

Summary

Polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP) is an insoluble adsorbent resin with two main properties of interest in winemaking:

- The specific adsorption capacity for heavy metals such as copper and iron, as well as for aluminium, allows higher concentrations to be removed, thereby preventing hazes in wines.
- By eliminating heavy metals, such as copper, it prevents the destruction of varietal thiols (A3MH, 3MH, 4MMP, etc.) present in several white varietals such as Sauvignon Blanc, as well in certain red wines.
- The prevention of browning through the adsorption of phenolic compounds such as 3,4-dihydroxycinnamic acid derivatives, which otherwise enter into enzymatic and nonenzymatic reactions. The products of these reactions are responsible for the undesirable brown discoloration of white and rosé wines.
- Prevention of pinking by eliminating catalysers of oxidation such as iron and copper and by reducing the polyphenolic content of wine.
- Iron and copper should not be completely removed, as traces of these elements (0.05 to 0.1 mg/L for copper and 2 to 3 mg/L for iron) are important for the oxido-reductive potential of wine(see for example Oenartis, 2015b).

The European Union requested the addition of PVI/PVP copolymer to the Annex of the Wine Agreement in late 2015. Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement. To ensure consistency with the Food Standards Code it is requested to amend the table to clause 14 (Permitted processing aids with miscellaneous functions) of Standard 1.3.3 of the Food Standards Code to include PVI/PVP copolymer. In the revised food Standards Code it would probably fall under Schedule 18-9 (Processing Aids that perform various technological purposes). It is also proposed to include PVI/PVP copolymer in the table to clause 4 of Standard 4.5.1 to permit the use of PVI/PVP copolymer as a processing aid by Australian producers.

PVI/PVP copolymer is insoluble in alcoholic beverages. The precipitates it forms with unwanted components in alcoholic beverages during processing are removed via filtration or similar processes. Therefore, no analytical method is needed to check for residues. Standard 1.3.4 requires that substances added to food, including processing aids, comply with relevant specifications as detailed in the Code. PVI/PVP copolymer meets the OIV specification which is one of the secondary references for specifications in Standard 1.3.4 (Identity and Purity). Therefore, no new specification is required for the Code.

**Part B: APPLICATION TO AMEND THE AUSTRALIA AND
NEW ZEALAND FOOD STANDARD CODE FOR THE INCLUSION OF Polyvinylimidazole-
Polyvinylpyrrolidone Copolymers (PVI/PVP) AS A PROCESSING AID FOR WINE**

Contents

Summary	3
3.1 GENERAL REQUIREMENTS	4
3.1.2 Applicant details.....	4
3.1.3 PURPOSE OF THE APPLICATION	5
3.1.4 JUSTIFICATION FOR THE APPLICATION	5
A. REGULATORY IMPACT INFORMATION	6
3.1.5 INFORMATION TO SUPPORT THE APPLICATION	6
A. Technical Information on the Processing Aid	7
1. <i>Information on the type of processing aid</i>	7
2. <i>Information on the identity of the processing aid</i>	7
3. <i>Information on the chemical and physical properties of the processing aid</i>	7
4. <i>Manufacturing process</i>	8
5. <i>Specification for identity and purity</i>	8
6. <i>Analytical method of detection</i>	8
B. Information Related to the Safety of a chemical processing aid	8
1. <i>General Information on the Industrial use of this chemical</i>	8
2. <i>General information on the use of the chemical as a food processing aid in other countries</i>	8
§173.50 Polyvinylpyrrolidone.	9
1. <i>Information on the toxicity of the chemical processing aid and, if necessary, its major metabolites</i> .10	
5. <i>Safety assessment reports prepared by international agencies or other national government agencies if available</i>	10
F. Information Related to the Dietary Exposure to the Processing Aid.	10
3.1.10 STATUTORY DECLARATION	13
3.1.11 CHECKLIST	13
References	14

Summary

Polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP) is an insoluble adsorbent resin with two main properties of interest in winemaking:

- The specific adsorption capacity for heavy metals such as copper and iron, as well as for aluminium, allows higher concentrations to be removed, thereby preventing hazes in wines.
- By eliminating heavy metals, such as copper, it prevents the destruction of varietal thiols (A3MH, 3MH, 4MMP, etc.) present in several white varietals such as Sauvignon Blanc, as well in certain red wines.
- The prevention of browning through the adsorption of phenolic compounds such as 3,4-dihydroxycinnamic acid derivatives, which otherwise enter into enzymatic and nonenzymatic reactions. The products of these reactions are responsible for the undesirable brown discoloration of white and rosé wines.
- Prevention of pinking by eliminating catalysers of oxidation such as iron and copper and by reducing the polyphenolic content of wine.
- Iron and copper should not be completely removed, as traces of these elements (0.05 to 0.1 mg/L for copper and 2 to 3 mg/L for iron) are important for the oxido-reductive potential of wine (see for example Oenartis, 2015b).

The European Union requested the addition of PVI/PVP copolymer to the Annex of the Wine Agreement in late 2015. Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement. To ensure consistency with the Food Standards Code it is requested to amend the table to clause 14 (Permitted processing aids with miscellaneous functions) of Standard 1.3.3 of the Food Standards Code to include PVI/PVP copolymer. In the revised food Standards Code it would probably fall under Schedule 18-9 (Processing Aids that perform various technological purposes). It is also proposed to include PVI/PVP copolymer in the table to clause 4 of Standard 4.5.1 to permit the use of PVI/PVP copolymer as a processing aid by Australian producers.

PVI/PVP copolymer is insoluble in alcoholic beverages. The precipitates it forms with unwanted components in alcoholic beverages during processing are removed via filtration or similar processes. Therefore, no analytical method is needed to check for residues. Standard 1.3.4 requires that substances added to food, including processing aids, comply with relevant specifications as detailed in the Code. PVI/PVP copolymer meets the OIV specification which is one of the secondary references for specifications in Standard 1.3.4 (Identity and Purity). Therefore, no new specification is required for the Code.

3.1 GENERAL REQUIREMENTS

3.1.2 Applicant details

Winemakers' Federation Australia

ABN: 38 359 406 467

Email: wfa@wfa.org.au

Level 1, Industry Offices

National Wine Centre

Botanic Road

Adelaide SA 5000

AUSTRALIA

Postal Address

PO Box 2414

Kent Town SA 5071

AUSTRALIA

Tel: +618 8133 4300

Fax: +618 8133 4366

Person Responsible

[REDACTED]

Winemakers' Federation of Australia

NFF House

14-16 Brisbane Avenue

Barton ACT 2600

AUSTRALIA

Postal Address

PO Box 3891

Manuka ACT 2603

AUSTRALIA

[REDACTED]

[REDACTED]

3.1.3 PURPOSE OF THE APPLICATION

The intention of this application is to allow polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP) as a processing aid for wine *via* amendment of Standard 4.5.1. Wine Production Requirements (Australia only) and as a permitted processing aid in food (Standard 1.3.3). The European Union requested the addition of PVI/PVP copolymer to the Annex of the Wine Agreement in late 2015. Provisional approval was granted for the use of these products in European wine exported to Australia under the Wine Agreement. To ensure consistency with the Food Standards Code it is requested to amend the table to clause 14 (Permitted processing aids with miscellaneous functions) of Standard 1.3.3 of the Food Standards Code to include PVI/PVP copolymer. In the revised food Standards Code it would probably fall under Schedule 18-9 (Processing Aids that perform various technological purposes). It is also proposed to include PVI/PVP copolymer in the table to clause 4 of Standard 4.5.1 to permit the use of PVI/PVP copolymer as a processing aid by Australian producers.

3.1.4 JUSTIFICATION FOR THE APPLICATION

a) Need for the Proposed Change.

PVI/PVP is an insoluble adsorbent resin with two main properties of interest in winemaking:

- The specific adsorption capacity for heavy metals such as copper and iron, as well as for aluminium, allows higher concentrations to be removed, thereby preventing hazes in wines.
- By eliminating heavy metals, such as copper, it prevents the destruction of varietal thiols (A3MH, 3MH, 4MMP, etc.) present in several white varietals such as Sauvignon Blanc, as well in certain red wines.
- The prevention of browning through the adsorption of phenolic compounds such as 3,4-dihydroxycinnamic acid derivatives, which otherwise enter into enzymatic and nonenzymatic reactions. The products of these reactions are responsible for the undesirable brown discoloration of white and rosé wines.
- Prevention of pinking by eliminating catalysers of oxidation such as iron and copper and by reducing the polyphenolic content of wine.
- Iron and copper should not be completely removed, as traces of these elements (0.05 to 0.1 mg/L for copper and 2 to 3 mg/L for iron) are important for the oxido-reductive potential of wine.

This will provide Australian winemakers with an additional method to remove undesirable trace elements from wine.

The European Union requested the addition of PVI/PVP copolymer to the Annex of the Wine Agreement in 2015.

b) Advantages of the Proposed Change Over the Status Quo

The change will enable Australia to meet its international obligations under the World Trade Organisation and the EU-Australia wine Agreement while giving Australian winemakers the opportunity to use this technology.

c) Status of Similar Application made in other Countries

No applications are being made by the applicant to other national jurisdictions.

A. REGULATORY IMPACT INFORMATION

1. Costs and benefits

These provide a cost effective and efficient alternative to removing copper and iron from wine without adversely affecting the sensory profile. This removes the need to use traditional bluefining¹.

a) Costs and benefits to the consumers

The use of PVI/PVP as wine fining agents are consistent with the increasing consumer expectations of achieving more sustainable industry practices and providing producers with a more sustainable alternative to bluefining.

b) Costs and Benefits to Industry and Business in General.

The use of PVI/PVP as a fining agent is voluntary. Individual wineries will consider the benefits in sustainability, process efficiencies, wine recovery and waste decrease against the cost of these PVI/PVP. It is expected there will be savings.

c) Costs and Benefits to Government.

There will be no increased regulatory or enforcement costs for the government.

2. Impact on International Trade

The change will enable Australia to meet its international obligations under the World Trade Organisation and the EU-Australia wine Agreement while giving Australian winemakers the opportunity to use this technology.

3.1.5 INFORMATION TO SUPPORT THE APPLICATION

1. General

There are no negative public health implications. PVI/PVP does not have any public health implications and the PVI/PVP complexes will be filtered out of the wine prior to bottling.

¹ In a process known as **blue fining**, potassium ferrocyanide is sometimes used to remove any copper and iron particles that have entered the wine from bentonite, metal winery and vineyard equipment, or vineyard sprays. Because potassium ferrocyanide may form hydrogen cyanide its use is highly regulated and, in many wine producing countries, illegal. Australian industry would prefer not to use potassium Ferrocyanide.

(a) Consumer Choice Issues

There are no consumer issues.

(b) Evidence of General Food Industry or Specific Company Support

The Winemaker's Federation of Australia (WFA) has submitted this application on behalf of the Australian wine sector. WFA is strongly committed to ensuring the integrity of Australia's Food Standards and the international trading system through compliance with World Trade Organisation obligations.

A. Technical Information on the Processing Aid

1. Information on the type of processing aid

Adsorbent copolymers of PVI/PVP are insoluble and slightly-hygroscopic powders. They are manufactured by "popcorn" polymerization of N-vinylimidazole (CAS no. 1072-63-5,) and N-vinyl-2-pyrrolidone (CAS no. 88-12-0,) with a ratio of 9:1. N,N'-divinylimidazolidin-2-one (CAS no. 13811-50,) is used as crosslinking agent at a level of less than 2% by weight of the total amount of the monomers (OIV, 2014).

PVI/PVP are used to fine wine, thus falling in category (d) – *Permitted decolourants, clarifying, filtration and adsorbent agents* in Standard 1.3.3.

2. Information on the identity of the processing aid

SYNONYMS of PVI/PVP are Terpolymer of 1-vinylimidazole, 1-vinylpyrrolidone, and 1,3-divinylimidazolidinone. Cross-linked copolymer of vinylimidazole/vinylpyrrolidone.

3. Information on the chemical and physical properties of the processing aid

PVI/PVP adsorbing copolymers are insoluble in practically all current solvents. It is therefore impossible to measure the molecular weight.

The following parameters are from Oenovitas (2015a)

PHYSICAL AND CHEMICAL PROPERTIES

Appearance and colour: Solid - Powder with a white to yellowish colour.

Odour: None

Odour threshold: N.A. pH: 4.01 (10%)

Melting point / freezing point: $\pm 220^{\circ}\text{C}$

Initial boiling point and boiling range: N.A.

Solid/gas flammability: N.A.

Upper/lower flammability or explosive limits:

N.A. Vapour density: N.A.

Flash point: $> 177^{\circ}\text{C } ^{\circ}\text{F}$

Evaporation rate: N.A.

Vapour pressure: N.A.
Relative density: 1.2 g/cm³
Solubility in water: 0.5 g/L
Solubility in oil: N.A.
Partition coefficient (n-octanol/water): N.A.
Auto-ignition temperature: 225 °C
Decomposition temperature: N.A.
Viscosity: N.A.
Miscibility: N.A.
Fat Solubility: N.A.
Conductivity: N.A.
Substance Groups relevant properties N.A.

4. *Manufacturing process*

Adsorbent copolymers of PVI/PVP are insoluble and slightly-hygroscopic powders. They are manufactured by "popcorn" polymerization of N-vinylimidazole (CAS no. 1072-63-5,) and N-vinyl-2-pyrrolidone (CAS no. 88-12-0,), with a ratio of 9:1. N,N'-divinylimidazolidin-2-one (CAS no. 13811-50,) is used as crosslinking agent at a level of less than 2% by weight of the total amount of the monomers (OIV 2014).

5. *Specification for identity and purity*

OIV (2014) has developed a specification for PVI/PVP. In the European Union, treatment with PVI/PVP copolymers can only occur after the purity and identification specifications for authorised copolymers have been laid down and published in the OIV's International Oenological Codex.

6. *Analytical method of detection*

OIV (2014) has developed analytical methods of detection for PVI/PVP. In the European Union, treatment with PVI/PVP copolymers must comply with the purity and identification specifications for authorised copolymers laid down and published in the OIV's International Oenological Codex.

B. Information Related to the Safety of a chemical processing aid

1. *General Information on the Industrial use of this chemical*

PVP and copolymers of PVP are widely used in the pharmaceutical industry (see for example BASF, 1998).

Copolymers of PVP are used in pharmaceuticals, cosmetics, to stabilise beverages; adhesives and textiles (Haaf, Sanner and Straub, 1985).

2. *General information on the use of the chemical as a food processing aid in other countries*

European Union

Under EC Regulation 606/2009 (as amended) the use of polyvinylimidazole/polyvinylpyrrolidone (PVI/PVP) copolymers is permitted to reduce the copper, iron and heavy metal content. The conditions of use are outlined in Annex 11:

Requirements for treatment with PVI/PVP copolymers

The purpose of this treatment is to reduce excessively high concentrations of metals and to prevent defects caused by this excessively high content, such as ferric casse, through the addition of copolymers that adsorb these metals.

Requirements

The added copolymers must be eliminated by filtering within two days at most of their addition to the wine, taking account of the precautionary principle.

In the case of musts, the copolymers must be added no earlier than two days before filtering.

The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

The adsorbant copolymers used must comply with the requirements of the International Oenological Codex published by the International Organisation of Vine and Wine, especially as regards the maximum monomer content.

United States

In the United States, PVI/PVP is permitted for beverages under Title 21 CFR 173.50:

Title 21: Food and Drugs

[PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION](#)
[Subpart A—Polymer Substances and Polymer Adjuvants for Food Treatment](#)

§173.50 Polyvinylpolypyrrolidone.

The food additive polyvinylpolypyrrolidone may be safely used in accordance with the following prescribed conditions:

- (a) The additive is a homopolymer of purified vinylpyrrolidone catalytically produced under conditions producing polymerization and cross-linking such that an insoluble polymer is produced.
- (b) The food additive is so processed that when the finished polymer is refluxed for 3 hours with water, 5 percent acetic acid, and 50 percent alcohol, no more than 50 parts per million of extractables is obtained with each solvent.
- (c) It is used or intended for use as a clarifying agent in beverages and vinegar, followed by removal with filtration.

3. Data on the toxicokinetics and metabolism of the chemical processing aid, and if necessary its metabolites

There are a large number of publications on the use of polyvinylpyrrolidone. A complete list with

assessments is to be found in “A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone” by Robinson, Sullivan, Borzelleca and Schwartz, published in 1990 in BASF (1998).

Because of the good tolerance of povidone, its Accepted Daily Intake (ADI) was adjusted to 0 – 50 mg/kg body weight by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) in 1987 [WHO Technical Report Series 751, FAO/WHO Report No. 30, 30 – 31 (1987)].

In 1983, the JECFA awarded crospovidone an ADI status of “not specified”, as on the basis of the available chemical, biochemical, toxicological and other data, the entire daily intake of the substance in the quantities to be expected did not represent any risk to health in the opinion of the JECFA. It therefore seemed unnecessary to set a numerical value for the ADI [FAO/WHO-Report No. 27 (1983) 26 – 27].

1. Information on the toxicity of the chemical processing aid and, if necessary, its major metabolites.

POLYVINYLPIRROLIDONE (PVP) was evaluated for acceptable daily intake for man (ADI) by the Joint FAO/WHO Expert Committee on Food Additives in 1966 and 1973. Toxicological monographs were issued in 1966 and 1973. Because of the nature of use of PVI/PVP filtered out before bottling, its insolubility and lack of toxicity, there are not seen to be any major issues with its use.

5. Safety assessment reports prepared by international agencies or other national government agencies if available

European Union

EFSA has evaluated PVP copolymers . See for example EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the safety of polyvinylpyrrolidone-vinyl acetate copolymer for the proposed uses as a food additive. EFSA Journal 2010;8(12):1948. [28 pp.] doi:10.2903/j.efsa.2010.1948. Available online: www.efsa.europa.eu/efsajournal.htm .

United States

n/a

F. Information Related to the Dietary Exposure to the Processing Aid.

PVI/PVP is an insoluble adsorbent resin with two main properties of interest in winemaking:

- The specific adsorption capacity for heavy metals such as copper and iron, as well as for aluminium, allows higher concentrations to be removed, thereby preventing hazes in wines.
- By eliminating heavy metals, such as copper, it prevents the destruction of varietal thiols (A3MH, 3MH, 4MMP, etc.) present in several white varietals such as Sauvignon Blanc, as well in certain red wines.
- The prevention of browning through the adsorption of phenolic compounds such as 3,4-dihydroxycinnamic acid derivatives, which otherwise enter into enzymatic and nonenzymatic reactions. The products of these reactions are responsible for the undesirable brown discoloration of white and rosé wines.

- Prevention of pinking by eliminating catalysers of oxidation such as iron and copper and by reducing the polyphenolic content of wine.
- Iron and copper should not be completely removed, as traces of these elements (0.05 to 0.1 mg/L for copper and 2 to 3 mg/L for iron) are important for the oxido-reductive potential of wine.

This will provide Australian winemakers with an additional method to remove undesirable trace elements from wine. However, there is not expected to be wide domestic use.

2. The levels of residues of the processing aids or its metabolites for each food or food group

PVI/PVP copolymer is insoluble in alcoholic beverages. The precipitates it forms with unwanted components in alcoholic beverages during processing are removed via filtration or similar processes.

3. Information on likely level of consumption

No information.

4. Percentage of food group to use processing aid

There is no information on the expected use of this processing aid in Australian wine or imported product currently being sold in Australia.

5. Information on residues in foods in other countries

There is no information on residues in wines where it is approved as a processing aid in other countries. However, residues are not expected to remain in the final product due to filtration.

6. Where consumption has changed, information on likely consumption

Not applicable

3.1.6 Assessment Procedure

This application seeks the appropriate assessment procedure is **General Procedure Level 1**.

3.1.7 CONFIDENTIAL COMMERCIAL INFORMATION

No confidential or commercial information is incorporated in this application.

3.1.8 EXCLUSIVE CAPTURABLE BENEFIT.

There is no exclusive capturable benefit to the applicant.

3.1.9 INTERNATIONAL AND OTHER STANDARDS

- A. Codex Alimentarius Commission (Codex) Standards

PVI/PVP is a processing aid.

B. Other National Standards

OIV

The OIV adopted the oenological practice to use PVI/PVP to reduce an excess of metals at its 12th General assembly in Mendoza on 14 November 2014 (Oenoppia 2015). The prescriptions for its use with musts and wine are attached (OIV, 2014b). Following the adoption of a product monograph in 2015 (OIV 2014a), the European Commission amended Regulation 606/2009 on 23 September 2015 to permit the use on PVI/PVP for musts and wines.

European Union

Under EC Regulation 606/2009 (as amended) the use of polyvinylimidazole/polyvinylpyrrolidone (PVI/PVP) copolymers is permitted to reduce the copper, iron and heavy metal content. The conditions of use are outlined in Annex 11:

Requirements for treatment with PVI/PVP copolymers

The purpose of this treatment is to reduce excessively high concentrations of metals and to prevent defects caused by this excessively high content, such as ferric casse, through the addition of copolymers that adsorb these metals.

Requirements

The added copolymers must be eliminated by filtering within two days at most of their addition to the wine, taking account of the precautionary principle.

In the case of musts, the copolymers must be added no earlier than two days before filtering.

The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

The adsorbant copolymers used must comply with the requirements of the International Oenological Codex published by the International Organisation of Vine and Wine, especially as regards the maximum monomer content.

United States

PVI/PVP has been approved for use in the United States for use in wine under part 24 of Title 27 of the Code of Federal Regulations (27 CFR part 24.250).

Polyvinyl-polypyr-rolidone (PVPP)/ polyvinylimadazole (PVI) polymer: To remove heavy metal ions and sulfides from wine.	The amount used to treat the wine must not exceed 80 grams per 100 liters of wine. 21 CFR 173.50 and FDA FCN No. 320.	Preliminary conclusion allowing use 8/25/2010.
--	---	--

3.1.10 *STATUTORY DECLARATION*

Attached

3.1.11 *CHECKLIST*

Attached

References

- BASF, (1998) Polyvinylpyrrolidone for the pharmaceutical industry, BASF Aktiengesellschaft Fine Chemicals, D-67056 Ludwigshafen, March 1998 (4th edition)
- EC (2007). Commission Delegated Regulation (EU) 2015/1576 of 6 July 2015 amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No. 436/2009 as regards registering of these practices in the wine sector registers Official Journal European Union 23.9.2015, (L246/1):1-4. Available at <http://faolex.fao.org/docs/pdf/eur148537.pdf>
- EC (2009) **COMMISSION REGULATION (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions.**
- EC (2015), **Commision Delegated Regulation (EU) 2015/1576 of 6 July 2015 amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC No 463/2009 as regards registering those practices in the wine sector registers**, Official Journal L 246/1-4.
- EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the safety of polyvinylpyrrolidone-vinyl acetate copolymer for the proposed uses as a food additive. EFSA Journal 2010;8(12):1948. [28 pp.] doi:10.2903/j.efsa.2010.1948.
- Haaf, F, Sanner, A. and Straub, F. (1985), Polymers of N-vinylpyrrolidone: Syntheses, Characterisation and Uses, Polymer Journal, Vol. 17, No. 1, pp 143-152
- Mira, H., Leite, P., Catarino, S., Ricardo-Da-Silva, J. and Curvelo-Garcia, A. (2007), Metal reduction in wine using PVI-PVP copolymer and its effects on chemical and sensory characters, Vitas 46 (3), 138-147.
- OENARTIS (2015a), **Safety Data Sheet STABYL-PVI/PVP**, Safety Data Sheet dated 6/30/2015
- OENARTIS (2015b), **Sheet Code: StabylPVIPVP/US**, Revision: n° 1, August 2015
- OENOPPIA (2015), **Information about wine regulation**, Press Release, Paris, 25 August 2015.
- OIV (2014a), **ADSORBENT COPOLYMERS OF POLYVINYLMIDAZOLE /POLYVINYLPYRROLIDONE (PVI/PVP)**, INTERNATIONAL OENOLOGICAL CODEX PVI/PVP, COEI-1-PVIPVP: 2014 E-COEI-1-PVIPVP 1 , pp.1-29
- OIV, (2014b) Adsorbant Copolymer Treatment PVI/PVP, International Code of Oenological Practices.
- Schubert, M and M (2010), Analysis and Chemistry of Migrants from Wine Fining Polymers, *J. Agric. Food Chem.*, 2010, 58 (14), pp 8300–8304.
- United States , (undated), Title 21 CFR 173.50, PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION Subpart A—Polymer Substances and Polymer Adjuvants for Food Treatment.

Attachments

- Attachment 1: Statutory Declaration
- Attachment 2: Checklist
- Attachment 3: Summary
- Attachment 4: Application
- Attachment 5: OENARTIS (2015b), **Sheet Code: Stabyl PVIPVP/US**, Revision: n° 1, August 2015
- Attachment 6: OIV (2014a), **ADSORBENT COPOLYMERS OF POLYVINYLMIDAZOLE /POLYVINYLPYRROLIDONE (PVI/PVP)**, INTERNATIONAL OENOLOGICAL CODEX PVI/PVP, COEI-1-PVIPVP: 2014 E-COEI-1-PVIPVP 1 , pp.1-29
- Attachment 7: OENARTIS (2015a), **Safety Data Sheet STABYL-PVI/PVP**, Safety Data Sheet dated 6/30/2015
- Attachment 8: United States , (undated), Title 21 CFR 173.50, PART 173—SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION Subpart A—Polymer Substances and Polymer Adjuvants for Food Treatment.
- Attachment 9: EC (2009) **COMMISSION REGULATION (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions.**
- Attachment 10: OIV, (2014b) Adsorbant Copolymer Treatment PVI/PVP, International Code of Oenological Practices.
- Attachment 11: OENOPPIA (2015), **Information about wine regulation**, Press Release, Paris, 25 August 2015.
- Attachment 12: EC (2015). Commission Delegated Regulation (EU) 2015/1576 of 6 July 2015 amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No. 436/2009 as regards registering of these practices in the wine sector registers Official Journal European Union 23.9.2015, (L246/1):1-4. Available at <http://faolex.fao.org/docs/pdf/eur148537.pdf>
- Attachment 13: Mira, H., Leite, P., Catarino, S., Ricardo-Da-Silva, J. and Curvelo-Garcia, A. (2007), Metal reduction in wine using PVI-PVP copolymer and its effects on chemical and sensory characters, *Vitas* 46 (3), 138-147.
- Attachment 14: BASF, (1998) Polyvinylpyrrolidone for the pharmaceutical industry, BASF Aktiengesellschaft Fine Chemicals, D-67056 Ludwigshafen, March 1998 (4th edition).
- Attachment 15: Haaf, F, Sanner, A. and Straub, F. (1985), Polymers of N-vinylpyrrolidone: Synthesis, Characterisation and Uses, *Polymer Journal*, Vol. 17, No. 1, pp 143-152.
- Attachment 16: EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS); Scientific Opinion on the safety of polyvinylpyrrolidone-vinyl acetate copolymer for the proposed uses as a food additive. *EFSA Journal* 2010;8(12):1948. [28 pp.] doi:10.2903/j.efsa.2010.1948.
- Attachment 17: Schubert, M and, M (2010), Analysis and Chemistry of Migrants from Wine Fining Polymers, *J. Agric. Food Chem.*, 2010, 58 (14), pp 8300–8304.

Abbreviations

ADI	Acceptable Daily Intake
CAS	Chemical Abstracts Serial number
EEC	European Economic Community
EINECS	European Inventory of Existing Chemical Substances
FAO	Food and Agriculture Organization (of the United Nations)

FDA	Food and Drug Administration (of the USA)
GRAS	Generally Recognized As Safe
INS	International Numbering System (Codex Alimentarius numbers for food additives)
IPPA	International Pectin Producers Association
JECFA	Joint Expert Committee for Food Additives (FAO/WHO)
SCF	Scientific Committee for Food (the European Union expert committee)



STABYL PVI/PVP

FOOD GRADE PVI/PVP

COMPOSITION

Cross-linked, insoluble co-polymer of vinylimidazole and vinylpyrrolidone in a ratio of 9:1.

GENERAL FEATURES

White to creamy, slightly hygroscopic powder with a mild odor. Cross-linked copolymer consisting of vinylimidazole (VI) and vinylpyrrolidone (VP), produced from an aqueous solution by means of a special polymerization technique. It is insoluble in water and all common solvents.

APPLICATIONS

Stabyl PVI/PVP is an insoluble adsorbant resin with two main properties of interest in winemaking:

- The specific adsorption capacity for heavy metals such as copper and iron, as well as for aluminium, allows higher concentrations to be removed, thereby preventing hazes in wines.
- By eliminating heavy metals, such as copper, it prevents the destruction of varietal thiols (A3MH, 3MH, 4MMP, etc.) present in several white varietals such as Sauvignon Blanc, as well in certain red wines.
- The prevention of browning through the adsorption of phenolic compounds such as 3,4-dihydroxycinnamic acid derivatives, which otherwise enter into enzymatic and non-enzymatic reactions. The products of these reactions are responsible for the undesirable brown discoloration of white and rosé wines.
- Prevention of pinking by eliminating catalysers of oxidation such as iron and copper and by reducing the polyphenolic content of wine.

Iron and copper should not be completely removed, as traces of these elements (0.05 to 0.1 mg/L for copper and 2 to 3 mg/L for iron) are important for the oxido-reductive potential of wine.

DOSAGE

40 – 80 g/hL

Preliminary laboratory trials are recommended to determine the correct dosage, in particular when treating wines.

INSTRUCTIONS FOR USE

STABYL PVI/PVP can be added as dry powder. Alternatively, it may be added in the form of a 5% suspension in the wine or must to be fined or in water. The suspension is prepared by repeated stirring. After it has been allowed to swell for at least 60 minutes at 65-68 °F, it can be added directly to the tank.

When used in musts, thorough mixing for periods of 20 to 60 minutes are recommended to ensure adequate contact time for the adsorption of the initial stages in the browning process.



STABYL PVI/PVP

In general, contact periods of 6 hours are recommended to remove traces of copper by fining, but a period as long as 24 hours must be allowed for the removal of iron traces.

During this period, it is absolutely essential that the polymer is continuously re-suspended, either through agitation, pump-over (without air) or other mixing methods. In the case of pump-over, it is recommended to return the wine or must through the bottom valve, in order to maintain the STABYL PVI/PVP in suspension.

After maintaining the STABYL PVI/PVP in suspension for the recommended time, it should be allowed to sediment for at least 48 hours and a maximum of 5 days, in order to achieve a turbidity less than 40 NTU. The wine should then be filtered through a plate with a maximum porosity of 3 microns (diatomaceous earth filter, or cellulose filter pads) and the pressure by filtration should not exceed 0.8 bars.

PACKAGING AND STORAGE

20 Kg in re-sealable plastic drums with polyethylene liners

The drums must be kept tightly sealed.

MAIN PHYSICAL-CHEMICAL FEATURES

The iron and the copper should not be removed completely, as traces of these elements (0.05 to 0.1 mg/L for copper and 2 to 3 mg/L for iron) are important for the oxido-reductive potential of wine.

Product approved for winemaking by the TTB.

Approved under 27 CFR 24.250

Legal Limit: The amount of STABYL PVI/PVP used shall not exceed 80 g/hL.

**ADSORBENT COPOLYMERS OF POLYVINYLMIDAZOLE
/POLYVINYLPYRROLIDONE (PVI/PVP)****N° C.A.S.: 87865-40-5
(Oeno 262/2014)****1. OBJECT, ORIGIN AND SCOPE OF APPLICATION**

Adsorbent copolymers of PVI/PVP are insoluble and slightly-hygroscopic powders. They are manufactured by "popcorn" polymerization of N-vinylimidazole (CAS no. 1072-63-5,) and N-vinyl-2-pyrrolidone (CAS no. 88-12-0,,) with a ratio of 9:1. N,N'-divinylimidazolidin-2-one (CAS no. 13811-50,) is used as crosslinking agent at a level of less than 2% by weight of the total amount of the monomers.

Adsorbent copolymers of PVI/PVP are added to must or wine in accordance with the files described in the Code of Oenological Practices of the OIV in amounts of less than 500 mg/l.

Adsorbent copolymers of PVI/PVP can be added to must or wine in order to prevent the defects caused by excessive metal contents or to reduce undesirably-high metal concentrations.

The must or wine must be filtered through a filter media with pores whose diameter is no greater than 3 microns and with a filtration pressure no greater than 0.8 bars.

2. SYNONYMS

Terpolymer of 1-vinylimidazole, 1-vinylpyrrolidone, and 1,3-divinylimidazolidinone. Cross-linked copolymer of vinylimidazole/vinylpyrrolidone.

3. LABELLING

The labelling must indicate that the PVI/PVP adsorbing copolymer is for oenological use. The storage and safety conditions must also be indicated.

The label must mention a 3-year use-by date.

4. CHARACTERS

Powder with a white to yellowish colour.

PVI/PVP adsorbing copolymers are insoluble in practically all current solvents. It is therefore impossible to measure the molecular weight.

5. TESTS

5.1 Loss on desiccation

Tare a metal capsule 50 mm in diameter. Place in the recipient between 0.8 and 1.4 g of PVI/PVP adsorbent copolymer, homogenised beforehand and weighed precisely in a closed balance. Dry in a drying oven at $140^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 1 hour. Allow to cool in a desiccator. Weigh again.

The loss on desiccation must be less than 5 %.

5.2 Ash

Heat a porcelain crucible until it is dark red; allow to cool in a desiccator and weigh. Place 1.5 g of PVI/PVP adsorbent copolymer in the crucible and incinerate at a constant weight in a muffle furnace at $800^{\circ}\text{C} \pm 25^{\circ}\text{C}$, allowing the crucible to cool in a desiccator after each incineration, the duration of the first incineration being 6 hours. If necessary, pre-incinerate the sample.

The weight of the ash must be less than 0.02 %.

5.3 Preparation of the solution for tests:

After weighing the ash, dissolve it in 1 ml of concentrated hydrochloric acid (R) and 10 ml of distilled water. Heat to activate dissolution. Make up to 20 ml with distilled water. 1 ml of this solution contains the mineral matter of 0.075 g of PVI/PVP adsorbent copolymer.

5.4 Zinc

Using the solution for tests prepared as in point 5.3, measure zinc according to the method described in Chapter II.

