Characterization of Omega Dietary Supplements by combining FT-IR and Pattern Recognition Tools

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Abstract
Omega-3 dietary supplements have been linked with health benefits including decreased cardiovascular disease mortality, anti-inflammatory effects, reduced blood pressure, and antiarrhythmic activity. The sources of these effects, polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) docosahexaenoic acid (DHA), are almost exclusively found in seafood products and are essential fatty acids for humans. The concentration of EPA and DHA in fish is highly variable depending on species, catch location, environment, season and processing parameters in determining the ratio of fatty acids found in the oil. Our objective was to characterize the composition of commercial Omega-3 dietary supplements using mid-infrared spectroscopy, gas chromatography, and chemometrics. Supplements were purchased from retailers that included a large amount of source, processing, and compositional variability. Fatty acid composition of oils was determined by fatty acid methyl esters gas chromatography analysis. The resulting fatty acid composition data was combined with spectral information using CHEMOMETRICS-soft independent modeling of class analogy (SIMCA)-pattern recognition analysis for screening and classification of fatty acid composition of fish oil supplements.

Introduction
Interest in omega-3 dietary supplements has increased due to their health and nutritional benefits. Scientific studies on these products have uncovered several health benefits such as decreased cardiovascular disease mortality, and the potential reduction in blood pressure. The compounds primarily responsible for these effects, polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA), 20:5, and docosahexaenoic acid (DHA), 22:6, are almost exclusively found in seafood products and are essential fatty acids for humans (dKH). Determination of EPA and DHA in fish depends on the type of fish, with salmon, herring, mackerel, tuna, and sardines having the highest concentrations. As each PUFA has a different role in health promotion and disease prevention, it is important to understand the variability in composition within fish oils, and whether they are supplied as triglycerides, esters, or fatty acids. Alkyl esters of omega-3 fatty acids are more prone to oxidation than their triglyceride counterparts, and some evidence suggests that the biochemical activity of alkyl esters is lower than that of intact triglycerides. Modern, rapid techniques to determine composition, authenticity, and quality of supplements are quickly becoming available, but development of Fourier transform infrared (FT-IR) and attenuated total reflectance (ATR) spectrometry equipment has opened the door to novel methods. FT-IR systems provide a more robust alternative to older dispersive systems, and ATR-IR systems can accommodate a wide variety of sample types, from gasses to liquids, to powders. Although the food and supplement industries have utilized near infrared (NIR) spectroscopy for decades, applications of mid-infrared (MIR) spectroscopy have only recently gained ground. Alkyl esters of fatty acids are non-destructive and rapid techniques that allow for analysis of the chemical bonds in a system. When combined with multivariate statistical analysis and modern computational power (collectively known as chemometrics), the large amount of information provided by MIR can be used to replace traditional techniques (Bosque-Sendra et al., 2012). In oil analysis, these traditional techniques are both time consuming and expensive. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are used in the analysis of oils, but the extensive sample preparation, long run-times, and destructive nature of the analysis has piqued interest in spectroscopic alternatives.

Objective
The objective of this research was to create a model for rapid determination of fatty acid composition and classification of fish oil supplements.

Methodology

25 Samples of Fish, Flaxseed, and GLO oils

Cary 630 portable benchtop FT mid-infrared spectrometer

Derivatization and methylation

Agilent 6890 gas chromatography system

Soft Independent Modeling of Class Analogy (SIMCA) Classification Model

Partial Least Square Regression (PLSR)

Discussion
• Analysis of fatty acid composition by gas chromatography revealed a wide range of EPA (6-65%) and DHA (34-60%) concentrations. These values are comparable to the label claims, with most samples having more EPA/DHA than claimed.

• With correlation coefficients of cross-validation of r 0.98 and SECVs of 2.73% and 1.76%, for EPA and DHA respectively, PLSR fatty acid determination is comparable to modern chromatographic techniques (Table 2). Ratio of performance deviation (RPD) of the models versus the reference data shows that this method is capable of quantifying EPA & DHA across a broad compositional range.

• SIMCA classification revealed clustering by oil source, as well as 3D spacing with regards to EPA/DHA content. This region responsible for this significant separation, 900-1300 cm⁻¹, is associated with C-C and C-O stretching. The band primarily responsible for distinction, 1038 cm⁻¹, has been found to correlate with the C-O stretching in esters.

• The Fish A group has a distinct carbonyl band shift (from 1745 cm⁻¹ to 1737 cm⁻¹) that is associated with alkyl esters. This may reflect the difference in the oil supplements as primarily fatty acid alkyl esters instead of triglycerides.

Conclusions
We have shown a fast, simple, and reliable method for determining the characterization of the fish oils in these supplements and for quantification of the important omega-3 fatty acids present, to support and validate label claims. The portable ATR-MIR method not only provides fatty acid composition information, but can be used to determine the state of these fatty acids; whether they be methyl/alkyl esters, free fatty acids, or triglycerides.

References