Efficient Fractionation and Analysis of Fatty Acids and their Salts in Fat, Oil and Grease (FOG) Deposits

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Abstract: A fractionation methodology of fat, oil and grease (FOG) deposits was developed based on the insolubility of fatty acid salts in dichloromethane (DCM) and the relatively high solubility of fatty acids and triglycerides in DCM. Using this method, coupled with spectral analysis, it was shown that fatty acids rather than fatty acid salts were the predominant species in FOG deposits obtained from three metropolitan locations in the United States and that fatty acid triglycerides were either not detected or were present in very small concentrations. This solubility-based fractionation approach also revealed the presence of nitrogen-containing compounds that had not been previously detected in FOG deposits including peptides and (or) proteins. The comparison of the ratios of stearic acid salts to stearic acid versus the ratio of palmitic acid salts to palmitic acid in FOG deposits may indicate that the initial step in FOG deposit formation is the preferential precipitation of stearic acid salts.

Key words: FOG deposits, fractionation, composition, FAME analysis, fatty acid salts, fatty acids

1 INTRODUCTION

Nationwide, about 40-50 percent of sanitary sewer overflows are caused by accumulations of hard, tough and adhesive materials referred to as fat, oil and grease (FOG) deposits (see Fig. 1) [1]. In 2000, sanitary sewer overflows in 18 states resulted in the release of an estimated 1.2 billion gallons of raw sewage into the environment [2]. Large neighborhoods have been flooded by sewage backups presumably caused by FOG deposits that caused long term evacuations [3]. Understanding the compositions of FOG deposits is the first step in understanding and mitigating their formation process.

Previous studies of FOG deposits have not addressed the specific chemical makeup of different fatty acid species (e.g., triglyceride esters, fatty acid salts and the fatty acid itself) potentially present that can be separated based on inherent chemical differences (e.g., solvent solubility). The objective of this study was to devise a simple and efficient fractionation approach to directly determine the weights and compositions of fatty acid salts, fatty acids, triglycerides and other components in FOG deposits. Determination of the percentages of individual fatty acid salts and fatty acids in separate fractions of FOG deposits can provide clues regarding the mechanism of their formation.

2 MATERIALS AND METHODS

2.1 Materials

Calcium stearate was acquired from Alfa Aesar. Palmitic acid and ammonium palmitate were acquired from Sigma Aldrich.

Abbreviations: FOG: fat, oil and grease; DCM: dichloromethane, FAME: fatty acid methyl ester; IR: infrared; NMR: nuclear magnetic resonance

Fig. 1 FOG deposit collected from Racine, WI lift station.

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2.2 Standard Analytical Methods

Standard procedures and official published methods from the Association of Official Analytical Chemists (AOAC) and American Public Health Association (APHA) were used as noted and modified as described.

2.3 Sampling of FOG Deposits

Three samples were obtained from sanitary sewers from three locations in Wisconsin, USA: Milwaukee Sanitary District (from bar rack), Racine (floating “egg” at a lift station), and the Mishawaka wastewater plant.

2.4 FOG Deposit Drying

Before the solvent-based fractionations were performed, FOG deposits were dried under vacuum at ambient temperature until samples approached constant weight. Without this treatment, the variable amounts of water randomly trapped within cavities in FOG deposits would interfere with obtaining an accurate measure of percent mass recovery of both the solvent-soluble and solvent-insoluble fractions. The Racine and Mishawaka FOG deposits were cut into small pieces before placing under vacuum to achieve faster drying. FOG deposits were stored at ambient temperature before and after drying.

2.5 Biohazard Considerations

FOG deposits were considered to be biohazards due to their probable contact with pathogens in municipal sewers. Safety protocols included storing and manipulating samples in a dedicated laboratory fume hood, retaining contaminated personal protective equipment (i.e., gloves) within the fume hood until bagging and disposal, and rinsing the surfaces of all equipment sequentially with 1% sodium hypochlorite and ethanol before removing from the hood.

