Additional file 1:



Figure S1 – Linearity of response of selected FAMEs in GC-MS analysis



Figure S2: GC chromatogram of aqueous phase, Aq1

 

Figure S3 – HPLC chromatogram of aqueous phase, Aq1



Figure S4 – 1D GC analysis of wax, Wax1, including expanded C20 region of the chromatogram



Figure S5 – Comparison of 1-alcohols in LHcL1 using 1D-GC and GCxGC, showing the 1:1 relationship expected as well as the linear regression fit to the data (forced to fit the origin)



Figure S6 – McLafferty rearrangement of long chain methyl ester to form the McLafferty ion



Figure S7 – 1H NMR of C14 standards, 1%w/w in CDCl3



Figure S8 – Repeatability of 1HMR analysis of a wax sample

The presence of a small amount of formate in 1H NMR spectra shown in Figure S9, indicated by the peak at approximately 8.2ppm, was caused by a small level of contamination originating from the vial lid during solvation of the wax in the CDCl3. This was an artefact and no formate was observed to be present in any further samples once this problem had been resolved.

Figure S9 – Comparison of 1H NMR and GCxGC analysis of 1-alcohols in FT waxes and light hydrocarbons

|  |  |  |
| --- | --- | --- |
| **Analyte** | **Relative Response**  | **RSD %** |
|   | Run 1 | Run 2 | Run 3 | Run 4 | Run 5 | Run 6 | Run 7 | Run 8 | Run 9 | Run 10 |   |
| Methyl hexanoate | 1.091 | 1.106 | 1.101 | 1.101 | 1.109 | 1.108 | 1.093 | 1.123 | 1.122 | 1.134 | 1.2 |
| Methyl Octanoate | 1.202 | 1.210 | 1.200 | 1.199 | 1.206 | 1.215 | 1.154 | 1.241 | 1.253 | 1.240 | 2.3 |
| Methyl decanoate | 1.575 | 1.591 | 1.574 | 1.557 | 1.562 | 1.589 | 1.515 | 1.624 | 1.652 | 1.619 | 2.5 |
| Methyl dodecanoate | 1.991 | 2.070 | 2.039 | 2.038 | 2.057 | 2.061 | 2.089 | 2.136 | 2.109 | 2.121 | 2.1 |
| Methyl tetradecanoate | 2.456 | 2.480 | 2.465 | 2.453 | 2.462 | 2.467 | 2.600 | 2.566 | 2.528 | 2.529 | 2.1 |
| Methyl hexadecanoate | 2.830 | 2.878 | 2.836 | 2.841 | 2.838 | 2.840 | 3.068 | 2.967 | 2.909 | 2.889 | 2.6 |
| Methyl octadecanoate | 3.200 | 3.253 | 3.201 | 3.200 | 3.159 | 3.166 | 3.444 | 3.286 | 3.235 | 3.207 | 2.6 |
| Methyl eicosanoate | 3.538 | 3.606 | 3.540 | 3.524 | 3.494 | 3.485 | 3.813 | 3.587 | 3.519 | 3.469 | 2.8 |
| Methyl docosanoate | 3.876 | 3.929 | 3.893 | 3.865 | 3.831 | 3.811 | 4.191 | 3.927 | 3.827 | 3.748 | 3.1 |
| Methyl tetracosanoate | 4.179 | 4.250 | 4.224 | 4.197 | 4.167 | 4.128 | 4.600 | 4.264 | 4.120 | 4.070 | 3.5 |

Table S1 – Relative standard deviation (RSD) established for FAME analyte response at a concentration of 10.0 µg mL-1 over 10 analyses for the methyl esters of C6 – C24 carboxylic acids in GC-MS analysis

|  |  |  |  |
| --- | --- | --- | --- |
|  | Recoveredµg/kg | Actualµg/kg | Recovery% |
| Hexanoic acid | 42 | 45 | 93 |
| Decanoic acid | 53 | 50 | 106 |
| Octadecanoic acid | 49 | 51 | 96 |

Table S2 – Recovery of carboxylic acid standards in GC-MS analysis

|  |  |
| --- | --- |
| Carboxylic acid measuredas methyl ester | Concentration mg/kg |
|  | Preparation 1 | Preparation 2 |
|  |  |  |
| Hexanoic acid | 34.9 | 33.5 |
| Heptanoic acid | 45.5 | 45.9 |
| Octanoic acid | 49.4 | 49.9 |
| Nonanoic acid | 56.5 | 56.8 |
| Decanoic acid | 60.0 | 61.1 |
| Undecanoic acid | 62.7 | 64.4 |
| Dodecanoic acid | 60.7 | 61.4 |
| Tridecanoic acid | 50.1 | 50.8 |
| Tetradecanoic acid | 37.0 | 37.7 |
| Pentadecanoic acid | 23.5 | 24.0 |
| Hexadecanoic acid | 26.0 | 24.4 |
| Heptadecanoic acid | 8.81 | 8.77 |
| Octadecanoic acid | 20.1 | 16.9 |
| Nonadecanoic acid | 3.48 | 3.47 |
| Icosanoic acid | 2.45 | 2.43 |
| Henicosanoic acid | 1.56 | 1.54 |
| Docosanoic acid | <1 | <1 |
| Tricosanoic acid | <1 | <1 |
| Tetracosanoic acid | <1 | <1 |
| Pentacosanoic acid | <1 | <1 |
| Hexacosanoic acid | <1 | <1 |
| Heptacosanoic acid | <1 | <1 |
| Octacosanoic acid | <1 | <1 |
| Nonacosanoic acid | <1 | <1 |
| Triacontanoic acid | <1 | <1 |

