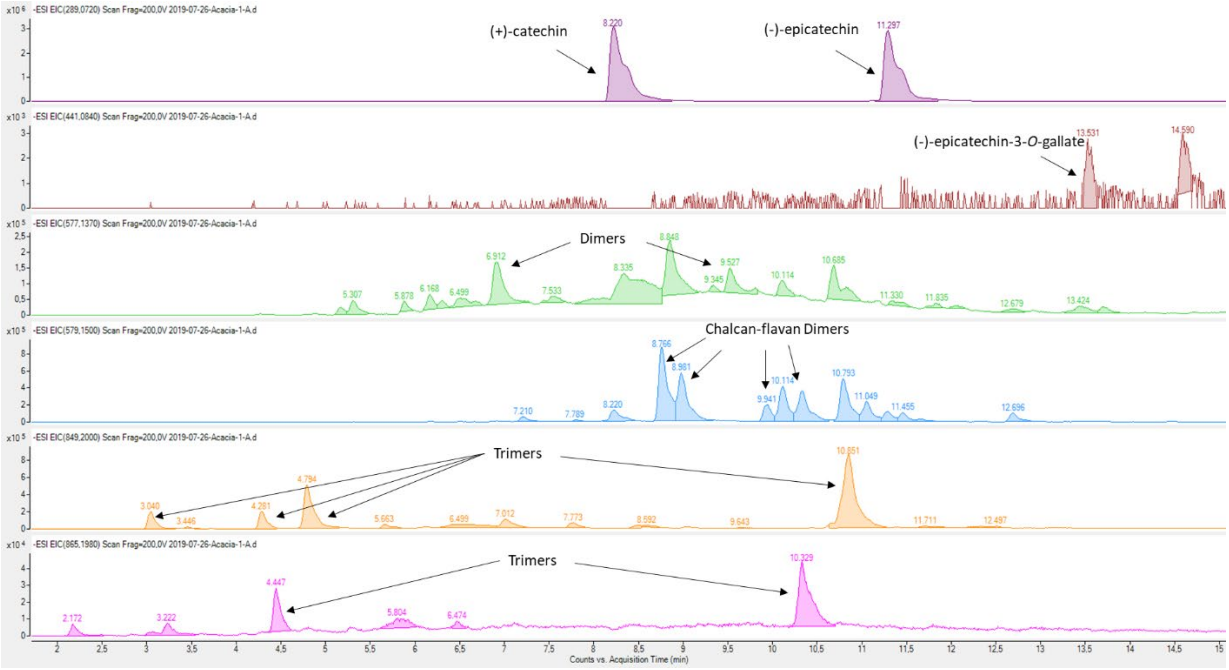

Figure 2: Example ESI-SIM scan of acacia tree

1.2. Conclusion

An oenological tannin is recognised as a profisetinidin/prorobitenidin when:

- **its total polyphenol content is higher than 65% (gravimetric method in Annex 1 of the general monograph 17-624),**
- **its molecular procyanidin/prodelphinidin content as characterised by the HPLC method is higher than 20 mg (for quebracho tannins) and 150 mg (for acacia tannins) equivalent of (+)-catechin per gram of oenological tannins.**

2. Methods of measurement of properties and functionalities

The following compliance methods and criteria are only applicable when the property/functionality is claimed on the preparation of tannins.

2.1. Antioxidant ability

2.1.1. Principle

Determination of profisetinidins/prorobitenidins' antioxidant ability to contribute to the protection of must and wine from oxidation.

2.1.2. Products

2.1.2.1. Antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl): MM = 394.32
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid): MM = 250.29
Methanol at 99.9% volume
96-well microplate reader (FLUOstar Omega - BMG Labtech, for example)

2.1.2.2. Direct oxygen consumption (OCR)

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Iron (III) chloride hexahydrate: MM = 270.30, CAS No. 7705-08-0
Copper (II) sulfate pentahydrate: MM = 249.68, CAS No. 7758-98-7
Clear glass bottles with inserted pills of 0.75-L capacity
NomaSens oximeter, for example

2.1.3. Protocols

2.1.3.1. Antioxidant capacity (DPPH assay)

0.15 g/L oenological tannin solution: dissolve 37.5 mg of oenological tannins in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5). Dilution of oenological tannins solution could be needed if the measurement absorbance is higher than 1 unit (in this case the dilution should be included in the calculation).

1mM Trolox solution: dissolve 125 mg of Trolox in 500 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Calibration curve: dissolve in 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mL of 1 mM Trolox solution into 0, 0.2, 0.4, 0.6, 0.8 and 0.9 mL of model wine solution. These quantities correspond to 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mM final concentration of Trolox respectively.

6.10^{-5} M DPPH solution: dissolve 2.36 mg of DPPH in 100 mL of methanol. The solution should be freshly prepared.

2.1.3.2. Direct oxygen consumption (OCR)

1 g/L oenological tannin solution: dissolve 0.75 g of oenological tannins in 750 mL of model wine solution.

Model wine solution: dissolve 4 g of tartaric acid, 2.25 mg of Iron (III) chloride hexahydrate and 0.225 mg of Copper (II) sulfate pentahydrate in 90 mL of ethanol and 660 mL of distilled water. The pH should be adjusted at 3.5.

2.1.4. Tests

2.1.4.1. Antioxidant capacity

First a blank containing solely the DPPH reagent (RB) is measured at 515 nm by placing 190 µL of DPPH solution (1.3.1) in all the wells of the plate. Then, add 10 µL of oenological tannin solution (samples), distilled water (blank) or Trolox curve solution (standards) into the wells and measure (MS) at 515 nm after 30 min.

See Figure 2 for an example of how to fill the plate.

The formula to be applied for the calculation of the antioxidant capacity is as follows:

1) $RB - MS = x$
2) $antioxidant\ capacity\ (mg\ eq. Trolox\ per\ g\ of\ tannins) = \frac{250.29\ (mg)}{0.15\ (g)}\ x \frac{x-b}{a}$

where “a” and “b” correspond respectively to the slope and the constant of the Trolox calibration curve: Absorbance = f ([Trolox]) → Absorbance = ax + b

In all cases, profisetinidins/prorobitenidins should demonstrate an antioxidant capacity, and more specifically they should have more than 450 ± 50 mg equivalent Trolox per gram of tannins (commercial extract).

	1	2	3	4	5	6	7	8	9	10	11	12
A	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
B	T 0.1	T 0.1	T 0.2	T 0.2	T 0.4	T 0.4	T 0.6	T 0.6	T 0.8	T 0.8	T 1	T 1
C	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
D	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
E	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
F	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
G	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11
H	Blank	OT 1	OT 2	OT 3	OT 4	OT 5	OT 6	OT 7	OT 8	OT 9	OT 10	OT 11

T = Trolox OT = Oenological Tannins

Figure 3: Example 96-well plate

2.1.4.2. Direct oxygen consumption (OCR)

First the model wine solution is saturated with oxygen at 8 mg/L by bubbling with air for 10 min at 20-25 °C. Then, add the oenological tannins to the model wine solution in the bottles filled to 0.75 L. Seal the bottles hermetically and shake to fully homogenise.

1) Measure the oxygen consumed every two days starting 1h after the filled of the bottles.
2) To determine the oxygen consumption rate, follow the pathway as shown in **Figure 2**:
   - represent the oxygen consumption versus the time,
   - then represent the inverse of the oxygen consumed versus the inverse of the time,
   - the oxygen consumption rate corresponds to the inverse of the slope coefficient:

     OCR t_0 mg of O_2 consumed per day and per g of tannins = 1/A, A being the slope coefficient

In all cases, profisetinidins/prorobitenidins should demonstrate an ability to consume the oxygen directly, and more specifically they should be able to consume at least 0.10 ± 0.05 mg of O_2 per litre, per day and per gram of tannins (commercial extract).

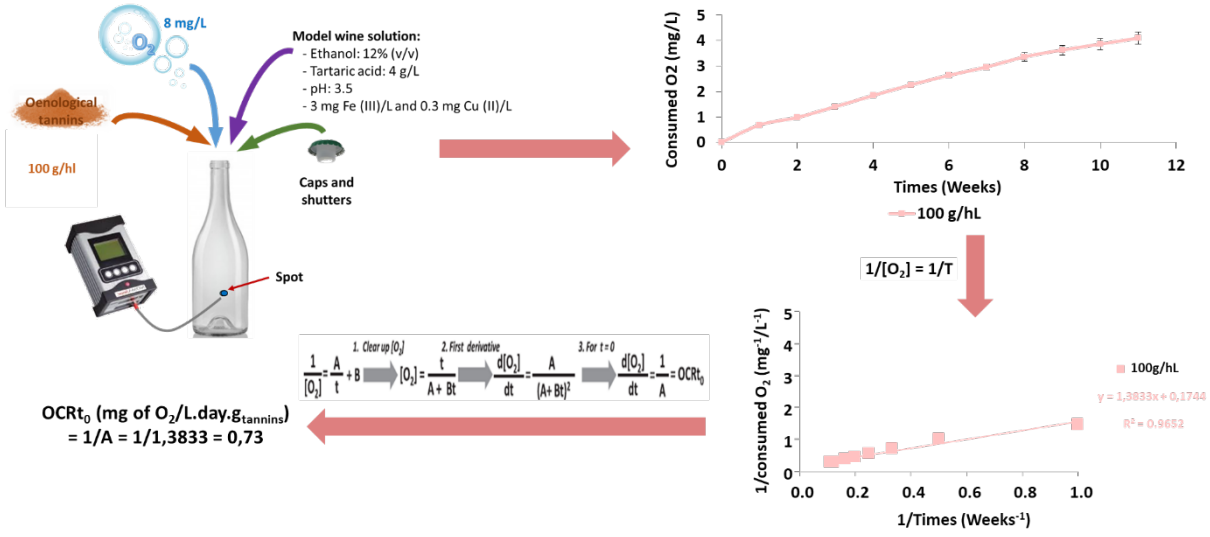

Figure 4: Pathway to determine oxygen consumption rate

2.2. Antioxidasic ability

2.2.1. Principle

Determination of profisetinidins/prorobitenidins' antioxidasic ability to contribute to antioxidasic protection in terms of the laccase activity of compounds in must and wine.

2.2.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Sodium acetate: MM = 82.03, CAS No. 6131-90-4
Syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine): MM = 360.36, CAS No. 14414-32-5
Polyvinylpolypyrrolidone: PVPP, CAS No. 25249-54-1
Must botrytised with laccase activity
Distilled water (HPLC quality)

2.2.3. Protocols

2 g/L oenological tannin solution: dissolve 200 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

Buffer solution (8.2 g/L): dissolve 410 mg of sodium acetate in 50 mL of distilled water.

Syringaldazine solution (0.06 g/L): dissolve 30 mg of syringaldazine in 500 mL of ethanol.

2.2.4. Tests

1) Add 4 mL of botrytised must to 1 mL of oenological tannin solution in tube, which will correspond to sample modality.
2) Add 4 mL of botrytised must to 1 mL of model wine solution in tube, which will correspond to control modality.
3) After 4 minutes (precisely), add 0.8 g of PVPP in both tube (sample and control modalities), stirred and centrifuged for 10 minutes at 8,500 rpm.
4) Recover 1 mL of the supernatant (for both sample and control modalities), into 1.4 mL of buffer solution and 0.6 mL of syringaldazine solution. Put the mixture into a plastic spectrophotometer cuvette (10 mm path length).
5) Measure the absorbance at 530 nm every minute for 5 minutes (including time measurements at 0 minutes).
6) Then determine the laccase activity and the residual laccase activity by using the following equations and **Figure 3**:

$$Laccase\ activity = 46.15\ x\ \Delta A\ \mu mol.\ L^{-1}.\ min^{-1} = 46.15\ x\ \Delta A\ UL$$

$$\%\ of\ residual\ activity = (laccase\ activity_{sample} / laccase\ activity_{control})\ x\ 100$$

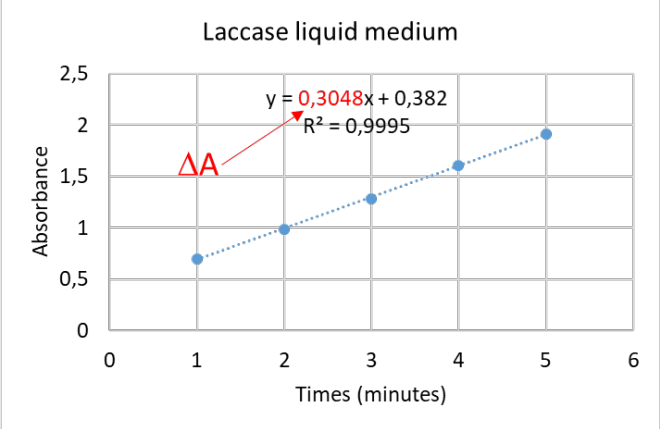

Figure 5: Example determination of ΔA.

In all cases, profisetinidins/prorobitenidins should demonstrate an antioxidasic ability, and more specifically they should be able to reduce the residual laccase activity by at least 40%. This value is valuable for must and wine containing less than 5 UL (units of laccases).

2.3. Colour stabilisation

2.3.1. Principle

Determination of profisetinidins/prorobitenidins' colour stabilisation properties to promote the expression, stabilisation and preservation of colour in red must and wine.

