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**Inspection of grain and oils - Determination of
phosphatide content**

粮油检验 磷脂含量的测定

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Inspection of grain and oils - Determination of phosphatide content

1 Scope

This Standard specifies the terms and definitions, principle, instruments and utensils, reagents and solutions, operation steps, result calculation and repeatability for the determination of phosphatide content in vegetable oil.

This Standard is applicable to vegetable crude oil, degummed oil and refined oil.

Method One of this Standard is arbitration method.

2 Normative references

The following documents are essential to the application of this Standard. For the dated documents, only the versions with the dates indicated are applicable to this document; for the undated documents, only the latest version (including all the amendments) are applicable to this Standard.

GB/T 5524, *Animal and vegetable fats and oils - Sampling*

GB/T 15687, *Animal and vegetable fats and oils - Preparation of test sample*

3 Terms and definitions

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

3.1 phosphatide

a compound composed of glycerin, fatty acid, phosphoric acid, amino alcohol and cyclic alcohol

Method One - Molybdenum blue colorimetry

4 Principle

Phospholipid in vegetable oil is burned into phosphorus pentoxide. It is turned

6.5 Sodium molybdate;

6.6 Hydrazine sulfate.

6.7 Potassium dihydrogen phosphate: Dry at 101°C for 2h before use.

6.8 2.5% sodium molybdate dilute sulfuric acid solution: Measure 140mL of concentrated sulfuric acid (6.4). Inject into 300mL of water. Cool to room temperature. Add 12.5g of sodium molybdate. After dissolving, use water to set volume to 500mL. Shake well. Leave it for 24h for future use.

6.9 0.015% hydrazine sulfate solution: Dissolve 0.15g of hydrazine sulfate (6.6) in 1L of water.

6.10 50% potassium hydroxide solution: Dissolve 50g of potassium hydroxide (6.3) in 50mL of water.

6.11 1:1 hydrochloric acid solution: Dissolve hydrochloric acid (6.1) in an equal volume of water.

6.12 Phosphate standard stock solution: Weigh 0.4387g of dry potassium dihydrogen phosphate (6.7). Use water to dissolve, dilute and set volume to 1000mL. This solution contains 0.1mg/mL phosphorus.

6.13 Phosphate standard solution for standard curve: Use a pipette 10mL of standard stock solution (6.12) to a 100mL volumetric flask. Add water to dilute and set volume. This solution contains 0.01mg/mL phosphorus.

7 Operation steps

7.1 Sampling

In accordance with GB/T 5524.

7.2 Specimen preparation

In accordance with GB/T 15687.

7.3 Drawing of standard curve

Take six colorimetric tubes (5.9) and turn them into six numbers: 0, 1, 2, 4, 6, and 8. According to the number order, respectively inject 0mL, 1mL, 2mL, 4mL, 6mL, 8mL of standard solution (6.13). Then according to the sequence, respectively add 10mL, 9mL, 8mL, 6mL, 4mL, 2mL of water. Then add 8 mL of hydrazine sulfate solution (6.9) and 2mL of sodium molybdate solution (6.8) into the six colorimetric tubes. Add stoppers. Shake 3~4 times. Remove stoppers. Put the colorimetric tubes into a boiling water bath (5.5) and heat for 10min.

X - Phospholipid content, in milligrams per gram (mg/g);

P - Phosphorus content of the test solution checked by the standard curve, in milligrams (mg);

m - Specimen mass, in grams (g);

V₁ - Diluted volume after the sample is ashed, in milliliters (mL);

V₂ - Volume of the test solution taken during color comparison, in milliliters (mL);

26.31 - Number of milligrams of each milligram of phosphorus equivalent to phospholipids.

When the extinction value of the tested solution is greater than 0.8, it needs to appropriately reduce the volume of the tested solution, so as to ensure that the extinction value of the tested solution is below 0.8.

Each sample shall be tested twice in parallel. When the result of parallel test meets the precision requirements, take its arithmetic mean as the result. The calculation result keeps three decimal places.

9 Repeatability

The absolute difference between two independent determination results obtained under repeated conditions shall not exceed 10% of the arithmetic mean.

Method Two - Weighing method

10 Principle

Phospholipid in vegetable oil swells with water. Density increases. Turn it from flocculent suspended matter to sediment. After the specimen is hydrated, use acetone to repeatedly wash and filter. Since phospholipid is not soluble in acetone, oil is soluble in acetone, so that the phospholipid can be separated from the oil. Weigh the mass of phospholipid. Calculate its content. The precipitated filtrate obtained by this method is not entirely phospholipids. There are other lipid substances that are insoluble in acetone.

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