2.6 FOG Deposit Fractionation

Dichloromethane (DCM) was used as the fractionation solvent based on its relatively high solubility for fatty acids (0.30 mL and 5.0 mL DCM were required to dissolve 1.0 g of oleic acid and palmitic acid, respectively, at ambient temperature) and its negligible solubility for fatty acid salts. FOG deposit fractionation was performed using a ratio of 22.3-23.1 mL DCM per gram dried FOG deposit which was well in excess of the volume/weight ratio needed to fully dissolve both saturated and unsaturated fatty acids. Calcium salts of fatty acids are insoluble in DCM and also less dense and thus float at the DCM surface allowing convenient and selective isolation.

The fractionation procedure began with loading an Erlemeyer flask containing a magnetic stir bar with accurate amounts of dried FOG deposit (typically 10-15 g) and then addition of an amount of DCM required to achieve the specified volume/weight ratio. The contents of the flask were stirred rapidly at ambient temperature for approximately 18 hours and the mixture was then allowed to sit for at least one hour to allow effective component separation. The following phases were typically observed within the extraction flask after the settling period (see Fig. 2):

- An upper, grey layer of floating and finely dispersed fatty acid salts plus other materials that are insoluble in DCM and less dense than DCM such as seeds,
- The middle DCM turbid solution that contains dissolved fatty acids and any other components that are soluble in DCM, and
- A lower sediment layer consisting of grit, rust or other components that are both insoluble and more dense than DCM.

The next step involved separation and removal of all floating material and the bulk of the DCM solution from the sediment at the bottom of the extraction flask to a separate flask (flask A). This separation was best performed by leaving the extraction flask stationary to avoid sediment dispersal while using a large volume pipet to remove the floating solid and DCM from the surface of the mixture. After removing all floating solid and a portion of the DCM solution, the sediment and remaining DCM solution in the extraction flask was passed through a 0.45 micron pore size nylon membrane filter under vacuum using fresh DCM to collect the sediment on the filter.

The mixture of floating solid and DCM solution in flask A and the filtrate from the above filtration were combined and filtered in portions through a pressure filtration device equipped with a 0.45 micron pore size nylon membrane filter while using fresh DCM to achieve complete transfer. The filter cake was removed from the filtration device and adhering powder was thoroughly collected from the inner walls of the pressure filter and combined with the filter cake. The collected powder was transferred to a tared vessel and placed in a vacuum oven at ambient temperature until constant weight was obtained. This material comprised the floating and DCM-insoluble fraction.

The filtrate from the pressure filtration was placed on a rotary evaporator to remove the solvent and the residue of DCM.
the solvent stripped filtrate was further dried under vacuum at ambient temperature until constant weight was obtained. This material comprised the DCM-soluble fraction.

2.7 Fraction Analysis

2.7.1 Fatty Acid Analyses

Fatty acids were determined by the fatty acid methyl ester (FAME) analytical approach using AOAC method 996.06 MOD which involves converting all carboxylic acids, triglycerides, diglycerides and monoglycerides to their methyl esters by a combination of esterification for fatty acids and transesterification for the other species. Thus, the FAME analysis provides total quantities of fatty acids plus the fatty acids present in triglycerides, diglycerides and monoglycerides without differentiating among these categories. This method incorporates the non-natural triglyceride of undecylenic acid as a surrogate for determining overall mass recovery. To ensure full account of the stearic acid content of authentic calcium stearate, the standard AOAC method was modified to include initial pre-acidification of both the DCM-insoluble and the DCM-soluble fractions to facilitate conversion of all salts of carboxylic acids to their conjugate acids prior to esterification. This modification provided close to the theoretical quantity of stearic acid from calcium stearate and was used for the FAME analysis of all DCM-insoluble and DCM-soluble fractions.

2.7.2 Metals, Sulfur and Nitrogen Analyses

Calcium, iron, magnesium and sulfur were determined using AOAC method 990.08/SW846 6010B which uses Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Nitrogen was determined using AOAC method 992.15 MOD which uses the Dumas method. Analyses were performed by Microbac Laboratories, Inc., Pittsburgh Division.

