AAFCO Update on AOCS Fatty Acid Composition Methods

26 July 2014

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AOCS Official Method Ce 2b-11

Direct Methylation of Lipids in Foods by Alkali Hydrolysis

DEFINITION

This method describes a simultaneous alkali hydrolysis and methylation procedure without prior digestion option for the preparation of fatty acid methyl esters (FAME) directly from food matrices. The incorporation of triacylglycerol (TAG) standards allows the quantification of total fat and fatty acids using Theoretical Correction Factors (TCFs) and Empirical Correction Factors (ECFs).

SCOPE

This method may be applicable for fat-containing matrices (e.g., food stuffs, beverages, tissues, and oils). The results of the collaborative study indicate that most matrices do not require acid pretreatment. Extruded cat, dog foods and oat based foods gave low results, thus, analysts are advised to test the chosen method against appropriate reference methods. Materials with high moisture contents do not require drying before analysis. However, if drying is required (except when freeze drying) antioxidants should be added to protect from oxidation. An appropriate grinding technique for homogenization of the test sample should be used, when necessary.

This method is not suitable for steryl ester concentrates and steryl ester-fortified food products.

AOCS Official Method Ce 2c-11
Direct Methylation of Lipids in Foods by Acid-Alkali Hydrolysis

DEFINITION

This method describes an acid-alkaline procedure for the preparation of fatty acid methyl esters (FAME) directly from food matrices. The fats or oils are released from the matrix by *in situ* acid digestion (hydrochloric acid in methanol) followed by alkali hydrolysis (sodium hydroxide in methanol) and methylation (with boron trifluoride in methanol as the catalyst).

SCOPE

This method is only required when AOCS Official Method Ce 2b-11 does not release all fatty acids quantitatively.

Some food matrices such as extruded cat and dog foods, oat based foods, and some encapsulated oils require acid pretreatment. Some long chain polyunsaturated fortified beverages show lower recoveries using this method. Since not all matrices could be evaluated, analysts are advised to test the chosen method against appropriate reference methods. Many materials with high moisture contents do not require drying before analysis. However, antioxidants should be added if drying is required (except when freeze drying) to protect from oxidation. An appropriate grinding technique for homogenization of the test sample should be used, when necessary.

Analysis of steryl ester concentrates and steryl ester-fortified food products are outside the scope of this method since greatly increased saponification times are required.

AOCS Official Method Ce 1j-07

Determination of *cis-, trans-,* Saturated, Monounsaturated, and Polyunsaturated Fatty Acids in Extracted Fats by Capillary GLC

DEFINITION

This method provides a gas–liquid chromatography (GLC) procedure for the determination of the fatty acid composition, including the *trans* fatty acid isomers of extracted fats. The fatty acid methyl esters (FAME) are separated on a capillary gas chromatography column having a highly polar stationary phase, according to their chain length (CL), degree of unsaturation, and geometry and position of the double bonds [DB(s)].

AOCS Official Method Ce 1j-07

Determination of *cis-, trans-,* Saturated, Monounsaturated, and Polyunsaturated Fatty Acids in Extracted Fats by Capillary GLC

SCOPE

This method is specially designed to evaluate, by a single capillary GLC procedure, the levels of *trans* isomers, saturated fatty acid (SFA), *cis*- and *trans*-monounsaturated fatty acid (MUFA), and *cis*- and *trans*-polyunsaturated fatty acid (PUFA) levels in dairy and ruminant based fat samples from the same sample and analysis. The method is not designed to provide detailed definitive isomer composition that may be desired for nutritional and/or biochemical purposes. To obtain more detailed isomeric composition information prior argentation chromatographic separations and/or additional GC analyses are required. For nutritional labeling purposes the total fat, saturated, *cis*-monounsaturated, *cis*-polyunsaturated and *trans* fatty acid contents are to be determined. This method may also determine *cis*-polyunsaturated fatty acids (PUFA), including arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).

This method utilizes a triacylglycerol (13:0 TAG) internal standard (IS) for determining the concentration of the individual fatty acids in the oil samples after methylation. The method is applicable to fats derived from dairy and ruminant products. This method is not applicable to products containing mixtures of both dairy and vegetable fats as the *trans* linolenic acid (18:3) isomers will coelute with the gondoic acid (20:1) isomers. In that case both this method coupled with AOCS Ce 1h-05 would be required for analysis. Conjugated linoleic acids (CLAs) will be present in dairy and ruminant fats and may be quantitated with this method, however for nutritional labeling purposes CLA are not included either as *cis*- or *trans*-PUFA (References 1 and 2). There is minor co-elution of *cis*- and *trans*- fatty acid isomers, particularly in the 16:1, 17:1, and 18:1 regions, using this technique.

Theoretical Correction Factors (TCFs) are used to quantitate all saturated, monounsaturated, and polyunsaturated fatty acids (PUFA) of 18 carbons. TCFs are also used for fatty acids, which lack pure standards. Empirical Correction Factors (ECFs) are used for very long chain PUFA of 20 carbons or more and three or more double bonds for which standards are readily available.

Data from collaborative studies here