Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information for

Analysis of wild and farmed salmon using ^{13}C solid-state NMR and MRI directly on fillet tissue

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^{13}C T_1 values and shift assignments

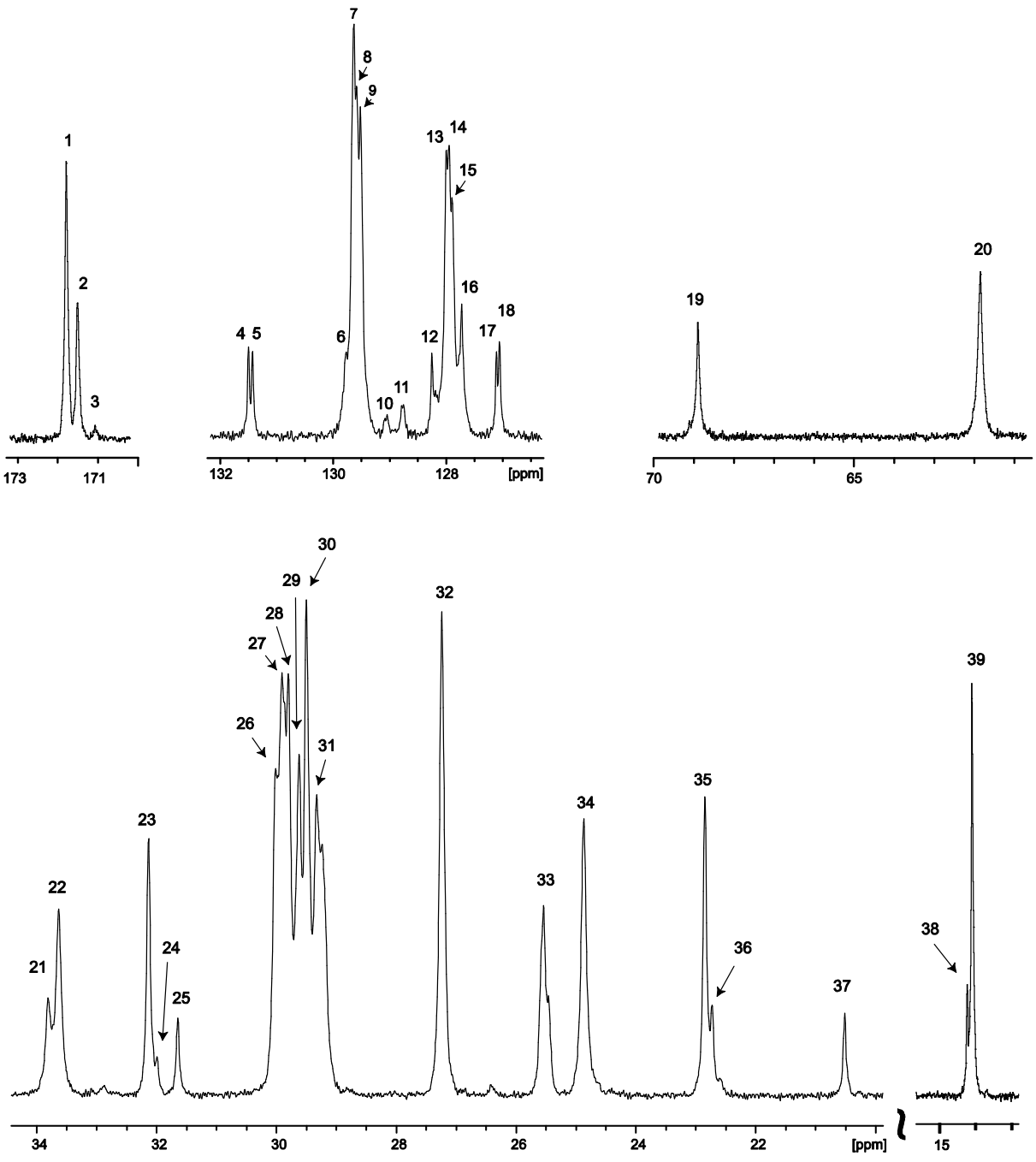


Figure S1. ^{13}C MAS spectrum of farmed salmon #1, labelled numerical in accordance with Table S1.