The zinc content must be less than 1 mg/kg.

5.5 Iron

Using the solution for tests prepared as in point 5.3, measure iron according to the method described in Chapter II.

The iron content must be less than 1 mg/kg.

5.6 Copper

Using the solution for tests prepared as in point 5.3, measure copper according to the method described in Chapter II.

The copper content must be less than 1 mg/kg.

5.7 Lead

Using the solution for tests prepared as in point 5.3, measure lead according to the method described in Chapter II.

The lead content must be less than 2 mg/kg.

5.8 Cadmium

Using the solution for tests prepared as in point 5.3, measure cadmium according to the method described in Chapter II.

The cadmium content must be less than 1 mg/kg.

5.9 Arsenic

Do not use the solution for tests prepared as in point 5.3.

Determine the arsenic according to the method described in Chapter II.

The arsenic content must be less than 2 mg/kg.

5.10 Mercury

Do not use the solution for tests prepared as in point 5.3.

Determine the mercury according to the method described in Chapter II.

The mercury content must be less than 1 mg/kg.

5.11 Organic impurities

Determine the organic impurities according to the method described in Appendix 1.

The limits of organic impurities must be as follows:

- The vinylpyrrolidone content must be less than 5 mg/kg
- The vinylimidazole content must be less than 10 mg/kg
- The divinylimidazolidinone content must be less than 2 mg/kg
- The pyrrolidone content must be less than 50 mg/kg
- The imidazole content must be less than 50 mg/kg

5.12 Measurement of total nitrogen

Place approximately 450 mg of PVI/PVP adsorbing copolymer (test portion *m* mg) in a mineralisation flask, add 10 g of Missouri Catalyst¹, and 3 glass beads. Wash all the particles that adhere to the neck of the flask with a small quantity of sulphuric acid (R). Add in total 20 ml of sulphuric acid (R), running it along the walls of the flask, and mix the contents by rotation. Continue the analysis according to the method described in Chapter II.

The total nitrogen content must lie between 26.0 and 29.0% with respect to the dry weight.

¹

Missouri Catalyst (= 49.9% K₂SO₄ + 49.8% Na₂SO₄ + 0.3% CuSO₄), Merck, Darmstadt or the equivalent

5.13 Solubility in an aqueous medium

Place 10 g of PVI/PVP adsorbent copolymer in a graduated 200-ml flask containing 100 ml of water. Shake the bottle and allow the contents to rest for 24 hours. Filter on a filter membrane with 2.5 µm diameter pores, and then on a filter membrane with 0.8 µm diameter pores. The dry residue remaining after evaporation of the filtrate on a water bath must be less than 0.5%.

5.14 Solubility in acid and alcohol

Introduce 1 g of PVI/PVP adsorbent copolymer into a bottle containing 500 ml of the following mixture:

Acetic acid	3 g
Ethanol	10 ml
Water	100 ml

Allow to rest for 24 hours. Filter on a filter membrane with 2.5 µm diameter pores, then on a filter membrane with 0.8 µm diameter pores. Concentrate the filtrate on a water bath. Finish the evaporation on a water bath in a calibrated silica capsule 70mm in diameter. The dry residue remaining after evaporation must be less than 1%, taking into account all the residue of the evaporation of the 500 ml of the mixture of acetic acid, ethanol and water.

5.15 Determination and content of monomers in musts and wines**5.15.1 Analytical method**

Proceed with the determination according to the analytical method in Appendix 2

5.15.2 Limits of monomers in musts and wines²

The vinylpyrrolidone content must be less than 10 µg/l

The vinylimidazole content must be less than 10 µg/l

The pyrrolidone content must be less than 25 µg/l

The imidazole content must be less than 150 µg/l

² The calculation of the upper limits was based on the results obtained from the migration tests with the recommended dosage of 0,5 g/l, the maximum application time of 48 hours, and a treatment temperature of 20 °C, multiplied by a factor of 2.

Under acidic conditions (at lower pH-values) divinylimidazolidinone (divinylethylene-urea) is not stable and hence degrades to imidazolidinone and vinyl alcohol. Furthermore imidazolidinone degrades to urea and ethylene glycol. Vinyl alcohol is in chemical equilibrium with acetaldehyde.

Imidazolidinone was included in the toxicological assessment as well as acetaldehyde, urea and ethylene glycol.

6. STORAGE

The PVI/PVP adsorbing copolymer must be kept in a cool place. The recipients must be dry and hermetically sealed.

Appendix 1

Determination by gas chromatography of the constitutive monomers and/or impurities liable to be found in copolymers of vinylpyrrolidone-vinylimidazole (vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole)

1. Principle

Detection and determination of the constitutive monomers and/or impurities liable to be found in copolymers of vinylpyrrolidone-vinylimidazole (vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole).

The analysis is carried out by capillary gas chromatography using a nitrogen specific detector (NSD). The substances to be analysed are extracted beforehand from the polymer by acetone.

2. Range of contents to be determined

Vinylimidazole:	2-55 µg/g
Vinylpyrrolidone:	2-50 µg/g
Pyrrolidone:	2-70 µg/g
Divinylethyleneurea:	2-33 µg/g
Imidazole:	2-50 µg/g

3. Reagents and reference material

- 3.1 Vinylpyrrolidone-vinylimidazole copolymers;
- 3.2 Vinylimidazole, $M(C_5H_6N_2) = 94.12$ g/mol
purity > 99% (GC), e.g. Fluka, item no. 95005
(R: 22-34, S: 26-36/37/39-45)
- 3.3 Vinylpyrrolidone (*1-vinyl-2-pyrrolidone*), $M(C_6H_9NO) = 111.14$ g/mol
purity = 99.8% (GC), e.g. Fluka, item no. 95060
(R: 20/21/22-36/37/38-40, S: 26-36/37/39)
- 3.4 Pyrrolidone, (*2-pyrrolidone*), $M(C_4H_7NO) = 85.11$ g/mol
purity > 99% (GC), e.g. Fluka, item no. 83300
(R: 36/37/38, S: 26-36)
- 3.5 Divinylethyleneurea (*N,N-divinylimidazolidone*), $M(C_7H_{10}N_2O) = 138.17$ g/mol
purity ≥ 99% (GC), BASF reference material

(R: 36/38-40, S: 26-36/37)

- 3.6 Imidazole, ($C_3H_4N_2$) = 68.08 g/mol
purity > 99.5% (GC), e.g. Fluka, item no. 56748
(R: 22-34, S: 26-36/37/39-45)
- 3.7 Benzonitrile,
purity > 99% (G), e.g. Merck-Schuchardt, item no. 801800
(R: 10-35, S: 23-26-45)
- 3.8 Acetone,
purity \geq 99% (GC), e.g. Fluka, item no. 00585
(R: 11, S: 9-16-23-33)

4. Apparatus

- 4.1 Capillary gas chromatograph with an automatic sampler, split injector, nitrogen specific detector (NSD).
- 4.2 Fused silica capillary column, with a polyethylene glycol film, (e.g. DB-Wax, J&W Scientific)
Length: 30 m
Internal diameter: 0.25 mm
Film thickness: 0.5 μ m
- 4.3 Data acquisition and processing system
- 4.4 Analytical balance accurate to 0.1 mg
- 4.5 Laboratory glassware and standard apparatus
- 4.6 Rotary mixer capable of housing small-capacity flasks, e.g. 50 ml.

5. Solutions

- 5.1 Internal standard solution

Benzonitrile, 250 μ g/ml in acetone (3.8)

- 5.2 Stock calibration solution

Prepare a stock calibration solution of different concentrations in acetone (3.8) containing vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole with amounts ranging from 250 mg/l to 1000 mg/l.

5.3 Calibration solutions

Prepare at least two calibration solutions with different concentrations in acetone (3.8). Each solution must contain a suitable quantity of the internal standard as well as vinylimidazole, vinylpyrrolidone, pyrrolidone, divinylethyleneurea and imidazole so that the calibration points include the values currently being measured.

Example: 4 µl-200 µl of stock solution (5.2) + 24 ml of acetone (3.8) + 1 ml of internal standard solution (5.1).

6. Example of chromatographic conditions

Temperatures:

Injector:	220°C
Oven:	160°C
- then programmed at a rate of 5°C/min up to 210°C	
Final isothermal period:	210°C, 7 min
Detector (NSD):	250°C

Carrier gas:	helium
Column head pressure:	140 kPa (1.4 bar)
Split flow:	10 ml/min
Septum purge:	5 ml/min
Volume injected:	1.0 µl

7. Preliminary check of the analytical system

7.1 Resolution

Prepare a solution of benzonitrile and vinylimidazole (10 and 2 µg/ml in acetone).

Inject this solution into the chromatograph under the conditions described in 6.

The analysis is considered satisfactory when the resolution of the two chromatographic peaks is at least 1.5 ($R > 1.5$), with a return to the baseline between the two peaks.

7.2 Sensitivity

To check the sensitivity:

- 1) Carry out a preliminary analysis of a sample (8.1) under the conditions described in section 6.
- 2) Add to the sample 2 µg/g divinylethyleneurea then repeat the analysis under the conditions described in section 6.

If the sample does not contain divinylethyleneurea the system is suitable when the peak of added divinylethyleneurea presents a signal-to-noise ratio of at least 10.

If the sample contained divinylethyleneurea a clear increase in the signal should be observed.

8. Procedure

8.1 Preparation of the samples

Weigh about 2g of sample, accurate to 0.1 mg, then mix it with 1 ml of internal standard solution (5.1) and 24 ml of acetone (3.8). Extract the sample for 4 h on the rotary mixer (4.6) then analyse the supernatant solution under the conditions described in point 6.

For routine determinations, analyse each sample twice.

8.2 Chromatograms

Extracted by acetone from a copolymer (fig. 1)

Extracted by acetone from a copolymer supplemented with analytes (fig. 2)

9. Calculation

9.1 Calibration factor

Chromatographic calibration factor $f(i)$:

$$f(i) = \frac{A(i)_0 \times m(I.S.)_0}{m(i)_0 \times A(I.S.)_0}$$

where:

$A(i)_0$ = peak area of analyte i in the chromatogram for the calibration solution (mVs)

$m(i)_0$ = initial weight of reference product i in the calibration solution [mg]

$A(I.S.)_0$ = peak area of the internal standard in the chromatogram of the calibration solution (mVs)

$m(I.S.)_0$ = initial weight of the internal standard in the calibration solution [mg]

The weight ratio $w(i)$ of analyte i is calculated in the following way:

$$w(i) = \frac{A(i) \times m(I.S.)}{A(I.S.) \times m(s) \times f'(i)}$$

where:

$w(i)$ = weight ratio of the analyte i [$\mu\text{g/g}$]

$A(i)$ = peak area of the analyte i in the chromatogram of the sample solution (mVs)

$A(\text{I.S.})$ = peak area of the internal standard in the chromatogram of the sample solution (mVs)

$m(\text{I.S.})$ = initial weight of the internal standard added to the sample [μg]

$m(s)$ = initial weight of sample [g]

$f'(i)$ = average chromatographic calibration factor

For routine determinations, the result is expressed as a whole number.

10. Characteristics of the method

10.1 Specificity, selectivity

In the chromatogram, the peaks are identified according to their retention time in comparison with the retention time of the solutions of pure analytes (3.2 to 3.6) injected under the same conditions.

Check that the components of the sample have a retention time different from that of the internal standard and that the resolution between peaks is always greater than 1.5.

10.2 Linearity

During calibration, the calibration factors were determined at 6 levels of concentration for each analyte. The calibration curves are straight lines (cf. fig. 3-7) with the following coefficients of determination:

Vinylimidazole $R^2 = 0.9987$

Vinylpyrrolidone $R^2 = 0.9999$

Pyrrolidone $R^2 = 0.9956$

Divinylethyleneurea $R^2 = 0.9937$

Imidazole $R^2 = 0.9982$

10.3 Limit of quantification

The calibration measurements were used to determine the following limits of quantification:

Vinylimidazole: 2 $\mu\text{g/g}$

Vinylpyrrolidone: 2 $\mu\text{g/g}$

Pyrrolidone: 2 $\mu\text{g/g}$

Divinylethyleneurea: 2 $\mu\text{g/g}$

Imidazole: 2 µg/g

10.4 Precision

To determine the precision under repeatability conditions, a copolymer sample was analysed 6 times: (Table 1)

Table 1

		Vinylimidazole	Vinylpyrrolidone	Pyrrolidone	Divinylethyleneurea	Imidazole
1. Determination	[µg/g]	nq*	nd**	4.1	nd	10.7
2. Determination	[µg/g]	nq	nd	4.3	nd	10.8
3. Determination	[µg/g]	nq	nd	4.2	nd	11.5
4. Determination	[µg/g]	nq	nd	4.3	nd	11.8
5. Determination	[µg/g]	nq	nd	3.9	nd	10.2
6. Determination	[µg/g]	nq	nd	3.9	nd	10.8
Average	[µg/g]	nq	nd	4.1	nd	11.0
Standard deviation	[µg/g]			0.2		0.6
Coeff. of variation	%			4.8		5.1
Measurement uncertainty	[µg/g]			0.6		1.7
Relative measurement uncertainty	%			14		15

*nq = not quantifiable

**nd= not detectable

In the sample, the vinylpyrrolidone and divinylethyleneurea could not be detected and the vinylimidazole could not be quantified.

10.4.1 Repeatability

The copolymer sample was supplemented with all the analytes then analysed 6 times. The accuracy under repeatability conditions can be deduced from the repeatability for vinylpyrrolidone, divinylethyleneurea and vinylimidazole. (Table 2)

Table 2

		Vinylimidazole	Vinylpyrrolidone	Pyrrolidone	Divinylethyleneurea	Imidazole
1. Determination	[%]	102.3	112.4	97.0	103.3	90.7
2. Determination	[%]	98.5	101.9	89.6	102.1	91.7
3. Determination	[%]	111.8*	111.5	105.7	111.1	112.6*
4. Determination	[%]	102.7	103.3	91.9	104.8	94.5
5. Determination	[%]	104.2	101.0	89.3	102.7	97.0
6. Determination	[%]	100.4	104.9	90.4	110.3	95.4
Average	[%]	101.6	105.8	94.0	105.7	93.9
Standard deviation	[%]	2.2	4.9	6.4	3.9	2.6
Coeff. of variation	[%]	2.2	4.7	6.8	3.7	2.8
Measurement uncertainty	[%]	6.6	14.8	19.2	11.8	7.8
Relative measurement uncertainty	[%]	7	14	20	11	8

* = outlier value according to the Dixon test

10.5 Addition recovery

The recovery can be calculated from table 2.

Vinylimidazole: 101.6 %

Vinylpyrrolidone: 105.8 %

Pyrrolidone: 94.0 %

Divinylethyleneurea: 105.7 %

Imidazole: 93.9 %

Note

Applicability to other copolymers of vinylpyrrolidone-vinylimidazole

The method was validated for Divergan HM. In principle, we can consider the determination is also valid for other copolymers of vinylpyrrolidone-vinylimidazole.

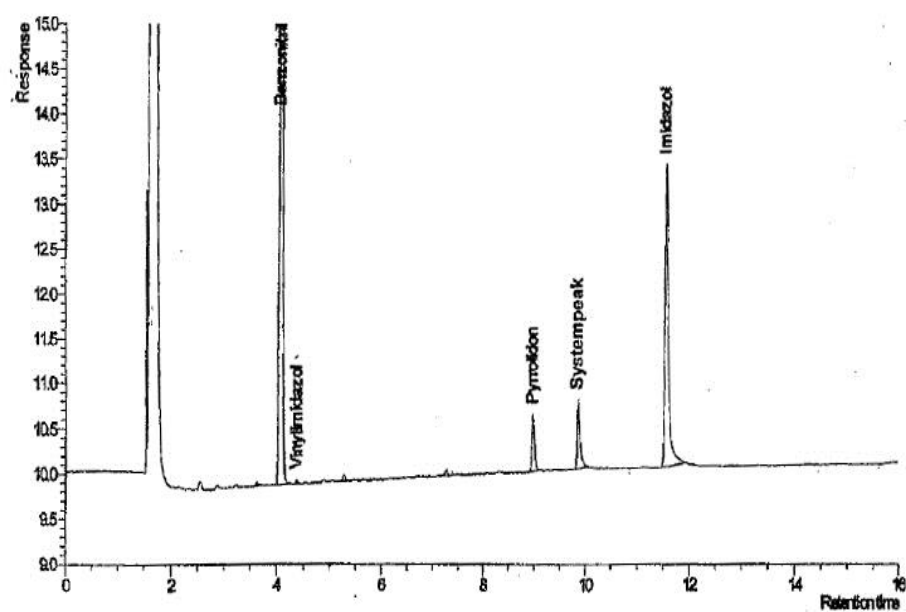


Fig. 1: Chromatogram of the copolymer extract (with internal standard)

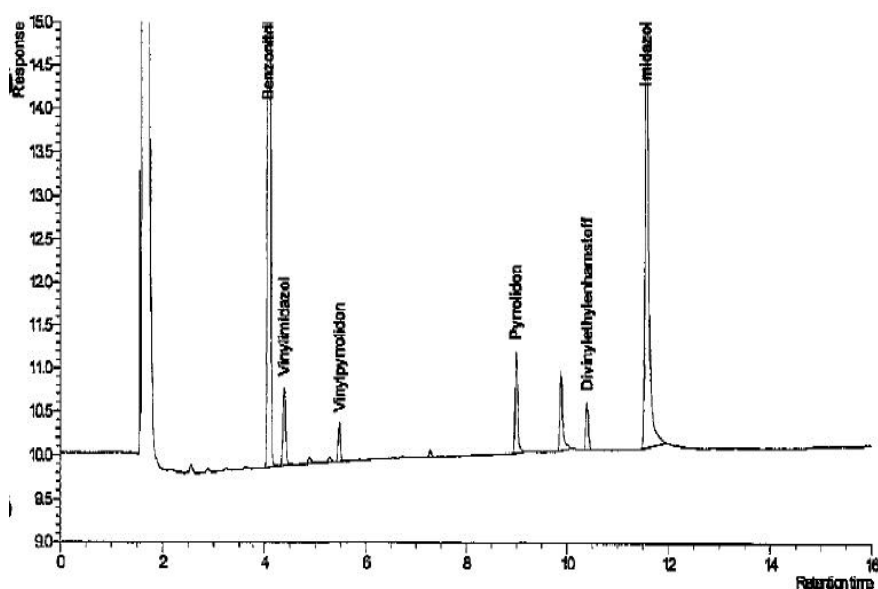


Fig. 2 Chromatogram of the copolymer extract (with internal standard), supplemented by 2.1 µg/g of vinylimidazole, 2.1 µg/g of vinylpyrrolidone, 3.9 µg/g of pyrrolidone, 2.1 µg/g of divinylethyleneurea, and 12.7 µg/g of imidazole.

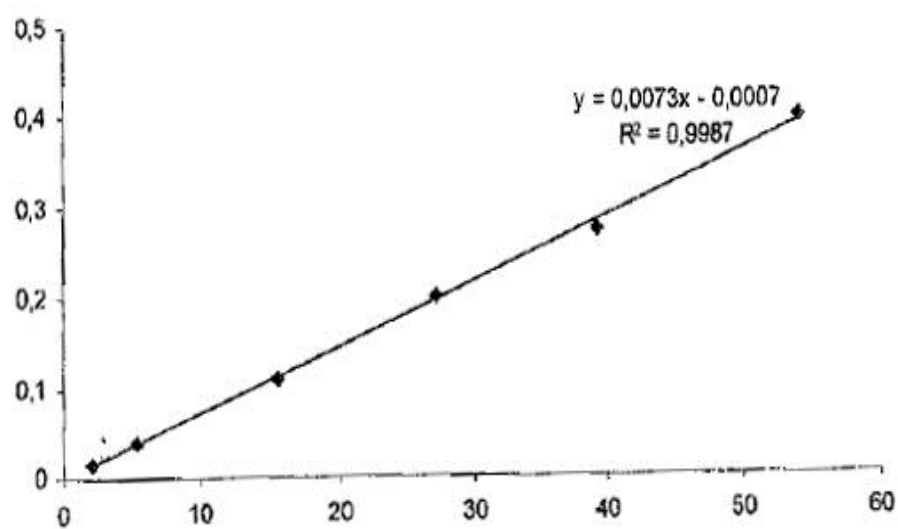


Fig. 3: calibration line for vinylimidazole

Analyte peak area*test sample (int. std.)

Peak area (int. std.) [mg]

Analyte test sample related to standard test sample [µg/g]

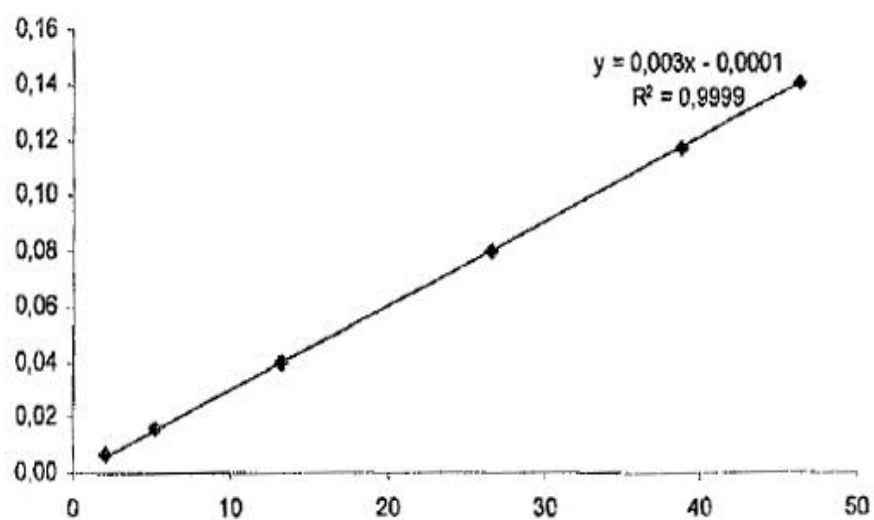


Fig. 4: calibration line for vinylpyrrolidone

Analyte peak area*test sample (int. std.)

Peak area (int. std.) [mg]

Analyte test sample related to standard test sample [µg/g]

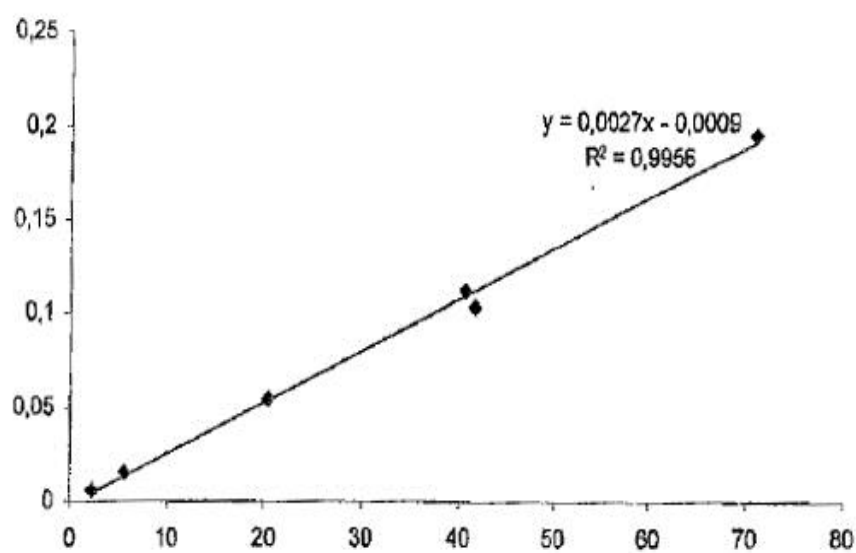


Fig. 5: calibration line for pyrrolidone

Analyte peak area*test sample (int. std.)

Peak area (int. std.) [mg]

Analyte test sample related to standard test sample [µg/g]

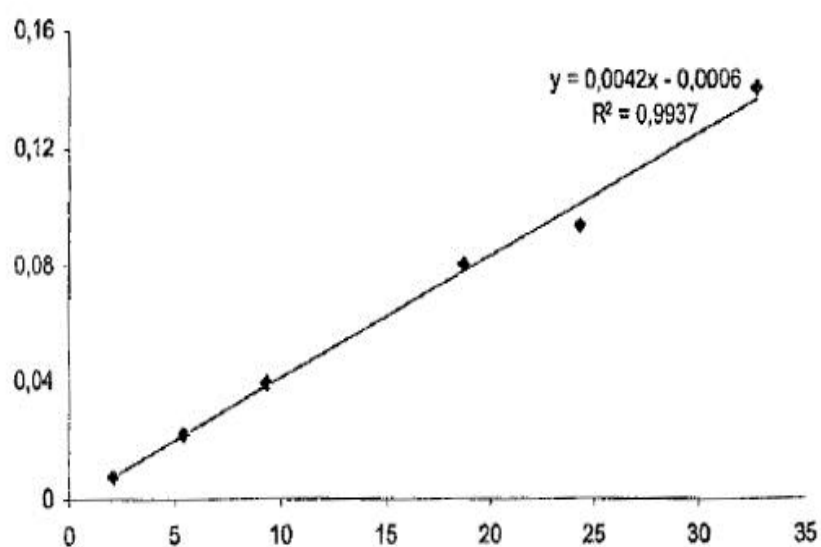


Fig. 6: calibration line for divinylethyleneurea

Analyte peak area*test sample (int. std.)

Peak area (int. std.) [mg]

Analyte test sample related to standard test sample [µg/g]

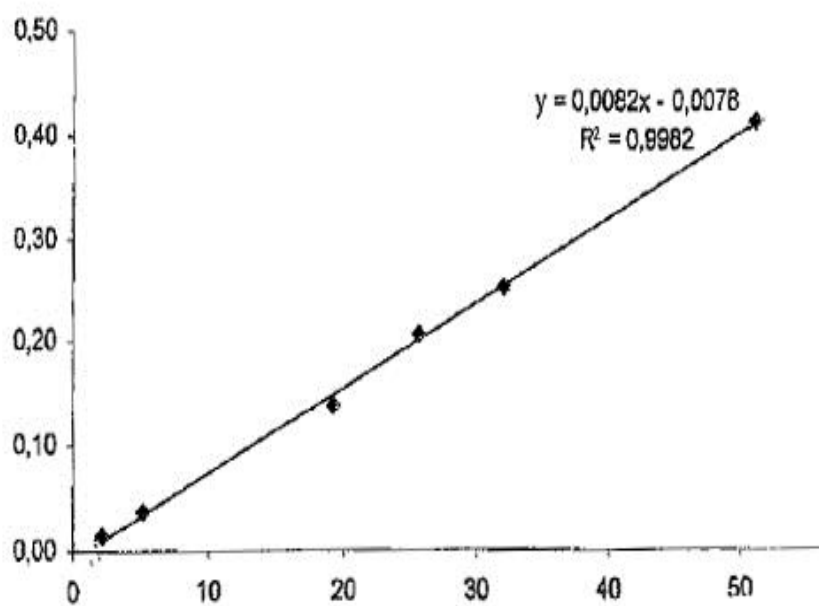


Fig. 7: calibration line for imidazole

Analyte peak area*test sample (int. std.)

Peak area (int. std.) [mg]

Analyte test sample related to standard test sample [µg/g]

Appendix 2

Analytical method for the detection of Imidazole, Pyrrolidone and residual monomers (Vinylpyrrolidone, Vinylimidazole, Divinylimidazolidinone) in wines and musts

1 Scope

The method described here is suitable for the determination of Imidazole, Pyrrolidone, Vinylimidazole and Vinylpyrrolidone in white, red, sweet and dry wines, and must.

Divinylimidazolidinone has a half-life of 3.75 min at pH-value of 3.7. Thus determination is not appropriate in wine and must.

The study described covers the concentration ranges of 5 to 125 µg/l for Imidazole, 25 to 250 µg/l for Pyrrolidone, 2 to 25 µg/l for Vinylimidazole and 2 to 12.5 µg/l for Vinylpyrrolidone.

2 Definitions

HPLC High performance liquid chromatography

LC-MS Liquid Chromatography – Mass spectrometry

MRM multiple-reaction monitoring

3 Principle

Samples are analyzed directly by LC-MS on a reversed-phase column (C18). Detection is then carried out in multiple-reaction monitoring mode.

4 Reagents and Materials

4.1 Chemicals

4.1.1 Methanol (LiChrosolv) (CAS: 67-56-1) quality for CL-SM

4.1.2 Bidistilled water

4.1.3 Heptafluorobutyric acid, puriss., ≥99,5% (CAS: 375-22-4)

4.2 Preparation of eluents

- 4.2.1** Solvent A:
Pipette 0.6 ml of heptafluorobutyric acid (4.1.3) into 1000 ml bidistilled water (4.1.2), shake and degas.
- 4.2.2** Solvent B:
Add 300 ml of bidistilled water (4.1.2) to 700 ml of methanol (4.1.1) and shake. Pipette 0.6 ml of heptafluorobutyric acid (4.1.3) into this solution, shake and degas.

4.3 Standards

- 4.3.1** Imidazole, $\geq 99,5$ % (CAS: 288-32-4)
- 4.3.2** Pyrrolidone, ≥ 99 % (CAS: 616-45-5)
- 4.3.3** Vinylimidazole, ≥ 99 % (CAS: 1072-63-5)
- 4.3.4** Vinylpyrrolidone, $\geq 99,8$ % (CAS: 88-12-0)

4.4 Preparation of standard solutions

- 4.4.1** Preparation of the stock standard solutions (1,00 g/l):
Weigh exactly 100 mg of standards (4.3.1-4.3.4), transfer them without loss into a 100 ml volumetric flask, fill with bidistilled water (4.1.2) to about 90 ml, shake and adjust to 100 ml.
- 4.4.2** Preparation of the mixed standard solution (Imidazole: 62.5 mg/l; Pyrrolidone: 62.5 mg/l; Vinylimidazole: 12.5 mg/l; Vinylpyrrolidone: 6.25 mg/l):
Pipette 6.25 ml of the Imidazole stock solution (4.4.1), 6.25 ml of the Pyrrolidone stock solution (4.4.1), 1.25 ml of the Vinylimidazole stock solution (4.4.1) and 0.625 ml of the Vinylpyrrolidone stock solution (4.4.1) to a 100 ml volumetric flask, fill with bidistilled water (4.1.2) to about 90 ml, shake and adjust to 100 ml.
- 4.4.3** Preparation of the working standard solution:
Pipette 40 μ l mixed standard solution (4.4.2) to a 25-ml volumetric flask, fill with bidistilled water to 25 ml and shake.

4.5 Preparation of the matrix calibration curve

Matrix-matched calibration solutions are prepared in an uncontaminated wine or must. Dilute the mixed standard solution (4.4.2) appropriately with the sample to give five working standards.
Calibration standards must be prepared just before measurement!

end volume	mixed standard	Imidazole	Pyrrolidone	Vinylimidazole	Vinylpyrrolidone
25 ml	0 µl	0 µg/l	0 µg/l	0 µg/l	0 µg/l
25 ml	10 µl	25 µg/l	25 µg/l	5 µg/l	2.5 µg/l
25 ml	20 µl	50 µg/l	50 µg/l	10 µg/l	5 µg/l
25 ml	30 µl	75 µg/l	75 µg/l	15 µg/l	7.5 µg/l
25 ml	40 µl	100 µg/l	100 µg/l	20 µg/l	10 µg/l
25 ml	50 µl	125 µg/l	125 µg/l	25 µg/l	12.5 µg/l

5 Apparatus

- 5.1 Analytical balance accurate to 0.1mg
- 5.2 Assorted precision pipettes and volumetric flasks
- 5.3 HPLC vials (4 ml)
- 5.4 High-performance liquid chromatograph with mass spectrometric detector (Applied Biosystems API 4000 or equivalent)
- 5.5 Knauer Eurospher 100-5 C18 column with an integrated pre-column or equivalent
Internal diameter: 4.6 mm
Length: 250 mm
Stationary Phase: C18, pore size: 100 Å, particle size: 5 µm, end-capped

6 Sample preparation

6.1 Model wine Solution

The model wine solution is prepared according to Martínez-Rodríguez and Polo, 2000 (Characterization of the Nitrogen Compounds Released during Yeast Autolysis in a Model Wine System).

Four grams of tartaric acid, 0.1 g of acetic acid, and 120 mL of ethanol are dissolved in 800 mL of water (bidistilled). After adjustment of the pH value to 3.2 with 2N sodium hydroxide, the solution is made up to 1000 mL. The model wine solution is brought to temperature at 20°C.

6.2 Sample preparation for migration analysis

The amount of 0.5 grams Divergan HM are added to 1 litre of model wine solution and stirred at 20°C for 48 hours (at approximately 150 rpm).

Prior to analysis the sample is centrifuged (approximately 3 min, 4500 rpm) and filtered through a 0.45 µm membrane filter.

6.3 Other samples (e.g. musts and wines)

Clear samples are filled directly into sample vials and ready for chromatography without any sample preparation. Cloudy wine samples are filtered through a 0.45 µm membrane filter before injection, and the first fractions of filtrate are discarded.