2.7.3 Fourier Transform Infrared (IR) Spectroscopy

Infrared (IR) spectra were obtained on a Digilab Fourier Transform FTS-7000c spectrometer equipped with a UMA 600 microscope. This instrument was run using 4 wave-number resolution and was scanned between 4000-450 wavenumbers to obtain 400 scans using a mercury, cadmium, telluride (MCT) detector cooled with liquid nitrogen. Samples were compressed into a thin film on a zinc selenide crystal positioned on a microscope stage and were run in the transmittance mode. Spectral deconvolution was performed using GRAMS software from Thermo Fisher Scientific, Inc.

2.7.4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Proton (^1H) NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer (500 MHz). Spectra were obtained using deuterochloroform as the solvent and tetramethylsilane (TMS) as an internal chemical shift reference standard.

3 RESULTS

3.1 Fractionation of FOG Deposits

FOG deposits from Milwaukee, Racine and Mishawaka sanitary systems were dried and then extracted with DCM to obtain nearly quantitative mass recovery as shown in Table 1. These high mass recoveries provided a basis on which to quantitate FOG deposit components present in DCM-insoluble and DCM-soluble fractions and also estimate non-identified components based on the analytical approaches described below.

3.2 Analysis of Separated FOG Deposit Fractions

Separated FOG deposit fractions were analyzed by a combination of IR and NMR spectroscopy, FAME analysis; metals, sulfur and nitrogen analyses and ninhydrin tests.

| Table 1  | Fractionation of FOG deposits using dichloromethane (DCM). |
|----------|----------------------------------------------------------|
| FOG Sample | Dried Starting Wt (g) | DCM Insoluble Fraction (g) | DCM Soluble Fraction (g) | Grit/Seeds (g) | Total Recovery (g) | Percent Recovery | % DCM Insoluble Material | % DCM Soluble Material |
| Milwaukee Met. San. District | 13.49 | 2.65 | 9.99 | 12.64 | 93.7 | 21.0 | 79.0 |
| Racine, Floating at Lift Station | 10.48 | 2.55 | 7.70 | 0.0720/0.0987 | 10.43 | 99.5 | 24.9 | 75.1 |
| Mishawaka Wastewater Plan | 12.58 | 4.11 | 6.73 | 1.53/0.30 | 12.67 | 100.7 | 37.9 | 61.1 |

a) Grit and seeds were not collected in this extraction.
b) Percentages were calculated excluding contributions from grit and seeds.
3.2.1 IR Analysis

The IR spectrum of the DCM-insoluble fraction obtained from the Racine FOG deposit and the IR spectrum of authentic calcium stearate are shown in Fig. 3. The IR spectrum of the DCM-soluble fraction from the Racine FOG deposit and the IR spectrum of authentic palmitic acid are shown in Fig. 4. Analysis of these IR spectra indicated that fatty acid salts were exclusively present in the DCM-insoluble fractions and fatty acids were exclusively present in the DCM-soluble fraction which indicates the high fractionation selectivity of this extraction approach. IR analysis of individual fractions also provides information concerning the presence of other potential FOG components such as proteins, peptides, amino acids and ammonium salts of fatty acids.

3.2.2 Fatty Acid Methyl Ester (FAME) Analysis

FAME analyses of DCM-insoluble and DCM-soluble fractions obtained from Milwaukee, Racine, and Mishawaka FOG deposits are shown in Table 2 which provides the total saturated fatty acid content and the individual fatty

![Fig. 3 Infrared Spectra of Racine DCM-Insoluble Fraction and Calcium Stearate.](image)

![Fig. 4 Infrared Spectra of Racine DCM-Soluble Fraction and Palmitic Acid.](image)

