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NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA

GB 5009.231-2016

**National Food Safety Standard - Determination of
Volatile Phenol Residues in Aquatic Products**

食品安全国家标准

水产品中挥发酚残留量的测定

Issued on: August 31, 2016

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National Food Safety Standard - Determination of Volatile Phenol Residues in Aquatic Products

1 Scope

This Standard specifies the spectrophotometric method for the determination of volatile phenol residues in aquatic products.

This Standard applies to the determination of volatile phenol residues in edible parts of aquatic products.

2 Principle

Use alkaline solution to destroy the structure of the sample; under acidic conditions, use water vapor to distill off the volatile phenolic compound; in the presence of potassium ferricyanide, it reacts with 4-aminoantipyrine to form an orange-red antipyrine dye; use trichloromethane to extract; measure the absorbance quantity at the wavelength of 460 nm.

3 Reagents and materials

Unless otherwise specified, all the reagents in this method are analytical reagents, the water is grade-3 water that is specified by GB/T 6682.

3.1 Reagents

3.1.1 Trichloromethane (CHCl_3).

3.1.2 Ammonia solution ($\text{NH}_3 \cdot \text{H}_2\text{O}$).

3.1.3 Sulfuric acid (H_2SO_4).

3.1.4 Sodium hydroxide (NaOH).

3.1.5 Potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$).

3.1.6 Copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).

3.1.7 Methyl orange ($\text{C}_{14}\text{H}_{15}\text{N}_3\text{NaO}_3\text{S}$).

3.1.8 Ammonium chloride (NH_4Cl).

3.4.2 Standard working solution: take an appropriate amount of phenol standard solution; use phenol-free water to dilute to 1.00 µg of phenol per millimeter; use it within 2 hours after preparation.

4 Instruments and apparatuses

4.1 Water vapor distiller or 500 mL glass water-vapor distilling apparatus (see Appendix A).

4.2 Balance: the sensitivity is 0.1mg and 0.01g.

4.3 500 mL (pear-shaped) separatory funnel.

4.4 Magnetic stirrer.

4.5 Spectrophotometer.

4.6 Constant-temperature electric furnace or thermostat electric heating sleeve.

4.7 Tissue masher.

5 Analysis steps

5.1 Sample preparation and storage

Use the tissue masher to homogenate the edible part of the aquatic products; stir the mixture uniformly; package; seal and refrigerate or freeze to store.

5.2 Sample processing

5.2.1 Distillation extraction

Weigh 10 g ~ 20 g of pretreated sample (accurate to 0.01g, and do a blank test at the same time) into the beaker; add 75 mL of sodium hydroxide solution; use a magnetic stirrer to stir for 10 min. Then, add 3 ~ 5 drops of methyl orange solution; use sulfuric acid solution to adjust the $\text{pH} \leq 4$ (the solution is orange-red); add 5 mL of copper sulphate solution; mix; then put it in a water-vapor distiller or a 500 mL distilling flask; use a small amount of phenol-free water to wash the flask; merge the washing liquid into a water-vapor distilling apparatus or a distilling flask, and add several glass beads.

Use the glass water-vapor distilling apparatus (or water-vapor distiller) to perform distillation - the water-vapor distilling apparatus shall be connected well to ensure strict gas leakage; take a 250 mL conical flask to receive the distillate; distillate at medium speed for 60 min ~ 90 min; until the distillate is about 240 mL, stop the distillation, and use 10 mL of phenol-free water to wash the condenser; merge the washing liquid into the distillate (which remains acidic during the distillation). The distillate shall be

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