Table S3 – Example of a duplicate sample preparations of a liquid hydrocarbon for carboxylic acid analysis

|  |  |  |
| --- | --- | --- |
| C14 Standard | Average calculated carbon number | Average class compound group per carbon chain |
| 1-olefin | 2- olefin | 1-Alcohol |
| Tetradecane | 14.0 | 0.00 | 0.00 | 0.00 |
| 1-Tetradecene | 14.1 | 0.98 | 0.00 | 0.00 |
| 1-Tetradecanol | 13.5 | 0.00 | 0.00 | 0.98 |

Table S4 – 1H NMR of C14 standards, calculation of average carbon number and class compound per carbon chain

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Wax sample replicate | Average carbon number | 1-olefin (mmol/g) | 2- olefin (mmol/g) | 1-alcohol (mmol/g) |
| A | 19.1 | 0.246 | 0.186 | 0.192 |
| B | 19.0 | 0.235 | 0.204 | 0.195 |
| C | 18.6 | 0.246 | 0.201 | 0.199 |

Table S5 – Concentration of class compounds in repeat analyses of a wax sample

**Calculations using 1H NMR peak integrations**

The response of all protons in the 1H NMR spectra were assumed to be constant and therefore the proportion of carbon atoms in different locations of the hydrocarbon structure and functional groups could be calculated. For example, if the Fischer-Tropsch product was a purely paraffinic product, being composed of only saturated linear chains, then the calculation of average carbon number, Nc, would simply be:

1. Nc = $([Total C as CH2]/[Total C as CH3] )x2 +2$

The 1H NMR inputs for the calculation of Nc would therefore be the intensity of the peaks associated with CH2 and the terminal CH3 which are assigned as F and G respectively in table 7. Considering the ratio of the protons on each carbon type the calculation becomes:

1. Nc = $([3xF])/([G])+2$

However, since 1-olefin, 2-olefin, 1-alcohol and aldehyde functionalities were detected in the 1H NMR analysis of the pilot plant products then the calculation becomes more complicated and the following equations should be used:

1. Total C within chain, $X=[A]+[C]+[E]/2+[F]/2$
2. Total C at chain ends$, Y=[B]/2+[C]/2+[D]/2+[G]/3+[H]$
3. Nc = $2x([X]/[Y] )+2$

Since the peak due to the methyl at the chain end of the 2-olefin group, at ~1.6ppm, is close to that of water, which is present to some degree in the most CDCl3 solvents, the accurate quantification of the contribution of this carbon is not possible. Therefore, the value of C is included again with an appropriate factor being applied, i.e. C/2. This then gives the contribution of the methyl chain end of the 2-olefin to the calculation of total chain ends, Y.

The concentration of functional groups, as mmol/g, is given by the following calculation.

1. Concentration (mmol/g) $=\left[Mole fraction of functional group\right]x1000/[Average Mw of product]$

 These units are chosen for quantification to allow for an accurate assessment rather than assuming each functional group is equally populated across the chain lengths, and then quoting these as mol%, which would certainly not be correct for the olefins. The appropriate number of carbon atoms contribution to the functionality group and the number of protons associated with these carbon atoms need to be considered in these calculations. For example, the calculation of 1-olefin calculation uses only the terminal CH2 group, that of the 2-olefin uses both CH parts of the olefinic double bond and the 1-alcohol uses the CH2 group associated with the terminal oxygenate functionality.

1. Average Mw of product, W, $=[Nc]x([A]x13+[B/2]x14+[C]x13+[C/2]x15+[D/2]x31+[E/2]x14+[F/2]x14+[G/3]x15+[H]x29)/(([X]+[Y]) )$
2. 1-olefin (mmol/g) $=(([B/2])x2/[Y])x1000/[W]$
3. 2-olefin (mmol/g) $=(([C/2])x2/[Y])x1000/[W]$
4. 1-alcohol (mmol/g) $=(([D/2])x2/[Y])x1000/[W]$

**Calculations using 13C NMR peak integrations**

The integration of the quantitative 13C NMR spectra, with peak assignments given in table 8 allows the average chain length and concentration of the specific groups to be calculated. Again, the functional group concentrations are given in units of mmolg-1. For the calculation of the concentration the total intensity of the 13C NMR peaks associated with the functional group and the total number of carbon atoms contributing are used as inputs.

1. Total C within chain, S $=\left[A\right]+\left[C\right]+\left[D\right]+\left[E\right]+\left[F\right]+\left[K\right]$
2. Total C at chain ends, T $=\left[B\right]+\left[G\right]+\left[H\right]+\left[I\right]+[J]$
3. Average carbon chain length, Nc2 $= 2x([S]/[T] )+2$
4. Average Mw, V $=[Nc2]x(\left(\left[A\right]+\left[C\right]+\left[D\right]+\left[E\right]+\left[F\right]\right)x13+\left(\left[B\right]+\left[G\right]+[K])x14\right)+(\left[G\right]x17)+\left([H\right]+\left[I\right]+[J])x15))/([S]+[T])$
5. 1-olefin (mmolg-1)$ = ((\left[A\right]+\left[B\right])/2)x1000/(\left[V\right]) $
6. 2-olefin (mmolg-1) $((\left[C\right]+\left[D\right]+\left[E\right]+[F])/2)x1000/(\left[V\right]) $
7. 1-alcohol (mmolg-1) $=[G]x1000/(\left[V\right]) $