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A COMPARATIVE STUDY OF CONVENTIONAL AND SIMPLIFIED TECHNIQUES FOR POLYUNSATURATED FATTY ACID ANALYSIS

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ABSTRACT

Many physiological processes rely on polyunsaturated fatty acids (PUFAs), which are present in cellular membranes. Understanding the roles and consequences of PUFAs in health and illness requires accurate study of these compounds. This research compares and contrasts traditional methods with more streamlined approaches to PUFA analysis, with an emphasis on the extraction and methylation processes. By merging the extraction and methylation processes into one, we were able to streamline the traditional procedure. The novel approach has been tested on a wide range of biological materials, including human specimens, animal tissues, and cultured cells. While both methods recover long chain fatty acids from tissue samples, statistical analysis shows that the streamlined approach is far more effective than the conventional method. However, there is no change in the relative content of fatty acids between the two methods. By streamlining the process, we can save a lot of time and resources while lowering the risk of sample contamination and loss.

Keywords: technique, fatty acids, gas chromatography, Conventional, Simplified.

I. INTRODUCTION

Polyunsaturated fatty acids (PUFAs) play an essential role in inflammation, cellular communication, energy consumption, and are essential components of cell membranes. There are mainly two groups of these fatty acids, the omega-3 (n-3) and the omega-6 (n-6). Because imbalances in this fatty acid profile have been linked to several chronic diseases, including cancer, diabetes, and cardiovascular disease, maintaining a healthy balance is crucial for maintaining excellent health. As a result, medical diagnosis and scientific inquiry rely on accurate PUFA evaluations in biological samples.

A number of stages are typically included in the conventional method of PUFA testing. Fatty acid methyl esters (FAMEs) are a byproduct of lipid extraction and methylation of biological materials; these FAMEs are suitable for study by gas chromatography (GC) or high-

performance liquid chromatography (HPLC). Despite their widespread use and verification, these techniques may be time-consuming and prone to sample loss and contamination because of all the handling that is necessary. In addition, the analysis is costly and has environmental effects since separate extraction and methylation techniques are required, which in turn need large volumes of solvents and reagents.

The hydrocarbon chain of polyunsaturated fatty acids is characterized by the presence of two or more double bonds. Among polyunsaturated fatty acids (PUFAs), alpha-linolenic acid (ALA, 18:3 n-3) and linoleic acid (LA, 18:2 n-6) stand out. Since these fatty acids are not synthesized by the human body, they are considered essential and must be obtained from diet. Eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), and arachidonic acid (AA, 20:4 n-6) are three of these fatty acids that have extended molecular structures and are integral to several physiological functions.

Critical to the structure and function of cell membranes, polyunsaturated fatty acids (PUFAs) influence the membrane's pliability and the activity of enzymes and receptors housed therein. Bioactive lipid mediators including prostaglandins, thromboxanes, leukotrienes, and resolvins are also formed from these substances. In order to control immune and inflammatory responses, these mediators play a key role. Many studies have shown that omega-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), can reduce inflammation, which may explain why they are protective against cardiovascular disease and other inflammatory disorders.

Multiple health benefits have been associated with polyunsaturated fatty acid (PUFA) intake. A lower risk of cardiovascular disease, better cognitive function, and overall better mental health have all been linked to increased consumption of omega-3 fatty acids. On the other hand, consuming too much omega-6 fatty acids compared to omega-3s might cause inflammation and raise the likelihood of developing chronic illnesses. Hence, precise evaluation of polyunsaturated fatty acid (PUFA) concentrations in biological specimens is crucial for epidemiological investigations, nutritional analysis, and clinical diagnostics.

Conventional Methods for PUFA Analysis

The traditional techniques for PUFA analysis have undergone significant improvements over many years and are widely regarded as the most reliable and accurate approaches in the field of lipidomics. The method usually starts with lipid extraction utilizing organic solvents. The Folch technique, which was created in the 1950s, continues to be one of the most extensively employed procedures. The process is blending the biological sample in a combination of chloroform and methanol, and then separating the phases to separate the lipid portion. This approach is exceedingly efficient, nevertheless, it necessitates meticulous management of volatile and possibly perilous solvents.

After the lipids are extracted, they undergo methylation. The transformation of fatty acids into FAMEs is essential for their analysis using GC-MS, as it increases the capacity of the fatty

acids to vaporize and withstand high temperatures. This enables improved separation and detection throughout the analysis process. Methylation is often conducted using acidic catalysts, such as sulfuric acid in methanol. However, other techniques including base or boron trifluoride are sometimes employed. Optimizing the methylation process is crucial to achieve full conversion of fatty acids into their methyl esters.

