Master thesis of EMQAL project

Evaluation of Extraction Methods for Recovery of Fatty Acids from Marine Products

Liping Xiao 肖丽平

Supervisor: Svein Are Mjøs, Nofima Ingredients,

Bjørn Grung, University of Bergen

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Abstract

The extraction efficiency of Soxhlet, acid hydrolysis and Bligh and Dyer were evaluated by using direct methylation on extracts and residues for calculating the mass balance of fatty acids for eight marine powders (fishmeals, krillmeals, cod filet, salmon filet and herring roe).

The results show that Soxhlet gave lowest extracted fatty acid content, especially for the samples which contain a high amount of phospholipid. Acid hydrolysis and Bligh and Dyer extract gave comparable extracted fatty acid contents with direct methylation. The mass balance of fatty acids in extract and residue is close to 100% for the three extraction methods which indicate that fatty acid was not lost during the extraction procedures. The difference of extracted fatty acids is mainly due to the different extracting efficiency.

The gravimetric lipid has limited correlation with total fatty acids, especially for Soxhlet.

Analyses of the fatty acid profiles showed that the Soxhlet extracts were different from the others. Extracts from the acid hydrolysis and Bligh and Dyer methods had similar fatty acid profiles as the direct methylation method.

The precision of fatty acid analysis by direct methylation method for marine powders were also validated. The coefficient of variation was 5.11% for solid samples and 1.21% for liquid sample.

Key words: direct methylation, one-step methylation, fatty acids, Soxhlet, acid hydrolysis, Bligh and Dyer

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List of Abbreviations

AA	Arachidonic acid (20:4 n-6)
AE	acid hydrolysis extraction
ALA	Alpha-linolenic acid (18:3 n-3)
AR	acid hydrolysis reconstructed
B&D	Bligh and Dyer
BE	Bligh and Dyer extraction
BR	Bligh and Dyer reconstructed
CADs	charged aerosol detectors
C.O.	control oil
CV	coefficient of variance
DAG	diacylglyerols
DDT	dichloro-diphenyl-trichloroethane
DHA	docosahexaenoic acid (22:6 n-3)
DM	direct methylation
EPA	eicosapentaenoic acid (20:5 n -3)
FA	fatty acid
FAME	fatty acid methyl ester
FFA	free fatty acid
FID	flame ionization detector
GC	gas chromatography
IS	Internal standard
LA	linoleic acid (18:3 n-3)
LC	liquid chromatography
LPC	lysophosphatidylcholine
LPE	lyso-phosphatidyl ethanolamine
HPLC	high-performance liquid chromatography
MAG	monoacylglyerols
MUFA	monounsaturated fatty acid
PC	phosphatidylcholine
PCA	principle Component analysis
PCB	polychlorinated biphenyls
PE	phosphatidylethanolamine
PI	phoshatidylinositol
PUFA	polyunsaturated fatty acids
PL	phospholipid
PS	phosphatidylserine
SE	Soxhlet extraction
SR	Soxhlet reconstructed
SFA	saturated fatty acids
SOX	Soxhlet
TAG	triacylglycerols

1 Introduction

1.1 Lipid nutrition in fish products

Fish and fish products play an important role in human's life. Fish lipids are excellent sources of the essential polyunsaturated fatty acids (PUFAs) in both the omega-3 and omega-6 families of fatty acids. Omega-6 PUFAs are also derived from vegetable oil, whereas long chain omega-3 PUFAs, such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) derive mainly from fish [1].

In recent years, the significance of polyunsaturated fatty acids analysis has gained much attention because of their various biological activities in health and disease, especially the n-3 and n-6 fatty acids. These fatty acids play an important role in the prevention and treatment of cardiovascular diseases, autoimmune diseases, eye sight and the improvement of learning ability [2]. The American Heart Association (AHA) recommends that patients with cardiovascular disease eat a variety of fish (preferably oily) at least twice a week, or to consume about 1g of EPA+DHA per day, preferably from oily fish [3].

Fishmeal and fish oil are basically made from small, bony, and oily fish that otherwise are not suitable for human consumption and some is manufactured from by-products of seafood processing industries. Fishmeal and fish oil are among the major internationally traded food and feed commodities in the world. The trade in world fishmeal and fish oil totals about 4.0-4.5 million tonnes, of which fishmeal represents about 85%-90% [4]. They are globally important to livestock production, fish farming and human health.

Although most of the oil usually gets extracted during processing of the fishmeal, the remaining lipid typically represents between 6% and 10% by weight but can range from 4% to 20%. The lipids in fishmeal not only impart an excellent source of essential fatty acids but also provide a high content of energy to the diet. The lipids in fishmeal are easily digested by all animals. The predominant omega-3 fatty acids in fishmeal and fish oil are linolenic acid, decosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Incorporation of DHA and EPA found in fish meal into the diets of fish and other farm animals is a convenient method to ensure a proper concentration of these important omega-3 fatty acids in the human diet [5].

1.2 Lipid soluble organic pollutants

The consumption of fish may also cause potential health risk because of the presence of lipophilic organic pollutants, such as DDT, dieldrin, heptachlor, PCBs and dioxines. These contaminants are present in low levels in lakes, rivers, seas and oceans, etc. However, the fish species can concentrate the

environmental contaminants by bioaccumulation and biomagnifications. The fat soluble environmental contaminants concentrate in fatty tissue of fish. Thus, high levels of environmental contaminants may be stored in fatty tissue of fish and fish consumption is an important source of human exposure to the above-mentioned environmental contaminants [6].

Recently, more and more attention has been paid to the problem of optimizing the balance between the risk and benefit of fish intake [7, 8, 9]. To study the contaminants in fish products, usually the crude fat is extracted for further analysis and the lipid content is a key parameter to interpret data on organic contaminants [10, 11].

It is therefore necessary to have a good method to determine the lipid content and lipid composition in fish products for the following reasons:

- To evaluate the nutrition of fish products;
- To meet the requirements of international trade;
- To manage the animal feeding;
- To inspect chemical contaminants in fish products.

This method should not only be accurate and reliable but also convenient, cost efficient and environmental sound.

1.3 Total lipid and fatty acid composition analysis

The lipid content is traditionally gravimetrically determined by solvent extractions. There are a large number of methods for lipid extraction. Soxhlet method [12], acid hydrolysis method [13], Bligh and Dyer [14] are most commonly used in fish industry. Different extraction methods vary in their lipid extraction efficiency. The total lipid by solvent extraction represents the content of crude fat, which may also contain non-fat material and often fails to accurately estimate nutritional values in biological materials.

Total fatty acids are generally a better alternative for assessment of nutritional value than extractable lipids, especially for determination of digestible energy. Fatty acid components need to be converted into fatty acid methyl ester (FAME) before analyzed by GC. FAME can either be prepared by multistep methods, consisting of lipid extraction followed by transmethylation, or by direct methylation methods. Direct methylation combines extraction and transmethylation into one step. It overcomes several limitations of the multistep methodology, giving rise to a simpler and faster analysis, consuming less organic solvent [15,16,17].

Another advantage by the direct methylation methods is that fatty acids are released from the matrix by breaking the ester bonds. In general, direct methylation is therefore more efficient than extraction for recovering fatty acids in lipids that is tightly bound to the matrix, such as samples rich in phospholipids [15]. The main focus of the present study was to evaluate the efficiency of conventional lipid extraction methods in eight marine powders

(including lean fish, fat fish, fish meals and Krill meals) by using direct methylation on extracts and residues for calculating the mass balance of the fatty acids. Three classical extraction methods were studied: Soxhlet method, acid hydrolysis method and Bligh and Dyer. The analysis procedure is illustrated in Fig. 1 where the amount of fatty acids in Result 3 should be equal to the sum of Results 1 and 2.

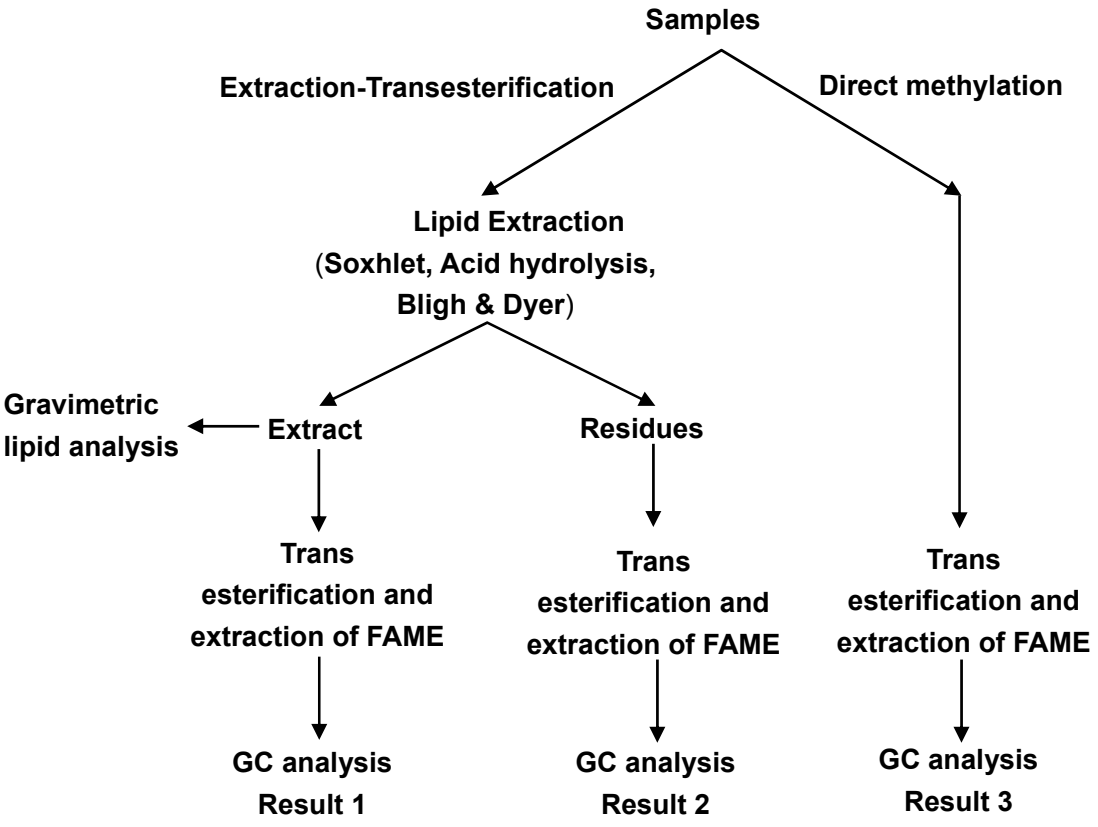


Figure 1. Flow diagram of analysis procedure

2 Theory

2.1 Lipids

The term “lipid” does not specify a particular chemical structure. Lipids are much more chemically diverse. There are operational and structural definitions of lipids.

A common structural definition is that lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. It includes cholesterol and bile acids, but does not include other steroids, fat-soluble vitamins, carotenoids, terpenes or mineral oil, except in rare circumstances [18]. Although the term *lipid* is sometimes used as a synonym for fats, fats are usually regarded as triacylglycerols, which is a subgroup of lipids [19].

Typical operational definitions of lipids are non-volatile substances that can be extracted from biological sources by solvents of low to medium polarity, where the conditions are further specified by the various methods. The lipid extracted by solvents is also called “crude fat” or “extractable fat”. Crude fat is heterogeneous material, consisting of a mixture of triacylglycerols, phospholipids, fatty acids, sterols, waxes and pigments. The gravimetrically determined content of crude lipids is usually referred to as “total lipid”.

Total lipid, as an estimate for energy content and nutritional values in biological material has been criticized because of the content of non-fat and non-digestible substances. The US Nutrition labeling and Education Act of 1990 (NLEA) has defined total fat as the sum of all fatty acids obtained from a total lipid extract expressed as triacylglycerols [20]. To avoid confusion with the total lipid by solvent extraction, “Total fatty acids” are used for the sum of the fatty acids expressed as triacylglycerols by direct methylation in this work.

2.2 Fatty acids

Fatty acids are compounds synthesized in nature via condensation of malonyl coenzyme A units by a fatty acid synthase complex. Fatty acids act as building blocks of lipids. In general, they contain even numbers of carbon atoms in straight chains (usually in the range C14 to C24),although the synthases can also produce odd- and branched chain fatty acids to some extent when supplied with the appropriate precursors; other substituent groups, including double bonds, are normally incorporated into the aliphatic chain later by different enzyme systems [18]. Fatty acids can either be saturated, monounsaturated or polyunsaturated depending on the number of double bonds.

Saturated fatty acids (SFAs)

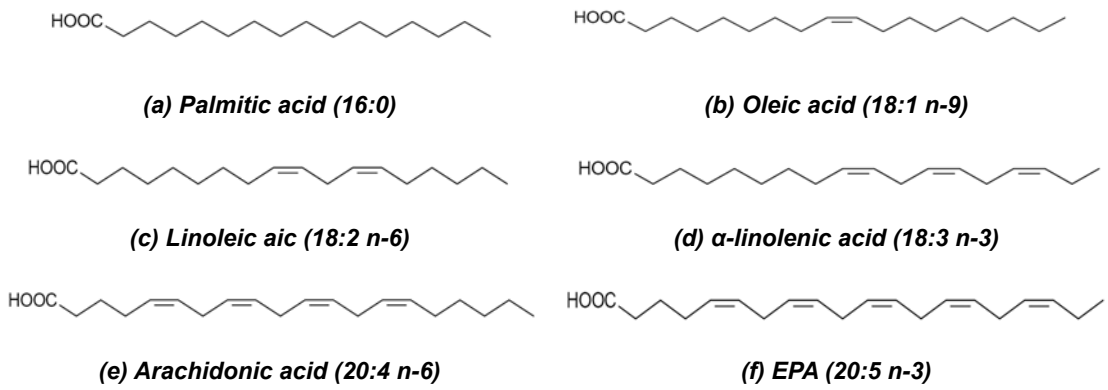
The most common and abundant saturated fatty acids in animal and plant tissues are straight chain compounds with 14, 16 and 18 carbon atoms: myristic acid (14:0), palmitic acid (16:0) (Fig. 2a) and stearic acid (18:0). But all the possible odd- and even- numbered homologues with 2 to 36 carbon atoms have been found in nature in esterified form.

Monounsaturated fatty acids (MUFAs)

Straight-chain even-numbered fatty acids with 10 to more than 30 carbon atoms and containing one cis-double bound have been characterized from natural sources. The most abundant monounsaturated fatty acid in tissue is cis-9-octadecenoic acid (18:1 n-9), also termed “oleic acid” (Fig. 2b).

Polyunsaturated fatty acids (PUFAs)

The polyunsaturated fatty acids (PUFAs) are fatty acids containing two or more double bonds. There are two principal families of PUFAs – the omega-3 and the omega-6 families in PUFAs. Their first double bond is located on the 3rd or 6th carbon-carbon bond, counting from the terminal methyl carbon (designated as n or ω) toward the carbonyl carbon, and double bonds are separated by one methylene unit. Since humans cannot synthesize double bonds at position 6 or lower, omega-3 (n-3) and omega-6 (n-6) PUFAs must be obtained from the diet. The omega-3 PUFAs are derived from fish and some plants, whereas the omega-6 PUFAs are derived mainly from vegetable oil. The parent compound of the n-6 family, linoleic aicd (LA) (18:2 n-6) (Fig.2c) is plentiful in nature. Alpha-linolenic acid (ALA) (18:3 n-3) (Fig.2d), the parent compound of the omega-3 family, is far less common. Both α-linolenic acid and linoleic acid can be elongated and desaturated to long-chain PUFAs: linoleic acid to arachidonic acid (AA) (20:4 n-6)(Fig.2e) and α-linolenic acid to eicosapentaenoic acid (EPA) (20:5 n-3)(Fig. 2f) and docosahexaenoic acid (DHA) (22:6 n-3). The fatty acids may be found in free form but in general they are combined in more complex molecules usually through ester bonds, but ether, amide or other bonds may also occur [21]. Good dietary source of 18:3 n-3 are seeds and vegetable oils, such asflaxseeds, flaxseed oil, Canola (rapeseed) oil, soybeans, soybean oil. The primary dietary source of 20:5 n-3 and 22:6 n-3 are fatty marine fish, such as salmon, mackerel, halibut, sardines, herrings, anchovies, tuna etc [1].



(a) Palmitic acid (16:0) *(b) Oleic acid (18:1 n-9)*

(c) Linoleic aic (18:2 n-6) *(d) α-linolenic acid (18:3 n-3)*

(e) Arachidonic acid (20:4 n-6) *(f) EPA (20:5 n-3)*

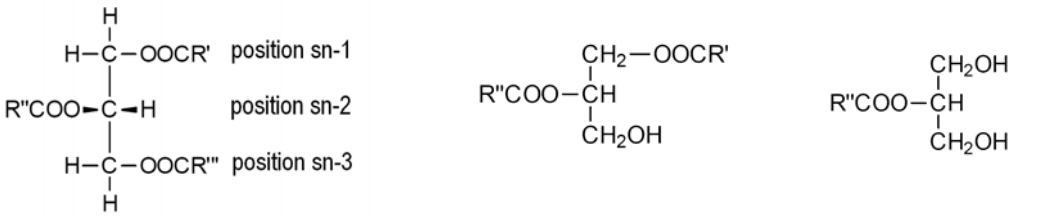
Figure 2. Structures of some important fatty acids

2.3 Lipid classes: simple lipids and complex lipids

The lipids are generally classified into the following two groups: simple lipids and complex lipids. Simple lipids (including fatty acids, triacylglycerols, sterols, sterol and wax ester) are those that yield on hydrolysis at most two types of primary products per mole; complex lipids (including glycerophospholipids, glyceroglycolipids, ether lipids and sphingolipids) yield three or more primary hydrolysis products per mole[18].

Triacylglyerols and related compounds

Nearly all the commercially important fats and oils of animal and plant origin consist almost exclusively of triacylglycerols(TAG)(Fig. 3a). They consist of a glycerol moiety with each hydroxyl group esterified to a fatty acid. Diacylglyerols(DAG) (Fig. 3b) and monoacylglyerols(MAG)(Fig. 3c) contain two moles and one mole of fatty acids per mole of glycerol, respectively, and are rarely present at greater than trace levels in fresh animal and plant tissues, but may be formed in stored products from hydrolysis of TAG.



(a)The structure of triacylglyerol *(b)1,2-/2,3-diacylglyerol* *(c)2-monoacylglyerol*

Figure 3. The structures of TAG, DAG and MAG

Sterols and sterol esters

Cholesterol (Fig.4) is by far the most common member of a group of sterols in animal tissues. It is found both in the free state, where it has an essential role in maintaining membrane fluidity, and in esterified form, i.e. as cholesterol esters. Other sterols are present in free and esterified form in animal tissues, but at trace levels only. In plants, cholesterol is rarely present in other than small amounts, but some other sterols are usually found, and they perform a similar function.

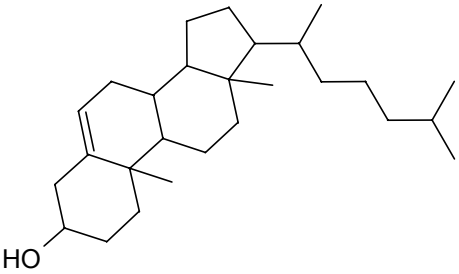


Figure 4. The structure of cholesterol

Waxes

In their most common form, wax esters consist of fatty acids esterified to long-chain alcohols with similar chain-lengths. The latter tend to be saturated or have one double bond only. Such compounds are found in animal, plant and microbial tissues and they have a variety of functions, such as acting as energy stores, waterproofing and lubrication.

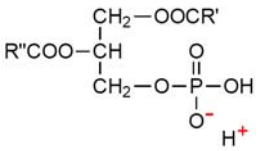
Free (unesterified) fatty acids (FFA)

Free fatty acids are minor constituents of living tissue but are of biological importance as precursors of lipids, as an energy source and as cellular messengers. Large amounts of FFA are usually indicative of artefactual hydrolysis during storage or extraction of the tissues.

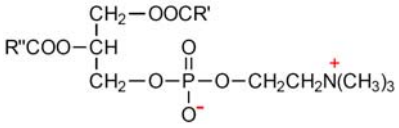
Glycerophosoholipids

Phosphatides or phospholipids are lipids which contain phosphorus and, in many instances, nitrogen. Phosphatidylcholine (PC) (Fig. 5b) is usually the most abundant lipid in the membranes of animal tissues, and it is often a major lipid component of plant membranes, but only rarely of bacteria. Together with the other choline-containing phospholipid, sphingomyelin, it comprises much of the lipid in the external monolayer of the plasma membrane of animal cells especially. Lysophosphatidylcholine(LPC) (Fig.5c), which contains only one fatty acid moiety in each molecule, generally in position *sn*-1, is sometimes present as a minor component of tissues. It is a powerful surfactant and is more soluble in water than most other lipids. Phosphatidylethanolamine(PE)(Fig.5d) is usually the second most abundant phospholipid class in animal and plant tissues, and can be the major lipid class

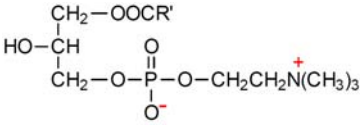
in microorganisms. Other phospholipids such as phosphatidic acid (Fig. 5a), phosphatidylintositol(Fig. 5e), phosphatidylserine (Fig. 5f) ect. are found naturally in trace amounts in tissue but are important metabolically.



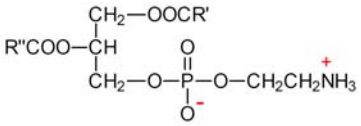
(a) Phosphatidic acid



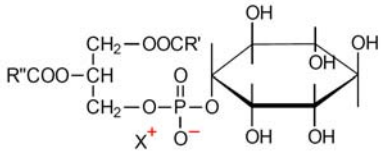
(b) Phosphatidylcholine(PC)



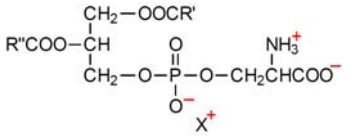
(c)Lysophosphatidylcholine(LPC)



(d)Phosphatidylethanolamine(PE)



(e) Phosphatidylinositol (PI)



(f) Phosphatidylserine (PS)

Figure 5. The structures of the principal glycerophosoholipids

Other lipids

Glycoglycerolipids, Sphingolipids and glycosohingolipids are of metabolic importance but usually only present in trace amounts in most tissues. They are therefore of limited relevance for this study.

2.4 Neutral and polar lipids

Alternatively to the terms simple and complex lipids, ‘neutral’ and ‘polar‘ lipids, respectively, are used frequently to define lipid groups, although they are less precise and can be misleading. For example, PE in Table 1 belongs to polar lipids, but it is less polar than some neutral lipids, FFAs is usually regarded as neutral lipids, but they typically have higher polarity than many polar lipids. This classification is convenient for chromatographic analysis. Neutral lipids mainly contain triacylglycerol(TAG) and cholesterol(C), free fatty acid (FFA), diacylglycerol (DAG) and monoacylglyerols (MAG) etc. The main polar lipids in most tissues are phosphatidylcoline (PC), phosphatidylethanolamin (PE), phoshatidylinositol (PI), phosphatidylserine (PS), lysopholipids. The polarity of lipids affect the solubility in solvents, thus affect the extraction efficiency of the solvents. Some lipids are acidic (free fatty acids, Phosphatidic acid, PI and PS), and their phase distribution is therefore highly dependent of the pH in the polar phase. See 2.5 for detailed discussion about the extraction efficiency.

Table 1. Polarity of some lipids

Material	Log P*
Neutral lipids:	
Saturated fatty acid: 16:0	7.15
Monounsatrurated fatty acid: 18-1 n-9	7.7
Diunsaturated fatty acid: n-6 series: 18:2 n-6	7.18
Polyunsaturated fatty acids, n-3 series: 20:5 n-3	6.23
Triacylglycerols (TAG): 1-Eicosapentaenoyl-2-octadecenoyl-3-tetradecanoyl-sn-glycerol	20.73
Diacylglyerol (DAG): 1-Eicosapentaenoyl-2-octadecenoyl-sn-glycerol	12.45
Monoacylglycerol (MAG): 2-octadecenoyl-sn-glycerol	6.71
Cholesterol	9.85
Polar lipids:	
Phosphatidylethanolamine (PE): 1-Docosahexaenoyl-2-hexadecanoyl-sn-glycero-3-phosphoethanolamine	13.33
Lyso-phosphatidyl ethanolamine (LPE):1-Docosahexaenoyl-sn-glycero-3-phosphoethanolamine	5.29
Plosphatidyl choline (PC):1-Docosahexaenoyl-2-hexadecanoyl-sn-glycero-3-phosphocholine	10.5
Lyso-phosphatidyl choline (LPC): 2-Docosahexaenoyl-sn-glycero-3-phosphocholine	2.46

logP: Rough estimate of polarity. Log P is the log 10 of the octanol/water partition coefficient, Calculated using ChemSketch (Advanced Chemistry Development, Toronto, Canada). Lower value means higher polarity

2.5 *Lipid extraction principle* [18, 23]

There are three main facets to any practical procedure for extracting lipids from tissue; firstly, exhaustive extraction and solubility of the lipids in organic solvent and secondly, removal of non-lipid contaminants from the extracts; thirdly, the potential toxicity of solvents to analysts.

Lipids occur in tissues in a variety of physical forms. The simple lipids are often part of large aggregates in storage tissues, from which they are relatively easily extractable. On the other hand, the complex lipids are usually constituents of membranes, where they occur in a close association with such compounds as proteins and polysaccharides, with which they interact, and they are not extracted so readily. Generally, lipids are linked to other cellular components by weak hydrophobic or Van der Waal's forces, by hydrogen bonds and by ionic bonds.

Pure lipids will dissolve in a variety of solvents, depending on the relative strengths of the interactions between the solvent and either the hydrophobic or the hydrophilic regions of the molecules. Lipids with functional groups of low polarity only, such as triacylglycerols or cholesterol esters, are very soluble in hydrocarbon solvents like hexane, cyclohexane or toluene, and in solvents of somewhat higher polarity, such as chloroform or ethers. They tend to be rather insoluble in polar solvents such as alcohols, and methanol especially;

solubility in polar solvents increases as the chain-length of the fatty acid moieties in these lipids decrease or as the chain-length of the solvent alcohol increases. Unsaturated lipids tend to dissolve in most solvents more readily than saturated and higher-melting analogues. In contrast, the polar complex lipids tend to be only sparingly soluble in hydrocarbon solvents, though dissolution can be aided by the presence of other lipids, but they do dissolve readily in more polar solvents such as chloroform, methanol and ethanol.

In order to extract lipids from tissues, it is necessary to find solvents that will not only dissolve the lipids readily but will disrupt the interactions between the lipids and the tissue matrix. The energy required to disrupt the weak forces is always less than 2 kcal/mole, while that for hydrogen bonds can be as much as 12 kcal/mole [18]; ionic bonds can only be disrupted by lowering the pH of the medium or by increasing its ionic strength. Some lipids can also be physically trapped within a tissue matrix; The cell wall in some organisms are less permeable than others to solvents; water then assists the extraction by causing swelling of the biopolymers and it is an essential component of many extraction systems. In some circumstances, it may be necessary to effect an appreciable denaturation of the other constituents of the cell walls by some means before a thorough extraction of the lipid is possible.

Lipid extracts from tissue tend to contain significant amounts of non-lipid contaminants, such as sugars, amino acids, urea and salts. These contaminants must be eliminated form the recovered lipids by washing or other solvent partition procedures, before the sample can be subjected to detailed analysis.

2.6 Extraction methods and total lipid determination

2.6.1 Lipid extraction methods

Though there are many methods to determine the total lipid of food products, gravimetric determination after solvent extraction are the most commonly used methods of isolating lipids from different matrices and determining the total lipid content of foods. Extraction methods can be classified into the following three categories: batch solvent extraction, semi-continuous solvent extraction and continuous solvent extraction [24].

Batch solvent extraction

These methods are based on mixing the sample and the solvent in a suitable container, *e.g.,* a separatory funnel. The container is shaken vigorously or homogenization of the sample and solvent is applied before the solvent and residues are separated by gravity, filtration or centrifugation. This procedure may have to be repeated a number of times to improve the efficiency of the extraction process, sometimes with solvents or solvent mixtures of different properties. Batch extractions are commonly applied with solvent mixtures that form two phases, one polar and one apolar, where the polar phase is usually

discarded in lipid determination. Folch method [25] and Bligh and Dyer methods [14] belong to this category.

Semi-continuous solvent extraction

The Soxhlet method is the most commonly used example of a semi-continuous method. The detailed description is in 2.6.2. A number of instrument manufacturers have designed modified versions of the Soxhlet method that can be used to determine the total lipid content more easily and rapidly (*e.g.* Soxtec [26]).

Continuous solvent extraction

The Goldfish method is similar to the Soxhlet method except that the extraction chamber is designed so that the solvent just trickles through the sample rather than building up around it. This reduces the amount of time required to carry out the extraction, but it has the disadvantage that *channeling* of the solvent can occur, *i.e.,* the solvent may preferentially take certain routes through the sample and therefore the extraction is inefficient. This is not a problem in the Soxhlet method because the sample is always surrounded by solvent.

Along with the demand for higher recovery, faster analysis, and increased possibilities for automation and reduced solvent usage, newer extraction techniques have been developed during the past decades including Microwave assisted extraction [27], accelerated solvent extraction [28, 29], supercritical fluid extraction[30,31] etc. Several studies have shown that these methods in many cases can be more efficient that the traditional methods. However, there is still limited information about how the methods perform with a broad range of sample types.

The choice of extraction procedure will depend on the nature of the tissue matrix. Another factor is the amount of information required from the sample; many simple extraction procedures can be used for triacylglycerol-rich tissues such as adipose tissue or oil seeds if the main lipid class only is required for analysis. On the other hand, if a detailed knowledge of every minor lipid class is required, few shortcuts are possible.

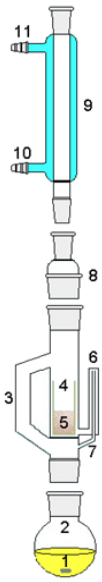
2.6.2 Commonly used methods

In this study, the extraction efficiency of three extraction methods were evaluated because of their widespread use: the Soxhlet method(SOX)[12], Acid hydrolysis method [13] and Bligh and Dyer (B&D) [14].

Soxhlet method

Soxhlet is recognized by the Association of Analytical Chemists (AOAC) as the standard method for crude fat analysis. Fat is extracted through repeated washing, or percolation, with an organic solvent under reflux in special glassware (Fig. 6). Extraction efficiencies for different compound classes are

highly dependent on the properties of the applied solvents. Lipid extraction by soxhlet is usually performed with apolar solvents such as hexane, ethylacetate or petroleum ether. Under these conditions, the method basically determines the content of triacylglycerols and has been reported to incompletely extract phospholipids in the samples [30, 32, 33].



1. **Stirrer bar/anti-bumping granules**
2. **Still pot (extraction pot)**
3. **Distillation path**
4. **Soxhlet Thimble**
5. **Extraction solid (residue solid)**
6. **Syphon arm inlet**
7. **Syphon arm outlet**
8. **Expansion adapter**
9. **Condenser**
10. **Cooling water in**
11. **Cooling water out**

Figure 6. A schematic representation of a Soxhlet extractor [34]

The most outstanding advantages of conventional Soxhlet are as follows: the sample is repeatedly brought into contact with the fresh portions of the solvent, thereby helping to displace the transfer equilibrium. The temperature of the system remains relatively high since the heat applied to the distillation flask reaches the extraction cavity to some extent. No filtration is required after the leaching step. Sample throughput can be increased by simultaneous extraction in parallel, since the basic equipment is inexpensive. It needs little specialized training and is non-matrix dependent.

The most significant drawbacks of Soxhlet extraction are the long time required for the extraction and the large amount of solvent wasted, which is not only expensive to dispose off but which can itself cause additional environmental problems. Samples are usually extracted at the boiling point of the solvent for a long period of time and the possibility of thermal decomposition of the target compounds cannot be ignored, when thermolabile analytes are involved. Due to the large amount of solvent used, an evaporation/concentration step after the extraction may be necessary. The technique is restricted to solvent selectivity and is not easily automated [35].

Modification of the conventional Soxhlet extractor has been developed to shorten the extraction time by using auxiliary energy and automation, for example, ultrasound-assisted Soxhlet extraction [36], microwave-assisted Soxhlet extraction[27] etc.

Acid hydrolysis method

In acid hydrolysis method, the sample is hydrolyzed with aim to release complex and/or bound fatty materials and permit extraction of total fat with an appropriate solvent. Thus, it is usually performed before the other extraction methods.

Many standard methods use acid hydrolysis to extract total fat of samples. For example, AOAC 996.01 for determination of total, saturated, polyunsaturated and monounsaturated fat in cereal-based products [37]. This method involves hydrolysis of the ground sample, extraction of fat into diethyl and petroleum ether solvents, evaporation of the solvents, methylation of the extracted fat, and quantification of fatty acids by gas chromatography. AOAC method 948.15, fat in seafood-Mojonnier technique and AOAC method 922.06, Fat in flour-Mojonnier technique also use hydrolysis to release the bound lipid in samples.

It has been reported that the method often include some non-lipid moieties that are freed during hydrolysis and extracted along with the lipid, causing the overestimation of total fat in foods[64]. This is the common disadvantage for all the conventional extraction methods. It has also been reported that acid hydrolysis extractions are extremely aggressive and produce extracts which are chemically degraded and unsuitable for fatty acid profiling [30].

A modification of ISO 6492:1999(E), “animal feeding stuffs-determination of fat content” [13] has been applied in this work. The method that was used involves an initial Soxhlet extraction with petroleum ether followed by acid-hydrolysis and repeated Soxhlet extraction with petroleum ether, before evaporation of the solvent and weighing.

Bligh and Dyer (1959) (B&D)

The Bligh and Dyer method is generally considered to be among best method for polar lipid extraction and is commonly used for determining lipid contents in environmental samples. The Bligh and Dyer method is a simple adaptation of the Folch procedure and was developed merely as an economical means (in terms of solvent volumes) of extracting lipids from tissue such as fish muscle, which contain relatively little lipid and a high proportion of water. The fat in the sample is extracted by a polar solvent mixture consisting of chloroform, methanol and water (1:2:0.8), which gives a one-phase system. After extraction, the one-phase system is separated into chloroform and methanol/water phases by addition of more chloroform and water. The lipids will follow the chloroform phase. The fat content is usually determined in an aliquot of the chloroform phase by weighing the lipids after evaporation of the solvent. The method will extract both neutral and polar lipids.

Since the introduction of this method many investigators have applied the method with many different modifications [38, 39, 40, 41]. The B&D method is operationally defined, altering the original procedure can easily lead to

deviating results. In 1994, a lipid intercalibration exercise using the B&D method was organized within QUASIMEME, a quality assurance project of the European community. The results from the 33 participating laboratories were discussed and evaluated. The variability of the intercomparison exercise (CV on the total lipid determination, 12.6%) could be explained in part by modification of the original method by the participants. The main variations between the different adaptions of the method is 1) Blind subsampling, measured subsampling or no subsampling and 2) single extraction or repeated extraction of the residue[42].

The disadvantage of the Bligh and Dyer is that it requires a highly skilled technician for repeatable results. Inadequately estimating the water content of the samples can lead to incomplete extractions. Also because of the negative effect of chlorinated solvents on the environment, the use of chloroform should be strongly discouraged. Following the Montreal Protocol, EU regulations have been developed to control strictly the use of chlorinated solvents. The use of chlorinated solvents will also add significantly to solvent disposal costs. Attempts have been made to adapt the Bligh and Dyer method to the use of non-chlorinated solvents [43, 44]. However, because of the high efficiency of Bligh and Dyer system for extracting both polar and nonpolar lipids, no alternative system has so far been fully successful. Bligh and Dyer are still widely used.

After extraction the lipids are often characterized further by analyzing fatty acid profiles by GC (2.7), lipid class composition by LC (2.8) or by analysis of sterols, vitamins or organic pollutants.

2.7 Total fatty acids and Fatty Acid profile analysis

The use of gas chromatography (GC) to characterize fatty acid profiles of lipids in biological materials after conversion to methyl ester is routine in laboratories of various scientific institutions and industrial organizations.

2.7.1 Transmethylation / methylation [45]

Fatty acid components need to be converted into fatty acid methyl esters (FAME) in order to improve their volatility and thus ensuring better gas chromatographic peak shape.

Formation of FAME is normally accomplished in the presence of a catalyst mixed with or dissolved in methanol. There are numerous catalysts for these reactions. The majority can be characterized as either acidic (HCl, H_2SO_4 and BF_3) or alkaline ($NaOCH_3$, KOH and NaOH).

Lipids are mainly mixtures of esters, and preparation of FAMEs actually involves converting one ester to another. The reaction is referred to as “transesterification” in general and “transmethylation” or simply “methylation” when the fatty acids are esterified to methanol. Both transeserification and

esterification of free acids are reversible reactions. However, transesterification can be catalyzed by either an acid or a base:

$$R'-CO-OR''+R-OH \xleftrightarrow{H^+ or\ ^-OCH_3} R'-CO-OR+R''-OH$$

Whereas esterification can only be esterified by acid catalysis:

$$R'-CO-OH+R-OH \xleftrightarrow{H^+} R'-CO-OR+HOH$$

To shift the equilibria to the right, it is necessary to use a large excess of the alcohol corresponding to the esters we wish to make, or else remove one of the products from the reaction.

Transesterification of fatty acids with anhydrous HCl/MeOH for gas chromatrography was introduced about 35 years ago. It is one of the milder reagents and has been claimed to be the best general purpose esterifying agent [15]. In this assay, this catalyst was used to obtain FAME.

2.7.2 Multistep method vs. direct methylation method

There are several different procedures to make FAME. These can be divided into multistep methods and direct methylation methods.

Multistep methods

These methods usually consist of extraction of lipids out of biological materials, followed by a methylation/ transmethylation of extracted lipids to fatty acid methyl esters (FAMEs) and extraction of FAME for the final GC determination [46,47].

Many of these methods are capable of producing reliable data if some special precautions are taken. However, due to involvement of multiple steps to complete each procedure, they are cumbersome, time consuming and cost-inefficient. Generally, it requires large volumes of reagents and large sample sizes. In addition, the multistep procedures may lead to introduction of contaminants and losses of esters. Therefore, it is impractical in those laboratories where the number of tests are relatively large and the amount of sample material is limited.

Direct methylation methods (DM)

Direct methylation methods are also called one-step extraction/methylation methods. This method combines extraction and transmethylation into one step. DM methods solve several of the limitations of the multistep methods, such as high solvent consumption and low extraction efficiencies and have been applied for fatty acid analysis in a wide range of sample types [14, 15, 48, 49, 17, 50].

It has been reported that direct methylation gave comparable fatty acid profile but higher fatty acid recovery of marine tissues than the conventional Folch

extraction followed by transesterification [15]. When direct methylation was applied in mammal tissue, higher fatty acid concentration than that of the conventional method was also obtained. It has successfully been used for several different biological matrices [51]. These researches indicated that the direct methylation methods have outstanding advantages over the conventional methods. It meets the demand of NLEA. It is simple, requires small sample amounts and gives high recovery of total fatty acids. It is also cost efficient and environmentally sound.

However, there are also disadvantages with DM methods. Large amount of matrix other than lipids may influence the result, and low sample size may cause sampling problems when non-homogeneous materials are analyzed. Also the formation of trans fatty acids has been reported [52].

2.7.3 GC analysis

Capillary columns and a flame ionization detector (FID) are most commonly used to characterize the fatty acid profile in biological materials.

FAME analysis of marine samples demands high chromatographic resolution because of the large number of positional and geometrical isomers of unsaturated fatty acids. This requirement made packed columns outdated for general applications. Whereas Fused-silica capillary columns can easily achieve high resolution, their temperature can be programmed or the flow velocity of the carrier gas can be raised to optimize resolution and reduce the analysis time [53].