7 LC-MS Analysis**7.1 Operating conditions for HPLC:**

Injection volume: 10 µl

Flow rate: 1 ml/min

Gradient:

85 : 15 (A : B) $\xrightarrow{10 \text{ min}}$ 85 : 15 $\xrightarrow{5 \text{ min}}$ 0 : 100 $\xrightarrow{10 \text{ min}}$ 0 : 100
 $\xrightarrow{5 \text{ min}}$ 85 : 15 $\xrightarrow{15 \text{ min}}$ 85 : 15

Column heater: 25 °C

Run time: 45 min

7.2 MS conditions:

Mass spectrometer: Applied Biosystems API 4000 or equivalent

Scan Type: MRM

Polarity: Positive

Ion Source: Turbo Spray

Duration: 20,005 min; 1364 Cycles

Curtain Gas: 40 psi

Ionspray Voltage: 2500 V

Temperature: 550 °C

Ion Source Gas 1: 60 psi

Ion Source Gas 2: 60 psi

Collision Gas: Medium

Entrance Potential: 10 V

Collar 2: 0

compound	Q1 Mass (amu)	Q3 mass (amu)	Dwell (msec)	Parameter	Start	Stop
Imidazole	69.08	42.20	75.00	DP	81.00	81.00

				CE	31.00	31.00
				CXP	2.00	2.00
Pyrrolidone	86.10	44.10	75.00	DP	66.00	66.00
				CE	31.00	31.00
				CXP	6.00	6.00
	86.10	69.00	75.00	DP	66.00	66.00
				CE	23.00	23.00
				CXP	4.00	4.00
Vinylimidazole	95.09	41.10	75.00	DP	71.00	71.00
				CE	33.00	33.00
				CXP	0.00	0.00
	95.09	69.20	75.00	DP	71.00	71.00
				CE	29.00	29.00
				CXP	12.00	12.00
Vinylpyrrolidone	112.08	69.20	75.00	DP	51.00	51.00
				CE	21.00	21.00
				CXP	4.00	4.00
	112.08	84.00	75.00	DP	51.00	51.00
				CE	17.00	17.00
				CXP	14.00	14.00

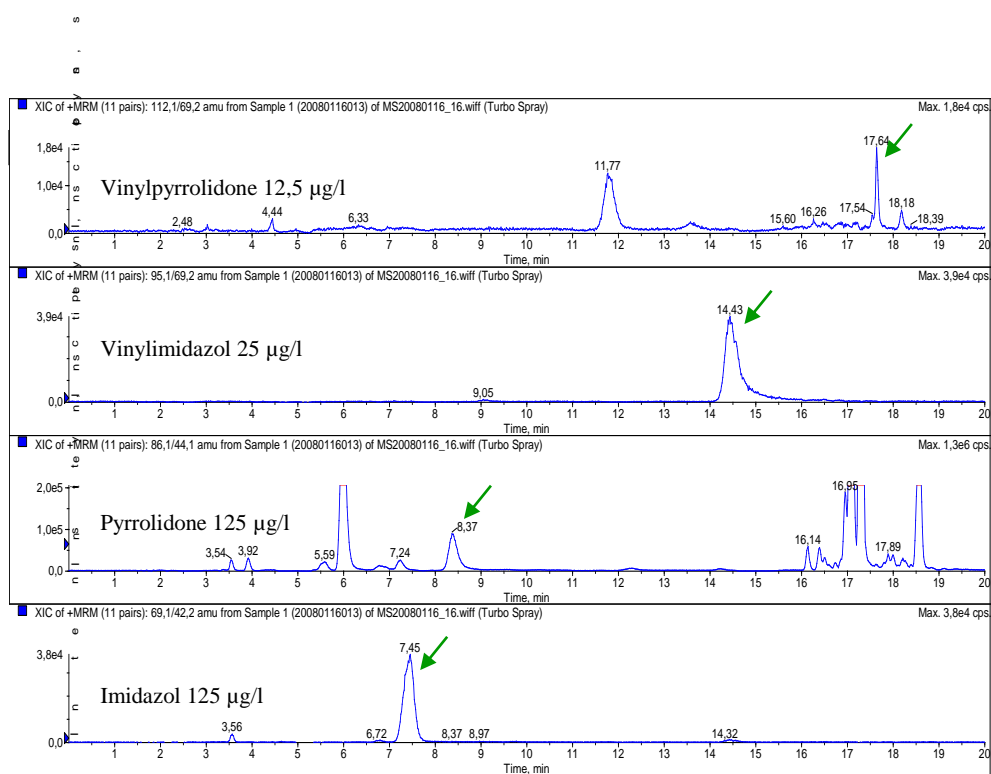
DP: Declustering Potential (in volts)
CE: Collision Energy (in volts)
CXP: Collision Cell Exit Potential (in volts)

8 Evaluation

8.1 Identification:

Inject 10 µl of working standard solution (4.4.3) to ascertain the retention times. Approximate retention times are:

compound	retention time
Imidazole	7.45 min
Pyrrolidone	8.37 min
Vinylimidazole	14.43 min
Vinylpyrrolidone	17.64 min



8.2 Quantification:

Mass transfers for quantification:

compound	mass transfer
Imidazole	69.1 → 42.2
Pyrrolidone	86.1 → 44.1
Vinylimidazole	95.1 → 69.2
Vinylpyrrolidone	112.1 → 69.2

Use the standard addition method for quantification.

8.3 Expression of results

Results should be expressed in µg/l for Imidazole, Pyrrolidone, Vinylimidazole and Vinylpyrrolidone with no decimals (e.g. 3 µg/l).

8.4 Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) depend on the individual measurement conditions of the chemical analysis and are to be determined by the user of the method.

The limit of detection (LOD) and the limit of quantification were estimated using the instrumentation and conditions mentioned as an example above (section 7) following the instructions in the resolution OENO 7-2000 (E-AS1-10-LIMDET) "*Estimation of the Detection and Quantification Limits of a Method of Analysis*". Following the "Logic Diagram for Decision-Making" in point 3 the "graph" approach should be applied following paragraph 4.2.1. For this purpose a window is drawn on the multiple reaction monitoring chromatogram, enclosing the range of a tenfold peak width at mid-height ($w_{1/2}$) either side at the retention time of an analyte peak in the relevant part of the chromatogram. Two parallel lines are then drawn which just enclose the maximum amplitude of the signal window. The separation between these two lines gives h_{max} , expressed in abundance units, which is multiplied by 3 for LOD, by 10 for LOQ, and finally converted into concentration units by implementing the individual response factor.

compound	limit of detection (LOD)	limit of quantification (LOQ)
Imidazole	5 µg/l	12 µg/l
Pyrrolidone	25 µg/l	83 µg/l
Vinylimidazole	2 µg/l	6 µg/l
Vinylpyrrolidone	2 µg/l	6 µg/l

9 Precision and trueness

As matrices three different wines (dry white wine, dry red wine and sweet red wine) and grape juice were used. Within-laboratory reproducibility, repeatability and recovery were calculated based on matrix calibration and three spikes (Imidazole: 40/60/80 µg/l; 2-Pyrrolidone: 40/60/80 µg/l; Vinylimidazole: 8/12/16 µg/l; Vinylpyrrolidone: 4/6/8 µg/l).

1.1 Imidazole

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SD_{wIR}):	40 µg/l	41	2	5 %	26
	60 µg/l	61	3	5 %	24
	80 µg/l	80	5	6 %	23
repeatability (SD_r):	40 µg/l	41	1	2 %	
	60 µg/l	61	2	3 %	
	80 µg/l	80	4	5 %	
recovery (WDF):	40 µg/l	102 %			
	60 µg/l	101 %			
	80 µg/l	101 %			
	0	101 %			

1.2 Pyrrolidone

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SD_{wIR}):	40 µg/l	42	9	22 %	26
	60 µg/l	60	9	15 %	24
	80 µg/l	81	9	11 %	23
repeatability (SD_r):	40 µg/l	42	5	12 %	
	60 µg/l	60	4	7 %	
	80 µg/l	81	8	9 %	
recovery (WDF):	40 µg/l	105 %			
	60 µg/l	100 %			
	80 µg/l	101 %			
	0	102 %			

1.3 Vinylimidazole

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SD_{wIR}):	8 µg/l	8	0	4 %	33
	12 µg/l	12	1	5 %	31
	16 µg/l	16	1	4 %	30
repeatability (SD_r):	8 µg/l	8	0	4 %	
	12 µg/l	12	0	3 %	
	16 µg/l	16	0	3 %	
recovery (WDF):	8 µg/l	101 %			
	12 µg/l	102 %			
	16 µg/l	102 %			
	0	102 %			

1.4 Vinylpyrrolidone

	fortification	mean of series	standard deviation	corresponding CV	Horwitz RSD %
within-laboratory reproducibility (SD_{wIR}):	4 µg/l	3	1	31 %	37
	6 µg/l	4	1	26 %	35
	8 µg/l	5	2	29 %	33
repeatability (SD_r):	4 µg/l	3	1	25 %	
	6 µg/l	4	1	22 %	
	8 µg/l	5	1	26 %	
recovery (WDF):	4 µg/l	66 %			
	6 µg/l	63 %			
	8 µg/l	66 %			
	0	65 %			

I

(Acts adopted under the EC Treaty/Euratom Treaty whose publication is obligatory)

REGULATIONS

COMMISSION REGULATION (EC) No 606/2009

of 10 July 2009

laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, oenological practices and the applicable restrictions

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Regulation (EC) No 479/2008 of 29 April 2008 on the common organisation of the market in wine, amending Regulations (EC) No 1493/1999, (EC) No 1782/2003, (EC) No 1290/2005, (EC) No 3/2008 and repealing Regulations (EEC) No 2392/86 and (EC) No 1493/1999 ⁽¹⁾, and in particular Articles 25(3) and 32 thereof,

Whereas:

- (1) The definition of wine given in the first indent of point (c) of the second subparagraph of paragraph 1 of Annex IV to Regulation (EC) No 479/2008 listing the categories of grapevine products provides for a total alcoholic strength of not more than 15 % vol. However, that limit may be increased to 20 % vol. for wines produced without enrichment in certain wine-growing areas that should be defined.
- (2) Chapter II of Title III of Regulation (EC) No 479/2008 and Annexes V and VI thereto lay down general rules on oenological practices and processes and refer for the rest to detailed implementing rules to be adopted by the Commission. The permitted oenological practices should be defined clearly and precisely, including the methods for sweetening wines, and limits on the use of certain substances and the conditions for using certain of those substances should be laid down.

- (3) Annex IV to Council Regulation (EC) No 1493/1999 of 17 May 1999 on the common organisation of the market in wine ⁽²⁾ listed authorised oenological practices. That list of authorised oenological practices, described more clearly and more coherently and supplemented to take account of technical progress, should be kept in a single annex.
- (4) Annex V A to Regulation (EC) No 1493/1999 lays down maximum levels of sulphites in wines produced in the Community that are higher than the limits laid down by the International Organisation of Vine and Wine (OIV). The limits should be aligned with those of the OIV, which are recognised internationally, and the derogations required for certain sweet wines produced in small quantities because of their higher sugar content and to ensure their good conservation should be kept. In the light of current scientific studies into the reduction and replacement of sulphites in wine and the sulphite intake from wine in the human diet, provision must be made for re-examining the maximum limits at a later date with a view to reducing them.
- (5) The procedures by means of which the Member States may authorise certain oenological practices and processes not provided for by Community rules for a definite period and for experimental purposes should be laid down.
- (6) The production of sparkling wines, quality sparkling wines and quality aromatic sparkling wines requires a number of specific practices in addition to the oenological practices permitted elsewhere. For reasons of clarity, those practices should be listed in a separate annex.

⁽¹⁾ OJ L 148, 6.6.2008, p. 1.

⁽²⁾ OJ L 179, 14.7.1999, p. 1.

- (7) The production of liqueur wines requires a number of specific practices in addition to the oenological practices permitted elsewhere and the production of liqueur wines with a protected designation of origin has certain particularities. For reasons of clarity, those practices and restrictions should be listed in a separate annex.
- (8) Coupage is a widespread oenological practice and, in view of its possible consequences for the quality of wine, its use must be strictly defined and regulated in order to prevent abuse and to ensure high quality wines at the same time as promoting a more competitive sector. As far as rosé wine production is concerned, for the reasons mentioned above, this practice must be regulated more particularly for certain wines which are not subject to specifications.
- (9) Purity and identification specifications of a large number of substances used in oenological practices are already laid down in the Community rules on foodstuffs and in the International Oenological Codex of the OIV. For the purposes of harmonisation and clarity, those specifications should be used in the first instance, while providing for additional rules specific to the situation in the Community.
- (10) Wine products that do not comply with the provisions of Chapter II of Title III of Regulation (EC) No 479/2008 or those to be laid down in this Regulation may not be placed on the market. However, some of these products may be used for industrial purposes and the conditions for their use should be laid down so as to ensure adequate monitoring of their final use. In addition, to avoid financial losses for operators with stocks of certain products produced before the date of application of that Regulation, it should be laid down that products made in accordance with the rules in force before that date may be released for consumption.
- (11) In accordance with paragraph 4 of point D of Annex V to Regulation (EC) No 479/2008, all enrichment, acidification and deacidification operations must be notified to the competent authorities. This also holds for quantities of sugar, concentrated grape must and rectified concentrated grape must held by the natural or legal persons undertaking such operations. The purpose of such notification is to allow the operations in question to be monitored. Notifications must therefore be addressed to the competent authority of the Member State on whose territory the operation is to take place and must be as accurate as possible. Where an increase in alcoholic strength is involved, the competent authority must be notified in sufficient time to permit it to carry out an effective check.
- (12) In the case of acidification and deacidification, a check after the operation is sufficient. For that reason and to simplify administrative procedures, it must therefore be possible to make such notification, except for the first notification in the wine year, by updating records regularly verified by the competent authority. In certain Member States, the competent authorities carry out systematic analytical checks of all batches of products turned into wine. As long as this continues to be the case, declarations of intention to enrich wine are not absolutely necessary.
- (13) Notwithstanding the general rule laid down in point D of Annex VI to Regulation (EC) No 479/2008, the pouring of wine [or grape must onto lees or grape marc or pressed 'aszú' or 'výber' pulp is an essential characteristic of the production of certain Hungarian and Slovak wines. The particular rules for that practice must be laid down in accordance with the national provisions in force in the Member States concerned on 1 May 2004.
- (14) Article 31 of Regulation (EC) No 479/2008 lays down that the analysis methods for establishing the composition of the products covered by that Regulation and the rules for checking whether those products have been subjected to processes in violation of authorised oenological practice are those recommended and published by the OIV in the Compendium of International Methods of Analysis of Wines and Musts. Where specific analysis methods are necessary for certain Community wine products and they have not been established by the OIV, those Community methods should be described.
- (15) So as to ensure greater transparency, a list of the analysis methods concerned and their description should be published at Community level.
- (16) Consequently, Commission Regulations (EEC) No 2676/90 of 17 September 1990 determining Community methods for the analysis of wines ⁽¹⁾ and (EC) No 423/2008 of 8 May 2008 laying down certain detailed rules for implementing Council Regulation (EC) No 1493/1999 and establishing a Community code of oenological practices and processes ⁽²⁾ should be repealed.
- (17) The measures provided for in this Regulation are in accordance with the opinion of the Regulatory Committee established by Article 113(2) of Regulation (EC) No 479/2008,
- HAS ADOPTED THIS REGULATION:
- Article 1*
Purpose
- This Regulation lays down detailed rules for the application of Title III, Chapters I and II of Regulation (EC) No 479/2008.
- ⁽¹⁾ OJ L 272, 3.10.1990, p. 1.
⁽²⁾ OJ L 127, 15.5.2008, p. 13.

*Article 2***Wine-growing areas where wines may have a maximum total alcoholic strength of 20 % vol.**

The wine-growing areas referred to in the first indent of point (c) of the second subparagraph of paragraph 1 of Annex IV to Regulation (EC) No 479/2008 shall be zones C I, C II and C III referred to in Annex IX to that Regulation and the areas of zone B in which white wines with the following protected geographical indications may be produced: 'Vin de pays de Franche-Comté' and 'Vin de pays du Val de Loire'.

*Article 3***Authorised oenological practices and restrictions**

1. The authorised oenological practices and restrictions applicable to the production and conservation of products covered by Regulation (EC) No 479/2008, referred to in Article 29(1) thereof, are laid down in Annex I hereto.
2. The authorised oenological practices and the conditions for and the limits on their use are set out in Annex I A.
3. The maximum sulphur dioxide contents of wines are given in Annex I B.
4. The maximum volatile acid contents are given in Annex I C.
5. The rules on sweetening are laid down in Annex I D.

*Article 4***Experimental use of new oenological practices**

1. For experimental purposes as referred to in Article 29(2) of Regulation (EC) No 479/2008, each Member State may authorise the use of certain oenological practices or processes not provided for in that Regulation or in this Regulation, for a maximum of three years, on condition that:
 - (a) the practices and processes concerned meet the requirements of Articles 27(2) and 30(b) to (e) of Regulation (EC) No 479/2008;
 - (b) such practices and processes are applied to quantities not exceeding 50 000 hectolitres per year for any one experiment;
 - (c) the Member State concerned informs the Commission and the other Member States at the beginning of the experiment of the terms of each authorisation;
 - (d) the processes shall be entered on the accompanying document referred to in Article 112(1) and in the register referred to in Article 112(2) of Regulation (EC) No 479/2008.

'Experiment' shall mean an operation or operations carried out in the context of a well-defined research project with a single experimental protocol.

2. The products obtained by the experimental use of such practices and processes may be placed on the market of a Member State other than the Member State concerned provided the Member State authorising the experiment gives prior notification to the competent authorities of the Member State of destination of the terms of the authorisation and the quantities involved.

3. During the three months following the end of the period referred to in paragraph 1, the Member State concerned shall forward to the Commission a report on the authorised experiment and the results thereof. The Commission shall notify the other Member States of those results.

4. Depending on these results, the Member State concerned may apply to the Commission for authorisation to continue the experiment, possibly with a larger quantity than in the original experiment, for a further maximum period of three years. The Member State shall submit an appropriate dossier in support of its application. The Commission, in accordance with the procedure referred to in Article 113(2) of Regulation (EC) No 479/2008, shall decide on the application to continue the experiment.

*Article 5***Oenological practices applicable to categories of sparkling wines**

The authorised oenological practices and restrictions, including enrichment, acidification and de-acidification, concerning sparkling wines, quality sparkling wines and quality aromatic sparkling wines, referred to in point (b) of the second paragraph of Article 32 of Regulation (EC) No 479/2008 are listed in Annex II hereto, without prejudice to the oenological practices and restrictions of general application laid down in Regulation (EC) No 479/2008 and in Annex I hereto.

*Article 6***Oenological practices applicable to liqueur wines**

The authorised oenological practices and restrictions concerning liqueur wines referred to in point (c) of the second paragraph of Article 32 of Regulation (EC) No 479/2008 are listed in Annex III hereto, without prejudice to the oenological practices and restrictions of general application laid down in Regulation (EC) No 479/2008 and in Annex I hereto.

*Article 7***Definition of coupage**

1. Within the meaning of point (d) of the second paragraph of Article 32 of Regulation (EC) No 479/2008, 'coupage' shall mean the mixing of wines or musts of different origins, different vine varieties, different harvest years or different categories of wine or of must.

2. The following shall be regarded as different categories of wine or must:

- (a) red wine, white wine and the musts or wines suitable for yielding one of these categories of wine;
- (b) wines without a protected designation of origin or geographical indication, wines with a protected designation of origin (PDO) and wines with a protected geographical indication (PGI) as well as musts or wines suitable for yielding one of these categories of wine.

For the purposes of this paragraph, rosé wine shall be regarded as red wine.

3. The following processes shall not be regarded as coupage:

- (a) enrichment by the addition of concentrated grape must or rectified concentrated grape must;
- (b) sweetening.

Article 8

General rules on blending and coupage

1. A wine may be obtained by blending or coupage only where the constituents of that blending or coupage possess the required characteristics for obtaining wine and comply with Regulation (EC) No 479/2008 and this Regulation.

Coupage of a non-PDO/PGI white wine with a non-PDO/PGI red wine cannot produce a rosé wine.

However, the second subparagraph does not exclude coupage of the type referred to therein where the final product is intended for the preparation of a cuvée as defined in Annex I to Regulation (EC) No 479/2008 or intended for the production of semi-sparkling wines.

2. Coupage of a grape must or a wine which has undergone the oenological practice referred to in paragraph 14 of Annex I A to this Regulation with a grape must or a wine which has not undergone that practice shall be prohibited.

Article 9

The purity and identification specifications of substances used in oenological practices

1. Where they are not laid down by Commission Directive 2008/84/EC ⁽¹⁾, the purity and identification specifications of substances used in the oenological practices referred to in point (e) of the second paragraph of Article 32 of Regulation (EC) No 479/2008 shall be those laid down and published in the International Oenological Codex of the International Organisation of Vine and Wine.

Where necessary, those purity criteria shall be supplemented by the specific requirements provided for in Annex I A hereto.

⁽¹⁾ OJ L 253, 20.9.2008, p. 1.

2. The enzymes and enzymatic preparations used in the authorised oenological practices and processes listed in Annex I A shall meet the requirements of Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on food enzymes ⁽²⁾.

Article 10

Conditions governing the holding, circulation and use of products not complying with Chapter II of Title III of Regulation (EC) No 479/2008 or this Regulation

1. Products not complying with Chapter II of Title III of Regulation (EC) No 479/2008 or this Regulation shall be destroyed. However, Member States may authorise the use of certain products, the characteristics of which they shall determine, by distilleries or vinegar factories or for industrial purposes.

2. Such products may not be held without legitimate cause by producers or traders and they may be moved only to distilleries, vinegar factories, or establishments using them for industrial purposes or products or elimination plants.

3. Member States may have denaturing agents or indicators added to wines as referred to in paragraph 1 in order to make them more easily identifiable. Where justified, they may also prohibit the uses provided for in paragraph 1 and have the products disposed of.

4. Wine produced before 1 August 2009 may be offered or supplied for direct human consumption provided that it complies with the Community or national rules in force prior to that date.

Article 11

General rules applicable to the enrichment, acidification and deacidification of products other than wine

The processes referred to in paragraph 1 of point D of Annex V to Regulation (EC) No 479/2008 must be carried out in a single operation. However, Member States may permit some of these processes to be carried out in more than one operation where this improves the vinification of the products concerned. In such cases, the limits laid down in Annex V to Regulation (EC) No 479/2008 shall apply to the whole operation concerned.

⁽²⁾ OJ L 354, 31.12.2008, p. 7.

Article 12

Administrative rules applicable to enrichment

1. Notifications as referred to in paragraph 4 of point D of Annex V to Regulation (EC) No 479/2008 relating to operations to increase alcoholic strength shall be made by the natural or legal persons carrying out the operations concerned and in compliance with suitable time limits and control conditions set by the competent authority of the Member State on whose territory the operation takes place.

2. Notifications as referred to in paragraph 1 shall be made in writing and shall include the following information:

- (a) the name and address of the person making the notification;
- (b) the place where the operation is to be carried out;
- (c) the date and time when the operation is to commence;
- (d) the description of the product undergoing the operation;
- (e) the process used for the operation, with details of the type of product to be used.

3. Member States may allow prior notifications covering several operations or a specified period to be sent to the competent authorities. Such notifications shall be accepted only if the person making the notification keeps a written record of each enrichment operation as provided for in paragraph 6 and of the information required by paragraph 2.

4. Where the person concerned is prevented by reasons of force majeure from carrying out the notified operation in due time, Member States shall specify the conditions under which that person is to submit a new notification to the competent authority so that the necessary checks can be carried out.

5. The notification referred to in paragraph 1 shall not be required in Member States in which the competent inspection authorities carry out systematic analytical checks of all batches of products turned into wine.

6. The particulars relating to operations to increase alcoholic strength shall be entered in the registers referred to in Article 112(2) of Regulation (EC) No 479/2008 immediately after the operation is completed.

In cases where prior notifications covering several operations do not indicate the date and time when the operations are to commence, an entry must also be made in those registers before each operation commences.

Article 13

Administrative rules applicable to acidification and deacidification

1. In the case of acidification and deacidification, operators shall make notifications as referred to in paragraph 4 of point D of Annex V to Regulation (EC) No 479/2008 not later than the second day following the first operation carried out in any wine year. Such notifications shall be valid for all operations in that wine year.

2. Notifications as referred to in paragraph 1 shall be made in writing and shall include the following information:

- (a) the name and address of the person making the notification;
- (b) the type of operation involved;
- (c) the place where the operation took place.

3. The particulars relating to each acidification and deacidification operation shall be entered in the registers referred to in Article 112(2) of Regulation (EC) No 479/2008.

Article 14

Pouring of wine or grape must to lees or grape marc or pressed 'aszú'/'výber' pulp

The pouring of wine or grape must to lees or grape marc or pressed 'aszú'/'výber' pulp, provided for in paragraph 2 of point D of Annex VI to Regulation (EC) No 479/2008, shall be carried out as follows, in accordance with the national provisions in force on 1 May 2004:

- (a) 'Tokaji fordítás' or 'Tokajský fordítás' shall be prepared by pouring must or wine on pressed 'aszú'/'výber' pulp;
- (b) 'Tokaji máslás' or 'Tokajský máslás' shall be prepared by pouring must or wine on the lees of 'szamorodni'/'samorodné' or 'aszú'/'výber'.

The products concerned must be from the same harvest year.

Article 15

Applicable Community analysis methods

1. The analysis methods referred to in the second paragraph of Article 31 of Regulation (EC) No 479/2008 applicable for the verification of certain wine products and certain limits laid down at Community level are set out in Annex IV hereto.

2. The Commission shall publish in the C Series of the *Official Journal of the European Union* the list and description of the analysis methods referred to the first paragraph of Article 31 of Regulation (EC) No 479/2008 and described in the Compendium of International Methods of Analysis of Wines and Musts of the International Organisation of Vine and Wine and applicable for verification of the limits and requirements laid down by Community rules for the production of wine products.

*Article 16***Repeal**

Regulations (EEC) No 2676/90 and (EC) No 423/2008 are repealed.

References to the repealed Regulations and to Regulation (EC) No 1493/1999 shall be construed as references to this Regulation

and shall be read in accordance with the correlation table in Annex V.

Article 17

This Regulation shall enter into force on the seventh day following its publication in the *Official Journal of the European Union*.

It shall apply from 1 August 2009.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 10 July 2009.

For the Commission
Mariann FISCHER BOEL
Member of the Commission

ANNEX I A

AUTHORISED OENOLOGICAL PRACTICES AND PROCESSES.

1		2	3
Oenological practice		Conditions of use ⁽¹⁾	Limits on use Applications
1	Aeration or oxygenation using gaseous oxygen		
2	Heat treatments		
3	Centrifuging and filtration with or without an inert filtering agent		Use of an agent must not leave undesirable residues in the treated product
4	Use of carbon dioxide, argon or nitrogen, either alone or combined, in order to create an inert atmosphere and to handle the product shielded from the air		
5	Use of yeasts for wine production, whether dry or in wine suspension	Only with fresh grapes, grape must, partially fermented grape must, partially fermented grape must obtained from raisined grapes, concentrated grape must and new wine still in fermentation and for the second alcoholic fermentation of all categories of sparkling wine.	
6	The use, to encourage yeast development, of one or more of the following substances, with the possible addition of microcrystalline cellulose as an excipient:		
	— addition of diammonium phosphate or ammonium sulphate	Only with fresh grapes, grape must, partially fermented grape must, partially fermented grape must obtained from raisined grapes, concentrated grape must and new wine still in fermentation and for the second alcoholic fermentation of all categories of sparkling wine.	No more than 1 g/l (expressed in salts) ⁽²⁾ or 0,3 g/l for the second fermentation of sparkling wines.
	— addition of ammonium bisulphite	Only with fresh grapes, grape must, partially fermented grape must, partially fermented grape must obtained from raisined grapes, concentrated grape must and new wine still in fermentation	No more than 0,2 g/l (expressed in salts) ⁽²⁾ and up to the limits set in point 7.
	— addition of thiamin hydrochloride	Only with fresh grapes, grape must, partially fermented grape must, partially fermented grape must obtained from raisined grapes, concentrated grape must and new wine still in fermentation and for the second alcoholic fermentation of all categories of sparkling wine.	No more than 0,6 mg/l (expressed in thiamin) for each treatment
7	Use of sulphur dioxide, potassium bisulphite or potassium metabisulphite, also called potassium disulphite or potassium pyrosulphite		Limits (i.e. maximum quantity in the product placed on the market) as laid down in Annex I B
8	Elimination of sulphur dioxide by physical processes	Only with fresh grapes, grape must, partially fermented grape must, partially fermented grape must obtained from raisined grapes, concentrated grape must, rectified concentrated grape must and new wine still in fermentation	

1		2	3
Oenological practice		Conditions of use ⁽¹⁾	Limits on use Applications
9	Treatment with charcoal for oenological use	Only for musts and new wines still in fermentation, rectified concentrated grape must and white wines	No more than 100 g of dry product per hl
10	Clarification by means of one or more of the following substances for oenological use: <ul style="list-style-type: none"> — edible gelatine, — plant proteins from wheat or peas, — isinglass, — casein and potassium caseinates, — egg albumin, — bentonite, — silicon dioxide as a gel or colloidal solution, — kaolin, — tannin, — pectolytic enzymes, — enzymatic preparations of beta-glucanase 	Conditions for using beta-glucanase laid down in Appendix 1	
11	Use of sorbic acid in potassium sorbate form		Maximum sorbic acid content in the product so treated and placed on the market: 200 mg/l
12	Use of tartaric L(+) acid, malic L acid, DL malic acid, or lactic acid for acidification purposes	Conditions and limits laid down in points C and D of Annex V to Regulation (EC) No 479/2008 and Articles 11 and 13 of this Regulation. Specifications for L(+) tartaric acid laid down in paragraph 2 of Appendix 2	
13	Use of one or more of the following substances for deacidification purposes: <ul style="list-style-type: none"> — neutral potassium tartrate, — potassium bicarbonate, — calcium carbonate, which may contain small quantities of the double calcium salt of L(+) tartaric and L(-) malic acids, — calcium tartrate, — L(+) tartaric acid — a homogeneous preparation of tartaric acid and calcium carbonate in equivalent proportions and finely pulverised 	Conditions and limits laid down in points C and D of Annex V to Regulation (EC) No 479/2008 and Articles 11 and 13 of this Regulation. Specifications for L(+) tartaric acid laid down in Appendix 2	
14	Addition of Aleppo pine resin	Under the conditions set out in Appendix 3	

1		2	3
Oenological practice		Conditions of use ⁽¹⁾	Limits on use Applications
15	Use of preparations from yeast cell walls		No more than 40 g/hl
16	Use of polyvinylpyrrolidone		No more than 80 g/hl
17	Use of lactic bacteria		
18	Addition of lysozyme		No more than 500 mg/l (where added to both the must and the wine, the total overall quantity must not exceed 500 mg/l)
19	Addition of L ascorbic acid		Maximum content in wine thus treated and placed on the market: 250 mg/l ⁽³⁾
20	Use of ion exchange resins	Only with grape must intended for the manufacture of rectified concentrated grape must under the conditions set out in Appendix 4	
21	Use in dry wines of fresh lees which are sound and undiluted and contain yeasts resulting from the recent vinification of dry wine	For the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	Quantities not exceeding 5 % of the volume of product treated
22	Bubbling using argon or nitrogen		
23	Addition of carbon dioxide	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 7 and 9 of Annex IV to Regulation (EC) No 479/2008	In the case of still wines the maximum carbon dioxide content in the wine so treated and placed on the market is 3 g/l, while the excess pressure caused by the carbon dioxide must be less than 1 bar at a temperature of 20 °C
24	Addition of citric acid for wine stabilisation purposes	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	Maximum content in wine thus treated and placed on the market: 1g/l
25	Addition of tannins	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Council Regulation (EC) No 479/2008	
26	The treatment: — of white and rosé wines with potassium ferrocyanide, — of red wines with potassium ferrocyanide or with calcium phytate	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008 under the conditions set out in Appendix 5	In the case of calcium phytate, no more than 8 g/hl
27	Addition of metatartaric acid	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	No more than 100 mg/l

1		2	3
Oenological practice		Conditions of use ⁽¹⁾	Limits on use Applications
28	Use of acacia	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	
29	Use of DL tartaric acid, also called racemic acid, or of its neutral salt of potassium, for precipitating excess calcium	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008 and under the conditions laid down in Appendix 5	
30	To assist the precipitation of tartaric salts, use of: — potassium bitartrate or potassium hydrogen tartrate, — calcium tartrate	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	In the case of calcium tartrate, no more than 200 g/hl
31	Use of copper sulphate or cupric citrate to eliminate defects of taste or smell in the wine	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	No more than 1 g/hl, provided that the copper content of the product so treated does not exceed 1 mg/l
32	Addition of caramel within the meaning of Directive 94/36/EC of the European Parliament and of the Council of 30 June 1994 on colours for use in foodstuffs ⁽⁴⁾ , to reinforce the colour	Only with liqueur wines	
33	Use of discs of pure paraffin impregnated with allyl isothiocyanate to create a sterile atmosphere	Only for partially fermented must for direct human consumption as such, and wine. Permitted solely in Italy as long as it is not prohibited under that country's legislation and only in containers holding more than 20 litres	No trace of allyl isothiocyanate must be present in the wine
34	Addition of dimethyldicarbonate (DMDC) to wine for microbiological stabilisation	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008 and under the conditions laid down in Appendix 6	No more than 200 mg/l with no detectable residues in the wine placed on the market
35	Addition of yeast mannoproteins to ensure the tartaric and protein stabilisation of wines	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008	

1		2	3
Oenological practice		Conditions of use ⁽¹⁾	Limits on use Applications
36	Electrodialysis treatment to ensure the tartaric stabilisation of the wine	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008 and under the conditions laid down in Appendix 7	
37	Use of urease to reduce the level of urea in the wine	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008 and under the conditions laid down in Appendix 8	
38	Use of oak chips in winemaking and ageing, including in the fermentation of fresh grapes and grape must	Under the conditions laid down in Appendix 9	
39	Use: — of calcium alginate, or, — of potassium alginate,	Only for the manufacture of all categories of sparkling and semi-sparkling wines obtained by fermentation in bottle and with the lees separated by disgorging	
40	Partial dealcoholisation of wine	Only with wine and under the conditions laid down in Appendix 10	
41	Use of polyvinylimidazole/polyvinylpyrrolidone (PVI/PVP) copolymers in order to reduce the copper, iron and heavy metal content	Under the conditions laid down in Appendix 11	No more than 500 mg/l (where added to both the must and the wine, the total overall quantity must not exceed 500 mg/l)
42	Addition of carboxymethylcellulose (cellulose gums) to ensure tartaric stabilisation	Only with wine and all categories of sparkling and semi-sparkling wine	No more than 100 mg/l
43	Treatment with cation exchangers to ensure the tartaric stabilisation of the wine	For partially fermented must for direct human consumption as such and the products defined in paragraphs 1, 3, 4, 5, 6, 7, 8, 9, 15 and 16 of Annex IV to Regulation (EC) No 479/2008 and under the conditions laid down in Appendix 12	

⁽¹⁾ Unless otherwise stated, the practice or process described may be used for fresh grapes, grape must, partially fermented grape must, partially fermented grape must from raisined grapes, concentrated grape must, new wine still in fermentation, partially fermented grape must for direct human consumption, wine, all categories of sparkling wine, semi-sparkling wine, aerated semi-sparkling wine, liqueur wines, wines made from raisined grapes and wines made from over-ripened grapes.