2.3.2. Products

Ethanol at 96% volume, CAS No. 64-17-5
Tartaric acid: MM = 150.09, CAS No. 87-69-4
Malvidin-3-*O*-glucoside: MM = 528.87, CAS No. 18470-06-9

2.3.3. Protocols

0.8 g/L oenological tannin solution: dissolve 80 mg of oenological tannins in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

0.1 g/L malvidin-3-*O*-glucoside solution: dissolve 10 mg of malvidin-3-*O*-glucoside in 100 mL of model wine solution (distilled water, 12% vol. of ethanol, 4 g/L of tartaric acid and pH adjusted to 3.5).

2.3.4. Tests

1) Place 0.75 mL of oenological tannin solution and 0.75 mL of model wine solution in one 2-mL stoppered conical tube – hereinafter a "tube" – and keep it in the dark at room temperature. This tube will be called "T_0".
2) Place 0.75 mL of malvidin-3-*O*-glucoside solution and 0.75 mL of model wine solution in one tube and keep it in the dark at room temperature. This tube will be called "M".
3) Place 0.75 mL of oenological tannin solution and 0.75 mL of malvidin solution in one tube and keep it in the dark at room temperature. This tube will be called "T_M".
4) After 7 days, measure the absorbance at 450, 520, 570 and 630 nm of the three tubes (T_M, T_0 and M).
5) Subtract the absorbance values of T_0 to T_M to obtain the absorbance avoiding the interferences due to the "natural" colour of the oenological tannin.
   $A(T_M) – A(T_0) = A(T)$

6) Then, determine the CIELAB coordinates (L*, a* and b*) corresponding to tannin solution with malvidin-3-*O*-glucoside (T) and malvidin-3-*O*-glucoside solution (M) with the free MSCV software (https://www.unirioja.es/color/descargas.shtml) or equivalent.

The formulas to be applied for the calculation of the copigmentation index are as follows:

$$1)\ \Delta E_{ab}.TS = \sqrt{(L^*_T - L^*_W) + (a^*_T - a^*_W) + (b^*_T - b^*_W)}$$

$$2)\ \Delta E_{ab}.CS = \sqrt{(L^*_M - L^*_W) + (a^*_M - a^*_W) + (b^*_M - b^*_W)}$$

$$3)\ \text{Copigmentation Index (\%)} = 100 \times \frac{\Delta E_{ab}.TS - \Delta E_{ab}.CS}{\Delta E_{ab}.CS}$$

ΔE_{ab}.TS: total colour difference between the solution of malvidin-3-*O*-glucoside containing commercial tannins (T) and a pure white colour solution (W).

ΔE_{ab}.CS: total colour difference between the solution of malvidin-3-*O*-glucoside (M) and a pure white colour solution (W).

The CIELAB coordinates of a pure white colour solution are L* = 100.00, a* = 0.00 and b* = 0.00.

In all cases, profisetinidins/prorobitenidins should demonstrate an ability to stabilise the colour, and more specifically their copigmentation index should read as higher than 3.0 ± 0.5% after 7 days.

Note: Alternative methods of determination can be used in place of any of the methods described, on the condition that these have been internally validated.

3. Bibliography

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FINAL DRAFT RESOLUTION
OENO-SPECIF 20-681 Et8

POWDERED CELLULOSE

THE GENERAL ASSEMBLY,

CONSIDERING Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING that microfibre-composed celluloses of recognised food-grade quality have been in existence for some years now, and that these could substitute microcrystalline cellulose at a lower cost, it has been proposed that the TECHNO Group make a decision on the possible use of these food celluloses or powder celluloses. This practice has been adopted, which is reflected in the addition of food cellulose to file 2.3.2 of the *Code* as a fermentation activator (OENO 633-2019).

CONSIDERING the work of the "Specifications of Oenological Products" Expert Group,

DECIDES, at the proposal of "Oenology" Commission II, to add the following monograph to Chapter I of the *International Oenological Codex*:

POWDERED CELLULOSE

$(C_{12}H_{20}O_{10})_n$
INS No.: 460 (ii)

CAS No.: 9004-34-6

1. Object, origin and scope of application

Powdered cellulose is cellulose of food-grade quality. Cellulose is a linear glucose homopolymer composed of glucopyranose units linked by **β-**1,4-glycosidic bonds; its degree of polymerisation (DP) is dependent on the origin of the cellulosic material.
Powdered cellulose is a non-modified, purified cellulose, obtained by the mechanical disintegration of alpha-cellulose procured in pulp form from fibrous plant material.
Powdered cellulose plays a "support" role in clarified fermentation media; it allows for improved "degassing" of carbon dioxide at the start of alcoholic fermentation and thus shortens the latency phase. It increases the fermentability of must.

2. Labelling

The following should be mentioned on the label:
- the identification of the cellulose and its use in food,
- the concentration of the product, including in the case of a mixture,
- safety and storage conditions
- the batch no.,
- the expiry date.

3. Characteristics

Powdered cellulose comes in the form of flakes or very fine fibres, and is whitish in colour, odourless and flavourless.

4. Test limits and methods

4.1 Identification

Place approx. 10 mg powdered cellulose on a watch glass and disperse into 2 mL iodinated zinc chloride solution (R). The solution will turn a blue-purple colour.

4.2 Solubility

Powdered cellulose is insoluble in water, ethanol, ether and diluted acids, and slightly soluble in sodium hydroxide solution.

4.3 Purity

The powdered cellulose content should not be less than 92%.

4.4 Size of fine particles

The size of fine particles should not be less than 5 µm; the number of particles smaller than 5µm should not exceed 10%.

4.5 pH

Mix approx. 10 g dry cellulose in 90 mL water free of carbon dioxide for 60 min. Centrifuge. The pH of the supernatant liquid should be between 5.0 and 7.5.

4.6 Water-soluble substances

Mix around 6 g of sample, dried beforehand, with 90 mL water that has recently been boiled then cooled. Leave to rest for 10 min. Filter using a membrane with porosity of 3 µm, throw away the first 10 mL of filtrate and pass the filtrate through the same filter a second time if necessary to obtain a clear filtrate.

Evaporate a 15-mL portion of filtrate to dryness in a tared evaporating dish over a water bath, and dry to 105 °C for 1 h. Weigh the dish containing the dry residue. Less than 15 mg of residue should be obtained.

4.7 Detection of starch

Add 90 mL demineralised water (R) to 10 g powdered cellulose and boil for 5 min. Filter while hot with a 25-µm membrane filter. Cool and add 0.1 mL 0.05 M iodine solution to the filtrate. No blue colouration should appear. With very fine grains, a light blue colour may be observed after the addition of the iodine solution but will disappear after 30 minutes.

4.8 Loss on drying

Place 1 g powdered cellulose in a tared dish in an oven at 100-105 °C for 3 hours. The loss on drying should not exceed 7.0%.

4.9 Ashes

Incinerate the residue obtained in point 4.8 at 800 ± 25 °C for 4 hours. The weight of the ashes should not be greater than 0.3%.

All of the limits set below relate to dry products.

5. Preparation of the test solution

After weighing, dissolve the ashes in 2 mL concentrated hydrochloric acid (R) and 10 mL water (R). Heat to activate dissolution and make up to 50 mL with water.

5.1 Iron

Determine the iron content of the solution prepared for testing purposes (5) by atomic absorption spectrophotometry according to the method described in Chapter II. The iron content should be less than or equal to 10 mg/kg.

5.2 Lead

Determine the lead content of the solution prepared for testing purposes (5) according to the method described in Chapter II. The lead content should be less than 2 mg/kg.

5.3 Mercury

Determine the mercury content of the solution prepared for testing purposes (5) according to the method described in Chapter II. The mercury content should be less than 1 mg/kg.

5.4 Cadmium

Determine the cadmium content of the solution prepared for testing purposes (5) according to the method described in Chapter II. The cadmium content should be less than 1 mg/kg.

5.5 Arsenic

Determine the arsenic content of the solution prepared for testing purposes (5) according to the method described in Chapter II. The arsenic content should be less than 1 mg/kg.

6. Storage

Powdered cellulose should be stored in a well-ventilated place in airtight packaging, away from any volatile substances that it could adsorb.

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FINAL DRAFT RESOLUTION[1]
OENO-SCMA 19-662A Et8

MEASURING OCHRATOXIN A IN GRAPE JUICE, RECONSTITUTED GRAPE JUICE, CONCENTRATED GRAPE JUICE, AND GRAPE NECTAR, AFTER GOING THROUGH AN IMMUNOAFFINITY COLUMN AND HIGH LIQUID PERFORMANCE CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

THE GENERAL ASSEMBLY,

IN VIEW ~~OF~~of Article 2, paragraph iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the "Methods of Analysis" Sub-Commission,

DECIDES to add the following

Measuring ochratoxin A in grape juice, reconstituted grape juice, concentrated grape juice, and nectar, after going through an immunoaffinity column and high liquid performance chromatography with fluorescence detection

Type IV method

1.1 For grape juice, reconstituted grape juice, and nectar: the application of the Method OIV-MA-AS315-10 of the Compendium of International Methods of Analysis of Wines and Musts.

1.2 For the concentrated grape juice: dilute the juices five times (m/m) with water, before initiate the item 5.1 of the Procedure. Consider this dilution in the final calculation of the OTA concentration (item 7), in the following equation:

COTA = MA × F/V1 × V3/V2, in the value of the dilution factor (F).

1.3 Method proposal:

[1] ~~All the project of resolutions concerning grape juices methods were kept in step 3 and they were not discussed one by one during the meeting.~~

~~Before discussing each method of analysis, it was decided to create a work group with the following objectives:~~

- ~~to draw up general principles for grape juices methods;~~
- ~~to assign each analyte to a category of method for its analysis (new method required; existing method available which needs to be reviewed and can be adopted; or no method required);~~
- ~~to discuss the need to creating a dedicated Compendium of analytical methods for grape juice analysis.~~

Measuring ochratoxin A in grape juice, concentrated grape juice, reconstituted grape juice, and nectar after going through an immunoaffinity column and high liquid performance chromatography with fluorescence detection

Type IV method

1. Scope

This document describes the method used for determining ochratoxin A (OTA) in grape juice, reconstituted grape juice, concentrated grape juice, and nectar, in concentrations ranging from 0.2 µg/L up to 10 µg/L.

2. Principle

This method use immunoaffinity column and high performance liquid chromatography (HPLC). OTA is eluted with methanol and quantified by HPLC in reverse phase with fluorimetric detection.

3. Reagents and materials

3.1 Reagents for separation of the OTA on an immunoaffinity column. The reagents listed below are examples. Suppliers of immunoaffinity columns may offer dilution solutions and eluents suitable for their products. If so, it is preferable to use these products.

3.1.1 Sodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$) CAS [10028-24-7].

3.1.2 Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) CAS [10049-21-5].

3.1.3 Sodium chloride (NaCl) CAS [7647-14-5].

3.1.4 Purified water for laboratories, for example EN ISO 3696 quality.

3.1.5 Phosphate buffer (dilution solution).

Dissolve 60.0 g of $Na_2HPO_4 \cdot 2H_2O$ (3.1.1) and 8.8 g of $NaH_2PO_4 \cdot H_2O$ (3.1.2) in 950 mL of water (3.1.4) and add more water (3.1.4) to make up to 1 litre.

3.1.6 Phosphate buffer saline (washing solution).

Dissolve 2.85 g of $Na_2HPO_4 \cdot 2H_2O$ (3.1.1), 0.55 g of $NaH_2PO_4 \cdot H_2O$ (3.1.2) and 8.70 g of NaCl (3.1.3) in 950 mL of water (3.1.4) and add more water to make up to 1 litre.

3.1.7 Methanol (≥ 99.9 % purity) (CH_3OH) CAS [67-56-1].

3.2 Reagents for HPLC

3.2.1 Acetonitrile for HPLC (CH_3CN) CAS [75-05-8].

3.2.2 Acetic acid (100 % purity) (CH_3COOH) CAS [64-19-7].

3.2.3 Mobile phase: water: acetonitrile: acetic acid, 99 : 99 : 2, v/v/v (approximate proportions)

Mix 990 ml of water (3.1.4) with 990 mL of acetonitrile (3.2.1) and 20 mL of acetic acid (3.2.2). In the presence of undissolved components, filter through a 0.45 µm filter. Degas (with helium, for example) unless the HPLC equipment used includes a degassing step.

3.3 Reagents for the preparation of the OTA stock solution

3.3.1 Toluene ($C_6H_5CH_3$) CAS [108-88-3].

3.3.2 OTA (≥ 99.5 % purity) CAS [303-47-9].

3.3.3 Solvent mixture (toluene: acetic acid, 99 : 1, v/v)

Mix 99 parts in volume of toluene (3.3.1) with one part volume of acetic acid (3.2.2).
A commercial solution of standard concentration (around 50 µg/mL~~l~~), with an analysis certificate stating the reference value and uncertainty of the concentration it is of preferential use than the use of OTA in solid form.

3.4 OTA stock solution

Dissolve 1 mg of OTA or the same content in a bulb, if the OTA was obtained in the form of film after evaporation, in the solvent mixture (3.3.3) to obtain a solution containing approximately 20 to 30 µg/mL of OTA.
To determine the exact concentration (if necessary), record the absorption spectrum between 300 and 370 nm in a quartz space with 1 cm of optical path while using the solvent mixture (3.3.3) as a blank. Identify maximum absorption and calculate the concentration of OTA (COTA) in µg/mL by using the following equation:
$COTA = Amax \times M \times 100 / \varepsilon \times \delta$
In which:
Amax = Absorption determined by the longest maximum wave (about 333 nm);
M = OTA molecular mass = 403.8 g/mol;
ε = OTA molar extinction coefficient in the solvent mixture (3.3.3) (ε = 544 m^2/mol);
δ = optical pathway (cm).
This solution is stable at -18 °C for at least 4 years.