The capillary columns typically used for the analysis of fatty acids are 10-100m long, with an inner diameter of about 0.1 to 0.35mm. Except for the moderately polar polyethylene glycols, most stationary phases used for the analysis of FAMEs consist of various proportions of the nonpolar methylpolysiloxanes and the very polar cyanoalkylpolysiloxanes. Identification of the fatty acids is based on the retention times of FAME and the most common fatty acids are available in commercial reference mixtures. To confirm the identity of an analyte, gas chromatography-mass spectrometry (GC-MS) can be utilized in order to compare the mass spectrum with a reference spectrum stored in a computer database [53].

2.8 Lipid class analysis

The lipids can be separated into the various classes by using a variety of chromatographic methods on the basis of their relative polarity. The solvent mixtures of increasing polarity are used to elute lipids off the bed or column in the order of their increasing polarity. Most studies of lipid classes are based on different thin-layer chromatography (TLC) methods [54,55,56] and solid phase extraction (SPE) procedures [57,58] Even though these techniques are well developed and partly automated, they still have the disadvantages of being

time-consuming or limited in terms of separation capacity.

In recent years, the application of HPLC for lipid analysis has become important. The use of HPLC instead of TLC offers the possibility of full automation and higher separation capacity. Another benefit of HPLC is that fractions containing single lipid classes can easily be collected for further identification and analysis of molecular species. Mass sensitive detectors, such as evaporative light-scattering detectors (ELSDs) [59, 60, 61] and charged aerosol detectors (CADs) [62,63] have greatly simplified the development of HPLC methods for lipid class separations. These types of detectors have been used with ternary gradient systems to separate all the simple and complex lipids from animal tissues in a single chromatographic run.

In this study, HPLC-CAD was applied to analyze the lipid class of the samples to give a brief overview of the lipid composition in the samples.

3. Experimental Section

3.1 Samples

Eight fish samples were chosen to represent a wide range of lipid content (Table 2). The samples were ground on Retch Grindomix GM 200 laboratory mill for about 20 sec at 8000 rpm. All the samples were fine powders except for Salmon. Because of high lipid content in salmon, it easily agglomerated. To avoid oxidation of the samples during the three month period of the experiment, every sample was divided into three parts and vacuum packed, one bag for Soxhlet method, one for acid hydrolysis method and one for the Bligh and Dyer method. Before every subsampling, the powders were shaken to ensure homogeneity.

Table 2. Information about the samples

Sample	Type	Source
A	Fishmeal	Commercial
B	Cod filet	Nofima
C	Salmon filet	Nofima
D	Herring roe	Nofima
F	Krill meal	Commercial
G	Fishmeal made from Blue whiting	Commercial
H	Fishmeal	Commericial [65]
I	Krill meal	Nofima

Among the eight samples, sample B, C and D contain all lipids originally present in the fish, while the majority of the TAG has been removed as oil in the processing step of the other samples.

Cod filet powder (B) was prepared by freeze drying frozen cod filets followed by grinding as described above. The cod filets were delivered from Nofima's feed production facility, where it is used as reference material for digestibility studies. Cod fish is a typical lean fish. The oil in lean fish is concentrated in the liver, rather than being distributed through the flesh. Their fat content is less than 5 percent (wet weight) and the flesh is mild and lightly colored.

Salmon filet (C) was freeze dried from fresh salmon and were ground. Salmon is typical fat fish and contains important quantities of PUFAs. Since the oil is distributed throughout the flesh of the fish, the flesh tends to be darker than that of leaner species. The sample was prepared from farmed salmon, which generally is fatter and have a different fatty acid composition than wild salmon.

Herring roe (D) was from the Norwegian spring-spawning herring, fished in February 2007. The roe was delivered block frozen (-20ºC) in boxes of about 20 kg of a mixture of roe and milt. Three boxes was thawed in July 2007 for the

sorting of roe and milt. 6.6 kg wet roe was then stored at -20ºC before freeze-drying soon after. By freeze drying nitrogen gas were used to reduce oxidation, and the roe were transferred to tight packages and stored at -80ºC. Freeze-dried roe were ground and sifted through a 1.18 mm analytical sieve for removing coarse particles such as fish scales and bones, and the powder was then vacuum-packed and stored at -80ºC until use.

Krill meal (I) was produced from frozen Antarctic krill (*Euphausia superba*) packed in cartons of 20 kg. The cartons were marked Okami Hiro, Hirohya and stamped Made in Poland, DAO 20217F. The frozen krill was thawed overnight to -5 – 0ºC. And meal was produced by a process similar to the conventional fish meal process. Portions of krill were heated to 80ºC and then pressed in a twin screw press. The press liquid was heated to 90ºC, particles were removed in a fluid roto sieve and the liquid was separated in an oil separator. The stickwater was concentrated to 40 % dry matter in a 4-stage falling film evaporator. The press liquid and press cake were mixed to 5.5% moisture in a hot air drier with mechanical fluidisation The product was stabilised with ethoxyquin (FEQ 500, Facet Pharma ApS, Hillerød, Denmark), 100 mg/kg dm in the press cake + 100 mg/kg in dry meal.

Samples A, F, G, and H were commercial products delivered from Nofima's feed production facility. Sample G was fishmeal made from blue whiting. Sample F was krill meal prepared by a process similar to the fish meal process, i.e. with separation of water and oil from the press cake followed by drying and milling to krill meal. Samples A and H are commercial fish meals made from various species. Commercial fish meals are stabilized either with ethoxyquine or by mixtures of natural antioxidants.

3.2 Water content

The water contents were determined by weighing 3 gram samples in a tared aluminum tray that was heated in the oven at 103ºC for 4.5 hours. The tray was weighed again after cooling in desiccators for 30 minutes and the water content was calculated as weight difference divided by the sample weight.

3.3 Extraction Methods

Four procedures were used to prepare FAME from fish samples. (a) Soxhlet fat extraction followed by methylation; (b) Acid hydrolysis fat extraction followed by methylation; (c) Bligh and Dyer fat extraction followed by methylation; (d) direct methylation. There are some modifications of the original methods for the purpose of checking the mass balance of fatty acids in extract and residue. The original methods are given in appendixes.

3.3.1 Soxhlet method

The original method used at Nofima BioLab is given in appendix F.

Modifications of original procedure are introduction of extra weighings to check the mass balance, analysis of the residue, and that an aliquot is taken from the extract for analysis of fatty acids. The latter requires corrections to the original equation for calculating total extracted lipids. Since all samples are dry powders, the initial drying step in the original method was also omitted to avoid possible oxidation.

4-7 gram of sample (W0) was accurately weighed and transfered to extraction tube. The soxhlet flasks were dried in the oven at 103ºC for one hour and then cooled in the desiccators for 30min and tared with (W1) and without the stopper (W2). The sample was covered with cotton wool in the extraction tube and placed the tube in the soxhlet apparatus with a tared flask. Approx 100 ml petroleum ether was added to the soxhlet flask, the sample was extracted over night with a condensation rate of 2-3 drops per second.

Treatment of extract

The Soxhlet flask was taken out, the extract was diluted to approx 100ml with petroleum ether. The Soxhlet flask with glass stopper (W3) was weighed. Two reaction tubes with internal standard were prepared as described in 3.4.2 and tared with cap (W4a, W5a). 4 ml extract was transferred to each of two reaction tubes and weighed with cap again (W4b, W5b). The soxhlet flask was weighed with glass stopper again (W6). The petroleum ether was distilled off, the flask was dried in the oven for 2 hours at103±1ºC. The soxhlet flask was cooled in the desiccators and weighed (W7). The two reaction tubes are subjected to methylation procedure described in 3.4.2

Treatment of residue

The extraction tube was taken out of the extractor, the solvent was evaporated under vacuum for 2 hrs. The residue (W8) was weighed. Approx 200mg of the residue was weighed to each of the two reaction tubes with internal standard for methylation (W9, W10). FAMEs were prepared as described in 3.4.2

Calculations

Gravimetric lipid content was calculated by the following equation

$$\text{Extracted lipid} = \frac{(W7 - W2) \times (W3 - W1)}{W0 \times (W6 - W1)} \times 100\% \qquad (1)$$

From the GC analyses, the extracted fatty acids M1 (g FA/100g extract) and residual fatty acids M3 (g FA/100g residue) were obtained. To calculate extracted fatty acids M2 (g FA/100g sample) and residual fatty acids M4 (g FA/100g sample), the following formulas were used:

$$M2 = M1 \times (W3 - W1) / W0 \qquad (3)$$

$$M4 = M3 \times W8 / W0 \qquad (4)$$

Validation

The measured and calculated masses the experiments can be validated as follows:

Weight of extract removed from Soxhlet flak for methylation should be equal to the weight transferred to the reaction tubes:

$$(W4b - W4a) + (W5b - W5a) = W3 - W6 \quad (5)$$

A mass balance for the extract that ideally should be 100% can therefore be calculated by:

$$\text{Mass balance} = 100 \times (W4b - W4a + W5b - W5a) / (W3 - W6) \quad (6)$$

Weight of extracted lipid plus weight of residue (W8) should be equal to the initial weight of sample (W0)

$$W0 = \frac{(W7 - W2) \times (W3 - W1)}{W6 - W1} + W8 \quad (7)$$

A mass balance for the residue that ideally should be 100% can therefore be calculated by:

$$\text{Mass balance} = \frac{W8}{W0 - \frac{(W7 - W2) \times (W3 - W1)}{W6 - W1}} \times 100\% \quad (8)$$

The above calculation assumes no net loss (or gain) of moisture from the sample.

3.3.2 Acid hydrolysis method

The original method used at Nofima BioLab is given in appendix G. Modifications of original procedure are the same as for the soxhlet method.

The first Soxhlet extraction step is the same as Soxhlet method described in 3.3.1, except that the extraction time is at least 2 hours instead of overnight. After the first extraction, the extraction tube was taken out, the flask with the extract was set in a fume hood until second extraction. The tube with the remnants of the sample was dried at 103ºC for approx. one hour. The remnants was transfered from the tube to a round-bottomed flask, the tube was marked for later use. 100 ml 3M hydrochloric acid was added, the condenser was amounted on the flask. The solution was boiled gently on a water bath in approximately one hour. The flask was shaken occasionally to avoid that the sample sticks to the glass wall. The sample was cooled. Minimum 3g of filter aid was added to the flask. The sample was filtered through a wetted, fat free, 150mm white band filter paper and washed with cold purified water (approx. 500ml) until the filtrate is neutral. The filter paper was folded and transferred to the same extraction tube that was applied in the first extraction and dried for 2

hours in the oven at 103ºC.

The sample was covered with cotton wool, the tube was placed in the Soxhlet extractor together with the flask from the first extraction. 100ml petroleum ether was added to the flask, the sample was extracted over night with a condensation rate of approximately 2-3 drops per second.

Treatment of extract

The same as the treatment of Soxhlet extract described in 3.3.1

Treatment of residue

The same as the treatment of Soxhlet residue described in 3.3.1

Calculation and validation

The same as described in 3.3.1 except for the validation of the mass balance of the residue. Because of the addition of filter aid, the mass balance of the residue could not be checked as for the Soxhlet method.

3.3.3 Modified Bligh and Dyer method

Different modified Bligh and Dyer methods are usually used by chemists. The original method used at Nofima BioLab is given in appendix H. It is also a modification of original Bligh and Dyer. Based on Nofima BioLab method, several modifications were done here to make it suitable for evaluation of extraction efficiency (re-extraction and recovery of the residue). These modifications were also more in accordance with the original procedure described by Bligh and Dyer [14].

Besides re-extraction and recovery of the residue, the modification of B&D of Nofima BioLab also includes: the weight of samples was reduced to get the similar lipid weight with the original B&D; to collect the residue, filter paper was used instead of cotton; measured subsampling was applied instead of blind subsampling.

2.5 gram sample (W0) (the water content of the sample has been determined as described in 3.2) was weighed in a 250 ml flask. Water was added so that the total water content (added water + water content of sample) is 16 ml. After adding 40 ml methanol and 20 ml chloroform, the sample was homogenized for 60 seconds on the homogenizer (IKA, Stanfen, Germany). To the mixture was then added Additional 20 ml chloroform and after homogenizing for 30 seconds, 20 ml water was added and homogenization continued for another 30 seconds. The flask was covered with the cap and cooled in water bath with ice. The sample was filtrated through black band filter paper (150 mm, Whatman, Dassel, Germany) in a glass funnel. The solvent was collected in a cylinder. The funnel was covered with a watch glass to reduce solvent evaporation during the filtration. After the filtration, the volume of water/methanol phase (upper phase) (V1) was recorded and aliquot was collected for further analysis. Then the leftover of water/methanol phase was removed by aspiration using a

water aspirator. The residue was transferred into the original flask, 20ml chloroform was added into the flask. After homogenization for 15 seconds, the sample was filtered again and the solvent was collected in the same cylinder. The total volume of chloroform phase (V2) was recorded.

Treatment of extract (chloroform phase)

20ml of the chloroform phase was transferred to a tared tray (W1) for evaporation using a volumetric pipette. The solvent was evaporated under an infrared lamp. After the tray had cooled down, the tray with lipid (W2) was weighed. Two reaction tubes with internal standard were prepared as described in 3.4.2. 3 ml or 1.5ml extracts were transferred to each of two reaction tubes for methylation. The two reaction tubes were subjected to methylation procedure described in 3.4.2

Treatment of water phase

Two reaction tubes with internal standard were prepared as described in 3.4.2. 3 ml extracts were transferred to each of two reaction tubes for methylation. The water was evaporated at 100ºC on Pierce React-therm 1# 1882 heating module under nitrogen. The two reaction tubes were subjected to the methylation procedure described in 3.4.2

Treatment of residue

The residue was collected and the solvent was evaporated under vacuum for 2 hrs. The dry residue (W3) was weighed. Approx 200mg of the residue was weighed to each of the two reaction tubes with internal standard for methylation (W4, W5). FAMEs were prepared as described in 3.4.2

Calculation

The calculation procedure is different from the method given in the appendix H, because measured subsampling was used instead of the blind subsampling. For details on measured versus blind subsampling, see section4.3.3 and [42].

Gravimetric lipid content was calculated by the following equation:

$$\text{Extracted lipid} = \frac{(W_2 - W_1) \times V_2}{W0 \times c} \times 100\% \qquad (9)$$

c- Chloroform aliquot for evaporation (20ml)

From the GC profile, total FAs in chloroform phase M1 (g FA/100ml extract), total FAs left in water/methanol phase M3 (g FA/100ml residue) and total FAs left in the residue M5 (g/100g residue) were obtained. To calculate Total FAs extracted in chloroform M2 (g FA/100g sample), total FAs left in water/methanol M4 (g FA/100g sample) and total FAs in residue M6 (g FA/100g sample), the following formulas were used:

$$M2 = M1 \times V2 / W0 \qquad (10)$$

$$M4=M3\times V1/W0 \quad (11)$$

$$M6=M5\times W3/W5 \quad (12)$$

3.4 Transmethylation/ methylation method

3.4.1 Preparation of Methylation detergent and internal standard

Dry HCl in methanol (2.5 M) was prepared in a special glass apparatus by slowly adding 25 ml concentrated HCl to 50 ml concentrated H_2SO_4 and bubbling the HCl gas through 100 ml ice cold methanol. The absorption of HCl to methanol was verified by weighing (the mass increases 10 g). Three parts methanolic HCl was then mixed with one part toluene to prepare the methylation reagent containing 25% toluene.

Internal standard, 2.0 mg/ml 23:0 FAME was prepared as follows: 100 mg Methyl Tricosanoate (Larodan AB, Malmo, Sweeden) with 99.5% purity was accurately weighed and transferred to 50 ml volumetric flask. Approximately half the flask was filled with isooctane, the FAME was dissolved with application of ultrasound and gentle heating. After the flask cooled to room temperature, the volumetric flask was filled to the mark. The internal standard mixture was transferred to smaller vials with teflon lined screw cap and stored in freezer (-16ºC).

3.4.2 Methylation procedure

Appropriate internal standard solution was added to a reaction tube. Solvent was evaporated to dryness on Pierce Reac-therm 1#1882 heating module (Thermo Fisher Scientific, USA) under a stream of nitrogen by Pierce Evaporating unit Model 18780 (Thermo Fisher Scientific, USA). For solid samples, approx. 200 mg sample was accurately weighed to the reaction tubes. For liquid samples, appropriate volume was transferred to the vial and weighed. The solvent was evaporated under nitrogen (the temperature was 70ºC for extract and 100ºC for water phase of Bligh and Dyer).

1.0 ml methylation reagent was added. The entire sample needed to be covered with reagent. The reaction tubes was flushed with N_2 and capped before heated at 100ºC in the oven for 2 hours. The tubes were cooled and opened carefully, and approximately 50% of the methylation reagent was evaporated by gentle heating under a stream of nitrogen. 1 ml water and 1 ml isooctane were added; the sample was mixed for approx 30 sec on the Retsch mixer. Centrifugation was applied if there was no clear separation between the phases. The apolar upper phase was then transferred to a 2ml vial with snap cap. Another 1 ml isooctane was added and the extraction was repeated. The upper phase was transferred to the same vial. Appropriate volume extract was transferred from the snap cap vial to GC vials containing 1.0 ml isooctane. The sample size, IS volume and dilution volumes for different samples have been listed in Table 3.

Table 3) Sample size, IS volume and dilution volume for different samples

	Sample size	IS	Dilution*	Sample size	IS	Dilution*
Marine powder	200mg	1.791mg	10µl			
oil	12mg	0.995mg	15µl			
	Soxhlet extract			Soxhlet residue		
A	4ml	0.995mg	10µl	200mg	0.4975mg	150µl
B					0.4975mg	150µl
C					0.4975mg	150µl
D					1.791mg	30µl
F					1.791mg	50µl
G					0.4975mg	150µl
H					0.4975mg	75µl
I					1.791mg	30µl
	acid hydrolysis extract			acid hydrolysis and B &D residue		
A	4ml	0.995mg	10µl	200mg	0.2488mg	150µl
B	4ml				0.2488mg	150µl
C	2ml				0.2488mg	150µl
D	4ml				0.4975mg	120µl
F	4ml				0.2488mg	150µl
G	4ml				0.2588mg	150µl
H	4ml				0.2488mg	250µl
I	2ml				0.4975mg	120µl
	Bligh and Dyer extract			Bligh and Dyer water phase		
A	3ml	0.995mg	10µl	3ml	0.0498mg	250µl
B	3ml					
C	1.5ml					
D	3ml					
F	3ml					
G	3ml					
H	3ml					
I	1.5ml					

*The dilution volume refers to the volume transferred to 1ml isooctane in GC vials.

3.5 Fatty acid analysis by GC

One microliter was injected splitless (the split was opened after 2 min) on a Trace GC gas chromatograph (Thermo Fisher Scientific) with flame ionization detector (GC–FID). The column was BPX-70 (cyano propyl) (SGE, p/n 054623) 60m×0.25mm capillary coated with 0.25µm film thickness. Helium 4.6 was used as mobile phase at the pressure of 2.20 bar. The injector temperature was 280ºC and the detector temperature 260ºC. The oven was programmed as

follows: 60ºC for 4 min, 30ºC/min to 166ºC, then 1.1ºC/min to 213ºC, then 120ºC/min to 250ºC/min where the temperature was held for 10 min. The total analytical time was about one hour.

Fatty acid methyl esters were identified by comparing the elution pattern and relative retention times of FAME with reference FAME mixture (GLC-793, Nu-Chek Prep Inc. Elysian MN, USA) that contains equal amounts of 28 common fatty acids. In addition, the control oil, where the fatty acids have been identified by GC-MS, was used for identification.

Calculations of fatty acids as g/100g sample are performed by the GC software (Chromeleon). The chromatographic areas are corrected by the use of empirical response factors (RF) calculated from chromatography of the reference mixture according to

$$RF_{emp} = \frac{A_{23:0}}{A_{FS}} \tag{13}$$

Where $A_{23:0}$ is the area in the reference mixture of the 23:0 FAME, and A_{FS} is the area for other fatty acids. The concentration of each fatty acid is thereafter calculated as follows:

$$C_{FS}(g/100g) = \frac{A_{FS} \cdot IS_W \cdot RF_{emp}}{A_{IS} \cdot W} \cdot 100 \tag{14}$$

A_{FS} = Area of the FAME

A_{IS} = Area of internal standard (23:0 FAME)

C_{FS} = g/100 g of the FAME

IS_W = Weight of added internal standard (mg)

RF_{emp} = Empirical response factor for the fatty acid calculated relative to 23:0

W = Weight of sample in mg

3.6 Lipid class analysis by LC

The applied method for lipid class analysis by LC is a modified version of [Reynold Homan, Maureen K. Anderson, Journal of Chrom. B. 708 (1998) 21-26] with application of CAD instead of ELSD as detector. The parameters are briefly explained below. Further details can be found in the Nofima Biolab procedure A88.

A suitable aliquot of the Bligh and Dyer extract was transferred to a tared reaction tube. The solvent was evaporated on Pierce Reac-therm 1#1882 heating module (Thermo Fisher Scientific, USA) under a stream of nitrogen by Pierce Evaporating unit Model 18780 (Thermo Fisher Scientific, USA). The

temperature of the heating module was 60ºC. The sample was weighed after evaporation of the solvent to determine the weight of the extracted fat, then diluted with an appropriate amount of chloroform.

The HPLC system (Perkin Elmer, Shelton, USA) consists of an Pump, and an autosampler, and column oven set at 45ºC and charged aerosol detector (CAD)(ESA, Chelmsford, USA).The nebulizer temperature in the detector is 30ºC. The gas pressure on the nitrogen line to the detector is at 36 psi. The precolumn is LichroCART 4-4, diol 5 µm and the LC column is LiChroCART 125-4, diol 5µm. Injection volume was 20 µl. Lipid classes were separated in a single chromatographic run by using the gradient program described in Table 4.

Table 4) Ternary gradient mobile phase composition

Step	Time(min)	Flow rate (ml/min)	Percent solvent		
			A	B	C
0	1	1.6	100	0	0
1	7	1.6	90	10	0
2	3	1.6	70	30	0
3	2	1.6	40	50	10
4	13.3	1.6	39	0	61
5	0.1	2	40	0	60
6	2	2	40	0	60
7	2.5	2	0	100	0
8	10	2	100	0	0
9	0				

A, isooctane; B, Acetone-dichloromethane (1:2, v/v); C, Isopropanol-methanol-acetic acid/ ethanolamine solution (85:7.5: 7.5, v/v)

Quantification is based on the calibration curves of external standards. The standard material of neutral lipid classes were purchased from Nu-Chek Prep and the polar lipid classes were purchased from Sigma.

3.7 Quality control

(1) The repeatability of direct methylation was checked by analyzing 6 replicates of the sample A and 7 replicates of soxhlet extract of sample A on the same day. The intermediate precision was evaluated by measuring samples (A-I) on different days throughout the study.

(2) Blank samples: To make sure that there is no cross contaminants between the samples and no interference from the reagents, blank samples were prepared every time when the methylations were done.

(3) Control oil sample was analyzed once a week. To make sure the whole procedure was under control, and that the equipment and the reagents were in good condition. The control oil is NorsalmOil from Vedde company [66]. It is

manufactured from selected species of fish, mainly capelin and herring. It was stored in freezer in 1.5ml vials. The lab of Nofima also use it daily to check stability of the fatty acid method.

(4) Quality control of GC: Blank sample (isooctane) was tested for every batch in GC. And the same control oil is used daily in the lab to check the stability of the routine procedure. The results are control by using the control chart. When something abnormal is detected through the control chart or after maintenance, the standard FAME mixture (GLC-793, Nu-Chek Prep Inc. Elysian MN, USA) is used to calibrate GC equipment.

3.8 Analysis of data

The results from the experiment were calculated in Microsoft Excel 2003. ANOVA, F-test and t-test were used to analyze the data at P-0.05 level. The data were also subjected to PCA by using the Sirus 8.0 software (Pattern Recognition Systems, Bergen, Norway).

3.9 Outline of the experiment

An outline of the experiment is given in Fig.7. A total of eight powders of marine origin were analyzed. For each sample, four methods were performed: Direct methylation (1) were performed in three replicates, three extraction method were performed in two replicates respectively. For each extract, residue or water phase (2-8), FA analyses were performed in two replicates. Thus, there are 248 fatty acid analyses and 48 gravimetric lipid content determinations for the samples. When checking the precision of the direct methylation, 6 replicates of sample A and 7 replicates of Soxhlet extract of sample A were performed with fatty acid analysis. Control oil was analyzed in two replicates every week, total 16 fatty acid analyses. Around 40 blank samples were performed with fatty acid analyses. The GC data are listed in appendixes.

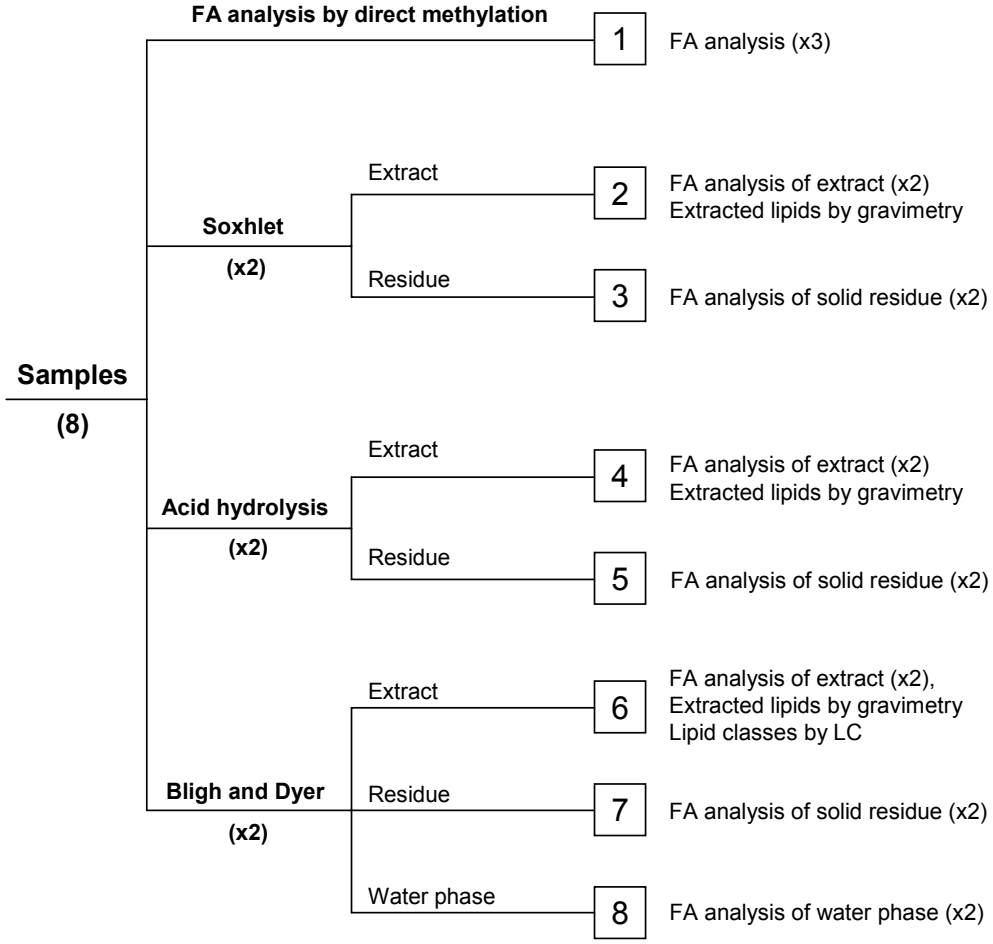


Figure 7. Outline of the experiment

4 Results and discussion

It is common to give the weight of lipids and fatty acids on different scales, the masses of fatty acids may be given relative to the total mass of the sample, relative to the lipid weight of the sample and relative to the sum of quantified fatty acids. A clarification of various concepts used in the result and discussion section is given in this paragraph.

Samples are the original marine powders which are subjected to extractions or fatty acid analysis by the direct methylation method. Extraction of the samples gives an organic *extract* and a *residue*, which is the non-extracted part of the sample. The Bligh and Dyer extraction will also yield a polar phase, which in general is referred to as a *water phase*.

The following analysis results will be given in this section:

1) *Gravimetric lipid content* (extracted) the content of all components in the organic extract determined by gravimetry. Results are generally given as g/100g sample.

2) Extracted fatty acids are the fatty acids in the extracts. Values are corrected for dilutions and subsampling and normalized against the original sample weight so that they are given as g extracted fatty acids per 100 g sample.

3) *Residual fatty acids* are the fatty acids in the residues after extractions. Values are corrected for dilutions and subsampling and normalized against the original sample weight so that they are given as g non-extracted fatty acids per 100 g sample. Fatty acids in the liquid phase after Bligh and Dyer extraction will also be treated as residual fatty acids.

4) *Reconstructed fatty acid composition* is the sum of the fatty acids in 3 and 4 and given as g fatty acids per 100 g sample.

5) Fatty acids by DM is the fatty acid composition analyzed by the direct methylation method. Results are given as g fatty acids per 100 g sample.

In addition to normalization against the total sample weight, the fatty acids may also be normalized against the sum of fatty acids in the sample, extract or residue (sum of fatty acids equals 100%). These results will usually be referred to as a fatty acid *profiles*. Results given as tables in the appendices may deviate from these definitions. Lipid class compositions determined by LC are given relative to the gravimetric lipid content as g /100g extracted fat by Bligh and Dyer.

4.1 Quality Control Results

The precision of the direct methylation method was determined by repeatability (within-day) and intermediate precision (between-day). The precision study was examined by analysing different samples (fish powers(A-I), control oil, extract of fishmeal A) by only one operator. The repeatability was evaluated by analyzing six or seven times within one day, whereas intermediate precision was evaluated by analyzing on different days during two months. Different batches of chemical reagents were used during this period, such as methylation reagent, internal standard etc.

4.1.1 Repeatability of the fatty acid analysis by DM

Repeatability of the fatty acid analysis by DM was evaluated by analyzing seven replicates of Sample A and six replicates taken from a single soxhlet extract of the same samples. The results reported in Table 5 and Table 6 demonstrate different method precision for powders and extracts. The coefficient of variation for the solid sample is 5.11%. For the specific fatty acids, C.V. range between 4.4% and 5.7%. The repeatability for the extract is 1.21%. For the specific fatty acids, C.V range between 0.8% and 2.1%.

There are two factors that can explain the higher variation for the solid samples. Solid samples are less homogeneous than liquid, and the matrix in solid samples are more complicated, the other components, such as proteins, carbohydrates may affect the methylation.

Table 5. The repeatability data of direct methylation method for fishmeal A

	DM repeatability for fishmeal A							(g FA/100g sample)	
FA	**GC 61***	**GC 62***	**GC 63***	**GC 64***	**GC 65***	**GC 66***	**GC 67***	**Average**	**C.V.(%)**
16:0	1.21	1.29	1.11	1.16	1.25	1.26	1.22	1.22	4.97
18:1	0.93	0.98	0.86	0.88	0.95	0.94	0.92	0.92	4.36
20:5 n3	0.66	0.70	0.60	0.64	0.69	0.68	0.67	0.66	5.25
22:6 n3	1.39	1.45	1.22	1.33	1.43	1.41	1.41	1.38	5.66
SFA	1.88	1.99	1.71	1.79	1.93	1.93	1.88	1.87	4.96
MUFA	3.34	3.49	3.00	3.18	3.44	3.36	3.33	3.31	4.99
PUFA	2.62	2.75	2.33	2.52	2.70	2.68	2.66	2.61	5.47
Total FA	7.84	8.23	7.05	7.49	8.07	7.96	7.87	7.79	5.11

*The numbers are references to GC-runs given in the appendixes.

Table 6. The repeatability data of direct methylation for Soxhlet extract of fishmeal A

	DM repeatability for Soxhlet extracts of fishmeal A (g FA/ 100g extract)							
FA	**GC 53***	**GC 54***	**GC 55***	**GC 57***	**GC 58***	**GC 59***	**Average**	**C.V.(%)**
16:0	0.054	0.053	0.055	0.056	0.056	0.056	0.055	2.036
18:1	0.050	0.049	0.050	0.050	0.052	0.050	0.050	1.836
20:5 n3	0.033	0.032	0.033	0.033	0.033	0.033	0.033	0.903
22:6 n3	0.056	0.056	0.056	0.057	0.057	0.057	0.056	0.778
SFA	0.090	0.088	0.092	0.092	0.094	0.093	0.092	2.237
MUFA	0.188	0.187	0.191	0.191	0.192	0.191	0.190	0.988
PUFA	0.123	0.121	0.123	0.123	0.124	0.122	0.123	0.934
Total FA	0.402	0.396	0.406	0.407	0.410	0.406	0.404	1.210

*The numbers are references to GC-runs given in the appendixes.

4.1.2 Intermediate precision for fish powder (A-I) and control oil (C.O)

The intermediate precision was evaluated by analyzing control oil on a weekly basis as a check for method stability. In addition, the samples were analysed by direct methylation three times thoughout the study to check for sample stability.

The coefficient of variation of the control oil is 1.85%. Since oil is also more homogeneous than the solids, lower CV can be expected. The results showed the direct methylation procedure is under good control. This guarantees the reliable result of the other samples.

For most of the samples, the intermediate precision of total fatty acids is around 5% (Table 7). The ANOVA statistical test *(P*-0.05 for the 95% confidence intervals) did not show any significant differences among the results of total FA between the three times. For each sample, there were no significant differences *(P*-0.05 for the 95% confidence intervals) in the proportions of SFA, MUFA or PUFA between these three times. That shows that the samples were stable, no degradation during time.

Table 7. Intermediate precision data for the marine powders (A-I) and the control oil (C.O.) analyzed by DM

	n		16:0	18:1	20:5	22:6	SFA	MUFA	PUFA	Total FA
C.O.	16	Average(g/100g)	11.55	9.07	6.29	10.33	20.24	39.22	27.76	87.22
		CV,%	2.89	2.22	2.20	1.46	2.82	1.72	1.63	1.85%
A	3	Average(g/100g)	1.24	0.93	0.67	1.36	1.91	3.28	2.62	7.81
		CV,%	4.71	4.25	3.00	2.37	4.74	3.07	3.07	3.29%
B	3	Average(g/100g)	0.42	0.27	0.29	0.80	0.56	0.37	1.28	2.20
		CV,%	3.47	4.81	7.53	6.66	3.26	10.08	7.24	6.58%
C	3	Average(g/100g)	3.96	13.81	1.34	2.31	6.22	18.08	11.92	36.22
		CV,%	7.12	7.09	6.16	5.38	7.11	6.89	6.50	6.80%
D	3	Average(g/100g)	2.16	1.59	1.19	3.04	2.82	2.45	4.85	10.13
		CV,%	2.41	2.44	7.48	7.83	2.56	2.22	7.61	4.59%
F	3	Average(g/100g)	3.47	2.96	2.06	1.20	5.44	4.32	4.42	14.18
		CV,%	2.52	1.98	2.61	1.96	2.46	1.97	2.37	1.89%
G	3	Average(g/100g)	0.88	0.93	0.36	0.60	1.25	2.68	1.25	5.17
		CV,%	5.65	3.28	6.63	4.83	5.62	3.44	4.93	4.16%
H	3	Average(g/100g)	1.55	1.36	0.56	1.10	2.39	4.34	2.20	8.92
		CV,%	4.36	4.60	3.88	2.40	4.52	5.18	3.21	4.24%
I	3	Average(g/100g)	3.03	2.43	2.67	1.49	4.47	2.23	6.00	13.71
		CV,%	2.80	4.33	3.51	3.31	2.94	4.71	2.92	2.76%

4.1.3 Comparison of the results of control oil

The control oil is also used for the routine quality control of the fatty acid analyses at Nofima BioLab. This lab follows the guidelines given in AOCS Official Method Ce 1b-89 [67], where methylationis done by alkaline transmethylation in NaOH followed by acid methylation by BF_3. The result obtained by the HCl catalysed method applied in this study was compared with the lab result of the same period (Table 8). Except for the differences in methylation, the samples from the two methods have been analysed under identical conditions (same GC applied in the same period)

From the table, it can be seen that though the two sets of results have difference in the precision in 20:2 n-6 and 20:5 n-3, the values obtained for the four components from the two methods are not significantly different (*P*-0.05 for the 95% confidence intervals).

Table 8. Comparison of the result of the control oil between the routine test in lab and direct methylation method (g/100g sample)

	Result from the lab (n=40)			Result from DM method (n=16)			Statistical comparison	
	Average	Std Dev.	RSD,%	Average	Std Dev	RSD,%	F-test for two variance	t -test for two means (2 tails)
14:0	6.87	0.23	3.4	6.87	0.19	2.77	S1=S2	$\bar{X}1=\bar{X}2$
20:2 n6	0.23	0.01	5.07	0.22	0.02	7.03	S1≠S2	$\bar{X}1=\bar{X}2$
20:5 n3	6.29	0.14	2.16	6.29	0.14	2.2	S1≠S2	$\bar{X}1=\bar{X}2$
22:6 n3	10.24	0.18	1.73	10.33	0.15	1.46	S1=S2	$\bar{X}1=\bar{X}2$

Note: the F-test and t-test were set P-0.05 for the 95% confidence intervals.

The four fatty acids in Table 8 are under control by using the control chart in Nofima BioLab. The comparison results demonstrate satisfactory results for fatty acid analysis by the direct methylation method. In the following discussion, the result of total FA obtained from direct methylation methods will be used as the benchmark to evaluate the extraction efficiency of the three methods: Soxhlet method, Acid hydrolysis method and Bligh and Dyer method.

4.1.4 Comparison of the results of extraction methods

Sample A is also used in Nofima BioLab as control sample for extraction methods. The comparison of extraction methods are as follows.

Soxhlet extraction:

The gravimetric lipid for sample A is 8.86g/100g sample and CV is 0.7% (n=18) in the lab, the difference between the two replicates of the same day range between 0.34% and 4.1%. While the gravimetric lipid in this study is 9.27g/100g sample, the difference of two replicates on different days is 2.32% (Table 11). The precision is similar with the lab, but the gravimetric lipid content is much higher than achieved with re routine procedure used on the lab. This may be because the drying step was removed in this study. Possible oxidation and degradation caused by heat and exposure to oxygen is therefore avoided. Another possible reason may be that the water (7.8%) in the sample change the properties of the matrix, e.g. swelling, so that the lipids are extracted more efficiently. The presence of water is for instance critical for the chloroform/methanol based extraction procedures.

Acid hydrolysis extraction:

The comparable results were obtained. The gravimetric lipid of acid hydrolysis in the lab is 10.45g/100g sample and CV is 2.26% (n=24). The differences between the two replicates of the same day range between 0 and 3.1%. In our study, the gravimetric lipid of acid hydrolysis is 10.66 g/ 100g sample (Table 12). It is within the range of control chart of the lab. The difference between the two replicates is 2.53%.

Bligh and Dyer:

The gravimetric lipid content for sample A in the lab is 11.7 g/100g sample (n=4), the differences between the two replicates on the same day are 0.43% and 1.0%. In this study, the gravimetric lipid content for sample A is 10.71g/100g and difference of two replicates are 0.68%. Since the modification was made in this study, and as we have discussed in 2.6.2, B&D method is operationally defined, altering the original procedure can easily lead to deviating results. The difference between our result and the lab was as expected. The difference between 11.7 g/100g sample and 10.71g/100g sample is 8.9%. It's lower than the variability of intercomparion exercise of 33 labs by QUASIMEME[42].

4.2 Samples

The fatty acid compositions by GC and lipid class compositions by LC for eight marine powders were determined. These eight samples are: fishmeal (A), herring roe (B), salmon filet (C), herring roe (D), krillmeal (F), fishmeal (G), fishmeal (I) and krillmeal (I). GC and LC results show that the eight samples vary greatly in lipid composition.

The moisture contents in the samples determined as described in 3.2 were as follows: A, 7.84%; B, 2.31%; C, 6.49%; D,1.5%; F, 6.09%; G, 10.48%; H, 7.47%; I, 6.39%.

4.2.1 Fatty acid composition by GC

Fatty acid compositions of the sample by direct methylation as described in section 3.4 are shown in Table 9.

