

# LECITHIN

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**SYNONYMS** Phosphatides, phospholipids; INS No. 322(i)

**DEFINITION** Usually prepared from oil-bearing seeds used for food, especially soybeans; may also be prepared from animal sources; a complex mixture of acetone-insoluble phosphatides which consists chiefly of phosphatidylcholine, phosphatidyl-ethanolamine, and phosphatidyl-inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates; refined grades may contain any of these components in varying proportions and combinations depending on the type of fractionation used; its oil-free forms, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex.

C.A.S. number 8002-43-5

Assay Not less than 60% of acetone-insoluble matter (phosphatides)

**DESCRIPTION** Consistency of both natural grades and refined grades may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents; from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached; odourless or has a characteristic, slight nut-like odour. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavour characteristics.

**FUNCTIONAL USES** Emulsifier, antioxidant

## CHARACTERISTICS

### IDENTIFICATION

Solubility (Vol. 4) Only partially soluble in water; readily hydrates to form emulsions; oil-free phosphatides are soluble in fatty acids, but practically insoluble in fixed oils

Test for phosphorus Ignite 1 g of the sample with 2 g of anhydrous sodium carbonate. Cool and dissolve the residue in 5 ml of water and 5 ml of nitric acid. Add 5 ml of ammonium molybdate TS and heat to boiling. A yellow precipitate is obtained.

Test for choline To 0.5 g of the sample, add 5 ml of diluted hydrochloric acid (1+1), heat in a water bath for 2 h, and filter. Use this solution as the test solution. Perform

*Paper Chromatography* with 10 µl of the test solution, using choline chloride solution (1+200) as the control solution and n-butanol-water-acetic acid mixture (4:2:1) as the developing solvent. A red-orange spot corresponding to the spot obtained from the control solution is observed. For the filter paper, use a No. 2 filter paper for chromatography. Stop the development when the developing solvent rises about 25 cm, air-dry, spray with Dragendorff TS to develop a colour, and observe in daylight.

Test for fatty acids Reflux 1 g of the sample for 1 h with 25 ml of 0.5 N ethanolic potassium hydroxide. When cooled to 0°, a precipitate of potassium soap is obtained.

Test for hydrolysis To a 800 ml beaker add 500 ml of water (30-35°). Then slowly add 50 ml of the sample with constant stirring. Hydrolyzed lecithin will form a homogeneous emulsion. Non-hydrolyzed lecithin will form a distinct mass of about 50 g.

#### PURITY

Loss on drying (Vol. 4) Not more than 2% (105°, 1 h)

Acid value Not more than 36  
See description under TESTS

Peroxide value Not more than 10  
See description under TESTS

Toluene-insoluble matter Not more than 0.3%  
See description under TESTS

Lead (Vol. 4) Not more than 2 mg/kg

Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

#### TESTS

Acid value Weigh accurately about 2 g of the well-mixed sample into a 250-ml Erlenmeyer flask. Dissolve in 50 ml of petroleum ether by shaking gently. Then add 50 ml of ethanol, previously neutralized to phenolphthalein with 0.1 N sodium hydroxide, and shake to mix. Add 4 drops of phenolphthalein TS and titrate while shaking with 0.1 N sodium hydroxide until the pink colour persists for 5 sec.

$$\text{Acid value} = \frac{\text{ml } 0.1 \text{ N NaOH} \times 5.6}{\text{weight of sample (g)}}$$

Peroxide value Reagents  
- Acetic acid-chloroform solution: Mix 3 volumes of acetic acid with 2

volumes of chloroform.

- Potassium iodide solution, saturated: Dissolve excess potassium iodide in freshly boiled water. Excess solid must remain. Store in the dark. Test daily by adding 0.5 ml to 30 ml of the acetic acid-chloroform solution, then add 2 drops of starch TS. If the solution turns blue, requiring more than 1 drop of 0.1 N sodium thiosulfate to discharge the colour, prepare a fresh solution.

#### Procedure

Weigh accurately about 5 g of the sample into a 250-ml Erlenmeyer flask. Add 30 ml of the acetic acid-chloroform solution and swirl to dissolve. Add 0.5 ml of the saturated potassium iodide solution, allow to stand with occasional shaking for 1 min, and add 30 ml of water. Slowly titrate with 0.01 N sodium thiosulfate with vigorous shaking until the yellow colour is almost gone. Add about 0.5 ml of starch TS, and continue the titration, shaking vigorously to release all the iodine from the chloroform layer, until the blue colour disappears.

Perform a blank determination and make any necessary correction.

$$\text{Peroxide value} = \frac{S \times N \times 1000}{W}$$

where

S = ml of N sodium thiosulfate

N = normality of sodium thiosulfate

W = weight of the sample (g)

Toluene-insoluble matter Weigh 10 g of the well-mixed sample into a 250-ml flask. Add 100 ml of toluene and shake until dissolved. Filter through a tared filter funnel G3 or equivalent with a porosity of 16-40  $\mu\text{m}$ . Wash the flask with 25-ml portions of toluene and pour the washings through the funnel. Place the funnel in a forced-draft oven and dry at 105° for 1 h. Weigh dried funnel and subtract tare to determine weight of toluene insoluble residue:

$$\frac{\text{weight of residue (g)}}{\text{weight of sample (g)}} \times 100 \%$$

## **METHOD OF ASSAY**

#### Purification of phosphatides

Wash about 10 g of the sample 3 times well with each 100 ml of acetone. The insoluble residue (phosphatides) is used. Residues (phosphatides) obtained from assays carried out previously can also be used. Dissolve 5 g of these phosphatides in 10 ml of petroleum ether, and add 25 ml of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-ml centrifuge tubes using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 ml with acetone, stir again, chill for 15 min in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the phosphatide-acetone solution. To saturate about 16 litres of acetone, 5 g of the purified phosphatides are required.

### Phosphatide acetone solution

Add a quantity of purified phosphatides to sufficient acetone, previously cooled to a temperature of about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h., shaking it vigorously at 15-min. intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

### Procedure

If lecithin is plastic or semisolid, soften a portion of the sample by warming it in a water bath at a temperature not exceeding 60° and then mixing it thoroughly. Transfer about 2 g of a well-mixed sample, accurately weighed, into a previously tared 40-ml centrifuge tube, containing a glass stirring rod, and add 15 ml of Phosphatide-Acetone Solution from a buret. Warm the mixture in a water bath until the lecithin melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, and then transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about one half of the required volume of Phosphatide-Acetone Solution, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 ml with chilled Phosphatide-Acetone Solution (5°), again stir, and return the tube and contents to the ice bath for 15 min. At the end of the 15-min chilling period, stir again while still in the ice bath, remove the stirring rod, temporarily supporting it in a vertical upside-down position, and centrifuge the mixture immediately at about 2000 rpm for 5 min. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the same stirring rod previously used, and refill the tube to the 40-ml mark with chilled (5°) Phosphatide-Acetone Solution and repeat the chilling, stirring, centrifugation, and decantation procedure previously followed. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the assigned stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min in a forced-draft oven, cool, and weigh.

Calculate the percentage of acetone-insoluble matter by the formula  $(100R/S) - B$ , in which R is the weight of residue, S is the weight of the sample, and B is the percentage of toluene-insoluble matter (see TESTS).