3.5 Standard OTA solution 2 µg/mL (toluene: acetic acid, 99 : 1, v/v)

Dilute the stock solution (3.4) with the solvent mixture (3.3.3) to obtain a standard solution of OTA with a concentration of 2 µg/mL.
This solution can be stored at ~~–~~4 °C in a refrigerator. The stability should be tested in use.

4. Apparatus

Usual laboratory equipment and in particular, the following equipment:

4.1 Glass tubes (4 mL).
4.2 ~~Calibrated~~ Aanalytical balance.
4.3 ~~Calibrated~~ Vvolumetric flasks.
4.4 ~~Calibrated~~ Vvolumetric pipettes.
4.5 ~~Calibrated~~ Micropipettes.
4.6 Solid Phase Extraction (SPE) system for immunoaffinity columns.
4.7 Reservoir and flow tube adapted to immunoaffinity columns.
4.8 Glass microfiber filters.
4.9 Immunoaffinity columns specifically for OTA.

The column should have the total link capacity of at least 100 ng OTA. This will allow for a purification yield of at least 85 % when a diluted solution of the sample containing 100 ng OTA is passed through.

4.10 Liquid chromatograph, with a pump for the mobile phase capable of achieving a constant flow rate of 1 mL/min isocratic. Injection system must be equipped with 100 µl loop.
4.11 Column of analytical HPLC in steel 150 × 4.6 mm (i.d.) filled with a stationary phase C18 (5 µm) preceded with a pre-column or a pre-filter (0.5 µm) containing an appropriate phase. Different size columns can be used provided that they guarantee a good base line and background noise enabling the detection of OTA peaks, among others.

4.12 Fluorescence detector is connected to the column and the excitation wavelength is set at 333 nm and the emitting wavelength at 460 nm.
4.13 Information retrieval system.
4.14 U.V. spectrophotometer.

5. Preparations of samples (given as example)

5.1 For grape juice, reconstituted grape juice, and nectar

Pour 10 ml of the sample in a 100 mL conical flask. Add 10 mL of the dilution solution (3.1.5). Mix vigorously. Filter through glass microfibre filter (4.8). Filtration is necessary for cloudy solutions or when there is precipitation after dissolving.

5.2 For concentrated grape juice

First, dilute the juices five times (m/m) with water (3.1.4). Consider this dilution in the final calculation of the OTA concentration (item ~~7~~8). Then, Pour 10 mL~~l~~ of the diluted sample in a 100 mL conical flask. Add 10 mL of the dilution solution (3.1.5). Mix vigorously. Filter through glass microfibre filter (4.8). Filtration is necessary for cloudy solutions or when there is precipitation after dissolving.

6. Procedure

6.1 Purification by immunoaffinity column (as an example)

Samples are diluted with a suitable solvent according to the instructions on the kit manufacturer. This solution is filtered and purified on an immunoaffinity column. Set up the immunoaffinity column (4.9) to the SPE system (4.6), and attach the reservoir (4.7)
Add 10 mL (equivalent to 5 mL of the sample) of the diluted solution in the reservoir. Put this solution through the immunoaffinity column at a flow of 1 drop per second. The immunoaffinity column should not become dry. Wash the immunoaffinity column with 5 mL of washing solution (3.1.6) and then with 5 ml of water (3.1.4) at a flow of 1 to 2 drops per second. Blow air through to dry column. Elute OTA in a glass tube (4.1) with 2 mL of methanol (3.1.7) at the rate of 1 drop per second. Evaporate the eluate to dryness at 50 °C with nitrogen. Dissolve again immediately in 250 µl of the HPLC mobile phase (3.2.3) and keep at 4 °C until the HPLC analysis.

6.2 HPLC analysis

Using the injection loop, inject 100 µl of reconstituted extract in the chromatography.
Operating conditions (as an example)
Flow: 1 mL/min.
Mobile phase: acetonitrile: water: acetic acid (99 : 99 : 2, v/v/v) (3.2.3)
Fluorescence detector: Excitation wavelength = 333 nm
Emitting wavelength = 460 nm
Volume of injection: 100 µL

7. Quantification of ochratoxin A (OTA)

The quantification of OTA should be calculated by measuring the area or the height of the peaks at the OTA retention time and compared to the calibration curve.

7.1 Calibration curve (as an example)

Prepare a calibration curve daily or every time chromatographical conditions change. Measure out 0.5 mL~~l~~ of the standard OTA solution (3.5) at 2 µg/mL in a glass tube (4.1) and evaporate the solvent using nitrogen. Dissolve again in 10 mL in the HPLC mobile phase (3.2.3) which was previously filtered using a 0.45 µm filter. This produces an OTA solution of 100 µg/L. Prepare at least 6 HPLC calibration solutions in five 5 mL calibrated volumetric flasks following Table 1 (as an example).
Complete each 5 mL standard solution with HPLC mobile phase (3.2.3).
Inject 100 µL of each solution in the HPLC.

Table 1. Calibration curve

Calibration standards	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
µL of mobile phase filtered HPLC (3.2.3)	4990	4975	4950	4900	4770	4500
µL ~~-~~of OTA solution at 100 µg/L	10	25	50	100	250	500
OTA concentration (µg/L)	0.2	0.5	1.0	2.0	5.0	10
Injected OTA (ng)	0.02	0.05	0.10	0.20	0.50	1.00

NOTES:

1. *If the quantity of OTA in the samples is outside the calibration range, an appropriate dilution should occur. In these cases, the calculation of the final concentration (8) should be reviewed on a case by case basis.*
2. *Check in the Guide to the critical points of the method of measuring ochratoxin A by immunoaffinity column (appendix III-* method OIV-MA-AS315-10).

8. Calculations

Calculate the quantity of OTA in the aliquot of the solution tested and injected in the HPLC column.
Calculate the concentration of OTA (COTA) in µg/L by using the following formula:
COTA = MA × F/V1 × V3/V2
Where:
MA is the mass of ochratoxin A (in µg) in the aliquot part of the template injected on the column and evaluated from the calibration curve.
F is the dilution factor
V1 is the sample volume to be analysed (0.01 L)
V2 the volume of the solution tested and injected in the column (100 µL)
V3 is the volume of solution used to dissolve the dry eluate (250 µL)

The recovery rate of the immunoaffinity columns must be taken into consideration in the case of direct calibration by synthetic solutions.

9. Expression of the results

The amount of OTA is expressed in micrograms per liter (µg/L) with two significant figures ~~decimals~~ taking into account uncertainty.

10. Characteristics of the method

An internal validation study was carried out for the purpose of assessing the suitability

of the method for grape juices, taking into account linearity, limits of detection and quantification and the accuracy of the method. The latter parameter was determined by defining the levels of precision and trueness of the method.

10.1 Linearity of the method

Based on the results obtained from the linear regression analysis, the method proved to be linear within the ranges shown in the Figure 1 and in the Table 2.

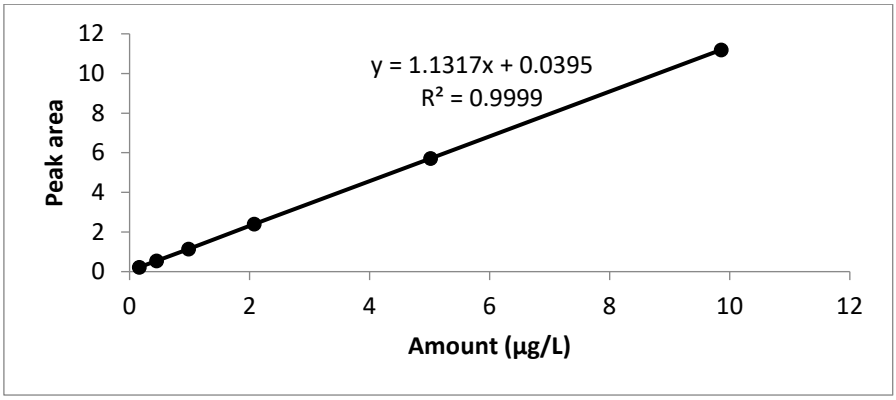

Figure 1. OTA calibration curve.

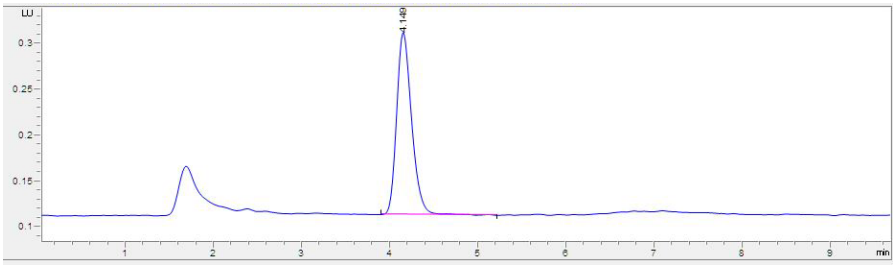

Figure 2. Chromatogram of 2.0 µg/L OTA standard.

10.2 Limit of detection and limit of quantification

The detection limit (LD) and the limit of quantification (LoQ) were calculated from a series of 8 analytical repetitions of a grape juice spiked with 0.10 µg/L of OTA and is equal to 3 standard deviations for the LD and 10 standard deviations for the LoQ (Table 2).

10.3 Precision of the method

The parameters taken into account were repeatability and reproducibility. Table 2 shows the values of these parameters. The repeatability was expressed as the relative standard deviation of measurements repeated in different concentrations found for grape juice. And the reproducibility was expressed as the average of the relative standard deviation of measurements of the same grape juice sample, made by different operators.

10.4 Trueness of the method

The percentage recovery was determined using a grape juice sample spiked in 6 concentrations levels of OTA, ranging from 0.10 µg/L to 10 µg/L. Each level was analyzed 5 times. An example of a recovery chromatogram is given in Figure 3.

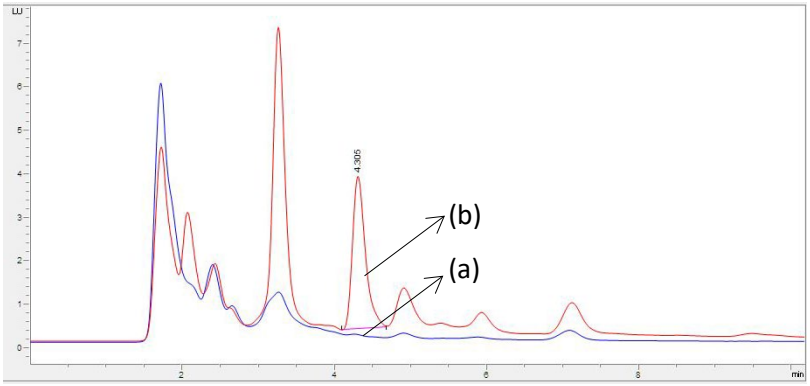

Figure 3. An example of a recovery chromatogram of a grape juice (a) and a grape juice spiked with 2 µg/L of OTA (b).

Table 2. Characteristics of the method.

Linearity range (µg/L)	Determination coefficient (r^2)	LD (µg/L)	LoQ (µg/L)	Repeatability (n=7) RSD%	Reproducibility (n=7) RSD%	Recovery (%)
0.20 - 10	0.9999	0.12	0.22	4.79	5.17	104.2 ± 2.9

11. Bibliography

OIV. Compendium of International Methods of Analysis of Wines and Musts. Method OIV-MA-AS315-10.

EN ISO 3696. Water for analytical laboratory use — Specification and test methods (ISO 3696:19958~~7~~).

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FINAL DRAFT RESOLUTION
OENO-SCMA 19-662C Et8[1]

DETERMINATION OF VOLATILE ACIDITY IN GRAPE JUICE, RECONSTITUTED GRAPE JUICE, CONCENTRATED GRAPE JUICE, AND GRAPE NECTAR

Type IV method

THE GENERAL ASSEMBLY,

IN VIEW ofOF Article 2, paragraph iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the "Methods of Analysis" Sub-Commission,

CONSIDERING that for grape juice, reconstituted grape juice, concentrated grape juice, and grape nectar is proposed the application of the Method OIV-MA-AS313-02 (Volatile Acidity) of the Compendium of International Methods of Analysis of Wines and Musts,

CONSIDERING that for concentrated grape juice a simple dilution need to be done before the analysis,

DECIDES to add the following method:

DETERMINATION OF VOLATILE ACIDITY IN GRAPE JUICE, RECONSTITUTED GRAPE JUICE, CONCENTRATED GRAPE JUICE, AND GRAPE NECTAR

Type IV method

1. Scope

This method applies to volatile acid determination in grape juice, reconstituted grape juice, concentrated grape juice, and grape nectar by titration, at the indicated concentrations ranging from 0.11 up to 1.09 g/L in acetic acid.

[1] All the project of resolutions concerning grape juices methods were kept in step 3 and they were not discussed one by one during the meeting.

Before discussing each method of analysis, it was decided to create a work group with the following objectives:

- to draw up general principles for grape juices methods;
- to assign each analyte to a category of method for its analysis (new method required; existing method available which needs to be reviewed and can be adopted; or no method required); discussing the need to creating a dedicated Compendium of analytical methods for grape juice analysis.