⁽²⁾ These ammonium salts may also be used in combination, up to an overall limit of 1g/l, without prejudice to the specific limits of 0,3 g/l or 0,2 g/l set above.

⁽³⁾ The use limit is 250 mg/l for each treatment.

⁽⁴⁾ OJ L 237, 10.9.1994, p. 13.

Appendix 1

Requirements for beta-glucanase

1. International code for beta-glucanase: E.C. 3-2-1-58
2. Beta-glucan hydrolase (breaking down the glucan in *Botrytis cinerea*)
3. Origin: *Trichoderma harzianum*
4. Area of application: breaking down the beta-glucans present in wines, in particular those produced from botrytised grapes
5. Maximum dose: 3 g of the enzymatic preparation containing 25 % total organic solids (TOS) per hectolitre
6. Chemical and microbiological purity specifications:

Loss on drying	Less than 10 %
Heavy metals	Less than 30 ppm
Pb	Less than 10 ppm
As	Less than 3 ppm
Total coliforms	Absent
<i>Escherichia coli</i>	Absent in 25 g sample
<i>Salmonella</i> spp	Absent in 25 g sample
Aerobic count:	Less than 5×10^4 cells/g

*Appendix 2***L(+) tartaric acid**

1. Tartaric acid, the use of which for deacidification purposes is provided for in paragraph 13 of Annex I A, may be used only for products that:

are from the Elbling and Riesling vine varieties; and

are obtained from grapes harvested in the following wine-growing regions in the northern part of wine-growing zone A:

- Ahr,
- Rheingau,
- Mittelrhein,
- Mosel,
- Nahe,
- Rheinhessen,
- Pfalz,
- Moselle luxembourgeoise.

2. Tartaric acid, the use of which is provided for in paragraphs 12 and 13 of this Annex, also called L(+) tartaric acid, must be of agricultural origin and extracted specifically from wine products. It must also comply with the purity criteria laid down in Directive 2008/84/EC.
-

*Appendix 3***Aleppo pine resin**

1. Aleppo pine resin, the use of which is provided for in paragraph 14 of Annex I A, may be used only to produce 'retsina' wine. This oenological practice may be carried out only:
 - (a) in the geographical territory of Greece;
 - (b) using grape must from grape varieties, areas of production and wine-making areas as specified in the Greek provisions in force at 31 December 1980;
 - (c) by adding 1 000 grams or less of resin per hectolitre of the product used, before fermentation or, where the actual alcoholic strength by volume does not exceed one third of the overall alcoholic strength by volume, during fermentation.
 2. Greece shall notify the Commission in advance if it intends to amend the provisions referred to in paragraph 1(b). If the Commission does not respond within two months of such notification, Greece may implement the planned amendments.
-

Appendix 4

Ion exchange resins

The ion exchange resins which may be used accordance with paragraph 20 of Annex I A are styrene and divinylbenzene copolymers containing sulphonic acid or ammonium groups. They must comply with the requirements laid down in Regulation (EC) No 1935/2004 of the European Parliament and of the Council ⁽¹⁾ and Community and national provisions adopted in implementation thereof. In addition, when tested by the analysis method laid down in paragraph 2, they must not lose more than 1 mg/l of organic matter into any of the solvents listed. They must be regenerated with substances permitted for use in the preparation of foodstuffs.

These resins may be used only under the supervision of an oenologist or technician and in installations approved by the authorities of the Member States on whose territory they are used. Such authorities shall lay down the duties and responsibility incumbent on approved oenologists and technicians.

Analysis method for determining the loss of organic matter from ion exchange resins:

1. SCOPE AND AREA OF APPLICATION

The method determines the loss of organic matter from ion exchange resins.

2. DEFINITION

The loss of organic matter from ion exchange resins. The loss of organic matter is determined by the method specified.

3. PRINCIPLE

Extracting solvents are passed through prepared resins and the weight of organic matter extracted is determined gravimetrically.

4. REAGENTS

All reagents shall be of analytical quality.

Extracting solvents.

4.1. Distilled water or deionised water of equivalent purity.

4.2. Ethanol, 15 % v/v. Prepare by mixing 15 parts of absolute ethanol with 85 parts of water (paragraph 4.1).

4.3. Acetic acid, 5 % m/m. Prepare by mixing 5 parts of glacial acetic acid with 95 parts of water (paragraph 4.1).

5. APPARATUS

5.1. Ion exchange chromatography columns.

5.2. Measuring cylinders, capacity 2 l.

5.3. Evaporating dishes capable of withstanding a muffle furnace at 850 °C.

5.4. Drying oven, thermostatically controlled at 105 ± 2 °C.

5.5. Muffle furnace, thermostatically controlled at 850 ± 25 °C.

5.6. Analytical balance, accurate to 0.1 mg.

5.7. Evaporator, hot plate or infra-red evaporator.

⁽¹⁾ OJ L 338, 13.11.2004, p. 4.

6. PROCEDURE

- 6.1. Add to each of three separate ion exchange chromatography columns (paragraph 5.1) 50 ml of the ion exchange resin to be tested, washed and treated in accordance with the manufacturer's directions for preparing resins for use with food.
- 6.2. For the anionic resins, pass the three extracting solvents (paragraphs 4.1, 4.2 and 4.3) separately through the prepared columns (paragraph 6.1) at a flow rate of 350 to 450 ml/h. Discard the first litre of eluate in each case and collect the next two litres in measuring cylinders (paragraph 5.2). For the cationic resins, pass only solvents referred to in paragraphs 4.1 and 4.2 through the columns prepared for this purpose.
- 6.3. Evaporate the three eluates over a hotplate or with an infrared evaporator (paragraph 5.7) in separate evaporating dishes (paragraph 5.3) which have been previously cleaned and weighed (m_0). Place the dishes in an oven (paragraph 5.4) and dry to constant weight (m_1).
- 6.4. After recording the constant weight (paragraph 6.3), place the evaporating dish in the muffle furnace (paragraph 5.5) and ash to constant weight (m_2).
- 6.5. Calculate the organic matter extracted (paragraph 7.1). If the result is greater than 1 mg/l, carry out a blank test on the reagents and recalculate the weight of organic matter extracted.

The blank test shall be carried out by repeating the operations referred to in paragraphs 6.3 and 6.4 but using two litres of the extracting solvent, to give weights m_3 and m_4 in paragraphs 6.3 and 6.4 respectively.

7. EXPRESSION OF THE RESULTS

7.1. Formula and calculation of results

The organic matter extracted from ion exchange resins, in mg/l, is given by:

$$500 (m_1 - m_2)$$

where m_1 and m_2 are expressed in grams.

The corrected weight (mg/l) of the organic matter extracted from ion exchange resins is given by:

$$500 (m_1 - m_2 - m_3 + m_4)$$

where m_1 , m_2 , m_3 and m_4 are expressed in grams.

- 7.2. The difference in the results between two parallel determinations carried out on the same sample must not exceed 0,2 mg/l.

*Appendix 5***Potassium ferrocyanide****Calcium phytate****DL tartaric acid**

Potassium ferrocyanide or calcium phytate, the use of which is provided for in paragraph 26 of Annex I A, or DL tartaric acid, the use of which is provided for in paragraph 29 of Annex I A, may be used only under the supervision of an oenologist or technician officially approved by the authorities of the Member State in whose territory the process is carried out, the extent of whose responsibility shall be fixed, if necessary, by the Member State concerned.

After treatment with potassium ferrocyanide or calcium phytate, the wine must contain traces of iron.

Supervision of the use of the product referred to in the first paragraph shall be governed by the provisions adopted by the Member States.

*Appendix 6***Requirements for dimethyldicarbonate****AREA OF APPLICATION**

Dimethyldicarbonate may be added to wine for the following purpose: microbiological stabilisation of bottled wine containing fermentable sugar.

REQUIREMENTS

- the addition must be carried out only a short time prior to bottling, defined as putting the product concerned up for commercial purposes in containers of a capacity not exceeding 60 litres,
 - the treatment may only be applied to wine with a sugar content of not less than 5 g/l,
 - the product used must comply with the purity criteria laid down in Directive 2008/84/EC,
 - this treatment is to be recorded in the register referred to in Article 112(2) of Regulation (EC) No 479/2008.
-

Appendix 7

Requirements for electrodialysis treatment

The purpose is to obtain tartaric stability of the wine with regard to potassium hydrogen tartrate and calcium tartrate (and other calcium salts) by extraction of ions in supersaturation in the wine under the action of an electrical field and using membranes that are either anion-permeable or cation-permeable.

1. MEMBRANE REQUIREMENTS

- 1.1. The membranes are to be arranged alternately in a 'filter-press' type system or any other appropriate system separating the treatment (wine) and concentration (waste water) compartments.
- 1.2. The cation-permeable membranes must be designed to extract cations only, in particular K^+ , Ca^{++} .
- 1.3. The anion-permeable membranes must be designed to extract anions only, in particular tartrate anions.
- 1.4. The membranes must not excessively modify the physico-chemical composition and sensory characteristics of the wine. They must meet the following requirements:
 - they must be manufactured according to good manufacturing practice from substances authorised for the manufacture of plastic materials intended to come into contact with foodstuffs as listed in Annex II to Commission Directive 2002/72/EC ⁽¹⁾,
 - the user of the electrodialysis equipment must show that the membranes used meet the above requirements and that any replacements have been carried out by specialised personnel,
 - they must not release any substance in quantities endangering human health or affecting the taste or smell of foodstuffs and must meet the criteria laid down in Directive 2002/72/EC,
 - their use must not trigger interactions between their constituents and the wine liable to result in the formation of new compounds that may be toxic in the treated product.

The stability of fresh electrodialysis membranes is to be determined using a simulant reproducing the physico-chemical composition of the wine for investigation of possible migration of certain substances from them.

The experimental method recommended is as follows:

The simulant is a water-alcohol solution buffered to the pH and conductivity of the wine. Its composition is as follows:

- absolute ethanol: 11 l,
- potassium hydrogen tartrate: 380 g,
- potassium chloride: 60 g,
- concentrated sulphuric acid: 5 ml,
- distilled water: to make up 100 litres,

This solution is used for closed circuit migration tests on an electrodialysis stack under tension (1 volt/cell), on the basis of 50 l/m² of anionic and cationic membranes, until 50 % demineralisation of the solution. The effluent circuit is initiated by a 5 g/l potassium chloride solution. Migrating substances are tested for in both the simulant and the effluent.

⁽¹⁾ OJ L 220, 15.8.2002, p. 18.

Organic molecules entering into the membrane composition that are liable to migrate into the treated solution will be determined. A specific determination will be carried out for each of these constituents by an approved laboratory. The content in the simulant of all the determined compounds must be less than 50 g/l.

The general rules on controls of materials in contact with foodstuffs must be applied to these membranes.

2. MEMBRANE UTILISATION REQUIREMENTS

The membrane pair is formulated so that the following conditions are met:

- the pH reduction of the wine is to be no more than 0,3 pH units,
- the volatile acidity reduction is to be less than 0,12 g/l (2 meq expressed as acetic acid),
- treatment must not affect the non-ionic constituents of the wine, in particular polyphenols and polysaccharides,
- diffusion of small molecules such as ethanol is to be reduced and must not cause a reduction in alcoholic strength of more than 0,1 % vol.,
- the membranes must be conserved and cleaned by approved methods with substances authorised for use in the preparation of foodstuffs,
- the membranes are marked so that alternation in the stack can be checked,
- the equipment is to be run using a command and control mechanism that will take account of the particular instability of each wine so as to eliminate only the supersaturation of potassium hydrogen tartrate and calcium salts,
- the treatment is to be carried out under the responsibility of an oenologist or qualified technician.

The treatment is to be recorded in the register referred to in Article 112(2) of Regulation (EC) No 479/2008.

Appendix 8

Requirements for urease

1. International code for urease: EC 3-5-1-5, CAS No: 9002-13-5.
2. Activity: urease activity (active at acidic pH), to break down urea into ammonia and carbon dioxide. The stated activity is not less than 5 units/mg, one unit being defined as the amount that produces one μmol of ammonia per minute at 37 °C from 5 g/l urea at pH 4.
3. Origin: *Lactobacillus fermentum*.
4. Area of application: breaking down urea present in wine intended for prolonged ageing, where its initial urea concentration is higher than 1 mg/l.
5. Maximum dose: 75 mg of enzyme preparation per litre of wine treated, not exceeding 375 units of urease per litre of wine. After treatment, all residual enzyme activity must be eliminated by filtering the wine (pore size < 1 μm).
6. Chemical and microbiological purity specifications:

Loss on drying	Less than 10 %
Heavy metals	Less than 30 ppm
Pb	Less than 10 ppm
As	Less than 2 ppm
Total coliforms	Absent
<i>Salmonella</i> spp	Absent in 25 g sample
Aerobic count	Less than 5×10^4 cells/g

Urease used in the treatment of wine must be prepared under similar conditions to those for urease as covered by the opinion of the Scientific Committee for Food of 10 December 1998.

*Appendix 9***Requirements for pieces of oak wood****PURPOSE, ORIGIN AND AREA OF APPLICATION**

Pieces of oak wood are used in winemaking and ageing, including in the fermentation of fresh grapes and grape must, to pass on certain characteristics of oak wood to wine.

The pieces of oak wood must come exclusively from the *Quercus* genus.

They may be left in their natural state, or heated to a low, medium or high temperature, but they may not have undergone combustion, including surface combustion, nor be carbonaceous or friable to the touch. They may not have undergone any chemical, enzymatic or physical processes other than heating. No product may be added for the purpose of increasing their natural flavour or the amount of their extractable phenolic compounds.

LABELLING

The label must mention the origin of the botanical species of oak and the intensity of any heating, the storage conditions and safety precautions.

DIMENSIONS

The dimensions of the particles of wood must be such that at least 95 % in weight are retained by a 2 mm mesh filter (9 mesh).

PURITY

The pieces of oak wood may not release any substances in concentrations which may be harmful to health.

This treatment is to be recorded in the register referred to in Article 112(2) of Regulation (EC) No 479/2008.

*Appendix 10***Requirements for the partial dealcoholisation of wine**

The aim of this treatment is to produce a partially dealcoholised wine, by eliminating some of the alcohol (ethanol) in it using physical separation techniques.

Requirements

- The wines treated must have no organoleptic faults and must be suitable for direct human consumption,
 - Elimination of alcohol from the wine cannot be carried out if one of the enrichment operations laid down in Annex V to Regulation (EC) No 479/2008 was applied to one of the wine products used in the preparation of the wine in question,
 - Reduction of the actual alcoholic strength by volume may not be more than 2 % vol. and the actual alcoholic strength by volume of the final product must comply with that defined in point (a) of the second subparagraph of paragraph 1 of Annex IV to Regulation (EC) No 479/2008.
 - The treatment is to be carried out under the responsibility of an oenologist or qualified technician,
 - This treatment is to be recorded in the register referred to in Article 112(2) of Regulation (EC) No 479/2008,
 - The Member States may require this treatment to be notified to the competent authorities.
-

*Appendix 11***Requirements for treatment with PVI/PVP copolymers**

The purpose of this treatment is to reduce excessively high concentrations of metals and to prevent defects caused by this excessively high content, such as ferric casse, through the addition of copolymers that adsorb these metals.

Requirements

- The added copolymers must be eliminated by filtering within two days at most of their addition to the wine, taking account of the precautionary principle.
- In the case of musts, the copolymers must be added no earlier than two days before filtering.
- The treatment is to be carried out under the responsibility of an oenologist or qualified technician.
- The adsorbant copolymers used must comply with the requirements of the International Oenological Codex published by the International Organisation of Vine and Wine, especially as regards the maximum monomer content ⁽¹⁾.

⁽¹⁾ Treatment with PVI/PVP copolymers can only occur after the purity and identification specifications for authorised copolymers have been laid down and published in the OIV's International Oenological Codex.

*Appendix 12***Requirements for treatment with cation exchangers to ensure the tartaric stabilisation of the wine**

The purpose is to obtain tartaric stability of the wine with regard to potassium hydrogen tartrate and calcium tartrate (and other calcium salts).

Requirements

1. The treatment must be limited to the elimination of excess cations.
 - The wine must first of all be cooled.
 - Only the minimum fraction of wine necessary to obtain stability must be treated with cation exchangers.
2. The treatment is to be carried out on acid-regenerated cation-exchanger resins.
3. All the operations are to be carried out under the responsibility of an oenologist or qualified technician. The treatment must be recorded in the register referred to in Article 112(2) of Regulation (EC) No 479/2008.
4. The cationic resins used must comply with the requirements of Regulation (EC) No 1935/2004 of the European Parliament and of the Council ⁽¹⁾, the Community and national provisions adopted thereunder and the analytical requirements laid down in Appendix 4 to this Regulation. Their use must not excessively modify the physico-chemical composition or the organoleptic characteristics of the wine and must comply with the limits set out in point 3 of the International Oenological Codex monograph 'Cation-exchange resins' published by the OIV.

⁽¹⁾ OJ L 338, 13.11.2004, p. 4.

ANNEX I B

THE MAXIMUM SULPHUR DIOXIDE CONTENT OF WINES

A. THE SULPHUR DIOXIDE CONTENT OF WINES

1. The total sulphur dioxide content of wines, other than sparkling wines and liqueur wines, on their release to the market for direct human consumption, may not exceed:
 - (a) 150 milligrams per litre for red wines;
 - (b) 200 milligrams per litre for white and rosé wines.
2. Notwithstanding paragraph 1(a) and (b), the maximum sulphur dioxide content shall be raised, as regards wines with a sugar content, expressed as the sum of glucose and fructose, of not less than five grams per litre, to:
 - (a) 200 milligrams per litre for red wines;
 - (b) 250 milligrams per litre for white and rosé wines;
 - (c) 300 milligrams per litre for:
 - wines entitled to the description 'Spätlese' in accordance with Community provisions,
 - white wines entitled to one of the following protected designations of origin: Bordeaux supérieur, Graves de Vayres, Côtes de Bordeaux-Saint-Macaire, Premières Côtes de Bordeaux, Côtes de Bergerac, Haut Montravel, Côtes de Montravel, Gaillac, Rosette and Savennières;
 - white wines entitled to the protected designations of origin Allela, Navarra, Penedès, Tarragona and Valencia and wines entitled to a protected designation of origin from the Comunidad Autónoma del País Vasco and described as 'vendimia tardia',
 - the sweet wines entitled to the protected designation of origin 'Binissalem-Mallorca',
 - wines originating in the United Kingdom produced in accordance with UK legislation where the sugar content is more than 45 g/l,
 - wines from Hungary with the protected designation of origin 'Tokaji' and described in accordance with Hungarian provisions as 'Tokaji édes szamorodni' or 'Tokaji szàraz szamorodni',
 - wines entitled to one of the following protected designations of origin: Loazzolo, Alto Adige and Trentino described by the terms or one of the terms: 'passito' or 'vendemmia tardiva',
 - wines entitled to the protected designation of origin: 'Colli orientali del Friuli' accompanied by the term 'Picolit',
 - wines entitled to the protected designations of origin 'Moscato di Pantelleria naturale' and 'Moscato di Pantelleria',
 - wines from the Czech Republic entitled to the description 'pozdni sběr',
 - wines from Slovakia entitled to a protected designation of origin and described by the term 'neskorý zber' and Slovak 'Tokaj' wines entitled to the protected designation of origin 'Tokajské samorodné suché' or 'Tokajské samorodné sladké',
 - wines from Slovenia entitled to a protected designation of origin and described by the term 'vrhunsko vino ZGP — pozna trgatev',
 - white wines with the following protected geographical indications, with a total alcoholic strength by volume of more than 15 % vol. and a sugar content of more than 45 g/l:
 - Vin de pays de Franche-Comté,
 - Vin de pays des coteaux de l'Auxois,
 - Vin de pays de Saône-et-Loire,

- Vin de pays des coteaux de l'Ardèche,
- Vin de pays des collines rhodaniennes,
- Vin de pays du comté Tolosan,
- Vin de pays des côtes de Gascogne,
- Vin de pays du Gers,
- Vin de pays du Lot,
- Vin de pays des côtes du Tarn,
- Vin de pays de la Corrèze,
- Vin de pays de l'Île de Beauté,
- Vin de pays d'Oc,
- Vin de pays des côtes de Thau,
- Vin de pays des coteaux de Murviel,
- Vin de pays du Val de Loire,
- Vin de pays de Méditerranée,
- Vin de pays des comtés rhodaniens,
- Vin de pays des côtes de Thongue,
- Vin de pays de la Côte Vermeille,
- sweet wines originating in Greece with an actual alcoholic strength by volume equal to or more than 15 % vol. and a sugar content equal to or more than 45 g/l and entitled to one of the following protected geographical indications:
 - Τοπικός Οίνος Τυρνάβου (Regional wine of Tyrnavos),
 - Αχαϊκός Τοπικός Οίνος (Regional wine of Ahaia),
 - Λακωνικός Τοπικός Οίνος (Regional wine of Lakonia),
 - Τοπικός Οίνος Φλώρινας (Regional wine of Florina),
 - Τοπικός Οίνος Κυκλάδων (Regional wine of Cyclades),
 - Τοπικός Οίνος Αργολίδας (Regional wine of Argolida),
 - Τοπικός Οίνος Πιερίας (Regional wine of Pieria),
 - Αγιορείτικος Τοπικός Οίνος (Regional wine of Mount Athos- Regional wine of Holy Mountain),
- sweet wines originating in Cyprus with an actual alcoholic strength by volume equal to or less than 15 % vol. and a sugar content equal to or more than 45 g/l and entitled to the protected designation of origin Κουμανδάρια (Commandaria),
- sweet wines originating in Cyprus produced from overripe grapes or from raisined grapes with a total alcoholic strength by volume equal to or more than 15 % vol. and a sugar content equal to or more than 45 g/l and entitled to one of the following protected geographical indications:
 - Τοπικός Οίνος Λεμεσός (Regional wine of Lemesos),
 - Τοπικός Οίνος Πάφος (Regional wine of Pafos),

- Τοπικός Οίνος Λάρνακα (Regional wine of Larnaka),
 - Τοπικός Οίνος Λευκωσία (Regional wine of Lefkosia);
- (d) 350 milligrams per litre for:
- wines entitled to the description 'Auslese' in accordance with Community provisions,
 - Romanian white wines entitled to one of the following protected designations of origin: Murfatlar, Cotnari, Târnave, Pietroasa, Valea Călugărească,
 - wines from the Czech Republic entitled to the description 'výběr z hroznů',
 - wines from Slovakia entitled to a protected designation of origin and described by the term 'výber z hrozna' and Slovak 'Tokaj' wines entitled to the protected designation of origin 'Tokajský mászlás' or 'Tokajský fordítás',
 - wines from Slovenia entitled to a protected designation of origin and described by the term 'vrhunsko vino ZGP — izbor';
- (e) 400 milligrams per litre for:
- wines entitled to the descriptions 'Beerenauslese', 'Ausbruch', 'Ausbruchwein', 'Trockenbeerenauslese', 'Strohwein', 'Schilfwein' and 'Eiswein' in accordance with Community provisions,
 - white wines entitled to one of the following protected designations of origin: Sauternes, Barsac, Cadillac, Cérons, Loupiac, Sainte-Croix-du-Mont, Monbazillac, Bonnezeaux, Quarts de Chaume, Coteaux du Layon, Coteaux de l'Aubance, Graves Supérieures, Sainte-Foy Bordeaux, Saussignac, Jurançon except where followed by the term 'sec', Anjou-Coteaux de la Loire, Coteaux du Layon followed by the name of the commune of origin, Chaume, Coteaux de Saumur, Pacherenc du Vic Bilh except where followed by the term 'sec', Alsace et Alsace grand cru followed by the term 'vendanges tardives' or 'sélection de grains nobles',
 - sweet wines originating in Greece produced from overripe grapes and from raisined grapes with a residual sugar content, expressed as sugar, equal to or more than 45 g/l and entitled to one of the following protected designations of origin: Σάμος (Samos), Ρόδος (Rhodes), Πατρα (Patras), Ρίο Πατρών (Rio Patron), Κεφαλονία (Kefallonia), Λήμνος (Limnos), Σητεία (Sitia), Σαντορίνη (Santorini), Νεμέα (Nemea), Δαφνές (Daphnes) and sweet wines produced from overripe grapes and from raisined grapes entitled to one of the following protected geographical indications: Σιάτιστας (Siatista), Καστοριάς (Kastoria), Κυκλάδων (Cyclades), Μονεμβάσιος (Monemvasia), Αγιορείτικος (Mount Athos — Holy Mountain),
 - wines from the Czech Republic entitled to the descriptions 'výběr z bobulí', 'výběr z cibéb', 'ledové víno' or 'slámové víno',
 - wines from Slovakia entitled to a protected designation of origin and described by the terms 'bobuľový výber', 'hrozienský výber', 'cibébový výber', 'ľadové víno' or 'slamové víno' and Slovak 'Tokaj' wines entitled to the protected designation of origin 'Tokajský výber', 'Tokajská esencia' or 'Tokajská výberová esencia',
 - wines from Hungary entitled to a protected designation of origin and described in accordance with Hungarian provisions as 'Tokaji mászlás', 'Tokaji fordítás', 'Tokaji aszúeszencia', 'Tokaji eszencia', 'Tokaji aszú' or 'Töppedt szőlőből készült bor',
 - wines entitled to the protected designation of origin 'Albana di Romagna' and described by the term 'passito',
 - Luxemburg wines entitled to a protected designation of origin and described by the terms 'vendanges tardives', 'vin de glace' or 'vin de paille',
 - white wines entitled to the protected designation of origin 'Douro' followed by the term 'colheita tardia',
 - wines from Slovenia entitled to a protected designation of origin and described by the terms 'vrhunsko vino ZGP — jagodni izbor', 'vrhunsko vino ZGP — ledeno vino' or 'vrhunsko vino ZGP — suhi jagodni izbor',
 - white wines originating in Canada entitled to the description 'Icewine'.

3. The lists of wines bearing a protected designation of origin or a protected geographical indication given in subparagraphs (c), (d) and (e) of paragraph 2 may be amended where the production conditions of the wines concerned are amended or the designation of origin or geographical indication is changed. The Member States shall provide the Commission, in advance, with all the necessary technical information for the wines concerned, including their product specifications and the annual quantities produced.
4. Where climate conditions make this necessary, the Commission may decide in accordance with the procedure referred to in Article 113(2) of Regulation (EC) No 479/2008 that in certain wine-growing areas of the Community the Member States concerned may authorise an increase of a maximum of 50 milligrams per litre in the maximum total sulphur dioxide levels of less than 300 milligrams per litre referred to in this point for wines produced within their territory. The list of cases in which the Member States may permit such an increase is given in Appendix 1.
5. Member States may apply more restrictive provisions to wines produced within their territory.

B. THE SULPHUR DIOXIDE CONTENT OF LIQUEUR WINES

The total sulphur dioxide content of liqueur wines, on their release to the market for direct human consumption, may not exceed:

150 mg/l where the sugar content is less than 5 g/l;

200 mg/l where the sugar content is not less than 5 g/l.

C. THE SULPHUR DIOXIDE CONTENT OF SPARKLING WINES

1. The total sulphur dioxide content of sparkling wines, on their release to the market for direct human consumption, may not exceed:
 - (a) 185 mg/l for all categories of quality sparkling wine; and
 - (b) 235 mg/l for other sparkling wines.
 2. Where climate conditions make this necessary in certain wine-growing areas of the Community, the Member States concerned may authorise an increase of up to 40 mg/l in the maximum total sulphur dioxide content for the sparkling wines referred to in paragraph 1(a) and (b) produced in their territory, provided that the wines covered by this authorisation are not sent outside the Member State in question.
-

*Appendix 1***Increase in the maximum total sulphur dioxide content where the climate conditions make this necessary**

(Annex I B to this Regulation)

	Year	Member State	Wine-growing areas(s)	Wines concerned
1.	2000	Germany	All wine-growing areas of Germany.	All wines obtained from grapes harvested in 2000.
2.	2006	Germany	The wine-growing areas in the regions of Baden-Württemberg, Bavaria, Hessen and Rhineland Palatinate.	All wines obtained from grapes harvested in 2006.
3.	2006	France	The wine-growing areas in the departments of Bas-Rhin and Haut-Rhin.	All wines obtained from grapes harvested in 2006.

ANNEX I C

THE MAXIMUM VOLATILE ACID CONTENT OF WINES

1. The volatile acid content may not exceed:
 - (a) 18 milliequivalents per litre for partially fermented grape must;
 - (b) 18 milliequivalents per litre for white and rosé wines; or
 - (c) 20 milliequivalents per litre for red wines.
2. The levels referred to in paragraph 1 shall apply:
 - (a) to products from grapes harvested within the Community, at the production stage and at all stages of marketing;
 - (b) to partially fermented grape must and wines originating in third countries, at all stages following their entry into the geographical territory of the Community.
3. Derogations from paragraph 1 may be granted:
 - (a) for certain wines with a protected designation of origin or a protected geographical indication:
 - where they have been aged for a period of at least two years, or
 - where they have been produced according to particular methods;
 - (b) wines with a total alcoholic strength by volume of at least 13 % vol.

The Member States must notify these derogations to the Commission, which must then inform the other Member States.

ANNEX I D

LIMITS AND CONDITIONS FOR THE SWEETENING OF WINES

1. The sweetening of wine may be authorised only if carried out using one or more of the following products:

- (a) grape must;
- (b) concentrated grape must;
- (c) rectified concentrated grape must.

The total alcoholic strength by volume of the wine in question may not be increased by more than 4 % vol.

2. The sweetening of imported wines intended for direct human consumption and bearing a geographical indication is forbidden within the territory of the Community. The sweetening of other imported wines shall be subject to the same conditions as wines produced in the Community.

3. The sweetening of a wine with a protected designation of origin may be authorised by a Member State only if it is carried out:

- (a) in accordance with the conditions and limits laid down in this Annex;
- (b) within the region in which the wine was produced or within an area in immediate proximity.

The grape must and concentrated grape must referred to in paragraph 1 must originate in the same region as the wine for the sweetening of which it is used.

4. The sweetening of wines shall be authorised only at the production and wholesale stages.

5. The sweetening of wines must be carried out in accordance with the following specific administrative rules:

- (a) Any natural or legal person intending to carry out a sweetening operation shall notify the competent authority of the Member State on whose territory the operation is to take place.
- (b) Notice shall be given in writing. It shall reach the competent authority at least forty-eight hours before the day on which the sweetening operation is to take place.
- (c) However, where an undertaking frequently or continuously carries out sweetening operations, Member States may allow a notification covering several operations or a specified period to be sent to the competent authorities. Such notification shall be accepted only on condition that the undertaking keeps a written record of each sweetening operation and records the information required by point (d).
- (d) Notifications shall include the following information:
 - the quantity and the total and actual alcoholic strengths of the wine to be sweetened,
 - the quantity and the total and actual alcoholic strengths of the grape must or the quantity and density of the concentrated grape must or rectified concentrated grape must to be added, as the case may be,
 - the total and actual alcoholic strengths of the wine after sweetening.

The persons referred to in point (a) shall keep goods inwards and outwards registers showing the quantities of grape must, concentrated grape must or rectified concentrated grape must which they are holding for sweetening operations.

ANNEX II

**AUTHORISED OENOLOGICAL PRACTICES AND RESTRICTIONS APPLICABLE TO SPARKLING WINES,
QUALITY SPARKLING WINES AND QUALITY AROMATIC SPARKLING WINES****A. Sparkling wine**

1. For the purposes of this point and points B and C of this Annex:
 - (a) 'tirage liqueur' means;

the product added to the cuvée to provoke secondary fermentation;
 - (b) 'expedition liqueur' means;

the product added to sparkling wines to give them special taste qualities.
2. The expedition liqueur may contain only:
 - sucrose,
 - grape must,
 - grape must in fermentation,
 - concentrated grape must,
 - rectified concentrated grape must;
 - wine, or
 - a mixture thereof,with the possible addition of wine distillate.
3. Without prejudice to enrichment authorised pursuant to Regulation (EC) No 479/2008 for the constituents of a cuvée, any enrichment of the cuvée shall be prohibited.
4. However, each Member State may, in respect of regions and varieties for which it is technically justified, authorise the enrichment of the cuvée at the place of preparation of the sparkling wines provided that:
 - (a) none of the constituents of the cuvée has previously undergone enrichment;
 - (b) the said constituents are derived solely from grapes harvested in its territory;
 - (c) the enrichment is carried out in a single operation;
 - (d) the following limits are not exceeded:
 - (i) 3 % vol. for a cuvée comprising constituents from wine-growing zone A;
 - (ii) 2 % vol. for a cuvée comprising constituents from wine-growing zone B;
 - (iii) 1,5 % vol. for a cuvée comprising constituents from wine-growing zone C;
 - (e) the method used is the addition of sucrose, concentrated grape must or rectified concentrated grape must.
5. The addition of tirage liqueur and expedition liqueur shall be considered neither as enrichment nor as sweetening. The addition of tirage liqueur may not cause an increase in the total alcoholic strength by volume of the cuvée of more than 1,5 % vol. This increase shall be measured by calculating the difference between the total alcoholic strength by volume of the cuvée and the total alcoholic strength by volume of the sparkling wine before any expedition liqueur is added.

6. The addition of expedition liqueur shall be carried out in such a way as not to increase the actual alcoholic strength by volume of the sparkling wine by more than 0,5 % vol.
7. Sweetening of the cuvée and its constituents shall be prohibited.
8. In addition to any acidification or deacidification of the constituents of the cuvée in accordance with Regulation (EC) No 479/2008, the cuvée may be subject to acidification or deacidification. Acidification and deacidification of the cuvée shall be mutually exclusive. Acidification may be carried out only up to a maximum of 1,5 grams per litre, expressed as tartaric acid, i.e. 20 milliequivalents per litre.
9. In years of exceptional climate conditions, the maximum limit of 1,5 grams per litre or 20 milliequivalents per litre may be raised to 2.5 grams per litre or 34 milliequivalents per litre, provided that the natural acidity of the products is not less than 3 g/l, expressed as tartaric acid, or 40 milliequivalents per litre.
10. The carbon dioxide contained in the sparkling wines may be produced only as a result of the alcoholic fermentation of the cuvée from which such wine is prepared.

