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Analytical Methods

Potential of spectroscopic techniques and chemometric analysis for rapid measurement of docosahexaenoic acid and eicosapentaenoic acid in algal oil

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ABSTRACT

Developing rapid methods for measuring long-chain ω-3 (n-3) poly-unsaturated fatty acid (LCPUFA) contents has been a crucial request from the algal oil industry. In this study, four spectroscopy techniques, namely visible and short-wave near infra-red (Vis–SNIR), long-wave near infra-red (LNIR), mid-infra-red (MIR) and nuclear magnetic resonance (NMR) spectroscopy, were exploited for determining the docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) contents in algal oil. The best prediction for both DHA and EPA were achieved by NMR spectroscopy, in which the determination coefficients of cross-validation (r^2_{CV}) values were 0.963 and 0.967 for two LCPUFAs. The performances of Vis–SNIR and LNIR spectroscopy were also accepted. The variable selection was proved as an efficient and necessary step for the spectral analysis in this study. The results were promising and implied that spectroscopy techniques have a great potential for assessment of DHA and EPA in algal oil.

1. Introduction

People began to understand the health benefits of consuming long-chain ω-3 (n-3) poly-unsaturated fatty acids (LCPUFA) since the epidemiological reports on Inuit living in Greenland published in 1970s (Igarashi et al., 2000). Consuming long-chain LCPUFA was reported having health benefits for humans, against such as cancer (Berquin et al., 2007), cardiovascular disease (Wang et al., 2006), Parkinson's disease (Bousquet et al., 2008), and cognitive decline (Morris, Evans, Tangney, Bienias, & Wilson, 2005). LCPUFA are obtained primarily from fatty fish, such as tuna, salmon and mackerel (Fedorova-Dahms, Marone, Bailey-Hall, & Ryan, 2011). However, algae are the primary producers of long-chain LCPUFA in the food chain (Arterburn et al., 2007). Fish do not synthesise long-chain LCPUFA de novo, lacking the required key enzymatic activities (Sargent & Tacon, 1999). Instead, fish consume microscopic algae or other algae-consuming fish (Stamey, Shepherd, de Veth, & Corl, 2012). Compared with fish oil, algal oil has further advantages, such as the lack of unpleasant odour, reduced risk of chemical contamination and better purification potential (Pulz & Gross, 2004).

Production of algal oil with the LCPUFA quality guaranteed is important for the modern algal oil industry to be successful in today's highly competitive market. LCPUFA content in the source of algal oil is subject to algae species and natural variations due to growth environment. Besides the source of algae, the LCPUFA content of concentrated algal oil depends on the process regimes and the levels of other ingredients in the algal oil. As the great variance in raw algae could result in highly variable products, it is a major challenge faced by the algal oil industry to improve consumer satisfaction by providing consistent quality of products. On the other hand, the products should be labelled with correct quality information. To make huge profit, mislabeling of LCPUFA levels has happened for some algal oil products, in which the amounts of LCPUFA shown on the packages do not match the actual contents. In order to guarantee consumers' rights and interests, all the algal oil products sold in the markets must be liable to high quality management. In fact, guaranteeing LCPUFA content in algal oil would encourage consumers to have brand loyalty.

Gas chromatography (GC) is generally used to determine fatty acids in foods and biological samples based on their methyl ester derivatives or directly as the ethyl esters (Curtis, 2007). However, there are many manipulation steps for GC analysis, which includes a lot of sample preparation and takes a long time to process. Besides the inspection of LCPUFA for algal oil products, it is also necessary to determine LCPUFA of algal oil during its process in a

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production plant environment. Real-time monitoring the progress of the enrichment and determination of the optimal end point are required (Azizian, Kramer, Ehler, & Curtis, 2010). However, GC methods are not suitable for rapid determination of LCPUFA in algal oil in a production plant environment. Rapid screening techniques to determine LCPUFA contents in algal oil are of great interest for both industry and suppliers.

Spectroscopy techniques may be such promising choices to predict LCPUFA in algal oil in an accurate and rapid way. Some typical spectroscopic techniques, such as visible and near infra-red (Vis–NIR) spectroscopy, mid-infra-red (MIR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy, have been accepted as powerful tools and increasingly applied for food control and analysis (Monakhova, Kuballa, & Lachenmeier, 2013; Sun, 2008). The principle of the first two techniques bases on the interaction between electromagnetic radiation emitted from lights and physicochemical materials existed in food. In general, the pigment and colour information of food samples could be reflected by measuring their visible spectra. The responses of the electromagnetic vibration of the molecular bonds C—H, O—H, N—H and C—O in chemical substances of food could be found in MIR spectra, while the overtones and combinations of these bonds are reflected in the NIR spectra. NMR spectroscopy could provide physical and chemical properties of molecules where the atoms are contained. In NMR measurement, the resonant frequencies of certain atomic nuclei present in different chemical surroundings are measured. Spectroscopic techniques have several advantages over the time-consuming GC method, such as simplicity, rapid analysis, and minimal sample preparation. These techniques do not alter the sample or produce hazardous wastes, so that they agree with the green chemistry principle. Currently, the applications of spectroscopic techniques for fatty acid determination of edible oil are mainly focused on fish oil (Azizian et al., 2010; Guillen, Carton, Goicoechea, & Uriarte, 2008; Igarashi et al., 2000), Camellia oleifera oil (Yuan, Wang, Chen, Zhou, & Ye, 2013), and olive oil (Lerma-García, Simó-Alfonso, Bendini, & Cerretani, 2011). Wu et al. (2009) successfully applied near infra-red spectroscopy to determine alpha-linolenic acid and linoleic acid in edible oils, which included grinding sesame oil, benne oil, purified benne oil, purified olive oil, peanut oil, camellia oil, soya oil, *Hippophae rhamnoides* L. fruit oil and seed oil. However, to the best of our knowledge, there is few works on rapid LCPUFA determination of algal oil using spectroscopic techniques in tandem with multivariate selection and calibration. Moreover, no direct comparison between Vis–NIR, MIR, and NMR spectroscopy for predicting LCPUFA in algal oil or other edible oils has been published yet.

