Process Biochemistry 44 (2009) 401–405

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

FTIR determination of free fatty acids in fish oils intended for biodiesel production

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ARTICLE INFO

Article history:
Received 7 February 2008
Received in revised form 25 September 2008
Accepted 1 December 2008

Keywords:
Fish oil
Free fatty acids
Sodium hydrogen cyanamide
Titration
Fourier transform infrared (FTIR) spectroscopy

ABSTRACT

Biodiesel is commonly derived from vegetable oils and animal (fish and livestock) fats by alkali- or lipase-catalyzed transesterification reactions. Since free fatty acid (FFA) content is a critical parameter in the conversion of fish oils to methyl esters, the performance of a Fourier transform infrared (FTIR) spectroscopic method was assessed as an alternative to the conventional AOCS titrimetric method. The FTIR method involves the simultaneous extraction of FFAs and their stoichiometric conversion to their salts using a weak base, sodium hydrogen cyanamide (NaHNCN) dissolved in methanol, followed by measurement of the carboxylate band, $\nu(COO^-)$, at 1573 cm^{-1} relative to a baseline at 1820 cm^{-1} in the differential spectrum of the methanol extract. With minor modifications, this method was found to be capable of responding linearly to oleic acid (0–6.5%) addition, producing a FFA calibration equation having a S.D. of ±0.014% FFA. FTIR and titrimetric analytical results were compared for samples prepared by standard addition as well as for fish oils extracted from salmon skin which had been stored up to 120 days at −20 °C. Both methods responded in a comparable manner; however, the FTIR method was more reproducible and accurate as well as simpler to carry out and was deemed to be a better primary method than the titrimetric method. The FFA content of Atlantic salmon (*Salmo salar*) skin lipids increased linearly from ~0.6% to 4.5% within 120 days, likely as a result of autoxidation. It was concluded that the NaHNCN-based FTIR method is a flexible, viable instrumental alternative to the AOCS titrimetric procedure for the determination of FFA content of fish tissue lipids destined for biodiesel production.

1. Introduction

The fish processing industry generates large quantities of tissue waste and byproducts which tend to be either discarded or retailed at low value for fertilizer or animal feed. Fish processing activities may generate byproducts such as: heads, trimmings (tail, fin), viscera and skin. In some fish species, such as salmon the skin is peeled off longitudinally before the flesh is sold or sent for canning or smoking. The skins like the other byproducts listed above are mostly not put to profitable use and are discarded or dumped as waste in landfills or in the ocean (Atkins, 2008, personal communication; Atkins et Frères Inc., Mont-Louis, Québec). In the processing of Atlantic salmon (*Salmo salar*), between 33% and 50% of the total body weight constitutes byproduct, much of which is discarded and ends up in the waste stream [1]. In studies conducted in our laboratory, the lyophilizing of salmon skins for 24 h resulted in 58% moisture loss and 61.53 ± 1.14% extractable lipids. Given the high volumes of fish skins generated by commercial fish processing plants such as Atkins et Frères Inc. (typical samples are shown in Fig. 1) fish skin and tissue discards could be a potential source of fats/oils for biodiesel production. However, due to the relatively high autolytic activity associated with fish tissue in combination with its high polyunsaturated fatty acid (PUFA) content, the lipids are very prone to both lipolysis and oxidation. Thus, the oils extracted from fish tissue will tend to have high levels of free fatty acids (FFAs), which are problematic during the conversion of oils to methyl esters for biodiesel. In general, the transesterification reaction is carried out in methanol with alkaline catalysis; however, if the lipid contains more than 0.5% FFA, soaps can form and the efficiency of the catalyst can be compromised [2]. As such, the FFAs first need to be removed by refining, and accurate determination of the FFA content of the refined oil is required to ensure that all the FFAs have been neutralized, to minimize any inhibition of the subsequent catalytic transesterification reaction with methanol to produce biodiesel.