Such fermentation, unless it is intended for processing grapes, grape must or partially fermented grape must directly into sparkling wine, may result only from the addition of tirage liqueur. It may take place only in bottles or in closed tanks.

The use of carbon dioxide in the case of the process of transfer by counter-pressure is authorised under supervision and on condition that the pressure of the carbon dioxide contained in the sparkling wine is not thereby increased.

11. In the case of sparkling wines other than sparkling wines with a protected designation of origin:
 - (a) the tirage liqueur intended for their preparation may contain only:
 - grape must,
 - grape must in fermentation,
 - concentrated grape must,
 - rectified concentrated grape must, or
 - sucrose and wine;
 - (b) the actual alcoholic strength by volume, including the alcohol contained in any expedition liqueur added, shall be not less than 9,5 % vol.

B. Quality sparkling wine

1. The tirage liqueur intended for the production of a quality sparkling wine may contain only:
 - (a) sucrose,
 - (b) concentrated grape must,
 - (c) rectified concentrated grape must,
 - (d) grape must or partially fermented grape must, or
 - (e) wine.
2. Producer Member States may define any supplementary or more stringent characteristics or conditions of production and circulation for the quality sparkling wines covered by this Title and produced in their territory.
3. The manufacture of quality sparkling wines is also covered by the rules referred to in:
 - paragraphs 1 to 10 of point A,
 - paragraph 3 of point C for the actual alcoholic strength, paragraph 5 of point C for the minimum excess pressure and paragraphs 6 and 7 of point C for the minimum length of the production process, without prejudice to paragraph 4(d) of this point,

4. As regards quality aromatic sparkling wines:
- (a) except by way of derogation, these may be obtained only by making exclusive use, when constituting the cuvée, of grape must or partially fermented grape must derived from wine varieties contained on the list given in Appendix 1. However, quality aromatic sparkling wine may be produced in the traditional way by using as constituents of the cuvée wines obtained from grapes of the 'Prosecco' variety harvested in the regions of Trentino-Alto Adige, Veneto and Friuli-Venezia Giulia;
 - (b) control of the fermentation process before and after the cuvée has been constituted, in order to render the cuvée sparkling, may be effected only by refrigeration or other physical processes;
 - (c) the addition of expedition liqueur shall be prohibited;
 - (d) the length of the production process for quality aromatic sparkling wines may not be less than one month.

C. Sparkling wines and quality sparkling wines with a protected designation of origin

1. The total alcoholic strength by volume of the cuvées intended for the preparation of quality sparkling wines with a protected designation of origin shall be not less than:
 - 9,5 % vol. in wine-growing zones C III,
 - 9 % vol. in other wine-growing zones.
2. However, the cuvées intended for the preparation of quality sparkling wines with the protected designations of origin 'Prosecco di Conegliano Valdobbiadene' and 'Montello e Colli Asolani' and prepared from a single vine variety may have a total alcoholic strength by volume of not less than 8,5 % vol.
3. The actual alcoholic strength by volume of quality sparkling wines with a protected designation of origin, including the alcohol contained in any expedition liqueur added, shall be not less than 10 % vol.
4. The tirage liqueur for sparkling wines and quality sparkling wines with a protected designation of origin may contain only:
 - (a) sucrose,
 - (b) concentrated grape must,
 - (c) rectified concentrated grape must;and:
 - (a) grape must,
 - (b) partially fermented grape must,
 - (c) wine;suitable for yielding the same sparkling wine or quality sparkling wine with a protected designation of origin as that to which the tirage liqueur is added.
5. Notwithstanding paragraph 5(c) of Annex IV to Regulation (EC) No 479/2008, when kept at a temperature of 20 °C in closed containers of a capacity of less than 25 cl., quality sparkling wines with a protected designation of origin must have an excess pressure of not less than 3 bar.
6. The duration of the process of making quality sparkling wines with a protected designation of origin, including ageing in the undertaking where they are made and reckoned from the start of the fermentation process designed to make the wines sparkling, may not be less than:
 - (a) six months where the fermentation process designed to make the wines sparkling takes place in closed tanks;
 - (b) nine months where the fermentation process designed to make the wines sparkling takes place in the bottles.

7. The duration of the fermentation process designed to make the cuvée sparkling and the duration of the presence of the cuvée on the lees shall not be less than:
 - 90 days,
 - 30 days if the fermentation takes place in containers with stirrers.
 8. The rules laid down in paragraphs 1-10 of point A and paragraph 2 of point B shall also apply to sparkling wines and quality sparkling wines with a protected designation of origin.
 9. As regards quality aromatic sparkling wines with a protected designation of origin:
 - (a) these wines may be obtained solely by using, for constituting the cuvée, grape must or partially fermented grape must of vine varieties on the list given in Appendix 1, provided that these varieties are recognised as suitable for the production of quality sparkling wines with a protected designation of origin in the region whose name the quality sparkling wines with a protected designation of origin bear. By derogation, a quality aromatic sparkling wine with a protected designation of origin may be produced by using as constituents of the cuvée wines obtained from grapes of the 'Prosecco' vine variety harvested in the regions of the designations of origin 'Conegliano-Valdobbiadene' and 'Montello e Colli Asolani';
 - (b) control of the fermentation process before and after the cuvée has been constituted, in order to render the cuvée sparkling, may be effected only by refrigeration or other physical processes;
 - (c) the addition of expedition liqueur shall be prohibited;
 - (d) the actual alcoholic strength by volume of quality aromatic sparkling wines with a protected designation of origin may not be less than 6 % vol.;
 - (e) the total alcoholic strength by volume of quality aromatic sparkling wines with a protected designation of origin may not be less than 10 % vol.;
 - (f) when kept at a temperature of 20 °C in closed containers, quality aromatic sparkling wines with a protected designation of origin must have an excess pressure of not less than 3 bar;
 - (g) notwithstanding paragraph 6 of point C, the duration of the process of producing quality aromatic sparkling wines with a protected designation of origin must not be less than one month.
-

Appendix 1

List of vine varieties grapes of which may be used to constitute the cuvée for preparing quality aromatic sparkling wines and quality sparkling wines with a protected designation of origin

Airén	All the Malvoisies
Aleatico N	Mauzac blanc and rosé
Alvarinho	Monica N
Ασύρτικο (Assyrtiko)	Μοσχοφίλερο (Moschofilero)
Bourboulenc B	Müller-Thurgau B
Brachetto N.	All the Muscatels
Busuioacă de Bohotin	Manzoni moscato
Clairette B	Nektár
Colombard B	Pálava B
Csaba gyöngye B	Parellada B
Cserszegi fűszeres B	Perle B
Devín	Piquepoul B
Fernão Pires	Poulsard
Freisa N	Prosecco
Gamay N	Ροδίτης (Roditis)
Gewürztraminer Rs	Scheurebe
Girò N	Tămăioasă românească
Γλυκερύθρα (Glykerythra)	Torbato
Huxelrebe	Touriga Nacional
Irsai Olivér B	Verdejo
Macabeu B	Zefír B

ANNEX III

AUTHORISED OENOLOGICAL PRACTICES AND RESTRICTIONS APPLICABLE TO LIQUEUR WINES AND LIQUEUR WINES WITH A PROTECTED DESIGNATION OF ORIGIN OR PROTECTED GEOGRAPHICAL INDICATION**A. Liqueur wines**

1. The products referred to in paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008 and used for preparing liqueur wines and liqueur wines with a protected designation of origin or a protected geographical indication may have undergone, where appropriate, only the oenological practices and processes referred to in Regulation (EC) No 479/2008 or this Regulation.
2. However,
 - (a) the increase in natural alcoholic strength by volume may be due only to the use of the products referred to in paragraph 3(e) and (f) of Annex IV to Regulation (EC) No 479/2008; and
 - (b) by derogation, Spain is authorised to permit the use of calcium sulphate for Spanish wines described by the traditional terms 'vino generoso' or 'vino generoso de licor' where this practice is traditional and provided that the sulphate content of the product so treated is not more than 2,5 g/l, expressed as potassium sulphate. These products may undergo additional acidification up to a maximum limit of 1,5 g/l.
3. Without prejudice to any provisions of a more restrictive nature which the Member States may adopt for liqueur wines and liqueur wines with a protected designation of origin or a protected geographical indication prepared within their territory, the oenological practices referred to in Regulation (EC) No 479/2008 and in this Regulation shall be authorised for those products.
4. The following are also authorised:
 - (a) sweetening, subject to a declaration and registration requirement, where the products used have not been enriched with concentrated grape must, by means of:
 - concentrated grape must or rectified concentrated grape must, provided that the increase in the total alcoholic strength by volume of the wine in question is not more than 3 % vol.,
 - concentrated grape must, rectified concentrated grape must or partially fermented grape must obtained from raisined grapes for Spanish wines described by the traditional term 'vino generoso de licor' and provided that the increase in the total alcoholic strength by volume of the wine in question is not more than 8 % vol.,
 - concentrated grape must or rectified concentrated grape must for liqueur wines with the protected designation of origin 'Madeira' and provided that the increase in the total alcoholic strength by volume of the wine in question is not more than 8 % vol.;
 - (b) the addition of alcohol, distillate or spirits, as referred to in paragraphs 3(e) and (f) of Annex IV to Regulation (EC) No 479/2008, in order to compensate for losses due to evaporation during ageing;
 - (c) ageing in vessels at a temperature not exceeding 50 °C, for liqueur wines with the protected designation of origin 'Madeira'.
5. The vine varieties from which the products referred to in paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008 used for the preparation of liqueur wines and liqueur wines with a protected designation of origin or a protected geographical indication are produced shall be selected from those referred to in Article 24(1) of Regulation (EC) No 479/2008.
6. The natural alcoholic strength by volume of the products referred to in paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008 used for the preparation of a liqueur wine other than a liqueur wine with a protected designation of origin or a protected geographical indication may not be less 12 % vol.

B. Liqueur wines with a protected designation of origin (provisions other than those laid down in point A of this Annex and concerning specifically liqueur wines with a protected designation of origin)

1. The list of liqueur wines with a protected designation of origin whose production involves the use of grape must or the mixture of grape must with wine, referred to in the fourth indent of paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008, is given in Appendix 1 A to this Annex.

2. The list of liqueur wines with a protected designation of origin to which the products referred to in paragraph 3(f) of Annex IV to Regulation (EC) No 479/2008 may be added is given in Appendix 1 B to this Annex.
3. The products referred to in paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008 and concentrated grape must and partially fermented grape must obtained from raisined grapes referred to in paragraph 3(f)(iii) of that Annex IV used for the preparation of liqueur wine with a protected designation of origin must come from the region whose name the liqueur wine with a protected designation of origin in question bears.

However, for liqueur wines with the protected designations of origin 'Málaga' and 'Jerez-Xérès-Sherry', the grape must, concentrated grape must or, pursuant to paragraph 4 of point B of Annex VI to Regulation (EC) No 479/2008, the partially fermented grape must obtained from raisined grapes referred to in paragraph 3(f)(iii) of Annex IV to Regulation (EC) No 479/2008 obtained from the 'Pedro Ximénez' vine variety may come from the Montilla-Moriles region.

4. The operations referred to in paragraphs 1 to 4 of point A of this Annex for the preparation of a liqueur wine with a protected designation of origin may be performed only within the region referred to in paragraph 3.

However, as regards the liqueur wine with a protected designation of origin for which the designation 'Porto' is reserved for the product prepared from grapes obtained from the region delimited as the 'Douro', the additional manufacturing and ageing processes may take place either in the aforementioned region or in Vila Nova de Gaia — Porto.

5. Without prejudice to any provisions of a more restrictive nature which the Member States may adopt for liqueur wines with a protected designation of origin prepared within their territory:

(a) the natural alcoholic strength by volume of the products referred to in paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008 used for the preparation of a liqueur wine with a protected designation of origin may not be less than 12 % vol. However, some liqueur wines with a protected designation of origin on one of the lists given in Appendix 2 A to this Annex may be obtained from:

(i) grape must with a natural alcoholic strength by volume of not less than 10 % vol. in the case of liqueur wines with a protected designation of origin obtained by the addition of spirit obtained from wine or grape marc with a designation of origin, possibly from the same holding; or

(ii) partially fermented grape must or, in the case of the second indent below, from wine with an initial natural alcoholic strength by volume of not less than:

— 11 % vol. in the case of liqueur wines with a protected designation of origin obtained by the addition of neutral alcohol, or of a distillate of wine with an actual alcoholic strength by volume of not less than 70 % vol., or of spirit of vinous origin,

— 10,5 % vol. for wines prepared from white grape must referred to in list 3 given in Appendix 2 A,

— 9 % vol. in the case of a Portuguese liqueur wine with the protected designation of origin 'Madeira', the production of which is traditional and customary in accordance with the national legislation, which makes express provision for such a wine;

(b) the list of liqueur wines with a protected designation of origin with, notwithstanding paragraph 3(b) of Annex IV to Regulation (EC) No 479/2008, a total alcoholic strength by volume of less than 17,5 % vol. but not less than 15 % vol., where national legislation applicable thereto before 1 January 1985 expressly so provides, is given in Appendix 2 B.

6. The specific, traditional terms 'οἶνος γλυκὺς φυσικός', 'vino dulce natural', 'vino dolce naturale' and 'vinho doce natural' shall be used only for liqueur wines with a protected designation of origin:

— obtained from harvests at least 85 % of which are of the vine varieties listed in Appendix 3,

— derived from musts with an initial natural sugar content of at least 212 grams per litre,

— obtained by adding alcohol, distillate or spirits, as referred to in paragraph 3(e) and (f) of Annex IV to Regulation (EC) No 479/2008 to the exclusion of any other enrichment.

7. Insofar as is necessary to conform to traditional production practices, Member States may, for liqueur wines with a protected designation of origin produced within their territory, stipulate that the specific traditional name 'vin doux naturel' is used only for liqueur wines with a protected designation of origin which are:
- made directly by producers harvesting the grapes and exclusively from their harvests of Muscatel, Grenache, Macabeo or Malvoisie grapes; however, harvests may be included which have been obtained from vineyards that are also planted with vine varieties other than the four indicated above provided these do not constitute more than 10 % of the total stock,
 - obtained within the limit of a yield per hectare of 40 hl of grape must referred to in the first and fourth indents of paragraph 3(c) of Annex IV to Regulation (EC) No 479/2008, any greater yield resulting in the entire harvest ceasing to be eligible for the description 'vin doux naturel',
 - derived from a grape must as referred to above with an initial natural sugar content of at least 252 grams per litre,
 - obtained, to the exclusion of any other enrichment, by the addition of alcohol of vinous origin amounting in pure alcohol to a minimum of 5 % of the volume of the grape must as referred to above used and a maximum represented by the lower of the following two proportions:
 - either 10 % of the volume of the abovementioned grape must used, or,
 - 40 % of the total alcoholic strength by volume of the finished product represented by the sum of the actual alcoholic strength by volume and the equivalent of the potential alcoholic strength by volume calculated on the basis of 1 % vol. of pure alcohol for 17,5 grams of residual sugar per litre.
8. The specific traditional name 'vino generoso' shall be used only for dry liqueur wines with a protected designation of origin developed totally or partly under flor and:
- obtained only from white grapes obtained from the Palomino de Jerez, Palomino fino, Pedro Ximénez, Verdejo, Zalema and Garrido Fino vine varieties,
 - released to the market after it has been matured for an average of two years in oak barrels.
- Development under flor as referred to in the first subparagraph means the biological process which, occurring when a film of typical yeasts develops spontaneously at the free surface of the wine after total alcoholic fermentation of the must, gives the product specific analytic and organoleptic characteristics.
9. The specific traditional name 'vinho generoso' shall be used only for liqueur wines with the protected designations of origin 'Porto', 'Madeira', 'Moscatel de Setubal' and 'Carcavelos' in association with the respective designation of origin.
10. The specific traditional name 'vino generoso de licor' shall be used only for liqueur wines with a protected designation of origin:
- obtained from 'vino generoso', as referred to in paragraph 8, or from wine under flor capable of producing such a 'vino generoso', to which either partially fermented grape must obtained from raisined grapes or concentrated grape must has been added,
 - released to the market after it has been matured for an average of two years in oak barrels,

Appendix 1

The list of liqueur wines with a protected designation of origin whose production involves special rules**A. LIST OF LIQUEUR WINES WITH A PROTECTED DESIGNATION OF ORIGIN WHOSE PRODUCTION INVOLVES THE USE OF GRAPE MUST OR A MIXTURE THEREOF WITH WINE**

(Paragraph B 1 of this Annex)

GREECE

Σάμος (Samos), Μοσχάτος Πατρών (Patras Muscatel), Μοσχάτος Ρίου Πατρών (Rio Patron Muscatel), Μοσχάτος Κεφαλληνίας (Kefallonia Muscatel), Μοσχάτος Ρόδου (Rhodes Muscatel), Μοσχάτος Λήμνου (Lemnos Muscatel), Σητεία (Sitia), Νεμέα (Nemea), Σαντορίνη (Santorini), Δαφνές (Dafnes), Μαυροδάφνη Κεφαλληνίας (Mavrodafne of Kefallonia), Μαυροδάφνη Πατρών (Mavrodafne of Patras)

SPAIN

Liqueur wines with a protected designation of origin	Description of product as established by Community rules or national legislation
Alicante	Moscatel de Alicante Vino dulce
Cariñena	Vino dulce
Jerez-Xérès-Sherry	Pedro Ximénez Moscatel
Malaga	Vino dulce
Montilla-Moriles	Pedro Ximénez Moscatel
Priorato	Vino dulce
Tarragona	Vino dulce
Valencia	Moscatel de Valencia Vino dulce

ITALY

Cannonau di Sardegna, Girò di Cagliari, Malvasia di Bosa, Malvasia di Cagliari, Marsala, Monica di Cagliari, Moscato di Cagliari, Moscato di Sorso-Sennori, Moscato di Trani, Masco di Cagliari, Oltrepó Pavese Moscato, San Martino della Battaglia, Trentino, Vesuvio Lacrima Christi.

B. LIST OF LIQUEUR WINES WITH A PROTECTED DESIGNATION OF ORIGIN WHOSE PRODUCTION INVOLVES THE ADDITION OF THE PRODUCTS REFERRED TO IN PARAGRAPH 3(f) OF ANNEX IV TO REGULATION (EC) No 479/2008

(Paragraph 2 of point B of this Annex)

1. List of liqueur wines with a protected designation of origin whose production involves the addition of wine alcohol or dried-grape alcohol with an actual alcoholic strength of not less than 95 % vol. and not more than 96 % vol.

(First indent of paragraph 3(f)(ii) of Annex IV to Regulation (EC) No 479/2008)

GREECE

Σάμος (Samos), Μοσχάτος Πατρών (Patras Muscatel), Μοσχάτος Ρίου Πατρών (Rio Patron Muscatel), Μοσχάτος Κεφαλληνίας (Kefallonia Muscatel), Μοσχάτος Ρόδου (Rhodes Muscatel), Μοσχάτος Λήμνου (Lemnos Muscatel), Σητεία (Sitia), Σαντορίνη (Santorini), Δαφνές (Dafnes), Μαυροδάφνη Πατρών (Mavrodafne of Patras), Μαυροδάφνη Κεφαλληνίας (Mavrodafne of Kefallonia).

SPAIN

Condado de Huelva, Jerez-Xérès-Sherry, Manzanilla-Sanlúcar de Barrameda, Málaga, Montilla-Moriles, Rueda, Terra Alta.

CYPRUS

Κουμανδαρία (Commandaria).

2. **List of liqueur wines with a protected designation of origin whose production involves the addition of spirits distilled from wine or grape marc with an actual alcoholic strength of not less than 52 % vol. and not more than 86 % vol.**

(Second indent of paragraph 3(f)(ii) of Annex IV to Regulation (EC) No 479/2008)

GREECE

Μαυροδάφνη Πατρών (Mavrodafne of Patras), Μαυροδάφνη Κεφαλληνίας (Mavrodafne of Kefallonia), Σητεία (Sitia), Σαντορίνη (Santorini), Δαφνές (Dafnes), Νεμέα (Nemea).

FRANCE

Pineau des Charentes or Pineau charentais, Flocc de Gascogne, Macvin du Jura.

CYPRUS

Κουμανδαρία (Commandaria).

3. **List of liqueur wines with a protected designation of origin whose production involves the addition of spirits distilled from dried grapes with an alcoholic strength of not less than 52 % vol. but less than 94,5 % vol.**

(Third indent of paragraph 3(f)(ii) of Annex IV to Regulation (EC) No 479/2008)

GREECE

Μαυροδάφνη Πατρών (Mavrodafne of Patras), Μαυροδάφνη Κεφαλληνίας (Mavrodafne of Kefallonia).

4. **List of liqueur wines with a protected designation of origin whose production involves the addition of partially fermented grape must obtained from raisined grapes**

(First indent of paragraph 3(f)(iii) of Annex IV to Regulation (EC) No 479/2008)

SPAIN

Liqueur wines with a protected designation of origin	Description of product as established by Community rules or national legislation
Jerez-Xérès-Sherry	Vino generoso de licor
Málaga	Vino dulce
Montilla-Moriles	Vino generoso de licor

ITALY

Aleatico di Gradoli, Giró di Cagliari, Malvasia delle Lipari, Malvasia di Cagliari, Moscato passito di Pantelleria

CYPRUS

Κουμανδαρία (Commandaria).

5. **List of liqueur wines with a protected designation of origin whose production involves the addition of concentrated grape must obtained by the action of direct heat, complying, with the exception of this operation, with the definition of concentrated grape must.**

(Second indent of paragraph 3(f)(iii) of Annex IV to Regulation (EC) No 479/2008)

SPAIN

Liqueur wines with a protected designation of origin	Description of product as established by Community rules or national legislation
Alicante	
Condado de Huelva	Vino generoso de licor
Jerez-Xérès-Sherry	Vino generoso de licor
Málaga	Vino dulce
Montilla-Moriles	Vino generoso de licor
Navarra	Moscatel

ITALY

Marsala

6. **List of liqueur wines with a protected designation of origin whose production involves the addition of concentrated grape must**

(Third indent of paragraph 3(f)(iii) of Annex IV to Regulation (EC) No 479/2008)

SPAIN

Liqueur wines with a protected designation of origin	Description of product as established by Community rules or national legislation
Málaga	Vino dulce
Montilla-Moriles	Vino dulce
Tarragona	Vino dulce

ITALY

Oltrepó Pavese Moscato, Marsala, Moscato di Trani.

Appendix 2

A. Lists referred to in paragraph 5(a) of Annex III B

1. **List of liqueur wines with a protected designation of origin produced from grape must with a natural alcoholic strength by volume of not less than 10 % vol. obtained by the addition of spirit obtained from wine or grape marc with a registered designation of origin, possibly from the same holding.**

FRANCE

Pineau des Charentes or Pineau charentais, Floc de Gascogne, Macvin du Jura.

2. **List of liqueur wines with a protected designation of origin produced from fermenting grape must with an initial natural alcoholic strength by volume of not less than 11 % vol. obtained by the addition of neutral alcohol or of a distillate of wine with a an actual alcoholic strength by volume of not less than 70 % vol., or of spirit of vinous origin.**

PORTUGAL

Porto — Port

Moscatel de Setúbal, Setúbal

Carcavelos

Moscatel do Douro.

ITALY

Moscato di Noto

Trentino

3. **List of liqueur wines with a protected designation of origin produced from wine with an initial natural alcoholic strength by volume of not less than 10,5 % vol.**

SPAIN

Jerez-Xérès-Sherry

Manzanilla-Sanlúcar de Barrameda

Condado de Huelva

Rueda

4. **List of liqueur wines with a protected designation of origin obtained from fermenting grape must with an initial natural alcoholic strength by volume of not less than 9 % vol.**

PORTUGAL

Madeira.

B. List referred to in paragraph 5(b) of Annex III B

List of liqueur wines with a protected designation of origin with a total alcoholic strength by volume of less than 17,5 % vol. but not less than 15 % vol., where national laws applicable thereto before 1 January 1985 expressly so provided

(Paragraph 3(b) of Annex IV to Regulation (EC) No 479/2008)

SPAIN

Liqueur wines with a protected designation of origin	Description of product as established by Community rules or national legislation
Condado de Huelva	Vino generoso
Jerez-Xérès-Sherry	Vino generoso
Manzanilla-Sanlúcar de Barrameda	Vino generoso
Málaga	Seco
Montilla-Moriles	Vino generoso
Priorato	Rancio seco
Rueda	Vino generoso
Tarragona	Rancio seco

ITALY

Trentino

PORTUGAL

Liqueur wines with a protected designation of origin	Description of product as established by Community rules or national legislation
Porto — Port	Branco leve seco

Appendix 3

List of varieties that may be used to produce liqueur wines with a protected designation of origin that bear the specific, traditional terms ‘vino dulce natural’, ‘vino dolce naturale’, ‘vinho doce natural’ and ‘οινος γλυκός φυσικός’

Muscats — Grenache — Garnacha Blanca — Garnacha Peluda — Listán Blanco — Listán Negro-Negramoll — Maccabéo — Malvoisies — Mavrodaphne — Assirtiko — Liatiko — Garnacha tintorera — Monastrell — Palomino — Pedro Ximénez — Albarola — Aleatico — Bosco — Cannonau — Corinto nero — Giró — Monica — Nasco — Primitivo — Vermentino — Zibibbo.

ANNEX IV

SPECIAL COMMUNITY ANALYSIS METHODS

A. ALLYL ISOTHIOCYANATE

1. Principle of the method

Any allyl isothiocyanate present in the wine is collected by distillation and identified by gas chromatography.

2. Reagents

- 2.1. Ethanol, absolute.
- 2.2. *Standard* solution: solution of allyl isothiocyanate in absolute alcohol containing 15 mg of allyl isothiocyanate per litre.
- 2.3. Freezing mixture consisting of ethanol and dry ice (temperature – 60 °C).

3. Apparatus

- 3.1. Distillation apparatus as shown in the figure. A stream of nitrogen is passed continuously through the apparatus.
- 3.2. Heating mantle, thermostatically controlled.
- 3.3. Flowmeter.
- 3.4. Gas chromatograph fitted with a flame spectrophotometer detector equipped with a selective filter for sulphur compounds (wavelength = 394 nm) or any other suitable detector.
- 3.5. Stainless steel chromatograph column of internal diameter 3 mm and length 3 m filled with Carbowax 20M at 10 % on Chromosorb WHP, 80 to 100 mesh.
- 3.6. Microsyringe, 10 µl.

4. Procedure

Put two litres of wine into the distillation flask, introduce a few millilitres of ethanol (paragraph 2.1) into the two collecting tubes so that the porous parts of the gas dispersion rods are completely immersed. Cool the two tubes externally with the freezing mixture. Connect the flask to the collecting tubes and begin to flush the apparatus with nitrogen at a rate of three litres per hour. Heat the wine to 80 °C with the heating mantle, distil and collect 45 to 50 ml of the distillate.

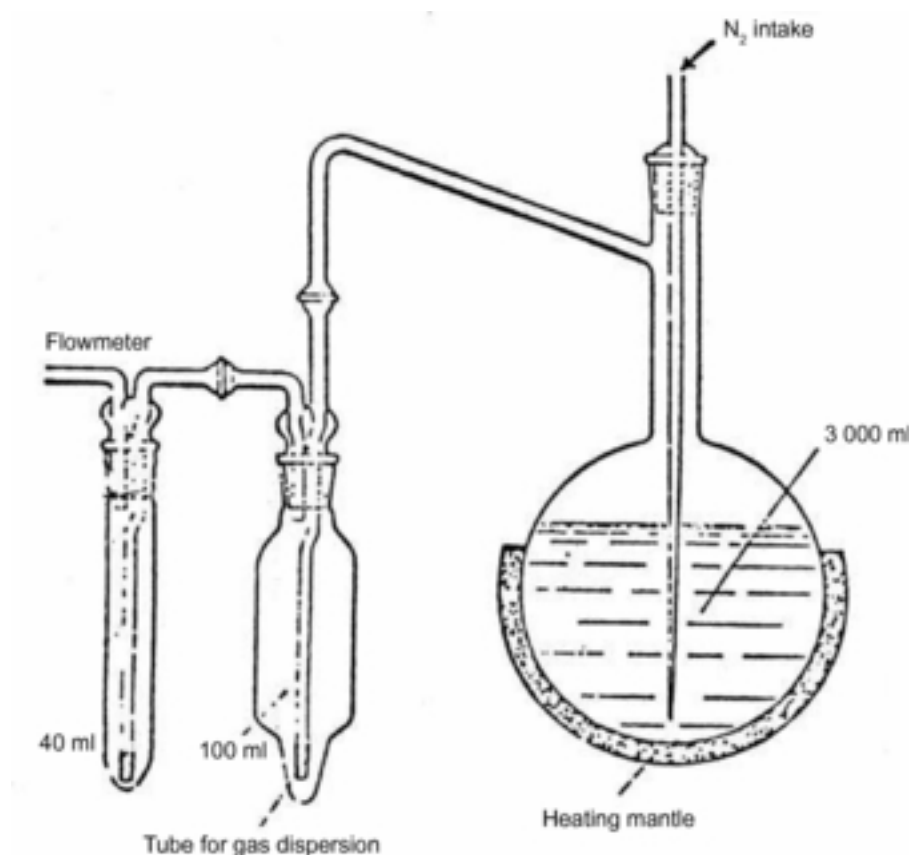
Stabilize the chromatograph. It is recommended that the following conditions are used:

- injector temperature: 200 °C,
- column temperature: 130 °C,
- helium carrier gas flow rate: 20 ml per minute.

With the microsyringe, introduce a volume of the *standard* solution such that the peak corresponding to the allyl isothiocyanate can easily be identified on the gas chromatogram.

Similarly introduce an aliquot of the distillate into the chromatograph. Check that the retention time of the peak obtained corresponds with that of the peak of allyl isothiocyanate.

Under the conditions described above, compounds naturally present in the wine will not produce interfering peaks on the chromatogram of the sample solution.

Apparatus for distillation under a current of nitrogen**B. SPECIAL ANALYSIS METHODS FOR RECTIFIED CONCENTRATED GRAPE MUST****(a) Total cations****1. Principle**

The test sample is treated by a strongly acid cation exchanger. The cations are exchanged with H⁺. Total cations are expressed by the difference between the total acidity of the effluent and that of the test sample.

2. Apparatus

2.1. Glass column of internal diameter 10 to 11 mm and length approximately 300 mm, fitted with a drain tap.

2.2. pH meter with a scale graduated at least in 0,1 pH units.

2.3. Electrodes:

- glass electrode, kept in distilled water,
- calomel/saturated potassium chloride reference electrode, kept in a saturated solution of potassium chloride, or
- a combined electrode, kept in distilled water,

3. Reagents

3.1. Strongly acid cation exchange resin in H⁺ form pre-swollen by soaking in water overnight.

3.2. Sodium hydroxide solution, 0,1 M.

3.3. Paper pH indicator.

4. Procedure

4.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v): introduce 200 g of accurately weighed rectified concentrated must into a 500 ml volumetric flask. Make up to the mark with water and homogenise.

4.2. Preparation of the ion exchange column

Introduce into the column approximately 10 ml pre-swollen ion exchanger in H + form. Rinse the column with distilled water until all acidity has been removed, using the paper indicator to monitor this.

4.3. Ion exchange

Pass 100 ml of the rectified concentrated must solution prepared as in paragraph 4.1 through the column at the rate of one drop every second. Collect the effluent in a beaker. Rinse the column with 50 ml of distilled water. Titrate the acidity in the effluent (including the rinse water) with the 0,1 M sodium hydroxide solution until the pH is 7 at 20 °C. The alkaline solution should be added slowly and the solution continuously shaken. Let n ml be the volume of 0,1 M sodium hydroxide solution used.

5. Expression of the results

The total cations are expressed in milliequivalents per kilogram of total sugar to one decimal place.

5.1. Calculations

— Acidity of the effluent expressed in milliequivalents per kilogram of rectified concentrated must:

Where E = The free sulphur dioxide in milligrams per litre is 2,5 n.

— Total acidity of the rectified concentrated must in milliequivalents per kilogram: a.

— Total cations in milliequivalents per kilogram of total sugars:

$$((2,5n-a)/(P)) \times 100$$

P = percentage concentration (m/m) of total sugars.

(b) Conductivity

1. Principle

The electrical conductivity of a column of liquid defined by two parallel platinum electrodes at its ends is measured by incorporating it in one arm of a Wheatstone bridge.

The conductivity varies with temperature and it is therefore expressed at 20 °C.

2. Apparatus

2.1. Conductivity meter enabling measurements of conductivity to be made over a range from 1 to 1 000 microsiemens per cm ($\mu\text{S cm}^{-1}$).

2.2. Waterbath for bringing the temperature of samples to be analysed to approximately 20 °C (20 ± 2 °C).

3. Reagents

3.1. Demineralised water with specific conductivity below $2 \mu\text{S cm}^{-1}$ at 20 °C.

3.2. Reference solution of potassium chloride

Dissolve 0,581 g of potassium chloride, KCl, previously dried to constant mass at a temperature of 105 °C, in demineralised water (paragraph 3.1). Make up to one litre with demineralised water (paragraph 3.1). This solution has a conductivity of $1\,000 \mu\text{S cm}^{-1}$ at 20 °C. It should not be kept for more than three months.

4. Procedure

4.1. Preparation of the sample to be analysed

Use the solution with a total sugar concentration of 25 % (m/m) (25° Brix): weigh a mass equal to 2 500/P and make up to 100 g with water (paragraph 3.1), where P = percentage (m/m) concentration of total sugars in the rectified concentrated must.

4.2. Determination of conductivity

Bring the sample to be analysed to 20 °C by immersion in a waterbath. Maintain the temperature to within $\pm 0,1$ °C.

Rinse the conductivity cell twice with the solution to be examined.

Measure the conductivity and express the result in $\mu\text{S cm}^{-1}$.

5. Expression of the results

The result is expressed in microsiemens per cm (μScm^{-1}) at 20 °C to the nearest whole number for the 25 % (m/m) (25° Brix) solution of rectified concentrated must.