| Sample                             | DCM Solubility | Total % FAs | C14:0 | C16:0 | C18:0 | C18:1 | C18:2 | % Sat. Fatty Acids | % Surrogate Recovery |
|------------------------------------|----------------|-------------|-------|-------|-------|-------|-------|-------------------|----------------------|
| Milwaukee Met. San. District       | DCM Insoluble  | 62.46       | 4.48  | 65.54 | 14.15 | 5.35  | 0.34  | 92.77             | 89.0                 |
|                                   | DCM Soluble    | 70.65       | 4.28  | 69.23 | 12.19 | 6.17  | 0.64  | 90.71             | 95.9                 |
| Racine, Floating at Lift Station  | DCM Insoluble  | 77.16       | 3.24  | 62.86 | 20.82 | 7.25  | 1.03  | 89.52             | 108.9                |
|                                   | DCM Soluble    | 87.99       | 4.31  | 69.32 | 14.73 | 6.60  | 0.79  | 90.33             | 92.35                |
| Mishawaka Wastewater Plant        | DCM Insoluble  | 79.73       | 3.70  | 68.23 | 19.12 | 4.07  | 0.41  | 93.62             | 78.08                |
|                                   | DCM Soluble    | 90.56       | 4.86  | 72.67 | 14.51 | 4.51  | 0.00  | 93.69             | 105.30               |

a) The percent that detected fatty acids are of each fraction.
b) The percent that each fatty acid is of the total fatty acids present.
c) A non-natural fatty acid surrogate is used to determine extraction and derivatization efficiencies.
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3.2.3 Analysis of Metals, Sulfur and Nitrogen

Metal analyses (calcium, iron and magnesium) as well as sulfur and nitrogen analyses of fractionated FOG deposits are shown in Table 4 and indicates that the major cation in fatty acid salts found in the DCM-insoluble fractions from the Milwaukee and Racine FOG deposits calcium (95.4% and 97.6%, respectively) while iron and magnesium were minor components in these salts. However, in the Mishawaka DCM-insoluble fraction, the calcium percentage was 67.1% while iron was the next most prevalent metal cation. The presence of nitrogen exclusively in the DCM-insoluble fractions is believed to be caused by peptides and/or proteins. The almost exclusive presence of metals in the DCM-insoluble fractions provides another measure of the high fractionation efficiency of the current method in separating

| Sample                     | DCM Solubility | Total % FAs | Surrogate Recovery % | Total % Corrected FAs | % Salts as Calcium Palmitate | % Salts as Metal Carboxylates | % Non-Identified Mass |
|----------------------------|----------------|-------------|----------------------|-----------------------|-----------------------------|-------------------------------|-----------------------|
| Milwaukee Met. San. District | DCM Insoluble  | 62.46       | 89.0                 | 70.2                  | 75.7                        | 75.5                          | 24.5                  |
|                            | DCM Soluble    | 70.65       | 95.9                 | 73.7                  | –                           | –                             | 26.3                  |
| Racine, Floating at Lift Station | DCM Insoluble | 77.16       | 108.9                | 70.9                  | 76.4                        | –                             | 23.6                  |
|                            | DCM Soluble    | 87.99       | 92.35                | 95.3                  | –                           | –                             | 4.7                   |
| Mishawaka Wastewater Plant  | DCM Insoluble  | 79.73       | 78.1                 | 102                   | 110.2                       | –                             | – 10.2                |
|                            | DCM Soluble    | 90.56       | 105.30               | 86.00                 | –                           | –                             | 14.0                  |

Table 3 Correction of FAME data and estimates of non-identified mass based on surrogate recovery factors.

| Sample                     | DCM Solubility | Percent Metals and Nitrogen | Percent Metal | Percent Nitrogen |
|----------------------------|----------------|------------------------------|---------------|-----------------|
| Milwaukee Met. San. District | DCM Insoluble  | Ca 5.81, Fe 0.136, Mg 0.107, S 0.136, N 0.81, Pot. % Protein 5.09 | 5.09          | 0.81            |
|                            | DCM Soluble    | 0.002, 0.012, 0.003, 0.046, 0.04, 0.26 | 0.26          |
| Racine, Floating at Lift Station | DCM Insoluble | 5.27, 0.045, 0.083, 0.019, 1.03, 6.43 | 6.43          | 1.03            |
|                            | DCM Soluble    | 0.000, 0.002, 0.001, 0.092, < 0.03, – | –             |
| Mishawaka Wastewater Plant  | DCM Insoluble  | 4.28, 2.03, 0.073, 0.335, 2.80, 17.5 | 17.5          | 2.80            |
|                            | DCM Soluble    | 0.004, 0.034, 0.034, 0.083, < 0.03, – | –             |

Table 4 Percent metals, sulfur and nitrogen in fractionated FOG deposits.

a) Obtained by dividing total fatty acid percentages by surrogate recovery fraction.
b) Obtained by dividing by 0.927, which is the fraction of palmitic acid in calcium palmitate.

a) Obtained by multiplying % nitrogen by 6.25, weight relationship between % nitrogen and "average" % protein, and factor is similar when applied to proteins, peptides, amino acids or any combination thereof.
fatty acids salts from fatty acids.

