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Approach to fat analysis of foods

Lea Hyvönen

Department of Food Technology, University of Helsinki, P.O. Box 27 (Viikki B), FIN-00014 University of Helsinki, Helsinki, Finland

Behind the nutritional labelling of food fat hides a problem of definition of fat, as well as an analytical problem. Although triacylglycerols are the prevailing structure of the food lipids in most cases, there are exceptions, too. Analytes called ether extract, crude fat, total fat and total lipids have been interpreted to food fat in nutritional labelling and food databases. However, the techniques traditionally used for fat determination in foods may vary considerably in their ability to recover the various lipid components. Diversity in the lipid composition of various foods and the effects of processing and storage on the diversity and availability of the fat make the correct nutritional labelling of fat problematic. The traditional methods, where the total fat content is determined by extracting the fat with an appropriate fat solvent or a solvent mixture, give good technical measures. The use of this extracted fat-soluble material as the nutritional concept of fat may be misleading. We have suggested (Hyvönen *et al.*, 1993, *J. Food Comp. Anal.*, **6**, 24–40) the use of the concept of net fat in nutritional food labelling. This definition of fat includes all the unchanged fatty acids from the various food lipids converted to triacylglycerols. In energy calculations 1 g of net fat corresponds to 9 kcal or 38 kJ. This concept reduces the energy values of foods because it eliminates the lipid components, which are not real fats, from the calculation. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The determination of fat content of food is one of the most common analyses performed in a food laboratory. The quantitative extraction of fat from samples, however, is not straightforward (Sheppard *et al.*, 1974). One of the difficulties in the determination of fat content is the definition of fat. What do we mean by fat? What is the difference between a fat and a lipid? Novel foods such as non-fat margarines probably confuse consumers as well as the scientists and legislators.

DEFINITIONS

Dictionaries typically define fats as glycerides of fatty acids and lipids as any of the various substances that are soluble in organic solvents including fats, waxes, phosphatides, cerebrosides and related and derived compounds. For an analyst it would be almost impossible to find an extraction procedure to determine only the glycerides of fatty acids in most foods. The above definition of lipids could include such organic solvent-soluble substances as carbohydrates and protein fractions and some food additives which analysts would not class as lipids.

Terms such as 'simple' and 'complex' lipids are also commonly used. Simple lipids are esters of fatty acids and alcohols, usually glycerol, but sterol and wax esters also belong to this group. Fat and simple lipids are also called 'neutral' lipids owing to their solubility in non-polar solvents. Complex lipids include phospholipids, glycolipids and lipoproteins. The term 'polar' lipid is used to describe complex lipids because of the polar nature of some of their functional groups and consequently their preferential solubility in polar solvents.

The terms ether extract, crude fat, total fat and total lipids are used interchangeably in compositional analysis of food. In other words, there are many definitions and classifications for fats and lipids, but how can analysts be sure that the fat they are determing complies with the definition without considerable additional analytical work? In practice the analysts often use standard procedures for the determination of fat in a particular food (e.g. meat, milk) or food type (dairy products, cereal products). When doing so they may not need to know what they are extracting and determining because the standard method itself will chemically define the fat content under the conditions of determination. However, this leads to a multitude of definitions of fat.

Table 1. Composition of total fat in various foods. Total fat as wt%, triacylglycerol (TAG) fraction and sum of other lipid fractions ('others') as percentage of total fat

	Total fat wt%	TAG	Others
Milk[1]	3.6	94	6
Soya[1]	23.0	88	12
Pork[2]	8.4	84	16
Egg[2]	33.5	65	35
Wheat[1]	1.5	41	59
Apple[1]	0.1	5	95

[1]Berlitz & Grosch (1987).
[2]Weihrauch *et al.* (1977).

FAT CONTENT OF FOOD FOR ENERGY CONTENT CALCULATIONS

The fat content of food is one of the most often utilized values of food databases and it is usually used to evaluate the energy content of food. In food labelling fat content has a similar role. Most often the total fat of food is converted to energy using the energy value 9 kcal/g of triglycerides for the calculation. The lipid composition of the total fat (extractable fat-soluble material) is inhomogeneous and dependent on the food source and processing, for example. The effect of the origin of the total fat on the lipid composition is shown in Table 1. It can be noted that the proportion of 'real fat', triacylglycerols, is dominant in milk and soya fat, but significantly less so in wheat and apple fat.

In our recent study we characterized the lipid components of 10 processed (baked and fried) fat-containing foods and found polymerization, oxidation and hydrolysation products in the extracted total fat fraction (Hopia *et al.*, 1994). The availability of the oxidized and polymerized lipid material for energy is questionable. In the conventional method the total fat may include this type of changed lipid material in addition to the constituents which can hardly be regarded as a part of the nutritional concept of 'fat'. Such compounds include sphingosines, phosphoric acid, organic bases, mono-, di- and oligosaccharides, ethane, propane and butane diols, fatty alcohols and sterols. In food analysis many of these compounds are determined separately or with other nutrients. Calculating energy values on the basis of the extractable fat material, total fat, is problematic (Miles *et al.*, 1984).

TOTAL FAT TO DETERMINE THE FATTY ACID CONTENT

The relative proportions of fatty acids have been determined from the total fat. In the past, the quantities of fatty acids were determined directly using the percentage data of fatty acids and the total fat content assuming that the total fat equalled the triacylglycerols. Later, food-specific correction factors (Weihrauch *et al.*, 1977; Holland *et al.*, 1991) were used to eliminate the effect of non-triacylglycerol material on the fatty acid content. Because of differences, for example, in (phospho)lipid composition, weight of (phospho)lipid classes and fatty acid weights in the lipid structures the conversion factors are, however, approximations. In addition, relevant correction factors for every food or food group are not available, they are tedious to produce and the relevance of the factors for composed and processed foods can be questioned.

