COMMISSION REGULATION (EEC) No 3220/90

of 7 November 1990

laying down conditions for the use of certain oenological practices provided for in Council Regulation (EEC) No 822/87

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Economic Community,

Having regard to Council Regulation (EEC) No 822/87 of 16 March 1987 on the common organization of the market in wine [1], as last amended by Regulation (EEC) No 1325/90 [2], and in particular Article 15 (6) thereof;

Whereas Regulation (EEC) No 822/87 provides that the conditions for the use of polyvinylpolypyrrolidone and lactic bacteria should be determined;

Whereas the measures provided for in this Regulation are in accordance with the opinion of the Management Committee for Wine,

HAS ADOPTED THIS REGULATION:

Article 1

1. Polyvinylpolypyrrolidone, the use of which is provided for in Annex VI (1) (p) and (3) (y) to Regulation (EEC) No 822/87, may be used only if it meets the requirements set out in Annex I hereto.

2. Lactic bacteria, the use of which is provided for in Annex VI (1) (q) and (3) (z) to Regulation (EEC) No 822/87, may be used only if they meet the requirements set out in Annex II hereto.

Article 2

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

It shall apply with effect from 1 September 1990.

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

Done at Brussels, 7 November 1990.

For the Commission

Ray MAC SHARRY

Member of the Commission

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[1] OJ No L 84, 27. 3. 1987, p. 1.
[2] OJ No L 132, 23. 5. 1990, p. 19.

ANNEX I

SPECIFICATIONS FOR PVPP

Polyvinylpolypyrrolidone, the use of which is provided for in Annex VI (1) (p) and (3) (y) to Regulation (EEC) No 822/87 is a statistically reticulated polymer of [1—(2—oxo—1—pyrrolidinylethylene)].

It is manufactured by polymerizing N—vinyl—2—pyrrolidone in the presence of a catalyst: either caustic soda or N'N—divinylimidazolidone.

CHARACTERISTICS:

Light powder, white to cream-coloured.

Insoluble in water and organic solvents.

Insoluble in strong mineral acids and alkalis.

TESTS:

1. **Loss on drying:**

   Less than 5 % in the following conditions:

   Place 2 g of PVPP in a silica capsule with a diameter of 70 mm; dry in oven at 100 to 105 °C for six hours.

   Leave to cool in a desiccator and weigh.

   Note:

   All the limits fixed below refer to dry weight.

2. **Ash**

   Weight of ash less than 0,5 % in the following conditions:

   Gradually ash the residue from test 1, without exceeding 500 to 550 °C, and weigh.

3. **Arsenic**

   Less than 2 parts per million in the following conditions:

   Preparation of the product to be tested:

   Place 0,5 g of PVPP into a round-bottom flask of borosilicate glass placed on a disk with a hole in the middle, with the neck inclined.

   Add 5 ml of pure sulphuric acid (AR quality) and 10 ml of pure nitric acid (AR quality) and heat gradually. When the mixture begins to turn brown, add a small quantity of nitric acid and continue to heat.

   Continue in this way until the liquid remains colourless and the flask fills with white SO_3 fumes. Leave to cool, take up in 10 ml of water and reheat to dispel the nitrous vapours until white fumes are obtained. This operation is repeated a second time; after taking up a third time, bring to the boil for a few seconds, cool and make up to 40 ml with water.

   Reagents (ARs)

   1. Concentrated arsenic solution (100 mg of arsenic per litre):

      Weigh exactly 0,132 g of arsenous anhydride, previously dried at 100 °C, into a 500 ml conical flask. Add 3 ml of sodium hydroxide and 20 ml of water. Shake until dissolved. Neutralize the arsenous liquor with 15 ml of sulphuric acid diluted to 10 % (w/w) and add saturated bromine water (AR quality) until the yellow colour of free bromine becomes stable (theoretically, 7 ml). Bring to the boil to dispel the excess bromine, transfer to a 1 000 ml volumetric flask and make up to quantity with distilled water.

