

## RESOLUTION OIV-OENO 571-2017

### MONOGRAPH ON GLUTATHIONE

THE GENERAL ASSEMBLY,

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

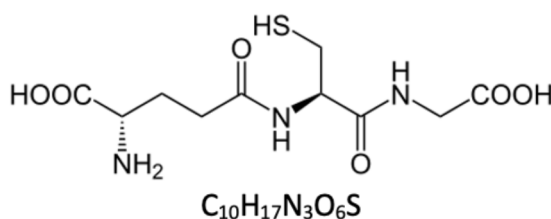
IN VIEW OF the work of the "Specifications of Oenological Products" Expert Group,

CONSIDERING Resolution OIV-OENO 445-2015 "Treatment of must with glutathione" and Resolution OIV-OENO 446-2015 "Treatment of wine with glutathione", both adopted in 2015,

DECIDES to supplement the International Oenological Codex with the following monograph:

#### Glutathione

Chemical name:  $\gamma$ -L-Glutamyl-L-cysteinyl-glycine



CAS number: 70-18-8

Molecular weight: 307.33 g/mol

## 1. OBJECT, ORIGIN AND SCOPE OF APPLICATION

Reduced glutathione (GSH) is a biologically active tripeptide consisting of L-glutamate, L-cysteine and glycine. Its antioxidant properties can fight against oxidation phenomena in musts and wines and protect aromatic compounds.

GSH is principally produced by microbial fermentation. The more onerous methods of production – chemically or by enzymatic reaction – are not used on an industrial scale.

Production by microbial fermentation frequently uses *Saccharomyces cerevisiae* and *Candida utilis* or other non-*Saccharomyces* microorganisms, and more generally their mutant forms. The GSH content in cultures of mutant yeast strains is usually high (3.5%-9% of dry cell weight).

When mutants used for GSH production come from genetically modified yeasts, they must be authorised for use beforehand by the relevant authorities.

## 2. LABELLING

The label must indicate:

- the name or sales denomination,
- the indication 'product for oenological use, limited use',
- the GSH content,□
- any additives,□
- instructions for use,
- the batch number as well as the expiry date, and the storage conditions in terms of temperature, humidity and ventilation conditions,
- the name of the genus and the species of microbial sources (only if produced by microbial fermentation),
- the indication that the GSH was produced by mutants obtained by genetic modification and the modified characteristic if such is the case (only if produced by microbial fermentation), the name or company name and address of the manufacturer, packager or supplier,
- the net weight

## 3. CHARACTERISTICS

GSH is usually available in white crystalline powder form soluble in water, which results in a clear and colourless aqueous solution with a light flavour of reduction. Precautions must be taken (points 4.3. and 6) to ensure the stability of GSH in order to avoid oxidation and oxidised glutathione (GSSG) production.

### **3.1. Identification**

#### **3.1.1. Rotatory power**

Specific rotatory power:  $-18.9^{\circ}$  ( $c=4.653\%$  at  $T=25^{\circ}\text{C}$ )

#### **3.1.2. Melting point**

190-195  $^{\circ}\text{C}$

## **4. LIMITS AND TEST METHODS**

### **4.1. GSH content**

Reduced glutathione (GSH) concentrations are measured by the capillary electrophoresis method described in the Annex.

The reduced glutathione content must be  $\geq 98\%$ .

### **4.2. Humidity**

Measured by the loss in the weight of 5 g of product, dried at 105  $^{\circ}\text{C}$  until the weight is constant (for approximately 3 hours). The maximum humidity of the solid form must be less than or equal to 0.5%.

### **4.3. Test solution**

Dissolve 1 g of GSH in 100 mL of type-I ultra-pure water (UPW). The GSH solution must be prepared each day and stored at low temperature (2-4  $^{\circ}\text{C}$ ) in a brown glass bottle.

### **4.4. Lead**

Proceed with determination according to the method indicated in Chapter II of the International Oenological Codex. The lead content must be less than 2 mg/kg of dry matter.

### **4.5. Mercury**

Proceed with determination according to the method indicated in Chapter II of the International Oenological Codex. The mercury content must be less than 1 mg/kg of dry matter.

### **4.6. Arsenic**

Proceed with determination according to the method indicated in Chapter II of the

International Oenological Codex. The arsenic content must be less than 3 mg/kg of dry matter.

#### **4.7. Cadmium**

Proceed with determination according to the method indicated in Chapter II of the International Oenological Codex. The cadmium content must be less than 1 mg/kg of dry matter.

#### **4.8. Living yeasts**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. The live yeast count must be less than or equal to  $10^2$  CFU/g.

#### **4.9. Moulds**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. The mould count must be less than  $10^2$  CFU/g.

#### **4.10. Lactic acid bacteria**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. The lactic bacteria count must be less than  $10^3$  CFU/g.

#### **4.11. Acetic acid bacteria**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. The acetic acid bacteria count must be less than  $10^3$  CFU/g.

#### **4.12. Salmonella**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. Absence must be checked on a sample of 25 g.

#### **4.13. Escherichia coli**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. Absence must be checked on a sample of 1 g.

#### **4.14. Staphylococci**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. Absence must be checked on a sample of 1 g.

#### **4.15. Coliforms**

Enumerate according to the method indicated in Chapter II of the International Oenological Codex. The coliform count must be less than 10 CFU/g.

### **5. ADDITIVES**

They must comply with the currently applicable regulations.

### **6. STORAGE**

Store in sealed packaging in a cool (2-8 °C), dry place. In all cases, refer to the manufacturer's instructions.

## **Annex Determination of glutathione (GSH) in commercial preparations by capillary electrophoresis**

This determination is carried out according to the method for the determination of glutathione in musts and wines (Resolution OIV-OENO 345-2009).

The glutathione samples to be determined are prepared by dilution of the test solution (point 4.3 of the glutathione monograph) so as to obtain a final concentration of around 20 mg/L (e.g. 200 µL in 100 mL of ultra-pure water if the level of glutathione in the commercial preparation is close to 100%). If necessary, this preparation should be clarified by centrifugation before being analysed.

### **1. METHOD CHARACTERISTICS**

Certain internal elements of validation were determined in the wine matrix (Resolution OIV-OENO 345-2009) to produce calibration curves and repeatability tests. Each concentration is calculated based on the average of three determinations obtained by using the regression line of the calibration curve. The results are expressed in mg/L. The linear regression and correlation coefficient are calculated according to the least squares method. The glutathione stock solution is produced from an HCl/EDTA solution, allowing it to be stored at +6 °C for several days with no loss. Successive dilutions of this solution allow the threshold limit of detection of the method to be estimated, for a signal-to-noise ratio of three or more.

The calibration curve is established between 0 and 40 mg/L, the linear regression equates to  $Y = 0.583X - 0.948$  and the correlation coefficient is 0.9966.

These analytical conditions make it possible to eliminate interference caused by MBB hydrolysis products.

The method's repeatability is calculated on the basis of 10 analyses of the same sample of wine. For a 10 mg/L concentration, the coefficient of variation is 6.0% for glutathione.

The limit of detection of glutathione is 20 µg/L (in the wine) and the limit of quantification is 60 µg/L.

## **2. BIBLIOGRAPHY**

See Resolution OIV-OENO 345-2009.