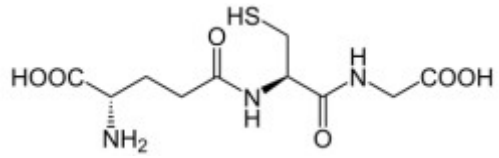
COEI-1-GLUTAT Glutathione

Chemical name: □-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 S*accharomyces 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:

$$[\alpha]_D^{25}: -18.9° \ (c = 4.653\% \ at \ T = 25°C$$

Melting point

- 190-195 °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 ≥ 98%.

4.2. Humidity

Measured by the loss in the weight of 5 g of product, dried at 105 °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 °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 of 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.