   2. Diluted arsenic solution (1 mg of arsenic per litre): Mix 10^4 ml of concentrated arsenic solution (100 mg per litre) with distilled water to make up 1 000 ml. 1 ml of this solution contains 1/1 000 mg of arsenic.

   3. Lead acetate cotton: Immerse absorbent cotton in a 5 % (w/v) lead acetate solution to which 1 % acetic acid has been added. Drain the cotton and leave to dry in the air. Keep in a well-sealed bottle.

   4. Absorbent cotton dried in an oven at 100 °C: Keep in a well-sealed bottle.

   5. Mercuric bromide paper: Place an alcoholic solution of mercuric bromide (5 %) in a rectangular basin. Immerse 80 g/m² white filter paper, cut into pieces of 15 × 22 cm and folded in two, in the solution. Drain the paper and leave to dry in a dark place hung over a non-metallic line. Cut at 1 mm away from the fold and 1 cm from lower edges. Cut the paper into 15 × 15 mm squares; keep in a well-sealed bottle, covered with black paper.

6. Stannous chloride solution : Cold attack 20 g of pure tin shot (analytical quality) with 100 ml of pure hydrochloric acid, d = 1,19. Keep in the presence of metallic tin in an airtight bottle with valve stopper.

7. Potassium iodide solution :

Potassium iodide	10^4 g
Water to make up to	100 ml

8. Nitric (acid) for the determination of arsenic (AR quality) : Acid with a density of 1,38 at 20 °C, containing 61,5 to 65,5 % nitric acid HNO_3. It should not leave a fixed residue of more than 0,0001 %. It may not contain lead detectable with dithizone, or more than 1 millionth of chlorine ion, 2 millionths of sulphuric ion, 2 millionths of orthophosphoric ion or one hundred millionth of arsenic.

9. Sulphuric acid for the determination of arsenic (AR quality) : Acid with a density of 1,831 to 1,835 at 20 % volume, containing at lest 95 % sulphuric acid H_2SO_4. It should not leave a fixed residue of more than 0,0005 %. It may not contain more than 2 millionths of heavy metals, one millionth of iron, one millionth of chlorine ion, one millionth of nitric ion, 5 millionths of ammonium ion, 2 hundred millionths of arsenic.

10. 20 % (v/v) diluted sulphuric acid solution :

(36 g H_2SO_4 per 100 ml)

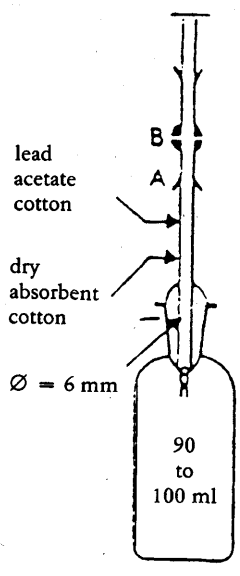
Mix 200 ml of pure sulphuric acid (AR quality) with distilled water to make up to 1 000 ml.

11. Platinized zinc :

Pure zinc, free of arsenic, in shot or cylinder form. Platinize the zinc by placing it in a cylindrical flask and covering it with a 1/20 000 platinum chloride solution. After two hours of contact, wash the zinc with distilled water, drain the platinized zinc on several thicknesses of blotting paper, dry and place in a dry bottle.

Verify that 5 g of this zinc, placed in the apparatus described below with 4,5 ml of pure sulphuric acid and made up to 40 ml with water, to which 2 drops of stannous chloride and 5 ml of 10 % potassium iodide solution are added, leave no stain after at least two hours on mercuric bromide paper. Check also that 1µg of arsenic used as indicated below leaves a discernible trace.

Description of the apparatus :



Use a 90 to 100 ml flask sealed with a glass stopper fitted with a 90 mm-long glass tube with an inner diameter of 6 mm. The lower part of the tube is tapered and pierced by a lateral hole (anti-entrainment device). The upper edge has a ground flat surface perpendicular to the tube's axis. A second glass tube with the same internal diameter and 30 mm in length with an upper edge having a ground flat surface like the first tube may be attached to the former and secured by two coil springs or rubber rings (see figure).