4 DISCUSSION
4.1 Fractionation of FOG Deposits

As shown in Table 1, recovery data of DCM-insoluble and DCM-soluble fractions after drying FOG samples demonstrate that nearly quantitative mass recovery was obtained from each FOG deposit. The weight percentages of the DCM-insoluble fractions from Milwaukee, Racine and Mishawaka were found to be 21.0%, 24.9% and 37.9%, respectively. The DCM-insoluble fractions were found to be predominantly fatty acid calcium salts and the DCM-soluble fractions were found to be predominantly fatty acids. These data unambiguously indicate that fatty acids are the major components while fatty acid salts are the minor components in the FOG deposits studies. A recent study provides a correlation between hard water content and increased calcium content in FOG deposits\(^{9}\) and potentially suggests that correlations should also exist between hard water content and the percentages of FOG deposit fatty acid calcium salts.

DCM-soluble fractions that are predominantly comprised of fatty acids are waxy and cohesive solids whereas the DCM-insoluble fractions that are predominantly comprised of fatty acid salts are powdery and non-cohesive solids. The potential implication of these observations is that fatty acids serve to “glue” FOG deposits together while fatty acid salts contribute to the tensile strengths of these materials.

4.2 IR Analysis of Fractionated FOG Deposits

4.2.1 DCM-Insoluble Fractions

It can be seen in Fig. 3 that the IR spectrum of the DCM-insoluble fraction from the Racine FOG deposit and that of authentic calcium stearate are essentially identical to each other. The spectrum of this FOG sample is characterized by the presence of two relatively high absorbance peaks at 1577 and 1541 wavenumbers and lower absorbance peaks at 1472, 1434, and 1419 wavenumbers that are characteristic of calcium salts of fatty acid carboxylic salts\(^{2}\). In agreement with this assignment, analysis of this sample by FAME analysis and metal analysis indicated these fractions are composed mainly of fatty acids and the major cation is calcium ion.

The IR spectrum of the DCM-insoluble fraction from Mishawaka was almost identical to the analogous IR spectrum from Racine. The IR spectrum of the DCM-insoluble fraction from the Milwaukee FOG deposit was similar to the IR spectra from the Racine and Mishawaka DCM-insoluble fractions but it also had multiple absorptions in the 1600-1730 wavenumber region. Deconvolution of this spectral region resulted in peaks at approximately 1626, 1660, 1685, and 1720 wavenumbers and these peaks had decreasing absorbencies with increasing frequency. At this time, the additional peaks in the deconvoluted IR spectrum are unidentified.

4.2.2 DCM-Soluble Fractions

It can be seen in Fig. 4 that the IR spectra of the DCM-soluble fraction from the Racine OG deposit and that of authentic palmitic acid are very similar to each other. The carbonyl group of the Racine sample was observed at 1701 wavenumber while that of palmitic acid was observed at 1704 wavenumbers. Since this DCM-soluble fraction was shown to be comprised mainly of fatty acids by FAME analysis and also to have a negligible metal content based on metal analysis, these combined data demonstrate this fraction was comprised mainly of fatty acids. The IR spectra of the DCM-soluble fractions from Milwaukee and Mishawaka were almost identical to the IR spectrum of the analogous fraction from Racine, thus indicating that each of these DCM-soluble fractions had very similar compositions.