Salmon (C) is typical fat fish, it contains highest amount of total fatty acids (36.22g/100g) of the eight samples. The main fatty acids were 18:1 fatty acids, 38.13%; 18:2, n-6, 11.10% and palmitic acid (16:0, 10.93%). The lean cod filet (B) represents the lowest content of total fatty acids (2.20g/100g). In the fatty acids, 22:6 n-3, 36.36%; 20:5 n-3, 13.18% and palmitic aicd, 19.09%. Herring roe (D) contains 10.13g/100g total fatty acids. The main fatty acids were 16:0, 21.32 %; 22:6 n-3, 30.01% ;20:5 n-3, 11.75%) and 18:1, 15.70%.

The two krill meals (F, I) have similar contents of total fatty acids (14.18g/100g, 13.71g/100g). The main fatty acids were 16:0 (F: 24.5%; I: 22.1%), 18:1 (F: 18.0%; I: 17.7%) and 20:5 n-3 (F: 14.5%; I: 19.5%). The three fish meals (A, G, H) have less than 10% of total fatty acids. The main fatty acids were 16:0, 18:1 fatty acids, 22:1 fatty acids and 22:6 n-3.

Table 9. Fatty acid composition of direct methylation (g/100g sample) analyzed by GC (n=3)

Fatty acid composition by direct methylation (g/100g sample)								
	A	**B**	**C**	**D**	**F**	**G**	**H**	**I**
12:0	0.01	n.a.	0.02	<0.01	0.03	n.a.	0.01	0.03
14:0	0.48	0.03	1.04	0.42	1.69	0.18	0.61	1.23
16:0	1.24	0.42	3.96	2.16	3.47	0.88	1.55	3.03
18:0	0.16	0.11	1.03	0.23	0.23	0.17	0.19	0.17
20:0	0.01	n.a.	0.11	n.a.	0.01	0.01	0.01	n.a.
22:0	<0.01	n.a.	0.06	n.a.	0.01	n.a.	n.a.	n.a.
16:1 n7	0.28	0.03	1.04	0.36	0.99	0.23	0.35	0.51
18:1	0.93	0.27	13.81	1.59	2.96	0.93	1.36	2.43
20:1	0.82	0.05	1.82	0.25	0.2	0.67	1.03	0.16
22:1	1.14	0.01	1.19	0.11	0.14	0.71	1.51	0.13
24:1	0.11	0.03	0.2	0.14	0.03	0.13	0.14	0.01
16:2 n4	0.02	n.a.	0.11	0.01	0.11	0.01	0.02	0.07
16:3 n4	0.02	n.a.	0.07	0.02	0.06	0.01	0.02	0.06
18:2 n6	0.1	0.02	4.02	0.12	0.24	0.06	0.11	0.24
18:3 n6	n.a.	n.a.	0.04	n.a.	0.02	n.a.	n.a.	0.01
20:2 n6	0.01	n.a.	0.38	0.01	0.01	0.01	0.02	0.01
20:3 n6	0.01	n.a.	0.07	0.01	0.01	n.a.	0.01	0.01
20:4 n6	0.04	0.11	0.13	0.07	0.06	0.04	0.04	0.04
18:3 n3	0.07	0.01	1.69	0.09	0.15	0.03	0.07	0.38
18:4 n3	0.17	0.01	0.26	0.1	0.29	0.06	0.12	0.72
20:3 n3	0.01	0.01	0.2	<0.01	0.01	0.01	0.01	0.03
20:4 n3	0.05	0.01	0.4	0.07	0.08	0.02	0.04	0.1
20:5 n3	0.67	0.29	1.34	1.19	2.06	0.36	0.56	2.67
21:5 n3	0.02	n.a.	0.09	0.01	0.06	0.01	0.01	0.1
22:5 n3	0.07	0.03	0.79	0.11	0.06	0.04	0.07	0.06
22:6 n3	1.36	0.8	2.31	3.04	1.2	0.6	1.1	1.49
SFA	1.91	0.56	6.22	2.82	5.44	1.25	2.39	4.47
MUFA	3.28	0.37	18.08	2.45	4.32	2.68	4.34	3.23
PUFA	2.62	1.28	11.92	4.85	4.42	1.25	2.2	6
Total FA	7.81	2.2	36.22	10.13	14.18	5.17	8.92	13.71

Principal component analysis (PCA) of the data in Table 9, with fattty acids as variables and samples as objects was used to give a clearer overview of the differences between the samples. PCA biplot is given in Figure 8. As expected, the three fish meal samples A, G, H group together in the plot, they contain a high percentage of fatty acids 22:1 and 20:1. The two Krill meals are quite similar. B and D are similar, they contain high percentage of 20:5 n-3 and 22:6 n-3. C is far away from the other samples. The most typical fatty acids for salmon are 18:1.

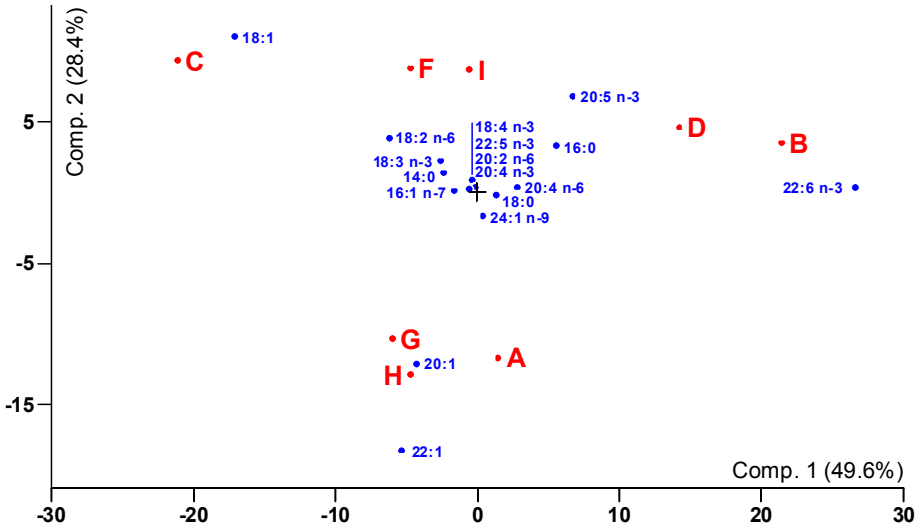


Figure 8. PCA biplot of fatty acids composition by DM

4.2.2 Lipid class composition by LC

Lipid class composition analyses by LC determined as described in section 3.6 are shown in Table 10.

Table 10) Lipid class analysis by LC (g/100g extracted fat)

	A	B	C	D	F	G	H	I
Triacylgcerol	54	2.3	87	17	45	25	57	39
Diacylglycerol	<0.5	<0.5	<0.5	<0.5	1.1	1.8	<0.5	<0.5
Monoacylglyerol	<1	<1	<1	<1	<1	1.4	<1	<1
free fatty acids	3.4	25	0.8	2.6	3.6	36	5.3	13.6
Cholesterol	2.1	6.7	<0.5	8.2	2	6.2	4.1	1.2
Cholesterol ester	<0.5	<0.5	0.5	<0.5	0.8	0.7	<0.5	<0.5
Phosphatidyletanolamin	6.2	12	<1	7	<1	<1	<1	1.2
Phosphatidylinositol	<1	<1	<1	<1	<1	<1	<1	<1
Phosphatidylserin	<1	<1	<1	<1	<1	<1	<1	<1
Phosphatidylcholin	16	29	<1	49	34	8	13	35
Lyso-Phoshatidylcholin	1.2	1.8	<1	<1	2.8	1.8	1	1.3
Total polar lipid	23.6	42.8	1.1	56.6	37.9	10.1	14.6	37.8
Total neutral lipid	59.7	35.3	88.6	27.6	52.1	71.2	67.2	54.4
Total sum lipid	83.3	78	89.7	84.2	90	80.9	81.8	92.3

Note: The crude lipids were extracted from the samples by the method of Bligh and Dyer as described in 3.3.3

Herring roe has a high percentage of phosphatidylcholine (PC, 49%).It was the main component in the PL class of herring roe. Cod filet also contains a high percentage of phospholipids (42.8%). About 99% of the lipid of salmon filet (sample C) exists as triacylgcerol.

Fish meal G and cod filet B have high content of FFA. This may suggest that hydrolysis of lipids have taken place in these two samples, most likely by the

activity of lipases. Since the lipid classes were analysed only at the end of the study it is not possible to know if hydrolysis has occurred during the time of the study. However, enzyme activity in dry powders is minimal, so most likely the hydrolysis has happened before preparation of the samples. The high FFA content was confirmed by an alternative method (titration by AOCS Ca 5A – 40 [68]) which showed levels of 27.5% and 28.6% for B and G respectively.

The sum of lipids qualtified by LC varied from 78-92% of the gravimetric lipid content determined by the Bligh and Dyer extraction. This difference may partly be caused by the presence of non-lipid material in the extract.

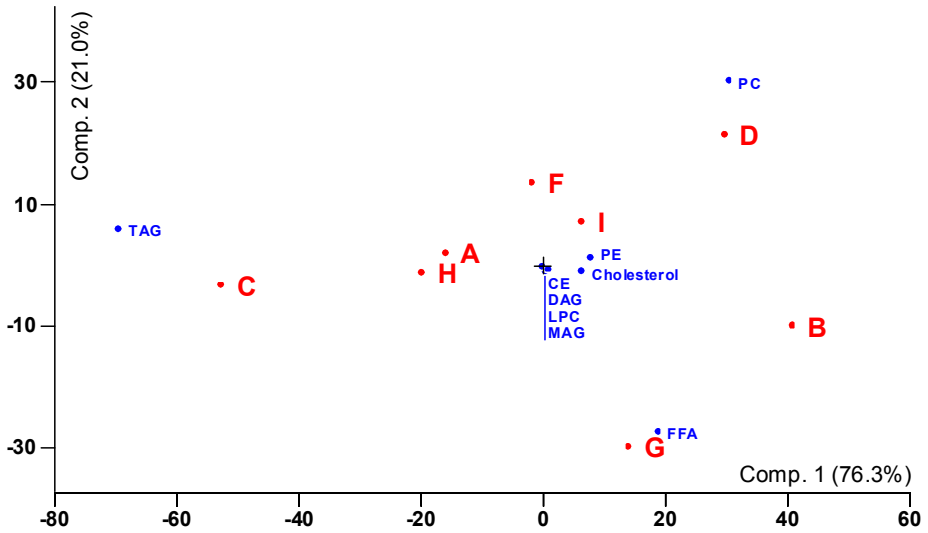


Figure 9. PCA biplot of lipid class composition

It can be seen from PCA plot of lipid class compositon (Fig. 9) that the most typical lipid for C (salmon) is triacylgerol, and for D (herring roe) is phosphatidylcholin. We can also see that C (fat fish) and B (lean fish) are negatively correlated in lipid class composition (correlation coefficient= -0.993).

4.3 Mass balance of the extraction methods

The main objective of this study was to evaluate the extraction efficiency of the three extraction methods, by comparing the amounts of extracted and residual fatty acids with the amounts found by direct methylation. The lipids in the eight samples were extracted by the using the three extraction methods: Soxhlet, acid hydrolysis and Bligh and Dyer. The fatty acids in extracts and residues were determined by GC. The analysis procedure has been illustrated in Fig.1. The mass balances to direct methylation were calculated. The sum of fatty acids in extracts and residues (reconstructed fatty acid composition) should ideally be equal to the amounts found by direct methylation.

4.3.1 Mass balance for the Soxhlet method

The Soxhlet method was compared with direct methylation method. The figures obtained are given in Table 11. The result clearly illustrated that the Soxhlet method gave lower yield of fatty acids than direct methylation, in some samples, much lower (cod filet and herring roe). The only satisfactory yield of fatty acids obtained by Soxhlet method is salmon filet, where the lipids are basically TAG. For most samples, substantial fatty acids were still left the residues. The total recovery of fatty acids in extract and residue are close to 100%. This indicates that fatty acid was not lost during extractions of samples and handling of the residues.

Table 11) Mass balance result for the Soxhlet method

Sample	Sample weight (g)	Gravimetr. lipid (g/100g sample)	Recovered FA (g/100g sample)			Mass balance rel. to DM		
			Extract	Residue	Sum	Extract	Residue	Sum
A1	4.81	9.37	6.88	1.32	8.2	88.15%	16.93%	105.08%
A2	4.36	9.16	7.08	1.41	8.49	90.64%	18.12%	108.76%
B1	4.2	1.43	0.82	1.46	2.28	37.33%	66.27%	103.59%
B2	4.26	1.3	0.84	1.44	2.28	38.25%	65.18%	103.43%
C1	4.12	38.62	35.5	0.83	36.33	98.00%	2.30%	100.30%
C2	4.02	38.96	35.98	0.83	36.81	99.32%	2.29%	101.62%
D1	4.87	5.35	3.18	7.59	10.77	31.37%	74.94%	106.31%
D2	4.24	5.18	2.95	6.75	9.7	29.12%	66.65%	95.77%
F1	4.49	15.89	11.37	3.02	14.4	80.23%	21.31%	101.54%
F2	4.02	16.36	10.81	2.93	13.74	76.25%	20.65%	96.90%
G1	4.51	6.41	4.52	1.06	5.58	87.46%	20.42%	107.90%
G2	4.11	6.34	4.41	1.17	5.57	85.21%	22.52%	107.32%
H1	4.82	10.86	8.24	1.57	9.81	92.42%	17.62%	110.04%
H2	4.7	10.81	8.29	1.68	9.98	92.97%	18.89%	111.86%
I1	4.28	16.59	11.57	2.75	14.32	84.42%	20.04%	104.47%
I2	4.22	16.04	11.18	2.83	14.01	81.53%	20.66%	102.19%

It has been reported that Soxhlet method failed to extract phopholipids in the samples [32, 30, 33]. This can be further proven here by comparing the the Soxhlet extracting efficiency with the neutral lipid proportion of the samples (Fig. 9).

Fig. 10 indicated a good agreement between Soxhlet extraction efficiency and the neutral lipid proportion of the samples: higher neutral lipid, higher Soxhlet extraction efficiency. Take salmon filet for example, it contains 97.73% triacylgcerol of the total lipid and high extraction efficiency was obtained by Soxhlet method (98.77% total FA in extract). While herring roe contains high amount of polar lipid (67.22% of total lipid), it can not be easily extracted by Soxhlet method, thus unacceptably low extraction levels of lipids were obtained (30.18%).This shows that Soxhlet methods with petroleum ether mainly extract

neutral lipids. It is not suitable for the samples that contain high polar lipids. It may be more appropriate to call the fat extracted by Soxhlet method ‘‘extractable lipids’’ than ‘‘the total lipids’’.

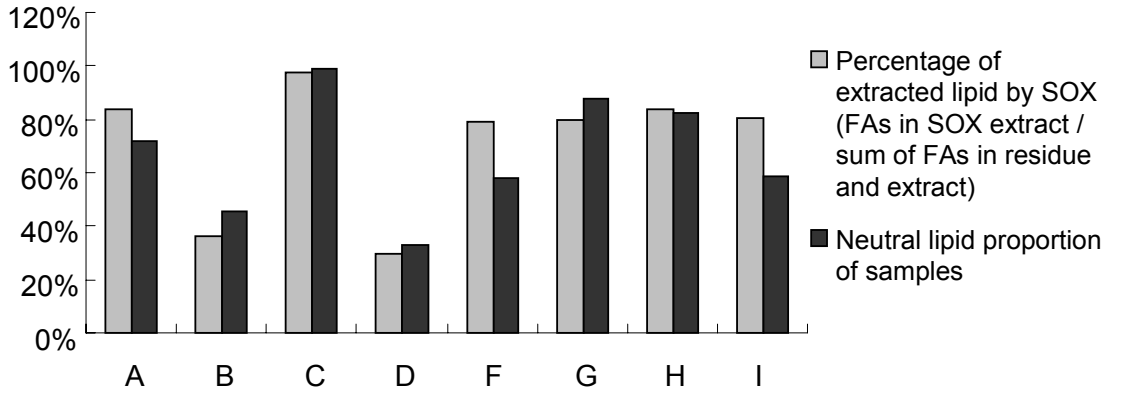


Figure 10. Comparison of the percentage of Soxhlet extracted fatty acids and neutral lipid proportion in the samples.

It needs to be pointed out that the neutral lipids can not be 100% extracted by Soxhlet and the polar lipids can not be 100% left in the samples. Goran Ewald *et al*. [10] have already illustrated this point. And some lipids contribute to extracted neutral lipids, but not to the fatty acids, for example Cholesterol. Thus it’s impossible that the Soxhlet extracting result corresponded with the neutral lipid proportion of the samples completely. But the selective extraction tendency of neutral lipids by soxhlet method has clearly been shown by this comparison.

4.3.2 Mass balance for acid hydrolysis method

The inefficient extraction of polar lipids by the Soxhlet method was an expected result in accordance with previous reports. Hydrolysis of the lipids prior to extraction is a common method to circumvent this problem. The acid hydrolysis method applied in this study is in principle identical to the soxhlet method, except that the residue after soxhlet is hydrolysed and extracted once more. It can therefore be used to evaluate whether hydrolysis is an efficient way to release the fatty acids that was not extracted by soxhlet.The results of acid hydrolysis method and comparison with direct methylation were shown in Table 12.

In acid hydrolysis method, acid hydrolysis step was taken after the Soxhlet extraction to release the bond lipid that can not be extracted by the Soxhlet. It was reported that the leftover phospholipids fraction was completely hydrolyzed into free fatty acids after acid hydrolysis step so that it can be extracted easily [69]. Thus the extraction efficiency of the acid hydrolysis method is higher than Soxhlet.

However, there were still substantial amounts of fatty acids left in the residue after extraction, over 5% in several cases, and as high as 17% in the case of the cod filet powder. This may be due to the incomplete hydrolysis of PL because of short hydrolysis time, or because the free fatty acids formed in the

hydrolysis are not efficiently extracted by the subsequent Soxhlet extraction. For further research, a Bligh and Dyer extraction of the residue followed by LC analysis of the extract would indicate if the residual fatty acids are bound in complex lipids or present as FFA. Increased hydrolysis time could also be used to test if the extraction efficiency is improved.

The total recovery of fatty acids in extract and residue are close to 100%. This indicates that the fatty acids were not lost during the acid hydrolysis process.

Table 12. Mass balance for the acid hydrolysis method

	Sample	Gravimetr. Lipid	Recovered FA (g/100g)			Mass balance rel. to DM		
Sample	weight (g)	(g/100g)	Extract	Residue	Sum	Extract	Residue	Sum
A1	4.67	10.55	8.04	0.18	8.22	103.05%	2.29%	105.34%
A2	4.61	10.77	8.79	0.23	9.02	112.64%	2.92%	115.57%
B1	4.73	2.58	1.81	0.38	2.2	82.29%	17.41%	99.70%
B2	4.22	2.58	1.97	0.38	2.35	89.35%	17.29%	106.64%
C1	4.34	39.21	37.16	0.34	37.5	102.58%	0.95%	103.53%
C2	4.47	39.25	37.02	0.24	37.26	102.19%	0.66%	102.86%
D1	4.61	12.24	9.41	0.59	10	92.93%	5.78%	98.71%
D2	4.71	12.43	10.06	0.68	10.75	99.38%	6.76%	106.14%
F1	4.53	18.95	13.83	0.26	14.09	97.56%	1.85%	99.41%
F2	4.5	18.77	13.61	0.59	14.2	96.02%	4.17%	100.19%
G1	4.84	7.58	5.39	0.3	5.69	104.19%	5.73%	109.92%
G2	4.75	7.45	5.4	0.36	5.76	104.39%	6.92%	111.30%
H1	4.92	12.73	9.64	0.18	9.82	108.05%	2.05%	110.10%
H2	4.53	12.58	9.66	0.26	9.92	108.30%	2.93%	111.24%
I1	4.38	19.08	13.56	0.35	13.91	98.91%	2.58%	101.49%
I2	4.87	19.24	14.2	0.29	14.49	103.61%	2.12%	105.72%

4.3.3 Mass balance for the Bligh and Dyer method

The results of Bligh and Dyer method and comparison with direct methylation are shown in Table 13. The total recovery of fatty acids in extract and residue are also close to 100%. For some samples, the sum of fatty acids relative to DM was much higher than 100%, even up to 120% for sample A. This may be partly due to the inaccuracies in our procedure caused by DM which may have sampling problem because of small sample size. Higher recovery of fatty acids in extract and residue can also be found in Soxhlet (Table 11) and acid hydrolysis (Table 12), but not as high as that in Bligh and Dyer. This indicates that there are additional problems causing the inaccuracies of the procedure of Bligh and Dyer, especially the subsampling step, where a small volume is sampled by pipette. The phase may not be completely homogeneous, or the sub-sampling of the organic solvents by usig the pipette may not be accurate. These problems also caused the worst repeatability of extracted FAs of B&D. See also section 4.3.4.

Also for the Bligh and Dyer method there was still fatty acids left in the residue.

This can be residual fatty acids, but also contributions from extracted fatty acids that are dissolved in solvents trapped in the residue. Especially in the residues of sample B and G, up to 10% fatty acids are left. This is most probably due to non-extracted lipids, since the samples that showed high amounts in residues after Bligh and Dyer also showed the highest levels in residues after the two other extraction methods. These two samples contain high amount of PL and FFAs that make the matrices more complicated than the others, thus substantial fatty acids are not extracted in these two samples. The percentage of FA lost due to trapping of solvent in the residue and filters, should be independent of lipid class composition and similar for all samples since the amount of residual matrix is similar. In sample C where most lipids are easily extracted, the residual lipid content was 1.6%, and lower than all other samples. This indicates that the majority of residual lipids are lipids that have not been released from the sample matrix.

Table 13) Mass balance result for Bligh and Dyer method

	Sample weight (g)	Gravimetr. Lipid (g/100g)	Recovered FA (g/100g)				Mass balance rel. to DM			
			Chloroform	Water	Residue	Sum	Chloroform	Water	Residue	Sum
A1	2.52	10.75	8.95	0.03	0.46	9.43	114.60%	0.38%	5.89%	120.74%
A2	2.56	10.67	7.97	0.03	0.52	8.52	102.05%	0.38%	6.66%	109.09%
B1	2.59	3.39	2.2	0.01	0.21	2.42	100.00%	0.45%	9.55%	110.00%
B2	2.54	3.29	1.98	0.01	0.23	2.23	90.00%	0.45%	10.45%	101.36%
C1	2.58	37.29	34.57	0.01	0.52	35.11	95.44%	0.03%	1.44%	96.94%
C2	2.51	37.46	33.17	0.02	0.66	33.85	91.58%	0.06%	1.82%	93.46%
D1	2.55	16.17	10.87	0.05	0.68	11.6	107.31%	0.49%	6.71%	114.51%
D2	2.56	16.3	9.87	0.02	0.61	10.5	97.43%	0.20%	6.02%	103.65%
F1	2.56	19.46	12.95	0.2	0.61	13.77	91.33%	1.41%	4.30%	97.11%
F2	2.57	19.08	12.17	0.39	0.52	13.08	85.83%	2.75%	3.67%	92.24%
G1	2.57	7.68	4.91	0.06	0.56	5.54	94.97%	1.16%	10.83%	107.16%
G2	2.55	7.68	4.78	0.05	0.4	5.23	92.46%	0.97%	7.74%	101.16%
H1	2.6	12.81	8.62	0.05	0.58	9.25	96.64%	0.56%	6.50%	103.70%
H2	2.51	12.77	8.28	0.05	0.5	8.83	92.83%	0.56%	5.61%	98.99%
I1	2.52	17.62	13.07	0.17	0.63	13.87	95.33%	1.24%	4.60%	101.17%
I2	2.52	17.67	11.88	0.15	0.41	12.45	86.65%	1.09%	2.99%	90.81%

In biphasic extraction system, some phopholipids can be retained in the water phase, especially the more hydrophilic phospholipids (*e.g.* lyso-phosphatidylcholine, lyso-phosphatidylethanolamine, or sphingomyelin) [70, 71]. Except for samples F, G and I, the amount of fatty acids in the aqueous phase was less than 1% of the levels in the organic phase. The small loss of fatty acids most likely correspond to a higher loss of lipids, since FA contribute with a limited percentage of the lipid mass in the most polar lipids such as lyso-lipids.

The wash with polar phase is basically used for removal of non-lipid material

when total lipids are determined by gravimetry. As long as loss of polar lipids are observed in Folch or Bligh and Dyer methods, one can question the use of these methods prior to determination of fatty acids or polar lipid classes by chromatographic methods that are not influenced by the presence of small amounts of non-lipids. The alternative methods based on single-phase solvent systems have proven to be more efficient than the biphasic extraction procedures on the isolation of PL from biological sources [70].

The results given in Table 13 determined by “measured subsampling”, where the recovered organic phase volume is measured and an aliquot is taken in which the lipid content is determined, the lipid content for the sample is then calculated for the total volume of the recovered organic phase. The alternative to measured subsampling is “blind subsampling” where the volume of the organic phase is expected to be equal to added chloroform. Both methods have advantages and disadvantages.

A single extraction and measured subsampling will underestimate the lipid content because of the amount of solvent that is trapped in the residue, filters and other places.

Blind subsampling is commonly applied with the Bligh and Dyer method [72, 42] and is also used by the routine method in Nofima Biolab as well (appendix H). In blind subsampling one assumes that the solvent that are trapped in residues and filters has the same lipid concentration as the sampled solvent, and that total solvent volume in the apolar phase is equal to added chloroform. This is more accurate than measured subsampling when a single extraction is applied. But the assumption that the concentration in the trapped liquid is identical to the concentration in the collected lipid can not be valid if repeated extractions are applied. Blind subsampling will also be affected by evaporation of solvents. The reason for using measured subsampling in this study is that we needed the second 'wash' of the residue for evaluation of the residual fatty acids. Then blind subsampling is not an option. But for the routine analysis by Bligh and Dyer, the second extraction will increase the nonlipid extraction, therefore not recommended.

4.3.4 Precision of the extraction methods

From table 11, 12 and 13, it can be seen that the repeatability of the gravimetric lipids for the three extraction methods is similar, below 5% for most samples. But for the repeatability of the extracted FAs, Bligh and Dyer method is worse than the other two methods.

There are several differences between the Bligh and Dyer and the other method that can lead to lower repeatability. There are numerous equilibria that need to be established in short time in the Bligh and Dyer procedure, e.g. between sample and solvent during extraction, between the two phases after phase separation, between filters and solvents, and between the chloroform wash and residue/filter. In addition there are the questions of dispersed droplets

of the other liquid phase in the two phases that may lead to sampling from non-homogenous phases. Other causess of non-homogeneity may be that lipids are amphiphatic, and some may concentrate in the interphase between the two phases. Filters may absorb lipids, there are also more equipment involved in general where lipids may be absorbed ore released (leading to cross contamination). The Bligh and Dyer method is also applied with volumetric measurements, while weight measurements were applied with the two other extraction methods in this study.

The repeatability of the gravimetric lipid for B&D was as good as the other two extraction methods. This may be because the volume of subsampling is large; the results were not affected too much by the non-homogeneity.

4.4 Extracted Fatty acids by the different methods

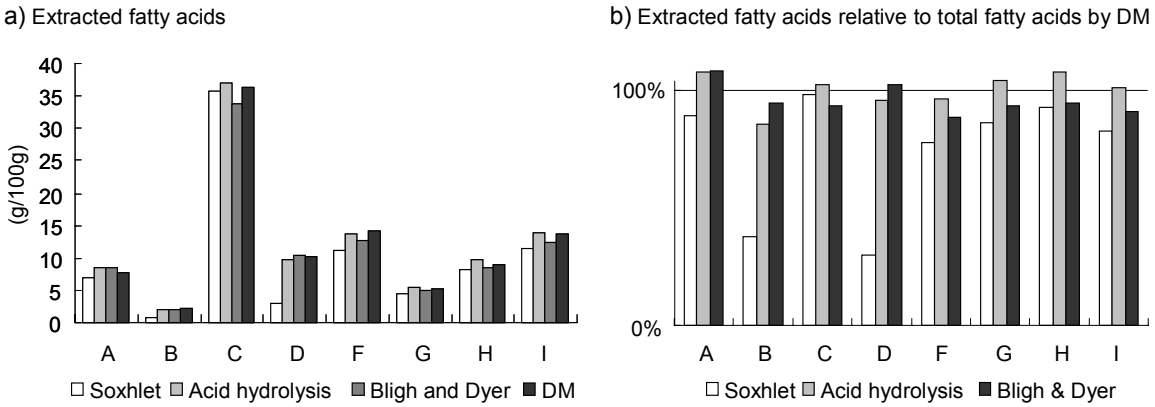


Figure 11. Comparison of the extracted fatty acids (g/100g) (a), and comparison of extracted FAs relative to total FAs by DM (%) (b)

Comparing the extracted fatty acids by the four methods (fig. 11), Soxhlet methods got the lower results than the other three methods for most samples, especially in cod filet (B) and herring roe (D). AVONA analysis shows there were no significant difference between the results obtained by Acid hydrolysis methods, Bligh and Dyer methods and direct methylation method *(P*-0.05 for the 95% confidence intervals).

4.5 Gravimetric lipid content by extraction methods

The comparison of the gravimetric lipid content by the three extraction methods are shown in Fig.12. For convenience, the levels are normalised to total FAs determied by direct methylation. But it should be emphasised that the amounts in this case should correspond to 100% only in cases where lipids are 100% TAG (since weight ratio of FAME to lipid is different than 1 for other lipid classes).

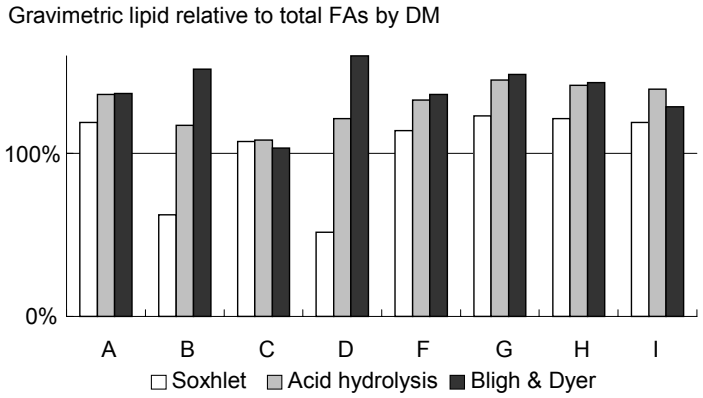


Figure 12. Comparison of gravimetric lipid content by three extraction methods

The lowest gravimetric lipid content was still obtained by using the Soxhlet method. For some samples, it was lower than the fatty acid content by DM because of the low extracting efficiency. The gravimetric lipid by Acid hydrolysis and B&D are higher than the total fatty acids by DM in the samples. Though there were no significant difference between the results obtained by Acid hydrolysis method and Bligh & Dyer method by t-test analysis *(P*-0.05 for the 95% confidence intervals), the gravimetric lipid content by Bligh and Dyer is a little higher than acid hydrolysis method for most samples. This is consistent with the result got by Adrianus J.de Koning et al [69].The reason may be due to the vigorous homogenization process used in the Bligh and Dyer. Another reason may be that acid hydrolysis releases the lipid from the connection with the other material and the washing step remove some non-fat material, thus less non-fat material was extracted in acid hydrolysis method. Also as was discussed above, triglycerides and phopholipids extracted from acid-hydrolyzed samples are recovered as free fatty acids whereas in the chloroform/methanol extraction method, triglycerides and phopholipids are recovered intact. A phospholipid will therefore contribute to more mass when extracted by the Bligh and Dyer method than extracted after acid hydrolysis. J.I. Rader *et al.* [38] also compared gravimetric lipid content by hydrolytic extraction with extracted fatty acids for 24 kinds of food. The result showed that total lipids by hydrolytic extraction were higher than the total fatty aicds determined chromatographically after hydrolytic extraction. This agrees with our observations as well. Therefore, it has been clear that the gravimetric lipid content by extraction methods is either higher or lower than the total fatty acids contents determined by DM.

Lipid is an important source of energy. Most fatty acids are excellent energy stores and easily oxidized to yield lots of energy. The correct description of energy value of fats is the content of total fatty acids not the gravimetric lipid content. But because of the lack of enough data about the fatty acids of different matrices, the gravimetric lipid determined by extraction sometimes are used to predict the dietary energy value by giving one factor [73,74].

The values of total lipids by three extraction methods and total fatty acids by

DM for the seven fish products were subjected to linear regression analysis to determine the relationship between the data pairs (Fig.13). Salmon was excluded because of its much higher lipid content than the other samples.

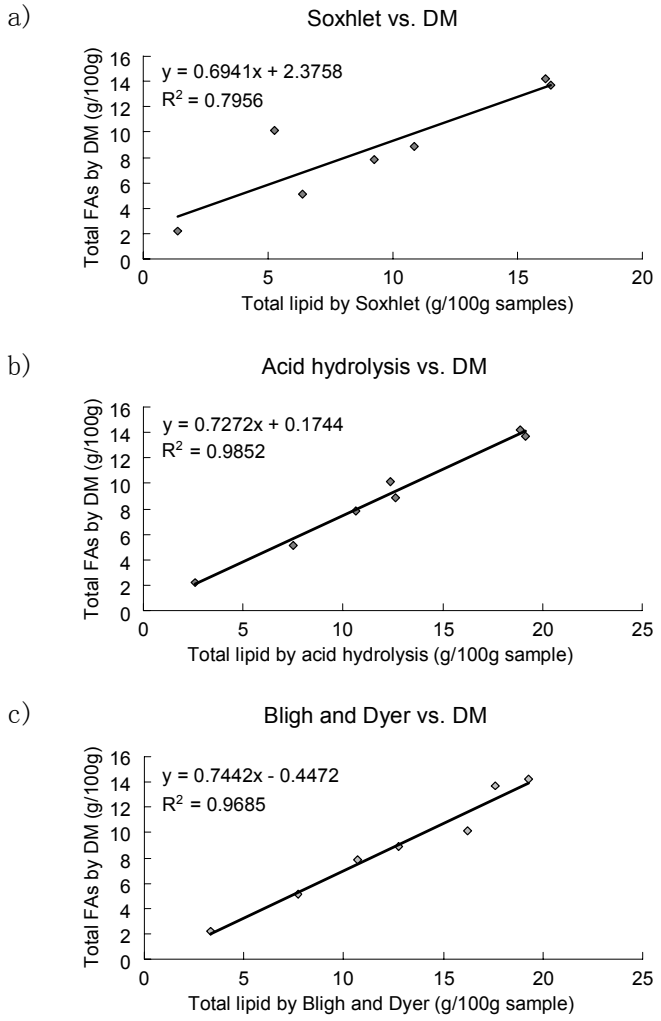


Figure. 13. Values for gravimetric lipids determined by different extraction methods were regressed on values for total fatty acids obtained by Direct methylation method of seven samples (salmon excluded). Gravimetric lipid by Soxhlet vs. total FAs by DM (a); Gravimetric lipid by acid hydrolysis vs. total FAs by DM (b); Gravimetric lipid by Bligh and Dyer vs. total FAs by DM (c)

The correlation of gravimetric lipid by Soxhlet and total FAs by DM (Fig. 13a) is the worst (R^2 =0.7956). That means that it is inappropriate to predict the energy value by using gravimetric lipid by Soxhlet. Acid hydrolysis and Bligh and Dyer gave better correlation. Gravimetric lipids by these two methods are therefore more suitable for the rough evaluation of energy values.

Recent research has showed that the dietary energy values of fat depends on the degree of saturation, free fatty acid content and chain length of the components of fats and oils[75,76]. It is therefore more accurate to use the fatty acid profiles to assess the energy value of fats. Next we would discuss how the extraction methods would influence the fatty acid profiles of the samples.

4.6 Influence of the extraction methods on fatty acid profiles

4.6.1 Introduction

Fatty acid profiles, where the values are normalized so that the sum of fatty acids is 100%, where used for evaluation of the influence of the different methods on the fatty acid composition. The advantage of using fatty acid profiles that are normalised to the sum of quantified fatty acids is that inaccuracies related to sample weights and the internal standard are eliminated. The effects on total fatty acid extraction are also discussed in sections 4.3 to 4.5, and a normalized fatty acid profile will not be affected by total extraction efficiency. Only fatty acids that constitute more than 1% of in least one of the samples (determined by DM) are included it the tables in this section. However, fatty acids below this threshold were included when calculating the partial sums (SFA, MUFA, PUFA).

The fatty acid profiles after direct methylation is given in Table 14. In the following discussion it will be compared to fatty acid profiles achieved by extractions followed by methylation.

Table 14. Fatty acid profile by DM (%)

	A	*B*	*C*	*D*	*F*	*G*	*H*	*I*
14:00	6.18	1.18	2.88	4.18	11.91	3.55	6.84	8.97
16:00	15.94	19.02	10.92	21.34	24.49	17.03	17.42	22.08
18:00	2.04	5.05	2.85	2.30	1.61	3.29	2.16	1.24
16:1	3.60	1.22	2.88	3.57	6.97	4.52	3.90	3.68
18:1	11.94	12.20	38.14	15.70	20.85	17.98	15.21	17.72
20:1	10.47	2.17	5.03	2.43	1.41	12.91	11.54	1.16
22:1	14.59	0.66	3.29	1.11	1.01	13.81	16.89	0.94
24:1 n9	1.38	1.37	0.56	1.37	0.20	2.51	1.61	0.09
18:2 n6	1.25	1.07	11.10	1.18	1.70	1.23	1.22	1.73
20:2 n6	0.17	0.16	1.05	0.12	0.09	0.24	0.17	0.10
20:4 n6	0.46	4.82	0.36	0.69	0.41	0.68	0.45	0.26
18:3 n3	0.93	0.34	4.67	0.89	1.05	0.52	0.76	2.78
18:4 n3	2.18	0.42	0.71	0.95	2.04	1.18	1.36	5.28
20:4 n3	0.63	0.52	1.09	0.65	0.57	0.43	0.48	0.70
20:5 n3	8.57	13.00	3.70	11.73	14.55	7.01	6.31	19.47
22:5 n3	0.87	1.55	2.19	1.12	0.41	0.77	0.79	0.43
22:6 n3	17.38	36.13	6.39	30.07	8.46	11.64	12.38	10.89
SFA	24.51	25.28	17.17	27.88	38.38	24.07	26.75	32.61
MUFA	41.99	16.70	49.91	24.18	30.44	51.74	48.61	23.59
PUFA	33.50	58.02	32.92	47.94	31.18	24.19	24.64	43.80

From Table 15 to Table 20, a similarity index is also given. The index is on the Euclidean distance between the profiles from DM and the extracts and is calculated by the following equation [22]:

$$d(p,q) = \sqrt{(p_1 - q_1)^2 + (p_2 - q_2)^2 + \cdots (p_n - q_n)^2} = \sqrt{\sum_{i=1}^{n} (p_i - q_i)^2} \quad (15)$$

$p = (p_1, p_2, ..., p_n)$ and $q = (q_1, q_2, ..., q_n)$ are two points in Euclidean n-space where p_i represents the percent of a fatty acid determined by DM and q_i represents the percent of the same fatty acid determined by other methods.

The partial sums (SFA, MUFA and PUFA) were not included in the calculation of the distance.

4.6.2 Fatty acid profile in extracts

Compared with acid hydrolysis and Bligh and Dyer method, Soxhlet method has largest difference to DM. Bligh and Dyer has smallest differences. For each method, various properties of the samples result in various extent of difference to DM. Cold filet (B) and herring roe (D) which have high content of phospholipids have bigger difference than the other samples. While salmon which contains basically triacylgcerol has smallest difference to DM.