Procedure :

In the outlet tube, at position A, place a plug of dry absorbent cotton, then a plug of lead acetate cotton.

Place a square of mercuric bromide paper between the two parts of the outlet tube at B and join the two parts of the tube.

Place 40 ml of sulphuric liquid, 2 drops of tin chloride solution and 5 ml of potassium iodide solution in the flask. Leave for 15 minutes. Add 5 g of platinized zinc and immediately seal the flask with the tube prepared in advance.

Allow the emission to continue until exhausted (at least two hours). Take apart the apparatus, immerse the square of mercuric bromide paper in 10 ml of potassium iodide solution for half an hour, shaking occasionally ; rinse generously and leave to dry.

The yellow or brown stain must be invisible, or paler than the stain obtained in a parallel test carried out with 1 ml of arsenic solution at 1µg per ml, to which 4,5 ml of pure sulphuric acid are added and made up to 40 ml with water, to which 2 drops of stannous chloride and 5 ml of 10 % potassium iodide solution are then added.

4. **Heavy metals**

Expressed as lead, less than 20 ppm in the following conditions:

After weighing, dissolve the ash in 1 ml of pure hydrochloric acid and 10 ml of distilled water. Heat to dissolve. Make up to 20 ml with distilled water. 1 ml of this solution contains the mineral matter of 0,10 g of PVPP.

Place 10 ml of ash solution in a 160 × 16 test tube with 2 ml of a 4 % pure sodium fluoride solution, 0,5 ml of pure ammonium, 3 ml of water, 0,5 ml of pure acetic acid and 2 ml of hydrogen sulphide saturated aqueous solution. No precipitation should take place. If a brown colour is produced, it should be less than the colour produced by the reference, prepared as follows:

Place 2 ml of a solution containing 0,01 g of lead (Pb) in 1 l (10 mg Pb per litre), 15 ml of water, 0,5 ml of 4 % (m/v) sodium fluoride, 0,5 ml of pure acetic acid and 2 ml of hydrogen sulphide saturated aqueous solution in a 160 × 16 test tube. The tube contains 20 µg of lead.

Note: At this concentration, the lead sulphide precipitates only in an acetic medium. Precipitation can be obtained in the presence of only 0,05 ml of hydrochloric acid for 15 ml, but this concentration is too delicate to be achieved exactly in practice.

If the 0,5 ml of acetic acid were replaced by 0,5 ml of hydrochloric acid, only copper, mercury, etc. would be precipitated.

Any iron present, generally in the ferric state, oxidizes hydrogen sulphide by producing a sulphur precipitate which conceals the colloidal lead sulphide precipitate. Complexed with 0,5 ml of sodium fluoride, iron oxidizes hydrogen sulphide more slowly.

This quantity is sufficient to complex 1 mg of iron (III). Increase the quantity of sodium fluoride if more iron is present.

For products containing calcium, filtration is required after the fluoride is added.

5. **Total nitrogen**

Between 11 and 12,8 % under the following conditions:

Apparatus

A. The apparatus is made up of:

1. A 1 l flask A of borosilicate glass as a heating vessel, fitted with a tap-funnel for filling. It can be heated on a gas or electric ring.
2. An extension C to collect the spent liquid from the bubbler B.
3. A 500 ml bubbler B with an inclined neck; the entry tube should reach the lower part of the flask. The exit tube is fitted with an anti-entrainment ball which constitutes the upper part of the bubbler. A tap-funnel E for introduction of the liquid to be treated and the alkaline solution.
4. A vertical condenser, 30 to 40 cm in length, with a fine-necked bulb at the end.
5. A 250 ml conical flask to collect the distillate.