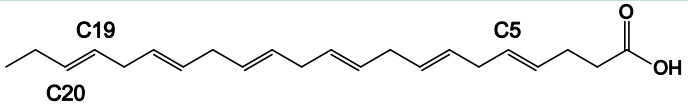
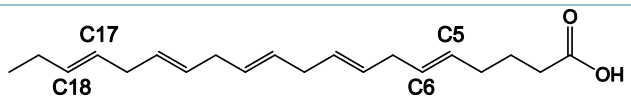
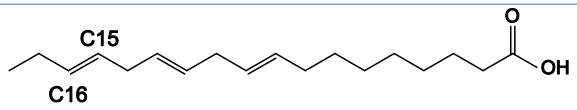
Table S1. ^{13}C T_1 and Chemical Shift Assignments of MAS spectra of regular farmed salmon. Assignments based on (M. Aursand & Grasdalen, 1992) and (M. Aursand, Rainuzzo, & Grasdalen, 1993).

Number	Description	Carbon	T_1 (sec) Farmed Salmon	T_1 (sec) Wild Salmon
1	Carbonyl α/γ	$C_{\alpha/\gamma}=O$	4.1	2.1
2	Carbonyl β	$C_{\beta}=O$	3.3	1.6
3	Carbonyl β w/DHA	$C_{\beta}=O$	-	2.2
4	EPA C_{18} / DHA C_{20}	-C=C-	4.9	5.2
5	ALA C_{16}	-C=C-	3.8	x
6	18:2 n-6 C_{13}	-C=C-	-	-*
7	ω9 20:1, C_{11-12} + 22:1 C_{13-14}	-C=C-	2.8	1.1*
8	ω7 16:1 C_9	-C=C-	-	-
9	ω9 18:1 C_9	-C=C-	2.5	-*
10	DHA C_5	-C=C-	2.3	1.0
11	EPA C_{5-6}	-C=C-	2.1	1.3
12	Highly unsaturated f.a.	-C=C-	4.3	2.7
13	Polyunsaturated f.a.	-C=C-	4.3	2.0
14	Polyunsaturated f.a.	-C=C-	4.0	2.0
15	18:2 n-6	-C=C-	-	-
16	Not identified	-C=C-	-	2.4
17	EPA C_{17} / DHA C_{19}	-C=C-	-	4.9
18	C_{15} – ALA	-C=C-	8.0	X
19	All f.a.	$(C_{\alpha}O)$-$(\underline{C}_{\beta}O)$-$(C_{\gamma}O)$	0.74	0.45
20	All f.a.	$(\underline{C}_{\alpha}O)$-$(C_{\beta}O)$-$(\underline{C}_{\gamma}O)$	0.48	0.25
21	All f.a.	$O=C$-CH_2-	0.99	0.51
22	All f.a.	$O=C$-CH_2-	1.08	0.54
23	Monounsaturated/saturated f.a.	$\underline{C}H_2$-CH_2-CH_3	4.14	1.9
25	18:2 n-6	$\underline{C}H_2$-CH_2-CH_3	-	-
26	f.a.	-CH_2-$\underline{C}H_2$-CH_2-	2.0	1.1**
27	f.a.	-CH_2-$\underline{C}H_2$-CH_2-	1.7	0.75**
28	f.a.	-CH_2-$\underline{C}H_2$-CH_2-	2.0	1.1**
29	f.a.	-CH_2-$\underline{C}H_2$-CH_2-	2.3	-**
30	f.a.	-CH_2-$\underline{C}H_2$-CH_2-	1.5	0.67**
31	f.a.	-CH_2-$\underline{C}H_2$-CH_2-	-	-**
32	Mono, di and 18:3	-$\underline{C}H_2$-CH=C	2.2	0.87
33	Unsaturated f.a.	=CH-$\underline{C}H_2$-CH=	4.6	1.9
34	C_3 – All F.a. except 22:6	-CH_2-	1.4	0.68
35	All f.a.	-$\underline{C}H_2$-CH_3	6.1	3.2
36	DHA C_3		-	-
37	All n-3 f.a.	-$\underline{C}H_2$-CH_3	9.8	2.33
38	Methyl ω3	-CH_3	-	-
39	Methyl	-CH_3	7.5	9.5

*Indistinguishable in wild salmon due to low occurrence

**This spectral region is quite different for farmed and wild salmon, and values are not directly comparable

Table S2. Omega-3 fatty acids analyzed in this study, with carbon resonances assigned in Figure 2 indicated in the molecular structures.