Table 15. Fatty acid profile in Soxhlet extract (%)

	A	B	C	D	F	G	H	I
14:0	6.97	2.13	2.98	6.89	14.55	3.97	7.73	10.88
16:0	13.82	27.07	10.82	18.96	22.97	16.27	15.33	21.70
18:0	1.56	4.70	2.75	1.67	1.68	2.71	1.70	1.32
16:1	4.18	1.51	2.96	5.61	8.55	4.92	4.33	4.42
18:1	12.46	14.98	38.73	25.00	22.99	17.64	15.14	19.57
20:1	12.18	2.76	5.06	3.53	1.61	14.06	13.20	1.28
22:1	16.99	0.96	3.29	1.77	0.91	15.54	19.53	0.89
24:1 n9	1.12	0.34	0.52	0.74	0.16	1.95	1.29	0.06
18:2 n6	1.36	1.50	11.42	2.25	1.70	1.23	1.29	1.84
20:2 n6	0.17	-	1.06	0.08	0.07	0.21	0.17	0.08
20:4 n6	0.36	3.82	0.31	0.46	0.33	0.59	0.36	0.24
18:3 n3	1.06	0.46	4.81	1.97	0.99	0.55	0.83	2.79
18:4 n3	2.64	0.43	0.73	1.89	2.23	1.26	1.62	5.74
20:4 n3	0.66	-	1.11	1.04	0.44	0.39	0.51	0.61
20:5 n3	8.12	11.80	3.55	9.17	11.26	6.74	5.56	16.92
22:5 n3	0.81	1.31	2.14	1.30	0.34	0.77	0.73	0.38
22:6 n3	14.11	25.95	5.47	16.82	6.75	10.34	9.42	8.69
SFA	22.69	33.69	17.11	27.87	39.66	23.15	25.14	34.34
MUFA	46.93	20.29	50.56	36.65	34.23	54.11	53.48	26.19
PUFA	30.38	45.36	32.32	35.48	26.11	22.73	21.38	39.47
Distance to DM	5.08	13.46	1.18	17.08	5.50	2.79	4.97	4.40
Mean distance				6.81				

Table 16. Fatty acid profile in acid hydrolysis extract (%)

	A	B	C	D	F	G	H	I
14:0	6.52	1.34	2.99	4.60	12.63	3.73	7.13	9.40
16:0	15.18	20.74	10.96	22.87	24.53	16.85	16.84	22.07
18:0	1.87	4.97	2.78	2.39	1.63	3.14	2.03	1.26
16:1	3.87	1.34	2.97	3.85	7.34	4.78	4.00	3.79
18:1	12.09	12.86	38.36	16.28	21.21	17.82	14.93	18.18
20:1	10.89	2.20	4.98	2.44	1.49	12.93	11.76	1.19
22:1	14.82	0.70	3.16	1.03	0.95	13.68	17.18	0.71
24:1 n9	1.24	1.01	0.50	1.26	0.19	2.28	1.54	0.07
18:2 n6	1.32	1.23	11.32	1.18	1.71	1.27	1.25	1.77
20:2 n6	0.18	0.19	1.05	0.11	0.09	0.24	0.19	0.10
20:4 n6	0.43	4.61	0.34	0.67	0.38	0.66	0.47	0.26
18:3 n3	0.99	0.37	4.79	0.88	1.04	0.54	0.78	2.80
18:4 n3	2.37	0.43	0.73	0.92	2.08	1.23	1.44	5.42
20:4 n3	0.64	0.40	1.09	0.62	0.55	0.45	0.51	0.68
20:5 n3	8.72	12.62	3.69	11.28	13.62	7.15	6.23	19.06
22:5 n3	0.89	1.72	2.17	1.26	0.44	0.89	0.84	0.51
22:6 n3	16.53	33.09	5.95	27.63	7.81	11.39	11.68	10.30
SFA	23.91	27.07	17.23	29.94	39.18	23.93	26.28	33.01
MUFA	42.91	18.11	49.97	24.87	31.18	51.49	49.42	23.94
PUFA	33.19	54.82	32.80	45.20	29.63	24.59	24.30	43.05
Distance to DM	1.37	3.61	0.60	3.02	1.44	0.60	1.09	1.01
Mean distance				1.59				

Table 17. Fatty acid profile in Bligh & Dyer extract (%)

	A	B	C	D	F	G	H	I
14:0	6.46	1.30	2.92	4.31	12.02	3.58	6.72	9.01
16:0	15.50	19.69	10.97	21.27	23.68	16.14	16.26	21.49
18:0	1.66	4.00	2.71	1.45	1.59	2.78	1.74	1.23
16:1	4.01	1.21	3.00	3.68	7.25	4.80	4.11	3.82
18:1	11.80	12.13	38.18	15.43	20.74	17.58	14.76	17.82
20:1	10.57	2.09	4.92	2.30	1.47	12.74	11.62	1.19
22:1	14.19	0.74	3.12	0.98	0.95	13.79	17.62	0.76
24:1 n9	1.22	1.21	0.50	1.15	0.19	2.36	1.49	0.07
18:2 n6	1.32	1.15	11.26	1.20	1.72	1.26	1.24	1.75
20:2 n6	0.17	0.18	1.04	0.10	0.09	0.21	0.18	0.09
20:4 n6	0.41	4.53	0.32	0.44	0.38	0.67	0.41	0.24
18:3 n3	1.00	0.36	4.81	0.96	1.06	0.53	0.77	2.80
18:4 n3	2.39	0.42	0.73	1.05	2.12	1.27	1.45	5.40
20:4 n3	0.68	0.42	1.10	0.67	0.56	0.47	0.49	0.67
20:5 n3	8.95	13.17	3.76	11.82	14.78	7.46	6.47	19.71
22:5 n3	0.92	1.73	2.18	1.51	0.53	1.05	0.89	0.44
22:6 n3	17.35	35.00	6.29	30.91	8.49	12.24	12.52	11.00
SFA	23.92	25.53	17.16	27.12	37.71	22.79	25.14	32.10
MUFA	41.79	17.38	49.71	23.55	30.60	51.26	49.60	23.63
PUFA	34.29	57.08	33.13	49.33	31.68	25.95	25.26	44.27
Distance to DM	1.01	1.74	0.38	1.37	0.92	1.42	1.55	0.70
Mean distance				1.13				

4.6.3 Reconstructed fatty acid profile

As shown above there are clear differences in the fatty acid profiles between the different extracts and between extracts and the DM result. However, one cannot conclude from these results if the differences are caused by incomplete extractions alone, or if there also are other causes, such as oxidation or other degradation reactions that will give a net loss of fatty acids. This question can be elucuidated by calculating a reconstructed fatty acid profiles. In the reconstructed profiles, the fatty acids in extracts and residues are summarized before the profiles are normalized to the sum of fatty acids. Since this represents the fatty acid profile of the entire sample (extract+residue) it may be used to evaluate whether the extraction process has caused a net loss of certain fatty acids, e.g. by oxidation. In the case of Bligh and Dyer, the contribution from the water phase (which was very low) was also included in the reconstructed profiles.

In general, reconstructed profiles are closer to DM than extracts. This shows that the differences in the fatty acid profiles in the extracts are compensated by the profiles of the residues. This compensation is most obvious by comparing Soxhlet extract and Soxhlet reconstructed profiles.

Table 18. Fatty acid profile for Soxhlet reconstructed (%)

	A	B	C	D	F	G	H	I
14:0	6.48	1.35	2.93	4.49	12.35	3.86	7.25	9.43
16:0	15.56	19.45	10.91	21.53	24.43	17.45	16.98	22.23
18:0	1.94	4.89	2.81	2.30	1.61	3.19	2.01	1.26
16:1	3.79	1.32	2.91	3.84	7.28	4.75	4.08	3.89
18:1	12.07	12.12	38.10	16.18	21.08	17.85	15.07	18.13
20:1	10.66	2.15	4.96	2.45	1.44	12.72	11.75	1.20
22:1	14.68	0.78	3.22	1.02	1.02	13.43	17.03	0.98
24:1 n9	1.37	1.09	0.54	1.31	0.19	2.25	1.49	0.07
18:2 n6	1.29	1.19	11.23	1.24	1.70	1.26	1.25	1.77
20:2 n6	0.17	0.12	1.04	0.10	0.07	0.21	0.17	0.09
20:4 n6	0.44	4.63	0.35	0.68	0.38	0.68	0.44	0.25
18:3 n3	0.98	0.39	4.76	0.96	1.04	0.54	0.78	2.78
18:4 n3	2.34	0.46	0.72	1.03	2.07	1.26	1.46	5.40
20:4 n3	0.65	0.32	1.10	0.64	0.53	0.40	0.51	0.66
20:5 n3	8.62	13.04	3.72	11.59	14.03	7.09	6.17	18.71
22:5 n3	0.82	1.49	2.16	1.15	0.39	0.76	0.75	0.42
22:6 n3	16.78	34.45	6.27	28.77	8.07	11.38	11.54	10.24
SFA	24.33	25.82	17.22	28.51	38.84	24.75	26.64	33.32
MUFA	42.58	17.36	49.74	24.81	30.98	50.99	49.43	24.23
PUFA	33.09	56.57	33.05	46.68	30.18	24.25	23.93	42.45
Distance to DM	0.86	1.80	0.25	1.47	0.88	0.84	1.11	1.21
Mean distance				1.05				

Table 19. Fatty acid profile for acid hydrolysis reconstructed (%)

	A	B	C	D	F	G	H	I
14:0	6.48	1.28	2.97	4.66	12.41	3.71	7.12	9.28
16:0	15.44	19.73	10.99	22.69	24.65	17.01	17.01	22.12
18:0	1.94	4.99	2.80	2.38	1.63	3.20	2.06	1.25
16:1	3.83	1.31	2.95	3.86	7.21	4.74	3.98	3.76
18:1	12.06	12.29	38.14	16.23	21.01	17.73	14.89	18.08
20:1	10.71	2.08	4.95	2.43	1.47	12.53	11.57	1.18
22:1	14.55	0.66	3.14	1.03	0.95	13.08	16.87	0.71
24:1 n9	1.26	1.07	0.51	1.32	0.19	2.30	1.53	0.07
18:2 n6	1.31	1.19	11.26	1.18	1.71	1.28	1.25	1.77
20:2 n6	0.18	0.18	1.04	0.11	0.10	0.23	0.19	0.10
20:4 n6	0.44	4.69	0.35	0.67	0.39	0.69	0.48	0.27
18:3 n3	0.98	0.37	4.78	0.89	1.04	0.55	0.77	2.80
18:4 n3	2.33	0.45	0.73	0.95	2.07	1.25	1.42	5.37
20:4 n3	0.64	0.41	1.09	0.62	0.56	0.46	0.51	0.69
20:5 n3	8.76	13.00	3.75	11.34	13.92	7.39	6.37	19.15
22:5 n3	0.88	1.69	2.18	1.26	0.44	0.90	0.84	0.51
22:6 n3	16.79	34.30	6.23	27.67	7.97	11.93	12.04	10.46
SFA	24.21	26.13	17.25	29.83	39.09	24.20	26.50	32.95
MUFA	42.38	17.41	49.68	24.86	30.84	50.38	48.84	23.80
PUFA	33.41	56.46	33.06	45.30	30.07	25.42	24.66	43.25
Distance to DM	0.94	2.00	0.35	2.88	1.00	1.06	0.71	0.77
Mean distance				1.21				

Table 20. fatty acid profile for Bligh and Dyer reconstructed (%)

	A	B	C	D	F	G	H	I
14:0	6.51	1.30	2.92	4.31	11.83	3.61	6.79	8.96
16:0	15.78	19.06	10.95	21.17	23.86	16.50	16.71	21.68
18:0	1.89	4.61	2.78	2.01	1.60	3.13	2.01	1.25
16:1	3.93	1.20	2.99	3.63	7.06	4.71	3.98	3.75
18:1	11.73	11.92	38.02	15.25	20.54	17.48	14.66	17.71
20:1	10.35	2.07	4.90	2.29	1.44	12.32	11.32	1.18
22:1	13.88	0.73	3.11	0.97	0.95	13.12	17.05	0.75
24:1 n9	1.28	1.26	0.51	1.20	0.19	2.34	1.57	0.07
18:2 n6	1.30	1.14	11.22	1.18	1.71	1.27	1.23	1.74
20:2 n6	0.17	0.18	1.04	0.10	0.09	0.21	0.18	0.09
20:4 n6	0.43	4.60	0.34	0.61	0.40	0.69	0.44	0.25
18:3 n3	0.98	0.36	4.81	0.94	1.06	0.52	0.76	2.79
18:4 n3	2.36	0.42	0.73	1.02	2.09	1.25	1.40	5.32
20:4 n3	0.67	0.41	1.09	0.66	0.56	0.45	0.48	0.68
20:5 n3	8.93	13.10	3.80	12.13	14.93	7.41	6.48	19.79
22:5 n3	0.91	1.69	2.18	1.48	0.52	0.99	0.89	0.44
22:6 n3	17.45	35.19	6.42	30.69	8.73	12.24	12.79	11.17
SFA	24.56	25.63	17.21	27.62	37.82	24.24	26.05	32.28
MUFA	41.17	17.18	49.52	23.34	30.18	49.97	48.54	23.44
PUFA	34.27	57.19	33.26	49.04	32.00	25.79	25.41	44.28
Distance to DM	1.01	1.13	0.37	1.05	0.87	1.43	1.06	0.61
Mean distance				0.94				

Since there is no expected loss of fatty acids due to low extraction efficiency in the reconstructed fatty acid profiles, we can compare the reconstructed fatty acids (Table18, 19 and 20) with DM fatty acids (Table 14) to evaluate if oxidation or other unwanted reactions has occurred during the process.

The most unsaturated fatty acids are the one most vulnerable to oxidation and other reactions on the double bonds, 20:5 and 22:6 were therefore used as markers together with total PUFA. From Fig.14, it can be seen that the recoveries of these fatty acids in Soxhlet and acid hydrolysis are lower than 100% for most samples, whereas the recoveries for Bligh & Dyer are higher than 100%, especially for 22:6. The lower amounts in the reconstructed profiles of Soxhlet and acids hydrolysis method was found significant for 22:6, but not for 20:5 (Table 21).

This indicates that the oxidation may happen in Soxhlet and acid hydrolysis. For Soxhlet, the result can not tell whether the oxidation occured in the extraction procedure or in the treatment of the residues. But for acid hydrolysis method, most probably oxidation happened in extraction method, since left-over fatty acid in residue is very little and the oxidation of residue won't affect the total result too much. This agrees with the conclusion drawn by Adrianus J.de Koning et al who have found that the acid hydrolysis method resulted in the oxidation of the polyunsaturated fatty acids [69]. Since hydrolysis step was applied after the Soxhlet extraction in this study, only small proportion of lipids were exposed to acid and heat, thus the oxidation and degradation may not be so evident. For some other acid hydrolysis methods which start with hydrolysis step, the oxidation would be expected to be more significant than this case.

Even though there is a significant difference for 22:6, it should be emphasized that this difference is small compared to the difference between samples, and the differences that can be seen as a results of varying extraction efficiencies. The average percent of 22:6 was 16.7% determined by Bligh and Dyer and 15.9% in the reconstructed profiles from the two extractions.

Compared to the DM, Bligh and Dyer shows increased levels of 20:5 and 22:6 and the difference is significant in the case of 20:5. This may indicate that there is loss of PUFA also in the direct methylation method. Even though the methylation conditions are identical for extracts and solid samples, the amount of matrix other than lipids is much higher with solid samples, and it is possible that compounds in the matrix may catalyse degradation of PUFA. Loss of 20:5 and 22:6 caused by formation of trans isomers has been reported to occur by direct methylation, but not with methylation of extracts from the same samples [52]. Other side reactions may be oxidation or chlorination of PUFA. However, also these differences are small. The average percentage of EPA was 10.5% in the DM profile and 10.8% in the reconstructed Bligh and Dyer profile.

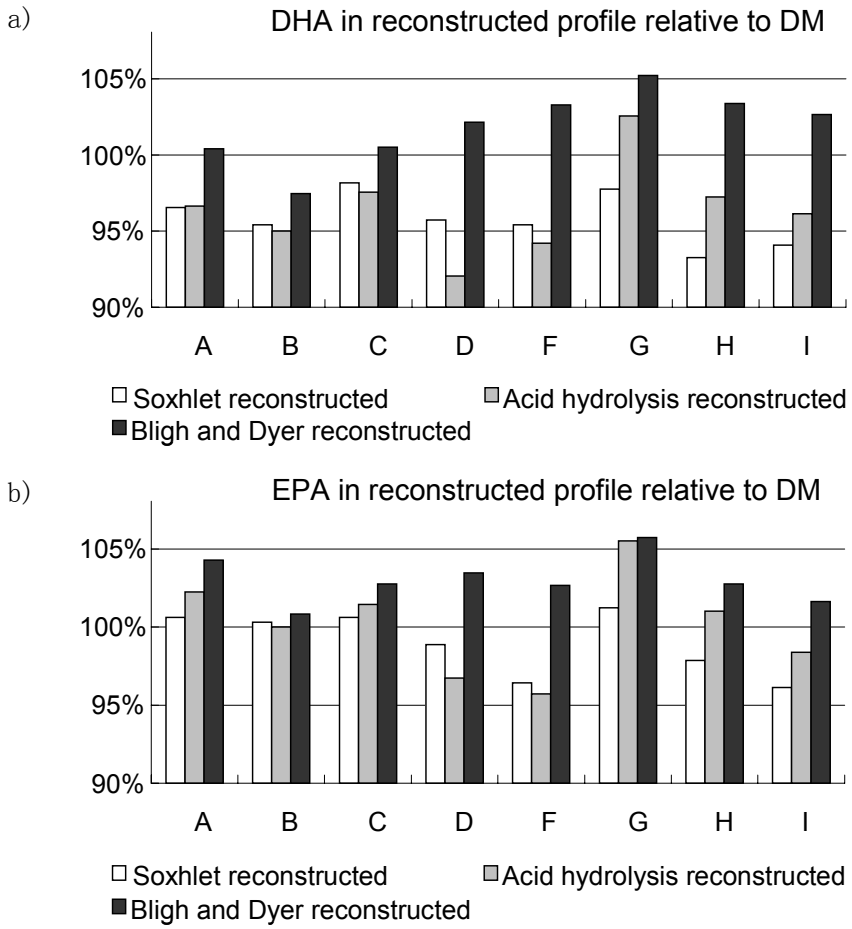


Figure. 14 Recovery of DHA in "reconstructed" fatty acid profile to DM fatty acid profile (a) and Recovery of EPA in "reconstructed" fatty acid profile DM fatty acid profile (b)

Table 21. Paired t-test for the reconstructed profile of the three extractions against DM ($t_{(0.05, 16)}$=2.12)

	DM vs. SOX	DM vs. Acid hydrolysis	DM vs. Bligh & Dyer
20:5 n-3	X1=X2	X1=X2	X1≠X2
22:6 n-3	X1≠X2	X1≠X2	X1=X2
PUFA	X1≠X2	X1=X2	X1≠X2

4.6.4 Multivariate evaluation of the profiles

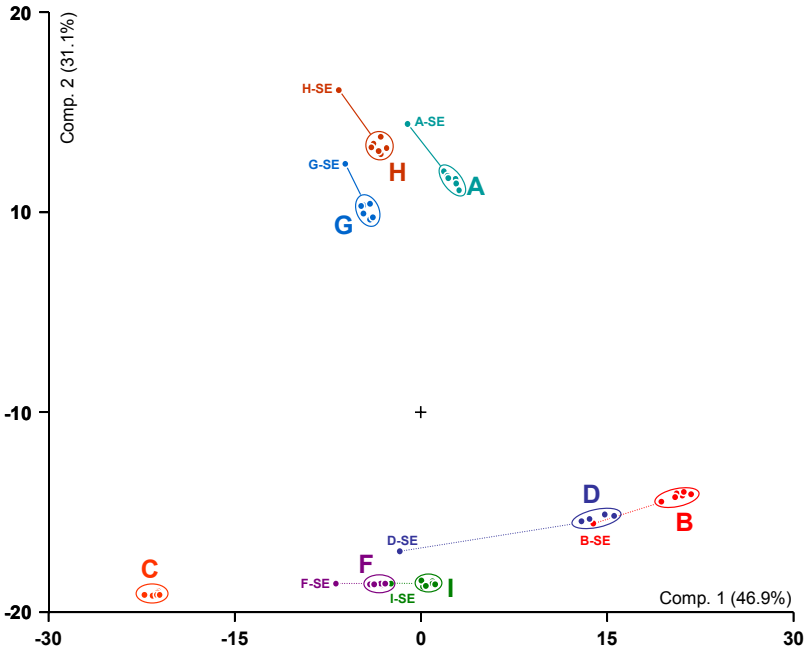
The fatty acid profile of Table 14-20 were subjected to PCA and to analysis of similarities based on dendrograms to check how the methods would affected the fatty acid profile and how big the differences are between the methods.

Scoreplot and Euclidian Dendrogram (Fig. 15) describe difference between the different FA profiles. Each column in the tables (14-20) is one object. The data matrix contains 56 objects ad 17 variables.

The scoreplot in Fig. 15a shows that the differences between the samples are

larger than the differences between the methods. Extract from Soxhlet has deviating profile in some cases, in particular sample D (herring roe) and B (Cod filet). This can also be seen in the dendrogram. It also suggested that dissimilarity between the methods is very little for salmon. It does not matter which methods are used, most fatty acids can be extracted for salmon. Whereas for the other sample, especially for the samples that are rich in polar lipids, the extraction method is more important. Except for Soxhlet, the dissimilarity between the other methods is low. And for most samples, B-E is closest to DM. This can be shown more clearly from Fig.15 b.

a)



b)

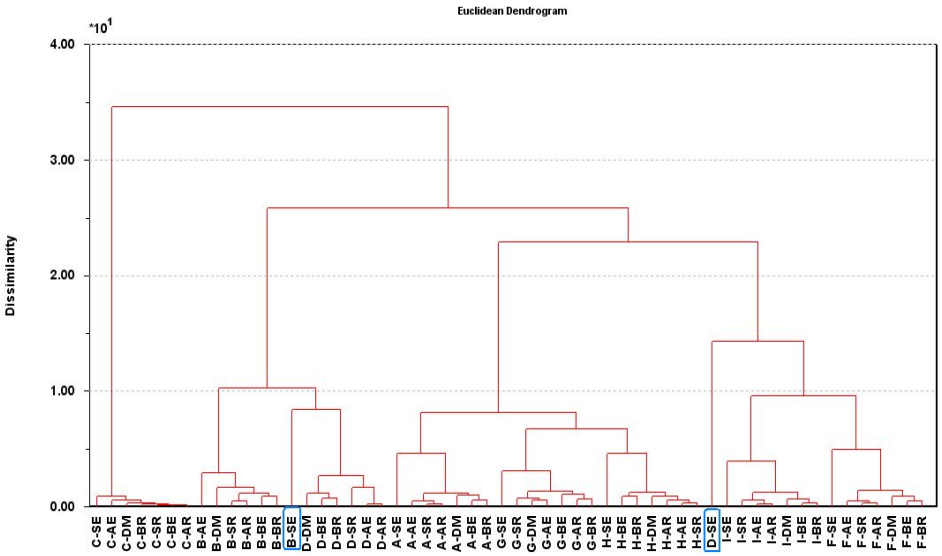
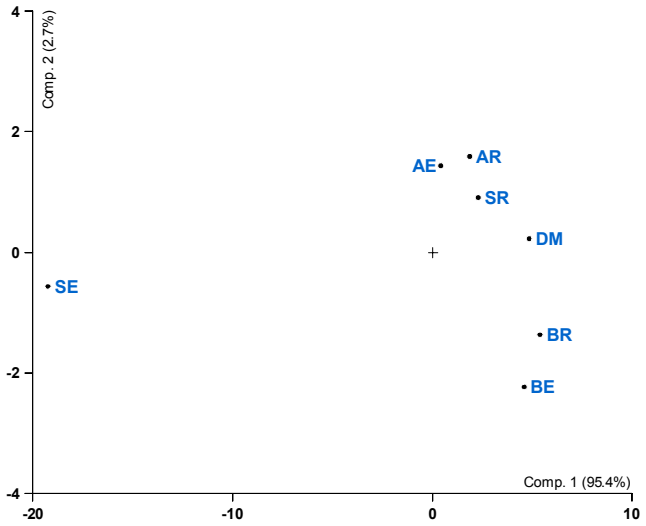


Figure 15. PCA scoreplot of fatty acid profiles (a) and Scores Euclidian Dendrogram (b). DM: Direct Methylation, SE: Soxhlet extract, SR: Soxhlet reconstructed, AE: Acid hydrolysis extract, AR: Acid hydrolysis reconstructed, BE: Bligh & Dyer extract, BR: Bligh and Dyer reconstructed.

The difference between the methods can be more clearly illustrated by using the 7 methods as objects (extracts and reconstructed profiles) and the 17 fatty acids for all 8 samples combined (146 variables) as variables (Fig. 16).

a)



b)

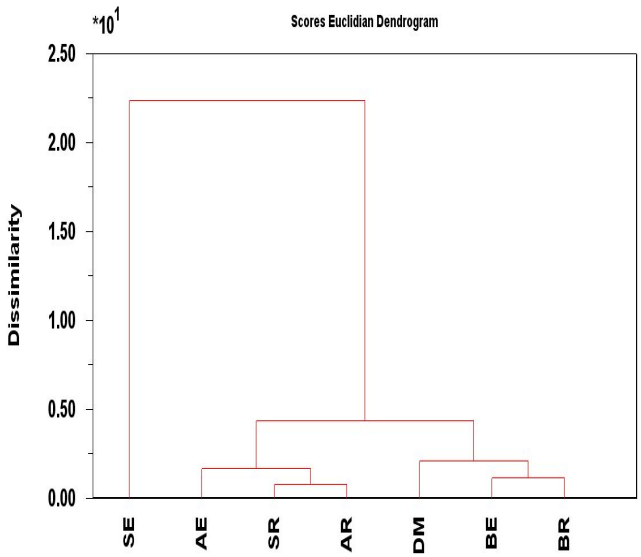


Figure 16. PCA scoreplot describing the differences between the methods (a) and Scores Euclidian Dendrogram describing the dissimilarities between the methods (b)

The scoreplot and Euclidian Dendrogram (Fig. 16) describe difference between the methods. The main difference is the difference between Soxhlet and the other methods. Since the profile is similar to the other when the contribution from the residue is included (SR), it shows that the reason why SE is different is caused by the low extraction efficiency for some lipids and not by net loss of fatty acids due to oxidation or other unwanted reactions. The dendrogram and euclidean distances also showed that BE and BR are closer to DM than the other methods.

5 Conclusions

DM method

Though the eight marine powders vary greatly in lipid composition and lipid content, the fatty acid analysis by DM seems fit for all these samples. The repeatability CV of fatty acids analysis by DM is 5.11% for fish powder and 1.21% for extracts. The intermediate precision is 1.85% for control oil and rang from 1.8% to 6.8% for fish powders. This demonstrates that DM is able to give a satisfactory result for determination of fatty acids in homogeneous fishmeal and similar products. The method also fulfills the U.S. Nutritional Labeling and Education Act (NLEA), in which fat is defined as the sum of all fatty acids obtained from a total lipid extract triacylglycerols. But because of low sample size and complicated matrix other than lipid, DM may have precision and sampling problems when non-homogeneous materials are analyzed.

Mass balances

The use of DM on the residues seem to be a suitable method for evaluating mass balances for the extraction methods (since most of the mass balance sums are quite close to 100%). The mass balance calculations for fatty acids show that extraction of polar lipids by Soxhlet is incomplete. Acid hydrolysis compensate for this to a large degree. Bligh and Dyer extraction gave similar results as acid hydrolysis. The loss of fatty acids by leaking of polar lipids to the water phase in Bligh and Dyer was below 1% for most samples and 2.8% at worst.

The gravimetric lipid content by Soxhlet is lower than that by acid hydrolysis and Bligh and Dyer. There is only limited correlation between gravimetric lipid by extraction and total fatty acids by DM, in particular for the soxhlet method. It is not accurate enough to predict the dietary energy value of lipid by using gravimetric lipid content.

Fatty acid profiles

PCA and Euclidean distances show that the fatty acid profile by Soxhlet is greatly different from the other methods because of incomplete extraction. This is compensated by including the fatty acids from the residues. It seems therefore not to be a net loss of fatty acids in the extraction. Bligh and Dyer is closest to DM.For salmon powder (C) which contains basically TAG, the dissimilarity between the methods is very small. While for herring roe (D) and cod filet (B) which have a large amount of polar lipids, the dissimilarity between the methods is very large. The choice of the extraction methods is more important for polar than neutral lipids.

The amounts of 20:5 n-3 and 22:6 n-3 in reconstructed fatty acid profiles from the extractions (extracts and residues combined) compared to direct methylation indicate some oxidation in soxhlet and and acid hydrolysis methods.

Comparison between direct methylation and Bligh and Dyer indicates that there may also be losses of PUFA in the direct methylation method. However, the differences caused by oxidation and other unwanted reactions seems small compared to the differences between samples and differences caused by the extraction efficiency.

B&D is marginally closer to direct methylation method than acid hydrlolysis. Both methods seem suitable for fatty acids analysis of marine samples.

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Thanks!

谢 谢!

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Appendixes

Appendix A. Data for Direct methylation (Table A1- A10)

Appendix B. Data for Soxhlet method (Table B1-B12)

Appendix C. Data for acid hydrolysis method (Table C1-C12)

Appendix D. Data for Bligh and Dyer method (Table D1-D12)

Appendix E. Data for control oil

Appendix F. Soxhlet procedure for Nofima BioLab

Appendix G. Acid hydrolysis procedure for Nofima BioLab

Appendix H. Bligh and dyer method for Nofima BioLab

All the data in the tables were calculated in Excel 2003 with many decimal numbers. In the tables, only two or three decimal numbers were given.

n.a in the tables means not available.

Appendix A. Data for Direct Methylation

Table A1. **DM for Sample A**(g/100g sample)

GC No.	113	174	340	average	C.V.(100%)
Fatty Acid	A1	A2	A3		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	0.01	0.01	0.01	0.01	10.00
12:0	<0.01	0.01	<0.01	0.01	47.78
14:0	0.47	0.51	0.47	0.48	5.15
16:0	1.22	1.31	1.20	1.24	4.71
18:0	0.16	0.17	0.16	0.16	3.37
20:0	0.01	0.01	0.01	0.01	10.52
22:0	<0.01	<0.01	<0.01	<0.01	20.52
16:1 n7	0.28	0.29	0.28	0.28	2.11
18:1 n9	0.91	0.98	0.91	0.93	4.25
20:1 n9	0.81	0.85	0.79	0.82	3.35
22:1 n11	1.14	1.17	1.11	1.14	2.69
24:1 n9	0.11	0.11	0.11	0.11	1.91
16:2 n4	0.02	0.03	0.02	0.02	14.72
16:3 n4	0.02	0.02	0.02	0.02	0.58
18:2 n6	0.09	0.10	0.10	0.10	4.46
18:3 n6	n.a.	<0.01	0.01	n.a.	n.a.
20:2 n6	0.01	0.01	0.01	0.01	4.65
20:3 n6	0.01	0.01	0.01	0.01	6.77
20:4 n6	0.04	0.04	0.04	0.04	1.53
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a
18:3 n3	0.07	0.08	0.07	0.07	3.91
18:4 n3	0.16	0.18	0.17	0.17	4.23
20:3 n3	0.01	0.02	0.01	0.01	59.30
20:4 n3	0.05	0.05	0.05	0.05	2.99
20:5 n3	0.65	0.69	0.67	0.67	3.00
21:5 n3	0.02	0.02	0.02	0.02	4.41
22:5 n3	0.06	0.07	0.07	0.07	3.55
22:6 n3	1.32	1.39	1.36	1.36	2.37
SFA	1.87	2.02	1.85	1.91	4.74
MUFA	3.25	3.39	3.19	3.28	3.07
PUFA	2.54	2.70	2.61	2.62	3.07
Total FA	7.66	8.10	7.66	7.81	3.29

Table A2. **DM for Sample B** (g/100g sample)

GC No.	114	175	341	average	C.V.(100%)
Fatty Acid	B1	B2	B3		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	n.a.	n.a.	n.a.	n.a.	n.a.
12:0	n.a.	<0.01	n.a.	n.a.	n.a.
14:0	0.03	0.02	0.03	0.03	6.65
16:0	0.42	0.40	0.43	0.42	3.47
18:0	0.11	0.11	0.11	0.11	2.71
20:0	n.a.	n.a.	n.a.	n.a.	n.a.
22:0	n.a.	n.a.	n.a.	n.a.	n.a.
16:1 n7	0.02	0.02	0.03	0.03	18.68
18:1 n9	0.27	0.26	0.28	0.27	4.81
20:1 n9	0.05	0.05	0.05	0.05	6.63
22:1 n11	0.01	0.02	0.01	0.01	4.91
24:1 n9	n.a.	n.a.	0.03	0.03	n.a.
16:2 n4	n.a.	n.a.	n.a.	n.a.	n.a.
16:3 n4	n.a.	n.a.	n.a.	n.a.	n.a.
18:2 n6	0.02	0.02	0.03	0.02	8.56
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.
20:2 n6	n.a.	<0.01	<0.01	n.a.	n.a.
20:3 n6	n.a.	n.a.	<0.01	n.a.	n.a.
20:4 n6	0.11	0.10	0.11	0.11	5.52
22:4 n6	<0.01	n.a.	0.01	<0.01	15.52
18:3 n3	0.01	0.01	0.01	0.01	25.31
18:4 n3	0.01	0.01	0.01	0.01	9.74
20:3 n3	n.a.	0.01	n.a.	0.01	n.a.
20:4 n3	n.a.	n.a.	0.01	0.01	n.a.
20:5 n3	0.28	0.27	0.31	0.29	7.53
21:5 n3	n.a.	n.a.	<0.01	n.a.	n.a.
22:5 n3	0.03	0.03	0.04	0.03	4.17
22:6 n3	0.79	0.75	0.85	0.80	6.66
SFA	0.56	0.54	0.57	0.56	3.26
MUFA	0.35	0.34	0.41	0.37	10.08
PUFA	1.25	1.20	1.38	1.28	7.24
Total FA	2.17	2.08	2.36	2.20	6.58

Table A3. **DM for Sample C**(g/100g sample)

GC No.	115	176	342	average	C.V.(100%)
Fatty Acid	C1	C2	C3		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	<0.01	n.a.	<0.01	n.a.	n.a.
12:0	0.02	0.02	0.01	0.02	28.36
14:0	1.01	1.12	1.00	1.04	6.58
16:0	3.88	4.27	3.72	3.96	7.12
18:0	1.01	1.12	0.96	1.03	7.55
20:0	0.11	0.12	0.11	0.11	7.62
22:0	0.06	0.06	0.06	0.06	5.22
16:1 n7	1.01	1.13	0.99	1.04	7.03
18:1 n9	13.58	14.89	12.97	13.81	7.09
20:1 n9	1.77	1.96	1.74	1.82	6.43
22:1 n11	1.16	1.28	1.15	1.19	6.05
24:1 n9	0.20	0.21	0.20	0.20	3.93
16:2 n4	0.10	0.12	0.10	0.11	9.18
16:3 n4	0.07	0.08	0.07	0.07	8.25
18:2 n6	3.94	4.34	3.78	4.02	7.27
18:3 n6	0.04	0.05	0.04	0.04	6.65
20:2 n6	0.37	0.41	0.36	0.38	6.74
20:3 n6	0.07	0.08	0.07	0.07	6.68
20:4 n6	0.13	0.14	0.12	0.13	5.81
22:4 n6	0.03	0.03	0.03	0.03	3.09
18:3 n3	1.65	1.82	1.60	1.69	7.03
18:4 n3	0.25	0.27	0.25	0.26	6.36
20:3 n3	0.20	0.22	0.18	0.20	8.87
20:4 n3	0.39	0.42	0.38	0.40	6.56
20:5 n3	1.30	1.43	1.28	1.34	6.16
21:5 n3	0.08	0.09	0.08	0.09	6.16
22:5 n3	0.76	0.84	0.77	0.79	5.09
22:6 n3	2.25	2.46	2.24	2.31	5.38
SFA	6.10	6.71	5.85	6.22	7.11
MUFA	17.72	19.46	17.05	18.08	6.89
PUFA	11.62	12.81	11.34	11.92	6.50
Total FA	35.45	38.98	34.24	36.22	6.80

Table A4. **DM for Sample D**(g/100g sample)

GC No.	116	177	343	average	C.V.(100%)
Fatty Acid	D1	D2	D3		
8:0	<0.01	n.a.	<0.01	n.a.	n.a.
10:0	n.a.	n.a.	<0.01	n.a.	n.a.
12:0	0.01	<0.01	n.a.	<0.01	27.17
14:0	0.40	0.42	0.44	0.42	4.68
16:0	2.12	2.14	2.22	2.16	2.41
18:0	0.23	0.23	0.24	0.23	1.35
20:0	n.a.	<0.01	n.a.	n.a.	n.a.
22:0	n.a.	n.a.	n.a.	n.a.	n.a.
16:1 n7	0.35	0.35	0.38	0.36	3.89
18:1 n9	1.57	1.57	1.63	1.59	2.44
20:1 n9	0.25	0.24	0.25	0.25	2.42
22:1 n11	0.12	0.11	0.11	0.11	4.57
24:1 n9	0.14	0.14	0.14	0.14	1.31
16:2 n4	0.01	0.01	0.02	0.01	30.53
16:3 n4	0.02	0.02	0.02	0.02	4.64
18:2 n6	0.12	0.11	0.12	0.12	5.16
18:3 n6	n.a.	n.a.	<0.01	n.a.	n.a.
20:2 n6	0.01	0.01	0.01	0.01	17.42
20:3 n6	0.01	0.01	n.a.	0.01	8.45
20:4 n6	0.07	0.07	0.07	0.07	5.68
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.09	0.08	0.10	0.09	7.37
18:4 n3	0.10	0.09	0.10	0.10	8.57
20:3 n3	0.01	n.a.	<0.01	<0.01	14.39
20:4 n3	0.07	0.06	0.07	0.07	9.06
20:5 n3	1.19	1.10	1.28	1.19	7.48
21:5 n3	0.01	0.01	0.01	0.01	6.53
22:5 n3	0.12	0.10	0.12	0.11	7.86
22:6 n3	3.10	2.78	3.25	3.04	7.83
SFA	2.76	2.80	2.90	2.82	2.56
MUFA	2.42	2.41	2.51	2.45	2.22
PUFA	4.92	4.46	5.19	4.85	7.61
Total FA	10.11	9.67	10.60	10.13	4.59

Table A5. **DM for Sample F**(g/100g sample)

GC No.	117	178	344	average	C.V.(100%)
Fatty Acid	**F1**	**F2**	**F3**		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	n.a.	n.a.	n.a.	n.a.	n.a.
12:0	0.03	0.03	0.04	0.03	5.60
14:0	1.73	1.65	1.69	1.69	2.44
16:0	3.57	3.40	3.45	3.47	2.52
18:0	0.23	0.22	0.23	0.23	2.61
20:0	0.01	0.01	0.01	0.01	5.26
22:0	0.01	0.01	0.01	0.01	3.00
16:1 n7	1.01	0.97	0.98	0.99	2.34
18:1 n9	3.02	2.90	2.95	2.96	1.98
20:1 n9	0.19	0.19	0.21	0.20	6.02
22:1 n11	0.15	0.15	0.12	0.14	11.66
24:1 n9	0.03	0.03	0.03	0.03	4.85
16:2 n4	0.12	0.11	0.11	0.11	2.04
16:3 n4	0.06	0.05	0.05	0.06	8.23
18:2 n6	0.24	0.24	0.24	0.24	1.54
18:3 n6	0.01	0.01	0.02	0.02	40.68
20:2 n6	0.01	0.01	0.01	0.01	4.04
20:3 n6	0.02	0.02	0.01	0.01	38.94
20:4 n6	0.06	0.06	0.06	0.06	4.25
22:4 n6	n.a.	n.a.	<0.01	n.a.	n.a.
18:3 n3	0.15	0.15	0.15	0.15	1.49
18:4 n3	0.29	0.28	0.30	0.29	2.88
20:3 n3	0.01	<0.01	0.01	0.01	32.72
20:4 n3	0.08	0.08	0.09	0.08	5.19
20:5 n3	2.06	2.01	2.12	2.06	2.61
21:5 n3	0.06	0.06	0.06	0.06	0.82
22:5 n3	0.06	0.05	0.07	0.06	10.02
22:6 n3	1.19	1.19	1.23	1.20	1.96
SFA	5.59	5.32	5.42	5.44	2.46
MUFA	4.41	4.24	4.30	4.32	1.97
PUFA	4.41	4.32	4.53	4.42	2.37
Total FA	14.40	13.88	14.25	14.18	1.89