B. A 300 ml egg-shaped mineralization flask with a long neck.

Substances required:

Pure sulphuric acid

Mineralization catalyst

30 % (m/m) sodium hydroxide

40 % (m/v) pure boric acid solution

0,1 N hydrochloric acid solution

A mixed indicator of bromocresol green and methyl red.

The heating vessel must be filled with water acidulated with 1/1 000 sulphuric acid. This liquid should be boiled before each operation, with the purge valve P open to dispel CO_2.

Procedure

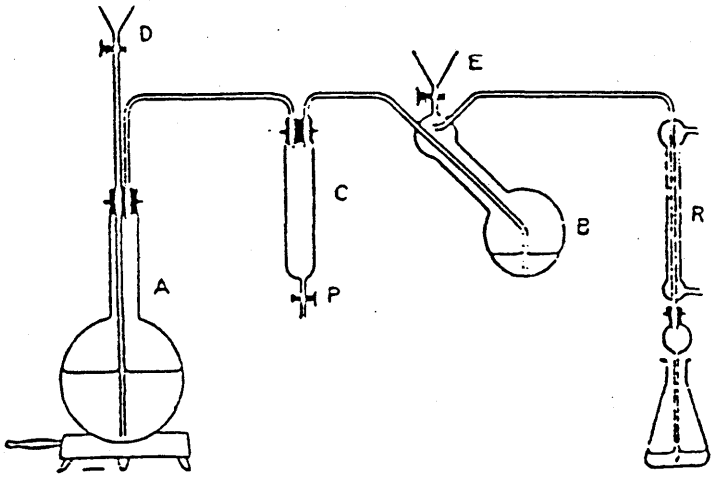
Place approximately 0,20 g of PVPP, weighed exactly, in the mineralization flask. Add 2 g of mineralization catalyst and 15 ml of pure sulphuric acid.

Heat over a naked flame, with the neck of the flask inclined, until the solution is colourless and the sides of the flask are free of carbonized substances.

After cooling, dilute with 50 ml of water and cool further ; place this liquid in the bubbler B via filter E ; next add 40 to 50 ml of 30 % sodium hydroxide, to obtain full alkalinization of the liquid and drive off the ammonia with the steam, collecting the distillate in 5 ml of boric acid solution placed in advance in the conical receiver flask with 10 ml of water, with the end of the bulb immersed in the liquid. Add 1 or 2 drops of mixed indicator and collect 70 to 100 ml of distillate. Titrate the distillate with the 0,1 N hydrochloric acid solution until the indicator turns pinkish violet.

1 ml of 0,1 N hydrochloric acid solution corresponds to 1,4 mg of nitrogen.

Apparatus for distilling ammonia in a stream of water vapour
(after Parnas and Wagner)



Tap-funnels P and E may be replaced by a plastic connector and a Mohr clamp.

6. Solubility in an aqueous medium

Less than 0,5 % in the following conditions :

Place 10 g of PVPP in a 200 ml flask containing 100 ml of distilled water. Shake and leave for 24 hours. Filter on a filter screen with a porosity of 2,5 µ, then on a filter screen with a porosity of 0,8 µ. The residue left by evaporating the filtrate over a water bath until dry must be less than 50 mg.

7. Solubility in an acid alcoholic medium

Less than 1 % in the following conditions :

Place 1 g of PVPP in a flask containing 500 ml of the following mixture :

Acetic acid	3 g
Ethanol	10 ml
Water to make up volume to	100 ml.

Leave for 24 hours. Filter on a filter screen with a porosity of 2.5 µ, then on a filter screen with a porosity of 0.8 µ. Concentrate the filtrate over a water bath. Finish evaporation over the water bath in a 70 mm diameter tared silica capsule. The dry residue remaining after evaporation must be less than 10 mg, taking account of any residue left by the evaporation of 500 ml of the acetic acid-ethanol mixture.

8. Effectiveness of PVPP in relation to the adsorption of phenolic compounds

The percentage of activity determined in the following conditions *must be 30 % or above.*

A. **Reagents :**

1. 0,1 N sodium hydroxide solution.
2. 0,1 N salicylic acid solution.