FFA determinations are traditionally carried out by titration, a reasonably sensitive, but cumbersome and subjective procedure if not carried out using an auto-titrator. FTIR spectroscopy has been advocated as an alternative means of determining a variety of oil quality parameters [3], including FFA content, by making use of the extensive functional group information available in the mid-infrared (MIR) portion of the spectrum. FTIR determination, of FFAs may be based on measurement of their characteristic functional group absorption at 1711 cm^{-1} (ν(C=O) of dimerized carboxylic acid groups) [4]. However, the partial overlap of this absorption

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doi:10.1016/j.procbio.2008.12.004

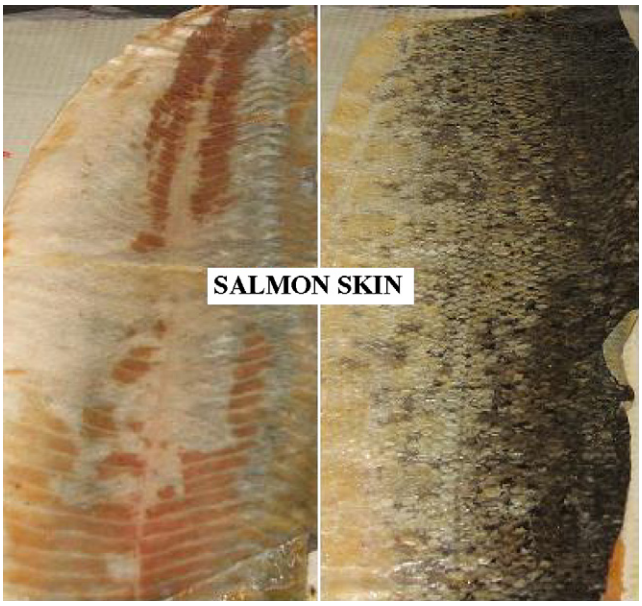

Fig. 1. Dissected salmon skin.

band with the strong ester ν(C=O) band of triacylglycerols gives rise to several limitations. Although this overlap is accounted for during calibration of the FTIR method, the intensity of the ester ν(C=O) band (and hence its contribution to the measured "FFA absorbance") depends on the saponification number of the oil, making it necessary to develop an individual calibration equation for each type of oil [5]. In addition, matrix effects arise because the position of the ester ν(C=O) band (and hence the extent to which it overlaps with the FFA ν(C=O) band) changes with changes in polarity of the oil, such as occur upon autoxidation [6]. These limitations may be circumvented by reacting the FFAs with base to convert them to carboxylate salts and measuring the absorption of the COO^- group, which occurs in a region where spectral interferences are minimal. The McGill IR Group has developed several methods based on the latter approach for the determination of FFA content in edible oils [4,7,8] as well as acid number (AN) in lubricants [9]. Originally, the base used was KOH [4], but errors due to saponification of triacylglycerols could be encountered if the analysis was not carried out very quickly [4,10]. Subsequently, saponification was avoided by using a weak base, potassium phthalimide, added to the oil in *n*-propanol [7]; however, this method required FTIR analysis of the sample both before and after treatment with the base in order to compensate for matrix effects. A more recently developed method [8], which uses a methanol solution of a weak base, sodium hydrogen cyanamide (NaHNCN), to extract the FFAs from the oil and convert them to their salts, avoids both saponification and the need to analyze two samples to obtain a single result. Furthermore, since the FFA salts are concentrated in the extraction solvent, analytical sensitivity is increased and automation of the FTIR analysis is facilitated because the samples are methanol solutions rather than viscous oils [8].

To date, none of the MIR FFA methods described above has been applied to FFA determination in fish oils whereas near-infrared (NIR) spectroscopy has been used to analyze for FFA content in mackerel oil [11] and in salmon fillets [12]. Although MIR and NIR spectroscopy are related analytical methodologies, NIR methods rely on advanced chemometrics, typically partial least squares (PLS) regression, to extract the pertinent data from overlapping overtone bands, and they characteristically require a large number of reference samples to obtain a representative calibration [13]. Thus, the NIR FFA methods [11,12] are secondary methods reliant on a primary reference method for calibration and applicable only to the oils represented by the calibration standards. In contrast, the MIR NaHNCN-based FFA method is a straightforward primary method in its own right, based on a simple, defined, stoichiometric reaction and measurement of a fundamental, well-defined absorption.