2. Definition

The volatile acidity is derived from the acids of the acetic series present in grape juice, reconstituted grape juice, concentrated grape juice, and grape nectar in its free state and combined as salts.

3. Principle

Volatile acids are separated from the grape juice, reconstituted grape juice, concentrated grape juice*, and grape nectar by steam distillation and titrated using sodium hydroxide solution of known concentration. The acidity of free and combined sulfur dioxide distilled under these conditions should be subtracted from the acidity of the distillate. The acidity of any sorbic acid, which may have been added to the grape juice, reconstituted grape juice, concentrated grape juice, and grape nectar must also be subtracted.
Note: Part of the salicylic acid used to stabilize the grape juices before analysis is present in the distillate. This must be determined and subtracted from the acidity as show on section 7.3.

4. Reagents and materials

4.1 Reagents

4.1.1 Tartaric acid 99.5 % ($C_4H_6O_6$) CAS [87-69-4].
4.1.2 Sodium hydroxide (NaOH) CAS [1310-73-2] solution 0.1 M.
4.1.3 Phenolphthalein 98 % ($C_20H_{14}O_4$) CAS [77-09-8].
4.1.4 Hydrochloric acid 37 % (HCl) CAS [7647-01-0].
4.1.5 Iodine (I_2) CAS [7553-56-2] solution 0.005 M.
4.1.6 Potassium iodide 99.0 % (KI) CAS [7681-11-0].
4.1.7 Starch $(C_6H_{10}O_5)_n$ CAS [9005-84-9] ~~5 g/L~~.
4.1.8 Sodium tetraborate 99.5 % ($Na_2B_4O_7.10H_2O$) CAS [1303-96-4].
4.1.9 Acetic acid 99.8 % (CH_3COOH) CAS [64-19-7] solution 0.1 M.
4.1.10 Lactic acid 85 % ($C_3H_6O_3$) CAS [50-21-5].
4.1.11 Sodium chloride 99.0 % (NaCl) [7647-14-5].
4.1.12 Sodium thiosulfate 99.5 % ($Na_2S_2O_3.5H_2O$) CAS [10102-17-7] solution 0.1 M or a commercial solution.
4.1.13 Iron (III) ammonium sulfate 99.0 % ($Fe(NH_4)_2(SO_4)_2.12H_2O$) CAS [7783-83-7] 10 % (m/v).
4.1.14 Sodium salicylate 99.5 % ($NaC_7H_5O_3$) CAS [54-21-7] solution 0.01 M.
4.1.15 Ethanol 96 % (C2H6O) CAS [64-17-5].
4.1.16 Water Type 1 (EN ISO 3696) or equivalente ultrapure.

4.2 Preparation of solutions

4.2.1 Phenolphthalein solution 1 % m/v
Mix 1 g of phenolphthalein (4.1.3) in 100 mL of ethanol (4.1.15).
Commercial solution can be used.
4.2.2 Starch indicator solution 5 g/L
Mix 5 g of starch (4.1.7) with about 500 mL of water (4.1.16). Bring to the boiling point, stirring continuously and boil for 10 min. Add 200 g of sodium chloride (4.1.11). When cool, complete to one liter of water (4.1.16).
4.2.3 Saturated solution of sodium tetraborate about 55 g/L
Mix 55 g of sodium tetraborate (4.1.8) in one liter of water (4.1.16).

4.2.4 Lactic acid solution 0.1 M - 90 g/L

Dilute 100 mL of lactic acid (4.1.10) in 400 mL of water (4.1.16). As an example, this solution is heated in an evaporating dish over a boiling water bath for four hours, topping up the volume occasionally with water (4.1.16). After cooling, complete to a liter of water (4.1.16). Titrate the lactic acid in 10 mL of sodium hydroxide solution (4.1.2). Adjust the concentration of the lactic acid solution for 90 g/L.

5. Apparatus

5.1 Steam distillation apparatus consisting of:

— a steam generator; the steam must be free of carbon dioxide;
— a flask with a steam pipe;
— a distillation column;
— a condenser.

This equipment must pass the three following tests:

(a) place 20 mL of distilled water free of CO_2 in the flask. Collect 250 mL of the distillate and add to it 0.1 mL of sodium hydroxide solution (4.1.2) and two drops of phenolphthalein solution (4.2.1). The pink coloration must be stable for at least 10 s (i.e. steam to be free of carbon dioxide);

(b) place 20 mL acetic acid solution (4.1.9), in the flask. Collect 250 mL of the distillate. Titrate with the sodium hydroxide solution (4.1.2) and two drops of phenolphthalein solution (4.2.1). The volume of the titer must be at least 19.9 mL (i.e. at least 99.5 % of the acetic acid entrained with the steam;

(c) place 20 mL lactic acid solution (4.2.4), in the flask. Collect 250 mL of the distillate and titrate the acid with the sodium hydroxide solution (4.1.2) and two drops of phenolphthalein solution (4.2.1). The volume of sodium hydroxide solution (4.1.2) added must be less than or equal to 1.0 mL (i.e. not more than 0.5 % of lactic acid is distilled).

Any apparatus (automated or not) or procedure, which passes these tests satisfactorily, fulfils the requirements of official international apparatus or procedures.

5.2 Volumetric material

5.3 Analytical balance verified and calibrated

5.4 ~~Calibrated~~ V~~v~~olumetric pipettes of 10 mL and 20 mL

5.5 ~~Calibrated~~ B~~b~~urette

5.6 Water bath~~.~~

6. Procedure

6.1 Preparation of sample

For gasified grape juices to eliminate CO_2:

place about 50 mL of grape juice, reconstituted grape juice, and grape nectar in a vacuum flask; apply vacuum to the flask with the water pump for one to two minutes while shaking continuously. Other CO_2 elimination systems may be used if the CO_2 elimination is guaranteed.

6.2 Steam distillation

Place 20 mL of grape juice, reconstituted grape juice, concentrated grape juice* and grape nectar into the flask. Add about 0.5 g of tartaric acid (4.1.1). Collect at least 250 mL of the distillate.

* The concentrated grape juice must be diluted (200 g to 500 mL, for example) and multiply the result by the dilution factor (F = 2.5, in this case).

6.3 Titration

Titrate with the sodium hydroxide solution (4.1.2), using two drops of phenolphthalein solution (4.2.1) as indicator. Let *n* mL be the volume of sodium hydroxide solution (4.1.2) used. Add four drops the hydrochloric acid (4.1.4) dilute 1/4 (v/v) with water (4.1.16), 2 mL of starch solution (4.2.2) and a few crystals of potassium iodide (4.1.6). Titrate the free sulfur dioxide with the iodine solution (4.1.5). Let *n'* mL be the volume used. Add the saturated solution of sodium tetraborate (4.2.3) until the pink coloration reappears. Titrate the combined sulfur dioxide with the iodine solution (4.1.5). Let *n"* mL be the volume used.

7. Calculation (Results)

7.1 Method of calculation

The volatile acidity, expressed in milliequivalents per liter to one decimal place, is given by:

$$5\ (n - 0.1\ n' - 0.05\ n'').$$

The volatile acidity, expressed in grams of acetic acid per liter to two decimal places, is given by:

$$0.30\ (n - 0.1\ n' - 0.05\ n'').$$

n = volume (mL) of sodium hydroxide solution (4.1.2~~mL~~) used.
n' = volume (mL) of iodine solution (4.1.5~~mL~~) used on the titration of the free sulfur dioxide.
n" = volume (mL) of iodine solution (4.1.5~~mL~~) used on the titration of the combined sulfur dioxide.
Note: In the case of the concentrated grape juice multiply the result by the dilution factor (F).

7.2 Samples with sorbic acid present

Since 96% of sorbic acid is steam distilled with a distillate volume of 250 mL, its acidity must be subtracted from the volatile acidity, knowing that 100 mg of sorbic acid corresponds to an acidity of 0.89 milliequivalents or 0.053 g of acetic acid and knowing the concentration of sorbic acid in mg/L as determined by other methods.

7.3 Samples with salicylic acid present

7.3.1 Identification of salicylic acid in the volatile acidity distillate

Immediately after the determination of the volatile acidity and the correction for free and combined sulfur dioxide, introduce into a conical flask 0.5 mL hydrochloric acid (4.1.4), 3 mL of the sodium thiosulfate solution (4.1.12) and 1 mL of the iron (III) ammonium sulfate solution (4.1.13). If salicylic acid is present, a violet coloration appears.

7.3.2 Determination the salicylic acid

On the above conical flask, indicate the volume of the distillate by a reference mark. Empty and rinse the flask. Subject a new test sample of 20 mL grape juice or reconstituted grape juice or concentrated grape juice (see section 6.2) or nectar to steam distillation and collect the distillate in the conical flask up to the reference mark. Ensure that the same volume of distillate is collected as in section 7.3.3. Add 0.3 mL hydrochloric acid (4.1.4) and 1 mL of the iron (III) ammonium sulfate solution (4.1.13). The contents of the conical flask turn violet. Into a conical flask identical to that carrying the reference mark, introduce distilled water (4.1.16) up to the same level as that of the distillate. Add 0.3 mL hydrochloric acid (4.1.4) and 1 mL of the iron (III) ammonium sulfate solution (4.1.13). Titrate the distillate water in the conical frask with sodium salicylate solution (4.1.14) until the violet coloration obtained has the same intensity as that of the conical flask containing the wine distillate. Let *n'''* mL be the volume of solution added from the burette.

7.3.3 Correction to the volatile acidity

Subtract the volume 0.1 x *n'''* mL from the volume *n* mL of sodium hydroxide solution (4.1.2) used to titrate the acidity of the distillate during the determination of volatile acidity.

8. Indicative characteristics of the method

A validation study with grape juice was carried out for the purpose of assessing the suitability of the method for the matrices in question, taking into account linearity, limits of detection and quantification and the accuracy of the method. The latter parameter was determined by defining the levels of precision and trueness of the method.

8.1 Linearity of the method

The method proved to be linear within the ranges 0.11 g/L – 1.09 g/L shown in the Table 1.

8.2 Limit of detection and limit of quantification

The detection limit (LD) and limit of quantification (LoQ) were calculated from 7 analytical repetitions of an 0.11 g/L aqueous acetic acid solution and is equal to 3 standard deviations for the LD and 10 standard deviations for the LoQ (Table 1).

8.3 Precision of the method

The parameters taken into account were repeatability and reproducibility. Table 1 shows the values of these parameters. The repeatability was expressed as Relative Standard Deviation (%RSD) of measurements repeated in different concentrations found for grape juice. And the reproducibility was expressed as the average of the Relative Standard Deviation (%RSD) of measurements made of the same grape juice sample, by different operators.

8.4 Trueness of the method

The percentage recovery was determined using a grape juice sample spiked ~~with~~ in 6 different levels of acetic acid, ranging from 0.11 g/L to 1.09 g/L.

Table 1. Characteristics of the method

Linearity range (g/L of acetic acid)	Correlation coefficient (r^2)	LD (g/L of acetic acid)	LoQ (g/L of acetic acid)	Repeatability (n = 7) %RSD~~%~~	Reproducibility (n = 7) %RSD~~%~~	Average recovery (%)
0.11 – 1.09	0.9905	0.09	0.16	2.75	3.15	103.63

9. Bibliography

OIV. Compendium of International Methods of Analysis of Wines and Musts. Method OIV-MA-AS313-02:R2015.
BS EN ISO 3696, Water for analytical laboratory use — Specification and test methods, 1995.

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				10/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SCMA 19-662H Et8

METHOD FOR $^{18}O/^{16}O$ ISOTOPE RATIO DETERMINATION OF WATER IN GRAPE JUICE

Type IV methods

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the "Methods of Analysis" Sub-Commission,

CONSIDERING that for grape juice the determination could be performed based on the method OIV-MA-AS2-12 of the *Compendium of international methods of wine and must analysis*, with a modification added at the points:

DECIDES to add the following method:

METHOD FOR $^{18}O/^{16}O$ ISOTOPE RATIO DETERMINATION OF WATER IN GRAPE JUICE

1. Scope

The method describes the determination of the isotopic ratio $^{18}O/^{16}O$ of water from grape juice after equilibration with CO_2, using the isotope ratio mass spectrometry.

2. Definitions

Oxygen isotopic ratio $^{18}O/^{16}O$ has been used to identify the water added in the grape juice.

3. Principle

The method is based on the equilibration of water from the grape juice with CO_2, according to the following isotopic exchange reaction:

$$C^{16}O_2 + H_2{}^{18}O \leftrightarrow C^{16}O^{18}O + H_2{}^{16}O$$

After equilibration the carbon dioxide in the gaseous phase is used for analysis by means of Isotopic Ratio Mass Spectrometry (IRMS) where the $^{18}O/^{16}O$ isotopic ratio is determined on the CO2 resulting from the equilibration.

4. Reagents and materials

The materials and consumables depend on the method used. The systems generally used are based on the equilibration of water in grape juice with CO_2.
The following reference materials, working standards and consumables can be used:

4.1 Reference materials

NAME	Issued by	$\delta^{18}O$ related to V-SMOW
VSMOW2	NIST/IAEA	0 ‰
GISP	NIST	-24.78 ‰
SLAP	NIST/IAEA	-55.5 ‰

4.2 Working Standards

4.2.1 Carbon dioxide used for equilibration in the case of continuous flow systems cylinders containing gas mixture helium-carbon dioxide can also be used).
4.2.2 Working Standards with calibrated $\delta^{18}O$ VSMOW values traceable to international reference materials.