CONCEPT OF NET FAT

In the late 1980s our research group analysed the fatty acid contents of about 300 commercial food items important as sources of fat in Finland (Hyvönen *et al.*, 1993; Hyvönen & Koivistoinen, 1994). Our main purpose was a direct quantitative fatty acid analysis. Therefore the most efficient extraction method for each food group was selected using as criterion the highest recovery of fatty acids in the extract. Owing to the awareness of the inhomogeneous nature of the total fat or extractable fat material and the possible presence of non-lipid material in it, we argued for using this indefinite analyte as a measure of fat in food databases once we knew the 'total fat' figures used for food energy calculations. From the literature (Krishnamoorthy *et al.*, 1979; Miles *et al.*, 1984) we found that the energy value 9 kcal/g is an average value for triglycerides. How relevant is it then to use the energy value of triglycerides for the extractable fat material of an unknown composition? We came to the conclusion that a concept of net fat, where all the quantitated fatty acids from various lipid structures of food are converted to triacylglycerols by calculation, is a nutritionally less ambiguous concept than the traditional extracted total fat. After direct quantitative determination of fatty acids we converted the fatty acid content to the corresponding amount of triacylglycerols using the conversion factor 1.046 (Hyvönen *et al.*, 1993).

In the United States, the Food and Drug Administration gave new regulations for nutritional labelling of food in 1993 (FDA, 1993). The new regulations define total fat as the sum of lipid fatty acids from all sources expressed as triglycerides. There are no agreed-upon methods for the measurement of individual fatty acid content of foods, however. The European Council Directive on nutrition labelling for foodstuffs (90/496) (EEC, 1990) defines fat as 'total lipids, and includes also phospholipids' in other words it is the traditional definition of extracted lipid material.

The relevance of the net fat concept is based on quantitative fatty acid analysis. This means reliable methodology from sampling to the quantitation of fatty acid data. We have analysed fatty acids as methyl esters by capillary gas chromatography. The fatty acid methyl esters were identified using a two-channel retention index monitoring (RIM) technique. Quantitation was based on an internal standard method using nonadecanoic (C19:0) acid methyl ester as the internal

standard. The results were corrected with the empirical response factors and methyl group correction factors. The conversion factor 1.046 was used to express the sum of quantitated fatty acids as net fat or triacylglycerol equivalents.

The molecular weight correction for $acid_i$ ($MW_{acid_i,corr} = \frac{(12.67+MW_{acid_i})}{MW_{acid_i}}$) in condensing a fatty acid to the triglyceride weight is proposed by House *et al.* (1994). Using specific factors for converting the fatty acids to triacylglycerols, instead of using just the single factor (1.046), improves further the accuracy of the net fat calculation. The use of theoretical response factors instead of empirical ones in conversion of area per cent to weight per cent is of benefit in fatty acid analysis, when the GC instrument is optimized. Craske (1993) concluded, on the basis of a collaborative study, that few analysts have spent sufficient time optimizing their chromatograph, however. The need for standards for determining possible errors in the methodology is also evident. Perhaps the major challenge will be in quantitating total fat in foods with very low fat content and/or those with complex carbohydrate ingredients.

TOTAL FAT VERSUS NET FAT

As an example of the difference between fat values obtained when expressed either as total fat or as net fat I will present our recent results. We analysed the total fat of 10 different processed foods using either hexane:isopropanol (1:1) or chloroform:methanol (1:1) one-phase extraction. Net fat was determined analysing the individual fatty acids by capillary GC, and the sum of the quantitated fatty acids was converted to triacylglycerols using the conversion factor 1.046. The methyl group correction and theoretical response factors were used in quantitation of fatty acids. Table 2 gives the results of the comparison. Net fat content of all the analysed foods was lower than the total fat content. Net fat content ranged from 4.2 to 26.6% of the fresh weight. The difference in fat content expressed as crude fat instead of net fat varied from 6.7 to 46%. Consequently almost half of the contents of the total fat in some foods can consist of other components and structures other than triacylglycerols. We characterized the structures of the non-triacylglycerol part of the total fat using HPSEC exclusion chromatography. Polymerization products of triacylglycerols, polar (oxidized) triacylglycerols, diglycerides and monoglycerides, which might have also oxidized structures, and another fraction, which contained the free sterols and fatty acids, were detected. The proportion of this polar, non-triacylglycerol fraction ranged from 2.0 to 19.8% of the total fat (Viinanen *et al.*, 1996).

Table 2. Crude fat (g/100 g), net fat (g/100 g) and net fat as a percentage of crude fat

	Crude fat	Net fat	Net fat as % of crude fat
Potato chips[1]	28.5	26.6	93
Cheese puffs[1]	30.6	25.6	84
Wiener[1]	20.9	16.8	80
Doughnut[1]	15.4	12.1	79
Meat pie[1]	11.4	9.6	84
Cinnamon roll[1]	9.6	7.7	80
French fries, prefried[2]	8.5	6.5	76
French fries, from restaurant[2]	14.4	11.7	81
Fish sticks[2]	7.8	4.2	54
Hamburgers[2]	18.0	13.2	73

[1]Fat extraction hexane:diethylether (1:1).
[2]Fat extraction chloroform:methanol (1:1).

CONCLUSION

The analytical data prove that the traditional extracted total fat values contain such inaccuracies that it is difficult to compare the energy content of fat origin in different foods. In our opinion the use of the net fat concept would express the fat content of the foods as a source of energy in a more commensurable manner. The other compounds extractable with triacylglycerols should be identified and quantitated as separate compounds or compound groups after which their nutritional significance could be evaluated more precisely.

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