GC-MS analysis entails the introduction of FAMEs into a gas chromatograph, where they undergo vaporization and separation according to their volatility and interaction with the stationary phase of the column. Subsequently, the isolated substances are recognized and measured utilizing mass spectrometry, a technique that offers comprehensive data on the molecular weight and structure of the fatty acids. GC-MS is a very sensitive and selective analytical technique that can identify fatty acids even at low concentrations and can differentiate between isomers.

Simplified Methods for PUFA Analysis

The objective of developing simpler techniques for PUFA analysis is to overcome the constraints of the traditional methodology. By integrating extraction and methylation into a unified phase, these techniques greatly diminish the intricacy and length of the analytical procedure. The simplified approach generally entails subjecting the biological sample to a combination of solvents and reagents that enable the extraction of lipids and the process of methylation to occur simultaneously.

As an illustration, a frequently used uncomplicated technique is immediately introducing a methanol-based reagent that contains an acid catalyst to the biological sample. This reagent performs a dual function of extracting lipids and converting fatty acids into FAMEs. Next, the mixture is heated briefly to guarantee full methylation. The resultant fatty acid methyl esters (FAMEs) are extracted using a non-polar solvent, such as hexane, and then evaluated using gas chromatography-mass spectrometry (GC-MS).

The simpler approach has several benefits. It decreases the number of procedural steps and the quantity of chemicals needed, resulting in cost reduction and decreased risk of sample contamination. The decreased manipulation also reduces the likelihood of sample loss, which is especially crucial when dealing with small or valuable biological samples. In addition, the streamlined approach may be executed more expeditiously, rendering it appropriate for high-throughput applications and routine analysis.

II. REVIEW OF LITERATURE

Akinyemi, Olufunmilola et al., (2017) Fatty acid profiling has emerged as a valuable and efficient method for diagnosing, preventing, and treating many illnesses, with a special emphasis on cardiovascular disease. To get precise results that would enhance the well-being of those afflicted by such illnesses, it is vital to employ not just exceptional laboratory techniques but also crucial monitoring of therapy responses.

Technol, Bioprocess et al., (2014) A reliable technique has been devised for precisely determining the concentrations of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in a range of marine-based oils. Attenuated total reflection Fourier transform infrared spectroscopy and multivariate data processing are the tools used in this procedure. There was no chemical treatment of the samples in order to get the spectrum data. Rather, the built spectrum analysis platform was used to directly handle the data. We recommend this method for regular use in the biotech, food research, and related fields since it is efficient, inexpensive, and well-suited to these fields. Using unsupervised pattern recognition methods such unsupervised hierarchical clustering and principal component analysis, the marine oils were categorized into several categories. When compared to the FA profiles found by conventional gas chromatography (GC) lipid analysis, these methods revealed both parallels and variations in the oils' FA compositions. We also measured the amounts of polyunsaturated fatty acids (PUFAs), EPA, and DHA using quantitative analysis.

Martínez, B. et al., (2012) Fatty acid (FA) derivatization and extraction from milk was made easier using a new, straightforward approach. A solution of H2SO4 and methanol was used for the lipid extraction of the milk. After two hours of methylation at 60°C, the FA methyl esters may be extracted using hexane for further chromatographic analysis. After fine-tuning the method's parameters, the straightforward approach was compared to the gold standard for extracting and methylating FAs from milk samples. Compared to the traditional technique, the simple method yielded either comparable or noticeably better recoveries for the majority of the 24 FAs identified. This straightforward technique can handle a large number of samples with little sample handling, contamination, and loss. In sum, the suggested approach is easy to implement, takes little time and money, and produces satisfactory outcomes.

Deore, Ashwini & Annapure, Uday. (2012) One promising biotechnological strategy for the manufacture of beneficial nutraceuticals is the fermentation of polyunsaturated fatty acids (PUFAs) by bacteria. We urgently want a dependable approach that can test several strains in a short amount of time. In this work, we provide a new, easier way to screen for and isolate PUFA-producing bacteria by seeing their growth in the H(2)O(2)-plate test. One way to visually differentiate between bacteria that make PUFAs (no zone of inhibition) and bacteria that do not produce PUFAs (zone of inhibition) is by looking at their oxidative stability in relation to additional H(2)O(2). We used Gas Chromatography-Mass Spectrometry (GCMS) to validate the test findings by injecting fatty acid methyl esters (FAMEs) produced by certain marine bacteria. This assay has the potential to save a lot of time, energy, and money compared to other methods for screening and isolating strains of bacteria that make PUFAs. It is also the most effective, cheap, and specific approach now available.

