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

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**Animal and vegetable fats and oils -- Determination of anisidine
value**

动植物油脂 茴香胺值的测定

(English Translation)

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1 Scope

This document describes the method for determining the anisidine value of animal and vegetable oils.

This document applies to the determination of aldehyde content (mainly alpha,beta-unsaturated aldehydes) in animal and vegetable oils.

This document does not apply to milk fat and milk fat products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, Animal and vegetable fats and oils -- Preparation of test sample

NOTE: GB/T 15687-2008, Animal and vegetable fats and oils -- Preparation of test sample (ISO 661:2003, IDT)

ISO 3696, Water for analytical laboratory use -- Specification and test methods

NOTE: GB/T 6682-2008, Water for analytical laboratory use - Specification and test methods (ISO 3696.1987, MOD)

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

anisidine value

Under specified test conditions, the increase in absorbance measured at 350 nm wavelength using a 10 mm cuvette after reacting with the sample with p-anisidine is magnified 100 times.

NOTE: The anisidine value has no unit; instead, it is measured as one unit when 1 g of sample is dissolved in a mixture of 100 mL of solvent and reagent.

4 Principle

The specimen is dissolved in isooctane (2,2,4-trimethylpentane) and reacted with an acetic acid solution of p-anisidine. The increase in absorbance is measured at 350 nm.

The anisidine value is calculated.

5 Reagents

Unless otherwise specified, all reagents used in this document are analytically pure.

Water shall meet the requirements for grade III water in ISO 3696.

5.1

Anhydrous sodium sulfate (Na_2SO_4).

5.2 Isooctane (2,2,4-trimethylpentane). absorbance not exceeding 0.01 with water as a

reference in the wavelength range of 300 nm~380 nm.

5.3 p-Anethole (4-anisole). anhydrous, milky white crystals.

WARNING. p-Anisidine is toxic and shall be avoided in contact with skin.

Store p-anisidine in a brown bottle. Store in the dark at 0°C~4°C. p-Anisidine shall be milky white. If it turns gray or pink, purify it as follows.

Dissolve 4 g of p-anisidine in 100 mL of distilled water at 75°C. Add 0.5 g of sodium sulfite and 2 g of activated carbon. Stir for 5 min. Filter with medium-speed filter paper to obtain a clear filtrate. Cool the filtrate to 0°C. Maintain this temperature for at least

4 h to obtain p-anisidine crystals. Filter or vacuum filter the crystals. Wash with a small amount of distilled water at 0°C. Dry the purified p-anisidine crystals in a vacuum desiccator.

5.4 Glacial acetic acid. the water content by mass fraction is not greater than 0.1%.

5.5 Anisidine reagent. anisidine is toxic and unstable. Prepare only the minimum amount required for the analysis on the same day. For example, a 50 mL reagent can be prepared as follows. accurately weigh 0.125 g of p-anisidine, dissolve in glacial acetic acid, and dilute to volume in a 50 mL volumetric flask, avoiding direct sunlight.

Before use, measure the absorbance using isooctane as a blank control. If the absorbance is above 0.2, the reagent shall not be used. Under no circumstances shall the prepared reagent be used on the same day.

6 Equipment

6.1

Spectrophotometer. usable in the 350 nm wavelength range, with a 10 mm cuvette.

When using a dual-beam spectrophotometer, it is advisable to use a pair of matching 10 mm cuvettes.

6.2

Volumetric flask. 25 mL.

6.3

Stoppered test tube. 10 mL.

6.4

Pipettes. 1 mL, 5 mL.

7 Sampling

Sampling method is described in ISO 5555.

Samples received by the laboratory shall be representative. They shall also be free from damage or deterioration during transportation or storage.

8 Preparation of specimens

Prepare the specimens according to ISO 661.

If the moisture content of the specimen is greater than 0.10% by mass, dry it according to the following method.

Because the presence of water can affect the reaction equilibrium, it is advisable to remove

moisture during specimen preparation. Add anhydrous sodium sulfate (5.1) at a ratio of 1 g~2 g per 10 g of sample. Mix thoroughly. If the specimen is a solid fat, it shall be melted first. The melting temperature shall not exceed 10°C above the melting point. After adding anhydrous sodium sulfate, stir thoroughly and filter. Maintain this temperature to prevent the sample from solidifying.

9.1 Preparation of test solution

Weigh an appropriate amount of the specimen (Chapter 8), accurate to 1 mg. Dissolve in 5 mL~10 mL of isooctane (5.2). Dilute to volume in a 25 mL volumetric flask. The solid specimen shall be preheated to above its melting point, but not exceeding 10°C below the melting point.

The sample mass is determined by the properties of the specimen and the spectrophotometer used, and ranges from 0.4 g~4.0 g. The absorbance of the specimen solution must not exceed the upper and lower limits of the spectrophotometer reading.

9.2 Determination of unreacted solution

Using a pipette (6.4), transfer 5 mL of the test solution (9.1) into a stoppered test tube (6.3). Using a pipette (6.4), add 1 mL of glacial acetic acid (5.4) solution. Stopper the tube and shake well. Incubate in the dark at $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 8 min.

Transfer the solution to a clean, dry cuvette in a spectrophotometer within 2 min. The total reaction time, starting from the addition of the glacial acetic acid solution, is $10 \text{ min} \pm 1 \text{ min}$. Measure the absorbance according to 9.5.

9.3 Determination of the reaction solution

Using a pipette (6.4), transfer 5 mL of the test solution (9.1) into a stoppered test tube (6.3). Using a pipette (6.4), add 1 mL of anisidine reagent (5.5). Stopper the tube and shake well. Incubate in the dark at $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 8 min.

Transfer the solution to a clean, dry cuvette in a spectrophotometer within 2 min. The total reaction time, starting from the addition of the anisidine reagent, is $10 \text{ min} \pm 1 \text{ min}$.

Measure the absorbance according to 9.5.

9.4 Preparation of blank test solution

Using a pipette (6.4), transfer 5 mL of isooctane (5.2) into a stoppered test tube (6.3).

Using a pipette (6.4), add 1 mL of anisidine reagent (5.5). Stopper the tube and shake well. Incubate in the dark at $23^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 8 min.

Transfer the solution to a clean, dry spectrophotometer cuvette within 2 min. The total reaction time, starting from the addition of the anisidine reagent, is $10 \text{ min} \pm 1 \text{ min}$.