The LCPUFA of interest include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). DHA and EPA are major components of neuronal membranes and have a wide range of functions, from modulating synaptic plasticity and neurochemistry, to neuroimmune-modulation and neuroprotection (Luchtman & Song, 2013). Currently, the challenge in the algal oil industry is to assess LCPUFA contents because they are crucial quality attributes of algal oil associated with consumer's health. Reliable methods are required for predicting LCPUFA in a rapid way. The main aim of this work was to investigate the potential of four spectroscopic techniques namely visible and short-wave near infra-red (Vis–SNIR), long-wave near infra-red (LNIR), MIR and NMR spectroscopy, for predicting DHA and EPA contents in algal oil. The successful outcome of the work will be of great importance in assuring buyers and consumers of algal oil consistently high quality and protecting their interests. The work was conducted by (1) measuring spectra of algal oil samples in Vis–SNIR (350–1050 nm), LNIR (870–2537 nm), MIR (4000–650 cm^{-1}), and NMR (−4.1791 to 16.4762 ppm) regions; (2) selecting optimal variables that are most useful for DHA/EPA prediction; (3) building multivariate calibration models using spectral data in the full spectral range or at the spectral bands of selected variables; and (4) comparing the performances of four spectroscopic techniques for DHA and EPA prediction in algal oil.

2. Materials and methods

2.1. Samples preparation and spectral measurements

In this study, algal oil samples from the products of Gold Nemans, Nemans, Zmarto, Bionuo, and USANA were obtained from local markets, resulting in 21 sample sets of algal oil obtained. Four samples were obtained from four batches with different production time for each set. Therefore, there were 84 samples (4 samples per set × 21 sample sets) obtained for Vis–SNIR, LNIR, MIR, and NMR spectral measurements, respectively. In other words, there were four groups of samples used for different spectroscopic measurements and each group had 84 samples. Vis–SNIR spectra in the wavelength range of 350–1050 nm were acquired using a USB4 000 miniature fibre optic spectrometer (Ocean Optics, Inc., USA) in transmittance mode. A NIR 256-2.5 spectrometer (Ocean Optics, Inc., USA) in transmittance mode was used to measure LNIR spectra, which covered the wavelength range of 870–2537 nm. Both spectrometers were equipped with a light source, SMA-terminated optical fibres and a cuvette holder (DH2000, P400-VIS/NIR, and CUV-UV Holder for 1-cm Cuvettes, respectively, Ocean Optics, Inc., USA). The quartz cell with a 1 cm path length was filled with oil sample and was inserted into the cuvette holder for the transmittance spectral measurement. The measurement of MIR spectra in the wavenumber range of 4000–650 cm^{-1} was achieved by using a FT-IR spectrometer Nicolet iS10 (Thermo Fisher Scientific Inc., Waltham, MA, USA) attached with a ZnSe ATR (attenuated total reflection) accessory. A Bruker AVANCE 500 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) operating at 500.17 MHz for the proton nucleus at 298 K was used for scanning ^{1}H NMR spectra of samples in the chemical shift from −4.1791 to 16.4762 ppm. Tetramethylsilane (TMS) was used as the reference for recording the full spectrum of each sample. Before the Fourier transformation, an exponential window function with a line-broadening factor of 1 Hz was applied to the free induction decay. The ^{1}H NMR spectra were phased and baseline-corrected using Topspin 2.1 (Bruker, Karlsruhe, Germany) and were automatically reduced by using the AMIX (version 2.5, Bruker GmbH, Karlsruhe, Germany).

2.2. GC analysis

Reference contents of DHA and EPA for multivariate calibration were measured using gas chromatography (GC) method. Algal oils were saponified and the fatty acids were esterified (with a 30% solution of BF_3 in methanol) (AOAC method 963.22, 2000). A gas chromatography (Agilent 6890N; Agilent Technologies Inc., USA) with a flame ionisation detector (FID) and an Agilent DB-1701 capillary column, 30 m × 0.32 mm × 0.25 μm (Agilent Technologies Inc., USA) was used to analyse the methyl esters. A 1 μL aliquot of treated sample was injected at a flow rate of 3.0 mL/min with nitrogen (99.995% purity) as carrier gas. After injection, the column temperature was maintained at 180 °C for 2 min and then increased at a rate of 10 °C/min to. After that the temperature was maintained at 240 °C for 10 min. The temperatures of the injector port and the detector were set at 250 °C. The flow rates of hydrogen and air were 45 mL/min and 450 mL/min, respectively. The retention time relative to commercial methyl esters standards (DHA and EPA) (Sigma, USA) were used to identify DHA and EPA. Analysis of standard mixtures and individual correction coefficients were used to calculate the contents of DHA and EPA. Table 1 shows the reference values of DHA and EPA in algal oil measured using GC analysis.