4.2.3 Effectiveness of DCM-Based Fractionation Approach

As seen in Figs. 3 and 4, the main absorptions in the IR spectra of dried calcium salts of fatty acids are two peaks at about 1577 and 1541 wavenumbers while dried fatty acids had strong absorptions at 1701-1704 wavenumbers. The effectiveness of DCM-based fractionation was demonstrated by the fact that IR absorptions characteristic of fatty acid salts were exclusively found in DCM-insoluble fractions while the carbonyl absorption characteristic of fatty acids was exclusively found in DCM-soluble fractions for each FOG deposit analyzed. The near absence of metals in DCM-soluble fractions provides additional support of the selective exclusion of metal salts from that phase. It is also noted that the IR absorptions in both fractions are well resolved in comparison to the relatively crowded IR spectrum of an unfraccionated FOG sample\(^{9}\).

4.3 Fatty Acid Methyl Ester (FAME) Analysis of Fractionated FOG Deposits

Palm, peanut, soybean, corn, and canola oils contain 49%, 19%, 15%, 14%, and 7% saturated fatty acids, respectively, while beef tallow, pig lard and chicken fat contain 48%, 42% and 33% saturated fatty acids, respectively\(^{10}\). Table 2 shows that the total saturated fatty acid percentages of both the DCM-insoluble and DCM-soluble fractions of each FOG deposit are about 90% or higher. These high levels of saturated fatty acids in FOG deposits indicate that selective deposition of both saturated acid salts and saturated fatty acids occurred during deposition of FOG deposits since typical cooking oils and animal fats contain significantly lower levels of saturated fatty acids.

Palmitic acid is the predominant saturated fatty acid in both cooking oils and animal fats\(^{10}\). However, it can be seen that the concentrations of palmitic acid and its salts were 4-5 times higher than concentrations of stearic acid...
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and its salts in these FOG deposits. It is instructive to compare the relative amounts of these two fatty acids in the DCM-insoluble fractions versus DCM-soluble fractions for each FOG deposit where in each FOG deposit the relative concentrations of stearic acid salts are higher than stearic acid concentrations while the concentrations of palmitic acid are higher than palmitic acid salt concentrations. One potential explanation of these inverted concentration ratios is that the initial step in FOG deposit formation involves preferential initial precipitation of stearic acid salts compared to precipitation of palmitic acid salts. In agreement with this hypothesis, the aqueous solubility of calcium stearate has been found to be somewhat lower than that of calcium palmitate at two different temperatures, but the high relative error in these measurements makes this a tentative conclusion\(^{11}\). We postulate that after FOG growth has been initiated by the selective preferential precipitation of stearic acid salts, continued deposition will be less selective and other available varieties of fatty acid salts and fatty acids can precipitate on these seed crystals based on their relative availabilities. Interestingly, it has been demonstrated that oleic acid, the major unsaturated fatty acid in both cooking oils and animal fats\(^{10}\), undergoes biologically induced cleavage/reduction in estuary sediments to form palmitic acid and stearic acid that are in equilibrium with each other\(^{12}\). If these phenomena occur in sewer systems, the stearic acid undergoing equilibration could be captured by available calcium ions to provide an additional source for preferential calcium stearate precipitation.

Table 3 provides the percent total fatty acids and the percent surrogate recovery of each fraction. The "Total % Corrected Fatty Acid" is calculated by dividing by the percent saturated fatty acids by the surrogate recovery fraction. For the DCM-insoluble fractions, the fatty acid percentage is converted to "% Salts as Calcium Palmitate" since calcium is the predominant cation and palmitic acid is the predominant fatty acid in the DCM-insoluble fractions. This conversion is performed by dividing by the mass fraction of palmitic acid in pure calcium palmitate (0.927).

This correction factor was refined for the DCM-insoluble sample from the Milwaukee Metropolitan District by incorporating the weighted averages of all 14 contributing fatty acids while arbitrarily assuming these fatty acids were exclusively paired with calcium to obtain an adjusted fatty acid content of 0.930. This assumption is reasonable due to the much smaller concentrations of iron and magnesium compared to calcium and the fact that the atomic weight of magnesium is less than that of calcium by the identical amount that the atomic weight of iron is greater than that of calcium. This adjusted fatty acid content was applied to the DCM-insoluble fraction which resulted in the slight correction shown in the column heading "Percent Salts as Metal Carboxylates". Given that this corrected fatty acid content only slightly modifies the calculated "% Salts as Calcium Palmitate", this approach, although more rigorous, was not applied to the other two DCM-insoluble fractions from Racine and Mishawaka.

