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ANALYSIS of FATS in FOODS

Technical Bulletin

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WHAT IS FAT?

Fats are primarily the triesters of fatty acids and glycerol, and thus are commonly called triglycerides. Solid triglycerides are referred to as fat while liquid triglycerides are called oils. Lipids, on the other hand, include all the “fatty” materials (i.e. those materials dissolved in a fat solubilizing solvent) in a food. This includes the sterols, mono, di, and triglycerides, phospholipids, glycolipids, free fatty acids, fat soluble vitamins, etc.

For nutrition labeling purposes, fat is defined as the sum of the fatty acids in the food, regardless of source, expressed as triglyceride equivalents (see 21CFR§101.9(c)(2)). These fatty acids may be present as free fatty acids, mono, di, and triglycerides, phospholipids, glycolipids, or sterol lipids. Individual fatty acids are classified according to their degree of unsaturation. These classifications include saturated, monounsaturated, polyunsaturated and *trans* fatty acids.

FAT METHODS

Historically, a number of methods have been developed for the analysis of fats in various foods and food products, based upon the ease or difficulty of removing fat from a given matrix and depending upon whether fat or total lipids are to be extracted from the sample.

The common methods used to determine fat are the gas chromatography (GC) method and the solvent extraction-gravimetric method. The GC method must be used for accurate nutritional labeling to include *trans*, and should be used for research projects. This method provides accurate quantitation of total fat as well as the individual fatty acids; saturated, mono and polyunsaturated fat and *trans* fat. The gravimetric method can be used for quick fat determinations and relative comparisons between samples, but the results can be approximately 10% higher than fat by GC results. This is due to certain nonfat components such as amino acids, organic acids, sugars, glycerol and low molecular weight carbohydrates that are typically extracted with the fat causing the results to be erroneously high.

Procedure:

FAT by GC (FATGC)

Triglycerides and other fatty acid containing molecules are released from food matrices using an acid or base hydrolysis, and extracted into a mixture of ethyl and petroleum ether. Pyrogalllic acid is added to minimize oxidative degradation of fatty acids. The fatty acids of the fatty acid-containing compounds are then esterified or transesterified with a solution of boron trifluoride in methanol (BF_3/MeOH) to form fatty acid methyl esters (FAMES). FAMES are quantitatively measured by capillary gas chromatography by comparing them to a known quantity of internal standard. Total fat is calculated as the sum of individual fatty acids expressed as their respective triglyceride equivalents. Saturated, monounsaturated, and polyunsaturated fats are calculated as the sum of the respective component fatty acids. *Cis and trans* fatty acids and omega 3's can be totaled as well.

GRAVIMETRIC PROCEDURES

ACID HYDROLYSIS

The acid hydrolysis method extracts fat from the sample by subjecting it to hydrochloric acid followed by extraction with mixed ethers. The hydrochloric acid breaks fatty acids from the glycerides, glyco- and phospholipids and sterol esters. Acid hydrolysis also breaks lipid-carbohydrate bonds, assists in the hydrolyzing of proteins and polysaccharides, and disrupts cell walls. All of this makes the lipids available for complete extraction with mixed ethers, the ether is evaporated and the extracted residue weighed. Samples run by the acid hydrolysis method are baked products, bread, cereal, cooked product, egg, fish, flour, food dressings, fruit, grain, mixes, pasta, product with cocoa, seafood, and vegetables.

BASE HYDROLYSIS

The base hydrolysis method, typically used for dairy matrices (Roese-Gottlib), extracts fat from the sample by treating it with ammonium hydroxide followed by extraction with mixed ethers. The ammonium hydroxide weakens lipid-protein bonds in order to disrupt the casein, breaks up fat emulsions, and neutralizes any endogenous acid prior to the extraction with mixed ethers. A centrifugation step also aids in the breaking of emulsions (common in dairy products) and in separating the ether layer from the aqueous phase. Samples are extracted into mixed ethers and total fat is calculated gravimetrically after evaporation and residue weighing. Samples run using the base hydrolysis method are dairy products such as cream cheese, milk, non-fat dry milk, yogurt, and whey.

ACID AND BASE HYDROLYSIS

The combined acid and base hydrolysis method, sometimes necessary for matrices where the fat is difficult to remove, extracts fat from the sample by treatment with ammonium hydroxide then by hydrochloric acid solution, followed by extraction with mixed ethers. The ammonium hydroxide weakens the lipid-protein bonds and the acid breaks the lipid-carbohydrate bonds making the lipid available for complete extraction. Samples are extracted into mixed ethers and total fat is calculated gravimetrically. Samples run by the combined acid and base hydrolysis method are cheese, cheese blends, entrees with cheese, products with cheese, pizza roll filling, and pizza.

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