# RESOLUTION OENO 12/2003

# CASEINS

THE GENERAL ASSEMBLY,

CONSIDERING Article 5 of the International Convention on the Unification of the Methods of Analysis and Appraisal of Wines of 13 October 1954,

UPON THE PROPOSAL of the Sub-Commission on Methods of Analysis and Appraisal of Wines,

DECIDES to replace the existing monograph in the International Oenological Codex by the following monograph:

# CASEINS

**(Lactic Casein or Caseina acids)**

## 1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Casein, a heteroprotein containing phosphorous, is found in milk in the state of calcium salt.

It is obtained by coagulating skim milk.

It is the fining agent indicated for the treatment of oxidations in wine. It can only be used in alkaline water with potassium carbonate or potassium hydrogenocarbonate.

Casein adsorbs polyphenols, in particular oxidised polyphenols.

## 2. LABELLING

The concentration of casein used for the preparation must be indicated on the label including in the case of a mixture, as well as the storage conditions.

## 3. CHARACTERISTICS

Casein is a yellowish white coloured powder. It is amorphous, odourless and insoluble in pure water and various organic solvents. It can have a slight lactic odour. In alkaline water or in saline solutions with alkaline reactions, it swells and produces a colloidal solution: 100 ml of alkaline water for 1 g of potassium hydroxide or sodium hydroxide, dissolve 10 g of casein in a water bath at 100°C. The solution diluted 20 times its



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volume in water is cloudy; it should be free of lumps.

The so-called soluble caseins are mixed with pure powder and/or potassium carbonate (maximum 25%), or potassium hydrogenocarbonate).

Caseins used in oenology are fit for human consumption.

# 4. IDENTIFYING CHARACTERISTICS

## 4.1. Casein doesn't precipitate by heating its alkaline solution. This solution precipitates by acidification once the pH is less than 5.

## 4.2. Casein ashes contain phosphates characterised by the nitromolybdic reagent (R).

# 5. TEST TRIALS

Casein should have no flavour, nor abnormal odour (rotten, mouldy, putrid, etc.)

## 5.1. Acidity

### 5.1.1. Principle

Determining free acidity in casein by an acidobasic determination of an aqueous extract of the product.

### 5.1.2. Reagents

- Sodium hydroxide 0.1 M
- Phenolphthalein, solution at 10 g/l in ethanol

### 5.1.3. Procedure

Preliminary test:

- Homogenise the product by shaking vigorously;
- Put 50 g of the product on a strainer (metal mesh strainer 200 mm in diameter, nominal size of 500 µm for the opening with a receptacle (Standard ISO 3310/1);
- If 50 g of the product passes through completely, use the product as it is;



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- If the 50 g of the product do not pass through, grind the product until 50 g do pass through.

During all these operations, avoid changing the water content of the product.
Preparation for the test trial solution:

- Take approximately 10 g to the nearest 10 mg of the 50 g passed through the strainer, or m of this mass.
- Put the mass m in a 250 ml conical flask
- Pour 200 ml of recently boiled distilled water brought to 60°C into the flask.
- Shake the closed flask.
- Allow to stand for 30 minutes in a water bath at 60 °C while shaking the flask every 10 minutes.
- Filter.

The filtrate at 20°C must be clear.
Carrying out the test:

- Take 100 ml of filtrate.
- Place the test sample in a 250 ml conical flask.
- Add 0.5 ml of phenolphthalein solution to the flask.
- Titrate using 0.1 M sodium hydroxide solution.
- Let V represent the volume used.

### 5.1.4. Calculation

Free acidity in casein expressed in meq/l is equal to:

$$\frac{20.V.T}{m}$$

- V is the volume in ml of sodium hydroxide used.



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- T is the exact mole fraction of the sodium hydroxide solution
- m is the mass density in g of the test trial sample.

Acidity expressed as lactic acid should be less than 1.6 g/l.