5.1. Calculations

If the apparatus does not have temperature compensation, correct the measured conductivity using Table I. If the temperature is below 20 °C, add the correction; if the temperature is above 20 °C, subtract the correction.

Table I

Corrections to be made to the conductivity for temperatures different from 20 °C ($\mu\text{S cm}^{-1}$)

Conductivity	Temperature (°C)									
	20,2 19,8	20,4 19,6	20,6 19,4	20,8 19,2	21,0 19,0	21,2 18,8	21,4 18,6	21,6 18,4	21,8 18,2	22,0 ⁽¹⁾ 18,0 ⁽²⁾
0	0	0	0	0	0	0	0	0	0	0
50	0	0	1	1	1	1	1	2	2	2
100	0	1	1	2	2	3	3	3	4	4
150	1	1	2	3	3	4	5	5	6	7
200	1	2	3	3	4	5	6	7	8	9
250	1	2	3	4	6	7	8	9	10	11
300	1	3	4	5	7	8	9	11	12	13
350	1	3	5	6	8	9	11	12	14	15
400	2	3	5	7	9	11	12	14	16	18
450	2	3	6	8	10	12	14	16	18	20
500	2	4	7	9	11	13	15	18	20	22
550	2	5	7	10	12	14	17	19	22	24
600	3	5	8	11	13	16	18	21	24	26

⁽¹⁾ Subtract the correction.

⁽²⁾ Add the correction.

(c) Hydroxymethylfurfural (HMF)

1. Principle of the methods

1.1. Colorimetric method

Aldehydes derived from furan, the main one being hydroxymethylfurfural, react with barbituric acid and paratoluidine to give a red compound which is determined by colorimetry at 550 nm.

1.2. High-performance liquid chromatography (HPLC)

Separation through a column by reversed-phase chromatography and determination at 280 nm.

2. Colorimetric method

2.1. Apparatus

2.1.1. Spectrophotometer for making measurements between 300 and 700 nm.

2.1.2. Glass cells with optical paths of 1 cm.

2.2. Reagents

2.2.1. Barbituric acid, 0,5 % solution (m/v).

Dissolve 500 mg of barbituric acid, $C_4O_3N_2H_4$, in distilled water and heat slightly over a waterbath at 100 °C. Make up to 100 ml with distilled water. The solution keeps for about a week.

2.2.2. Paratoluidine solution, 10 % (m/v).

Place 10 g of paratoluidine, $C_6H_4(CH_3)NH_2$, in a 100 ml volumetric flask; add 50 ml of isopropanol, $CH_3CH(OH)CH_3$, and 10 ml of glacial acetic acid, CH_3COOH ($\rho_{20} = 1,05$ g/ml). Make up to 100 ml with isopropanol. This solution should be renewed daily.

2.2.3. Ethanal (acetaldehyde), CH_3CHO , 1 % (m/v) aqueous solution.

Prepare just before use.

2.2.4. Hydroxymethylfurfural, $C_6O_3H_6$, 1 g/l aqueous solution.

Prepare successive dilutions containing 5, 10, 20, 30 and 40 mg/l. The 1 g/l and the diluted solutions must be freshly prepared.

2.3. Procedure

2.3.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v): introduce 200 g of accurately weighed rectified concentrated must into a 500 ml volumetric flask. Make up to the mark with water and homogenise. Carry out the determination on 2 ml of this solution.

2.3.2. Colorimetric determination

Into each of two 25 ml flasks *a* and *b* fitted with ground glass stoppers place 2 ml of the sample prepared as in paragraph 2.3.1. Place in each flask 5 ml of paratoluidine solution (paragraph 2.2.2); mix. Add 1 ml of distilled water to flask *b* (control) and 1 ml barbituric acid solution (paragraph 2.2.1) to flask *a*. Shake to homogenize. Transfer the contents of the flasks into spectrophotometer cells with optical paths of 1 cm. Zero the absorbance scale using the contents of flask *b* for a wavelength of 550 nm. Follow the variation in the absorbance of the contents of flask *a*; record the maximum value *A*, which is reached after two to five minutes.

Samples with hydroxymethylfurfural concentrations above 30 mg/l must be diluted before the analysis.

2.3.3. Preparation of the calibration curve

Place 2 ml of each of the hydroxymethylfurfural solutions with 5, 10, 20, 30 and 40 mg/l (paragraph 2.2.4) into two sets of 25 ml flasks *a* and *b* and treat them as described in paragraph 2.3.2.

The graph representing the variation of absorbance with the hydroxymethylfurfural concentration in mg/l is a straight line passing through the origin.

2.4. Expression of results

The hydroxymethylfurfural concentration in rectified concentrated musts is expressed in milligrams per kilogram of total sugars.

2.4.1. Method of calculation

The hydroxymethylfurfural concentration *C* mg/l in the sample to be analysed is that concentration on the calibration curve corresponding to the absorbance *A* measured on the sample.

The hydroxymethylfurfural concentration in milligrams per kilogram of total sugars is given by:

$$250 \times ((C)/(P))$$

P = percentage (m/m) concentration of total sugars in the rectified concentrated must.

3. High-performance liquid chromatography

3.1. Apparatus

3.1.1. High-performance liquid chromatograph equipped with:

- a loop injector, 5 or 10 µl,
- spectrophotometer detector for making measurements at 280 nm,
- column of octadecyl-bonded silica (e.g.: Bondapak C₁₈ — Corasil, Waters Ass.),
- a recorder and, if required, an integrator,

Flow rate of mobile phase: 1,5 ml/minute.

3.1.2. Membrane filtration apparatus, pore diameter 0,45 µm.

3.2. Reagents

3.2.1. Doubly distilled water.

3.2.2. Methanol, CH₃OH, distilled or HPLC quality.

3.2.3. Acetic acid, CH₃COOH, (ρ₂₀ = 1,05 g/ml).

3.2.4. Mobile phase: water-methanol (paragraph 3.2.2)-acetic acid (paragraph 3.2.3) previously filtered through a membrane filter (0,45 µm), (40:9:1 v/v).

This mobile phase must be prepared daily and outgassed before use.

3.2.5. Reference solution of hydroxymethylfurfural, 25 mg/l (v/v).

Into a 100 ml volumetric flask, place 25 mg of hydroxymethylfurfural, C₆H₃O₆, accurately weighed, and make up to the mark with methanol (paragraph 3.2.2). Dilute this solution 1/10^e with methanol (paragraph 3.2.2) and filter through a membrane filter (0,45 µm).

If kept in a hermetically sealed brown glass bottle in a refrigerator, this solution will keep for two to three months.

3.3. Procedure

3.3.1. Preparation of sample

Use the solution obtained by diluting the rectified concentrated must to 40 % (m/v) (introduce 200 g of accurately weighed rectified concentrated must into a 500 ml volumetric flask. Make up to the mark with water and homogenise) and filter it through a membrane filter (0,45 µm).

3.3.2. Chromatographic determination

Inject 5 (or 10) µl of the sample prepared as described in paragraph 3.3.1. and 5 (or 10) µl of the reference hydroxymethylfurfural solution (paragraph 3.2.5) into the chromatograph. Record the chromatogram.

The retention time of hydroxymethylfurfural is approximately six to seven minutes.

3.4. Expression of results

The hydroxymethylfurfural concentration in rectified concentrated musts is expressed in milligrams per kilogram of total sugars.

3.4.1. Method of calculation

Let the hydroxymethylfurfural concentration in the 40 % (m/v) solution of the rectified concentrated must be C mg/l.

The hydroxymethylfurfural concentration in milligrams per kilogram of total sugars is given by:

$$250 \times ((C)/(P))$$

P = percentage (m/m) concentration of total sugars in the rectified concentrated must.

(d) **Heavy metals**

1. **Principle**

I. *Rapid method for evaluation of heavy metals*

Heavy metals are revealed in the suitably diluted rectified concentrated must by the coloration produced by the formation of sulphides. They are assessed by comparison with a standard lead solution corresponding to the maximum admissible concentration.

II. *Determination of lead content by atomic absorption spectrophotometry*

The chelate given by lead with ammonium pyrrolidinedithiocarbamate is extracted with methylisobutylketone and the absorbance measured at 283,3 nm. The lead content is determined by using known additional amounts of lead in a set of reference solutions.

2. **Rapid method for evaluation of heavy metals**

2.1. *Reagents*

2.1.1. Dilute hydrochloric acid, 70 % (m/v).

Take 70 g of hydrochloric acid, HCl ($\rho_{20} = 1,16$ to $1,19$ g/ml), and make up to 100 ml with water.

2.1.2. Dilute hydrochloric acid, 20 % (m/v).

Take 20 g of hydrochloric acid, HCl ($\rho_{20} = 1,16$ to $1,19$ g/ml), and make up to 100 ml with water.

2.1.3. Dilute ammonia.

Take 14 g of ammonia, NH_3 ($\rho_{20} = 0,931$ to $0,934$ g/ml) and make up to 100 ml with water.

2.1.4. pH 3,5 buffer solution.

Dissolve 25 g of ammonium acetate ($\text{CH}_3\text{COONH}_4$), in 25 ml of water and add 38 ml of dilute hydrochloric acid (paragraph 2.1.1). Adjust the pH if necessary with the dilute hydrochloric acid (paragraph 2.1.2) or the dilute ammonia (paragraph 2.1.3) and make up to 100 ml with water.

2.1.5. Thioacetamide solution, ($\text{C}_2\text{H}_5\text{NS}$), 4 % (m/v).

2.1.6. Glycerol solution, ($\text{C}_3\text{H}_8\text{O}_3$), 85 % (m/v)

($n_D^{20\text{ }^\circ\text{C}} = 1,449$ to $1,455$).

2.1.7. Thioacetamide reagent.

To 0,2 ml of thioacetamide solution (paragraph 2.1.5) add 1 ml of a mixture of 5 ml of water, 15 ml of 1 M sodium hydroxide solution and 20 ml of glycerol (paragraph 2.1.6). Heat over a waterbath at $100\text{ }^\circ\text{C}$ for 20 seconds. Prepare just before use.

2.1.8. Solution containing 0,002 g/l of lead.

Prepare a 1 g/l lead solution by dissolving 0,400 g of lead nitrate, $\text{Pb}(\text{NO}_3)_2$, in water and making up to 250 ml with water. At the time of use, dilute this solution with water to two parts in 1 000 (v/v) in order to obtain a 0,002 g/l solution.

2.2. *Procedure*

Dissolve a test sample of 10 g of the rectified concentrated must in 10 ml of water. Add 2 ml of the pH 3,5 buffer solution (paragraph 2.1.4); mix. Add 1,2 ml of the thioacetamide reagent (paragraph 2.1.7). Mix at once. Prepare the control under the same conditions by using 10 ml of the 0,002 g/l lead solution (paragraph 2.1.8).

After two minutes, any brown coloration of the rectified concentrated must solution should not be more intense than that of the control.

2.3. *Calculations*

Under the conditions of the above procedure, the control sample corresponds to a maximum admissible heavy metal concentration expressed as lead of 2 mg/kg of rectified concentrated must.

3. **Determination of lead content by atomic absorption spectrophotometry**

3.1. *Apparatus*

3.1.1. Atomic absorption spectrophotometer equipped with an air-acetylene burner.

3.1.2. Lead hollow cathode lamp.

3.2. *Reagents*

3.2.1. Dilute acetic acid.

Take 12 g of glacial acetic acid ($\rho_{20} = 1,05$ g/ml) and make up to 100 ml with water.

3.2.2. Solution of ammonium pyrrolidinedithiocarbamate, $C_5H_{12}N_2S_2$, 1 % (m/v).

3.2.3. Methylisobutylketone, $(CH_3)_2CHCH_2COCH_3$.

3.2.4. Solution containing 0,010 g/l of lead.

Dilute the 1 g/l lead solution (paragraph 2.1.8) to 1 % (v/v).

3.3. *Procedure*

3.3.1. Preparation of solution to be examined

Dissolve 10 g of rectified concentrated must in a mixture of equal volumes of dilute acetic acid (paragraph 3.2.1) and water, and make up to 100 ml with this mixture.

Add 2 ml of ammonium pyrrolidinedithiocarbamate solution (paragraph 3.2.2) and 10 ml of methylisobutylketone (paragraph 3.2.3). Shake for 30 seconds while protected from bright light. Leave the two layers to separate. Use the methylisobutylketone layer.

3.3.2. Preparation of reference solutions

Prepare three reference solutions containing, in addition to 10 g of rectified concentrated must, 1, 2 and 3 ml respectively of the solution containing 0,010 g/l of lead (paragraph 3.2.4). Treat these in the same way as the solution to be examined.

3.3.3. Control

Prepare a control by proceeding under the same conditions as in paragraph 3.3.1, but without the addition of the rectified concentrated must.

3.3.4. Determination

Set the wavelength to 283,3 nm.

Atomise the methylisobutylketone from the control sample in the flame and zero the absorbance scale.

By operating with their respective solvent extracts, determine the absorbences of the solution to be examined and the reference solutions.

3.4. *Expression of results*

Express the lead content in milligrams per kilogram of rectified concentrated must to one decimal place.

3.4.1. Calculations

Plot the curve giving the variation in absorbance as a function of the lead concentration added to the reference solutions, zero concentration corresponding to the solution to be examined.

Extrapolate the straight line joining the points until it cuts the negative part of the concentration axis. The distance of the point of intersection from the origin gives the lead concentration in the solution to be examined.

(e) **Chemical determination of ethanol**

This method is used for the determination of the alcoholic strength of low-alcohol liquids such as musts, concentrated musts and rectified concentrated musts.

1. **Principle**

Simple distillation of the liquid. Oxidation of the ethanol in the distillate by potassium dichromate. Titration of the excess dichromate with an iron (II) solution.

2. **Apparatus**

- 2.1. Distillation apparatus used to measure the alcoholic strength

3. **Reagents**

- 3.1. *Potassium dichromate solution.*

Dissolve 33,600 g of potassium dichromate, ($\text{K}_2\text{Cr}_2\text{O}_7$), in sufficient quantity of water to make one litre of solution at 20 °C.

One millilitre of this solution oxidizes 7,8924 mg of alcohol.

- 3.2. *Iron (II) ammonium sulphate solution.*

Dissolve 135 g of iron (II) ammonium sulphate, $\text{FeSO}_4 \cdot (\text{NH}_4)_2\text{SO}_4 \cdot 6 \text{H}_2\text{O}$ in sufficient quantity of water to make one litre of solution and add 20 ml of concentrated sulphuric acid, (H_2SO_4), ($\rho_{20} = 1,84 \text{ g/ml}$). This solution more or less corresponds to half its volume of dichromate solution when just prepared. Subsequently, it oxidizes slowly.

- 3.3. *Potassium permanganate solution.*

Dissolve 1,088 g of potassium permanganate, KMnO_4 , in a sufficient quantity of water to make one litre of solution.

- 3.4. *Sulphuric acid, diluted 1:2 (v/v).*

A little at a time and stirring continuously, add 500 ml of sulphuric acid, (H_2SO_4) ($\rho_{20} = 1,84 \text{ g/ml}$) to 500 ml of water.

- 3.5. *Ferrous orthophenanthroline reagent.*

Dissolve 0,695 g of ferrous sulphate, $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, in 100 ml of water, and add 1,485 g of orthophenanthroline monohydrate, $\text{C}_{12}\text{H}_8\text{N}_2 \cdot \text{H}_2\text{O}$. Heat to help the dissolution. This bright red solution keeps well.

4. **Procedure**

- 4.1. *Distillation*

Place 100 g of rectified concentrated must and 100 ml of water in the distillation flask. Collect the distillate in a 100 ml volumetric flask and make up to the mark with water.

- 4.2. *Oxidation*

Take a 300 ml flask with a ground glass stopper and with a widened neck enabling the neck to be rinsed without loss. In the flask, place 20 ml of the titrant potassium dichromate solution (paragraph 3.1) and 20 ml of the 1:2 (v/v) dilute sulphuric acid (paragraph 3.4) and shake. Add 20 ml of the distillate. Stopper the flask, shake, and wait at least 30 minutes, shaking occasionally. (This is the 'measurement' flask.)

Carry out the titration of the iron (II) ammonium sulphate solution (paragraph 3.2) with respect to the potassium dichromate solution by placing in an identical flask the same quantities of reagents but replacing the 20 ml of distillate by 20 ml of distilled water. (This is the 'control' flask.)

- 4.3. *Titration*

Add four drops of the orthophenanthroline reagent (paragraph 3.5) to the contents of the 'measurement' flask. Titrate the excess dichromate by adding to it the iron (II) ammonium sulphate solution (paragraph 3.2). Stop adding the ferrous solution when the mixture changes from green-blue to brown.

To judge the end-point more precisely, change the colour of the mixture back from brown to green-blue with the potassium permanganate solution (paragraph 3.3). Subtract a tenth of the volume of this solution used from the volume of the iron (II) solution added. Let the difference be $n \text{ ml}$.

Proceed in the same way with the 'control' flask. Let n' ml be the difference here.

5. Expression of the results

The ethanol is expressed in grams per kilogram of total sugars and is quoted to one decimal place.

5.1. Method of calculation

n' ml of ferrous solution reduces 20 ml of dichromate solution which oxidizes 157,85 mg of pure ethanol.

One millilitre of iron (II) solution has the same reducing power as:

$((157,85)/(n))$ mg of ethanol

$n - n'$ ml of iron (II) solution have the same reducing power as:

$157,85 \times ((n' - n)/(n))$ mg of ethanol.

Ethanol concentration in g/kg of rectified concentrated must is given by:

$7,892 \times ((n' - n)/(n))$

Ethanol concentration in g/kg of total sugars is given by:

$789,2 \times ((n' - n)/(n' \times P))$

P = percentage (m/m) concentration of total sugars in the rectified concentrated must.

(f) *Meso-inositol, scyllo-inositol and sucrose*

1. Principle

Gas chromatography of silylated derivatives.

2. Reagents

2.1. Internal standard: xylitol (aqueous solution of about 10 g/l to which a spatula tip of sodium azide is added)

2.2. Bis(trimethylsilyl)trifluoroacetamide — BSTFA — ($C_8H_{18}F_3NOSi_2$)

2.3. Trimethylchlorosilane (C_3H_9ClSi)

2.4. Pyridine p.A. (C_5H_5N)

2.5. Meso-inositol ($C_6H_{12}O_6$)

3. Apparatus

3.1. Gas chromatograph equipped with:

3.2. Capillary column (e.g. in fused silica, coated with OV 1, film thickness of 0,15 μ , length 25 m and internal diameter of 0,3 mm).

Operating conditions: carrier gas: hydrogen or helium

— carrier gas flow rate: about 2 ml/minute,

— injector and detector temperature: 300 °C,

— programming of temperature: 1 minute at 160 °C, 4 °C per minute to 260 °C, constant temperature of 260 °C for 15 minutes,

— splitter ratio: about 1:20.

3.3. Integrator.

3.4. Microsyringe, 10 μ l.

- 3.5. Micropipettes, 50, 100 and 200 µl.
- 3.6. 2 ml flasks with Teflon stopper.
- 3.7. Oven.

4. **Procedure**

An accurately weighed sample of about 5 g of rectified concentrated must is placed in a 50 ml flask. 1 ml of standard solution of xylitol (paragraph 2.1) is added and water added to capacity. After mixing, 100 µl of solution is taken and placed in a flask (point 3.6) where it is dried under a gentle stream of air. 100 µl of absolute ethyl alcohol may be added if necessary to facilitate evaporation.

The residue is carefully dissolved in 100 µl of pyridine (paragraph 2.4) and 100 µl of bis(trimethylsilyl)trifluoroacetamide (paragraph 2.2) and 10 µl of trimethylchlorosilane (paragraph 2.3) are added. The flask is closed with the Teflon stopper and heated at 60 °C for one hour.

Draw off 0,5 µl of clear fluid and inject using a heated hollow needle in accordance with the stated splitter ratio.

5. **Calculation of results**

- 5.1. A solution is prepared containing:

60 g/l of glucose, 60 g/l of fructose, 1 g/l of meso-inositol and 1 g/l of sucrose.

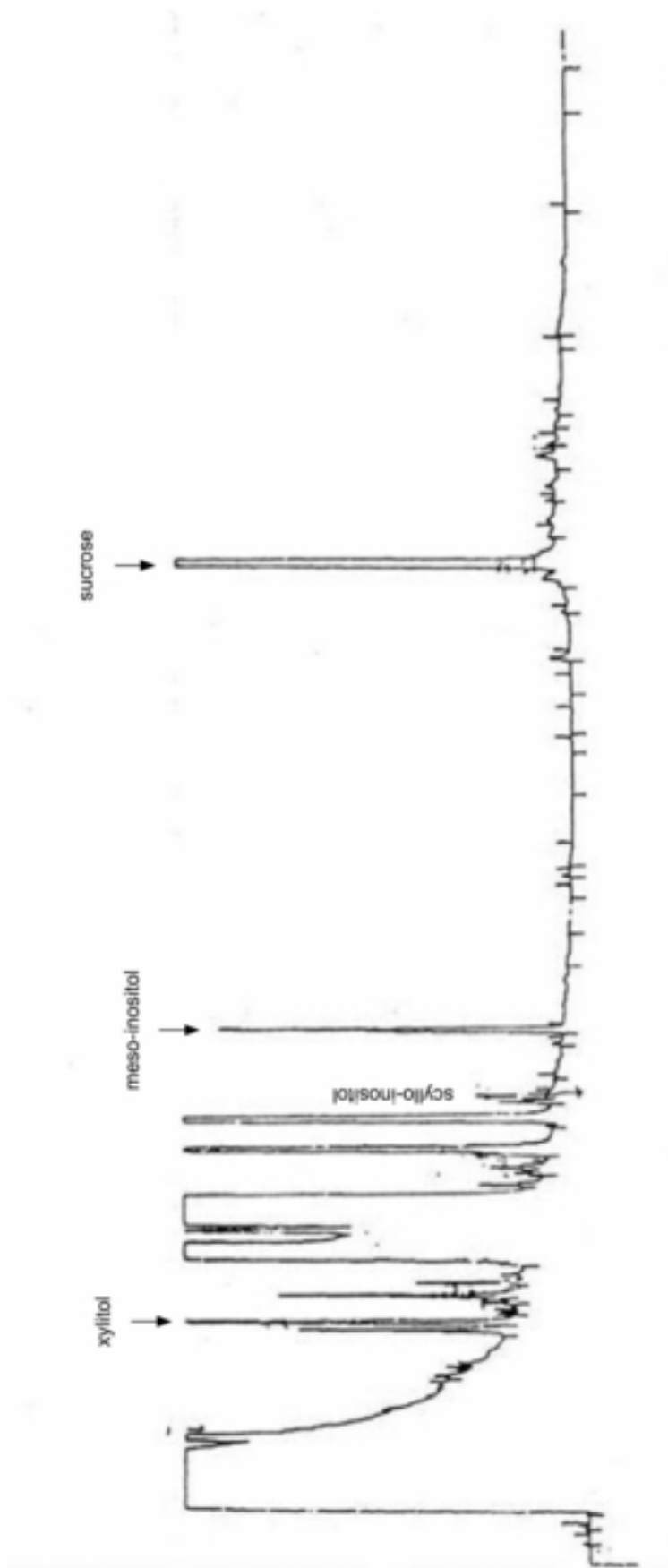
5 g of the solution is weighed and the procedure at paragraph 4 followed. The results for meso-inositol and sucrose with respect to xylitol are calculated from the chromatogram.

In the case of scyllo-inositol, which is not commercially available and has a retention time lying between the last peak of the anomeric form of glucose and the peak for meso-inositol (see diagram), the same result as for meso-inositol is taken.

6. **Expression of the results**

- 6.1. Meso-inositol and scyllo-inositol are expressed in milligrams per kilogram of total sugars.

Sucrose is expressed in grams per kilogram of must.



ANNEX V

CORRELATION TABLE REFERRED TO IN THE SECOND PARAGRAPH OF ARTICLE 16

Regulation (EC) No 1493/1999	Regulation (EC) No 2676/90	Regulation (EC) No 423/2008	This Regulation
—	—	Article 1	Article 1
—	—	—	Article 2
Article 43(1)	—	Article 5	Article 3(1)
Article 43(2), first indent	—	Article 23	Article 3(2)
Article 43(2), first indent	—	Article 24	Article 3(3)
Article 43(2), first indent	—	Articles 34, 35 and 36	Article 3(4)
—	—	Article 44	Article 4
Article 43(2), second indent	—	—	Article 5
Article 43(2), third indent	—	—	Article 6
—	—	Article 38	Article 7
Article 42(6)	—	Article 39	Article 8
—	—	Article 6	Article 9
—	—	Article 46	Article 10(1)
—	—	Article 45	Article 10(2)
—	—	Article 32	Article 11
—	—	Article 29	Article 12
—	—	Article 30	Article 13
—	—	Article 21	Article 14
—	Article 1(1)	Article 47	Article 15
—	—	Article 48	Article 16
Annex IV	—	Articles 7 and 12	Annex I A
—	—	Article 10	Annex I A, Appendix 1
—	—	Article 8	Annex I A, Appendix 2
—	—	Article 9	Annex I A, Appendix 3
—	—	Article 13	Annex I A, Appendix 4
—	—	Articles 14, 15 and 16	Annex I A, Appendix 5
—	—	Article 17	Annex I A, Appendix 6
—	—	Article 18	Annex I A, Appendix 7
—	—	Article 19	Annex I A, Appendix 8
—	—	Article 22	Annex I A, Appendix 9
Annex V A	—	—	Annex I B
Annex V B	—	—	Annex I C
Annex V F	—	—	Annex I D
Annex V H	—	Article 28	Annex II A
Annex V I	—	Article 4	Annex II B
Annex VI K	—	—	Annex II C
Annex V J	—	Articles 25 and 37	Annex III A
—	—	Article 43	Annex III A
Annex VI L	—	Articles 40 and 41	Annex III B
—	Annex, paragraph 39	—	Annex IV-A
—	Annex, paragraph 42	—	Annex IV-B

3. WINES

3.2 CLARIFICATION OF WINE

3.2.1 FINING (OENO 7/99), (OENO 6/04), (OENO 9/04) (OIV-OENO 339A-2009), (OIV-OENO 339B-2009, OENO 417-2011)

Definition :

Clarification of wine by addition of substances that precipitate particles in suspension :

- Whether by promoting the natural sedimentation of the former, or
- By coagulating around the particles to be eliminated and by entraining them in sediments.

Objectives :

- a) To complete spontaneous clarification on those occasions when it is unsatisfactory.
- b) To soften red wines by removing from them some of the tannins and polyphenols.
- c) To clarify wines with haze problems, stirred up lees, insoluble coloured matter, etc.

Prescriptions :

- a) For clarifying agents promoting simply the sedimentation of particles, refer to Treatment with bentonites¹¹
- b) For the coagulating clarifying agents, only the following products are admissible: gelatin, albumin and white of egg, isinglass, skim milk, casein, alginates, colloidal solution of silicon dioxide, kaolin, potassium caseinate, proteins of plant origin, chitosan, chitin-glucan, yeast protein extracts.
- c) The substances used shall to comply with the prescriptions of the International Oenological Codex.

Recommendation of the OIV :

¹¹ This definition applies solely to wines stricto sensu such as defined in the Part I, chap.3 "Wines" of the present Code.

Accepted.

3. WINES

3.2.2 FILTRATION (2/89) U

Definition :

Physical process consisting of passing the wine through appropriate filters that retain particles in suspension.

Objectives :

- a) Clarifying the wine, if necessary by successive stages (clarifying filtration) .
- b) Obtaining biological stability of the wine by elimination of microorganisms (sterilising filtration).

Prescriptions :

Filtration can be undertaken :

- a) By continuous deposition, using appropriate additives such as diatomaceous earth, perlite, cellulose...,
- b) On pads of cellulose or other appropriate materials,
- c) On mineral or organic membranes of a porosity greater than or equal to 0.2 µm (microfiltration).

The filter materials used shall comply with the prescriptions of the *International Oenological Codex* .

Recommendation of OIV:

Refer to the practices and treatments mentioned hereafter.

3. WINES

3.2.2.1 FILTRATION BY CONTINUOUS DEPOSITION (1/90) ⓘ

Definition :

Filtration of the wine, after formation of a filter bed, which is then fed constantly by a continuous addition of filter material into the wine to be clarified.

Objective :

To obtain a suitable level of clarity, to a given technological stage, by the elimination of substances in suspension in the wine.

Prescriptions :

- a) The nature of the filter material (such as diatomaceous earth, perlite and cellulose) and the necessary dose are determined by the turbidity of the wine and the clarification sought.
- b) The filter materials used shall comply with the prescriptions of the *International Oenological Codex* .

Recommendation of OIV:

Accepted.

3. WINES

3.2.2.2 STERILISING FILTRATION (1/90) ⓘ

Definition :

Filtration of wines on materials allowing the elimination of microorganisms.

Objective :

To obtain biologically stable wines for bottling.

Prescriptions :

a) The objective can be achieved by the use of the following, having regard to conditions of pressure, flow and recommended duration:

- Special prefabricated pads of cellulose or other materials ;
- Membranes having a maximum average pore diameter of 0.65 µm.

b) The filtration equipment must be sterilised beforehand by passing hot water or steam through them.

c) The materials used shall comply with the prescriptions of the *International Oenological Codex* .

Recommendation of OIV :

Accepted.

3. WINES

3.2.3 RACKING (16/70), (OENO 6/02)

Definition :

Operation involving the transfer of wine from one wine container to another by allowing the separation of solid deposits from the liquid.

Objectives:

- a) Separate wine from the lees, and/or the deposits resulting from the addition of clarifying agents, deposited at the bottom of the container
- b) Separate the wine from the micro-organisms at the end of the alcoholic and/or malolactic fermentation, or the bacterial or yeast sediment
- c) Enable the carrying out of all wine making operations, treatment or transportation of wines.
- d) Enable the tartaric stabilization by cooling and the separation of tartrate crystals (potassium bitartrate and calcium tartrate).

Prescriptions :

Racking can occur:

- a) Either in the absence of air to avoid oxidation,
- b) Or with aeration to eliminate hydrogen sulfide or to reduce carbon dioxide or to create controlled oxidation,
- c) Or at room temperature, or after cooling to avoid possible carbonic gas loss,
- d) Or using the law of interconnected vessels, either with pumps or hand containers
- e) In the case of racking in the absence of air, the container to be filled must be rendered inert with carbon dioxide, nitrogen or with argon. These gases must comply with International Oenological Codex prescriptions

Recommendation of OIV :

Accepted.

3. WINES

3.2.4 TREATMENT WITH SILICON DIOXIDE (1/91)

Definition :

Addition to wine of a colloidal solution (gel) of silicon dioxide coupled with the addition of a gelatin solution or, possibly, with other proteinaceous finings.

Objective :

To achieve the flocculation of the gelatin and possibly other proteinaceous finings, with a view to clarification.

Prescriptions :

- a) The product is added to young white wines and rosés and occasionally to red wines.
- b) Preliminary tests are necessary to determine optimal doses of the colloidal solutions of silicon dioxide and gelatin or possibly, of other proteinaceous finings.
- c) The products shall comply with the prescriptions of the *International Oenological Codex* .

Recommendation of OIV:

Accepted

3. WINES

3.2.5 DECANTING (RACKING) (5/88), (OENO 1/04)

Definition :

Operation consisting of transferring from one wine tank to another:

- A still wine at normal pressure,

Objectives :

- a) To separate wines from their lees, deposited on the bottom of the container.
- b) To prepare for mixing or blending operations.
- c) To prepare for physical clarification by filtration, centrifugation, etc.
- d) To achieve bulk transportation of the wine.

Prescriptions :

Decanting can be performed:

- a) Either under protection from air so as to avoid all oxidation, or
- b) With aeration, to eliminate hydrogen sulphide or to reduce the carbon dioxide or to create a managed oxidation.

Recommendation of OIV:

Accepted.

3. WINES

3.2.6 TANNIN ADDITION (16/70)

Definition :

Addition of tannin to wine.

Objectives :

- a) To facilitate the clarification of new wines by partial precipitation of excess proteinaceous matter.
- b) To facilitate fining.

Prescription :

The tannins used shall comply with the prescriptions of the *International Oenological Codex* .

Recommendation of OIV:

Accepted.

3. WINES

3.2.7 FINING USING PROTEINS OF PLANT ORIGIN (OENO 8/04)

Objectives :

Use of protein matter of plant origin for the fining of wines in order to improve their clarity, stability and gustatory properties.

Prescriptions:

1. The doses to be used are determined after a preliminary test trial. The maximum usage dose should be less than 50 g/hl. After racking, the wines are analysed (turbidity, colour, absorbance at 280nm) and tasted. The dose retained corresponds to the sample which clarifies the wine without excess and gives a better result for tasting.
2. Proteins of plant origin can be used with other admitted products such as tannins, bentonite, silica gel
3. Proteins of plant origin must comply with the prescriptions of the *International Oenological Codex* .

Recommendation of OIV:

Accepted.

3. WINES

3.2.8 USE OF ENZYMES FOR IMPROVING FILTERABILITY (OENO 15/04)

Definition:

The addition to wine of enzymatic preparations with in particular, polygalacturonase, pectin lyase, pectinmethylesterase and/or glucanase activities that catalyse the degradation of pectic polysaccharides and/or fungal β -glucans.

Objective:

To improve the filterability of wines by specific hydrolysis of colloids.

Prescription:

The enzymes used must comply with the prescriptions of the *International Oenological Codex*.

Recommendation of OIV:

Accepted.

3. WINES

3.2.9 USE OF ENZYMES FOR THE RELEASE OF FLAVOURING SUBSTANCES (OENO 17/04)

Definition:

The addition to wine of enzymatic preparations with, in particular, glycosidase activities.

Objective:

To enhance the aromatic potential in wine, from glycosylated precursors from grapes

Prescription:

The enzymes used must comply with the prescriptions of the *International Oenological Codex*.

Recommendation of OIV:

Accepted.

3. WINES

3.2.10 USE OF ENZYMES FOR IMPROVING THE SOLUBILISATION OF YEAST COMPOUNDS (OENO 18/04)

Definition:

The addition to wine during winemaking on lees of enzymatic preparations notably with β -glucanase activities that catalyse the degradation of yeast cell walls.