The objective of this study is to evaluate the suitability of the NaHNCN-based FFA method [8] for FFA determination in fish oils destined for biodiesel production. This method was originally designed to measure the low levels of FFA (<0.1%) in refined edible oils, and its performance at the high FFA levels commonly encountered in oils extracted from fish tissue has not previously been examined. Thus, this study assesses the reproducibility and accuracy of the NaHNCN-based FFA method relative to the AOCS titrimetric method in relation to tracking the changes in FFA content of lipids extracted from dried fish skin stored over time and compares their relative performance in that regard.

2. Materials and methods

2.1. Materials

Sodium hydrogen cyanamide (NaHNCN 99+%), oleic acid (90% technical grade), activated silica gel, sodium hydroxide and potassium acid phthalate were all obtained from Sigma–Aldrich Canada (Oakville, ON). Anhydrous methanol (MeOH) and ethanol were purchased from Fisher Scientific (Fair Lawn, NJ), and refined canola oil was purchased locally. Salmon skin samples from salmon fillets with almost no adherent tissue (muscle/flesh) were obtained from a fish market in Montreal (Waldman Plus). The skin was cut into smaller pieces, lyophilized for 24 h and stored at −20 °C. The lyophilized skin was extracted for 1 h with hexane using a SER 148 Velp solvent extractor (Velp Scientifica, Usmate, Italy) at a sample: solvent ratio of 1:10 (w/v). Following evaporation of the solvent, the salmon skin oil (SSO) was stored at −20 °C until needed for analysis. The NaHNCN–MeOH reagent was prepared by dissolving 4 g of NaHNCN in 1 L of anhydrous MeOH and was kept dry over 4 Å molecular sieves at all times. Prior to use, the solution was allowed to age [8] at room temperature (25 °C) until the ν(C≡N) band at 2100 cm^{-1} had completely disappeared (~4 days).

2.2. Instrumentation

The FTIR spectrometer employed for this study was a Bomem WorkIR spectrometer (Bomem, Québec City, PQ) equipped with a deuterated triglycine sulfate (DTGS) detector and purged with dry air from a Balston dryer (Balston, Lexington, MA). The spectrometer was controlled by an IBMcompatible Pentium 150-MHz PC running under proprietary Windows-based UMPIRE® (Universal Method Platform for InfraRed Evaluation) software (Thermal-Lube, Pointe-Claire, PQ). Sample analysis was carried out using a 100-μm CaF_2 transmission flow cell (International Crystal Laboratories, Garfield, NJ); the output line was connected to a trap and vacuum, and the input line was equipped with a valve to allow samples to be loaded by aspiration. All spectra were collected by co-adding 16 scans at a resolution of 8 cm^{-1} and a gain of 1.0 and were ratioed against an open-beam background spectrum.

2.3. Sample preparation/analytical protocol

A 1:5 (w/v) ratio of oil to NaHNCN–MeOH reagent solution was used for all analyses. Five grams of oil were weighed into a 50-mL tarred centrifuge tube to which 25 mL of the NaHNCN–MeOH solution was added. The sample was vortexed for 30 s and then centrifuged at 6000 × *g* for 15 min to separate the oil and solvent layers, with the upper layer being the analyte. The analytical protocol is summarized in Fig. 2. Prior to analysis, the cell and transfer lines were rinsed with methanol. The NaHNCN–MeOH solution and the upper solvent layer in the centrifuged sample were then sequentially aspirated into the transmission flow cell to collect their spectra. Prior to the spectral subtraction step shown in Fig. 2, an intermediate step was added to compensate for any dilution of the solvent layer of the sample by oil, whereby a dilution factor was determined by dividing the height of the methanol overtone band at 2045 cm^{-1} (measured relative to a baseline point at 2136 cm^{-1}) in the spectrum of the sample by its height in the spectrum of the NaHNCN–MeOH solution. Following multiplication of the spectrum of the sample by the inverse of this dilution factor, the spectrum of the NaHNCN–MeOH solution was subtracted from it to produce the differential spectrum of the analyte, the spectral features of the solvent being removed in the process. The absorbance of the carboxylate [$\nu(COO^-)$] band was then measured at 1573 cm^{-1} relative to a single baseline point at 1820 cm^{-1}.

2.4. IR calibration and titration

FFA calibration standards were prepared by gravimetrically adding 0–6.5% (w/w) oleic acid to refined canola oil, which had been passed through a column of activated