## 5.2. pH

Shake 10 g of casein in 100 ml of water for a few minutes. Decant; the pH of the solution should be less than or equal to 5 for pure casein.

## 5.3. Loss by dessication

Determine the weight loss of 2 g of the test trial sample by drying to constant weight at 100°C-105°C . Weight loss of casein must be less than 12%.

All the limits set below apply to dried products.

## 5.4. Ashes

Incinerate the residue left in the weight loss determination by dessication, without exceeding 600 °C.

The rate of the ashes should be less than 3% for casein acid and less than 11% for the casein acid and potassium carbonate or potassium hydrogenocarbonate mixture.

## 5.5. Preparation of test trial solution

After determining the weight of the ashes, dissolve them in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat to dissolve and add water until reaching a volume equal to 25 times the weight of dried casein. 1 ml of this solution contains 0.04 g of dried casein mineral matters.

## 5.6. Iron

Take 10 ml of the test trial solution (5.5), and add 1 ml of concentrated hydrochloric acid (R), 3 drops of hydrogen peroxide solution at 3 volumes(R) and 2 ml of potassium thiocyanate solution at 5% (R).

If a red colouration appears, it must be lighter than the control prepared with 8 ml of iron solution (III) at 0.01 g of iron per litre (R), 2 ml of water and the same volumes of concentrated hydrochloric acid (R) and potassium thiocyanate solution at 5% (R).

Iron content should be less than 200 mg/kg.

This determination can also be carried out by atomic absorption spectrophotometry.



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## 5.7. Lead

On the test trial solution (5.5), determine the lead according to the method described in Chapter II of the International Oenological Codex.

Lead content should be less than 5 mg/kg.

## 5.8. Cadmium

On the test trial solution (5.5), determine the cadmium according to the method described in Chapter II of the International Oenological Codex.

Cadmium content should be less than 1 mg/kg.

## 5.9. Mercury

Determine the mercury according to the method described in Chapter II of the International Oenological Codex.

Mercury content should be less than 1 mg/kg.

## 5.10. Arsenic

On the test trial solution (5.5), determine the arsenic according to the method described in Chapter II of the International Oenological Codex.

Arsenic content should be less than 3 mg/kg.

## 5.11. Total nitrogen

Introduce approximately 0.20 g of casein precisely weighed in a mineralisation flask with 15 ml of concentrated sulphuric acid (R) and 2 g of mineralisation catalyst (R) and continue the operation according to the method in chapter II of the International Oenological Codex.

Total nitrogen content must be more than 13%.

## 5.12. Proteins

Protein content should not be less than 82% of weight (total nitrogen 6.38).

## 5.13. Fat content

Determine the fat content using the gravimetric Schmid-Bondzynski-Ratslaff method (standard ISO 5543).

Fat content should be less than 2%.



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## 5.14. Bacteriological monitoring

Proceed as indicated in chapter II of the International Oenological Codex.

Limit: total viable microorganisms: less than 3 x $10^4$ CFU/g.

## 5.15. Coliforms

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

Absence must be checked on a sample of 25 g.

## 5.16. Staphylococci

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

The number of staphylococci (ß-hemolytiques positive coagulase) must be less than or equal to 1 per g.

## 5.17. Escherichia Coli

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

Absence must be checked on a sample of 1 g.

## 5.18. Salmonella

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

The number of salmonella should be less than 1 per 100 g.

## 5.19. Yeasts

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

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

## 5.20. Lactic bacteria

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

Content limit: $10^2$ CFU/g of preparation.



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### 5.21. Lactobacillus sp.*

Content limit: 10 CFU/g of preparation.

### 5.22. Pediococcus sp.*

Content limit: absence in a 10 g preparation sample.

### 5.23. 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

### 5.24. Mould

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

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

## 6. STORAGE

Casein must be stored in watertight bags between 5°C and 20°C with relative humidity less than 65%. Its shelf life is 24 months.

## 7. REFERENCES

Standard ISO 5543.

*Method to be defined later on.



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