# COEI-1-GELATI Gelatine

# Proteinum ossii

# Gelatina

## 1. Object, origin and field of application

Gelatine is the result of the partial hydrolysis of collagen contained in the skins, connective tissue and bones of animals. Gelatine comes in the form of roll sheets, flexible sheets, sprinkles, grains or colourless or slightly yellowish brown powder.

Certain gelatines are intentionally hydrolysed more than usual edible gelatines so as to be presented in ready-to-use colloidal solutions or in the form of atomised powder, soluble when cold. These products do not have the characteristic of becoming gel with water.

The structure and the iso-electric point of bovine skin gelatine proteins are different from gelatine from pork bones and rind.

Taking into account available scientific data, international standards and directives, gelatine must come from animals sources in compliance with recommendations from the International Office of Epizootics (IOE).

Gelatines are used as fining and clarification agents for wine. Gelatines react with wine tannins or additions and certain cations depending on their origin, the extraction process and their final degree of hydrolysis at the time of use in wine.

For the same quality of gelatine, the hydrolysis quality and the different phases of hydrolysis will produce products with very different behaviour concerning fining.

There is no single parameter to characterise the different types of gelatine due to their diversity.

## 2. Labelling

The origin of basic edible gelatine must be indicated as well as the optimal storage conditions, expiration date and the concentration of $SO_2$.

## 3. Solubility

Basic edible gelatine swells in cold water. It dissolves in hot water (80°C to 90°C) and the solution jellifies upon cooling.

## 4. Test trials

4.1. Taste test

The solution in warm water should not have an unpleasant odour nor taste.

4.2. pH

Evaluate the pH on a 1% solution at 40°C,

The colloidal solution pH level is between 3 to 4,

The solutions prepared from powder or grain products have a pH level between 5 to 7.

4.3. Loss through dessication

4.3.1. Solid form gelatine:

Place 2 g of gelatine in a 70 mm diameter silica capsule with a lid. Dry in an incubator at 100°C–105°C for 6 hours. Allow to cool in a covered capsule and a desiccator. Weigh. Let the quantity of dry residue be p g. Weight loss should not exceed 15%.

4.3.2. Liquid form gelatine:

Put about 10 g of colloidal gelatine solution in a 70 mm diameter silica capsule. Weigh exactly this quantity in a covered capsule and dry over a water bath at 100°C for 4 hours. Then proceed by drying in an incubator at 100°C–105°C for 3 hours. Allow to cool in a covered capsule and a dessicator. Weigh the amount of dry residue. Given p g of this quantity. In relation to 100 g of the colloidal solution, the dry residue must reach a minimum of 5%.

**All the limits set above are for the dry product.**

4.4. Ashes

Incinerate the dry residue from point 4.3 by slowly heating to 600°C in a muffle furnace after sprinkling gelatine with 0.2 to 0.3g of paraffin without ashes to avoid over spilling. Total ash content should not exceed 2.0%.

4.5. Preparation of test trial solution

After being weighed, dissolve ashes in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat to activate the dissolving and add distilled water until a volume equal to 25

times the weight of dried gelatine is reached. 1 ml of this solution contains mineral matter of 0.04 g of dried gelatine.

4.6. Iron

Add 1 ml of concentrated hydrochloric acid (R), one drop of concentrate potassium permanganate at 1% (R), 2 ml of potassium thiocyanate at 5% (R) to 10 ml of the test trial solution (4.5).

If a red colouration appears, it must be lighter than the control sample prepared with 2 ml of iron solution (III) at 0.010 g per litre (R), 5.2 ml of water and the same amounts of concentrated hydrochloric acid (R) and potassium thiocyanate at 5% (R).

Iron content should be less than 50 mg/kg.

It is also possible to determine iron by atomic absorption spectrophotometry. (See method described in Chapter II of the International Oenological Codex).

4.7. Chromium

Put 10 ml of test trial solution (4.5), 1 ml of ammonia persulfate solution at 15% (R), 0.5 ml of silver nitrate solution at 1% into a 50 ml conical flask. Heat and add potassium permanganate solution at 3% (R) drop by drop until the solution reaches a stable pink colour. Add a couple more drops and simmer 10 minutes. If the solution changes colour while boiling, add more potassium permanganate. After 10 minutes, add 1/10 diluted hydrochloric acid (R) until the solution is completely discoloured.

After cooling, transfer to a 20 ml graduated flask and add 2 ml of newly made 0.05% diphenylcarbazide solution in alcohol (R). Bring to 20 ml.