Objectives:

- a) To facilitate the release of yeast-soluble constituents into wine.
- b) To improve the colloidal stability of wines.

Prescription:

The enzymes used must comply with the prescriptions of the *International Oenological Codex*.

Recommendation of OIV:

Accepted.

3. WINES

3.2.11 USE OF ENZYMES FOR THE CLARIFICATION OF WINES (OENO 12/04)

Definition:

The addition to wine of enzymatic preparations with in particular, polygalacturonase, pectin lyase, pectinmethylesterase and/or β -glucanase activities that catalyse the degradation of pectic polysaccharides and/or fungal β -glucans.

Objective:

To facilitate the clarification of wines.

Prescription:

The enzymes used must comply with the prescriptions of the *International Oenological Codex*.

Recommendation of the OIV

Admitted

3. WINES

3.2.12 FINING USING CHITOSAN (OIV-OENO 337A-2009)

Definition:

Addition of chitosan of fungal origin for the purpose of fining wines

Objectives:

- a) To reduce turbidity by precipitating particles in suspension.
- b) To carry out a treatment to prevent protein haze by the partial precipitation of excess proteinaceous matter.

Prescriptions:

- a) The doses to be used are determined after preliminary testing. The maximum dose used must not exceed 100 g/hl.
- b) Sediments are eliminated by physical procedures.
- c) Chitosan of fungal origin may be used alone or together with other admitted products.
- d) Chitosan must comply with the requirements of the International Oenological Codex.

Recommendation of the OIV

Admitted

3. WINES

3.2.13 FINING USING CHITIN-GLUCAN (OIV-OENO 337B-2009)

Definition:

Addition of chitin-glucan of fungal origin for the purpose of fining wines

Objectives:

- a) To reduce turbidity by precipitating particles in suspension
- b) To carry out a treatment to prevent protein haze by the partial precipitation of excess proteinaceous matter.

Prescriptions:

- a) The doses to be used are determined after preliminary testing.
The maximum dose used must not exceed 100 g/hl.
- b) Sediments are eliminated by physical procedures.
- c) Chitin-glucan of fungal origin may be used alone or together with other admitted products.
- d) Chitin-glucan must comply with the requirements of the International Oenological Codex.

Recommendation of the OIV

Admitted

3. WINES

3.2.14 FINING USING YEAST PROTEIN EXTRACTS (OENO 417-2011)

Definition:

Addition of yeast protein extracts for fining wines

Objectives:

- a) Reduce turbidity of wines by precipitating suspended particles
- b) Preserve chromatic characteristics of wines
- c) Eliminate excess tannin
- d) Improve wine filterability

Prescriptions:

- a) The doses used are determined beforehand by laboratory trials (fining point)
- b) Maximum dose to be used as determined by an efficiency test conducted in laboratory must not exceed 60 g/hl for red wine and 30 g/hl for white and rosé wine
- c) The yeast protein extracts can be used alone or associated with other authorised fining products
- d) The deposits from the fining are to be eliminated from wine by physical procedures
- e) The yeast protein extracts must comply with the prescriptions of the International Oenological Codex

Recommendation of the OIV:

Admitted.

II

(Non-legislative acts)

REGULATIONS

COMMISSION DELEGATED REGULATION (EU) 2015/1576

of 6 July 2015

amending Regulation (EC) No 606/2009 as regards certain oenological practices and Regulation (EC) No 436/2009 as regards the registering of those practices in the wine sector registers

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007 ⁽¹⁾, and in particular Article 75(2) and (3)(g) and Article 147(3)(e) thereof,

Whereas:

- (1) In accordance with Article 3 of Commission Regulation (EC) No 606/2009 ⁽²⁾, authorised oenological practices are laid down in Annex I A to that Regulation. The International Organisation of Vine and Wine (OIV) has adopted resolutions allowing three new oenological practices. In order to take account of technical progress and to provide Union producers with the same possibilities as those available to third-country producers, those new oenological practices should be authorised in the Union under the conditions of use defined by the OIV.
- (2) Certain oenological practices are particularly exposed to the risk of fraudulent use and must be recorded in the registers pursuant to Article 41 of Commission Regulation (EC) No 436/2009 ⁽³⁾. For this reason, the three new oenological practices, that is the treatment of wines using a membrane technology coupled with activated carbone, the use of polyvinylimidazole-polyvinylpyrrolidone copolymers and the use of silver chloride, the last two substances being processing aids, should be entered in the registers.
- (3) Regulations (EC) No 606/2009 and (EC) No 436/2009 should therefore be amended accordingly,

HAS ADOPTED THIS REGULATION:

Article 1

Amendment of Regulation (EC) No 606/2009

Annex I A to Regulation (EC) No 606/2009 is amended in accordance with the Annex to this Regulation.

⁽¹⁾ OJ L 347, 20.12.2013, p. 671.

⁽²⁾ Commission Regulation (EC) No 606/2009 of 10 July 2009 laying down certain detailed rules for implementing Council Regulation (EC) No 479/2008 as regards the categories of grapevine products, enological practices and the applicable restrictions (OJ L 193, 24.7.2009, p. 1).

⁽³⁾ Commission Regulation (EC) No 436/2009 of 26 May 2009 laying down detailed rules for the application of Council Regulation (EC) No 479/2008 as regards the vineyard register, compulsory declarations and the gathering of information to monitor the wine market, the documents accompanying consignments of wine products and the wine sector registers to be kept (OJ L 128, 27.5.2009, p. 15).

*Article 2***Amendment of Regulation (EC) No 436/2009**

In the first subparagraph of Article 41(1) of Regulation (EC) No 436/2009, the following points are added:

- '(x) treatment using a membrane technology coupled with activated carbon;
- (y) use of polyvinylimidazole-polyvinylpyrrolidone copolymers;
- (z) use of silver chloride.'

*Article 3***Entry into force**

This Regulation shall enter into force on the seventh day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 6 July 2015.

For the Commission

The President

Jean-Claude JUNCKER

ANNEX

Annex I A to Regulation (EC) No 606/2009 is amended as follows:

(1) in the table, the following rows 53, 54 and 55 are added:

1		2	3
Oenological practice		Conditions of use	Limits on use Applications
53	Treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol	For wines and under the conditions laid down in Appendix 19	
54	Use of polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)	For musts and wines and under the conditions laid down in Appendix 20	No more than 500 mg/l (where added to both the must and the wine, the total overall quantity must not exceed 500 mg/l)
55	Use of silver chloride	For wines and under the conditions laid down in Appendix 21	No more than 1 g/hl, residue in the wine < 0,1 mg/l (silver)

(2) the following Appendices 19, 20 and 21 are added:

Appendix 19

Requirements for the treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol

The aim of the treatment is to reduce the content of 4-ethylphenol and 4-ethylguaiacol of microbial origin that constitutes organoleptic defects and masks the aromas of the wine.

Requirements:

- (1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.
- (2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.
- (3) The membranes used must comply with the requirements of Regulations (EC) No 1935/2004 and (EC) No 10/2011 and with the national provisions adopted for the implementation thereof. They must comply with the requirements of the International Oenological Codex published by the OIV.

Appendix 20

Requirements for polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)

The purpose of the use of PVI/PVP is to prevent defects caused by too high metal contents and to reduce undesirable high concentration of metals.

Requirements:

- (1) Copolymers must be eliminated by filtration no later than two days after their addition taking into account the precautionary principle.
- (2) In the case of cloudy musts, the copolymer must be added no earlier than a maximum of two days before filtration.

- (3) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.
 - (4) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.
-

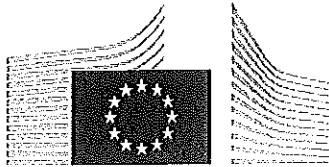
Appendix 21

Requirements for silver chloride

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (caused by reduction reactions characterised by the presence of hydrogen sulphide and thiols).

Requirements:

- (1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.
 - (2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.
 - (3) The silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure and must be treated by specialised sector.'
-



EUROPEAN
COMMISSION

Brussels, 6.7.2015
C(2015) 4510 final

ANNEX 1

ANNEX

to the

COMMISSION DELEGATED REGULATION

**amending Regulation (EC) No 606/2009 as regards certain oenological practices and
Regulation (EC) No 436/2009 as regards the registering of those practices in the wine
sector registers**

ANNEX

Annex I A to Regulation (EC) No 606/2009 is amended as follows:

(1) in the table, the following rows 53, 54 and 55 are added:

"

1		2	3
'Oenological practice		Conditions of use	Limits on use Applications
53	Treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol	For wines and under the conditions laid down in Appendix 19	
54	Use of polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)	For musts and wines and under the conditions laid down in Appendix 20	No more than 500 mg/l (where added to both the must and the wine, the total overall quantity must not exceed 500 mg/l)
55	Use of silver chloride	For wines and under the conditions laid down in Appendix 21	No more than 1 g/hl, residue in the wine <0,1 mg/l (silver)

"

(2) the following Appendices 19, 20 and 21 are added:

"Appendix 19

Requirements for the treatment of wines using a membrane technology coupled with activated carbon to reduce excess 4-ethylphenol and 4-ethylguaiacol

The aim of the treatment is to reduce the content of 4-ethylphenol and 4-ethylguaiacol of microbial origin that constitutes organoleptic defects and masks the aromas of the wine.

Requirements:

(1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.

(2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

(3) The membranes used must comply with the requirements of Regulations (EC) No 1935/2004 and (EC) No 10/2011 and with the national provisions adopted for the implementation thereof. They must comply with the requirements of the International Oenological Codex published by the OIV.

Appendix 20

Requirements for polyvinylimidazole-polyvinylpyrrolidone copolymers (PVI/PVP)

The purpose of the use of PVI/PVP is to prevent defects caused by too high metal contents and to reduce undesirable high concentration of metals.

Requirements:

- (1) Copolymers must be eliminated by filtration no later than two days after their addition taking into account the precautionary principle.
- (2) In the case of cloudy musts, the copolymer must be added no earlier than a maximum of two days before filtration.
- (3) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.
- (4) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.

Appendix 21

Requirements for silver chloride

Silver chloride is used for the treatment of wines to remove fermentation and storage-related abnormal odours (caused by reduction reactions characterised by the presence of hydrogen sulphide and thiols).

Requirements:

- (1) The treatment is to be carried out under the responsibility of an oenologist or qualified technician.
- (2) The treatment must be recorded in the registers referred to in Article 147(2) of Regulation (EU) No 1308/2013.
- (3) The silver chloride added to wine must be applied to an inert support, like kieselguhr (diatomaceous earth), bentonite, kaolin, etc. The precipitate must be eliminated by any appropriate physical procedure and must be treated by specialised sector."

Metal reduction in wine using PVI-PVP copolymer and its effects on chemical and sensory characters

H. MIRA¹⁾, P. LEITE^{1), 2), 3)}, S. CATARINO³⁾, J. M. RICARDO-DA-SILVA²⁾ and A. S. CURVELO-GARCIA³⁾

¹⁾Escola Superior Agrária de Santarém, Santarém, Portugal ²⁾Universidade Técnica de Lisboa, Instituto Superior de Agronomia, Laboratório Ferreira Lapa (Sector de Enologia), Lisboa, Portugal

³⁾INIAP, Estação Vitivinícola Nacional, Quinta da Almoíña, Dois Portos, Portugal

Summary

We studied the influence of an adsorbent PVI-PVP resin (a copolymer of vinylimidazole and vinylpyrrolidone), on the removal of heavy metals in wines, mainly copper (Cu), iron (Fe), lead (Pb), cadmium (Cd) and aluminium (Al). The study also investigated the influence of PVI-PVP on the physical-chemical and sensory characteristics of white and red wines, comparing its effect when applied in the must and in the wine. The removal of metals was more effective when PVI-PVP was applied to the wine than to the must. The removal of Fe and Pb was more effective in white wines than in red wines, while the removal of Cu and Al was higher in red wines. In general, the higher the PVI-PVP dose, the greater the quantity of metallic elements (copper, iron, lead and aluminium) that are removed. PVI-PVP had a minor effect on phenolic composition. The wines showed some decrease in total acidity and an increase in pH with PVI-PVP. The application of PVI-PVP at the dose rates employed here did not affect the wine's sensory characteristics significantly.

Key words : PVI-PVP, vinylimidazole, vinylpyrrolidone, metals, wine.

Introduction

The mineral constituents of must and of wine are present in ionic form and also as colloidal complexes with other compounds such as some organic acids and polysaccharides. Their primary presence is due to the natural transfer from soil-to-root-to-grapevine, in which the elements are absorbed by the roots according to their bio-availability in the soil, these being afterwards metabolised by the plant and further modified in the winemaking process and during the aging of the wine. These natural levels can also be increased by contamination (SEPPI and SPERANDIO 1978; MEDINA and SUDRAUD 1979; MCKINNON *et al.* 1992; OUGH 1993; ESCHNAUER *et al.* 1996; BAUER *et al.* 2001). A number of different factors can be responsible for the presence of exogenous metal contaminants in wines such as plant protection products, the winemaking equipment, and accidental contaminations of the must.

With technological progress, and especially with increased use of stainless steel in oenological equipment a

significant decrease in the level of residual Fe in wine has resulted. According to EDER *et al.* (2003) clouding problems are often the result of Cu levels above about 0.5 mg·l⁻¹. The use of copper-containing plant protection products can result in grape residues which are at least partially transferred to the wine. It is also possible that levels of this cation can be further raised by treatment of the wine with copper sulphate to eliminate unpleasant smells caused by sulphydric acid and by thiols (MATTIVI *et al.* 2000).

The presence of Pb in wine can originate from atmospheric pollution, from industrial emissions and from road traffic (presumably from the old, leaded petrol) (TEISSÈDRE *et al.* 1993 a; AUGAGNEUR *et al.* 1997; FOURNIER *et al.* 1998), or from plant protection and oenological products containing Pb as an impurity (TEISSÈDRE *et al.* 1993 b; MINGUEZ *et al.* 1997).

Contamination with Cd is the result mainly of contact with zinc materials rich in Cd and from some stainless steels (CURVELO-GARCIA 1988). GILBERT (1979) and MEDINA and SUDRAUD (1979) refer to a possible cadmium enrichment in musts from grapes growing close to major roads. ESCHNAUER *et al.* (1996) cite also plant protection products as possible sources of Cd.

High levels of metal contamination in wine such as Fe, Cu, Pb or Cd can influence the physico-chemical stability of the wine (BOULTON *et al.* 1995), causing browning, clouding and the formation of precipitates. This is quite separate from issues of food safety and of breaching legal upper limits. The present OIV (International Organisation of Vine and Wine) upper limits for Pb in wine are 150 µg·l⁻¹; for Cd 10 µg·l⁻¹, and for Cu 1 mg·l⁻¹ (OIV 2005, 2006) making it sometimes necessary to remove excess metals so as to lower the concentrations to below these legal limits. This also brings their contents to levels that are both safe for the consumer and favourable for the stability of the wine. These OIV limits are accepted within the European Community but not necessarily outside.

The classic removal process for excess metals in wine (mainly iron, but also sometimes copper, zinc, manganese, nickel, silver, lead, cadmium and aluminium) is by treatment with potassium ferrocyanide (K₄[Fe(CN)₆]·3H₂O). This is sometimes called 'blue fining'. The use of potassium ferrocyanide in wines is a technique which has limitations such as the need to carry out preliminary assays to determine the right dose rate. Obviously, 'over fining' by the addition of too much potassium ferrocyanide can result in the formation of free cyanide ions so that potassium

ferrocyanide use must be very carefully controlled indeed. Potassium ferrocyanide residues also raise environmental problems in disposal of waste. The addition of potassium ferrocyanide to the must is not allowed. From a technological viewpoint, this method has not anyway been very effective in eliminating Cu in wines that also contain low levels of Fe.

Because of these limitations, alternative methods have been explored such as those using adsorbent or exchanger resins (FENG *et al.* 1997; PALACIOS *et al.* 2001; EDER *et al.* 2001, 2003; BENITEZ *et al.* 2002). Many authors have developed methods based on the use of just one adsorbent resin to lower the levels of all of the contaminant metals present in wines (MATTIVI *et al.* 1994, 2000; EDER *et al.* 2001, 2003; NICOLINI *et al.* 2004), to values which are both safe for the consumer and which enhance the stability of the wine. The adsorbent resin referred to by FUSSNEGGER *et al.* (1992) is known as PVI-PVP or just PVI, and is a vinylimidazole and vinylpyrrolidone copolymer (ratio 9:1). PVI-PVP is insoluble in water and in most other liquids, and it has a number of functional imidazole groups which connect selectively to the various metals. Research has been carried out into the use of different doses of PVI-PVP in the must and in the wine with different times of contact, and its influence on wine colour, and on the various phenolic and aroma compounds of wine.

Besides its effect on the metals, PVI-PVP seems to have a relevant action in wine clarification. Here, as well as decreasing the level of hydroxycinnamic acid derivatives, mainly caftaric acid (MATTIVI *et al.* 1994, 2000; NICOLINI *et al.* 2004; EDER *et al.* 2001, 2003) it also minimises browning.

The treatment of musts and/or wines with PVI-PVP for lowering levels of metals such as Fe, Cu, Zn and Al, has been under discussion at OIV by experts under both the "Wine Technology" and, also the "Food Security" groups. The authorisation dossier is currently under evaluation. In May 2006, the French Agency of Food Security in an advice (AFSSA 2006) commented that utilisation of PVI-PVP at a dosage of 80g/hL did not appear to present any risk to consumer health.

The aim of this work was to study the influence of PVI-PVP on metal reduction (Fe, Cu, Pb, Cd, Al) and, especially to confirm that there were no important changes in the chemical and sensory characteristics of either white or red wines. We also wanted to compare its effects on wine characteristics when it was used either in the must or, later in the wine.

Material and Methods

Fining treatment applied to must and wine: **Wines:** White wines were prepared using grapes of the variety 'Fernão Pires' (*Vitis vinifera* L.), and red wines using 'Castelão' (*Vitis vinifera* L.). Grapes were harvested in 2002 from the Ribatejo region, Portugal.

PVI-PVP: The PVI-PVP (Divergan®HM, BASF, Germany) was applied at 10 and 20 g·hl⁻¹ (must) and 30 and 50 g·hl⁻¹ (wine). Experiments are summarised in Fig. 1. These application doses are those recommended by the supplier.

For the musts, the PVI-PVP was introduced before fermentation. They were immediately shaken for 20 min,

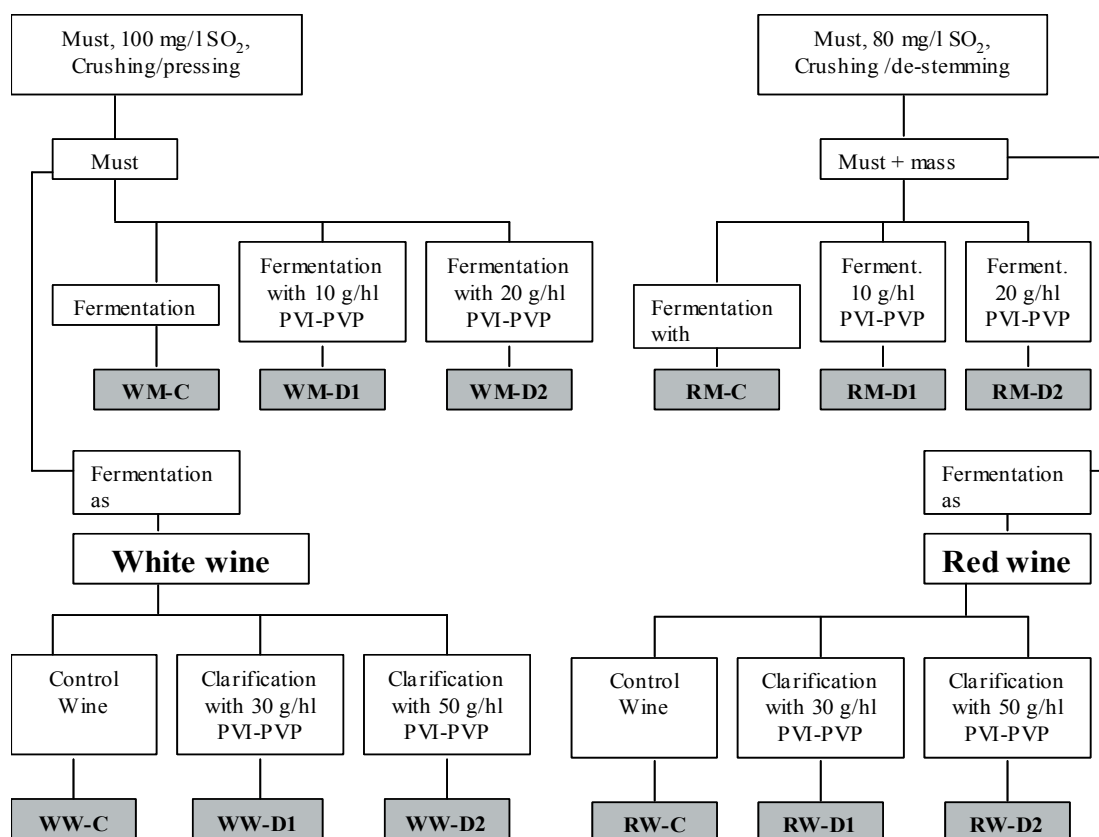


Fig. 1: Flowchart of the fining treatment (PVI-PVP) applied and wines.

after which they remained at rest during fermentation. Separation occurred at first racking. The wines were shaken for 15 min immediately after introduction of the PVI-PVP, after which they too remained at rest for 48 h.

Fining treatment applied in wine with metal additions: With the aim of confirming the influence of PVI-PVP on Fe and Cu we added these elements to matured white and red wines: 'Addition A' (5 mg·l⁻¹ of Fe and 0.5 mg·l⁻¹ of Cu). 'Addition B' (15 mg·l⁻¹ of Fe and 1 mg·l⁻¹ of Cu). We also added two levels of PVI-PVP 25 and 50 g·hl⁻¹ (the amounts '1' and '2' respectively).

Mineral composition: The concentration of potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), iron (Fe) and copper (Cu) was assayed by Flame Atomic Absorption Spectrometry (FAAS) using a Varian Spectra 10/20 (Victoria, Australia), according to the official method of OIV (OIV 1990).

The Pb, Cd and Al content were determined by Electrothermal Atomic Absorption Spectrometry (ETAAS), according to the method described by CATARINO (2000) and CATARINO *et al.* (2002).

Colour and phenolic composition: The total content of phenolic compounds was measured by the absorbance at 280 nm (RIBÉREAU-GAYON 1970); colour intensity by the sum ($A_{420} + A_{520} + A_{620}$), where tonality is defined by the ratio (A_{420}/A_{520}). For the white wine, colour intensity was measured using absorbance at 420 nm. Colour measurements of the wines were also performed according to the CIELAB 76 method (McLAREN 1980). Spectral readings, transmittance every 10 nm over the visible spectrum 380–770 nm, were performed with a UV4 Unicam Visible Spectrometer (Cambridge, UK), using quartz cells of path length 1 mm (red wine), and 10 mm (white wine). The software *Chroma 2.0 colour measurement* was used to calculate the CIELAB coordinates directly. The L*, a* and b* values describe a three-dimensional colour space.

For the red wine, the total pigments were estimated using the method of SOMERS and EVANS (1977); the polymeric pigments index was determined by a method proposed by GLORIES (1978) and total anthocyanins were evaluated according to the method of RIBÉREAU-GAYON and STONESTREET (1965).

Organic acid composition: Organic acids (tartaric, malic, citric, lactic and shikimic acid) were analysed by High Performance Liquid Chromatography (HPLC) using a method described by TUSSEAU and BENOIT (1986 a, b). Two columns with reverse phase Lichrospher 100 RP 8 (Merck, Darmstadt, Germany) (particle size 5 µm, 250 x 4 mm) were used. Detection was made with a UVIS 206 PHD (KONIK Instruments, Barcelona, Spain) set at 210 nm, and the peak areas were determined with Konikchrom 5.2 software.

Current analysis: pH, titratable and volatile acidity were determined by Fourier Transform Infrared Spectrometry – FTIR (WineScan FT120, Foss, Slangerupgade, Denmark) (MOREIRA *et al.* 2002 a, b).

Sensory analysis: Sensory analyses were performed by six expert panellists who were members of "Comissão Vitivinícola Regional of Ribatejo (CVRR)".

These persons were all properly trained and had good previous experience. We asked panellists to look for differences between the control wine and each sample of treated wine. The wine attributes included visual, nose and taste senses, as well as overall (global) appreciation.

Statistic analysis: The data were analyzed by Analysis of Variance using SPSS 12.0 for Windows. The treatment means were separated by the Scheffé test at the 5 % significance level.

Results and Discussion

Fining treatment applied in must and wine: **Must and white wine:** The results of applying PVI-PVP in must and wines were analysed separately. The summary of our variance analysis results with white wine is shown in Tab. 1.

For phenolic composition we confirm that PVI-PVP, either in the must or in wine caused a slight, but significant decrease in the level of phenolic compounds in the wine. This was expressed in a reduction in the total phenols index (Ipt). However, no significant differences appeared between the two PVI-PVP dose rates used. This is consistent with the observations of MATTIVI *et al.* (2000). According to these authors, PVI-PVP removes also some phenolic compounds from the wine and, in particular the low molecular weight ones. It is these that are mainly involved in the oxidative browning of white wines. Also NICOLINI *et al.* (2001, 2004) and EDER *et al.* (2001, 2003) refer to the influence of PVI-PVP on the reduction of hydroxycinnamates, thus improving colour stability.

In fact, with regard to the wines' chromatic characteristics, we confirm a slight, but not significant decrease in A420 and in the co-ordinates L*, a* and b*.

As for mineral composition, we found that PVI-PVP had no effect on Ca, Mg and Na contents, regardless of whether the product was used in the must or, later in the wine. This result is in line with that of other authors (EDER *et al.* 2001, 2003; NICOLINI *et al.* 2004). However, the application of PVI-PVP in the must did have a slight, but significant effect on the potassium content when compared with the control. This seems to indicate that the application of PVI-PVP in the must causes a premature elimination of this cation, and this probably contributes to tartaric stabilization in the subsequent wine.

In the particular case of Cu, the application of PVI-PVP in the must or in the wine did not have a significant effect on the content of this metal. This result may be due to the very low copper content of our wine, or to our experimental conditions. Also, the discontinuous shaking may have resulted in insufficient contact between PVI-PVP and the wine. Our result could be explained by that of NICOLINI *et al.* (2004), who found that removal was faster and more complete when mixing was more thorough. Also, EDER *et al.* (2003), suggest that metal removal can occur quite rapidly allowing PVI-PVP to be separated from the wine after only 16 h of contact.

Under our conditions, no effect on Fe content was detected with PVI-PVP either in white must or in wine;

Table 1

Effects of PVI-PVP application in must and wine on some white wine characteristics

	Effect		WM-C	WM-D1	WM-D2	WW-C	WW-D1	WW-D2	Effect
Colour and phenolic composition:									
Total phenol index (ua)	**	$\bar{\chi}$	10.8b	10.4a	10.2a	10.8c	9.6b	9.2a	**
		s	0.1	0.0	0.1	0.0	0.1	0.1	
A420 (ua)	ns	$\bar{\chi}$	0.113a	0.104a	0.116a	0.113a	0.103a	0.103a	ns
		s	0.035	0.007	0.009	0.002	0.001	0.005	
L*	ns	$\bar{\chi}$	98.1a	97.8a	98.2a	98.9a	98.1a	98.1a	ns
		s	0.2	0.3	0.0	1.7	0.1	0.0	
a*	ns	$\bar{\chi}$	-0.9a	-0.7a	-1.0a	-0.3a	-0.7a	-0.6a	ns
		s	0.14	0.06	0.24	0.45	0.16	0.05	
b*	ns	$\bar{\chi}$	7.6a	7.5a	8.0a	4.0a	6.9a	6.6a	ns
		s	0.08	0.10	0.98	0.75	0.09	0.04	
Mineral composition:									
Potassium (mg·l ⁻¹)	*	$\bar{\chi}$	605b	578a	565a	565a	565a	568a	ns
		s	10	3	4	10	13	0	
Calcium (mg·l ⁻¹)	ns	$\bar{\chi}$	72a	69a	70a	71a	70a	69a	ns
		s	1	1	1	0	1	0	
Sodium (mg·l ⁻¹)	ns	$\bar{\chi}$	47a	46a	47a	48a	48a	48a	ns
		s	1	0	0	1	0	1	
Magnesium (mg·l ⁻¹)	ns	$\bar{\chi}$	93a	91a	92a	91a	90a	91a	ns
		s	1	1	1	1	1	1	
Copper (mg·l ⁻¹)	ns	$\bar{\chi}$	0.1a	0.1a	0.1a	0.1a	0.1a	0.1a	ns
		s	0.1	0.1	0.1	0.1	0.1	0.1	
Iron (mg·l ⁻¹)	ns	$\bar{\chi}$	0.4a	0.4a	0.4a	0.4a	0.3a	0.3a	ns
		s	0.1	0.0	0.0	0.0	0.0	0.1	
Lead (µg·l ⁻¹)	*	$\bar{\chi}$	79.0b	48.5a	50.0a	34.4b	25.5a	19.6a	*
		s	10.7	4.2	1.1	1.2	0.8	2.1	
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$	< 0.3a	< 0.3a	< 0.3a	< 0.3a	< 0.3a	< 0.3a	ns
		s	0.45	0.00	0.92	0.30	0.04	0.00	
Acid composition:									
Tartaric ac. (g·l ⁻¹)	ns	$\bar{\chi}$	2.4a	2.4a	2.3a	2.4a	2.4a	2.4a	ns
		s	0.0	0.0	0.0	0.0	0.0	0.0	
Malic ac. (g·l ⁻¹)	ns	$\bar{\chi}$	1.3a	1.4a	1.3a	1.2a	1.2a	1.2a	ns
		s	0.0	0.1	0.0	0.0	0.0	0.0	
Shikimic ac. (mg·l ⁻¹)	ns	$\bar{\chi}$	33a	34a	33a	34a	33a	34a	ns
		s	0	0	0	0	0	0	
Lactic ac. (g·l ⁻¹)	ns	$\bar{\chi}$	0.6a	0.7a	0.6a	0.8a	0.6a	0.6a	ns
		s	0.1	0.0	0.0	0.0	0.1	0.0	
Citric ac. (mg·l ⁻¹)	ns	$\bar{\chi}$	744a	707a	709a	724a	673a	683a	ns
		s	45	29	2	0	32	17	
Current analysis:									
Density (g·ml ⁻¹)	ns	$\bar{\chi}$	0.9912a	0.9904a	0.9897a	0.9927a	0.9905a	0.9903a	ns
		s	0.0005	0.0004	0.000	0.0001	0.0001	0.0001	
Alcohol content (% v/v)	ns	$\bar{\chi}$	12.7a	12.8a	12.8a	12.8a	12.7a	12.8a	ns
		s	0.1	0.1	0.1	0.0	0.0	0.1	
Titrate acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$	5.7b	5.4a	5.3a	5.5b	5.3a	5.2a	**
		s	0.0	0.0	0.1	0.0	0.1	0.0	
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$	0.52a	0.49a	0.50a	0.52b	0.45a	0.47ab	*
		s	0.10	0.01	0.01	0.02	0.01	0.01	
pH	ns	$\bar{\chi}$	3.08a	3.11a	3.14a	3.14a	3.17ab	3.20b	*
		s	0.01	0.00	0.03	0.12	0.01	0.01	

The two experiments (must and wine) were analysed in separate. $\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffé test at the 5% significance level. Different letters in the same line indicate statistically significant differences: * (p≤0.05), ** (p≤0.01), ns-not significant; nd - not detected. WM-C: white wine control for PVI-PVP must applications; WM-D1 or D2: white wine, resulting from the PVI-PVP application in must; WW-C: white wine control for PVI-PVP wine applications; WW-D1 or D2: white wine, resulting from the PVI-PVP application in wine. C – control wine; D1 - amount 1 (low); D2 - amount 2 (high). L*.a*.b* - CIELAB coordinates, au – absorbance units.

however the Fe content in the wine was also very low. This may explain the partial disagreement with previous work (MATTIVI *et al.* 2000, EDER *et al.* 2003, NICOLINI *et al.* 2004). This difference too, may be related to an inadequate contact between PVI-PVP and the wine.

For Pb, we see a decrease in content with PVI-PVP, both in the must and in the wine. However, increasing the PVI-PVP dosage did not bring about a corresponding increase in Pb removal.

For Cd, all must and wine samples showed concentrations below our detection limit for this metal, so no conclusion about their removal is possible. Portuguese wines generally have very low cadmium concentrations (CATARINO 2000).

We did not find significant changes in the content of organic acids when the PVI-PVP was applied to the must or to the wine at either dosage. For the white wine, we observed a slight but significant decrease in total acidity, but an increase in pH, with PVI-PVP when this was added either to the must or the wine. This confirmed the results of EDER *et al.* (2001, 2003). PVI-PVP did not seem to have any influence on wine density or alcohol content.

The sensory analysis results from the test panel did not find significant differences between the wines regardless of PVI-PVP dosage and of whether it was added to the must or to the wine (Tab. 2). This represents an important technological result.

Table 2

Sensory analysis results for the wine. Values of the scores from 1 (minimum) to 5 (maximum) for each attribute, except global appreciation that is scaled from 1 to 20

		Visual	Nose	Taste	Global appreciation
White wine	WM-C	3.0	3.0	2.9	13.5
	WM-D1	3.4	2.9	2.8	13.2
	WM-D2	3.3	2.9	2.8	12.9
	WW-C	3.3	3.0	3.0	13.5
	WW-D1	3.2	2.7	2.7	12.6
	WW-D2	3.5	2.8	2.9	13.2
Red wine	RM-C	3.7	3.0	2.3	12.6
	RM-D1	3.6	2.8	2.5	13.0
	RM-D2	3.5	2.9	2.4	12.7
	RW-C	3.8	3.0	2.6	13.5
	RW-D1	3.9	3.3	2.4	13.1
	RW-D2	3.7	3.2	2.5	13.1

Legend: WM-C: white wine control for PVI-PVP must application; WM-D1 or D2: white wine, resulting from the PVI-PVP application in must; WW-C: white wine control for PVI-PVP wine applications; WW-D1 or D2: white wine, resulting from the PVI-PVP application in wine. RM-C: red wine control for PVI-PVP must application; RM-D1 or D2: red wine, resulting from the PVI-PVP application in must; RW-C: red wine control for PVI-PVP wine applications; RW-D1 or D2: red wine, resulting from the PVI-PVP application in wine. C – control wine; D1 - amount 1 (low); D2 - amount 2 (high).