Barison, Andersson et al., (2010) Here we propose a simple method for determining the fatty acid composition of edible oils by use of (1)H NMR. Glycerol is esterified to all fatty acid chains, so this method doesn't need any mathematical equations to quantify it; instead, it can be done directly in the (1)H NMR spectra by comparing the areas of the characteristic signals

of the fatty acids and the glycerol moiety. Results from using the technique to determine the fatty acid content of various food oils were similar to those from using the AOAC Official gas chromatography method. One major advantage is that it doesn't need any sample pre-treatment processes, such as derivatization or extraction, and it's also quite easy to use.

III. RESEARCH METHODOLOGY

Two methods that integrated extraction and methylation into one step were the subject of our investigation: the conventional method and the simplified technique. Cultured cells, tissue homogenates, and RBCs were the two parts of the materials that were examined. Both the conventional method and the streamlined approach were applied to the samples under the same chromatographic conditions. When we compared the two methods' results using an unpaired t-test, we considered the P values to be significant if they were less than 0.05. Standard deviations are included with the findings to show how they were averaged.

IV. RESULTS AND DISCUSSION

A comparison was made between the results obtained utilizing the standard procedures and the simplified approaches. Under most circumstances, and especially with smaller samples, the results produced by the simplified approach—which does not need previous extraction—are on par with or even superior to those produced by the conventional method. It is evident that the simplified method considerably enhances the recovery of long chain fatty acids from tissue samples when compared to the standard method, which shows no change in the relative composition of fatty acids (Table 1). Fatty acids having 16 or more carbons in their chain were unharmed, despite the potential destruction of certain medium and short chain fatty acids (<16C). Results were consistent among investigations that used cultured cells, RBCs, and mice tails. Based on these findings, it is not necessary to remove the lipid prior to methylation in order to conduct a comprehensive analysis of long chain fatty acid composition using GC. We also found that the analytical findings were impacted when the liquid sample water content was less than 5% by volume of the BF3 solution used. The simplified method works with both solid and liquid materials. However, the less complicated method is limited to detecting shifts in the treated samples' total lipid fatty acid composition. Prior to analysis, lipids (including phospholipids and triglycerides) must be isolated and purified. This is necessary for studies that need knowledge of the fatty acid composition of certain lipid classes.

Table 1: Comparing the Quantitative results of lipid analysis

Fatty Acids Identified	**Quantity (Area of Peak: × 1,000 counts)**		**Composition (% of total LCFA identified)**	
	Conventional Method	**Simplified Method**	**Conventional Method**	**Simplified Method**
16:0	26.5 ± 0.7	30.5 ± 0.8	14.7 ± 0.2	14.4 ± 0.5

18:0	34.2 ± 1.3	37.6 ± 0.3	18.5 ± 0.3	18.4 ± 0.2
18:1(9)	18.6 ± 0.7	21.4 ± 0.6	10.2 ± 0.2	10.1 ± 0.3
18:2(6)	29.7 ± 0.8	35.6 ± 0.4	16.5 ± 0.3	16.6 ± 0.5
20:4(6)	12.9 ± 0.6	15.2 ± 0.3	7.1 ± 0.1	7.2 ± 0.1
22:5(3)	4.3± 0.1	4.9± 0.5	2.2 ± 0.3	2.4 ± 0.4
22:6(3)	55.2 ± 1.7	64.6 ± 1.2	30.5 ± 0.4	30.6 ± 0.4
23:0 (std)	42.6 ± 1.7	47.5 ± 1.1		

V. CONCLUSION

This study shows that a simple method is much better than traditional methods for analyzing polyunsaturated fatty acids (PUFAs). The streamlined technique reduces analytical complexity, cost, and time while offering greater recovery rates, equivalent accuracy, and increased reproducibility by the integration of extraction and methylation into a single step. Because of these advantages, the streamlined approach is a good choice for both large-scale investigations and regular laboratory usage as a replacement for PUFA profiling. Dietary recommendations, illness processes, and ecological lipid profiles can all benefit from more precise and efficient PUFA analysis, which is essential for environmental, medical, and nutrition-related studies.

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