   (13,81 g of salicylic acid are dissolved in 500 ml of methanol and diluted in 1 litre of water).

B. **Procedure :**

1. Weigh 2 to 3 g of PVPP into a 250 ml conical flask and note the weight, W, accurate to 0,001 g.
2. Calculate the dry matter of the sample (solid percentage) and note P, expressed as a percentage accurate to 1 decimal point.
3. Add the 0,1 N salicylic acid solution using the following formula :
   $43 \times W \times P$ = ml of solution to be added.
4. Close the flask and shake for five minutes.
5. Pour the mixture, heated to 25 °C, into a Buchner funnel fitted with a filter connected to a 250 ml flask ; wait for it to empty until enough filtrate has been obtained to take a 50 ml sample (the filtrate must be clear).
6. Pipette 50 ml of the filtrate into a 250 ml conical flask.
7. Determine the neutralization point with phenophthalein, using a 0,1 N soda solution and note the volume V_s.
8. Titrate 50 ml of salicylic acid as reference in the same way and note the volume V_b.
9. Calculation :

$$\% \text{ of activity} = \frac{V_b - V_s}{V_b} \times 100$$

Note : All the limits fixed in points 2 to 8 refer to the dry matter.

9. **Free N-vinylpyrrolidone — not more than 0,1 %**

Method

Suspend 4,0 g of the sample with 30 ml of water, stir for 15 minutes, pass through a sintered glass filter of 9 to 15 µm (= type G 4) into a 250 ml conical flask. Wash the residue with 100 ml of water, add 500 mg of sodium acetate to the combined filtrates and titrate with 0,1 N iodine until the colour of the iodine no longer fades. Add an additional 3,0 ml of 0,1 N iodine, allow to stand for 10 minutes and titrate the excess iodine with 0,1 N sodium thiosulphate, adding 3 ml of starch TS as the end point is approached. Perform a blank determination. Not more than 0,72 ml of iodine is consumed, corresponding to not more than 0,1 % vinylpyrrolidone.

10. **Free N'N-divinylimidazole — not more than 2 mg/kg**

Principle

Free N,N'-divinylimidazolidone migrating from insoluble PVP into a solvent (acetone) is determined by capillary column gas chromatography.

Internal standard solution

Dissolve 100 mg of heptanoic acid nitrile (oenanthic acid nitrile) weighed out to within 0,1 mg in 500 ml of acetone.

Preparation of the specimen

Weigh out about 2 to 2,5 g of the polymer to within 0,2 mg into a 50 ml conical flask. Using a pipette, add 5 ml of internal standard solution. Next, run in about 20 ml of acetone. Shake the mixture for four hours or let it equilibrate for at least 15 hours and analyse the supernatant solution by gas chromatography.

Calibration solution

Weigh out about 25 mg of N,N'-divinylimidazolidone to within 0,2 mg into a flask and make up to 100 ml with acetone. Using a pipette, transfer 2,0 ml of this solution into another 50 ml calibration flask, make up to 50 ml with acetone. Transfer 2 ml of this solution to another flask, add 5 ml of the internal standard solution (see above) and make up to 25 ml with acetone.

Gas chromatography conditions :

Column :	capillary (fused silica) 'DB-Wax' (cross-linked Carbowax 20 ml), length 30 mm, internal diameter 0,25 mm, film thickness 0,5 µm.
Column oven temp. :	programmed, 140 °C to 240 °C, 4°/minute
Injector :	split injector, 220 °C split effluent 30 ml
Detector :	thermonomic detector (optomized in accordance with maker's instructions) 250 °C
Carrier gas :	Helium, 1 bar (over pressure)
Amount injected :	1 µl of supernatant solution of specimen or calibration solution.

Procedure:

Obtain a reliable determination of the calibration factor for the specific conditions of analysis by means of repetitive injections of the calibration solution. Analyse the sample. The content of N,N'-divinylimidazolidone in insoluble PVP may not be more than 0,1 %.