Table A6. **DM for Sample G** (g/100g sample)

GC No.	118	179	345	average	C.V.(100%)
Fatty Acid	**G1**	**G2**	**G3**		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	n.a.	n.a.	n.a.	n.a.	n.a.
12:0	n.a.	0.01	<0.01	n.a.	n.a.
14:0	0.17	0.19	0.18	0.18	5.71
16:0	0.84	0.94	0.86	0.88	5.65
18:0	0.16	0.18	0.17	0.17	4.36
20:0	0.01	0.01	0.01	0.01	3.21
22:0	<0.01	<0.01	n.a.	n.a.	n.a.
16:1 n7	0.21	0.25	0.24	0.23	8.40
18:1 n9	0.90	0.95	0.94	0.93	3.28
20:1 n9	0.64	0.69	0.67	0.67	3.88
22:1 n11	0.70	0.74	0.71	0.71	2.55
24:1 n9	0.13	0.13	0.13	0.13	2.17
16:2 n4	0.01	0.01	0.02	0.01	19.16
16:3 n4	0.01	0.01	0.01	0.01	5.24
18:2 n6	0.06	0.07	0.06	0.06	5.09
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.
20:2 n6	0.01	0.01	0.01	0.01	13.10
20:3 n6	n.a.	<0.01	n.a.	n.a.	n.a.
20:4 n6	0.03	0.04	n.a.	0.04	6.55
22:4 n6	n.a.	n.a.	<0.01	0.00	n.a.
18:3 n3	0.03	0.03	0.03	0.03	4.48
18:4 n3	0.06	0.06	0.06	0.06	6.40
20:3 n3	0.01	0.01	0.01	0.01	9.68
20:4 n3	0.02	0.02	0.02	0.02	7.50
20:5 n3	0.34	0.37	0.38	0.36	6.63
21:5 n3	0.01	0.01	0.01	0.01	12.42
22:5 n3	0.04	0.04	0.04	0.04	9.32
22:6 n3	0.57	0.61	0.63	0.60	4.83
SFA	1.19	1.32	1.23	1.25	5.62
MUFA	2.58	2.76	2.69	2.68	3.44
PUFA	1.18	1.29	1.28	1.25	4.93
Total FA	4.95	5.37	5.20	5.17	4.16

Table A7. **DM for Sample H**(g/100g sample)

GC No.	119	180	346	average	C.V.(100%)
Fatty Acid	H1	H2	H3		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	0.01	0.01	0.01	0.01	5.26
12:0	<0.01	0.01	0.01	0.01	58.51
14:0	0.59	0.65	0.60	0.61	4.99
16:0	1.51	1.63	1.52	1.55	4.36
18:0	0.19	0.20	0.19	0.19	2.00
20:0	0.01	0.01	0.01	0.01	5.55
22:0	<0.01	<0.01	n.a.	n.a.	n.a.
16:1 n7	0.34	0.36	0.34	0.35	3.50
18:1 n9	1.30	1.43	1.34	1.36	4.60
20:1 n9	1.01	1.07	1.01	1.03	3.63
22:1 n11	1.50	1.58	1.44	1.51	4.51
24:1 n9	0.14	0.14	n.a.	0.14	0.67
16:2 n4	0.02	0.02	0.03	0.02	6.97
16:3 n4	0.02	0.02	0.02	0.02	9.30
18:2 n6	0.10	0.11	0.11	0.11	4.41
18:3 n6	n.a.	<0.01	0.01	n.a.	n.a.
20:2 n6	0.01	0.01	0.02	0.02	11.48
20:3 n6	0.01	0.01	n.a.	0.01	12.51
20:4 n6	0.04	0.04	n.a.	0.04	1.75
22:4 n6	n.a.	n.a.	<0.01	<0.01	n.a.
18:3 n3	0.06	0.07	0.07	0.07	4.72
18:4 n3	0.12	0.13	0.12	0.12	3.80
20:3 n3	0.01	0.02	0.01	0.01	74.73
20:4 n3	0.04	0.05	0.04	0.04	5.36
20:5 n3	0.54	0.58	0.58	0.56	3.88
21:5 n3	0.01	0.01	0.01	0.01	0.87
22:5 n3	0.07	0.07	0.08	0.07	7.22
22:6 n3	1.07	1.12	1.12	1.10	2.40
SFA	2.31	2.51	2.34	2.39	4.52
MUFA	4.29	4.58	4.14	4.34	5.18
PUFA	2.12	2.27	2.20	2.20	3.21
Total FA	8.72	9.36	8.68	8.92	4.24

Table A8. **DM for Sample I** (g/100g sample)

GC No.	120	181	347	average	C.V.(100%)
Fatty Acid	I1	I2	I3		
8:0	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	n.a.	n.a.	<0.01	n.a.	n.a.
12:0	0.04	0.03	0.03	0.03	13.17
14:0	1.22	1.28	1.19	1.23	3.56
16:0	2.97	3.12	2.98	3.03	2.80
18:0	0.17	0.18	0.17	0.17	4.35
20:0	<0.01	<0.01	0.01	n.a.	n.a.
22:0	0.01	0.01	<0.01	n.a.	n.a.
16:1 n7	0.50	0.53	0.49	0.51	3.87
18:1 n9	2.40	2.55	2.34	2.43	4.33
20:1 n9	0.16	0.17	0.14	0.16	7.77
22:1 n11	0.14	0.14	0.10	0.13	17.96
24:1 n9	0.01	0.01	0.01	0.01	7.89
16:2 n4	0.08	0.08	0.07	0.07	3.35
16:3 n4	0.06	0.06	0.06	0.06	3.64
18:2 n6	0.23	0.24	0.23	0.24	2.91
18:3 n6	0.01	0.01	0.02	0.01	27.32
20:2 n6	0.01	0.01	0.01	0.01	10.70
20:3 n6	0.02	0.02	0.01	0.01	28.66
20:4 n6	0.03	0.04	0.04	0.04	5.01
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.37	0.39	0.38	0.38	2.84
18:4 n3	0.71	0.74	0.72	0.72	2.42
20:3 n3	0.03	0.04	0.03	0.03	14.09
20:4 n3	0.09	0.10	0.10	0.10	2.20
20:5 n3	2.56	2.70	2.74	2.67	3.51
21:5 n3	0.10	0.10	0.10	0.10	2.19
22:5 n3	0.06	0.07	0.05	0.06	10.50
22:6 n3	1.44	1.52	1.52	1.49	3.31
SFA	4.40	4.62	4.39	4.47	2.94
MUFA	3.22	3.39	3.09	3.23	4.71
PUFA	5.80	6.12	6.09	6.00	2.92
Total FA	13.42	14.14	13.57	13.71	2.76

Table A9. DM Repeatability for sample A (g FA/ 100g sample)

FA	GC 61	GC 62	GC 63	GC 64	GC 65	GC 66	GC 67	Average	C.V (%)
8:0	0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	6.52
12:0	0.01	0.01	<0.01	<0.01	<0.01	0.01	0.01	0.01	17.21
14:0	0.47	0.50	0.43	0.45	0.48	0.48	0.47	0.47	5.12
16:0	1.21	1.29	1.11	1.16	1.25	1.26	1.22	1.22	4.97
18:0	0.16	0.17	0.15	0.15	0.17	0.16	0.16	0.16	4.77
20:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	11.56
22:0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	21.43
16:1 n7	0.28	0.30	0.25	0.27	0.29	0.29	0.28	0.28	5.46
18:1	0.93	0.98	0.86	0.88	0.95	0.94	0.92	0.92	4.36
20:1	0.83	0.87	0.75	0.80	0.86	0.84	0.83	0.82	5.07
22:1	1.18	1.24	1.05	1.13	1.21	1.17	1.18	1.16	5.32
24:1 n9	0.12	0.12	0.10	0.11	0.12	0.11	0.12	0.11	6.94
16:2 n4	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	9.61
16:3 n4	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	4.86
18:2 n6	0.10	0.10	0.09	0.09	0.10	0.10	0.10	0.10	5.38
18:3 n6	n.a.	0.00	n.a.	<0.01	n.a.	n.a.	n.a.	<0.01	37.77
20:2 n6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	7.16
20:3 n6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	8.58
20:4 n6	0.03	0.04	0.03	0.03	0.04	0.04	0.04	0.04	5.60
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	4.38
18:4 n3	0.17	0.17	0.15	0.16	0.18	0.17	0.17	0.17	5.09
20:3 n3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	22.47
20:4 n3	0.05	0.05	0.04	0.05	0.05	0.05	0.05	0.05	6.56
20:5 n3	0.66	0.70	0.60	0.64	0.69	0.68	0.67	0.66	5.25
21:5 n3	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	9.75
22:5 n3	0.07	0.07	0.06	0.06	0.06	0.07	0.06	0.06	5.72
22:6 n3	1.39	1.45	1.22	1.33	1.43	1.41	1.41	1.38	5.66
SFA	1.88	1.99	1.71	1.79	1.93	1.93	1.88	1.87	4.96
MUFA	3.34	3.49	3.00	3.18	3.44	3.36	3.33	3.31	4.99
PUFA	2.62	2.75	2.33	2.52	2.70	2.68	2.66	2.61	5.47
Total FA	7.84	8.23	7.05	7.49	8.07	7.96	7.87	7.79	5.11

Table A10.DM repeatibility for Soxhlet Extract of sample A (g FA/100g extract)

FA	GC 53	GC 54	GC 55	GC 57	GC 58	GC 59	Average	C.V (%)
8:0	0.001	0.001	0.001	0.001	0.001	0.001	0.001	11.818
10:0	<0.001	n.a.	n.a.	n.a.	<0.001	<0.001	<0.001	33.852
12:0	0.001	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	77.553
14:0	0.028	0.027	0.028	0.029	0.029	0.029	0.028	2.351
16:0	0.054	0.053	0.055	0.056	0.056	0.056	0.055	2.036
18:0	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.991
20:0	0.001	<0.001	0.001	<0.001	<0.001	0.001	0.001	9.141
22:0	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	19.932
16:1 n7	0.016	0.016	0.018	0.017	0.017	0.017	0.017	4.083
18:1	0.050	0.049	0.050	0.050	0.052	0.050	0.050	1.836
20:1	0.049	0.049	0.050	0.050	0.050	0.050	0.050	1.026
22:1	0.069	0.069	0.069	0.069	0.070	0.070	0.069	0.423
24:1 n9	0.004	0.004	0.004	0.004	0.004	0.004	0.004	4.775
16:2 n4	0.002	0.002	0.002	0.002	0.002	0.001	0.002	13.358
16:3 n4	0.001	0.001	0.001	0.001	0.001	0.001	0.001	4.862
18:2 n6	0.006	0.005	0.006	0.006	0.006	0.006	0.006	4.074
18:3 n6	<0.001	n.a.	<0.001	<0.001	n.a.	<0.001	<0.001	26.194
20:2 n6	0.001	0.001	0.001	0.001	0.001	0.001	0.001	11.296
20:3 n6	<0.001	<0.001	<0.001	<0.001	n.a.	<0.001	<0.001	7.841
20:4 n6	0.001	0.001	0.002	0.001	0.001	0.001	0.001	11.319
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.004	0.004	0.004	0.004	0.004	0.004	0.004	2.041
18:4 n3	0.011	0.011	0.011	0.011	0.011	0.011	0.011	1.193
20:3 n3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	20.420
20:4 n3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	2.993
20:5 n3	0.033	0.032	0.033	0.033	0.033	0.033	0.033	0.903
21:5 n3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	5.679
22:5 n3	0.003	0.003	0.003	0.003	0.003	0.003	0.003	2.698
22:6 n3	0.056	0.056	0.056	0.057	0.057	0.057	0.056	0.778
SFA	0.090	0.088	0.092	0.092	0.094	0.093	0.092	2.237
MUFA	0.188	0.187	0.191	0.191	0.192	0.191	0.190	0.988
PUFA	0.123	0.121	0.123	0.123	0.124	0.122	0.123	0.934
Total FA	0.402	0.396	0.406	0.407	0.410	0.406	0.404	1.210

Appendix B. Data for Soxhlet method

Tabel B1. Calculation for Soxhlet method (sample A, B)

Sample Code	A		A		B		B	
sample weight(g):W0	4.8125		4.3586		4.2005		4.2645	
Flask(g): W2	54.0786		55.5625		56.5371		56.6895	
Flask + stopper (g): W1	70.252		71.736		74.02		74.172	
falsk+stopper+ extract(g): W3	146.1696		131.6170		144.7725		135.4988	
Extract for GC(g): W4b-W4a, W5b-W5a	3.2691	2.6676	2.6384	2.6126	2.6574	2.6115	2.6661	2.6131
GC No.	81	82	129	130	83	84	158	159
flak+stopper+extact left(g):W6	140.1184		126.2389		136.2053		130.1172	
flask+fat(g):W7	54.4936		55.9257		56.5899		56.7402	
residue(g): W8	4.16295		3.7577		4.2871		4.3275	
Residue for GC(g): W9, W10	0.2052	0.1907	0.1981	0.2014	0.2031	0.1993	0.2085	0.1969
GC No.	90	91	122	123	92	93	167	168
Extracted lipid (g/100g)[1]:	9.37		9.16		1.43		1.30	
Average of extracted lipid (g/100g sample)	9.26				1.37			
Relative error of extracted lipid %	2.32				9.29			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.11		97.64		-		98.10	
Mass balance for Residue(%)[2]:	95.45		94.90		103.54		102.82	
Total FA in extract (g FA/100g extract): M1	0.4376	0.4348	0.5097	0.5204	0.0491	0.0486	0.0586	0.0587
Total FA extracted (g FA/ 100g Sample) : M2= M1*(W3-W1)/W0	6.90	6.86	7.00	7.15	0.828	0.818	0.843	0.844
Average of Total FA extracted (g FA/ 100g Sample): $\overline{M2}$	6.98				0.83			
Relative error of total FA extracted %	1.84				1.50			
Total FA in Residue(g FA/100g residue):M3	1.5302	1.5256	1.6483	1.6336	1.4401	1.4225	1.3599	1.47176
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	1.32	1.32	1.42	1.41	1.47	1.45	1.38	1.49
Average of total FA left in sample(g/100g sample): $\overline{M4}$	1.37				1.45			
relative error of total FA left in sample%	3.95				3.38			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	8.35				2.28			

1. Extracted lipid= $\frac{(W7-W2)\times(W3-W1)}{W0\times(W6-W1)}\times 100\%$

2. Mass balance of residue= $\frac{W8}{W0-\frac{(W7-W2)\times(W3-W1)}{W6-W1}}\times 100\%$

3. M1, M3 was calculated by using the original GC profile. By using the same formula of M2 and M4, The original GC profiles (g FA/ 100g extract or g FA/ 100g residue) was converted into GC profile (g FA/ 100g samples).

4. The same calculation as in table B2, B3 and B4

Tabel B2. Calculation for Soxhlet method (sample C, D)

Sample Code	C		C		D		D	
sample weight(g):W0	4.1186		4.0151		4.8747		4.2383	
Flask(g): W2	58.3239		58.2848		56.7956		55.8697	
Flask + stopper (g): W1	75.339		75.301		74.514		73.588	
falsk+stopper+ extract(g): W3	146.8232		135.7998		150.0585		139.4826	
Extract for GC(g): W4b-W4a, W5b-W5a	2.6606	2.6349	2.6484	2.6766	2.6577	2.5979	2.6250	2.6373
GC No.	85	86	131	132	87	88	133	134
flak+stopper+extact left(g):W6	141.4512		130.3588		144.7215		134.1257	
flask+fat(g):W7	59.7951		59.7085		57.038		56.0715	
residue(g): W8	2.37627		2.3367		4.8019		4.1752	
Residue for GC(g): W9, W10	0.1975	0.1976	0.2076	0.1928	0.2029	0.2010	0.1925	0.1926
GC No.	94	95	124	125	96	97	126	127
Extracted lipid (g/100g)[1]:	38.62		38.96		5.35		5.18	
Average of extracted lipid (g/100g sample)	38.79				5.27			
Relative error of extracted lipid %	-0.87				3.19			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.58		97.87		98.48		98.23	
Mass balance for Residue(%)[2]:	94.00		95.35		104.08		103.90	
Total FA in extract (g FA/100g extract): M1	2.052	2.038	2.373	2.402	0.205	0.205	0.190	0.189
Total FA extracted (g FA/ 100g Sample) : M2= M1*(W3-W1)/W0	35.62	35.38	35.76	36.20	3.18	3.18	2.96	2.94
Average of Total FA extracted (g FA/ 100g Sample): $\overline{M2}$	35.74				3.06			
Relative error of total FA extracted %	0.97				4.29			
Total FA in Residue(g FA/100g residue):M3	1.4199	1.4717	1.4148	1.4418	7.7220	7.6860	6.9765	6.7251
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	0.82	0.85	0.82	0.84	7.61	7.57	6.87	6.62
Average of total FA left in sample(g/100g sample): $\overline{M4}$	0.83				7.17			
relative error of total FA left in sample%	1.67				6.91			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	36.57				10.23			

Tabel B3. Calculation for Soxhlet method (sample F, G)

Sample Code	F		F		G		G	
sample weight(g):W0	4.4899		4.017		4.5073		4.1073	
Flask(g): W2	57.4855		55.1501		56.8617		54.1919	
Flask + stopper (g): W1	72.236		69.899		75.941		73.27	
falsk+stopper+ extract(g): W3	139.5607		137.9138		146.5175		142.6586	
Extract for GC(g): W4b-W4a, W5b-W5a	2.6632	2.6518	2.6573	2.6225	2.6424	2.6340	2.6587	2.6164
GC No.	99	100	147	148	101	102	185	186
flak+stopper+extact left(g):W6	134.1693		132.5462		141.1639		137.2906	
flask+fat(g):W7	58.1418		55.7555		57.1285		54.432	
residue(g): W8	3.65615		3.2392		4.0025		3.5983	
Residue for GC(g): W9, W10	0.1943	0.2066	0.1938	0.2012	0.1905	0.1947	0.1906	0.2027
GC No.	106	107	138	139	108	109	196	197
Extracted lipid (g/100g)[1]:	15.89		16.36		6.41		6.34	
Average of extracted lipid (g/100g sample)	16.13				6.37			
Relative error of total fat%	-2.93				1.09			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.58		98.36		98.56		98.27	
Mass balance for Residue(%)[2]:	96.81		96.41		94.88		93.53	
Total FA in extract (g FA/100g extract): M1	0.772	0.745	0.638	0.639	0.290	0.287	0.258	0.264
Total FA extracted (g FA/ 100g Sample) : M2= M1*(W3-W1)/W0	11.57	11.17	10.80	10.82	4.55	4.50	4.36	4.46
Average of Total FA extracted (g FA/ 100g Sample): $\overline{M2}$	11.09				4.47			
Relative error of total FA extracted %	3.29				1.80			
Total FA in Residue(g FA/100g residue):M3	3.6906	3.7294	3.4322	3.8307	1.1852	1.1968	1.3369	1.3227
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	3.01	3.04	2.77	3.09	1.05	1.06	1.17	1.16
Average of total FA left in sample(g/100g sample): $\overline{M4}$	2.97				1.11			
relative error of total FA left in sample%	4.78				5.61			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	14.07				5.58			

Tabel B4. Calculation for Soxhlet method (sample H, I)

Sample Code	H		H		I		I	
sample weight(g):W0	4.8197		4.7044		4.2829		4.2153	
Flask(g): W2	55.8247		59.5284		55.0476		57.0621	
Flask + stopper (g): W1	74.315		78.02		74.425		76.439	
falsk+stopper+ extract(g): W3	133.3663		145.7262		144.9619		146.2283	
Extract for GC(g): W4b-W4a, W5b-W5a	2.6436	2.6314	2.6761	2.6152	2.6387	2.6340	2.6643	2.6470
GC No.	103	104	187	188	153	154	189	190
flak+stopper+extact left(g):W6	128.0274		140.3343		139.5723		140.8203	
flask+fat(g):W7	56.3006		59.9966		55.7039		57.6857	
residue(g): W8	4.04197		3.9732		3.5145		3.4325	
Residue for GC(g): W9, W10	0.2099	0.2079	0.1960	0.1994	0.1913	0.2057	0.1970	0.2018
GC No.	110	111	198	199	144	145	200	201
Extracted lipid (g/100g)[1]:	10.86		10.81		16.59		16.04	
Average of extracted lipid (g/100g sample)	10.83				16.31			
Relative error of extracted lipid %	0.39				3.40			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.80		98.13		97.83		98.21	
Mass balance for Residue(%)[2]:	94.08		94.70		98.38		96.98	
Total FA in extract (g FA/100g extract): M1	0.6715	0.6742	0.5686	0.5837	0.7015	0.7038	0.6810	0.6691
Total FA extracted (g FA/ 100g Sample) : M2= M1*(W3-W1)/W0	8.23	8.26	8.18	8.40	11.55	11.59	11.28	11.08
Average of Total FA extracted (g FA/ 100g Sample): $\overline{M2}$	8.27				11.37			
Relative error of total FA extracted %	1.14				2.14			
Total FA in Residue(g FA/100g residue):M3	1.8518	1.8960	1.9800	2.0098	3.2726	3.4238	3.4040	3.5501
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	1.55	1.59	1.67	1.70	2.69	2.81	2.77	2.89
Average of total FA left in sample(g/100g sample): $\overline{M4}$	1.63				2.79			
relative error of total FA left in sample%	4.17				3.06			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	9.90				14.16			

Table B5. Soxhlet Result for Sample A **(g/100g sample)**

Fatty Acid	A1				A2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 81	GC 82	GC 90	GC 91	GC 129	GC 130	GC 122	GC 123			
8:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
10:0	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
12:0	<0.01	<0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
14:0	0.48	0.47	0.05	0.05	0.48	0.50	0.06	0.06	0.49	0.06	0.54
16:0	0.96	0.94	0.33	0.33	0.96	1.00	0.35	0.33	0.96	0.33	1.30
18:0	0.11	0.11	0.05	0.05	0.11	0.11	0.05	0.05	0.11	0.05	0.16
20:0	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
22:0	n.a.	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
16:1 n7	0.28	0.28	0.02	0.02	0.30	0.31	0.03	0.02	0.29	0.02	0.32
18:1 n9	0.86	0.84	0.14	0.14	0.88	0.90	0.14	0.14	0.87	0.14	1.01
20:1 n9	0.84	0.83	0.04	0.04	0.86	0.87	0.04	0.04	0.85	0.04	0.89
22:1 n11	1.17	1.17	0.04	0.04	1.20	1.20	0.04	0.04	1.19	0.04	1.23
24:1 n9	0.08	0.08	0.04	0.03	0.08	0.08	0.04	0.04	0.08	0.04	0.11
16:2 n4	0.02	0.02	<0.01	<0.01	0.02	0.02	<0.01	<0.01	0.02	<0.01	0.02
16:3 n4	0.02	0.02	<0.01	<0.01	0.02	0.02	<0.01	<0.01	0.02	<0.01	0.02
18:2 n6	0.09	0.09	0.01	0.01	0.09	0.10	0.01	0.01	0.10	0.01	0.11
18:3 n6	n.a.	0.00	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	n.a.	<0.01
20:2 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
20:3 n6	0.00	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
20:4 n6	0.02	0.03	0.01	0.01	0.03	0.03	0.01	0.01	0.03	0.01	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
18:3 n3	0.07	0.07	0.01	0.01	0.07	0.08	0.01	0.01	0.07	0.01	0.08
18:4 n3	0.18	0.18	0.01	0.01	0.18	0.19	0.01	0.01	0.18	0.01	0.20
20:3 n3	0.00	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
20:4 n3	0.05	0.04	0.01	0.01	0.05	0.05	0.01	0.01	0.05	0.01	0.05
20:5 n3	0.57	0.56	0.15	0.15	0.56	0.58	0.16	0.16	0.57	0.15	0.72
21:5 n3	0.02	0.02	<0.01	<0.01	0.02	0.02	<0.01	<0.01	0.02	<0.01	0.02
22:5 n3	0.06	0.06	0.01	0.01	0.05	0.06	0.01	0.01	0.06	0.01	0.07
22:6 n3	0.98	0.99	0.40	0.40	0.98	0.98	0.43	0.44	0.98	0.42	1.40
SFA	1.57	1.54	0.44	0.44	1.58	1.65	0.47	0.45	1.58	0.45	2.03
MUFA	3.23	3.21	0.27	0.27	3.31	3.35	0.29	0.29	3.28	0.28	3.55
PUFA	2.10	2.11	0.61	0.62	2.11	2.15	0.67	0.67	2.12	0.64	2.76
Total FA	6.90	6.86	1.32	1.32	7.00	7.15	1.42	1.41	6.98	1.37	8.35

Table B6. Soxhlet Result for Sample B **(g/100g sample)**

Fatty Acid	B1				B2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 83	GC 84	GC 92	GC 93	GC158	GC 159	GC 167	GC 168			
8:0	n.a.	n.a.	<0.01	<0.01		n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
10:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
12:0	0.005	0.004	<0.01	<0.01	0.003	0.002	n.a.	n.a.	0.004	n.a.	<0.01
14:0	0.017	0.017	0.02	0.01	0.018	0.019	0.01	0.01	0.018	0.01	0.03
16:0	0.220	0.222	0.23	0.22	0.227	0.233	0.21	0.22	0.225	0.22	0.44
18:0	0.038	0.038	0.07	0.07	0.039	0.041	0.07	0.07	0.039	0.07	0.11
20:0	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
22:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
16:1 n7	0.012	0.011	0.02	0.02	0.016	0.011	0.01	0.02	0.013	0.02	0.03
18:1 n9	0.125	0.119	0.16	0.15	0.126	0.130	0.14	0.15	0.125	0.15	0.28
20:1 n9	0.023	0.021	0.03	0.03	0.023	0.025	0.03	0.03	0.023	0.03	0.05
22:1 n11	0.008	0.007	0.01	0.01	0.010	0.007	0.01	0.01	0.008	0.01	0.02
24:1 n9	n.a.	n.a.	0.02	0.02	0.003	n.a.	0.02	0.02	0.003	0.02	0.02
16:2 n4	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
16:3 n4	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	n.a.	n.a.	<0.01	<0.01
18:2 n6	0.013	0.012	0.02	0.01	0.012	0.013	0.01	0.02	0.012	0.01	0.03
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
20:2 n6	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
20:3 n6	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
20:4 n6	0.032	0.031	0.07	0.08	0.031	0.033	0.07	0.08	0.032	0.07	0.11
22:4 n6	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
18:3 n3	0.004	0.003	0.01	<0.01	0.005	0.003	<0.01	0.01	0.004	0.01	0.01
18:4 n3	0.004	0.004	0.01	0.01	0.003	0.003	0.01	0.01	0.004	0.01	0.01
20:3 n3	n.a.	n.a.	<0.01	<0.01	n.a.	0.003	<0.01	<0.01	0.003	<0.01	<0.01
20:4 n3	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	0.01	n.a.	0.01	0.01
20:5 n3	0.099	0.098	0.20	0.20	0.098	0.098	0.19	0.21	0.098	0.20	0.30
21:5 n3	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
22:5 n3	0.008	0.013	0.02	0.02	0.014	0.009	0.02	0.02	0.011	0.02	0.03
22:6 n3	0.220	0.217	0.56	0.58	0.215	0.213	0.55	0.59	0.216	0.57	0.79
SFA	0.280	0.281	0.32	0.31	n.a.	n.a.	0.29	0.31	0.281	0.31	0.59
MUFA	0.168	0.158	0.24	0.22	0.177	0.173	0.21	0.23	0.169	0.23	0.40
PUFA	0.379	0.379	0.906	0.923	0.377	0.376	0.877	0.946	0.378	0.91	1.29
Total FA	0.828	0.818	1.47	1.45	0.843	0.844	1.38	1.49	0.833	1.45	2.28

Table B7. Soxhlet Result for Sample C (g/100g sample)

Fatty Acid	C1				C2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 85	GC 86	GC 94	GC 95	GC 131	GC 132	GC 124	GC 125			
8:0	n.a.	n.a.	0.004	0.005	n.a.	n.a.	0.002	0.005	n.a.	n.a.	<0.01
10:0	0.01	0.01	n.a.	n.a.	0.01	0.01	n.a.	0.001	0.01	0.001	0.01
12:0	0.02	0.02	<0.001	0.001	0.02	0.02	0.001	0.001	0.02	0.001	0.02
14:0	1.06	1.05	0.005	0.005	1.07	1.08	0.007	0.007	1.07	0.006	1.07
16:0	3.84	3.80	0.121	0.130	3.89	3.94	0.119	0.123	3.87	0.123	3.99
18:0	0.98	0.97	0.044	0.046	0.99	1.00	0.043	0.044	0.98	0.044	1.03
20:0	0.11	0.11	<0.001	<0.001	0.11	0.12	<0.001	n.a.	0.11	<0.001	0.11
22:0	0.06	0.06	<0.001	<0.001	0.06	0.06	0.001	<0.001	0.06	<0.001	0.06
16:1 n7	1.06	1.05	0.006	0.006	1.07	1.05	0.006	0.007	1.06	0.006	1.06
18:1 n9	13.74	13.64	0.089	0.092	13.90	14.08	0.093	0.094	13.84	0.092	13.93
20:1 n9	1.80	1.80	0.005	0.005	1.80	1.83	0.005	0.005	1.81	0.005	1.81
22:1 n11	1.18	1.18	0.002	0.002	1.17	1.18	0.002	0.002	1.18	0.002	1.18
24:1 n9	0.19	0.18	0.014	0.013	0.18	0.19	0.013	0.013	0.18	0.013	0.20
16:2 n4	0.11	0.11	0.001	<0.001	0.11	0.12	0.001	0.001	0.11	0.001	0.11
16:3 n4	0.07	0.07	<0.001	n.a.	0.07	0.07	<0.001	<0.001	0.07	<0.001	0.07
18:2 n6	4.07	4.04	0.023	0.025	4.07	4.14	0.026	0.026	4.08	0.025	4.11
18:3 n6	0.04	0.05	n.a.	n.a.	0.04	0.04	n.a.	n.a.	0.04	n.a.	0.04
20:2 n6	0.38	0.38	0.003	0.003	0.38	0.38	0.003	0.003	0.38	0.003	0.38
20:3 n6	0.07	0.07	0.002	0.002	0.07	0.07	0.001	0.002	0.07	0.002	0.08
20:4 n6	0.11	0.11	0.015	0.015	0.11	0.11	0.015	0.015	0.11	0.015	0.13
22:4 n6	0.03	0.03	0.001	0.001	0.03	0.03	0.001	0.001	n.a.	n.a.	0.00
18:3 n3	1.72	1.71	0.022	0.023	1.71	1.73	0.023	0.024	1.72	0.023	1.74
18:4 n3	0.26	0.26	0.002	0.002	0.26	0.26	0.002	0.002	0.26	0.002	0.26
20:3 n3	0.19	0.19	0.003	0.002	0.20	0.21	0.004	0.003	0.20	0.003	0.20
20:4 n3	0.40	0.40	0.008	0.008	0.39	0.40	0.008	0.008	0.39	0.008	0.40
20:5 n3	1.28	1.27	0.087	0.091	1.26	1.27	0.088	0.091	1.27	0.089	1.36
21:5 n3	0.09	0.09	0.001	0.001	0.09	0.09	0.001	0.001	0.09	0.001	0.09
22:5 n3	0.77	0.77	0.027	0.027	0.76	0.76	0.027	0.027	0.76	0.027	0.79
22:6 n3	1.98	1.98	0.336	0.343	1.92	1.95	0.332	0.332	1.96	0.336	2.29
SFA	6.08	6.01	0.175	0.188	6.14	6.23	0.173	0.182	6.12	0.179	6.30
MUFA	17.97	17.85	0.115	0.118	18.13	18.33	0.119	0.121	18.07	0.118	18.19
PUFA	11.57	11.51	0.53	0.54	11.48	11.64	0.53	0.54	11.55	0.535	12.09
Total FA	35.62	35.38	0.819	0.849	35.76	36.20	0.823	0.839	35.74	0.833	36.57

Table B8. Soxhlet Result for Sample D (g/100g sample)

Fatty Acid	D1				D2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 87	GC 88	GC 96	GC 97	GC 133	GC 134	GC 126	GC 127			
8:0	n.a.	0.01	0.01	0.01	n.a.	n.a.	0.00	n.a.	n.a.	n.a.	<0.01
10:0	0.01	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
12:0	0.01	0.00	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
14:0	0.21	0.22	0.26	0.25	0.21	0.21	0.25	0.23	0.21	0.25	0.46
16:0	0.58	0.59	1.64	1.62	0.57	0.58	1.66	1.57	0.58	1.62	2.20
18:0	0.05	0.05	0.18	0.18	0.05	0.05	0.19	0.18	0.05	0.18	0.24
20:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
22:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01
16:1 n7	0.18	0.17	0.24	0.22	0.17	0.17	0.21	0.22	0.17	0.22	0.39
18:1 n9	0.78	0.78	0.90	0.88	0.75	0.76	0.91	0.87	0.77	0.89	1.66
20:1 n9	0.11	0.11	0.15	0.14	0.11	0.10	0.14	0.14	0.11	0.14	0.25
22:1 n11	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.10
24:1 n9	0.02	0.02	0.11	0.11	0.03	0.02	0.11	0.11	0.02	0.11	0.13
16:2 n4	0.01	0.01	0.01	0.01	0.01	0.01	0.01	<0.01	0.01	0.01	0.02
16:3 n4	0.01	0.01	n.a.	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.02
18:2 n6	0.07	0.07	0.06	0.06	0.07	0.06	0.06	0.05	0.07	0.06	0.13
18:3 n6	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
20:2 n6	n.a.	n.a.	0.01	0.01	<0.01	n.a.	0.01	0.01	<0.01	0.01	0.01
20:3 n6	n.a.	<0.01	0.01	0.01	<0.01	n.a.	<0.01	<0.01	<0.01	0.01	0.01
20:4 n6	0.01	0.02	0.06	0.06	0.01	0.01	0.05	0.05	0.01	0.05	0.07
22:4 n6	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
18:3 n3	0.06	0.06	0.04	0.04	0.06	0.06	0.03	0.03	0.06	0.04	0.10
18:4 n3	0.06	0.06	0.05	0.05	0.05	0.05	0.04	0.04	0.06	0.05	0.10
20:3 n3	n.a.	n.a.	0.01	0.01	n.a.	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
20:4 n3	0.04	0.03	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.07
20:5 n3	0.30	0.30	1.00	1.01	0.26	0.26	0.82	0.79	0.28	0.91	1.19
21:5 n3	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	0.01	0.01
22:5 n3	0.04	0.04	0.09	0.09	0.04	0.03	0.07	0.07	0.04	0.08	0.12
22:6 n3	0.56	0.55	2.67	2.70	0.48	0.47	2.19	2.15	0.52	2.43	2.94
SFA	0.86	0.87	2.09	2.07	0.84	0.84	2.11	1.99	0.85	2.06	2.92
MUFA	1.15	1.14	1.45	1.40	1.10	1.11	1.42	1.39	1.12	1.42	2.54
PUFA	1.17	1.17	4.06	4.10	1.01	0.99	3.35	3.25	1.09	3.69	4.78
Total FA	3.18	3.18	7.61	7.57	2.96	2.94	6.87	6.62	3.06	7.17	10.23

Table B9. Soxhlet Result for Sample F (g/100g sample)

Fatty Acid	F1				F2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 99	GC 100	GC 106	GC 107	GC 147	GC 148	GC 138	GC 139			
8:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01
10:0	n.a.	n.a.	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
12:0	0.03	0.03	<0.01	<0.01	0.03	0.03	<0.01	<0.01	0.03	<0.01	0.04
14:0	1.71	1.63	0.13	0.13	1.56	1.55	0.11	0.13	1.61	0.12	1.74
16:0	2.67	2.57	0.91	0.91	2.48	2.48	0.81	0.93	2.55	0.89	3.44
18:0	0.19	0.19	0.04	0.04	0.18	0.18	0.04	0.04	0.19	0.04	0.23
20:0	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	n.a.	<0.01	<0.01
22:0	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	n.a.	<0.01	<0.01
16:1 n7	1.00	0.96	0.08	0.08	0.92	0.92	0.07	0.08	0.95	0.08	1.02
18:1 n9	2.66	2.57	0.42	0.42	2.49	2.49	0.39	0.43	2.55	0.42	2.97
20:1 n9	0.19	0.18	0.02	0.03	0.17	0.18	0.02	0.02	0.18	0.02	0.20
22:1 n11	0.10	0.10	0.05	0.05	0.10	0.10	0.04	0.04	0.10	0.04	0.14
24:1 n9	0.02	0.02	0.01	0.01	0.02	0.02	n.a.	n.a.	0.02	0.01	0.03
16:2 n4	0.12	0.12	0.01	0.01	0.10	0.11	0.01	0.01	0.11	0.01	0.12
16:3 n4	0.05	0.05	0.01	0.01	0.05	0.05	n.a.	n.a.	0.05	0.01	0.06
18:2 n6	0.19	0.19	0.05	0.05	0.18	0.19	0.05	0.05	0.19	0.05	0.24
18:3 n6	0.01	0.02	<0.01	<0.01	0.01	0.02	<0.01	<0.01	n.a.	n.a.	<0.01
20:2 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
20:3 n6	<0.01	n.a.	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	0.01	0.01
20:4 n6	0.04	0.04	0.02	0.02	0.04	0.04	0.02	0.02	0.04	0.02	0.05
22:4 n6	n.a.	n.a.	0.00	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00
18:3 n3	0.11	0.11	0.04	0.04	0.11	0.11	0.03	0.04	0.11	0.04	0.15
18:4 n3	0.26	0.25	0.05	0.05	0.24	0.24	0.04	0.05	0.25	0.05	0.29
20:3 n3	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	n.a.	<0.01	<0.01
20:4 n3	0.05	0.05	0.03	0.03	0.05	0.05	0.02	0.03	0.05	0.03	0.07
20:5 n3	1.29	1.26	0.72	0.73	1.22	1.23	0.68	0.76	1.25	0.72	1.97
21:5 n3	0.04	0.04	0.02	0.02	0.04	0.04	0.02	0.02	0.04	0.02	0.06
22:5 n3	0.04	0.04	0.02	0.02	0.03	0.04	0.02	0.02	0.04	0.02	0.06
22:6 n3	0.76	0.76	0.38	0.38	0.74	0.74	0.38	0.40	0.75	0.39	1.14
SFA	4.62	4.43	1.09	1.10	4.27	4.27	0.97	1.11	4.40	1.07	5.46
MUFA	3.97	3.82	0.57	0.58	3.70	3.70	0.52	0.57	3.80	0.56	4.36
PUFA	2.99	2.92	1.35	1.37	2.83	2.84	1.28	1.40	2.90	1.35	4.25
Total FA	11.57	11.17	3.01	3.04	10.80	10.82	2.77	3.09	11.09	2.98	14.07

Table B10. Soxhlet Result for Sample G (g/100g sample)