The last column in Table 3 is "Percent Non-Identified Mass" which is obtained for DCM-insoluble fractions by subtracting the "Percent Salts as Calcium Palmitate" or "Percent Salts as Metal Carboxylates" from 100 percent. For DCM-soluble fractions, the "Percent Non-Identified Mass" is obtained by subtracting the "Total Corrected Fatty Acids" from 100 percent. These calculations provide estimates of masses present in each type of fraction that are not determined by FAME analysis. The non-identified mass was found to be in the range of 5-26% for the Racine and Milwaukee samples. The over-recovery in the DCM-insoluble fraction obtained for the Mishawaka sample is unexplained, and may warrant, as shown by additional irregular results described below, re-examination of the Mishawaka FOG deposit.

To help identify the nature and quantities of this non-identified mass, \(^1\)H NMR spectroscopy was applied to DCM-soluble fractions but not to DCM-insoluble fractions due to the insolubility of calcium fatty acid salts even in polar NMR solvents. A large number of small NMR absorptions were observed in these fractions. Candidate compound classes for this missing mass include triglycerides, diglycerides and monoglycerides that were found to be relatively soluble in DCM in our studies. These compounds are potential precursors of the fatty acids and fatty acid salts that comprise the bulk of FOG deposits. However, these NMR studies indicated that triglycerides were present in only small quantities (nil to 1 percent) while diglycerides and monoglycerides were conclusively shown not to be present in DCM-soluble fractions. These findings were supported by obtaining authentic samples of each type glyceride that served as analytical standards. We currently do not have plausible candidates for the small NMR absorptions.

The relative absence of triglycerides in each FOG deposit studied herein is in contrast with results provided in a recent study of FOG deposits\(^6\), where FOG deposits were extracted with an 80:20 mixture of hexane/tert.-butyl ether according to APHA 5520B. It is hypothesized that the extracted "oil" as reported in the study may have contained mainly fatty acids rather than triglycerides. This hypothesis is supported by the fact that the potential palmitic acid in their sample is fully soluble in less relative solvent than used in their study. Furthermore, the fact that their FOG mixtures were acidified to achieve pH 2 before extraction indicates that at least a portion of the fatty acid salts that were present would be converted to their fatty acids and be extracted by this solvent system.
4.4 Analysis of Metals, Sulfur and Nitrogen

4.4.1 Metals Analysis

Metal analysis provided in Table 4 indicates that calcium was the major cation in fatty acid salts present in DCM-insoluble fractions while iron and magnesium were minor components in the Milwaukee and Racine FOG deposits. Calcium concentrations in these fractions were found to be 5.81 and 5.27 percent while iron and magnesium were 2-4 percent of calcium concentrations. The theoretical calcium percentage of calcium palmitate (the predominant metal cation and fatty acid anion) is 7.27 percent so it could appear that the observed calcium percentages in these two samples were appreciably less than the calcium percentage required for calcium palmitate. However, this is due to the fact that, as shown in Table 3, the fatty acid salt percentages in these two fractions were close to 76 percent if the salts were primarily calcium palmitate (which they are). Dividing these observed calcium percentages by this fraction (0.76) provide values close to the expected calcium percentages.

On the other hand, in the DCM-insoluble fraction from the Mishawaka FOG deposit, the calcium concentration was appreciably lower (4.28 percent) and the iron concentration was significantly higher (2.03 percent) than the concentrations observed in the Milwaukee and Racine samples. Correction of the calcium concentration as above is not feasible due to the anomalous value of the "% Salts as Calcium Palmitate" (greater than 100) for this fraction. Efforts to determine if other fatty acid salt cations were present in this sample are provided below.