Calculation of the calibration factor:

$$f = \frac{W_D \times A_{St}}{W_{St} \times A_D}$$

W_D = amount of N,N'-divinylimidazolidone taken (mg)
W_{St} = amount of internal standard (mg)
A_{St} = area of peak of internal standard
A_D = area of peak for N,N'-divinylimidazolidone

Calculation of the content of N,N'-divinylimidazolidone

$$C_D = \frac{1\,000 \times f \times A_D \times W_{St}}{A_{St} \times W_S} \text{ (mg/kg)}$$

C_D = concentration of N,N'-divinylimidazolidone (mg/kg)
F = calibration factor
A_D = area of peak for N,N'-divinylimidazolidone
W_{St} = amount of internal standard added to the sample (mg)
A_{St} = area of peak of internal standard
W_S = amount of specimen taken (g).

ANNEX II

LACTIC BACTERIA

Specifications

Lactic bacteria, the use of which is provided for in Annex VI (1) (q) and (3) (z) to Regulation (EEC) No 822/87 must belong to the genera *Leuconostoc*, *Lactobacillus* and/or *Pedococcus*. They must convert the malic acid in must or wine into lactic acid and not affect the taste.

They must have been isolated from grapes, must, wine or products made from grapes. The name of the genus and species and the reference of the strain must be shown on the label, with the origin and the strain breeder.

Genetic manipulation of lactic bacteria must be granted prior authorization.

Form

They must be used in liquid or frozen form or as a powder obtained by lyophilization, in pure culture or associated culture.

Immobilized bacteria

The carrier medium for a preparation of immobilized lactic bacteria must be inert and must be permitted for use in winemaking.

Controls

— Chemical :

the same requirements as regards screened substances as in other œnological preparations, and heavy metals in particular.

— Microbiological :

- — the level of revivifiable lactic bacteria must be 10_8/g or 10_7/ml or more,
- — the level of lactic bacteria of a species different from the strain or strains indicated must be less than 0,01 % of the total revivifiable lactic bacteria,
- — the level of aerobic bacteria must be less than 10_3/g of powder or per millilitre,
- — the total yeast content must be less than 10_3 per gram of powder or per millilitre,
- — the mould content must be less than 10_3 per gram of powder or per millilitre.

Additives

Additives used in preparing the culture or reactivation of lactic bacteria must be substances permitted for use in foodstuffs and must be mentioned on the label.

Date of production

The manufacturer must indicate the date on which the product left the factory.

Use

The manufacturer must indicate instructions for use or the reactivation method.

Preservation

The storage conditions must be clearly marked on the label.

Analysis methods

— lactic bacteria : medium A(1) B (2) or C (3) with the utilization method for the strain as indicated by the producer,

— aerobic bacteria : Bacto-Agar medium,

— yeasts : Malt-Wickerham medium,

— mould : Malt-Wickerham or Czapeck medium.

(1)

Yeast extract	5	g
Meat extract	10	g
Trypsic peptone	15	g
Sodium acetate	5	g
NH_4-citrate	2	g
Tween 80	1	ml
$MnSO_4$	0,050	g
$MgSO_4$	0,200	g
Glucose	20	g
Water q.s.p.	1 000	ml
pH	5,4	

(2)

Tomato juice	250	ml
Difco-yeast extract	5	g
Peptone	5	g
L malic acid	3	g
Tween 80	1 drop	
$MgSO_4$	0,050	g
$MnSO_4$	0,050	g
Water q.s.p.	1 000	ml
pH	4,8	

(3)

Glucose	5	g
Difco tryptone	2	g
Difco peptone	5	g
Liver extract	1	g
Tween 80	0,05	g
Tomato juice diluted 4,2 times filtered with Whatman No 1	1 000	ml
pH with PO_4H_3 or KOH	5,5	g
Glucose	20	g