4.3 Consumables

- Helium for analysis (CAS 07440-59-7);
- Carbon dioxide for analysis, used as a reference gas (CAS 00124-38-9);

5. Apparatus

The equipment and interfaces depend on the method and can be used as follow

5.1 Isotope Ratio Mass Spectrometer (IRMS)

Isotope ratio mass spectrometer (IRMS) is able for determining the relative ^{18}O content of CO_2 naturally occurring with an internal accuracy of 0.05 ‰ or better expressed as a relative value. The mass spectrometer used to measure isotope ratios is generally equipped with a triple collector to simultaneously measure intensities for m/z = 44, 45 and 46.

5.2 Equipment and Materials

The equipment and materials used must meet stated requirements of the used method/apparatus (as specified by the manufacturer). However, all equipmentand materials can be replaced by items with similar performance.

5.2.1 Vials with septa appropriate for the used system;
5.2.2 Volumetric pipettes with appropriate tips;
5.2.3 Temperature controlled system to carry out the equilibration at constant temperature, typically within ±1 °C;
5.2.4 Vacuum pump (if needed for the used system);
5.2.5 Autosampler (if needed for the used system);

5.2.6 Syringes for sampling (if needed for the used system);
5.2.7 GC Column to separate CO2 from other elementary gases (if needed for the used system).
5.2.8 Water removal device (e.g. selective permeable membrane)

6. Sampling

Juice samples as well as a reference material are used for analysis without any pre-treatment. Possible fermentation must be avoided adding benzoic acid (or another anti fermentation product) or filtered by membrane with pore diameter of 0.22 µm.

7. Procedure

The descriptions that follow refer to procedures generally used for the determination of the $^{18}O/^{16}O$ isotopic ratios by means of equilibration of water with a CO_2 working standard and the subsequent measurement by IRMS. These procedures can be altered according to changes of equipment and instrumentation provided by the manufacturers as various kind of equilibration devices are available, implying various conditions of operation. Two main technical procedures can be used for introduction of CO_2 into the IRMS either through a dual inlet system or using a continuous flow system. The technical systems and the corresponding operation conditions are given as an example
Note: all values given for volumes, temperatures, pressures and time periods are only indicative. Appropriate values must be obtained from specifications provided by the manufacturer and/or determined experimentally.

7.1 Manual equilibration

A defined volume of the sample/standard is transferred into a flask using a pipette.
The flask is then attached tightly to the manifold.
Each manifold is cooled down to below -80°C to deep-freeze the samples (manifold equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated. After reaching a stable vacuum the gaseous CO_2 working standard is allowed to expand into the various flasks. For the equilibration process each manifold is placed in a temperature controlled water bath typically at 25°C (± 1 °C) for 12 hours (overnight). It is crucial that the temperature of the water-bath is kept constant and homogeneous.
After the equilibration process is completed, the resulting CO_2 is transferred from the flasks to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet.
This approach is repeated till the last sample of the sequence has been measured.

7.2 Use of an automatic equilibration apparatus

A defined volume of the sample/standard is transferred into a vial using a pipette.
The sample vials are attached to the equilibration system and cooled down to below -80°C to deep-freeze the samples (systems equipped with capillary opening tubes do not require this freezing step). Subsequently, the whole system is evacuated.
After reaching a stable vacuum the gaseous CO_2 working standard is expanded into the vials. Equilibrium is reached at a temperature of typically 22 ± 1 °C after a minimum period of 5 hours and with moderate agitation (if available). Since the equilibration duration depends on various parameters (e.g. the vial geometry, temperature, applied agitation ...), the minimum equilibrium time should be determined experimentally.

After the equilibration process is completed, the resulting CO_2 is transferred from the vials to the sample side bellow of the dual inlet system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet.
This approach is repeated till the last sample of the sequence has been measured.

7.3 Manual preparation manual and automatic equilibration and analysis with a dual inlet IRMS

A defined volume of sample / standard (eg. 200 µL) is introduced into a vial using a pipette. The open vials are then placed in a closed chamber filled with the CO_2 used for equilibration (4.2.1). After several purges to eliminate any trace of air, the vials are closed and then placed on the thermostated plate of the sample changer. The equilibration is reached after at least 8 hours at 40 °C. Once the process of equilibration completed, the CO_2 obtained is dried and then transferred into the sample side of the dual inlet introduction system. The measurements are performed by comparing several times the ratios of the CO_2 contained in the sample side and the standard side (CO_2 reference standard gas) of the dual inlet.
This approach is repeated till the last sample of the sequence has been measured.

7.4 Use of an automatic equilibration apparatus coupled to a continuous flow system

A defined volume of the sample/standard is transferred into a vial using a pipette.
The sample vials are placed into a temperature controlled tray.
Using a gas syringe the vials are flushed with mixture of He and CO_2. The CO_2 remains in the headspace of the vials for equilibration.
The temperature and the time for equilibration should be specified according to the manufacturer and/or determined experimentally. The equilibrium is normally reached at a temperature typically of 25 ± 1 °C after a minimum period of 18 hours.
After the equilibration process is completed, the resulting CO_2 is transferred by means of the continuous flow system into the ion source of the mass spectrometer.
CO_2 reference gas is also introduced into the IRMS by means of the continuous flow system. The measurement is carried out according to a specific protocol for each kind of equipment.

8. Calculation

The intensities for m/z = 44, 45, 46 are recorded for each sample and reference materials analysed. The $^{18}O/^{16}O$ isotope ratios are then calculated by the computer and the software of the IRMS instrument. In practice the $^{18}O/^{16}O$ isotope ratios are measured against a working standard previously calibrated against the V-SMOW. Small variations may occur while measuring online due to changes in the instrumental conditions. In such a case the $\delta^{18}O$ of the samples must be corrected according to the difference in the $\delta^{18}O$ value from the working standard and its assigned value, which was calibrated beforehand against V-SMOW. Between two measurements of the working standard, the variation is the correction applied to the sample results that may be assumed to be linear. Indeed, the working standard must be measured at the beginning and at the end of all sample series. Therefore, a correction can be calculated for each sample using linear interpolation between two values (the difference between the assigned value of the working standard and the measurements of the obtained values).

$$\delta^{18}O_{VSMOW} = \left[\frac{\left(\frac{18O}{16O}\right)_{Sample} - \left(\frac{18O}{16O}\right)_{VSMOW}}{\left(\frac{18O}{16O}\right)_{VSMOW}}\right] \times 1000 \quad [‰]$$

The $\delta^{18}O$ value normalized versus the V-SMOW/SLAP scale is calculated using the following equation:

$$\delta^{18}O_{VSMOW/SLAP} = \left[\frac{\delta^{18}O_{sample} - \delta^{18}O_{VSMOW}}{\delta^{18}O_{VSMOW} - \delta^{18}O_{SLAP}}\right] \times 55.5 \quad [‰]$$

9. Characteristics of the method

A validation study was carried out for the purpose of assessing the suitability of the method for the matrices in question, taking into account linearity, limits of detection and quantification and the accuracy of the method. The latter parameter was determined by defining the levels of precision and trueness of the method.

9.1 Precision of the method

The parameters taken into account were repeatability and intralaboratory reproducibility. Table 1 shows the values of these parameters. The repeatability was expressed as ‰ of measurements repeated in the same conditions and in the same day for all the grape juice. And the reproducibility was expressed as the average of a ‰ of measurements of the same grape juice sample, made by two operators.

9.2 Trueness of the method

The recovery percentage was determined using a grape juice sample spiked with 6 concentrations of tap water, ranging from 20% to 99.5%. Each level was analyzed 5 times. The trueness was also expressed in terms of bias using a reference material and calculating the relative error.

Table 1. Characteristics do the method

Correlation coefficient (R2)	Repeatability (n=16) ‰	Reproducibility (n=7) ‰	Recovery (%) ± SD	Trueness (RM) (‰)
0.998	0.20	0.29	101± 2.16	0.17

10. Bibliography

OIV. Method for $^{18}O/^{16}O$ isotope ratio determination of water in wines and must. Method OIV-MA-AS2-12.

STEP	1	2	3	4	5	6	7	8
DATE		04/2019	10/2019	10/2020	10/2020	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
OENO-SCMA 19-665 Et8

Determination of sweeteners in white wine and white wine-based beverages by high performance liquid chromatography coupled with a diode array detector and a charged aerosol detector

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL OF the "Methods of Analysis" Sub-Commission,

DECIDES to add the following method to Annex A of the *Compendium of International Methods of Wine and Must Analysis*:

Determination of sweeteners in white wine and white wine-based beverages by high performance liquid chromatography coupled with a diode array detector and a charged aerosol detector

Type IV method

1. Scope of application

This method makes it possible to determine five artificial sweeteners (acesulfame-K, aspartame, saccharine, sodium cyclamate and sucralose) in white wine (and white-wine-based beverages), within concentration ranges of up to 50 mg/L for saccharine, 125 mg/L for acesulfame-K and 250 mg/L for sucralose, sodium cyclamate and aspartame.

For greater concentrations, dilution of the sample is necessary.

Note: The presence of anthocyanins interferes with the determination of these sweeteners in rosé wine and red wines.

2. Principle

The five sweeteners are analysed by high performance liquid chromatography through separation on a C18 reverse-phase column coupled with a diode array detector and charged aerosol detector connected in series (HPLC/UV-CAD).

3. Reagents and solutions

3.1. Reagents:

3.1.1 Water compliant with EN ISO 3696 or equivalent
3.1.2 Acesulfame-K (purity ≥ 99%) (CAS No. 55589-62-3)
3.1.3 Aspartame (purity ≥ 98%) (CAS No. 22839-47-0)
3.1.4 Sodium cyclamate (purity ≥ 98%) (CAS No. 139-05-9)
3.1.5 Saccharine sodium salt dihydrate (purity ≥ 98%) (CAS No. 6155-57-3)
3.1.6 Sucralose (purity ≥ 98%) (CAS No. 56038-13-2)
3.1.7 Formic acid (purity ≥ 98%) (CAS No. 64-18-6)
3.1.8 Ammonium bicarbonate (purity ≥ 98%) (CAS No. 1066-33-7)
3.1.9 HPLC-grade methanol (purity ≥ 99.9%) (CAS No. 67-56-1)
3.1.10 HPLC-grade acetone (purity ≥ 99.8%) (CAS No. 67-64-1)

3.2. Preparation of buffer solution

Prepare an ammonium bicarbonate (3.1.8) buffer solution at 0.4 g/L in water (3.1.1) and adjust the pH to 4.6 with formic acid (3.1.7).

This solution can be kept for 1 month at room temperature.

4. Apparatus

4.1 Everyday laboratory equipment
4.2 pH meter
4.3 Stirrer
4.4 Ultrasound bath
4.5 Analytical balance with precision of ± 0.01 mg
4.6 Class A volumetric flasks
4.7 0.45 µm regenerated cellulose syringe filters (for example)
4.8 C18 HPLC column (15 cm in length, 4.6 mm in internal diameter, 5 µm)
4.9 Chromatography system composed of:
- pump system with a minimum of three channels,
- thermostatically-controlled sampler,
- column oven,
- diode array or UV-VIS detector,
- charged aerosol detector (CAD),
- system of data acquisition, integration and calculation.

5. Procedure

5.1 Preparation of samples

- Filter the sample with syringe filters before placing it in a vial,
- if the sample is too concentrated, dilute with the buffer solution to bring the concentration within the above-specified range.

5.2 Preparation of standard solutions

5.2.1 Preparation of the stock solution, L3 (given by way of example)

In a 100-mL flask, add the following approximate amounts (weighed and recorded accurately):

- 12.5 mg acesulfame-K (3.1.2),
- - 25 mg aspartame (3.1.3),
- - 25 mg sodium cyclamate (3.1.4),
- 5 mg saccharine (3.1.5),
- 25 mg sucralose (3.1.6).

Dissolve in approx. 20 mL water/ethanol (1:1), then make up to the mark with the buffer solution. Use ultrasonic processing if necessary.

This solution, L3, can be stored for 6 months at between 2 °C and 8 °C.

5.2.2 Preparation of working solutions, L2 and L1

Prepare two other concentration levels from solution L3:

- L2: solution L3 diluted to 1/2 its initial concentration. For example, add 10 mL L3 solution to a 20 mL flask and make up to volume with the buffer solution.
- L1: solution L3 diluted to 2/25 its initial concentration. For example, add 2 mL L3 solution to a 25 mL flask and make up to volume with the buffer solution.
  Filter the standard solutions with syringe filters and place the vials in the sampler.