4.4.2 Nitrogen and Sulfur Analysis

It can be seen in Table 4 that appreciable nitrogen and smaller amounts of sulfur were observed in the DCM-insoluble fractions of the three FOG deposits while much smaller or trace amounts of nitrogen and sulfur were observed in the three DCM-soluble fractions. The highest nitrogen concentration was observed in the DCM-insoluble fraction of the Mishawaka FOG deposit. One potential source of nitrogen is ammonia which has been measured at about 1668 wavenumbers and also broad peaks close to 3370 wavenumber that are slightly more intense than similar broad absorptions in calcium stearate (see Fig. 2). Proteins are reported to have amide I absorptions in the 1600-1700 wavenumber region and NH absorptions near 3300 wavenumbers while peptides have amide I absorptions close to 1680 wavenumbers. Thus, IR spectroscopy provides some support that proteins and (or) peptides are present in DCM-insoluble fractions. Amino acids are not present since their NH absorptions are found at about 3070 wavenumbers and this absorption was not present in the IR spectra of all DCM-insoluble fractions in this region.

More conclusive evidence for the presence of proteins and (or) peptides in FOG deposits was obtained by performing ninhydrin tests on both DCM-soluble and DCM-insoluble samples of FOG deposits from Milwaukee and Racine. Ninhydrin is a reagent that turns blue-purple when heated in the presence of peptides and proteins. Each of the DCM-insoluble fractions tested gave a purple color while the corresponding DCM-soluble fractions did not undergo a color change. These results suggest that peptides and (or) proteins could be at least partial sources of the nitrogen content in DCM-insoluble fractions.

AOAC method 992.15 MOD for nitrogen analysis provides the factor 6.25 to multiply by the percent nitrogen to approximate general protein and peptide content if these species were the sole source of nitrogen. The presence of these compounds could partially explain the presence of sulfur in these FOG deposits due to presence of sulfur-containing-amino acids such as cysteine or cystine. Sulfur-containing ionic surfactants, such as sodium lauryl sulfate, are other potential sulfur sources and these surfactants would be found in DCM-insoluble fractions due to their expected low solubilities in DCM (as are the salts of fatty acids).

The DCM-insoluble fraction from the Mishawaka FOG deposit had an appreciably higher nitrogen and sulfur content than the corresponding fractions from Milwaukee and Racine. It is possible that these relatively high nitrogen and sulfur contents correlate with the relatively high weight percentage of this fraction.

5 CONCLUSIONS

A method to fractionate FOG deposits with DCM was developed to aid analysis of FOG deposits by separation and quantitation of the fatty acid salts and fatty acids present in these materials. This method is based on the insolubility of fatty acid salts and the relatively high solubility of fatty acids in DCM. A key benefit of using DCM as the extraction solvent is that not only are fatty acid salts insoluble in DCM but they are also less dense and hence float to the surface which allows their separation from grit and sand that are also insoluble in this solvent. These combined properties
allow facile separation and determination of these classes of compounds and components. The effectiveness of this fractionation method was demonstrated by near 100 percent mass recovery and spectrally by showing that only fatty acid salts were found in DCM-insoluble fractions and only fatty acids were found in DCM-soluble fractions obtained from different FOG samples.

Other analytical techniques on these fractionated samples enabled additional insight to the FOG composition. Using pre-acidification which ensured conversion of calcium salts to the fatty acid, FAME analyses indicated that saturated fatty acids were the predominant type fatty acids and constituted at least 90 percent of both the DCM-soluble and DCM-insoluble fractions. Metal analyses indicated that calcium was by far the major metal (cation) in fatty acid salts in DCM-insoluble fractions. Based on variations in stearic acid concentrations in DCM-soluble and DCM-insoluble fractions, the hypothesis is made that the initial step in FOG deposit formation involves the preferential precipitation of stearic acid salts. Percent nitrogen was determined in both type DCM fractions and it was found that significant quantities of nitrogen were present in the DCM-insoluble fractions while very little or no nitrogen was found in DCM-soluble fractions. Proteins and peptides are one potential source for nitrogen. IR spectroscopy and positive ninhydrin tests for each DCM-insoluble fraction tested (Milwaukee and Racine samples) supported the presence of proteins and (or) peptides in these fractions. Proton NMR spectroscopy, used to analyze the DCM-soluble fractions, conclusively showed that monoglycerides and diglycerides were not present in these fractions and that triglycerides were shown only to be present in very low concentrations ranging from nil to less than 1 percent.

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