Fatty Acid	G1				G2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 101	GC 102	GC 108	GC 109	GC 185	GC 186	GC 196	GC 197			
8:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01
10:0	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
12:0	n.a.	n.a.	<0.01	<0.01	0.01	n.a.	<0.01	<0.01	0.01	<0.01	0.01
14:0	0.18	0.18	0.03	0.03	0.17	0.18	0.04	0.04	0.18	0.04	0.22
16:0	0.75	0.73	0.24	0.24	0.70	0.72	0.26	0.26	0.73	0.25	0.97
18:0	0.12	0.12	0.06	0.06	0.12	0.12	0.06	0.06	0.12	0.06	0.18
20:0	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01
22:0	n.a.	n.a.	<0.01	<0.01	<0.01	<0.01	n.a.	<0.01	n.a.	<0.01	<0.01
16:1 n7	0.22	0.22	0.04	0.04	0.20	0.23	0.05	0.05	0.22	0.04	0.26
18:1 n9	0.81	0.80	0.20	0.20	0.76	0.78	0.21	0.22	0.79	0.21	1.00
20:1 n9	0.63	0.64	0.08	0.08	0.62	0.63	0.08	0.08	0.63	0.08	0.71
22:1 n11	0.70	0.69	0.05	0.05	0.69	0.70	0.06	0.06	0.69	0.05	0.75
24:1 n9	0.09	0.08	0.04	0.04	0.09	0.09	0.04	0.04	0.09	0.04	0.13
16:2 n4	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
16:3 n4	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
18:2 n6	0.06	0.06	0.01	0.01	0.05	0.05	0.02	0.02	0.06	0.02	0.07
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
20:2 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
20:3 n6	n.a.	n.a.	n.a.	<0.01	n.a.	0.00	<0.01	<0.01	<0.01	<0.01	<0.01
20:4 n6	0.03	0.03	0.01	0.01	0.03	0.03	0.01	0.01	0.03	0.01	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
18:3 n3	0.03	0.02	0.01	0.01	0.02	0.03	0.01	0.01	0.02	0.01	0.03
18:4 n3	0.06	0.06	0.01	0.01	0.05	0.06	0.02	0.02	0.06	0.01	0.07
20:3 n3	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01
20:4 n3	0.02	0.02	<0.01	<0.01	0.02	0.02	0.01	0.01	0.02	<0.01	0.02
20:5 n3	0.31	0.31	0.08	0.09	0.29	0.30	0.10	0.10	0.30	0.09	0.40
21:5 n3	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
22:5 n3	0.03	0.04	0.01	0.01	0.03	0.04	0.01	0.01	0.03	0.01	0.04
22:6 n3	0.47	0.46	0.16	0.16	0.46	0.45	0.18	0.18	0.46	0.17	0.63
SFA	1.06	1.04	0.33	0.33	1.00	1.03	0.36	0.36	1.03	0.35	1.38
MUFA	2.45	2.43	0.41	0.42	2.36	2.42	0.44	0.44	2.42	0.43	2.84
PUFA	1.03	1.03	0.31	0.32	1.00	1.00	0.37	0.36	1.02	0.34	1.35
Total FA	4.55	4.50	1.05	1.06	4.36	4.46	1.17	1.16	4.47	1.11	5.58

Table B11. Soxhlet Result for Sample H (g/100g sample)

Fatty Acid	H1				H2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 103	GC 104	GC 110	GC 111	GC 187	GC 188	GC 198	GC 199			
8:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01
10:0	n.a.	0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
12:0	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
14:0	0.64	0.64	0.07	0.07	0.63	0.65	0.08	0.08	0.64	0.08	0.72
16:0	1.27	1.26	0.39	0.40	1.25	1.29	0.43	0.43	1.27	0.41	1.68
18:0	0.14	0.14	0.06	0.06	0.14	0.14	0.06	0.06	0.14	0.06	0.20
20:0	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	n.a.	<0.01	<0.01
22:0	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01
16:1 n7	0.36	0.36	0.04	0.04	0.35	0.37	0.05	0.05	0.36	0.05	0.40
18:1 n9	1.25	1.25	0.23	0.24	1.22	1.28	0.25	0.25	1.25	0.24	1.49
20:1 n9	1.08	1.09	0.07	0.07	1.09	1.10	0.07	0.07	1.09	0.07	1.16
22:1 n11	1.59	1.62	0.07	0.07	1.61	1.64	0.07	0.07	1.61	0.07	1.69
24:1 n9	0.11	0.11	0.04	0.04	0.11	0.11	0.04	0.04	0.11	0.04	0.15
16:2 n4	0.02	0.02	<0.01	<0.01	0.02	0.02	<0.01	<0.01	0.02	<0.01	0.03
16:3 n4	0.02	0.02	<0.01	<0.01	0.02	0.02	<0.01	<0.01	0.02	<0.01	0.02
18:2 n6	0.10	0.11	0.02	0.02	0.11	0.11	0.02	0.02	0.11	0.02	0.12
18:3 n6	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01
20:2 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.02
20:3 n6	0.01	0.01	<0.01	<0.01	0.01	n.a.	<0.01	<0.01	0.01	<0.01	0.01
20:4 n6	0.03	0.03	0.01	0.01	0.03	0.03	0.01	0.02	0.03	0.01	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00
18:3 n3	0.07	0.07	0.01	0.01	0.07	0.07	0.01	0.01	0.07	0.01	0.08
18:4 n3	0.13	0.13	0.01	0.01	0.13	0.14	0.01	0.01	0.13	0.01	0.14
20:3 n3	<0.01	0.01	<0.01	<0.01	0.02	0.02	<0.01	0.01	n.a.	<0.01	<0.01
20:4 n3	0.04	0.04	0.01	0.01	0.04	0.04	0.01	0.01	0.04	0.01	0.05
20:5 n3	0.46	0.46	0.14	0.14	0.46	0.47	0.16	0.16	0.46	0.15	0.61
21:5 n3	0.02	0.02	<0.01	<0.01	0.01	0.02	0.00	0.00	0.02	<0.01	0.02
22:5 n3	0.06	0.06	0.01	0.01	0.06	0.06	0.02	0.01	0.06	0.01	0.07
22:6 n3	0.78	0.78	0.35	0.36	0.77	0.78	0.37	0.38	0.78	0.36	1.14
SFA	2.07	2.08	0.52	0.54	2.05	2.12	0.58	0.58	2.08	0.56	2.64
MUFA	4.39	4.41	0.45	0.46	4.39	4.50	0.48	0.48	4.42	0.47	4.89
PUFA	1.76	1.77	0.58	0.58	1.75	1.78	0.61	0.63	1.77	0.60	2.37
Total FA	8.23	8.26	1.55	1.59	8.18	8.40	1.67	1.70	8.27	1.63	9.90

Table B12. Soxhlet Result for Sample I (g/100g sample)

Fatty Acid	I1				I2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 153	GC 154	GC 144	GC 145	GC 189	GC 190	GC 200	GC 201			
8:0	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01
10:0	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
12:0	0.04	0.04	<0.01	0.01	0.04	0.03	<0.01	<0.01	0.04	<0.01	0.04
14:0	1.25	1.26	0.09	0.10	1.23	1.20	0.10	0.10	1.24	0.10	1.34
16:0	2.52	2.53	0.65	0.68	2.44	2.39	0.68	0.71	2.47	0.68	3.15
18:0	0.16	0.15	0.03	0.03	0.15	0.14	0.03	0.03	0.15	0.03	0.18
20:0	<0.01	<0.01	n.a.	<0.01	<0.01	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01
22:0	<0.01	0.01	<0.01	<0.01	<0.01	n.a.	<0.01	<0.01	n.a.	<0.01	<0.01
16:1 n7	0.51	0.51	0.04	0.05	0.50	0.49	0.05	0.05	0.50	0.05	0.55
18:1 n9	2.26	2.27	0.33	0.34	2.21	2.17	0.34	0.36	2.23	0.34	2.57
20:1 n9	0.15	0.15	0.02	0.02	0.14	0.15	0.02	0.02	0.15	0.02	0.17
22:1 n11	0.10	0.10	0.04	0.04	0.10	0.10	0.04	0.04	0.10	0.04	0.14
24:1 n9	n.a.	0.01	n.a.	<0.01	0.01	n.a.	<0.01	<0.01	0.01	<0.01	0.01
16:2 n4	0.07	0.08	<0.01	<0.01	0.07	0.07	<0.01	0.01	0.07	<0.01	0.08
16:3 n4	0.05	0.05	0.01	0.01	0.05	0.05	0.01	0.01	0.05	0.01	0.06
18:2 n6	0.21	0.21	0.04	0.04	0.21	0.20	0.04	0.04	0.21	0.04	0.25
18:3 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	n.a.	n.a.	<0.01
20:2 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.01
20:3 n6	0.01	0.01	<0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	0.02
20:4 n6	0.03	0.03	0.01	0.01	0.03	0.03	0.01	0.01	0.03	0.01	0.03
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
18:3 n3	0.32	0.32	0.07	0.08	0.31	0.31	0.08	0.08	0.32	0.08	0.39
18:4 n3	0.66	0.66	0.11	0.11	0.65	0.64	0.11	0.12	0.65	0.11	0.76
20:3 n3	0.03	0.03	0.01	0.01	0.03	0.03	0.01	0.01	n.a.	0.01	0.01
20:4 n3	0.07	0.07	0.02	0.02	0.07	0.07	0.02	0.02	0.07	0.02	0.09
20:5 n3	1.95	1.96	0.70	0.74	1.90	1.88	0.72	0.75	1.92	0.72	2.65
21:5 n3	0.07	0.07	0.03	0.03	0.07	0.07	0.03	0.03	0.07	0.03	0.10
22:5 n3	0.05	0.04	0.02	0.02	0.04	0.04	0.02	0.02	0.04	0.02	0.06
22:6 n3	1.00	1.00	0.45	0.47	0.98	0.97	0.45	0.48	0.99	0.46	1.45
SFA	3.98	3.99	0.78	0.81	3.88	3.78	0.82	0.85	3.91	0.81	4.72
MUFA	3.01	3.03	0.43	0.45	2.96	2.91	0.45	0.47	2.98	0.45	3.43
PUFA	4.56	4.57	1.47	1.55	4.44	4.39	1.50	1.57	4.49	1.52	6.01
Total FA	11.55	11.59	2.69	2.81	11.28	11.08	2.77	2.89	11.37	2.79	14.16

Appendix C. Data for acid dydrolysis Method

Table C1. Calculation for acid hydrolysis method (sample A, B)

Sample Code	A		A		B		B	
sample weight(g): W0	4.669		4.6061		4.7282		4.2244	
Flask(g): W2	59.5732		56.6774		58.278		55.0388	
Flask + stopper (g): W1	75.746		72.85		75.7594		72.52	
falsk+stopper+ extract(g): W3	147.9617		147.5531		146.0755		145.4247	
weight of filter aid(g)	3.77		3.7727		3.8229		3.7896	
Extract for GC(g): W4b-W4a, W5b-W5a	2.6548	2.6033	2.6479	2.6486	2.6872	2.6753	2.6639	2.7481
GC No.	276	277	369	370	239	240	371	372
flak+stopper+extact left(g): W6	142.6087		142.1703		140.6259		140.0396	
flask+fat(g): W7	60.0272		57.1378		58.3907		55.1396	
residue(g): W8	3.8706		4.1421		4.1664		4.3498	
Residue for GC(g): W9, W10	0.2094	0.2043	0.2021	0.1957	0.1999	0.1991	0.2051	0.1928
GC No.	289	290	380	381	250	251	382	383
Extracted lipid (g/100g)[1]	10.50		10.77		2.58		2.58	
Average of extracted lipid (g/100g sample)	10.64				2.58			
Relative error of total fat, %	-2.53				0.29			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.23		98.40		98.40		100.50	
TOtal FA in Extract(g FA/100g extract):M1	0.5349	0.5054	0.5444	0.5399	0.1197	0.1243	0.1190	0.1092
Total FA extracted (g FA/ 100g Sample): M2= M1*(W3-W1)/W0	8.27	7.82	8.83	8.76	1.78	1.85	2.05	1.89
Average of total FA extracted (g FA/100g sample): $\overline{M2}$	8.42				1.89			
Relative error of total FA extracted %	5.60				6.15			
Total FA in Residue(g FA/100g residue)	0.2135	0.2173	0.2530	0.2543	0.4561	0.4149	0.3544	0.3861
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	0.18	0.18	0.23	0.23	0.40	0.37	0.36	0.40
Average of total FA left in sample(g/100g sample): $\overline{M4}$	0.20				0.38			
Relative error of total FA left in sample%	14.07				5.22			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	8.62				2.27			

1. Extracted lipid= $\frac{(W7-W2)\times(W3-W1)}{W0\times(W6-W1)}\times 100\%$

2. M1, M3 was calculated by using the original GC profile. By using the same formula of M2 and M4, The original GC profiles (g FA/ 100g extract or g FA/ 100g residue) was converted into GC profile (g FA/ 100g samples).

3. Same calculation as in Table C2, C3 and C4

Table C2. Calculation for acid hydrolysis method (sample C,D)

Sample Code	C		C		D		D	
sample weight(g): W0	4.3397		4.4718		4.6131		4.7136	
Flask(g): W2	56.5587		54.7905		56.554		57.5789	
Flask + stopper (g): W1	73.574		71.805		74.27		75.295	
falsk+stopper+ extract(g): W3	147.4955		141.8719		148.2471		150.7266	
weight of filter aid(g)	3.8051		3.8016		3.8264		3.8232	
Extract for GC(g): W4b-W4a, W5b-W5a	1.28883	1.3209	1.2936	1.3161	2.6515	2.6365	2.6695	2.6197
GC No.	304	305	373	374	375	376	394	395
flak+stopper+extact left(g): W6	144.834		139.2033		142.8474		145.3445	
flask+fat(g): W7	58.1991		56.4789		57.0776		58.1229	
residue(g): W8	4.0214		3.9116		4.6581		5.005	
Residue for GC(g): W9, W10	0.2098	0.192	0.1959	0.197	0.1960	0.2073	0.1964	0.1923
GC No.	313	314	384	385	386	387	403	404
Extracted lipid (g/100g)[1]	39.21		39.25		12.24		12.43	
Average of extracted lipid (g/100g sample)	39.23				12.34			
Relative error of extracted lipid, %	-0.10				-1.49			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.05		97.79		97.93		98.27	
TOtal FA in Extract(g FA/100g extract):M1	2.1269	2.2360	2.3534	2.3717	0.5784	0.5952	0.6349	0.6228
Total FA extracted (g FA/ 100g Sample): M2= M1*(W3-W1)/W0	36.23	38.09	36.87	37.16	9.28	9.54	10.16	9.97
Average of total FA extracted (g FA/100g sample): $\overline{M2}$	37.09				9.74			
Relative error of total FA extracted %	2.08				4.11			
Total FA in Residue(g FA/100g residue)	0.3722	0.3712	0.2667	0.2813	0.6171	0.5422	0.6014	0.6883
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	0.34	0.34	0.23	0.25	0.62	0.55	0.64	0.73
Average of total FA left in sample(g/100g sample): $\overline{M4}$	0.29				0.63			
Relative error of total FA left in sample%	20.79				11.86			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	37.38				10.37			

Table C3. Calculation for acid hydrolysis method (sample F, G)

Sample Code	F		F		G		G	
sample weight(g): W0	4.5346		4.5023		4.8412		4.7453	
Flask(g): W2	55.5514		54.5702		55.7687		53.6385	
Flask + stopper (g): W1	70.3001		69.3192		74.8467		72.717	
falsk+stopper+ extract(g): W3	136.4652		141.9606		145.2885		149.1877	
weight of filter aid(g)	3.795		3.7665		3.8184		3.8234	
Extract for GC(g): W4b-W4a, W5b-W5a	2.6579	2.5985	2.6771	2.6352	2.6316	2.5801	2.6476	2.6165
GC No.	241	242	351	352	243	244	353	354
flak+stopper+extact left(g): W6	131.13131		136.5551		139.9872		143.7929	
flask+fat(g): W7	56.3414		55.3524		56.108		53.9669	
residue(g): W8	4.2191		4.4715		3.9969		4.226	
Residue for GC(g): W9, W10	0.2078	0.1929	0.1941	0.1977	0.1993	0.1988	0.2057	0.1916
GC No.	252	253	360	361	254	255	362	363
Extracted lipid (g/100g)[1]	18.95		18.77		7.58		7.45	
Average of extracted lipid (g/100g sample)	18.86				7.51			
Relative error of extracted lipid, %	0.95				1.77			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.55		98.28		98.31		97.58	
TOtal FA in Extract(g FA/100g extract):M1	0.9342	0.9616	0.8507	0.8368	0.3749	0.3659	0.3390	0.3312
Total FA extracted (g FA/ 100g Sample): M2= M1*(W3-W1)/W0	13.63	14.03	13.72	13.50	5.46	5.32	5.46	5.34
Average of total FA extracted (g FA/100g sample): $\overline{M2}$	13.72				5.40			
Relative error of total FA extracted %	1.65				1.38			
Total FA in Residue(g FA/100g residue)	0.2838	0.2801	0.5265	0.6637	0.3654	0.3527	0.3882	0.4155
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	0.26	0.26	0.52	0.66	0.30	0.29	0.35	0.37
Average of total FA left in sample(g/100g sample): $\overline{M4}$	0.43				0.33			
Relative error of total FA left in sample%	46.35				11.34			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	14.15				5.72			

Table C4. Calculation for acid hydrolysis method (sample F, I)

Sample Code	H		H		I		I	
sample weight(g): W0	4.9196		4.526		4.3778		4.8716	
Flask(g): W2	61.7366		54.8236		56.9102		56.6604	
Flask + stopper (g): W1	80.2275		73.3152		76.287		76.0375	
falsk+stopper+ extract(g): W3	150.994		146.5638		147.3476		150.8469	
weight of filter aid(g)	3.746		3.8153		3.77		3.8167	
Extract for GC(g): W4b-W4a, W5b-W5a	2.6582	2.6496	2.6549	2.641	1.2862	1.2957	1.2877	1.3041
GC No.	245	246	355	356	282	283	357	358
flak+stopper+extact left(g): W6	145.601		141.1887		144.6984		148.2469	
flask+fat(g): W7	62.315		55.351		57.7143		57.5651	
residue(g): W8	3.9245		4.2167		4.266		4.2626	
Residue for GC(g): W9, W10	0.1904	0.1949	0.1915	0.1934	0.1956	0.1925	0.1911	0.2028
GC No.	256	257	364	365	295	296	366	367
Extracted lipid (g/100g)[1]	12.73		12.58		19.08		19.24	
Average of extracted lipid (g/100g sample)	12.65				19.16			
Relative error of extracted lipid, %	1.20				-0.84			
Mass balance for extract(%): 100*(W4b-W4a+W5b-W5a)/(W3-W6)	98.42		98.53		97.46		99.68	
TOtal FA in Extract(g FA/100g extract):M1	0.6719	0.6681	0.5967	0.5972	0.8321	0.8385	0.9087	0.9410
Total FA extracted (g FA/ 100g Sample): M2= M1*(W3-W1)/W0	9.67	9.61	9.66	9.66	13.51	13.61	13.95	14.45
Average of total FA extracted (g FA/100g sample): $\overline{M2}$	9.65				13.88			
Relative error of total FA extracted %	0.27				3.06			
Total FA in Residue(g FA/100g residue)	0.2259	0.2327	0.2826	0.2793	0.344	0.382	0.3335	0.3292
Total FA left in sample (g FA/ 100g Sample): M4=M3*W8/W0	0.18	0.19	0.26	0.26	0.34	0.37	0.29	0.29
Average of total FA left in sample(g/100g sample): $\overline{M4}$	0.22				0.32			
Relative error of total FA left in sample%	20.51				12.38			
Sum of FA in extract &residue(g FA/100g sample) : $\overline{M2}+\overline{M4}$	9.87				14.20			

Table C5. Acid hydrolysis Result for Sample A **(g/100g sample)**

Fatty Acid	A1				A2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 276	GC 277	GC 289	GC 290	GC 369	GC 370	GC 380	GC 381			
8:0	n.a.	n.a.	0.002	0.002	n.a.	n.a.	0.002	0.003	n.a.	0.002	<0.01
10:0	0.01	0.01	<0.001	0.001	0.01	0.01	n.a.	<0.001	0.01	<0.001	0.01
12:0	0.01	0.01	<0.001	n.a.	0.01	0.01	n.a.	n.a.	0.01	<0.001	0.01
14:0	0.53	0.51	0.007	0.007	0.58	0.58	0.012	0.011	0.55	0.009	0.56
16:0	1.23	1.17	0.050	0.052	1.36	1.35	0.057	0.056	1.28	0.054	1.33
18:0	0.15	0.14	0.011	0.011	0.17	0.17	0.009	0.009	0.16	0.010	0.17
20:0	0.01	0.01	<0.001	<0.001	0.01	0.01	n.a.	n.a.	0.01	<0.001	0.01
22:0	0.01	0.01	<0.001	<0.001	n.a.	n.a.	n.a.	n.a.	0.01	<0.001	0.01
16:1 n7	0.32	0.30	0.003	0.004	0.34	0.34	0.004	0.005	0.33	0.004	0.33
18:1 n9	0.98	0.93	0.023	0.024	1.09	1.07	0.020	0.021	1.02	0.022	1.04
20:1 n9	0.91	0.87	0.008	0.008	0.95	0.93	0.005	0.006	0.92	0.007	0.92
22:1 n11	1.26	1.19	0.008	0.008	1.27	1.27	0.004	0.004	1.25	0.006	1.25
24:1 n9	0.10	0.10	n.a.	n.a.	0.11	0.11	0.004	0.005	0.10	0.004	0.11
16:2 n4	0.03	0.03	n.a.	n.a.	0.03	0.03	<0.001	<0.001	0.03	<0.001	0.03
16:3 n4	0.02	0.02	n.a.	n.a.	0.02	0.02	<0.001	<0.001	0.02	<0.001	0.02
18:2 n6	0.11	0.10	0.002	0.002	0.12	0.11	0.002	0.002	0.11	0.002	0.11
18:3 n6	0.01	0.01	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
20:2 n6	0.02	0.01	<0.001	<0.001	0.02	0.02	n.a.	n.a.	0.02	<0.001	0.02
20:3 n6	0.01	0.01	n.a.	<0.001	0.01	0.01	n.a.	n.a.	0.01	<0.001	0.01
20:4 n6	0.04	0.03	0.001	0.001	0.04	0.04	0.002	0.002	0.04	0.001	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00
18:3 n3	0.08	0.08	0.001	0.001	0.09	0.09	0.001	0.001	0.08	0.001	0.08
18:4 n3	0.20	0.19	0.001	0.001	0.21	0.20	0.002	0.002	0.20	0.002	0.20
20:3 n3	0.01	0.01	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
20:4 n3	0.05	0.05	0.001	0.001	0.05	0.06	0.001	0.001	0.05	0.001	0.06
20:5 n3	0.73	0.69	0.014	0.013	0.76	0.76	0.029	0.029	0.73	0.021	0.76
21:5 n3	0.03	0.02	<0.001	<0.001	0.02	0.02	<0.001	n.a.	0.02	<0.001	0.02
22:5 n3	0.08	0.06	<0.001	n.a.	0.08	0.08	0.001	0.001	0.07	0.001	0.08
22:6 n3	1.36	1.29	0.043	0.043	1.46	1.45	0.071	0.070	1.39	0.057	1.45
SFA	1.94	1.85	0.070	0.073	2.14	2.13	0.079	0.079	2.01	0.075	2.09
MUFA	3.57	3.38	0.043	0.044	3.76	3.73	0.038	0.041	3.61	0.042	3.65
PUFA	2.76	2.59	0.06	0.06	2.92	2.90	0.11	0.11	2.79	0.087	2.88
Total FA	8.27	7.82	0.177	0.180	8.83	8.76	0.227	0.229	8.42	0.203	8.62

Table C6. Acid hydrolysis Result for Sample B **(g/100g sample)**

Fatty Acid	B1				B2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 239	GC 240	GC 250	GC 251	GC 371	GC 372	GC 382	GC 383			
8:0	n.a.	n.a.	0.002	0.003	n.a.	n.a.	0.002	0.003	n.a.	0.003	<0.01
10:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12:0	0.00	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
14:0	0.02	0.02	0.004	0.004	0.03	0.03	0.003	0.004	0.03	0.004	0.03
16:0	0.37	0.39	0.058	0.051	0.42	0.39	0.056	0.060	0.39	0.056	0.45
18:0	0.09	0.10	0.017	0.015	0.10	0.09	0.023	0.023	0.09	0.019	0.11
20:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
22:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16:1 n7	0.02	0.02	0.004	0.004	0.03	0.03	0.005	0.005	0.03	0.004	0.03
18:1 n9	0.23	0.24	0.035	0.032	0.26	0.23	0.037	0.040	0.24	0.036	0.28
20:1 n9	0.04	0.04	0.005	0.005	0.04	0.04	0.007	0.006	0.04	0.006	0.05
22:1 n11	0.01	0.01	0.001	0.001	0.01	0.01	0.002	0.002	0.01	0.002	0.02
24:1 n9	0.02	0.02	0.004	0.004	0.02	0.01	0.006	0.007	0.02	0.005	0.02
16:2 n4	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	n.a.	0.000	n.a.	<0.001	<0.01
16:3 n4	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	n.a.	n.a.	n.a.	<0.001	<0.01
18:2 n6	0.02	0.02	0.004	0.003	0.03	0.02	0.004	0.004	0.02	0.004	0.03
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20:2 n6	<0.01	<0.01	0.001	0.001	<0.01	<0.01	0.001	0.001	<0.01	0.001	<0.01
20:3 n6	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	<0.001	n.a.	n.a.	<0.001	<0.01
20:4 n6	0.08	0.08	0.021	0.019	0.09	0.09	0.019	0.020	0.09	0.020	0.11
22:4 n6	n.a.	n.a.	0.001	<0.001	<0.01	<0.01	0.001	0.001	n.a.	0.001	<0.01
18:3 n3	0.01	0.01	0.002	0.001	0.01	0.01	0.001	0.001	0.01	0.001	0.01
18:4 n3	0.01	0.01	0.002	0.002	0.01	0.01	0.002	0.002	0.01	0.002	0.01
20:3 n3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20:4 n3	0.01	0.01	0.002	n.a.	0.01	0.01	0.002	0.002	0.01	0.002	0.01
20:5 n3	0.21	0.22	0.063	0.058	0.27	0.25	0.050	0.056	0.24	0.057	0.30
21:5 n3	<0.01	<0.01	<0.001	<0.001	n.a.	<0.01	<0.001	<0.001	<0.01	<0.001	<0.01
22:5 n3	0.04	0.04	0.006	0.006	0.03	0.02	0.005	0.006	0.03	0.006	0.04
22:6 n3	0.58	0.59	0.169	0.156	0.69	0.64	0.140	0.153	0.63	0.154	0.78
SFA	0.49	0.51	0.081	0.073	0.55	0.50	0.084	0.091	0.51	0.082	0.59
MUFA	0.33	0.34	0.050	0.045	0.37	0.33	0.057	0.061	0.34	0.053	0.40
PUFA	0.96	0.99	0.27	0.25	1.14	1.05	0.22	0.25	1.04	0.247	1.28
Total FA	1.78	1.84	0.402	0.366	2.05	1.89	0.365	0.398	1.89	0.382	2.27

Table C7. Acid hydrolysis Result for Sample C (g/100g sample)

Fatty Acid	C1				C2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 304	GC 305	GC 313	GC 314	GC373	GC 374	GC 384	GC 385			
8:0	n.a.	n.a.	0.001	0.002	n.a.	n.a.	0.001	0.003	n.a.	0.002	<0.01
10:0	n.a.	n.a.	<0.001	0.001	n.a.	n.a.	n.a.	n.a.	n,a,	<0.001	<0.01
12:0	0.01	0.02	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
14:0	1.09	1.14	0.003	0.003	1.10	1.11	0.002	0.002	1.11	0.002	1.11
16:0	3.98	4.18	0.049	0.049	4.03	4.08	0.031	0.034	4.07	0.041	4.11
18:0	1.01	1.06	0.016	0.016	1.02	1.03	0.012	0.014	1.03	0.015	1.05
20:0	0.12	0.11	n.a.	n.a.	0.11	0.11	n.a.	n.a.	0.11	n.a.	0.11
22:0	0.06	0.05	n.a.	n.a.	0.06	0.06	n.a.	n.a.	0.06	n.a.	0.06
16:1 n7	1.08	1.13	0.003	0.003	1.09	1.10	0.002	0.002	1.10	0.002	1.10
18:1 n9	13.87	14.71	0.034	0.034	14.09	14.23	0.025	0.027	14.23	0.030	14.26
20:1 n9	1.81	1.88	0.002	0.002	1.86	1.84	0.001	0.001	1.85	0.002	1.85
22:1 n11	1.16	1.19	<0.001	<0.001	1.17	1.18	<0.001	<0.001	1.17	<0.001	1.17
24:1 n9	0.19	0.19	0.004	0.004	0.18	0.18	0.003	0.003	0.19	0.004	0.19
16:2 n4	0.11	0.11	<0.001	<0.001	0.11	0.11	<0.001	<0.001	0.11	<0.001	0.11
16:3 n4	0.07	0.08	<0.001	<0.001	0.07	0.07	n.a.	n.a.	0.07	<0.001	0.07
18:2 n6	4.10	4.32	0.011	0.011	4.17	4.21	0.007	0.008	4.20	0.009	4.21
18:3 n6	0.04	0.04	n.a.	n.a.	0.04	0.04	n.a.	n.a.	0.04	n.a.	0.04
20:2 n6	0.38	0.40	0.001	0.001	0.39	0.39	0.001	0.001	0.39	0.001	0.39
20:3 n6	0.07	0.08	0.001	0.001	0.08	0.08	<0.001	<0.001	0.08	<0.001	0.08
20:4 n6	0.12	0.12	n.a.	0.006	0.13	0.13	0.004	0.004	0.12	0.005	0.13
22:4 n6	0.03	0.03	n.a.	n.a.	0.03	0.03	n.a.	n.a.	0.03	n.a.	0.03
18:3 n3	1.74	1.83	0.011	0.011	1.76	1.78	0.007	0.007	1.78	0.009	1.79
18:4 n3	0.26	0.28	0.001	0.001	0.27	0.27	0.001	0.001	0.27	0.001	0.27
20:3 n3	0.20	0.20	0.001	0.001	0.20	0.21	<0.001	<0.001	0.20	0.001	0.20
20:4 n3	0.40	0.41	0.003	0.003	0.40	0.41	0.002	0.002	0.40	0.003	0.41
20:5 n3	1.33	1.39	0.043	0.042	1.37	1.38	0.028	0.028	1.37	0.035	1.40
21:5 n3	0.09	0.09	<0.001	<0.001	0.09	0.09	n.a.	<0.001	0.09	<0.001	0.09
22:5 n3	0.79	0.82	0.011	0.010	0.80	0.81	0.007	0.008	0.81	0.009	0.81
22:6 n3	2.15	2.21	0.147	0.143	2.23	2.23	0.098	0.100	2.21	0.122	2.33
SFA	6.26	6.56	0.070	0.071	6.33	6.40	0.046	0.053	6.39	0.060	6.45
MUFA	18.10	19.11	0.044	0.043	18.40	18.53	0.031	0.033	18.53	0.038	18.57
PUFA	11.87	12.42	0.23	0.23	12.15	12.23	0.16	0.16	12.17	0.194	12.36
Total FA	36.23	38.09	0.345	0.344	36.87	37.16	0.233	0.246	37.09	0.292	37.38

Table C8. Acid hydrolysis Result of Sample D **(g/100g sample)**

Fatty Acid	D1				D2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 394	GC 395	GC 403	GC 404	GC 375	GC 376	GC 386	GC 387			
8:0	0.01	0.01	0.002	0.003	n.a.	n.a.	0.001	0.002	0.01	0.002	0.01
10:0	<0.01	<0.01	<0.001	0.001	n.a.	n.a.	n.a.	<0.001	<0.01	0.001	<0.01
12:0	<0.01	<0.01	<0.001	0.001	<0.01	<0.01	n.a.	<0.001	<0.01	<0.001	<0.01
14:0	0.44	0.43	0.035	0.040	0.45	0.46	0.035	0.032	0.45	0.036	0.48
16:0	2.22	2.17	0.123	0.137	2.23	2.29	0.139	0.107	2.23	0.127	2.35
18:0	0.24	0.23	0.014	0.015	0.23	0.24	0.016	0.012	0.23	0.014	0.25
20:0	0.00	n.a.	n.a.	n.a.	0.00	0.00	n.a.	n.a.	<0.01	n.a.	<0.01
22:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16:1 n7	0.38	0.37	0.025	0.028	0.37	0.38	0.024	0.022	0.38	0.025	0.40
18:1 n9	1.60	1.57	0.097	0.118	1.55	1.62	0.091	0.086	1.59	0.098	1.68
20:1 n9	0.24	0.24	0.016	0.018	0.23	0.24	0.013	0.012	0.24	0.015	0.25
22:1 n11	0.10	0.10	0.007	0.008	0.10	0.10	0.005	0.005	0.10	0.006	0.11
24:1 n9	0.12	0.12	0.015	0.017	0.12	0.12	0.012	0.012	0.12	0.014	0.14
16:2 n4	0.01	0.02	0.001	0.001	0.02	0.02	0.001	0.001	0.02	0.001	0.02
16:3 n4	0.02	0.02	0.001	0.001	0.02	0.02	0.001	0.001	0.02	0.001	0.02
18:2 n6	0.12	0.12	0.008	0.009	0.11	0.11	0.007	0.006	0.11	0.008	0.12
18:3 n6	0.00	0.00	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	n.a.	<0.01
20:2 n6	0.01	0.01	<0.001	0.001	0.01	0.01	n.a.	<0.001	0.01	<0.001	0.01
20:3 n6	0.01	0.01	0.001	0.001	0.01	0.01	n.a.	n.a.	0.01	0.001	0.01
20:4 n6	0.07	0.07	0.004	0.005	0.06	0.06	0.004	0.003	0.06	0.004	0.07
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.09	0.09	0.007	0.008	0.08	0.08	0.006	0.006	0.09	0.007	0.09
18:4 n3	0.10	0.09	0.008	0.010	0.08	0.09	0.008	0.007	0.09	0.008	0.10
20:3 n3	0.01	0.00	n.a.	n.a.	<0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
20:4 n3	0.06	0.06	0.005	0.005	0.06	0.06	0.003	0.003	0.06	0.004	0.06
20:5 n3	1.19	1.16	0.077	0.089	1.01	1.03	0.076	0.070	1.10	0.078	1.18
21:5 n3	0.01	0.01	n.a.	0.001	0.01	0.01	n.a.	<0.001	0.01	0.001	0.01
22:5 n3	0.15	0.15	0.008	0.010	0.09	0.09	0.006	n.a.	0.12	0.008	0.13
22:6 n3	2.95	2.91	0.183	0.205	2.43	2.47	0.174	0.159	2.69	0.180	2.87
SFA	2.91	2.84	0.175	0.197	2.91	3.00	0.191	0.154	2.91	0.179	3.09
MUFA	2.44	2.40	0.159	0.188	2.37	2.47	0.146	0.137	2.42	0.158	2.58
PUFA	4.81	4.72	0.30	0.35	3.99	4.08	0.29	0.26	4.40	0.298	4.70
Total FA	10.16	9.97	0.639	0.731	9.28	9.54	0.623	0.547	9.74	0.635	10.37

Table C9. Acid hydrolysis Result for Sample F (g/100g sample)

Fatty Acid	F1				F2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 241	GC 242	GC 252	GC 253	GC 351	GC 352	GC 360	GC 361			
8:0	n.a.	n.a.	0.002	0.004	n.a.	n.a.	0.003	0.002	n.a.	0.003	<0.01
10:0	n.a.	n.a.	<0.001	0.001	n.a.	n.a.	0.001	<0.001	n.a.	0.001	<0.01
12:0	0.03	0.03	<0.001	<0.001	0.04	0.03	0.001	0.001	0.03	<0.001	0.03
14:0	1.71	1.77	0.012	0.012	1.74	1.71	0.030	0.037	1.73	0.023	1.76
16:0	3.34	3.46	0.063	0.067	3.36	3.30	0.157	0.199	3.37	0.121	3.49
18:0	0.22	0.23	0.003	0.004	0.23	0.22	0.008	0.009	0.22	0.006	0.23
20:0	0.01	0.01	n.a.	n.a.	0.01	0.01	<0.001	n.a.	0.01	<0.001	0.01
22:0	0.01	0.01	n.a.	n.a.	0.01	0.01	0.001	0.001	0.01	0.001	0.01
16:1 n7	1.00	1.03	0.007	0.007	1.01	0.99	0.017	0.022	1.01	0.013	1.02
18:1 n9	2.87	2.96	0.032	0.033	2.93	2.88	0.080	0.106	2.91	0.063	2.97
20:1 n9	0.20	0.19	0.001	0.002	0.21	0.21	0.005	0.006	0.20	0.003	0.21
22:1 n11	0.14	0.15	0.001	0.002	0.12	0.11	0.006	0.007	0.13	0.004	0.13
24:1 n9	0.03	0.03	<0.001	<0.001	0.02	0.03	0.001	0.002	0.03	0.001	0.03
16:2 n4	0.11	0.12	0.001	0.001	0.11	0.11	0.002	0.002	0.11	0.001	0.11
16:3 n4	0.05	0.06	0.001	0.001	0.05	0.05	0.001	0.002	0.05	0.001	0.06
18:2 n6	0.23	0.24	0.004	0.004	0.24	0.23	0.010	0.012	0.23	0.008	0.24
18:3 n6	0.02	0.03	n.a.	<0.001	0.02	0.02	<0.001	0.001	0.02	<0.001	0.02
20:2 n6	0.01	0.01	n.a.	n.a.	0.01	0.01	<0.001	0.001	0.01	0.001	0.01
20:3 n6	0.02	0.01	n.a.	<0.001	0.01	0.01	<0.001	<0.001	0.01	<0.001	0.01
20:4 n6	0.05	0.05	0.001	0.001	0.05	0.05	0.003	0.003	0.05	0.002	0.05
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.14	0.15	0.003	0.003	0.14	0.14	0.007	0.008	0.14	0.005	0.15
18:4 n3	0.28	0.28	0.005	0.005	0.29	0.28	0.008	0.009	0.29	0.007	0.29
20:3 n3	0.01	0.01	n.a.	n.a.	0.01	0.01	<0.001	<0.001	0.01	<0.001	0.01
20:4 n3	0.07	0.08	0.002	0.002	0.08	0.08	0.005	0.006	0.08	0.003	0.08
20:5 n3	1.87	1.91	0.075	0.068	1.87	1.83	0.116	0.142	1.87	0.100	1.97
21:5 n3	0.05	0.06	0.002	0.002	0.05	0.05	n.a.	n.a.	0.06	0.002	0.06
22:5 n3	0.06	0.06	0.001	0.001	0.05	0.06	n.a.	0.003	0.06	0.002	0.06
22:6 n3	1.08	1.09	0.044	0.042	1.05	1.06	0.063	0.078	1.07	0.057	1.13
SFA	5.33	5.52	0.082	0.087	5.39	5.28	0.200	0.249	5.38	0.155	5.53
MUFA	4.24	4.36	0.042	0.044	4.30	4.21	0.109	0.142	4.28	0.084	4.36
PUFA	4.07	4.15	0.14	0.13	4.04	4.01	0.21	0.27	4.07	0.188	4.25
Total FA	13.63	14.03	0.264	0.261	13.72	13.50	0.523	0.659	13.72	0.427	14.15

Table C10. Acid hydrolysis Result for Sample G (g/100g sample)