Must and red wine: When PVI-PVP was added to the must, the phenolic composition of the wine was not significantly affected (data not shown). On the other hand, when it was added to the wine (Tab. 4), we saw a small but significant reduction in the total phenol index, in the colour tonality, total pigments and in the total anthocyanin content. The latter effect usually reveals a decrease in the value of all of the parameters analysed. PVI-PVP dosage had no significant effect. MATTIVI *et al.* (2000) also found a slight decrease in total phenols and anthocyanins in red wine.

PVI-PVP did not remove significant amounts of K, Ca, Mg or Na when added to either the must or the wine (Tabs 3 and 4). This confirms the results of EDER *et al.* (2001, 2003) for must and for white wine.

Table 3

Effects of PVI-PVP application in red must on mineral composition

	Effect		RM-C	RM-D1	RM-D2
Potassium (mg·l ⁻¹)	ns	$\bar{\chi}$	785a	771a	767a
		s	13	1	1
Calcium (mg·l ⁻¹)	ns	$\bar{\chi}$	83a	82a	87a
		s	0	3	1
Sodium (mg·l ⁻¹)	ns	$\bar{\chi}$	14a	14a	14a
		s	0	1	1
Magnesium (mg·l ⁻¹)	ns	$\bar{\chi}$	70a	70a	70a
		s	0	2	0
Copper (mg·l ⁻¹)	*	$\bar{\chi}$	0.04b	0.02ab	0.01a
		s	0.01	0.00	0.00
Iron (mg·l ⁻¹)	ns	$\bar{\chi}$	7.3a	6.6a	6.0a
		s	0.4	0.2	0.5
Lead (mg·l ⁻¹)	*	$\bar{\chi}$	91.9b	64.4a	64.4a
		s	8.3	2.2	3.0
Cadmium (mg·l ⁻¹)	ns	$\bar{\chi}$	< 0.3a	< 0.3a	< 0.3a
		s			

$\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffé test at the 5% significance level. Different letters in the same line indicate statistically significant differences: * ($p \leq 0.05$), ** ($p \leq 0.01$), ns-not significant. RM-C: red wine control for PVI-PVP must applications; RM-D1 or D2: red wine, resulting from the PVI-PVP application in must; C - control wine; D1 - amount 1 (low); D2 - amount 2 (high).

We found a significant decrease in Cu content when PVI-PVP was added to the must.

When added to the wine, we observed a tendency, but not statistically significant, for decrease in Cu content. Copper levels were lowered to about 57 % of the initial value, with greater removal in red wine than in white. The probable explanation for this is copper's association with the anthocyanins as metal complexes (ESPARZA *et al.* 2004). The decrease in total anthocyanin content with PVI-PVP supports this hypothesis - see Tab. 4.

Table 4

Effects of PVI-PVP application in wine on some red wine characteristics

	Effect		RW-C	RW-D1	RW-D2
Colour and phenolic composition:					
Total phenol index (ua)	**	$\bar{\chi}$ s	63.6b 0.3	61.1a 0.8	60.9a 0.2
Color intensity (ua)	ns	$\bar{\chi}$ s	19.73a 0.51	19.14a 0.31	19.01a 0.46
Tonality (ua)	**	$\bar{\chi}$ s	0.569b 0.052	0.549a 0.063	0.555a 0.017
L*	ns	$\bar{\chi}$ s	58.4a 0.7	57.6a 0.4	58.0a 0.3
a*	ns	$\bar{\chi}$ s	46.4a 1.00	47.2a 0.31	46.5a 0.36
b*	ns	$\bar{\chi}$ s	0.31a 0.30	0.35a 0.19	0.43a 0.22
Total anthocyanins (mg dm ⁻³ Mv-3-glc)	**	$\bar{\chi}$ s	367.5b 6.8	358.0ab 5.3	346.6a 2.2
Total pigments colour (au)	*	$\bar{\chi}$ s	26.54b 1.33	23.86ab 0.10	22.72a 1.21
Polymerised pigments index (%)	ns	$\bar{\chi}$ s	56.31a 1.59	57.47a 0.77	56.81a 0.36
Mineral composition:					
Potassium (mg·l ⁻¹)	ns	$\bar{\chi}$ s	955a 8	951a 8	957a 8
Calcium (mg·l ⁻¹)	ns	$\bar{\chi}$ s	78a 1	78a 1	77a 1
Sodium (mg·l ⁻¹)	ns	$\bar{\chi}$ s	15a 1	15a 0	16a 1
Magnesium (mg·l ⁻¹)	ns	$\bar{\chi}$ s	84a 1	85a 1	85a 0
Copper (mg·l ⁻¹)	ns	$\bar{\chi}$ s	0.23a 0.13	0.10a 0.11	0.10a 0.08
Iron (mg·l ⁻¹)	ns	$\bar{\chi}$ s	6.6b 0.2	5.6a 0.2	5.1a 0.6
Lead (µg·l ⁻¹)	**	$\bar{\chi}$ s	133.6a 30.2	121.3a 15.2	124.2a 37.9
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ s	< 0.3a	< 0.3a	< 0.3a
Acid composition:					
Tartaric ac. (g·l ⁻¹)	*	$\bar{\chi}$ s	2.5b 0.0	2.4a 0.0	2.4a 0.0
Malic ac. (g·l ⁻¹)	ns	$\bar{\chi}$ s	0.2a 1.4	0.2a 0.0	0.2a 0.0
Shikimic ac. (mg·l ⁻¹)	ns	$\bar{\chi}$ s	55a 1	56a 0	55a 3
Lactic ac. (g·l ⁻¹)	ns	$\bar{\chi}$ s	4.4a 1.4	4.5a 0.2	4.4a 0.1
Citric ac. (mg·l ⁻¹)	ns	$\bar{\chi}$ s	973a 10	967a 12	972a 3
Current analysis:					
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ s	0.9947a 0.0002	0.9946a 0.0001	0.9945a 0.0001
Alcohol content (% v/v)	ns	$\bar{\chi}$ s	11.4a 0.1	11.4a 0.0	11.4a 0.0
Titration acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ s	7.6c 0.0	7.4b 0.0	7.3a 0.2
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ s	0.28a 0.05	0.31a 0.01	0.32a 0.01
pH	**	$\bar{\chi}$ s	3.12a 0.02	3.15a 0.00	3.18a 0.01

When PVI-PVP was added to the must, although the decrease in Fe content was not statistically significant it represented about a 15 % reduction for the lower dosage and 23% for the higher one. When added to the wine, PVI-PVP caused a very significant decrease in Fe content and this was enhanced by increasing the PVI-PVP dosage.

Lead levels were significantly lowered (30 %) by PVI-PVP when this was added to the must, but when added to the wine a non-significant reduction of 9 % was found (NB Pb determinations were very variable and standard deviations were high).

For Cd, the contents were below our detection limits for red wines, so no conclusions can be drawn. The same was true for must and for white wine.

No effect on organic acid content (tartaric, malic, lactic, citric or shikimic) was detected with PVI-PVP when this was added either to the must or to the wine. However, a significant effect was detected in wine on total acidity and on pH. The significant decrease in total acidity and the increase in pH had already been observed in the assay with white wine (Tab. 1), and in agreement with the results of EDER *et al.* (2003). The density and alcohol content were not affected by PVI-PVP.

The taste panel could not find significant differences between the wines for the attributes evaluated by them. This can be considered a good result from a technological point of view with PVI-PVP not affecting sensory characteristics at either of the dosages used in this study (Tab. 2).

Finishing treatment applied in wine with metals addition: To confirm the influence of PVI-PVP on Fe and Cu content of the wine, these metal elements were added to a white and a red wine as described above. In addition, we have analysed also the content of the other metals (Cd, Pb, Al).

White wine: The physical-chemical test results are shown in Tab. 5. As noted above, PVI-PVP had some influence on the total phenol index, on the optical density at 420 nm, and in the lightness of the wine (L*). Titrable acidity was significantly reduced by increasing PVI-PVP dosage. This was accompanied by an increase in pH, again confirming our earlier results (Tab. 1). EDER *et al.* (2001, 2003) also found a slight reduction in titrable acidity with PVI-PVP. The other physical-chemical parameters, density, alcohol content, and volatile acidity were not significantly affected.

Relatively to the remove of the metals in study (Cu, Fe, Cd, Pb and Al), we obtained a more effective reduction in Cu with the higher PVI-PVP dosage and with the highest Cu addition (59 %), as seen in Fig. 2. This result was similar to that of MATTIVI *et al.* (2000). The removal of Fe was very significant. The removal percent was nearly 70 % (except with the addition B having the lower level of PVI-

$\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffée test at the 5 % significance level. Different letters in the same line indicate statistically significant differences: * (p ≤ 0.05), ** (p ≤ 0.01), ns-not significant. RW-C: red wine control for PVI-PVP wine applications; RW-D1 or D2: red wine, resulting from the PVI-PVP application in wine. C – control wine; D1 – amount 1 (low); D2 – amount 2 (high). L* a* b* – CIELAB coordinates; au – absorbance units.

Table 5

Effects of PVI-PVP application on some white wine characteristics

		Addition A Fe: 5 mg·l ⁻¹ Cu: 0.5 mg·l ⁻¹			Addition B Fe: 15 mg·l ⁻¹ Cu: 1 mg·l ⁻¹			
	Effect	Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	Effect
Colour and phenolic composition:								
Total phenol index (ua)	**	$\bar{\chi}$ 7.9c s 0.0	7.4b 0.0	7.1a 0.1	7.9c 0.1	7.4b 0.0	7.1a 0.1	**
A420 (ua)	**	$\bar{\chi}$ 0.160c s 0.002	0.126b 0.001	0.117a 0.001	0.165c 0.001	0.131b 0.001	0.121a 0.002	**
L*	*	$\bar{\chi}$ 97.5a s 0.3	98.5b 0.0	98.7b 0.2	97.4a 0.1	98.2ab 0.3	98.4b 0.2	*
a*	ns	$\bar{\chi}$ -1.1a s 0.07	-1.2a 0.00	-1.2a 0.06	-1.4a 0.09	-1.2a 0.00	-1.3a 0.04	ns
b*	**	$\bar{\chi}$ 9.6c s 0.27	7.9b 0.03	7.2a 0.06	10.2b 0.37	8.0a 0.03	7.6a 0.01	**
Mineral composition:								
Copper (mg·l ⁻¹)	*	$\bar{\chi}$ 0.5b s 0.0	0.3a 0.0	0.3a 0.0	0.7b 0.0	0.3a 0.1	0.3a 0.0	**
Iron (mg·l ⁻¹)	**	$\bar{\chi}$ 4.9b s 0.0	1.5a 0.1	1.4a 0.4	9.4b 0.4	5.3ab 0.9	2.6a 1.6	*
Lead (µg·l ⁻¹)	**	$\bar{\chi}$ 35.6b s 1.3	29.4a 0.4	26.6a 4.7	35.4b 0.1	29.8a 0.6	27.0a 1.1	**
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ 0.7a s 0.0	0.7a 0.1	0.6a 0.0	0.7a 0.0	0.8a 0.0	0.7a 0.1	ns
Aluminium (µg·l ⁻¹)	**	$\bar{\chi}$ 414b s 8	334a 25	267a 16	429b 6	345ab 26	259a 13	*
Current analysis:								
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ 0.9902a s 0.0000	0.9901a 0.0000	0.9901a 0.0000	0.9903a 0.0001	0.9902a 0.0001	0.9901a 0.0000	ns
Alcohol content (% v/v)	ns	$\bar{\chi}$ 11.7a s 0.0	11.8a 0.0	11.8a 0.0	11.6a 0.1	11.7a 0.0	11.7a 0.0	ns
Titrate acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ 6.3b s 0.0	6.2b 0.0	6.0a 0.0	6.3b 0.0	6.2b 0.0	6.0a 0.0	**
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ 0.4a s 0.01	0.4a 0.00	0.4a 0.00	0.4a 0.00	0.4a 0.01	0.4a 0.01	ns
pH	**	$\bar{\chi}$ 3.16a s 0.00	3.20b 0.00	3.24c 0.01	3.16a 0.00	3.19b 0.01	3.24c 0.01	**

The two experiments (addition A and addition B) were analysed separately. $\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffée test at the 5 % significance level. Different letters in the same line indicate statistically significant differences: * ($p \leq 0.05$), ** ($p \leq 0.01$), ns-not significant. Control wine – wine without PVI-PVP application. L* a* b* – CIELAB coordinates; au – absorbance units.

PVP). In wine with addition A, increasing levels of PVI-PVP did not result in more effective Fe removal.

Lastly, PVI-PVP seems to have some capacity to deplete Pb and Al. The lowest dosage had some Pb reduction effect (18 %) and this was improved by the highest dosage. This fitted with the previous results for Pb. Nevertheless, PVI-PVP was not effective in removing Cd. Aluminium depletion (from 19 % to 40 %) has also been reported by NICOLINI *et al.* (2001, 2004).

Red wine: The results of PVI-PVP additions on the removal of metals and on other physical-chemical characteristics of red wine are presented in Tab. 6.

PVI-PVP affected the total phenols index of the wines slightly but still significantly with removal percentages of about 6 %. However, it did not significantly affect anthocy-

anin content, or the polymeric pigment, or the total pigments (expressed as the colour due to the total pigments). These results have confirmed those of our previous assays (Tab. 4). Confirming our previous results (Tab. 4), PVI-PVP reduces the total acidity of the wine, and significantly increases pH. As for metal removal, there was an efficient reduction of Cu, Fe, Pb and Al by PVI-PVP, but the greatest reduction was found for Cu (74 %). This agrees with MATTIVI *et al.* (2000) and NICOLINI *et al.* (2004). The removal percentage increased with dosage (Fig. 3) and was greater in the wines and with addition B. These results are consistent with those of Mattivi *et al.* (2000), in which adsorption efficiency is greater when the metal content is higher.

PVI-PVP also showed some ability to remove Pb, which confirms the results obtained in the previous assays.

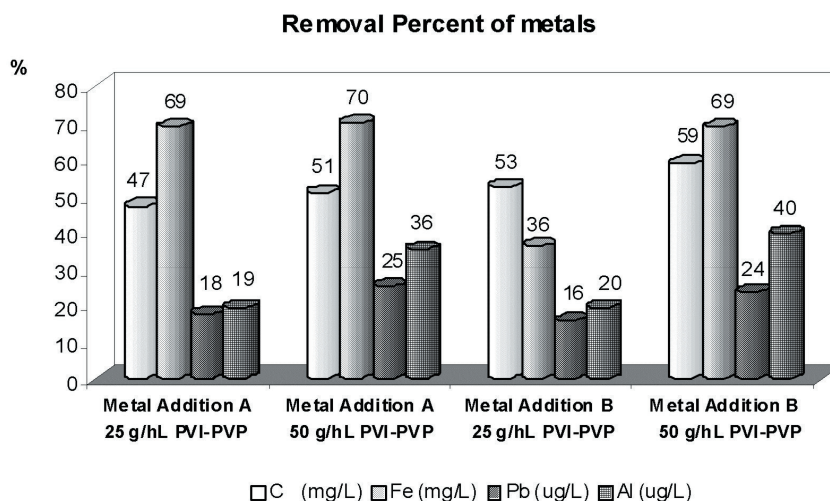


Fig. 2: Removal percent of copper, iron, lead and aluminium in white wine (metal addition A: 5 mg·ml⁻¹ of iron and 0.5 mg·ml⁻¹ of copper; metal addition B: 15 mg·ml⁻¹ of Fe and 1 mg·ml⁻¹ of copper).

However, in wines with a higher content of metals (addition B) the percentage of removal was slightly lower compared to the wines having the lower metal content addition A (Fig. 3).

Conclusion

Generally, PVI-PVP removed Cu, Fe, Pb and Al more effectively when it was added at the highest dosage and when these metal contents were initially high. However, PVI-PVP had no significant effect on the contents of Ca, Mg, Na and Cd. However, the wines used in this study were very low in Cd, lower than our detection limit as is usual in Portuguese wines.

The removal of metals was more efficient when PVI-PVP was used in the wine than in the must. This probably reflects the complex macromolecular composition of the must. In the metal addition assays, the removal of Fe and Pb was more efficient in white wine than in red, while the

removal of Cu and Al was more efficient for the red wine than for the white. For Pb, the complexes formed with pectic polysaccharide Rhamnogalacturonan II (PELLERIN and O'NEILL 1998), which are very important in red wines, could explain the smaller removal of Pb in red wines compared to white wines. Again, the percentage removal of all of these metallic elements increased with increasing dosages of PVI-PVP. In future work other (greater and lesser) application doses will be assessed.

PVI-PVP application had only a slight influence on the colour and phenolic composition of the wines. Organic acid content (tartaric, malic, lactic, citric and shikimic acids) of the wine was not influenced by PVI-PVP, either in the must or in the wine. The wines showed significant decreases in total acidity and increases in pH, with PVI-PVP additions. However, sensory analysis did not reveal significant differences between any of the wines from any of the many attributes evaluated by the test panel. The application of PVI-PVP in must or wine seems not to affect its sensory characteristics, for the dosages used in this study which is a

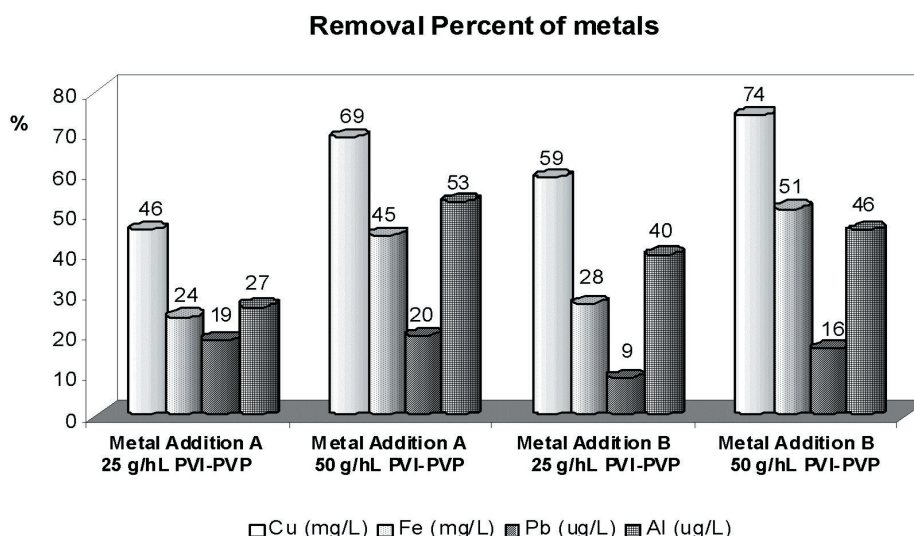


Fig. 3: Removal percent of copper, iron, lead and aluminium in red wine. (metal addition A: 5 mg·ml⁻¹ of iron and 0.5 mg·ml⁻¹ of copper; metal addition B: 15 mg·ml⁻¹ of Fe and 1 mg·ml⁻¹ of copper).

Table 6

Effects of PVI-PVP application on some red wine characteristics

		Effect	Addition A Fe: 5 mg·l ⁻¹ Cu: 0.5 mg·l ⁻¹			Addition B Fe: 15 mg·l ⁻¹ Cu: 1 mg·l ⁻¹			Effect
			Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	Control wine	PVI-PVP 25 g·hl ⁻¹	PVI-PVP 50 mg·l ⁻¹	
Colour and phenolic composition:									
Total phenol index (ua)	*	$\bar{\chi}$ s	57.4b 1.0	55.1ab 0.1	54.1a 0.6	57.3b 0.4	54.1ab 1.4	53.5a 0.1	*
Colour intensity (ua)	ns	$\bar{\chi}$ s	10.97c 0.05	10.72b 0.09	10.52a 0.09	11.02a 0.04	10.96a 0.24	10.68a 0.09	ns
Tonality (ua)	ns	$\bar{\chi}$ s	0.790b 0.006	0.785a 0.007	0.784a 0.004	0.792a 0.002	0.795a 0.010	0.797a 0.006	ns
L*	*	$\bar{\chi}$ s	51.5a 0.1	52.2ab 0.2	52.7b 0.2	51.4a 0.0	52.1a 0.1	52.7a 0.1	**
a*	ns	$\bar{\chi}$ s	43.03a 0.02	42.58a 0.31	42.07 0.56	42.44a 0.09	41.45a 0.39	41.03a 0.26	ns
b*	ns	$\bar{\chi}$ s	15.73a 0.01	15.61a 0.10	15.44a 0.13	15.77a 0.10	15.68a 0.03	15.59a 0.07	ns
Total anthocyanins (mg dm ⁻³ Mv-3-glc)	ns	$\bar{\chi}$ s	47.8a 1.5	50.2a 2.8	51.3a 2.6	49.0a 0.65	50.9a 2.1	47.3a 1.3	ns
Polymerised pigments index (%)	ns	$\bar{\chi}$ s	73.43a 0.50	73.74a 0.36	74.66a 0.23	75.40a 0.25	75.58a 0.78	75.42a 1.97	ns
Total pigments colour (ua)	ns	$\bar{\chi}$ s	12.28a 0.18	11.98a 0.11	11.95a 0.14	12.08a 0.18	12.08a 0.04	11.70a 0.14	ns
Mineral composition:									
Copper (mg·l ⁻¹)	**	$\bar{\chi}$ s	0.48c 0.03	0.26b 0.03	0.15a 0.01	0.97b 0.10	0.40a 0.02	0.25a 0.01	*
Iron (mg·l ⁻¹)	**	$\bar{\chi}$ s	4.9c 0.03	3.8b 0.01	2.8a 0.20	10.0b 0.71	7.9ab 1.26	5.3a 0.09	*
Lead (µg·l ⁻¹)	*	$\bar{\chi}$ s	41.4b 2.3	33.9a 1.9	33.7a 1.0	40.6b 2.6	36.9a 0.9	35.0a 0.1	*
Cadmium (µg·l ⁻¹)	ns	$\bar{\chi}$ s	0.3a 0.0	0.4a 0.0	0.4a 0.0	0.3a 0.0	0.4a 0.1	0.4a 0.2	ns
Aluminium (µg·l ⁻¹)	*	$\bar{\chi}$ s	928b 11	679ab 129	437a 18	752b 130	454ab 33	409a 31	*
Current analysis:									
Density (g·ml ⁻¹)	ns	$\bar{\chi}$ s	0.9926a 0.0000	0.9926a 0.0000	0.9926a 0.0001	0.9928a 0.0001	0.9927a 0.0001	0.9925a 0.0000	ns
Alcohol content (% v/v)	ns	$\bar{\chi}$ s	13.1a 0.0	13.1a 0.0	13.0a 0.0	13.0a 0.1	13.0a 0.0	13.0a 0.0	ns
Titrate acidity (g·l ⁻¹ tartaric ac.)	**	$\bar{\chi}$ s	6.1c 0.0	5.9b 0.0	5.8a 0.0	6.1c 0.0	5.9b 0.0	5.8a 0.0	**
Volatile acidity (g·l ⁻¹ acetic ac.)	ns	$\bar{\chi}$ s	1.1a 0.01	1.1a 0.01	1.1a 0.01	1.1a 0.01	1.1a 0.01	1.1a 0.00	ns
pH	**	$\bar{\chi}$ s	3.42a 0.01	3.46b 0.00	3.49b 0.00	3.43a 0.00	3.47b 0.00	3.50c 0.01	**

The two experiments (addition A and addition B) were analysed separately. $\bar{\chi}$ – mean; s – standard error. Treatment means were separated by the Scheffé test at the 5% significance level. Different letters in the same line indicate statistically significant differences: * ($p \leq 0.05$), ** ($p \leq 0.01$), ns – not significant. Control wine – wine without PVI-PVP application; L*.a*.b* – CIELAB coordinates; au – absorbance units.

useful result. In our opinion, these findings make an important, practical contribution, showing that oenologists could use this product without major impact on the chemical and sensory properties of their wines. Finally, PVI-PVP efficiently removes Cu and Fe from wines and does not seem

to have some of the limitations and dangers associated with traditional potassium ferrocyanide treatment. In addition, PVI-PVP does not impact negatively upon the principal characteristics of the wine making this copolymer a useful likely replacement.

Acknowledgements

The authors thank the AGRO Program (project nº23, measure 8.1 of the Ministry of Agriculture, Portugal) for financial support for this work; also, PRODEP – Action 5.3 – Advanced training of university teachers (Ministry of Education, Portugal); also, Comissão Vitivinícola Regional do Ribatejo (CVRR) for providing the wines and, lastly BASF, Germany for supplying the PVI-PVP copolymer.

References

- AFSSA (Agence Française de Sécurité Sanitaire des Aliments); 2006: Avis de l'Agence de Sécurité Sanitaire des Aliments Relatif à l'Emploi d'un Copolymère Polyvinylpyrrolidone en tant qu'Auxiliaire Technologique Destiné à Lier des Métaux Lourds dans le Vin. Saisine no. 2005-SA-0059, Maisons-Alfort.
- BAUER, K. M.; ESCHNAUER, H. R.; GEORGTES, S.; 2001: "Indicator Elements in Wine Analysis. Abstract book of 2nd Symposium "In Vino Analytica Scientia, 14-16 June 2001. Bordeaux.
- BENITEZ, P.; CASTRO, R.; BARROSO, C. G.; 2002: Removal of iron, copper and manganese from white wines through ion exchange techniques: Effects on their organoleptic characteristics and susceptibility to browning. *Anal. Chim. Acta* **458**, 197-202.
- BOULTON, R.; SINGLETON, V.; BISSON, L.; KUNKEE, R.; 1995: Principles and Practices of Winemaking, 320-351. Chapman and Hall, New York.
- CATARINO S. C. G.; 2000: A Ocorrência de Chumbo e Cádmio em Vinhos. Tese de Mestrado em Viticultura e Enologia. Faculdade de Ciências da Universidade do Porto. Porto.
- CATARINO S.; CURVELO-GARCIA A. S.; BRUNO DE SOUSA, R.; 2002: Determination of aluminium in wine by graphite furnace AAS: Validation of analytical method. *At. Spect.* **23**, 196-200.
- CEE; 1990: Regulamento (CEE) no. 2676/90 de 1990-09-17. Jornal Oficial das Comunidades Europeias, 3 de Outubro de 1990.
- CURVELO-GARCIA, A. S.; 1988: Controlo de Qualidade dos Vinhos. Química Enológica - Métodos Analíticos. Ed. Instituto da Vinha e do Vinho, Lisboa.
- EDER, R.; SCHREINER, A.; SCHLAGER, G.; WENDELIN, S.; 2001: Verminderung der Metallgehalte von Weinen durch Anwendung selektiver Harze. *Mitt. Klosterneuburg* **51**, 182-193.
- EDER, R.; SCHREINER, A.; SCHLAGER, G.; WENDELIN, S.; 2003: Réduction de la teneur en métaux dans les vins à l'aide de résines sélectives. *Bull. O I V (Off. Int. Vigne Vin)* **76**, 243-260.
- ESCHNAUER, H. R.; OSTAPCZUK, J.; SCOLLARY, G. R.; 1996: Cadmium, toxisches Schwermetall. *Das Deutsche Weinmagazin* **14**, 29-30.
- ESPARZA I.; SALINAS I.; CABALLERO I.; SANTAMARIA C.; CALVO I.; GARCÍA-MINA J. M.; FERNÁNDEZ J. M.; 2004: Evolution of metal and polyphenol content over a 1-year period of vinification: Sample fractionation and correlation between metals and anthocyanins. *Anal. Chim. Acta*, **524**, 215-224.
- FENG, M. H.; HU, S. W.; JANNEY, S.; CARRUTHERS, J.; HOLBEIN, B.; HUBER, A.; KIDBY, D.; 1997: Selective removal of iron from grape juice using an iron (III) chelating resin. *Separ. Purif. Tech.* **11**, 127-135.
- FOURNIER, J. B.; EL HOURCH, M.; MARTIN, G. J.; 1998: Analyse du zinc, du cuivre et du plomb dans le vin. Rapport entre les concentrations totales analysées par spectrométrie d'absorption atomique et les concentrations des ions libres déterminées par polarographie. *J. Int. Sci. Vigne Vin* **32**, 45-50.
- FUSSNEGGER, B.; MAURER, R.; DETERING, J.; 1992: Unlösliche komplexbildende Polymere als potentielle Substitutions-Produkte für Kaliumhexacyanoferrat (II) zur Schwermetallverminderung in Wein. *Vitic. Enol. Sci.* **47**, 8-23.
- GILBERT, E.; 1979: Teneurs en Cadmium et en Plomb dans les Moûts de Raisin et les Vins. *Feuillet Vert de l'OIV (Off. Int. Vigne Vin, No. 694)*.
- GLORIES, Y.; 1978: Recherches sur la Matière Colorante des Vins Rouges. Thèse Doct. Univ. Bordeaux II, Bordeaux.
- MATTIVI, F.; NICOLINI, G.; VERSINI, G.; 1994: Un nuovo polimero ad azione demetallizzante: Isultati do alcune prove applicative sperimentali. *L'Enotecnico* **10**, 75-84.
- MATTIVI, F.; VERSINI, G.; NICOLINI, G.; 2000: Interesting side-effects of the use of a PVI-PVP copolymer for removing heavy metals in white and red wines on polyphenols and colour characteristics. *Vitic. Enol. Sci.* **55**, 73-79.
- MEDINA, B.; SUDRAUD, P.; 1979: Dosage du Plomb et du Cadmium dans les Vins. *Feuillet Vert de l'OIV (Off. Int. Vigne Vin)*.
- McKINNON, A. J.; CATTRALL, R. W.; SCOLLARY, G. R.; 1992: Aluminium in wine - Its measurement and identification of major sources. *Am. J. Enol. Vitic.* **43**, 166-170.
- McLAREN, K.; 1980: Food Colourimetry. In: J. WALFORD (Ed.): Developments in Food Colours, vol. 1, 27-45. Appl. Sci. Publ., London, U.K.
- MÍNGUEZ, S.; HERNÁNDEZ, P.; GARCIA, J.; MÉDINA, B.; BARTRA, E.; VILAVELLA M.; ESPINÁS, E.; 1997: Presence du Plomb dans les Vins et Efficacité des Techniques Soustractives, Doc. OIV (Groupe d'Experts 'Technologie du vin').
- MOREIRA, J. L.; MARCOS, A. M.; BARROS, P.; 2002 a: Analysis of Portuguese wines by Fourier Transform Infrared Spectrometry (FTIR). *Ciência Tec. Vitiv.* **17**, 27-33.
- MOREIRA, J. L.; MARCOS, A. M.; BARROS, P.; 2002 b: Proficiency test on FTIR wine analysis. *Ciência e Tec. Vitiv.* **17**, 41-51.
- NICOLINI, G.; LARCHER, R.; MATTIVI, F.; 2001: ICP-OES and ICP-MS measurement of mineral elements in grapes and wines: analytical, compositional and enological aspects, 1-12. *Proc. VIIIth Viticult. Enol. Latin-American Congr. Montevideo, Uruguay*.
- NICOLINI, G.; LARCHER, R.; MATTIVI, F.; 2004: Experiments concerning metal depletion in must and wine by Divergan HMTM. *Mitt. Klosterneuburg* **54**, 25-32.
- OIV; 1990: Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts. O.I.V. (Off. Int. Vigne Vin), Paris.
- OIV; 2005: Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts. Org. Int. Vigne Vin, Paris.
- OIV; 2006: Résolution Oeno 13/2006. Org. Int. Vigne Vin, Paris.
- OUGH, C. S.; 1993: Lead in wines - A review of recent reports. *Am. J. Enol. Vitic.* **44**, 464-467.
- PALACIOS, V. M.; CARO, I.; PÉREZ, L.; 2001: Application of ion exchange techniques to industrial process of metal ions removal from wine. *Adsorption* **7**, 131-138.
- PELLERIN, P.; O'NEILL, M. A.; 1998: The interaction of the pectic polysaccharide Rhamnogalacturonan II with heavy metals and lanthanides in wines and fruit juices. *Analisis* **26**, M32-M36.
- RIBÉREAU-GAYON, P.; STONESTREET, E.; 1965: Les dosages des anthocyanes dans le vin rouge. *Bull. Soc. Chim.* **9**, 2649-2652.
- RIBÉREAU-GAYON, P.; 1970: Le dosage des composés phénoliques totaux dans les vins rouges. *Chim. Anal.* **52**, 627-631.
- SEPPI, A.; SPERANDIO, A.; 1978: I metalli dei vini. Teoria e verifica sperimentale della corrosione delle attrezzature metalliche di cantina. *Vini d'Italia* **20**, 35-42.
- SOMERS, T. C.; EVANS, M. E.; 1977: Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO₂, "Chemical age". *J. Sci. Food Agric.* **28**, 279-287.
- TEISSÉDRE, P. L.; CABANIS, M. T.; CHAMPAGNOL, F.; CABANIS, J. C.; 1993 a: Étude de la teneur en plomb des feuilles et de quelques organes du cep de vigne. *Bull. O I V (Off. Int. Vigne Vin)* **66**, 843-854.
- TEISSÉDRE, P. L.; CABANIS, M. T.; DAUMAS, F.; CABANIS, J. C.; 1993 b: Evolution de la teneur en plomb au cours de l'élaboration des vins des Côtes du Rhône et de la Vallée du Rhône. *Rev. Franc. Oenol. (Cahiers Scientifiques)* **14**, 6-18.
- TUSSEAU, D.; BENOIT, C.; 1986 a: Analyse Collaborative des Acides Organiques des Moûts et des Vins par HPLC. *Feuillet Vert de l'OIV (Off. Int. Vigne Vin, No. 800)*.
- TUSSEAU, D.; BENOIT, C.; 1986 b: Analyse Collaborative des Acides Organiques des Moûts et des Vins par HPLC. *Feuillet Vert de l'OIV (Off. Int. Vigne Vin, No. 813)*.

Received November 30, 2006