Summary table of standard solutions (given by way of example):

Sweeteners	***Stock solution (Level 3)***			***Working solution (L2)***			***Working solution (L1)***		
	Weight (mg)	Flask volume	Concentration in mg/L	Sampled volume of L3	Flask volume	Concentration in mg/L	Sampled volume of L3	Flask volume	Concentration in mg/L
Acesulfame-K	12.5	100 mL	125	10 mL	20 mL	62.5	2 mL	25 mL	10
Aspartame	25		250			125			20
Na cyclamate	25		250			125			20
Saccharine	5		50			25			4
Sucralose	25		250			125			20

5.3 Chromatography conditions

By way of example, the conditions used to achieve the performance described in the annex are as follows:

- Column oven temperature: 30 °C

- Sampler temperature: 20 °C
- Composition of the mobile phase (HPLC-quality reagents):
    - Phase A: 72% methanol / 25% buffer / 3% acetone
    - Phase B: 12% methanol / 88% buffer
- UV detection wavelength: 210 nm
- Flow rate: 1 mL/min
- Injection volume: 10 µL
- CAD parameters:
    - Filter: 3.6
    - Data collection rate: 10 Hz
    - Temperature: high (50 °C)
    - Power function: 0-13 min: 1.50; 13-40 min: 1.48

- Elution gradient to be applied:

Temps (min)	Percentage of A	Percentage of B
0	0%	100%
4	0%	100%
11	53%	47%
18.5	82%	18%
25	82%	18%
27	100%	0%
31	100%	0%
32	0%	100%
40	0%	100%

Stabilise the column for as long as necessary with mobile phase B, in addition to the CAD and DAD.

6. Calculations

- The results are calculated by external calibration according to the peak area of each sweetener and expressed in mg/L, according to the calculation formula:

$$Concentration\ of\ sample\ (mg/L) = \frac{A_s - Int}{P} \times Dilution$$

Where A_s is the peak area of the sample, *Int* is the intercept of the calibration curve and *P* is the slope of the calibration curve.

7. Expression of results

For concentrations of < 10 mg/L, the results can be expressed in mg/L to one significant figure after the decimal point.

For concentrations of ≥ 10 mg/L, the results can be expressed in mg/L to the nearest whole number.

8. Bibliography

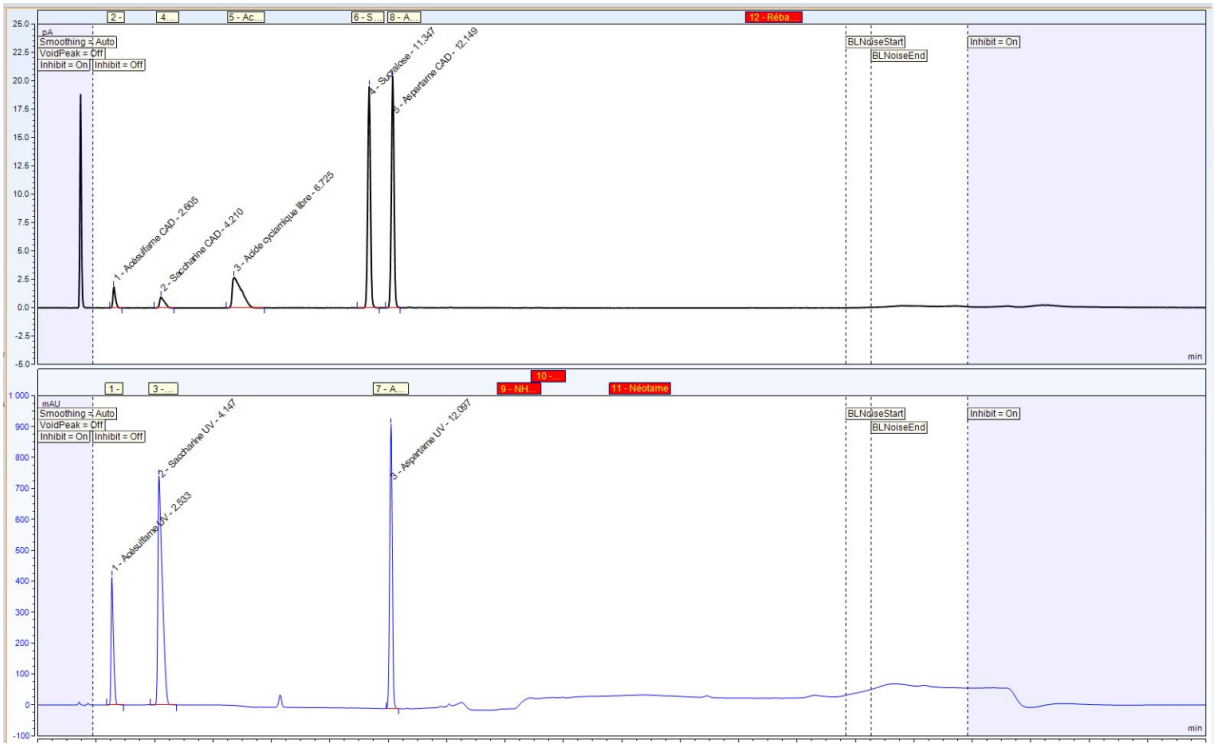
International Organisation of Vine and Wine (OIV), *Compendium of International Methods of Analysis of Wines and Musts*, Vol. 1 and 2.

NF EN 15911: Simultaneous determination of nine sweeteners by high performance liquid chromatography and evaporative light scattering detection in beverages and canned fruits.

ISO 3696: Water for analytical laboratory use — Specification and test methods.

ISO 11352: Water quality — Estimation of measurement uncertainty based on validation and quality control data.

Annex 1: Example chromatogram for a level 3 standard solution



~~Note: For information, this method can be used to highlight other sweeteners such as neotame, advantame, rebaudioside A and NHDC (neohesperidin dihydrochalcone). Their determination has nevertheless not been validated.~~

Annex 2: Example internal validation

The method has been the object of a performance evaluation and an internal validation study has been carried out for the white wine matrix and the white-wine-based beverage.
The method characteristics obtained as a result of this work are summarised in the following table:

Sweetener	Repeatability in % (r%)	Interlaboratory reproducibility in % (R%)	Limit of Detection applied in mg/L	Limit of Quantification applied in mg/L	Linearity range in mg/L
Acesulfame -K	2.5	8.5	2	5	5-125

Saccharine	1.7	6.8	1	2	2-50
Aspartame	1.9	8.7	4	10	10-250
Na Cyclamate	5.5	12.8	4	10	10-250
Sucralose	6.6	12.9	4	10	10-250

The Limit of Quantification applied corresponds to level 1; this is the first point within the calibration range.
The Limit of Detection applied corresponds to 1/3 of the Limit of Quantification.

The linearity has been verified by analysis of 5 concentration levels for each sweetener from 5 repetitions per level on different days.

The repeatability and reproductivity were obtained by analysis of each sweetener in each type of matrix on 5 different days with 2 repetitions each time, being on 3 concentration levels chosen in the linearity range of the method.

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FINAL DRAFT RESOLUTION
OENO-SCMA 20-667 Et8

WARNING: T**his draft resolution** amend~~modifie~~**s the following resolution:**
AG 8/78-OEN

UPDATE METHOD OIV-MA-AS-2-07B – CHROMATIC CHARACTERISTICS

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL OF the "Methods of Analysis" Sub-Commission,

CONSIDERING that the proposed modifications are intended to improve the method performance,

CONSIDERING that the scope of application of the current method, OIV-MA-AS2-07B, does not include must and sulphited must,

CONSIDERING that the current method, OIV-MA-AS2-07B, is not applicable to red wines with high colour intensity, even using a cell with an optical path length of 0.1cm,

DECIDES to modify the resolution AG 8/78-OEN and consequently the method OIV-MA-AS2-07B in Annex A of the *Compendium of International Methods of Analysis of Wines and Musts* by adding the following annex:

Operative instructions for the determination of Chromatic Characteristics of wines and/or musts obtained by grape varieties characterized by high concentrations of colouring pigments and/or high sulfur dioxide levels

1. Principle of the method

1.1 Field of application

Applicable to red wine with high concentrations of colouring pigments, must, and must with high sulphur dioxide levels.

A spectrophotometric method whereby chromatic characteristics are expressed, conventionally, as given below:

- The intensity of colour is given by the sum of absorbencies (or O.D. = Optical Densities) using a 1 cm optical path and radiation of wavelengths 420, 520 and 620 nm.
- The shade is expressed as the ratio of absorbance at 420 nm to absorbance at 520 nm.

For grape varieties characterized by high concentrations of colouring pigments, given the nature of the chemical structure of these substances, the determination of the chromatic characteristics requires the

dilution of the sample with a buffered solvent at pH 3.2. The use of a buffered solvent compared to dilution with water reduces the effect of the matrix and normalizes the O.D as the dilution increases.

2. Method

2.1. *Apparatus*

2.1.1 Spectrophotometer enabling measurements to be made between 300 and 700 nm.

2.1.2 Glass cuvettes or single use plastic cuvettes with optical path equal to 1 cm.

2.1.3 Volumetric glassware with variable volume according to needs

2.1.4 Syringe filter 0.45 µm

2.2 Reagents

2.2.1 Type II water for analytical use, ISO 3696 standard, or of equivalent purity

2.2.2 Tartaric acid ≥ 99.5% (CAS 87-69-4)

2.2.3 Sodium hydroxide NaOH 1 N (CAS 1310-73-2)

2.2.4 Hydrogen peroxide -30%- w/w (CAS 7722-84-1)

2.3 Working solutions

2.3.1 Buffer at pH 3.20

Daily preparation: weigh 7 ± 0.1 g of tartaric acid (2.2.2) in a 1000 mL volumetric flask, add 35 ml of NaOH 1 N (2.2.3) and make up to 1000 mL with water (2.2.1). Check the pH with a pH meter and verify that pH is 3.20±0.05. The solution should be checked and filtered (2.1.4) at the time of use.

2.3.2 Hydrogen peroxide 3% (v/v)

Dilute 1.0 mL of ~~30%~~ hydrogen peroxide (2.2.4) to 10 ml. The solution should be prepared at the time of use.

2.4. *Preparation of the sample*

If the sample is cloudy, clarify it by centrifugation (10 min, 2500 rcf~~rpm~~); If there is carbon dioxide remove it by agitation under vacuum (or similar systems).

In the case of grape must whose alcoholic fermentation is inhibited by adding sulphur dioxide, add 0.1 mL of 3% hydrogen peroxide solution (2.3.2) per mL of sample used and make up to volume, depending on the dilution chosen, with the buffer solution at pH 3.2 (2.3.1). Wait 20 minutes, then proceed with spectrophotometric reading.

2.5 Spectrophotometric reading for wine and must with high colour intensity or high sulphur dioxide levels

Take the spectrophotometric measurements of the samples: the absorbance (*A*) will fall between 0.3 and 1.0 (the absorbance acceptability range can be extended if instrumental technology allows it) If the *A*-value is above the maximum limit, make an appropriate number of dilutions (*d*) of the sample using the buffer solution (2.3.1) to meet the acceptability criteria.

Take the spectrophotometric measurements using the buffer solution as the reference liquid to set the absorbance scale of the apparatus to zero at the wavelengths of 420, 520 and 620 nm.

2.6 *Calculations*

Calculate the optical densities (O.D.) for each of the three wavelengths by multiplying the detected absorbances (A_{420}, A_{520} and A_{620}) by the number of dilutions made (*d*):

- $\text{D. O. a } 420 \text{ nm } = A_{420} \times d$
- $\text{D. O. a } 520 \text{ nm } = A_{520} \times d$
- $\text{D. O. a } 620 \text{ nm } = A_{620} \times d$

2.7 *Expression of results*

The colour intensity (*I*) is conventionally given by:

$I = A_{420} + A_{520} + A_{620}$

and is expressed to three decimal places.

The shade (*N*) is conventionally given by:

$N = A_{420} / A_{520}$

and is expressed to three decimal places.

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FINAL DRAFT RESOLUTION
OENO-SCMA 20-683 Et8

IMPORTANT: This draft resolution modifies the following resolution:
- OIV-OENO 13-2002

UPDATE TO METHOD OIV-MA-AS323-02B – Quantification of total nitrogen according to the Dumas method (musts and wines)

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL OF the "Methods of Analysis" Sub-Commission,

CONSIDERING that the proposed modifications are intended to improve the method performance,

DECIDES to modify the resolution OENO 13-2002 and consequently the method OIV-MA-AS323-02B in Annex A of the *Compendium of International Methods of Analysis of Wines and Musts* by making the following amendments:

Method OIV-MA-AS323-02B Type IV Method

UPDATE TO METHOD OIV-MA-AS323-02B

Quantification of total nitrogen according to the Dumas method
(musts and wines)
(Resolution Oeno 13/2002)

1 - Field of application
This method applies to the analysis of total nitrogen in musts and wine up to 1000 mg/L.

2 - Principle
The analysis of total nitrogen in an organic matrix can be carried out using the Dumas method. This involves the total combustion of the matrix in the presence of oxygen at a temperature higher than 900°C, followed by an additional oxidation. The nitrogen oxides are then reduced in dinitrogen, which is quantified using a thermal conductivity detector (katharometer), after elimination of the other oxides, water traces and carbon dioxide.
The nature and positioning of the traps varies according to the type of material.