Fatty Acid	G1				G2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 243	GC 244	GC 254	GC 255	GC 353	GC 354	GC 362	GC 363			
8:0	n.a.	n.a.	0.003	0.003	n.a.	n.a.	0.002	0.003	n.a.	0.003	<0.01
10:0	n.a.	n.a.	0.001	0.000	n.a.	n.a.	<0.001	0.001	n.a.	<0.001	<0.01
12:0	<0.01	<0.01	<0.001	<0.001	<0.01	<0.01	<0.001	<0.001	<0.01	<0.001	<0.01
14:0	0.20	0.20	0.010	0.010	0.21	0.20	0.012	0.013	0.20	0.011	0.21
16:0	0.92	0.89	0.059	0.056	0.92	0.90	0.069	0.074	0.91	0.065	0.97
18:0	0.17	0.17	0.012	0.011	0.17	0.17	0.016	0.017	0.17	0.014	0.18
20:0	0.01	0.01	n.a.	n.a.	0.01	<0.01	<0.001	<0.001	0.01	<0.001	0.01
22:0	<0.01	<0.01	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	n.a.	<0.01
16:1 n7	0.26	0.25	0.012	0.011	0.26	0.25	0.013	0.015	0.26	0.013	0.27
18:1 n9	0.97	0.94	0.048	0.046	0.99	0.95	0.058	0.061	0.96	0.054	1.01
20:1 n9	0.70	0.68	0.016	0.016	0.70	0.70	0.023	0.023	0.70	0.019	0.72
22:1 n11	0.75	0.74	0.008	0.008	0.74	0.73	0.013	0.013	0.74	0.011	0.75
24:1 n9	0.13	0.13	0.006	0.006	0.12	0.12	0.011	0.011	0.12	0.009	0.13
16:2 n4	0.01	0.01	0.001	0.001	0.02	0.01	0.001	0.001	0.01	0.001	0.02
16:3 n4	0.01	0.01	0.001	0.001	0.01	0.01	0.001	0.001	0.01	0.001	0.01
18:2 n6	0.07	0.07	0.004	0.004	0.07	0.07	0.005	0.005	0.07	0.005	0.07
18:3 n6	n.a.	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
20:2 n6	0.01	0.01	<0.001	<0.001	0.01	0.01	0.001	0.001	0.01	0.001	0.01
20:3 n6	<0.01	<0.01	<0.001	n.a.	<0.01	<0.01	n.a.	n.a.	<0.01	<0.001	<0.01
20:4 n6	0.04	0.04	0.004	0.004	0.04	0.04	0.004	0.004	0.04	0.004	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.03	0.03	0.002	0.002	0.03	0.03	0.002	0.002	0.03	0.002	0.03
18:4 n3	0.07	0.06	0.005	0.005	0.07	0.07	0.005	0.006	0.07	0.005	0.07
20:3 n3	<0.01	<0.01	<0.001	n.a.	n.a.	0.01	n.a.	n.a.	<0.01	n.a.	<0.01
20:4 n3	0.03	0.02	0.001	0.002	0.03	0.02	0.002	0.002	0.02	0.002	0.03
20:5 n3	0.38	0.37	0.036	0.035	0.40	0.39	0.037	0.040	0.39	0.037	0.42
21:5 n3	0.01	0.01	0.001	0.001	0.01	0.01	0.001	0.001	0.01	0.001	0.01
22:5 n3	0.06	0.06	0.004	0.003	0.04	0.03	0.003	0.003	0.05	0.003	0.05
22:6 n3	0.62	0.61	0.067	0.065	0.62	0.61	0.067	0.073	0.61	0.068	0.68
SFA	1.31	1.27	0.085	0.081	1.31	1.28	0.100	0.108	1.29	0.094	1.38
MUFA	2.81	2.74	0.091	0.087	2.81	2.75	0.119	0.124	2.78	0.105	2.88
PUFA	1.34	1.31	0.13	0.12	1.34	1.31	0.13	0.14	1.33	0.128	1.45
Total FA	5.46	5.32	0.302	0.291	5.46	5.34	0.346	0.370	5.40	0.327	5.72

Table C11. Acid hydrolysis Result for Sample H (g/100g sample)

Fatty Acid	H1				H2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 245	GC 246	GC 256	GC 257	GC 355	GC 356	GC 364	GC 365			
8:0	n.a.	n.a.	0.002	0.003	n.a.	n.a.	0.003	0.003	n.a.	0.003	<0.01
10:0	0.01	0.01	<0.001	0.001	n.a.	n.a.	0.001	<0.001	0.01	<0.001	0.01
12:0	0.01	0.01	<0.001	<0.001	0.01	0.01	<0.001	<0.001	0.01	<0.001	0.01
14:0	0.68	0.68	0.011	0.011	0.70	0.69	0.018	0.018	0.69	0.015	0.70
16:0	1.62	1.61	0.044	0.040	1.63	1.63	0.066	0.066	1.62	0.054	1.68
18:0	0.20	0.19	0.007	0.006	0.19	0.20	0.009	0.009	0.20	0.008	0.20
20:0	0.01	0.01	<0.001	n.a.	0.01	0.01	<0.001	<0.001	0.01	<0.001	0.01
22:0	n.a.	0.01	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
16:1 n7	0.39	0.38	0.005	0.005	0.39	0.39	0.008	0.008	0.39	0.007	0.39
18:1 n9	1.44	1.43	0.023	0.021	1.44	1.46	0.037	0.037	1.44	0.029	1.47
20:1 n9	1.13	1.13	0.006	0.005	1.14	1.14	0.010	0.010	1.13	0.008	1.14
22:1 n11	1.66	1.66	0.005	0.004	1.66	1.65	0.009	0.009	1.66	0.007	1.66
24:1 n9	0.15	0.15	0.003	0.003	0.14	0.14	n.a.	n.a.	0.15	0.003	0.15
16:2 n4	0.03	0.03	<0.001	<0.001	0.03	0.03	0.001	0.001	0.03	<0.001	0.03
16:3 n4	0.02	0.02	<0.001	<0.001	0.02	0.02	<0.001	<0.001	0.02	<0.001	0.02
18:2 n6	0.12	0.12	0.002	0.002	0.12	0.12	0.003	0.003	0.12	0.002	0.12
18:3 n6	0.01	<0.01	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
20:2 n6	0.02	0.02	<0.001	n.a.	0.02	0.02	<0.001	<0.001	0.02	<0.001	0.02
20:3 n6	0.01	0.01	n.a.	<0.001	0.01	0.01	n.a.	n.a.	0.01	<0.001	0.01
20:4 n6	0.05	0.04	0.002	0.001	0.05	0.05	n.a.	n.a.	0.05	0.002	0.05
22:4 n6	n.a.	n.a.	n.a.	n.a.	0.00	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.08	0.07	0.001	0.001	0.08	0.07	0.002	0.002	0.08	0.001	0.08
18:4 n3	0.14	0.14	0.002	0.002	0.14	0.14	n.a.	n.a.	0.14	0.002	0.14
20:3 n3	0.01	<0.01	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.01	n.a.	0.01
20:4 n3	0.05	0.05	0.001	0.001	0.04	0.05	n.a.	n.a.	0.05	0.001	0.05
20:5 n3	0.60	0.60	n.a.	0.023	0.61	0.60	0.030	0.030	0.60	0.027	0.63
21:5 n3	0.02	0.02	<0.001	n.a.	0.02	0.02	n.a.	n.a.	0.02	<0.001	0.02
22:5 n3	0.10	0.10	0.002	0.002	0.06	0.07	n.a.	n.a.	0.08	0.002	0.08
22:6 n3	1.13	1.12	0.062	0.055	1.13	1.12	0.065	0.064	1.13	0.062	1.19
SFA	2.53	2.52	0.065	0.061	2.55	2.54	0.098	0.096	2.54	0.080	2.62
MUFA	4.77	4.75	0.042	0.038	4.77	4.78	0.065	0.065	4.77	0.052	4.82
PUFA	2.36	2.34	0.07	0.09	2.33	2.34	0.10	0.10	2.34	0.090	2.43
Total FA	9.67	9.61	0.180	0.186	9.65	9.66	0.263	0.260	9.65	0.222	9.87

Table C12. Acid hydrolysis Result for Sample I (g/100g sample)

Fatty Acid	I1				I2				Average		
	extract 1		residue 1		extract 2		residue 2		extract	residue	sum
	GC 282	GC 283	GC 295	GC 296	GC 357	GC 358	GC 366	GC 367			
8:0	n.a.	n.a.	0.004	0.003	n.a.	n.a.	0.001	0.001	n.a.	0.002	<0.01
10:0	<0.01	<0.01	0.001	0.001	n.a.	n.a.	n.a.	<0.001	<0.01	0.001	<0.01
12:0	0.03	0.03	0.001	0.001	0.03	0.03	0.001	<0.001	0.03	0.001	0.03
14:0	1.23	1.26	0.014	0.015	1.33	1.39	0.012	0.012	1.30	0.013	1.32
16:0	2.93	2.97	0.082	0.092	3.12	3.23	0.070	0.069	3.06	0.078	3.14
18:0	0.17	0.17	0.004	0.004	0.18	0.18	0.003	0.003	0.17	0.004	0.18
20:0	<0.01	<0.01	n.a.	n.a.	0.00	0.01	n.a.	n.a.	<0.01	n.a.	<0.01
22:0	0.01	<0.01	<0.001	<0.001	n.a.	0.01	<0.001	<0.001	<0.01	<0.001	<0.01
16:1 n7	0.51	0.52	0.007	0.008	0.53	0.55	0.006	0.006	0.53	0.007	0.53
18:1 n9	2.43	2.46	0.048	0.053	2.56	2.64	0.039	0.039	2.52	0.045	2.57
20:1 n9	0.16	0.17	0.003	0.003	0.15	0.18	0.002	0.002	0.17	0.002	0.17
22:1 n11	0.10	0.09	0.003	0.003	0.10	0.10	0.002	0.002	0.10	0.002	0.10
24:1 n9	0.01	0.01	0.001	0.001	0.01	0.01	<0.001	n.a.	0.01	0.001	0.01
16:2 n4	0.07	0.07	0.001	0.001	0.08	0.08	0.001	0.001	0.08	0.001	0.08
16:3 n4	0.06	0.06	0.001	0.001	0.06	0.06	0.001	0.001	0.06	0.001	0.06
18:2 n6	0.24	0.24	0.005	0.006	0.25	0.26	0.005	0.005	0.25	0.005	0.25
18:3 n6	0.02	0.02	n.a.	<0.001	0.02	0.02	<0.001	<0.001	0.02	<0.001	0.02
20:2 n6	0.01	0.01	<0.001	<0.001	0.01	0.02	<0.001	<0.001	0.01	<0.001	0.01
20:3 n6	0.01	0.02	<0.001	<0.001	0.01	0.01	<0.001	n.a.	0.01	<0.001	0.01
20:4 n6	0.04	0.04	0.001	0.001	0.04	0.04	0.001	0.001	0.04	0.001	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.38	0.38	0.009	0.010	0.39	0.41	0.008	0.008	0.39	0.008	0.40
18:4 n3	0.73	0.74	0.011	0.013	0.76	0.78	0.011	0.011	0.75	0.011	0.76
20:3 n3	0.03	0.03	0.001	0.001	0.03	0.04	0.000	0.001	0.03	0.001	0.03
20:4 n3	0.10	0.09	0.003	0.003	0.09	0.10	0.002	0.002	0.09	0.003	0.10
20:5 n3	2.63	2.63	0.073	0.083	2.62	2.70	0.070	0.068	2.65	0.073	2.72
21:5 n3	0.10	0.10	0.003	0.003	0.10	0.10	0.003	0.003	0.10	0.003	0.10
22:5 n3	0.08	0.07	0.001	0.002	0.07	0.07	0.001	0.002	0.07	0.002	0.07
22:6 n3	1.45	1.44	0.058	0.065	1.40	1.43	0.052	0.052	1.43	0.057	1.49
SFA	4.37	4.44	0.106	0.115	4.66	4.85	0.087	0.086	4.58	0.099	4.68
MUFA	3.21	3.24	0.061	0.068	3.35	3.49	0.049	0.048	3.32	0.057	3.38
PUFA	5.92	5.93	0.17	0.19	5.94	6.11	0.15	0.15	5.98	0.166	6.14
Total FA	13.51	13.61	0.335	0.372	13.95	14.45	0.292	0.288	13.88	0.322	14.20

Appendix D. Data for Bligh and Dyer method

Table D1. Calculation for Bligh and Dyer method (sample A, B)

Sample Code	A		A		B		B	
sample weight(g): W0	2.521		2.561		2.588		2.538	
volume of water/methanol phase(ml): V1	60.5		60.8		60.6		60.0	
volume of chloroform phase(ml): V2	48.6		50.3		56.4		54.7	
weight of tray(g): W1	1.9791		1.9814		1.9843		1.9649	
weight of tray with fat (g): W2	2.0906		2.0901		2.0154		1.9954	
Extract for GC(ml)	3	3	3	3	3	3	3	3
GC No.	446	447	492	493	448	449	494	495
Water phase for GC(ml)	3	3	3	3	3	3	3	3
GC No.	463	464	503	504	465	466	505	506
residue(g): W3	2.6704		2.7063		3.9204		3.3427	
Residue for GC(g): W4, W5	0.2008	0.1918	0.1927	0.1936	0.1993	0.1943	0.2017	0.2027
GC No.	454	455	511	512	456	457	513	514
Extracted lipid (g/100g sample)[1]:	10.75		10.67		3.39		3.29	
Average of extracted lipid (g/100g sample)	10.71				3.34			
Relative error, %	0.68				3.06			
Total FA in chloroform phase(g/100ml): M1	0.4623	0.4660	0.4026	0.4093	0.1029	0.0992	0.0913	0.0926
Total FA extracted in chloroform (g/100g sample): M2=M1*V2/ W0	8.91	8.98	7.91	8.04	2.24	2.16	1.97	2.00
Average of total FA extracted (g/100g)	8.46				2.09			
C.V. of total FA extracted, %	6.69				6.30			
Total FA left in water/methanol phase(g/100ml): M3	0.0011	0.0012	0.0011	0.0011	0.0006	0.0006	0.0006	0.0006
Total FA left in water/methanol (g/100g sample): M4=M3*V1/ W0	0.020	0.028	0.026	0.027	0.015	0.014	0.013	0.014
Average of total FA left in water/methanol (g/100g sample)	0.027				0.014			
Total FA left in residue(g/100g residue): M5	0.4254	0.4404	0.4560	0.5277	0.1405	0.1309	0.1795	0.1759
Total FA in residue(g/100g sample): M6=M5* W3/W0	0.451	0.467	0.482	0.558	0.213	0.198	0.236	0.232
Average of total FA left in residue(g/100g sample)	0.489				0.220			
sum FA(in chloroform, water and residue) (g/100g sample): M2+M4+M6	9.39	9.48	8.42	8.62	2.47	2.37	2.22	2.24
Average of total FA (g/100g sample)	8.98				2.33			
C.V. of total FA, %	5.97				5.08			

1: Extracted lipid= $\frac{(W2-W1)\times V2}{W0\times 20}\times 100\%$

2. M1, M3, M5 were calculated by using the original GC profile. By using the same formula of M2 and M4 and M6, The original GC profiles (g FA/ 100ml chloroform or g FA/ 100ml water or g FA/ 100g residue) were converted into GC profile (g FA/ 100g samples).

3. same calculation as in Table D2, D3 and D4.

Table D2. Calculation for Bligh and Dyer method (sample C, D)

Sample Code	C		C		D		D	
sample weight(g): W0	2.577		2.507		2.551		2.564	
volume of water/methanol phase(ml): V1	64.7		65.2		62.7		65.7	
volume of chloroform phase(ml): V2	53		54		52.1		51	
weight of tray(g): W1	1.9765		1.9815		1.9774		1.9816	
weight of tray with fat (g): W2	2.3391		2.3293		2.1357		2.1455	
Extract for GC(ml)	3	3	1.5	1.5	1.5	1.5	3	3
GC No.	450	451	496	497	452	453	498	499
Water phase for GC(ml)	3	3	3	3	3	3	3	3
GC No.	467	468	507	508	469	470	509	510
residue(g): W3	1.7835		1.485		2.8354		2.4797	
Residue for GC(g): W4, W5	0.1942	0.1966	0.1966	0.2003	0.1938	0.1981	0.1957	0.2031
GC No.	458	459	515	516	460	461	517	518
Extracted lipid (g/100g sample)[1]:	37.29		37.46		16.17		16.30	
Average of extracted lipid (g/100g sample)	37.37				16.23			
Relative error, %	-0.46				-0.83			
Total FA in chloroform phase(g/100ml): M1	1.6178	1.7445	1.5299	1.5498	0.5413	0.5231	0.5006	0.4918
Total FA extracted in chloroform (g/100g sample): M2=M1*V2/ W0	33.27	35.88	32.95	33.38	11.06	10.68	9.96	9.78
Average of total FA extracted (g/100g)	33.87				10.37			
C.V. of total FA extracted, %	3.98				5.80			
Total FA left in water/methanol phase(g/100ml): M3	0.0004	0.0005	0.0008	0.0008	0.0021	0.0021	0.0009	0.0008
Total FA left in water/methanol (g/100g sample): M4=M3*V1/ W0	0.011	0.013	0.021	0.021	0.050	0.050	0.023	0.023
Average of total FA left in water/methanol (g/100g sample)	0.017				0.036			
Total FA left in residue(g/100g residue): M5	0.9327	0.5731	1.0817	1.1382	0.6261	0.5920	0.7253	0.5417
Total FA in residue(g/100g sample): M6=M5* W3/W0	0.646	0.397	0.641	0.674	0.696	0.658	0.701	0.524
Average of total FA left in residue(g/100g sample)	0.589				0.645			
sum FA(in chloroform, water and residue) (g/100g sample): M2+M4+M6	33.93	36.29	33.62	34.08	11.80	11.39	10.68	10.33
Average of total FA (g/100g sample)	34.48				11.05			
C.V. of total FA, %	3.54				6.05			

Table D3. Calculation for Bligh and Dyer method (sample F,G)

Sample Code	F		F		G		G	
sample weight(g): W0	2.555		2.565		2.566		2.549	
volume of water/methanol phase(ml): V1	60.5		59.1		64.3		63.8	
volume of chloroform phase(ml): V2	46		46.2		55		57.5	
weight of tray(g): W1	1.9645		1.9725		1.9703		1.9799	
weight of tray with fat (g): W2	2.1807		2.1844		2.042		2.048	
Extract for GC(ml)	3	3	3	3	3	3	3	3
GC No.	420	421	520	521	422	423	522	523
Water phase for GC(ml)	3	3	3	3	3	3	3	3
GC No.	429	430	529	530	431	432	531	532
residue(g): W3	3.082		2.5638		3.1376		3.2154	
Residue for GC(g): W4, W5	0.1978	0.1929	0.2037	0.2089	0.21	0.2087	0.202	0.2044
GC No.	438	439	537	538	440	441	539	540
Extracted lipid (g/100g sample)[1]:	19.46		19.08		7.68		7.68	
Average of extracted lipid (g/100g sample)	19.27				7.68			
Relative error, %	1.97				0.04			
Total FA in chloroform phase(g/100ml): M1	0.7119	0.7271	0.6771	0.6742	0.2299	0.2286	0.2137	0.2101
Total FA extracted in chloroform (g/100g sample): M2=M1*V2/ W0	12.82	13.09	12.20	12.14	4.93	4.90	4.82	4.74
Average of total FA extracted (g/100g)	12.56				4.85			
C.V. of total FA extracted, %	3.72				1.76			
Total FA left in water/methanol phase(g/100ml): M3	0.0084	0.0083	0.0170	0.0167	0.0023	0.0022	0.0018	0.0018
Total FA left in water/methanol (g/100g sample): M4=M3*V1/ W0	0.200	0.198	0.391	0.386	0.059	0.056	0.046	0.045
Average of total FA left in water/methanol (g/100g sample)	0.293				0.051			
Total FA left in residue(g/100g residue): M5	0.5188	0.4985	0.5115	0.5336	0.4259	0.4962	0.3156	0.3235
Total FA in residue(g/100g sample): M6=M5* W3/W0	0.626	0.601	0.511	0.533	0.521	0.607	0.398	0.408
Average of total FA left in residue(g/100g sample)	0.568				0.483			
sum FA(in chloroform, water and residue) (g/100g sample): M2+M4+M6	13.64	13.89	13.10	13.06	5.51	5.56	5.26	5.19
Average of total FA (g/100g sample)	13.42				5.38			
C.V. of total FA, %	3.05				3.37			

Table D4. Calculation for Bligh and Dyer method (sample H, I)								
Sample Code	H		H		I		I	
sample weight(g): W0	2.597		2.512		2.52		2.518	
volume of water/methanol phase(ml): V1	65.5		64.8		64.0		65.5	
volume of chloroform phase(ml): V2	54.0		55.2		52.6		54.0	
weight of tray(g): W1	1.9663		1.9732		1.9809		1.9759	
weight of tray with fat (g): W2	2.0895		2.0894		2.1497		2.1407	
Extract for GC(ml)	3	3	3	3	1.5	1.5	1.5	1.5
GC No.	424	425	524	525	480	481	526	527
Water phase for GC(ml)	3	3	3	3	3	3	3	3
GC No.	433	434	533	534	489	490	535	536
residue(g): W3	3.3558		2.6231		2.0731		2.1438	
Residue for GC(g): W4, W5	0.1935	0.1938	0.1951	0.1939	0.2001	0.2029	0.1982	0.2094
GC No.	442	443	541	542	484	485	543	544
Extracted lipid (g/100g sample)[1]:	12.81		12.77		17.62		17.67	
Average of extracted lipid (g/100g sample)	12.79				17.64			
Relative error, %	0.32				-0.31			
Total FA in chloroform phase(g/100ml): M1	0.4095	0.4192	0.3763	0.3774	0.6215	0.6311	0.5567	0.5510
Total FA extracted in chloroform (g/100g sample): M2=M1*V2/ W0	8.51	8.72	8.27	8.29	12.97	13.17	11.94	11.82
Average of total FA extracted (g/100g)	8.45				12.48			
C.V. of total FA extracted, %	2.49				5.59			
Total FA left in water/methanol phase(g/100ml): M3	0.0020	0.0020	0.0018	0.0018	0.0065	0.0067	0.0060	0.0058
Total FA left in water/methanol (g/100g sample): M4=M3*V1/ W0	0.051	0.051	0.047	0.046	0.164	0.169	0.156	0.150
Average of total FA left in water/methanol (g/100g sample)	0.049				0.160			
Total FA left in residue(g/100g residue): M5	0.4199	0.4815	0.4911	0.4753	0.7334	0.7871	0.4982	0.4766
Total FA in residue(g/100g sample): M6=M5* W3/W0	0.543	0.622	0.513	0.496	0.603	0.647	0.424	0.406
Average of total FA left in residue(g/100g sample)	0.543				0.520			
sum FA(in chloroform, water and residue) (g/100g sample): M2+M4+M6	9.11	9.39	8.83	8.84	13.74	13.99	12.52	12.37
Average of total FA (g/100g sample)	9.04				13.16			
C.V. of total FA, %	2.96				6.30			

Table D5. Bligh and Dyer Result of Sample A (g/100g sample)

FA	A1						A2						average			
	chloroform		water		Residue		chloroform		water		Residue		chloroform	Water	Residue	Sum
	GC 446	GC 447	GC 463	GC 464	GC 454	GC 455	GC 492	GC 493	GC 503	GC 504	GC 511	GC 512				
8:0	0.01	0.01	<0.001	0.001	0.002	0.012	0.01	0.01	0.001	0.001	n.a.	0.000	0.01	0.001	0.005	0.01
10:0	n.a.	n.a.	<0.001	<0.001	0.001	0.004	n.a.	n.a.	<0.001	<0.001	<0.001	0.001	n.a.	<0.001	0.002	<0.01
12:0	0.01	0.01	<0.001	n.a.	0.001	0.001	0.01	0.01	<0.001	<0.001	0.001	0.001	0.01	<0.001	0.001	0.01
14:0	0.58	0.59	0.006	0.006	0.031	0.032	0.50	0.51	0.006	0.006	0.030	0.037	0.55	0.006	0.033	0.58
16:0	1.39	1.42	0.012	0.012	0.090	0.091	1.21	1.22	0.012	0.012	0.090	0.103	1.31	0.012	0.093	1.42
18:0	0.15	0.15	0.004	0.004	0.025	0.025	0.13	0.13	0.003	0.003	0.026	0.028	0.14	0.003	0.026	0.17
20:0	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	n.a.	n.a.	<0.001	<0.001	0.01	n.a.	<0.001	0.01
22:0	n.a.	0.00	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	<0.01
16:1 n7	0.36	0.36	<0.001	<0.001	0.014	0.012	0.32	0.32	<0.001	<0.001	0.012	0.015	0.34	<0.001	0.013	0.35
18:1 n9	1.10	1.10	0.001	0.001	0.049	0.050	0.84	0.95	0.001	0.001	0.053	0.062	1.00	0.001	0.054	1.05
20:1 n9	0.94	0.94	<0.001	<0.001	0.029	0.029	0.85	0.85	<0.001	<0.001	0.036	0.041	0.89	<0.001	0.034	0.93
22:1 n11	1.24	1.23	<0.001	<0.001	0.039	0.038	1.17	1.16	<0.001	<0.001	0.050	0.056	1.20	<0.001	0.046	1.25
24:1 n9	0.10	0.10	n.a.	n.a.	0.010	0.010	0.10	0.10	<0.001	<0.001	0.012	0.013	0.10	<0.001	0.011	0.11
16:2 n4	0.03	0.03	n.a.	n.a.	0.001	0.001	0.03	0.03	n.a.	n.a.	0.001	0.001	0.03	n.a.	0.001	0.03
16:3 n4	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
18:2 n6	0.12	0.12	n.a.	n.a.	0.005	0.005	0.10	0.11	<0.001	n.a.	0.005	0.006	0.11	<0.001	0.005	0.12
18:3 n6	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	n.a.	n.a.	<0.001	n.a.	0.01	n.a.	<0.001	0.01
20:2 n6	0.01	0.01	n.a.	n.a.	0.001	0.000	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	n.a.	0.001	0.02
20:3 n6	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	n.a.	n.a.	0.001	n.a.	0.01	n.a.	0.001	0.01
20:4 n6	0.04	0.04	<0.001	n.a.	0.004	0.004	0.03	0.04	<0.001	<0.001	0.004	0.005	0.03	<0.001	0.004	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
18:3 n3	0.09	0.09	n.a.	n.a.	0.003	0.003	0.08	0.08	n.a.	n.a.	0.003	0.004	0.08	n.a.	0.004	0.09

18:4 n3	0.21	0.22	n.a.	n.a.	0.009	0.009	0.19	0.19	n.a.	n.a.	0.009	0.012	0.20	n.a.	0.010	0.21
20:3 n3	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	n.a.	n.a.	0.01
20:4 n3	0.06	0.06	n.a.	n.a.	0.002	0.002	0.05	0.06	n.a.	n.a.	0.003	0.003	0.06	n.a.	0.003	0.06
20:5 n3	0.80	0.81	0.001	0.001	0.040	0.042	0.72	0.71	0.001	0.001	0.043	0.050	0.76	0.001	0.044	0.80
21:5 n3	0.02	0.02	n.a.	n.a.	0.001	n.a.	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
22:5 n3	0.08	0.08	n.a.	n.a.	0.003	0.003	0.07	0.08	n.a.	n.a.	0.004	0.004	0.08	n.a.	0.004	0.08
22:6 n3	1.51	1.53	0.001	0.001	0.089	0.091	1.42	1.42	0.001	0.001	0.096	0.113	1.47	0.001	0.097	1.57
SFA	2.15	2.19	0.022	0.023	0.150	0.166	1.86	1.89	0.021	0.022	0.147	0.171	2.02	0.022	0.158	2.20
MUFA	3.74	3.74	0.002	0.003	0.141	0.138	3.28	3.38	0.003	0.003	0.163	0.186	3.54	0.003	0.157	3.70
PUFA	3.02	3.05	0.002	0.002	0.159	0.163	2.76	2.77	0.002	0.002	0.171	0.201	2.90	0.002	0.173	3.08
Total FA	8.91	8.98	0.026	0.028	0.451	0.467	7.91	8.04	0.026	0.027	0.482	0.558	8.46	0.027	0.489	8.98

Table D6. Bligh and Dyer Result of Sample B (g/100g sample)

FA	B1						B2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 448	GC 449	GC 465	GC 466	GC 456	GC 457	GC 495		GC 505	GC 506	GC 513	GC 514	chloroform	Water	Residue	Sum
8:0	0.01	0.01	0.001	<0.001	0.001	<0.001	0.01	0.01	0.001	<0.001	<0.001	<0.001	0.01	0.001	0.001	0.01
10:0	n.a.	n.a.	<0.001	<0.001	0.004	0.002	n.a.	n.a.	<0.001	<0.001	0.001	0.003	n.a.	<0.001	0.002	<0.01
12:0	n.a.	n.a.	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	<0.001	<0.01
14:0	0.03	0.03	0.001	0.001	0.002	0.002	0.03	0.03	0.001	0.001	0.002	0.003	0.03	0.001	0.002	0.03
16:0	0.45	0.42	0.004	0.004	0.028	0.026	0.39	0.39	0.003	0.003	0.031	0.027	0.41	0.003	0.028	0.44
18:0	0.09	0.09	0.001	0.001	0.024	0.023	0.08	0.08	0.001	0.001	0.023	0.021	0.08	0.001	0.023	0.11
20:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
22:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
16:1 n7	0.03	0.02	<0.001	<0.001	0.002	0.002	0.02	0.02	<0.001	<0.001	0.002	0.003	0.03	<0.001	0.002	0.03

18:1 n9	0.28	0.26	0.001	0.001	0.023	0.021	0.24	0.25	0.001	0.001	0.025	0.021	0.25	0.001	0.022	0.28
20:1 n9	0.05	0.04	<0.001	<0.001	0.004	0.004	0.04	0.04	<0.001	<0.001	0.005	0.004	0.04	<0.001	0.004	0.05
22:1 n11	0.02	0.02	<0.001	<0.001	0.001	0.001	0.01	0.01	<0.001	<0.001	0.002	0.002	0.02	<0.001	0.001	0.02
24:1 n9	0.03	0.03	<0.001	n.a.	0.003	0.004	0.02	0.02	<0.001	n.a.	0.004	0.004	0.03	<0.001	0.004	0.03
16:2 n4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
16:3 n4	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:2 n6	0.03	0.02	<0.001	n.a.	0.002	0.002	0.02	0.02	<0.001	<0.001	0.003	0.002	0.02	<0.001	0.002	0.03
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
20:2 n6	<0.01	<0.01	n.a.	n.a.	<0.001	n.a.	n.a.	n.a.	n.a.	n.a.	<0.001	<0.001	0.00	n.a.	<0.001	<0.01
20:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
20:4 n6	0.10	0.10	0.001	0.001	0.011	0.011	0.09	0.09	0.001	0.001	0.012	0.012	0.09	0.001	0.011	0.11
22:4 n6	<0.01	<0.01	n.a.	n.a.	n.a.	0.000	<0.01	<0.01	n.a.	n.a.	0.001	n.a.	<0.01	n.a.	<0.001	<0.01
18:3 n3	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	n.a.	0.001	0.01
18:4 n3	0.01	0.01	<0.001	n.a.	0.001	0.001	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	<0.001	0.001	0.01
20:3 n3	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
20:4 n3	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	0.01	n.a.	n.a.	0.001	n.a.	0.01	n.a.	0.001	0.01
20:5 n3	0.29	0.28	0.002	0.002	0.024	0.022	0.26	0.27	0.002	0.002	0.028	0.034	0.28	0.002	0.027	0.30
21:5 n3	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.000	n.a.	<0.01	n.a.	<0.001	<0.01
22:5 n3	0.04	0.04	n.a.	n.a.	0.003	0.003	0.04	0.02	n.a.	n.a.	0.003	0.003	0.04	n.a.	0.003	0.04
22:6 n3	0.76	0.76	0.004	0.004	0.077	0.072	0.69	0.71	0.003	0.004	0.091	0.090	0.73	0.004	0.083	0.82
SFA	0.58	0.55	0.006	0.006	0.059	0.054	0.50	0.51	0.006	0.006	0.058	0.054	0.53	0.006	0.056	0.60
MUFA	0.40	0.37	0.002	0.001	0.034	0.032	0.33	0.35	0.002	0.001	0.038	0.033	0.36	0.002	0.034	0.40
PUFA	1.26	1.24	0.007	0.006	0.120	0.112	1.13	1.14	0.006	0.007	0.141	0.144	1.19	0.007	0.129	1.33
Total FA	2.24	2.16	0.015	0.014	0.213	0.198	1.97	2.00	0.013	0.014	0.236	0.232	2.09	0.014	0.220	2.33

Table D7. Bligh and Dyer Result of Sample C (g/100g sample)

FA	C1						C2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 450	GC 451	GC 467	GC 468	GC 458	GC 459	GC 496	GC 497	GC 507	GC 508	GC 515	GC 516	chloroform	Water	Residue	Sum
8:0	0.03	0.02	0.001	0.001	<0.001	<0.001	0.02	0.03	0.001	0.001	<0.001	<0.001	0.02	0.001	<0.001	0.02
10:0	n.a.	n.a.	n.a.	n.a.	<0.001	0.001	n.a.	n.a.	n.a.	n.a.	<0.001	0.001	n.a.	n.a.	0.001	0.00
12:0	0.01	0.01	<0.001	n.a.	<0.001	<0.001	0.01	0.01	<0.001	<0.001	0.001	0.001	0.01	<0.001	<0.001	0.01
14:0	0.96	1.06	0.001	0.001	0.017	0.009	0.94	0.98	0.001	0.001	0.019	0.022	0.99	0.001	0.017	1.01
16:0	3.64	3.98	0.002	0.002	0.069	0.041	3.57	3.67	0.003	0.003	0.062	0.063	3.72	0.003	0.058	3.78
18:0	0.90	0.97	0.001	0.001	0.043	0.036	0.89	0.91	0.001	0.001	0.040	0.041	0.92	0.001	0.040	0.96
20:0	0.10	0.11	n.a.	n.a.	0.002	0.001	0.10	0.10	n.a.	n.a.	0.002	0.002	0.10	n.a.	0.002	0.10
22:0	0.05	0.05	n.a.	n.a.	0.001	0.001	0.06	0.04	n.a.	n.a.	0.001	0.001	0.05	n.a.	0.001	0.05
16:1 n7	0.99	1.09	<0.001	<0.001	0.015	0.009	0.98	1.00	<0.001	<0.001	0.016	0.019	1.01	<0.001	0.015	1.03
18:1 n9	12.72	13.75	0.002	0.003	0.207	0.115	12.54	12.70	0.004	0.005	0.193	0.182	12.93	0.003	0.174	13.11
20:1 n9	1.64	1.76	<0.001	<0.001	0.027	0.015	1.64	1.63	<0.001	<0.001	0.023	0.024	1.67	<0.001	0.022	1.69
22:1 n11	1.04	1.08	<0.001	<0.001	0.018	0.010	1.05	1.05	<0.001	<0.001	0.018	0.015	1.06	<0.001	0.015	1.07
24:1 n9	0.17	0.17	n.a.	n.a.	0.005	0.004	0.17	0.17	n.a.	n.a.	0.005	0.005	0.17	n.a.	0.005	0.17
16:2 n4	0.10	0.11	n.a.	n.a.	0.002	0.001	0.09	0.10	n.a.	n.a.	0.002	0.003	0.10	n.a.	0.002	0.10
16:3 n4	0.06	0.07	n.a.	n.a.	0.001	0.001	0.06	0.07	n.a.	n.a.	0.001	0.002	0.07	n.a.	0.001	0.07
18:2 n6	3.75	4.07	0.001	0.001	0.059	0.032	3.70	3.74	0.001	0.001	0.059	0.062	3.81	0.001	0.053	3.87
18:3 n6	0.04	0.04	n.a.	n.a.	0.001	n.a.	0.04	0.04	n.a.	n.a.	0.001	0.001	0.04	n.a.	0.001	0.04
20:2 n6	0.35	0.37	n.a.	n.a.	0.006	0.003	0.35	0.35	n.a.	n.a.	0.006	0.005	0.35	n.a.	0.005	0.36
20:3 n6	0.07	0.07	n.a.	n.a.	0.001	0.001	0.07	0.07	n.a.	n.a.	0.001	0.001	0.07	n.a.	0.001	0.07
20:4 n6	0.11	0.12	<0.001	<0.001	0.008	0.007	0.11	0.10	<0.001	<0.001	0.008	0.011	0.11	<0.001	0.009	0.12
22:4 n6	0.03	0.02	n.a.	n.a.	<0.001	<0.001	0.02	0.03	n.a.	n.a.	<0.001	n.a.	0.02	n.a.	<0.001	0.03

18:3 n3	1.60	1.74	<0.001	<0.001	0.028	0.016	1.58	1.60	0.001	0.001	0.031	0.037	1.63	<0.001	0.028	1.66
18:4 n3	0.24	0.26	n.a.	n.a.	0.004	0.002	0.24	0.24	n.a.	n.a.	0.005	0.007	0.25	n.a.	0.005	0.25
20:3 n3	0.17	0.18	n.a.	n.a.	0.003	0.002	0.18	0.17	n.a.	n.a.	0.003	0.003	0.18	n.a.	0.003	0.18
20:4 n3	0.37	0.39	n.a.	n.a.	0.006	0.003	0.36	0.37	n.a.	n.a.	0.007	0.007	0.37	n.a.	0.006	0.38
20:5 n3	1.24	1.35	0.001	0.001	0.030	0.020	1.24	1.27	0.002	0.003	0.036	0.045	1.27	0.002	0.033	1.31
21:5 n3	0.08	0.08	n.a.	n.a.	0.001	0.001	0.08	0.08	n.a.	n.a.	0.001	0.001	0.08	n.a.	0.001	0.08
22:5 n3	0.72	0.75	n.a.	<0.001	0.015	0.010	0.73	0.74	<0.001	0.001	0.016	0.017	0.74	<0.001	0.014	0.75
22:6 n3	2.09	2.19	0.002	0.003	0.076	0.058	2.12	2.12	0.005	0.005	0.082	0.099	2.13	0.004	0.079	2.21
SFA	5.69	6.20	0.005	0.005	0.132	0.088	5.60	5.75	0.006	0.005	0.124	0.131	5.81	0.005	0.119	5.93
MUFA	16.57	17.85	0.003	0.003	0.272	0.152	16.38	16.56	0.005	0.005	0.256	0.244	16.84	0.004	0.231	17.07
PUFA	11.01	11.82	0.004	0.006	0.241	0.157	10.98	11.08	0.010	0.010	0.261	0.299	11.22	0.008	0.239	11.47
Total FA	33.27	35.88	0.011	0.013	0.646	0.397	32.95	33.38	0.021	0.021	0.641	0.674	33.87	0.017	0.589	34.48

Table D8. Bligh and Dyer Result of Sample D (g/100g sample)

FA	D1						D2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 452	GC 453	GC 469	GC 470	GC 460	GC 461	GC 498	GC 499	GC 509	GC 510	GC 517	GC 518	chloroform	Water	Residue	Sum
8:0	0.01	0.01	0.001	0.001	0.003	0.007	0.01	0.01	0.001	0.001	0.001	<0.001	0.01	0.001	0.003	0.01
10:0	n.a.	n.a.	<0.001	<0.001	0.002	0.002	<0.01	0.00	<0.001	<0.001	<0.001	0.001	<0.01	<0.001	0.001	<0.001
12:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	n.a.	n.a.	<0.001	<0.01	n.a.	<0.001	<0.01
14:0	0.48	0.45	0.005	0.005	0.024	0.023	0.43	0.42	0.004	0.004	0.026	0.026	0.45	0.005	0.025	0.48
16:0	2.38	2.25	0.014	0.014	0.123	0.114	2.13	2.06	0.008	0.008	0.124	0.128	2.21	0.011	0.123	2.34
18:0	0.16	0.15	0.002	0.002	0.071	0.066	0.14	0.15	0.001	0.001	0.069	0.073	0.15	0.001	0.070	0.22
20:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
22:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01