If a purplish red colouration appears, it must be lighter than the colour obtained when treating 4 ml of potassium dichromate solution at 0.001g of chrome per litre with 2 ml of sulphuric acid at 5% (R), 5 ml of distilled water, and after mixing add 2 ml of 0.05% diphenylcarbazide solution in alcohol (R) and bringing it up to 20 ml.

Chromium content should be less than 10 mg/kg.

It is also possible to determine chrome by atomic absorption spectrophotometry. (See method described in Chapter II of the International Oenological Codex).

4.8. Copper

Put 2.5 ml of test trial solution (4.5) in a test tube and add 7.5 ml of water, 0.5 ml of hydrochloric citric solution (R), 1 ml 5M ammonia hydroxide (R), 0.5 ml of sodium diethyldthiocarbamate reagent (R). If a yellow colouration appears, it must not be darker than the solution obtained when adding the same volumes of the same reagents to 3.5 ml of a copper solution at 1 mg per litre (R) brought to 10 ml.

Copper content should be below 30 mg/kg.

It is also possible to determine copper by atomic absorption spectrophotometry (See method described in Chapter II of the International Oenological Codex).

4.9. Zinc

Put 3.75 ml of distilled water, 5 ml of buffer acetate solution (R), 1 ml of sodium thiosulfate solution at 25% (m/v) (R), 5 ml of dithizone solution at 25 mg per litre in the dichloromethane (R) in 1.25 ml of test trial solution (4.5). Shake for 2 minutes. Separate the organic phase. The colouration must be lighter than the colour obtained

when treating the same volumes of the same reagents, 2.5 ml of zinc solution at 1 mg per litre (R).

Zinc content should be less than 50 mg/kg.

It is also possible to determine zinc by atomic absorption spectrophotometry. (See method described in Chapter II of the International Oenological Codex).

4.10. Lead

Using the test trial solution (4.5), determine the lead according to the method in described in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Lead content should be less than 5 mg/kg.

4.11. Mercury

Determine the mercury according to the method described in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Mercury content should be less than 0.15 mg/kg.

4.12. Arsenic

Determine the arsenic according to the method in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Arsenic content should be less than 1 mg/kg.

4.13. Cadmium

Determine the cadmium according to the method described in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Cadmium content should be less than 0.5 mg/kg.

4.14. Determining total nitrogen

Determine the total nitrogen according to the method in Chapter II of the International Oenological Codex. Total nitrogen must be more than 14% of the weight of dry gelatine.

4.15. Sulphur dioxide

**Gelatine in dried form**

Sulphur dioxide, freed by a little excess of phosphoric acid, starts to boil under the reflux of a flow of nitrogen. Which is oxidised and set by a hydrogen peroxide solution and measured by an acid meter in the presence of bromophenol blue, according to the reference method in the Compendium of International Methods of Analysis of Wines and Musts. This is done with a sample of 2 g of solid gelatine and on 10 ml of diluted solution at 10% of gelatine.

Sulphur dioxide content should not exceed 50 mg/kg.

**Gelatine in colloidal solution form**

Liquid forms are stabilised with $SO_2$ and should not contain benzylic alcohol; sulphur dioxide content should not exceed 4 g/litre.

4.16. Urea

Determine urea using the Boehringer enzymatic method.

Content should be less than 2.5 g/kg.

4.17. Bacteria monitoring

Proceed as is indicated in Chapter II of the International Oenological Codex.

Limit: total viable micro-organisms: less than $10^4$ CFU/g

4.18. Escherichia coli

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence checked on a sample of 1 g.

4.19. Salmonella

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence of salmonella is checked on a 25 g sample.

4.20. Coliforms

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence of coliform bacteria is checked on a 1 g sample.

4.21. Spores of anaerobic sulphite-reducing micro-organisms[1]

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence is checked on a 1 g sample.

4.22. Clostridium perfringen spores[2]

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence is checked on a 1 g sample.

4.23. Staphylococci (Staphylococcus aureus)

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence is checked on a 1 g sample.

4.24. Yeasts

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: $10^3$ CFU/g of preparation.

4.25. Total lactic bacteria

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: $10^3$ CFU/ g of preparation.

4.26. Acetic bacteria

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: $10^3$ CFU/ g of preparation.

4.27. Mould

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: $10^3$ CFU/g of preparation.

**5. Storage**

Solid gelatine must be stored in closed containers or in a humidity-proof bag under temperate conditions.

Gelatine in ready-to-use colloidal solutions may contain preservatives authorised in wines and their concentrations must be indicated on the label.

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[1] Method to be defined later on by the experts' group "Wine microbiology".

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