

**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 4675.1-2016

Determination of glycerol in wine for export--Enzymatic method

出口葡萄酒中甘油的测定 酶法

(English Translation)

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Foreword

SN/T 4675 "Methods for Quality and Safety of Exported Wines" is divided into 30 parts.

---SN/T 4675.1 Determination of glycerol in exported wines;

---SN/T 4675.2 Determination of 2,3-butanediol in exported wines by gas chromatography;

---SN/T 4675.3 Determination of the stable carbon isotope ratio of ethanol in exported wines;

---SN/T 4675.4 Determination of lactic acid in exported wines;

--- SN/T 4675.5 Determination of organic acids in exported wines by ion chromatography;

---SN/T 4675.6 Determination of glucose, fructose and sucrose in exported wines;

--- SN/T 4675.7 Determination of acetaldehyde in exported wines by gas chromatography-mass spectrometry;

--- SN/T 4675.8 Determination of 5-hydroxymethylfurfural in exported wines by liquid chromatography;

--- SN/T 4675.9 Determination of diethylene glycol in exported wines by gas chromatography-mass spectrometry;

---SN/T 4675.10 Determination of ochratoxin A in exported wines by liquid chromatography-mass spectrometry/mass spectrometry;

---SN/T 4675.11 Determination of seven anthocyanins in exported wines by ultra performance liquid chromatography;

---SN/T 4675.12 Determination of lysozyme in exported wines by liquid chromatography;

--- SN/T 4675.13 Determination of 2,4,6-trichlorotoluene residues in exported wines by gas chromatography-mass spectrometry;

---SN/T 4675.14 Determination of natamycin in exported wines by liquid

chromatography-mass spectrometry/mass spectrometry;

---SN/T 4675.15 Determination of salicylic acid, dehydroacetic acid and p-chlorobenzoic acid in exported wines by liquid chromatography;

---SN/T 4675.16 Determination of fumaric acid in exported wines by liquid chromatography-mass spectrometry/mass spectrometry;

--- SN/T 4675.17 Determination of butyl tin content in exported wines by gas chromatography - mass spectrometry/mass spectrometry;

--- SN/T 4675.18 Determination of residual dithiocarbamate in exported wines.

---SN/T 4675.19 Determination of sodium, magnesium, potassium, calcium, chromium, manganese, iron, copper, zinc, arsenic, selenium, silver, cadmium and lead in exported wines;

---SN/T 4675.20 Determination of rare earth elements in exported wines by inductively coupled plasma mass spectrometry;

--- SN/T 4675.21 Determination of soluble inorganic salts in exported wines by ion chromatography;

---SN/T 4675.22 Determination of total sulfur dioxide in exported wines;

---SN/T 4675.23 Determination of ammonia nitrogen in exported wines and grape juices - Continuous flow analyzer method;

---SN/T 4675.24 Determination of the export wine Fulin-Shoka index spectrophotometer method;

--- SN/T 4675.25 Determination of the color of exported wine CIE1976 (L * a * b *) color space method;

---SN/T 4675.26 Determination of turbidity of exported wines by scattered light method;

---SN/T 4675.27 Determination of alkaline ash in exported wines;

---SN/T 4675.28 Counting bacteria, mold and yeast for exporting wine;

---SN/T 4675.29 Real-time fluorescent PCR method for wine yeast test in exported wine;

---SN/T 4675.30 In the wine, the Bayesian yeast test real-time fluorescent PCR method.

This part is the first part of SN/T 4765.

This part is drafted in accordance with the rules given in GB/T 1.1-2009.

This section is equivalent to the International Grape and Wine Organization (OIV) method OIV-MA-AS312-05 "glycerol". This part is in technology the content is consistent with this method.

1 Scope

This part of SN/T 4675 specifies the enzymatic method for the determination of glycerol in wine.

This section applies to the determination of glycerin content in wine.

2 Normative references

The following documents are indispensable for the application of this document. For dated references, only dated versions apply to this article.

Pieces. For undated references, the latest edition (including all amendments) applies to this document.

GB/T 6682 Analytical laboratory water specifications and test methods

3 Principle of the method

Glycerol in wine can be phosphorylated by adenosine triphosphate (ATP) under the catalysis of glycerol kinase (GK).

Glycerol-3-phosphate and adenosine diphosphate (ADP).

Reaction (1). Glycerin $\text{ATP} \rightarrow \text{GK}$ Glycerol-3-phosphate ADP the produced adenosine diphosphate (ADP) is converted to ATP by phosphoenolpyruvate (PEP) under the action of pyruvate kinase (PK).

Simultaneous production of pyruvate.

Reaction (2). $\text{ADP PEP} \rightarrow \text{PK ATP}$ pyruvate Pyruvate is reduced to lactic acid by the reducing agent nicotinamide adenine dinucleotide (NADH) under the action of lactate dehydrogenase (LDH).

Reaction (3). $\text{Pyruvate NADH H} \rightarrow \text{LDH lactate NAD}$ the amount of NAD produced during this reaction is proportional to the amount of glycerol. The degree of oxidation of NADH is through its wavelength the degree of attenuation at 334 nm, 340 nm or 365 nm was measured.

The sample is diluted with water, and then the buffer, the reaction solution, the sample diluent and the enzyme solution are sequentially added to the cuvette to carry out the reaction, and the spectroscopic light is used.

The absorbance change was measured by a metric meter and quantified by the difference in absorbance.

4 Reagents and materials

Unless otherwise specified, the reagents used were of analytical grade and the test water was the first grade water specified in GB/T 6682.

4.1

Sodium hydroxide (NaOH).

4.2

Glycyl glycine (C₄H₈N₂O₃).

4.3

Magnesium sulfate (MgSO₄·7H₂O).

4.4

Reduced nicotinamide adenine dinucleotide disodium (NADH).

4.5

adenosine triphosphate (ATP).

4.6

Phosphoenolpyruvate (PEP).

4.7

Sodium bicarbonate (NaHCO₃).

4.8

Pyruvate kinase (PK).