3 - Reagents and materials
3.1 - Nitrogen (technical quality)

3.2 - High-purity helium for GC (e.g. $H_2O \leq 3$ ppm; $O_2 \leq 2$ ppm; CnHm ≤ 1 ppm; $N_2 \leq 5$ ppm)
3.3 - High-purity oxygen for GC (e.g. $H_2O \leq 3$ ppm; Ar ≤ 3 ppm; CnHm ≤ 0,2 ppm; $N_2 \leq 5$ ppm)
3.4 - Demineralised water (e.g. ISO 3696 type I or HPLC grade)
3.5 - Oxidant (e.g. copper oxide [1317-38-0], chromium sesquioxide [1308-38-9] or silvered cobaltous/cobaltic oxide [1308-06-1])
3.6 - Reducer (e.g. copper [7440-50-8])
3.7 - Dehydrating agents (e.g. sodium hydroxide on silica or mixed with quartz crystals [1310-73-2], anhydrous magnesium perchlorate [10034-81-8] or calcium sulfate [7778-18-9])
3.8 – Product for calibration range (e.g. tris(hydroxymethyl)aminomethane [77-86-1] or atropine [51-55-8])
3.9 - Internal standard (e.g. glutamic acid hydrochloride [138-15-8], or a sample from an inter-laboratory proficiency-testing programme)

4 - Apparatus

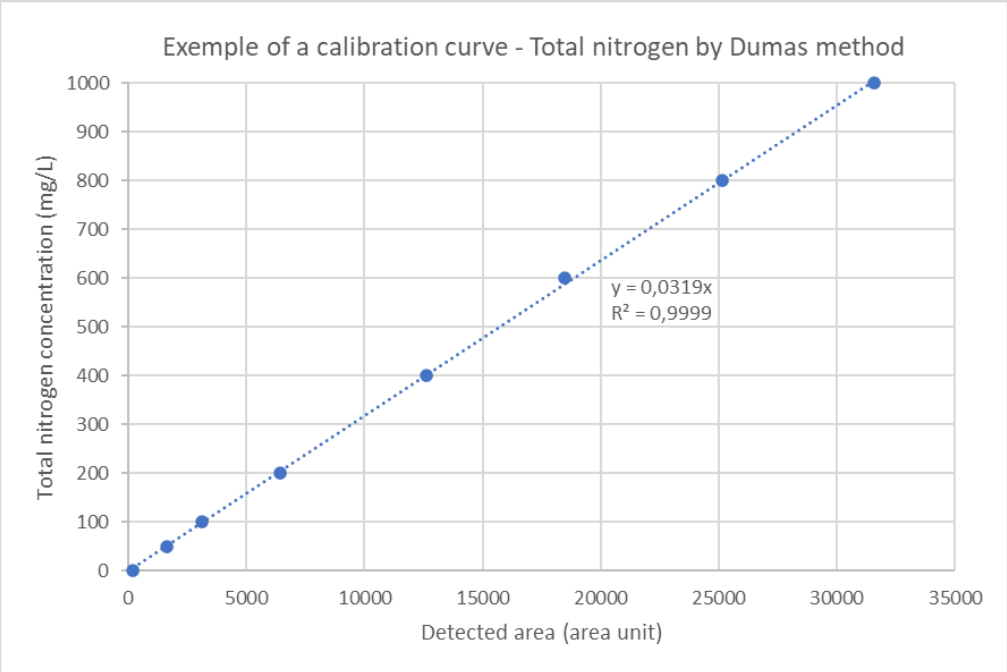
4.1 – Centrifuge
4.2 – ultrasonic bath
4.3 - Total nitrogen analyser optionally equipped with a sample changer
4.4 - Precision balance with precision of ± 0.01 mg between 0.5 mg and 30 g

5 - Sampling

If the sample contains a significant amount of carbon dioxide, degas for example by bubbling nitrogen (3.1) for 5 to 10 min or by using an ultrasound bath. For musts, centrifuge (4.1).

6 - Procedure

6.1 - Preparation of the calibration range (given by way of example)
From a 1 g N/L solution, prepare five samples of TRIS (3.8) at 800, 500, 250, 100 and 50 mg N/L. The calibration curve therefore passes through seven points, from a blank to the 1 g N/L solution and follows a linear model. It is recorded in the instrumental method. Analyse the 500 mg N/L standard before starting each analytical sequence, and as soon as necessary to adjust the analyser.



6.2 - Preparation of control samples (given by way of example)
Control samples are used regularly at the beginning and in the middle of analysis series. They can be made up using the hydrochloride form of glutamic acid (3.9) at 150, 300 and 600 mg N/L in demineralised water (3.4).

$$C_{glutamic\ acid} = \frac{M_{glutamic\ acid} \times C_N}{M_N}$$

With :

- $C_{glutamic\ acid}$ the glutamic acid concentration in the solution, expressed in g/L
- C_N the nitrogen concentration in the solution, expressed in g/L
- $M_{glutamic\ acid}$ the molar mass of glutamic acid. $M_{glutamic\ acid}$ = 183,59 g/mol
- M_N the molar mass of nitrogen. M_N= 14,007 g/mol

Weigh (4.4) 7.864 g of glutamic acid (3.9) and dilute in demineralised water (3.4) and make up to 1 L to obtain a 600 mg N/L solution. Dilute by 50% by transferring 250 mL of this solution and diluting to 500 mL to obtain a 300 mg N/L solution. Repeat with 250 mL of the 300 mg N/L solution to 500 mL to obtain a 150 mg N/L solution.

6.3 - Preparation of samples
Weigh (to the nearest 0.01 mg) the volume defined for the analysis of the must or wine with the precision balance (4.4). Place the samples on the analyser awaiting analysis.

6.4 – Analysis of samples
Carry out the analyses of samples and standards as per instrument manufacturer's instructions.

7 - Expression of results

The results are expressed in mg/L of nitrogen, rounded to the nearest whole number.

8 - Precision

Number of laboratories	Average contents	Repeatability r	Reproducibility R
11	591 mg/L	43 mg/L	153 mg/L

9 - Bibliography

DUMAS, A.: *Annales de chimie*, vol. 33, 1826, p.342.
Buckee, G. K.: *Determination of total nitrogen in Barley, Malt and Beer by Kjeldahl procedures and the Dumas combustion method*, Collaborative trial. J. Inst. Brew., vol. 100, 1994, pp. 57-64.
ISO 3696: Water for analytical laboratory use — Specification and test methods.
Instrument manufacturer's instruction manual, or equivalent.

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DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
CST-SCMA 20-668 Et8

OPINION OF THE OIV ON TOTAL DRY EXTRACT (TOTAL DRY EXTRACT, TOTAL DRY EXTRACT WITHOUT SUGARS, RESIDUAL EXTRACT)

THE GENERAL ASSEMBLY,

CONSIDERING Article 2 paragraph 2) iv) of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING the decision of the Steering Committee of the OIV in its session of 17 October 2019,

CONSIDERING the decision of the Executive Committee to do not apply the accelerated procedure under Rule 21 of the Rules of Procedure,

CONSIDERING that historically, the dry extract has been used from time to time as indicator of certain not permitted practices,

CONSIDERING that the dry extract can be used together with other parameters to evaluate the quality of wines and possible fraud,

CONSIDERING the opinion of the experts of the Subcommittee on Methods of Analysis of the OIV, in their session of April 2019, who indicated that the analysis of the dry extract of wines is still considered for the detection of frauds in some countries, being understood that the minimal limit of total dry extract, if considered in isolation, is an obsolete parameter in this context.

ON THE PROPOSAL of the Sub-Commission on Methods of Analysis,

DECIDES to complete Annex D of the International Analysis of Wine and Musts the following opinion:

dry extract (total dry extract also called total dry matter, sugar free dry extract also called non-reducing extract, residual extract

The dry extract of musts and wines includes all matter that is non-volatile when measured under specified physical conditions. These physical conditions must be such that the matter forming the dry extract undergoes as little alteration as possible while the analyse is being carried out.

The total dry extract, sugar free dry extract and residual extract are defined and measured as described in the OIV COMPENDIUM OF INTERNATIONAL METHODS OF ANALYSIS in Method OIV-MA-AS2-03A and Method OIV-MA-AS2-03B.

The value obtained for the dry extract depends solely on a physical measurement of non-volatile compounds of musts and wines (sugars, acids, minerals ...) that vary from one harvest to another, from one harvesting location to another, could be impacted by seasonal and regional weather

conditions and irrigation practices, and is also influenced by the wine making process including the use of permitted additives.
It is therefore not possible to establish fixed reference values which allow detection of wine frauds solely through measurement of total dry extract or, sugar free dry extract or residual extract, while it can be used in conjunction with other parameters to evaluate a possible fraud.

The OIV declares that the sole measurement of the total dry extract, total dry matter, sugar free dry extract or residual extract, if considered in isolation, it's not sufficient for the detection of possible frauds.

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DATE		06/2020	10/2020	03/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
ECO-DROCON 20-685 Et8

WARNING: this resolution amends the following resolution:
ECO 1/88 "List of optional indications for wine labelling"

UPDATE OF THE INTERNATIONAL STANDARD FOR WINE LABELLING: MEDALS AND DISTINCTIONS

THE GENERAL ASSEMBLY

IN VIEW of the article 2, paragraph 2 iii of the Agreement of 3rd April 2001, establishing the International Organisation of Vine and Wine,

CONSIDERING the decision of Executive Committee of April 2017 regarding the necessity to update the International Standard for Wine Labelling,

CONSIDERING The resolution ECO 332A/2009 "OIV standard for international wine and spirituous beverages of vitivinicultural origin competitions" and in particular its article 3 "Products eligible to compete",

CONSIDERING the international practice of conduction of international competitions of wines and the widely admitted possibility for wines non entitled to a Geographical Indication or an Appellation of Origin to take part in such competitions,

CONSIDERING that the requirement of indication of the batch identification is specified in the article 2.7 "Batch Identification" of the OIV International Standard for labelling of wine,

ON THE PROPOSAL of the Commission Economy and Law,

THE GENERAL ASSEMBLY

DECIDES TO modify the article 3.1.9 "Medals and distinctions" of the OIV International Standard for labelling of wines as well as the resolution ECO 1/88 as follows:

3.1.9. Medals and Distinctions

- In the first bullet point delete the following phrase: "having the entitlement to a recognised appellation of origin or a recognised geographic indication"
- Replace the second bullet point by the following text: "documentary proof exists, referring to the batch (article 2.7) concerned"
- Delete the third bullet point "the label shows the volume of the batch of wine, subject of the distinction, or an official control number"
- Add the following new bullet point: "Medals and distinctions received by an individual wine bear the name of the competition, the year of award and as a general rule shall only be applied to wines bearing the name under which the sample was entered. However, in recognition that a wine could be entered in a competition before a wine has been finally allocated to a Brand,

portability of an award so that it can stay with the wine which won it shall be permitted providing the wine competition regulations so allow"

Current version	**Version after the application of proposed amendments**
Article 3.1.9 Medals and Distinctions	**Article 3.1.9 Medals and Distinctions**
The indication relating to medals or distinctions depends on whether: - they were attributed in a non-restricted competition, according to the standards concordant with the criteria defined by the O.I.V., and on a homogenous and defined quantity of wine having the entitlement to a **recognised appellation of origin or a recognised geographic indication**, - documentary proof exists, - the label shows the volume of the batch of wine, subject of the distinction, or an official control number.	The indication relating to medals or distinctions depends on whether: - they were attributed in a non-restricted competition, according to the standards concordant with the criteria defined by the O.I.V., and on a homogenous and defined quantity of wine ~~having the entitlement to a **recognised appellation of origin or a recognised geographic indication**,~~ - documentary proof exists, referring to the batch (article 2.7) concerned ~~- the label shows the volume of the batch of wine, subject of the distinction, or an official control number.~~ - Medals and distinctions received by an individual wine bear the name of the competition, the year of award and as a general rule shall only be applied to wines bearing the name under which the sample was entered. However, in recognition that a wine could be entered in a competition before a wine has been finally allocated to a Brand, portability of an award so that it can stay with the wine which won it shall be permitted providing the wine competition regulations so allow.
	This resolution modified the resolution ECO 1/88

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DATE		03/2021	10/2021			05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
SECSAN-SECUAL 21-701 Et8

WARNING: This resolution amends the following resolutions:
- AG 11/67-OEN
- AG 2/53-OEN

REVISION OF THE MAXIMUM LIMIT OF ARSENIC IN WINE

IN VIEW of the article 2 paragraph iv of the Agreement of 3rd April 2001 establishing the International Organisation of Vine and Wine,

ON THE PROPOSAL of the " Food Safety " Expert group,

IN VIEW of the Resolution OENO 11/1967 which establishes the limits for Arsenic,

CONSIDERING the works of the Food Safety Expert group, and especially the collective expertise document on *"Arsenic and wine: a review"* published in 2021,

CONSIDERING the evaluation from the JECFA, specifically the new toxicological evaluation of Arsenic in food,

DECIDES, on the proposal of Commission IV " Safety and Health", to amend Part B of the Resolution OENO 11/1967 regarding the Arsenic limit of 0.2 mg/L specifically,

DECIDES to replace in all relevant OIV documents the existing Arsenic limit with the following:

* 0.1 mg/L for wine made, starting from the 2023 harvest year,

ENCOURAGES the member states to disseminate pertinent information as widely as possible in order to maintain Arsenic contents at the technologically lowest possible levels.