16:1 n7	0.41	0.39	0.002	0.002	0.019	0.018	0.37	0.36	0.001	0.001	0.018	0.018	0.38	0.001	0.018	0.40
18:1 n9	1.71	1.64	0.007	0.006	0.082	0.077	1.54	1.50	0.002	0.002	0.082	0.085	1.60	0.004	0.082	1.69
20:1 n9	0.25	0.24	0.001	0.001	0.014	0.013	0.23	0.23	<0.001	<0.001	0.014	0.014	0.24	0.001	0.014	0.25
22:1 n11	0.11	0.11	<0.001	<0.001	0.006	0.004	0.10	0.10	<0.001	<0.001	0.005	0.006	0.10	<0.001	0.005	0.11
24:1 n9	0.12	0.12	0.001	0.001	0.012	0.012	0.12	0.12	n.a.	0.000	0.013	0.013	0.12	0.001	0.012	0.13
16:2 n4	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
16:3 n4	0.02	0.02	<0.001	n.a.	0.001	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	<0.001	0.001	0.02
18:2 n6	0.13	0.13	<0.001	<0.001	0.006	0.006	0.12	0.12	<0.001	0.000	0.006	0.006	0.12	<0.001	0.006	0.13
18:3 n6	<0.001	<0.001	n.a.	n.a.	n.a.	n.a.	0.00	n.a.	n.a.	n.a.	n.a.	n.a.	<0.001	n.a.	n.a.	<0.01
20:2 n6	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	n.a.	0.001	0.01
20:3 n6	0.01	0.01	n.a.	n.a.	<0.001	0.001	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	n.a.	0.001	0.01
20:4 n6	0.05	0.05	<0.001	<0.001	0.022	0.021	0.04	0.04	n.a.	n.a.	0.022	0.022	0.05	<0.001	0.022	0.07
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01
18:3 n3	0.11	0.10	<0.001	<0.001	0.004	0.004	0.09	0.09	n.a.	n.a.	0.004	0.004	0.10	<0.001	0.004	0.10
18:4 n3	0.12	0.11	<0.001	<0.001	0.004	0.004	0.10	0.10	<0.001	n.a.	0.004	0.004	0.11	<0.001	0.004	0.11
20:3 n3	0.01	0.00	n.a.	n.a.	n.a.	n.a.	0.00	0.00	n.a.	n.a.	<0.001	n.a.	0.00	n.a.	<0.001	<0.01
20:4 n3	0.08	0.07	<0.001	<0.001	0.003	0.003	0.06	0.06	n.a.	n.a.	0.003	0.003	0.07	<0.001	0.003	0.07
20:5 n3	1.31	1.27	0.005	0.005	0.109	0.105	1.17	1.15	0.002	0.001	0.115	0.117	1.23	0.003	0.111	1.34
21:5 n3	0.01	0.01	n.a.	n.a.	<0.001	n.a.	0.01	0.01	n.a.	n.a.	0.001	n.a.	0.01	n.a.	<0.001	0.01
22:5 n3	0.16	0.16	<0.001	n.a.	0.007	0.006	0.16	0.15	n.a.	n.a.	0.007	n.a.	0.16	<0.001	0.006	0.16
22:6 n3	3.37	3.34	0.011	0.011	0.183	0.171	3.06	3.05	0.003	0.003	0.184	n.a.	3.21	0.007	0.179	3.39
SFA	3.04	2.86	0.022	0.022	0.223	0.213	2.72	2.64	0.014	0.013	0.221	0.229	2.81	0.018	0.221	3.05
MUFA	2.61	2.50	0.011	0.011	0.133	0.124	2.36	2.30	0.004	0.003	0.132	0.136	2.44	0.007	0.131	2.58
PUFA	5.41	5.32	0.018	0.018	0.340	0.321	4.88	4.84	0.005	0.005	0.348	0.159	5.12	0.011	0.292	5.42
Total FA	11.06	10.68	0.050	0.050	0.696	0.658	9.96	9.78	0.023	0.022	0.701	0.524	10.37	0.036	0.645	11.05

Table D9. Bligh and Dyer Result of Sample F (g/100g sample)

FA	F1						F2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 420	GC 421	GC 429	GC 430	GC 438	GC 439	GC 520	GC 521	GC 529	GC 530	GC 537	GC 538	chloroform	Water	Residue	Sum
8:0	0.01	0.01	<0.001	<0.001	0.029	0.020	0.01	0.01	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	0.012	0.02
10:0	n.a.	n.a.	n.a.	<0.001	0.009	0.006	n.a.	n.a.	<0.001	<0.001	<0.001	0.001	n.a.	<0.001	0.004	<0.01
12:0	0.03	0.03	<0.001	<0.001	0.002	0.002	0.03	0.03	0.001	0.001	0.001	0.001	0.03	0.001	0.002	0.03
14:0	1.56	1.59	0.020	0.020	0.052	0.055	1.45	1.43	0.044	0.044	0.037	0.037	1.51	0.032	0.045	1.59
16:0	3.06	3.12	0.068	0.067	0.151	0.138	2.88	2.84	0.108	0.107	0.130	0.142	2.97	0.087	0.140	3.20
18:0	0.20	0.21	0.003	0.003	0.011	0.010	0.19	0.19	0.006	0.006	0.010	0.010	0.20	0.005	0.010	0.22
20:0	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	<0.001	0.01
22:0	0.01	0.01	<0.001	<0.001	<0.001	0.001	0.01	0.01	<0.001	n.a.	0.001	0.001	0.01	<0.001	0.001	0.01
16:1 n7	0.94	0.97	0.009	0.009	0.025	0.022	0.87	0.86	0.024	0.024	0.018	0.019	0.91	0.017	0.021	0.95
18:1 n9	2.66	2.70	0.030	0.030	0.109	0.100	2.55	2.51	0.073	0.072	0.093	0.099	2.61	0.051	0.100	2.76
20:1 n9	0.18	0.20	0.002	0.002	0.006	0.005	0.19	0.17	0.005	0.004	0.005	0.005	0.19	0.003	0.005	0.19
22:1 n11	0.14	0.14	0.002	0.002	0.005	0.004	0.09	0.11	0.003	0.004	0.005	0.005	0.12	0.003	0.005	0.13
24:1 n9	0.02	0.02	0.001	0.001	n.a.	n.a.	0.02	0.02	0.001	0.001	0.002	0.002	0.02	0.001	0.002	0.03
16:2 n4	0.10	0.10	0.001	0.001	0.003	0.004	0.10	0.09	0.003	0.003	0.003	0.002	0.10	0.002	0.003	0.10
16:3 n4	0.05	0.05	0.001	0.001	0.002	0.002	0.04	0.04	0.002	0.001	0.002	0.002	0.05	0.001	0.002	0.05
18:2 n6	0.22	0.22	0.003	0.003	0.009	0.009	0.21	0.21	0.006	0.006	0.008	0.009	0.22	0.005	0.009	0.23
18:3 n6	0.02	0.02	n.a.	0.000	n.a.	n.a.	0.02	0.03	0.001	0.001	0.001	0.001	0.02	<0.001	0.001	0.03
20:2 n6	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	<0.001	<0.001	0.001	0.001	0.01	<0.001	0.001	0.01
20:3 n6	0.02	0.02	n.a.	<0.001	n.a.	n.a.	0.01	0.02	<0.001	<0.001	n.a.	<0.001	0.01	<0.001	<0.001	0.01
20:4 n6	0.05	0.05	0.001	0.001	0.005	0.004	0.05	0.05	0.002	0.002	0.004	0.005	0.05	0.001	0.004	0.05
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00

18:3 n3	0.14	0.14	0.002	0.002	0.006	0.006	0.13	0.13	0.004	0.004	0.005	0.005	0.13	0.003	0.006	0.14
18:4 n3	0.27	0.28	0.003	0.003	0.010	0.012	0.26	0.26	0.008	0.008	0.008	0.007	0.27	0.005	0.009	0.28
20:3 n3	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	<0.001	n.a.	<0.001	<0.001	0.01	<0.001	<0.001	0.01
20:4 n3	0.07	0.07	0.001	0.001	0.003	0.003	0.06	0.07	0.002	0.003	0.004	0.004	0.07	0.002	0.003	0.08
20:5 n3	1.85	1.90	0.035	0.034	0.102	0.113	1.83	1.85	0.061	0.060	0.092	0.092	1.86	0.047	0.100	2.00
21:5 n3	0.06	0.06	0.001	0.001	0.002	0.002	0.05	0.06	0.001	0.001	0.002	0.002	0.06	0.001	0.002	0.06
22:5 n3	0.07	0.07	<0.001	<0.001	0.002	0.002	0.07	0.07	0.002	0.002	0.002	0.002	0.07	0.001	0.002	0.07
22:6 n3	1.06	1.09	0.016	0.016	0.083	0.084	1.05	1.07	0.032	0.031	0.080	0.081	1.07	0.024	0.082	1.17
SFA	4.89	4.97	0.092	0.091	0.255	0.230	4.58	4.52	0.160	0.158	0.179	0.193	4.74	0.125	0.214	5.08
MUFA	3.94	4.04	0.044	0.043	0.144	0.130	3.72	3.67	0.106	0.105	0.122	0.129	3.84	0.075	0.132	4.05
PUFA	3.99	4.09	0.064	0.064	0.227	0.241	3.90	3.95	0.124	0.122	0.210	0.211	3.98	0.093	0.222	4.30
Total FA	12.82	13.09	0.200	0.198	0.626	0.601	12.20	12.14	0.391	0.386	0.511	0.533	12.56	0.293	0.568	13.42

Table D10. Bligh and Dyer Result of Sample G (g/100g sample)

FA	G1						G2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 422	GC 423	GC 431	GC 432	GC 440	GC 441	GC 522	GC 523	GC 531	GC 532	GC 539	GC 540	chloroform	Water	Residue	Sum
8:0	0.01	0.01	<0.001	0.001	0.024	0.084	0.01	0.01	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	0.027	0.03
10:0	n.a.	n.a.	<0.001	<0.001	0.007	0.034	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	n.a.	<0.001	0.021	0.02
12:0	<0.01	<0.01	<0.001	<0.001	0.001	0.001	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	<0.001	0.001	<0.01
14:0	0.18	0.18	0.003	0.003	0.024	0.024	0.17	0.17	0.003	0.003	0.011	0.012	0.17	0.003	0.018	0.19
16:0	0.81	0.79	0.020	0.020	0.094	0.095	0.77	0.75	0.018	0.017	0.078	0.082	0.78	0.019	0.087	0.89
18:0	0.14	0.14	0.008	0.008	0.026	0.026	0.13	0.13	0.007	0.007	0.026	0.027	0.13	0.007	0.026	0.17
20:0	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	0.001	<0.01	n.a.	0.001	<0.01
22:0	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	n.a.	<0.001	n.a.	<0.01	n.a.	<0.001	<0.01

16:1 n7	0.24	0.24	0.003	0.002	0.024	0.024	0.23	0.22	0.002	0.002	0.013	0.014	0.23	0.002	0.019	0.25
18:1 n9	0.87	0.87	0.011	0.009	0.085	0.083	0.84	0.83	0.008	0.007	0.074	0.078	0.85	0.009	0.080	0.94
20:1 n9	0.63	0.62	0.003	0.002	0.043	0.044	0.62	0.60	0.002	0.002	0.043	0.045	0.62	0.002	0.043	0.66
22:1 n11	0.67	0.67	0.001	0.001	0.037	0.036	0.67	0.66	0.001	0.001	0.036	0.037	0.67	0.001	0.036	0.71
24:1 n9	0.11	0.11	0.001	0.001	0.010	0.009	0.11	0.11	0.001	0.001	0.012	0.012	0.11	0.001	0.011	0.13
16:2 n4	0.01	0.02	<0.001	<0.001	0.003	0.002	0.01	0.01	n.a.	n.a.	0.001	0.002	0.01	<0.001	0.002	0.02
16:3 n4	0.01	0.01	n.a.	<0.001	0.001	0.001	0.01	0.01	n.a.	<0.001	<0.001	<0.001	0.01	<0.001	0.001	0.01
18:2 n6	0.06	0.06	0.001	0.001	0.008	0.008	0.06	0.06	0.001	0.001	0.006	0.006	0.06	0.001	0.007	0.07
18:3 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01	n.a.	n.a.	<0.01
20:2 n6	0.01	0.01	n.a.	n.a.	0.001	n.a.	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	n.a.	0.001	0.01
20:3 n6	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	<0.001	n.a.	<0.01	n.a.	<0.001	<0.01
20:4 n6	0.03	0.04	<0.001	<0.001	0.004	0.006	0.03	0.03	<0.001	<0.001	0.004	0.004	0.03	<0.001	0.004	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
18:3 n3	0.03	0.03	<0.001	<0.001	0.003	0.003	0.03	0.03	<0.001	<0.001	0.002	0.002	0.03	<0.001	0.002	0.03
18:4 n3	0.06	0.06	<0.001	<0.001	0.007	0.008	0.06	0.06	<0.001	<0.001	0.003	0.003	0.06	<0.001	0.005	0.07
20:3 n3	<0.01	<0.01	n.a.	n.a.	n.a.	n.a.	<0.01	<0.01	n.a.	n.a.	n.a.	<0.001	<0.01	n.a.	<0.001	<0.01
20:4 n3	0.02	0.02	n.a.	n.a.	0.002	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
20:5 n3	0.36	0.36	0.002	0.002	0.044	0.043	0.36	0.37	0.001	0.001	0.026	0.026	0.36	0.002	0.035	0.40
21:5 n3	0.01	0.01	n.a.	n.a.	n.a.	n.a.	0.01	0.01	n.a.	n.a.	<0.001	<0.001	0.01	n.a.	<0.001	0.01
22:5 n3	0.05	0.05	<0.001	n.a.	0.004	0.002	0.05	0.05	n.a.	<0.001	0.004	0.000	0.05	<0.001	0.003	0.05
22:6 n3	0.59	0.59	0.004	0.004	0.070	0.071	0.60	0.59	0.002	0.002	0.055	0.055	0.59	0.003	0.062	0.66
SFA	1.14	1.12	0.032	0.031	0.177	0.264	1.09	1.06	0.028	0.028	0.116	0.122	1.10	0.030	0.170	1.30
MUFA	2.52	2.51	0.018	0.016	0.198	0.197	2.47	2.43	0.013	0.012	0.178	0.185	2.48	0.015	0.190	2.69
PUFA	1.26	1.27	0.01	0.008	0.146	0.145	1.26	1.25	0.005	0.005	0.104	0.101	1.26	0.006	0.124	1.39
Total FA	4.93	4.90	0.059	0.056	0.521	0.607	4.82	4.74	0.046	0.045	0.398	0.408	4.85	0.051	0.483	5.38

Table 11. Bligh and Dyer Result of Sample H (g/100g sample)

FA	H1						H2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 424	GC 425	GC 433	GC 434	GC 442	GC 443	GC 524	GC 525	GC 533	GC 534	GC 541	GC 542	chloroform	Water	Residue	Sum
8:0	0.01	0.01	<0.001	<0.001	0.003	0.023	0.01	0.01	n.a.	<0.001	0.004	n.a.	0.01	<0.001	0.010	0.02
10:0	0.01	0.01	<0.001	<0.001	<0.001	0.009	0.01	0.01	0.000	<0.001	0.001	0.001	0.01	<0.001	0.003	0.01
12:0	0.01	0.01	<0.001	<0.001	<0.001	0.001	0.01	0.01	n.a.	<0.001	0.001	0.001	0.01	<0.001	0.001	0.01
14:0	0.58	0.60	0.011	0.011	0.040	0.042	0.54	0.54	0.011	0.010	0.031	0.031	0.57	0.011	0.036	0.61
16:0	1.41	1.45	0.018	0.018	0.137	0.123	1.31	1.33	0.017	0.017	0.108	0.108	1.37	0.017	0.119	1.51
18:0	0.15	0.15	0.005	0.005	0.034	0.030	0.14	0.15	0.004	0.004	0.029	0.029	0.15	0.005	0.030	0.18
20:0	0.01	0.01	n.a.	n.a.	n.a.	0.001	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	n.a.	0.001	0.01
22:0	0.00	0.00	n.a.	n.a.	0.001	<0.001	<0.01	<0.01	n.a.	n.a.	<0.001	<0.001	<0.01	n.a.	<0.001	0.01
16:1 n7	0.36	0.37	0.001	0.001	0.002	0.017	0.33	0.33	0.001	0.001	0.013	0.013	0.35	0.001	0.011	0.36
18:1 n9	1.26	1.31	0.005	0.005	0.062	0.085	1.20	1.21	0.004	0.004	0.075	0.075	1.25	0.005	0.074	1.33
20:1 n9	1.01	1.02	0.001	0.001	0.042	0.044	0.98	0.91	0.001	0.001	0.038	0.037	0.98	0.001	0.040	1.02
22:1 n11	1.47	1.49	0.002	0.002	0.054	0.056	1.48	1.51	0.002	0.002	0.047	0.046	1.49	0.002	0.051	1.54
24:1 n9	0.12	0.13	0.001	0.001	n.a.	0.016	0.13	0.13	0.001	0.001	0.015	0.015	0.13	0.001	0.015	0.14
16:2 n4	0.02	0.02	n.a.	n.a.	0.002	0.002	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
16:3 n4	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
18:2 n6	0.11	0.11	0.000	0.000	0.007	0.007	0.10	0.10	<0.001	<0.001	0.006	0.006	0.10	0.000	0.006	0.11
18:3 n6	0.00	0.00	n.a.	n.a.	n.a.	n.a.	0.01	0.01	n.a.	n.a.	<0.001	n.a.	<0.01	n.a.	v	0.01
20:2 n6	0.01	0.02	n.a.	n.a.	0.001	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
20:3 n6	0.01	0.01	n.a.	n.a.	0.001	0.000	0.01	0.01	n.a.	n.a.	0.000	0.000	0.01	n.a.	<0.001	0.01
20:4 n6	0.03	0.03	0.000	0.000	n.a.	0.006	0.04	0.03	<0.001	<0.001	0.005	n.a.	0.03	<0.001	0.005	0.04
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.01

18:3 n3	0.07	0.07	<0.001	<0.001	0.004	0.004	0.06	0.06	<0.001	<0.001	0.003	0.003	0.07	<0.001	0.003	0.07
18:4 n3	0.12	0.13	<0.001	<0.001	n.a.	0.006	0.12	0.12	<0.001	<0.001	0.004	0.004	0.12	<0.001	0.004	0.13
20:3 n3	0.01	0.01	n.a.	n.a.	0.001	n.a.	0.01	<0.01	n.a.	n.a.	<0.001	<0.001	0.01	n.a.	<0.001	0.01
20:4 n3	0.04	0.05	n.a.	n.a.	n.a.	0.003	0.04	0.04	n.a.	n.a.	0.002	n.a.	0.04	n.a.	0.003	0.04
20:5 n3	0.54	0.54	0.002	0.002	0.042	0.042	0.54	0.56	0.001	0.001	0.034	0.034	0.55	0.002	0.038	0.59
21:5 n3	0.01	0.02	n.a.	n.a.	n.a.	0.001	0.02	0.02	n.a.	n.a.	0.001	0.001	0.02	n.a.	0.001	0.02
22:5 n3	0.08	0.08	n.a.	<0.001	n.a.	0.005	0.07	0.07	n.a.	n.a.	0.004	n.a.	0.08	<0.001	0.005	0.08
22:6 n3	1.04	1.06	0.004	0.004	0.109	0.099	1.05	1.08	0.003	0.003	0.086	0.089	1.06	0.003	0.096	1.16
SFA	2.18	2.24	0.035	0.035	0.215	0.229	2.02	2.05	0.033	0.032	0.175	0.171	2.12	0.034	0.198	2.35
MUFA	4.22	4.33	0.010	0.010	0.160	0.218	4.13	4.09	0.009	0.009	0.189	0.186	4.19	0.010	0.188	4.39
PUFA	2.12	2.15	0.006	0.006	0.167	0.175	2.12	2.15	0.005	0.005	0.148	0.139	2.13	0.006	0.157	2.30
Total FA	8.51	8.72	0.051	0.051	0.543	0.622	8.27	8.29	0.047	0.046	0.513	0.496	8.45	0.049	0.543	9.04

Table D12. Bligh and Dyer Result of Sample I (g/100g sample)

FA	I1						I2						average			
	chloroform		water		Residue		chloroform		water		Residue					
	GC 480	GC 481	GC 489	GC 490	GC 484	GC 485	GC 526	GC 527	GC 535	GC 536	GC 543	GC 544	chloroform	Water	Residue	Sum
8:0	0.01	0.02	<0.001	<0.001	<0.001	0.001	0.02	0.01	<0.001	<0.001	0.001	0.001	0.02	<0.001	0.001	0.02
10:0	n.a.	n.a.	0.001	0.001	<0.001	<0.001	n.a.	n.a.	0.001	0.001	<0.001	<0.001	n.a.	0.001	<0.001	<0.01
12:0	0.03	0.03	0.002	0.002	0.001	0.002	0.02	0.02	0.002	0.002	0.001	0.001	0.03	0.002	0.001	0.03
14:0	1.18	1.21	0.023	0.023	0.040	0.042	1.05	1.04	0.021	0.020	0.025	0.025	1.12	0.022	0.033	1.18
16:0	2.82	2.87	0.047	0.048	0.146	0.153	2.52	2.51	0.044	0.042	0.101	0.102	2.68	0.045	0.126	2.85
18:0	0.16	0.17	0.003	0.004	0.009	0.010	0.15	0.14	0.003	0.002	0.006	0.006	0.15	0.003	0.008	0.17
20:0	n.a.	n.a.	n.a.	n.a.	<0.001	<0.001	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	<0.001	<0.01
22:0	<0.01	n.a.	n.a.	n.a.	<0.001	<0.001	0.01	<0.01	n.a.	n.a.	<0.001	<0.001	<0.01	n.a.	<0.001	<0.01

16:1 n7	0.50	0.51	0.006	0.006	0.017	0.018	0.45	0.44	0.005	0.005	0.010	0.001	0.48	0.005	0.011	0.49
18:1 n9	2.33	2.36	0.020	0.021	0.103	0.110	2.14	2.07	0.019	0.018	0.068	0.069	2.22	0.020	0.087	2.33
20:1 n9	0.15	0.15	0.001	0.001	0.006	0.007	0.14	0.15	0.001	<0.001	0.004	0.005	0.15	0.001	0.006	0.15
22:1 n11	0.12	0.10	0.001	0.001	0.005	0.006	0.08	0.08	0.001	0.001	0.003	0.000	0.09	0.001	0.004	0.10
24:1 n9	n.a.	0.01	n.a.	<0.001	0.001	0.001	0.01	0.01	n.a.	<0.001	0.001	0.001	0.01	<0.001	0.001	0.01
16:2 n4	0.07	0.07	0.001	0.001	0.002	0.003	0.07	0.07	0.001	0.001	0.002	0.002	0.07	0.001	0.002	0.07
16:3 n4	0.05	0.06	0.001	0.001	0.002	0.002	0.06	0.05	0.001	0.001	0.002	0.002	0.05	0.001	0.002	0.06
18:2 n6	0.23	0.23	0.002	0.002	0.010	0.011	0.21	0.21	0.002	0.002	0.007	0.007	0.22	0.002	0.009	0.23
18:3 n6	0.02	0.02	<0.001	<0.001	0.001	0.001	0.02	0.01	<0.001	<0.001	n.a.	n.a.	0.02	<0.001	0.001	0.02
20:2 n6	0.01	0.01	n.a.	n.a.	0.001	0.001	0.01	0.01	n.a.	n.a.	0.000	0.001	0.01	n.a.	0.001	0.01
20:3 n6	0.01	0.02	n.a.	<0.001	0.001	0.001	0.01	0.01	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	0.001	0.01
20:4 n6	0.03	0.03	<0.001	0.001	0.002	0.002	0.03	0.03	0.001	0.001	n.a.	n.a.	0.03	0.001	0.002	0.03
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00
18:3 n3	0.36	0.37	0.004	0.004	0.016	0.017	0.33	0.33	0.004	0.004	0.010	n.a.	0.35	0.004	0.014	0.37
18:4 n3	0.70	0.72	0.009	0.009	n.a.	n.a.	0.64	0.64	0.009	0.008	0.017	0.017	0.67	0.009	0.017	0.70
20:3 n3	0.03	0.02	<0.001	<0.001	0.002	0.001	0.03	0.02	<0.001	<0.001	n.a.	n.a.	0.03	<0.001	0.002	0.03
20:4 n3	0.09	0.09	0.001	0.001	0.005	0.005	0.08	0.08	0.001	0.001	n.a.	n.a.	0.08	0.001	0.005	0.09
20:5 n3	2.54	2.53	0.030	0.032	0.130	0.142	2.39	2.38	0.031	0.030	0.090	0.091	2.46	0.031	0.113	2.60
21:5 n3	0.09	0.10	0.001	0.001	0.005	0.005	0.09	0.09	0.001	0.001	0.003	0.003	0.09	0.001	0.004	0.10
22:5 n3	0.04	0.06	n.a.	n.a.	n.a.	0.003	0.06	0.05	n.a.	n.a.	n.a.	n.a.	0.05	n.a.	0.003	0.06
22:6 n3	1.40	1.41	0.011	0.011	0.097	0.103	1.33	1.35	0.011	0.010	0.072	0.073	1.37	0.011	0.086	1.47
SFA	4.21	4.30	0.077	0.078	0.198	0.208	3.77	3.74	0.070	0.067	0.135	0.136	4.00	0.073	0.169	4.25
MUFA	3.10	3.13	0.028	0.029	0.132	0.142	2.82	2.75	0.025	0.025	0.086	0.075	2.95	0.027	0.109	3.08
PUFA	5.67	5.74	0.060	0.063	0.273	0.298	5.35	5.33	0.061	0.059	0.203	0.195	5.52	0.061	0.242	5.83
Total FA	12.97	13.17	0.164	0.169	0.603	0.647	11.94	11.82	0.156	0.150	0.424	0.406	12.48	0.160	0.520	13.16

Appendix E. Data for control Oil

GC data for Control Oil Sample																(g/100g sample)
weithgt(g)	**0.0121**	**0.0127**	**0.0105**	**0.0120**	**0.0120**	**0.0115**	**0.0120**	**0.0121**	**0.0146**	**0.0115**	**0.0110**	**0.0142**	**0.0131**	**0.0123**	**0.0129**	**0.0126**
FA	**GC 77**	**GC 78**	**GC 135**	**GC 136**	**GC 191**	**GC 192**	**GC 246**	**GC 247**	**GC 286**	**GC 287**	**GC 348**	**GC 349**	**GC 377**	**GC 378**	**GC 471**	**GC 472**
8:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.08	0.10
10:0	0.27	0.28	0.28	0.27	0.26	0.27	0.31	0.31	0.29	0.29	0.27	0.28	0.29	0.28	0.04	0.04
12:0	0.08	0.08	0.08	0.17	0.14	0.14	0.09	0.13	0.09	0.09	0.10	0.09	0.10	0.09	0.10	0.10
14:0	6.92	6.68	7.14	7.05	6.93	7.17	6.80	6.84	6.56	6.65	6.90	6.67	7.12	6.96	6.67	6.83
16:0	11.69	11.17	12.20	12.00	11.71	11.96	11.37	11.46	11.10	11.19	11.49	11.29	11.87	11.71	11.23	11.43
18:0	1.36	1.29	1.41	1.43	1.34	1.39	1.33	1.37	1.34	1.35	1.35	1.35	1.42	1.40	1.34	1.31
20:0	0.08	0.07	0.09	0.08	0.08	0.07	0.06	0.07	0.08	0.07	0.08	0.07	0.08	0.08	0.09	0.09
22:0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.02	0.03	0.02	n.a.	0.02	n.a.	n.a.	n.a.	0.02
16:1 n7	4.29	4.12	4.43	4.41	4.30	4.39	4.36	4.48	4.32	4.34	4.41	4.34	4.59	4.32	4.45	4.40
18:1 n9	9.19	8.79	9.34	9.32	8.99	9.26	8.94	8.92	8.86	8.93	9.06	9.01	9.36	9.34	8.83	8.89
20:1 n9	10.53	10.24	10.83	10.86	10.43	10.65	10.45	10.35	10.39	10.45	10.55	10.44	10.80	10.85	10.24	10.26
22:1 n11	14.69	14.38	14.89	14.88	14.66	14.79	14.64	14.32	14.44	14.53	14.63	14.56	14.94	14.88	14.28	14.27
24:1 n9	0.66	0.60	0.64	0.64	0.62	0.56	0.72	0.65	0.69	0.67	0.68	0.69	0.68	0.63	0.67	0.65
16:2 n4	0.36	0.38	0.42	0.44	0.40	0.42	0.44	0.42	0.42	0.40	0.43	0.41	0.43	0.43	0.40	0.44
16:3 n4	0.31	0.31	0.32	0.32	0.31	0.31	0.31	0.32	0.30	0.32	0.31	0.30	0.32	0.32	0.31	0.31
18:2 n6	1.81	1.74	1.85	1.83	1.78	1.80	1.75	1.76	1.73	1.75	1.77	1.74	1.81	1.82	1.74	1.74
18:3 n6	0.07	0.07	0.07	0.07	0.07	0.05	0.07	0.07	0.09	0.11	0.08	0.11	0.10	0.12	0.12	0.12
20:2 n6	0.21	0.23	0.21	0.21	0.20	0.21	0.20	0.25	0.23	0.24	0.24	0.23	0.24	0.24	0.23	0.23
20:3 n6	0.05	0.05	0.06	0.05	0.04	0.03	0.06	0.05	0.08	0.07	0.07	0.07	0.07	0.06	0.06	0.04
20:4 n6	0.25	0.24	0.24	0.26	0.23	0.24	0.26	0.25	0.25	0.25	0.25	n.a.	0.27	0.26	0.24	0.24
22:4 n6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.02	0.02	0.02	n.a.

18:3 n3	1.78	1.73	1.84	1.81	1.76	1.79	1.76	1.76	1.73	1.74	1.78	1.74	1.81	1.82	1.72	1.75
18:4 n3	4.16	4.01	4.26	4.26	4.10	4.18	4.11	4.10	3.99	4.06	4.09	4.06	4.24	4.26	4.01	4.07
20:3 n3	0.17	0.21	0.28	0.32	0.33	0.34	0.23	0.16	0.19	0.17	0.21	0.20	0.24	0.19	0.18	0.19
20:4 n3	0.90	0.90	0.92	0.93	0.89	0.90	0.90	0.88	0.88	0.94	0.89	0.88	0.90	0.92	0.91	0.91
20:5 n3	6.32	6.12	6.44	6.41	6.21	6.25	6.27	6.13	6.32	6.28	6.37	6.30	6.51	6.54	6.04	6.20
21:5 n3	0.34	0.34	0.35	0.35	0.34	0.34	0.35	0.33	0.34	0.32	0.33	0.33	n.a.	0.35	0.33	0.33
22:5 n3	0.66	0.65	0.72	0.66	0.67	0.66	0.75	0.75	0.62	0.78	0.65	0.74	0.77	0.64	0.72	0.70
22:6 n3	10.39	10.17	10.41	10.42	10.20	10.30	10.28	10.17	10.31	10.40	10.37	10.28	10.60	10.65	10.20	10.10
SFA	20.40	19.58	21.21	20.99	20.47	21.00	19.96	20.20	19.48	19.66	20.19	19.78	20.88	20.51	19.54	19.91
MUFA	39.36	38.14	40.13	40.11	39.01	39.66	39.11	38.71	38.71	38.93	39.33	39.04	40.37	40.02	38.47	38.46
PUFA	27.76	27.12	28.38	28.32	27.53	27.82	27.74	27.39	27.49	27.83	27.83	27.38	28.33	28.65	27.23	27.37
Total FA	87.52	84.84	89.71	89.42	87.01	88.48	86.81	86.30	85.68	86.42	87.35	86.21	89.58	89.19	85.24	85.74
average of total FA								87.22								
C.V. of total FA, %								1.85								

Appendix F

Nofima	Analytical Procedures BioLab Analyse	Version : 1.6 Valid from : 24.03.09	Doc.id Method No. A 03
	FAT, SOXHLET	Responsible : L-alab	Page 1 of 2

FAT, SOXHLET PROCEDURE

1) Applications

Fishmeal and dry feed

2) Reference:

AOCS Ba 3 - 38

3) Pinciple:

Fat extraction by petroleum ether. The method will basically determine the content of triacylglycerols.

4) Equipment/chemicals:

1. Petroleum ether, p.a., boiling point 40-60ºC
2. Extraction tubes
3. Soxhlet extractor consisting of soxhlet flask, extraction apparatus and condenser
4. Indirectly heated water bath with temperature control
5. Analytical balance with accuracy 0.1 mg
6. Laboratory oven thermostatted at 103ºC
7. Purified cotton wool
8. Dessicator

5) Procedure:

1. Accurately weigh 4-7 gram of sample and transfer to extraction tube.
2. Dry the tube with the sample in the oven at 103ºC for two hours.
3. Dry the soxhlet flasks in the oven at 103ºC for one hour.
4. Let the soxhlet flasks cool in the exicator for 30 min. and tare.
5. Cover the sample with cotton wool in the extraction tube and place the tube in the soxhlet apparatus with a tared flask.
6. Add approximately 100 mL petroleum ether to the soxhlet flask and extract over night with a condensation rate of 2-3 drops per second.
7. Let the petroleum ether distill off and dry the flask in the oven for 2 hours at 103±1ºC.
8. Let the soxhlet flask cool in the dessicator and weigh.

6) Calculations

$$\text{Fat soxhlet} = \frac{(a-b)\cdot 100}{w} \quad \%$$

a = weight (gram) of flask + fat extract

b = weight (gram) of flask

w = weight (gram) of sample

Results are given with one decimal

Less than 0.05% are given as <0.1%

7) Comments:

1. Be aware the risk of cross contamination when weighing samples with large differences in fat content.

2. Petroleum ether is highly flammable. No electrical equipment is allowed in the fume hood where extraction takes place*.

3. Petroleum ether is toxic and can affect the central nervous system*.

4. Wear gloves and eye protection.

5. Use a fume hood when performing 5.6 and 5.7.

* Source: Kartotek for kjemiske stoffer
(Forlaget VITA-DATA A/S 2nd edn. 1991)

Appendix G

Nofima	Analytical methods BioLab Analyse	Version no: 1.5 Valid from : 24.03.09	Dok.id Method No. A 38
	FAT, ACIDIC HYDROLYSIS	Responsible : L-alab	Page 1 of 2

FAT, ACIDIC HYDROLYSIS

1) Applications

Fishmeal and feed / feed ingredients

2) Reference

98/64/EC

3) Principle

Fat extraction by petroleum ether before and after hydrolysis with hydrochloric acid

4) Equipment and chemicals

1. Petroleum ether, p.a., poiling point 40-60ºC
2. Hydrochloric acid, HCl, fuming, 37%, p.a.
3. Hydrochloric acid, HCL, 3 M.
   Dillute 245 mL 37% HCl to 1000 mL with purified water
4. Filter aid, e.g. kiselgur og Hyflo-supercel
5. Round bottomed flasks with condensers, 250 mL.
6. Filter papers, white band, 150 mm
7. Extraction tubes
8. Soxhlet extractor consisting of extraction apparatus and condenser
9. Indirectly heated water bath with temperature control
10. Analytical balance with accuracy 0.1 mg
11. Laboratory oven thermostatted at 103ºC
12. Purified cotton wool

5) Procedure

1. Weigh accurately 4 – 7 gram sample and transfer to extraction tube.
2. Dry the flasks in the oven at 103ºC for one hour.
3. Let the flasks cool in the dessicator for 30 min. and tare.
4. Cover the sample with cotton wool in the extraction tube and place the tube in the soxhlet apparatus with a tared flask.
5. Add approximately 100 mL petroleum ether to the flask and extraxt for at least two hours with a condensation rate of 2-3 drops per second.
6. Let the petroleum ether distil off and set the flask with the extract in a fume hood until second extraction.
7. Dry the tube with the remnants of the sample at 103ºC for approx. one hour.

8. Transfer the remnants from the tube to a round-bottomed flask, mark the tube for later use.
9. Add 100 mL 3M hydrochloric acid and mount the condenser on the flask.
10. Boil the solution gently on a water bath in approximately 1 hour. Shake occasionally to avoid that the sample sticks to the glass wall.
11. Let the sample cool.
12. Add filter aid to the flask, minimum 3 g.
13. Filter the sample through a wetted, fat free, 150 mm white band filter paper. Wash with cold purified water until the filtrate is neutral (approx. 500 mL)
14. Fold the filter paper, transfer it to the same extraction tube that was applied in the first extraction and dry for 2 hours in the oven at 103ºC.
15. Cover the sample with cotton wool and place the tube in the soxhlet extractor together with the steel tube from the first extraction.
16. Add 100 mL petroleum ether to the flask and extract over night with a condensation rate of approximately 2-3 drops per second.
17. Let the petroleum ether distil off and dry the flask in the oven for 2 hours at 103±1ºC
18. Let the flask cool in the exicator and weigh.

6) Calculation

$$\text{Fat} = \frac{a-b}{w} \cdot 100 \ \%$$

a = weight (gram) of tube with fat extract.

b = weight (gram) of tube (tara)

w =initial weight (gram) of sample

Results are given with one decimal

Less than 0.05% are given as <0.1%

7) Comments

1. Wet samples (>12% water) are weighed in tubes and dried at approx. 103ºC for two hours prior to extraction.

2. The tube with the sample remnants after the first extraction (see 5.5) can be left for the next day for drying.

3. Petroleum ether is highly flammable. No electrical equipment is allowed in the fume hood where extraction takes place*.

4. Petroleum ether is toxic and can affect the central nervous system*.

5. Hydrcochloric can cause severe burns to skin and eyes. Wear gloves and eye protection.

6. Point 5.10 and 5.11 must be done in a fume hood.

* Source: Kartotek for kjemiske stoffer
(Forlaget VITA-DATA A/S 2nd edn. 1991)

Appendix H

Fiskeriforskning	Analysemetoder Alab Fiskeriforskning	Utgave nr : 1.1 Gyldig fra : 15.08.2003	Dok.id Metode nr. A 56
	FAT BY BLIGH AND DYER	Ansvarlig : L-alab	Side 1 av 2

FAT BY BLIGH & DYER EXTRACTION

1) Applications:

Fishmeal, feeds, fish, and intermediates in fishmeal production.

2) Reference:

E.G. Bligh & W.J. Dyer : A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. Vol 37 (1959)

3) Principle:

The fat in the sample is extracted by a polar solvent mixture consisting of chloroform, methanol and water (1:2:0.8), which gives a one-phase system.

After extraction, the one-phase system is separated into chloroform and methanol/water phases by addition of chloroform and water. The lipids will follow the chloroform phase.

The fat content is determined in an aliquot of the chloroform phase by weighing the lipids after evaporation of the solvent. The method will extract both neutral and polar lipids.

4) A. Equipment:

1. Laboratory balance with 1 mg accuracy
2. 250 mL flasks with screw cap
3. Dispensers, 25 and 100 mL
4. Homogenizer
5. Reaction tubes, 100 mL
6. Glass funnels
7. Purified cotton
8. Analytical balance with 0.1 mg accuracy
9. Trays for evaporation, steel or aluminium foil, minimum 40 mL.
10. Volumetric pipet, 5, 10 or 20 mL, grade A or AS.
11. Infrared lamps, 150 W

B: Chemicals:

1. Methanol, pa
2. Chloroform, pa
3. Purified water

5) Procedure:

1. Weigh 5 to 20 gram sample (determined by the fat and water content of the sample) in a 250 mL flask.
2. Add water so that the total water content (added water + water content of sample) is 16 mL.
3. Add 40 mL methanol and 20 mL chloroform
4. Homogenize for 60 seconds.
5. Add additional 20 mL chloroform.
6. Homogenize for 30 seconds.
7. Add 20 mL water
8. Homogenize for 30 seconds.
9. Cover the flask with the cap and cool in water bath with ice.
10. Remove the residue of the sample by filtering through a piece of cotton in a glass funnel. Collect the solvent in a reaction tube. Cover the funnel with a watch glass to reduce solvent evaporation during the filtration.
11. Remove the water/methanol phase (upper phase) by aspiration using a water aspirator.
12. Transfer an accurate volume (5 – 20 mL) of the chloroform phase to a tared tray for evaporation using the volumetric pipet.
13. Let the solvent evaporate under an infrared lamp.
14. Let the tray cool and weigh the tray with lipid.

6) Calculation:

$$\text{Fat by Bligh \& Dyer} = \frac{d * b * 100}{w * (c - \frac{d}{0{,}92})} \%$$

b = mL added chloroform

c = mL chloroform aliquot for evaporation

d = weight of fat in the tray (gram)

0.92 = specific gravity of fat, g/mL

w = weight of sample (gram)

The result is reported with one decimal. Less than 0.05% is reported as <0.1%.

7. Comments:

1. Chloroform is poisonous by ingestion, skin contact, and inhalation. *
2. Methanol is highly flammable and poisonous by ingestion and inhalation. *
3. To determine the amount of water to be added in 5.2, the water content of the extraction must be known before the fat is determined.
4. Evaporation of chloroform must be performed in a fume hood.

5. If emulsion is formed during homogenization, the filtering in 5.10 can be replaced by centrifugation. If the emulsion is not broken by centrifugation, the analysis must be repeated with application of water containing 2% NaCl in 5.7.

* Source: “kartotek for kjemiske stoffer” (Forlaget VITA-DATA A/S 2nd edn. 1991)