STEP	1	2	3	4	5	6	7	8
DATE		10/2021	10/2021			05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
SECSAN-SECUAL 21-709 Et8

WARNING: This resolution amends the following resolution:
- OIV/OENO 427/2010

UPDATE TO THE RESOLUTION OIV-/OENO 427-/2010 ON CRITERIA FOR THE QUANTIFICATION OF ALLERGENS

The GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 iv of the Agreement establishing the International Organisation of Vine and Wine,

AT THE PROPOSAL of the "Methods of Analysis" Sub-Commission,

CONSIDERING that the OIV's activities are designed to help to protect consumer health and contribute to food safety,

CONSIDERING Resolution OIV-OENO-SECSAN 520-2014, "Code of good fining practices for wine to be applied in the use of proteinaceous wine fining agents with allergenic potential (casein and egg white)",

CONSIDERING Resolution OENO-COMEX 502-2012, "Revision of the limit of detection and limit of quantification related to potentially allergenic residues of fining agent proteins in wine",

Indicating that these criteria should be reconsidered according to the decisions of other international bodies,

Recognises that no specific methods for the determination of protein fining agents in wine have been defined and several ELISA methods are already available and applicable,

DECIDES to modify the Resolution OIV-OENO 427-2010, "Criteria for the methods of quantification of potentially allergenic residues of fining agent proteins in wine", in particular Table 1, "Performance criteria for methods of analyses for potentially allergenic fining agent proteins in wine", as follows, including the deletion of the part indicated below in bold and italics in the "Applicability" row.

Table 1: Performance criteria for methods of analyses for potentially allergenic fining agent proteins in wine

Parameter	**Value/Comment**
Applicability	Suitable for determining fining agents in wine.
Limit of detection	(expressed in mg/L) Casein caseinates: at least 0.25 Ovalbumin: at least 0.25 Isinglass : at least 0.25 Lysozyme : at least 0.25
Limit of quantification	(expressed in mg/L) Casein-caseinates: at least 0.5 mg/L Isinglass : at least 0.5 mg/L Lysozyme : at least 0.5 mg/L Ovalbumin : at least 0.5 mg/L
Precision	HORRAT values of less than or equal to 2 in the validation collaborative trial
Recovery	80% - 105% (as indicated in the collaborative trial)
Specificity	Free from matrix interferences
Trueness	$\left\|\bar{x} - m\right\| < 1.96 * \sqrt{s_{R(lab)^2} - s_{r(lab)^2} * \left(1 - 1/n\right)}$ where *m* is the certified value of the wine reference material and $\bar{x}$ is the average of *n* measurements of compound content in this wine, within the same laboratory. $S_r(lab)$ are standard deviations, calculated from results within the same laboratory under repeatability conditions. $S_R(lab)$ are standard deviations, calculated from results within different laboratories under reproducibility conditions.

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FINAL DRAFT RESOLUTION
SECSAN-SECUAL 21-710 Et8

WARNING: This resolution amends the following resolution:
- OIV-OENO-SECSAN 520-2014

UPDATE TO THE RESOLUTION OIV-OENO-SECSAN 520-2014 ON THE CODE OF GOOD FINING PRACTICES FOR WINE TO BE APPLIED IN THE USE OF PROTEINACEOUS WINE FINING AGENTS WITH ALLERGENIC POTENTIAL

THE GENERAL ASSEMBLY,

IN VIEW OF Article 2, paragraph 2 b) ii of the Agreement of 3 April 2001 establishing the International Organisation of Vine and Wine,

CONSIDERING that the labelling regulations of certain countries impose the mention of allergens if these have been used during production and can be determined in the finished food product, which applies to implementation of these regulations for wine,

CONSIDERING that without a legal limit food allergens proteins must be declared on the label when allergenic protein(s) from food is/are present and detectable in the finished vitivinicultural product by any analytical method,

IN VIEW OF the work of the “Food Safety” Expert Group,

DECIDES to modify point 5 of Resolution OIV-OENO-SECSAN 520-2014 by deleting the initial phrase below crossed out in bold and italics and adding the final part indicated in bold and italics:

~~***While regulations do not provide a specific threshold level,***~~
Typical analytical methods for food allergens can detect residues in the low parts per million (ppm) range. If these methods do not detect any allergenic protein in the wine, then it could be considered that no residue above the detection limit is present. ***The choice of the analytical method to be applied and of the relative limits of detection and quantification is the producer’s responsibility. If there is a national/supranational limit, the use of the criteria suggested by the OIV for methods of quantification of potentially allergenic residues of fining agent proteins in wine is recommended to Member States and producers, provided that the limits of detection and quantification are compatible.***

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DATE		06/2020	10/2020	04/2021	10/2021	05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
SECSAN- CONUSA 20-679 Et8

RECOMMENDATIONS ON THE DISSEMINATION OF INFORMATION ON WINE CONSUMPTION

THE GENERAL ASSEMBLY,

CONSIDERING that article 2 of the Agreement of 3rd April 2001, establishes the objects and functions of the OIV, such as to inform its members of measures whereby the concerns of producers, consumers and other players in the vine and wine products sector may be taken into consideration. To achieve these objectives, the OIV's activities shall be:

g) to help protect the health of consumers and to contribute to food safety, by promoting and guiding research into appropriate nutritional and health aspects; and

n) to gather, process and disseminate the most appropriate information and to communicate it;

CONSIDERING the OIV's Strategic Plan 2020-2024 (Axe III. A) to encourage research and to collect and disseminate scientific information on the effects of the wine consumption, grapes and other vine-based products on health, together and consistent with the WHO and the other relevant organizations;

CONSIDERING that consumers of wine should be fully informed about the characteristics and quality of what they consume, and the consequences for their health of their consumption, in order to make informed choices;

CONSIDERING that the "Safety and Health" Commission, has been gathering, processing and disseminating information on the health effects of alcohol consumption with the main objective to facilitate informed decisions about wine consumption;

CONSIDERING the World Health Organization's (WHO) Global Strategy[1] to Reduce the Harmful Use of Alcohol, endorsed by the WHO Assembly in 2010, contains information about health risks associated with

[1]The WHO global strategy to reduce the harmful use of alcohol, 2010;
http://www.who.int/substance_abuse/msbalcstragegy.pdf

alcohol consumption and provides a menu of policy options based on international consensus, which the OIV has considers as a starting point to identify a set of recommendations to facilitate informed decisions about wine consumption;

CONSIDERING the above mentioned, these recommendations presents results from systematic reviews and meta-analyses[2] and aims to facilitate for wine consumers to make informed decisions about their consumption. It aims to inform wine drinkers that they should consume wine with meals and in accordance with national drinking guidelines, and in certain circumstances abstain from drinking alcoholic beverages altogether (e.g. when underage, driving or during pregnancy);

CONSIDERING that public health is an area that falls within the competence of Member States, these recommendations should be implemented **at the discretion of each Member State in line with national public health priorities**, taking into account national circumstances, such as religious and cultural contexts, national public health priorities, as well as resources, capacities and capabilities[3]

Recognises that the identified literature suggests that:

~~1.~~ the risk of harm associated with alcohol consumption is dependent on the frequency and quantity of ethanol consumption and frequency of binge drinking, and the consumption occasion, for instance with or without a meal.

~~1.~~

2. the risk of harm associated with alcohol consumption is individual and varies with genetic and environmental factors including sex, age, health status and socioeconomic position.
3. the risk of harm associated with alcohol consumption is influenced by conscious choices including choice of diet (e.g. Mediterranean diet), exercise and smoking.

TAKING INTO CONSIDERATION the following OIV resolutions:

[2] OIV has not followed PRISMA guidelines on systematic reviews

[3] Global strategy to reduce the harmful use of alcohol, WHO, 2010 https://www.who.int/publications/i/item/9789241599931

- Resolution OENO 1/98, which indicates the importance of developing systems of education and information that take into account the legislation specific to the member countries;
- Resolution ECO 4/99, which indicates the factors that may influence alcohol consumption, such as genetic characteristics, culture, religion, family, peer group, media, education and the availability of products; which indicates that most educational programs focus on negative consequences without proposing recommendations on a healthy lifestyle and on how one who chooses to drink can do so properly and with appropriate control of risk; which underlines that all information concerning the effects of wine on our health must be presented in a competent and balanced manner;
- Resolution OENO 72/2000, which indicates that further research is needed in the field of wine and health;
- Resolution OENO 73/2000, which recommends that Member States, if they choose to publicly highlight the potential benefits to human health of regular and moderate wine consumption, accompany and balance any such statement with caveats highlighting the adverse effects and harms of excessive alcohol consumption to the individual and to the community;

TAKING INTO ACCOUNT the works of other organizations on the effects of alcohol consumption on health and in particular:

- WHO Global Strategy to Reduce the Harmful use of Alcohol which defines objectives and measures to reduce the negative health and social consequences of harmful use.
- WHO Europe which recommends to reduce consumption of beverage alcohol .
- WHO Europe Health Evidence Network Synthesis Report which explores the implementation and effectiveness of policies based on the New Nordic and Mediterranean diets.
- The American Heart Association, American Diabetic Association and Mayo Clinic which recommend not to drink wine or any other form of alcohol to gain potential health benefits and to consume alcoholic beverages only in moderation if at all.
- The World Cancer Research Fund which recommends for cancer prevention to limit alcohol consumption l, not to exceed their national guidelines.

- The Alzheimer Society which states that moderate alcohol consumption has not been conclusively linked to risk of dementia or cognitive decline.

Reccomends that communication directed to wine consumers should contain:

- information on the risk associated with harmful consumption alcoholic beverages beyond national drinking guidelines ;
- information tailored at risk groups, for example pregnant women or lactating women;
- actionable information to mitigate risk, for example recommend limiting consumption to meals and alternate with water; and
- information on drinking limits according to national drinking guidelines;

Recognises that more research is warranted on for example:

- mechanisms underlying the association between wine consumption and health ;
- consumers' perceptions of wine consumption being compatible with a healthy lifestyle; and
- association between wine consumption with meals and as part of a healthy diet and health.

STEP	1	2	3	4	5	6	7	8
DATE		10/2021	10/2021			05/2022	11/2022	11/2022

FINAL DRAFT RESOLUTION
SECSAN-CONUSA 21-711 Et8

RECOMMENDATION ON THE EVALUATION/ASSESSMENT AND THE COMMUNICATION OF EPIDEMIOLOGICAL STUDIES CONCERNING THE CONSUMPTION OF GRAPES, WINE AND OTHER VITIVINICULTURAL PRODUCTS

CONSIDERING that Article 2 of the Agreement of 3rd April 2001, establishes the objects and functions of the OIV, such as to inform its members of measures whereby the concerns of producers, consumers and other players in the vine and wine products sector may be taken into consideration. To achieve these objectives, the OIV's activities shall be:
g) to help protect the health of consumers and to contribute to food safety, by promoting and guiding research into appropriate nutritional and health aspects; and
n) to gather, process and disseminate the most appropriate information and to communicate it,

CONSIDERING the OIV's Strategic Plan 2020-2024 (Axe III. A) to encourage research and to collect and disseminate scientific information on the effects of wine consumption, grapes and other vine-based products on health, together and consistent with the WHO and other relevant organizations,

CONSIDERING that public health is an area that falls within the competence of Member States, these recommendations should be implemented at the discretion of each Member State in line with national public health priorities,

CONSIDERING that consumers of wine should be fully informed about the characteristics and quality of what they consume,

CONSIDERING that the "Safety and Health" Commission, has been gathering, processing and disseminating information on the health effects of alcohol consumption with the main objective to facilitate informed decisions about wine consumption,

CONSIDERING the importance of epidemiology and its use as defined by WHO: epidemiology is the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems. Various methods can be used to carry out epidemiological investigations: surveillance and descriptive studies can be used to study distribution; analytical studies are used to study determinants.

CONSIDERING that the "Safety and Health" Commission's works take into account the expertise document on the Evaluation and use of epidemiological evidence for grapes, wine and other vitivinicultural products consumption research,

RECOMMENDS that with assessment/ evaluation of and communication of epidemiological studies concerning the consumption of grapes, wine and other vitivinicultural products the following criteria should be taken into account:

1. Analysis and limits

A statistical analysis should be completed with biological interpretation. The data collected or obtained during an epidemiological study must be analysed in conformity with the study protocol. Nonetheless, data collected in a study may legitimately be analysed to evaluate hypotheses that were not explicitly formulated in the initial protocol, or for a secondary purpose different from that originally intended. Any significant change from the statistical methodology described in the study protocol must be expressly mentioned in any publication or presentation of the study results.

2. Quality control for the study

The quality of the data collected, obtained, produced or published during or as part of an epidemiological study should be guarantee.

3. Scientific integrity

All study results regardless of whether the funders are public or private, are under the scientific supervision of the epidemiologist who is the principal investigator, and not of the funder, and the results should be published if their scientific validity is sufficient. Before submitting for publication any requests to hide the results or change or attenuate the content of a report or to delay publication must be categorically rejected.

As generally request, the authors of epidemiological articles should report their possible conflicts of interest and inform who funded the research.

In order to ensure the above criteria, an assessment of the risk of bias in accordance with the well-acknowledged guidelines per study design should be conducted.

Recommend_for “any communication on epidemiological studies concerning the consumption of grapes, wine and other viti-vinicultural products” that the following criteria should be taken into account:

1. Impartiality

Users of published epidemiological studies should consider that results generally constitute only a small part of the information available, and some bias may affect the choice of data published, in selecting results that agree with the epidemiologist's point of view and not mentioning those that contradict it. This type of partiality or bias must be avoided.

2. Balance

Any communication must describe every aspect of the epidemiological study in an honest and balanced manner, without taking any other interests, especially non-scientific, into account.

3. Good practices

Communication should use clear, precise, sincere and confident information.

Communication should be trustworthy and transparent and impartial.

Communication should take into account that science is a process of collecting data and building strong evidence and must not contain speculations on what may be or not may be relevant in the future.