Fatty acid	Molecular Strucutre
ω-3 (DHA), docosahexaenoic acid, (22:6)	
ω-3 (EPA), eicosapentaenoic acid, (20:5)	
ω-3 (ALA), α-linolenic acid, (18:3)	

Additional ^{13}C MAS NMR spectra

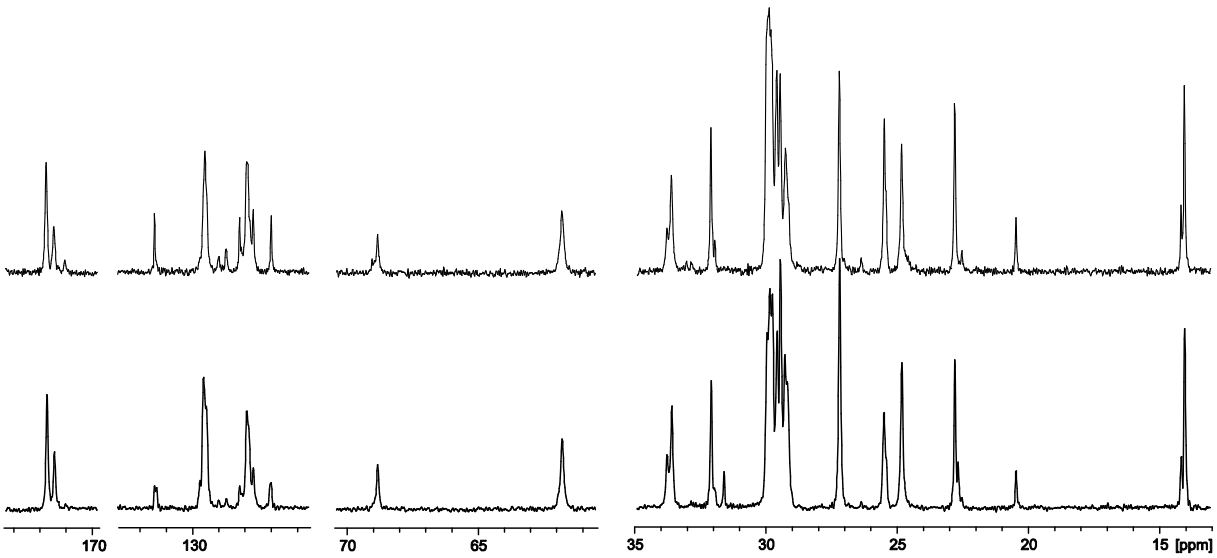


Figure S2. Spectra of farmed (bottom) and wild (top) salmon recorded with 256 transients, relaxation delay of 10 seconds, 50 msec acquisition time and high-power proton decoupling. The total experiment time is 20 minutes.

^{1}H T_1 values and spectra

Table S3. ^{1}H T_1 values and shift assignments of MAS spectra (Figure S3)

Number	Description	Carbon	T_1 (sec) Farmed Salmon	T_1 (sec) Wild Salmon
1	Unsaturated f.a.	-C<u>H</u>=C<u>H</u>-	0.91	0.91
2	water		1.08	0.69
3	glyceryl	-C<u>H$_2$</u>-O-	0.69	0.72
4	glyceryl	-C<u>H$_2$</u>-O-	0.71	0.71
5	Polyunsaturated f.a.	=CH-C<u>H$_2$</u>-CH=	0.62	0.66
6	All F.a. except 22:6	O=C-C<u>H$_2$</u>-CH$_2$-	0.58	0.58
7	Unsaturated f.a.	-<u>C</u>H$_2$-CH=CH-	0.57	0.58
8	All f.a. except DHA	O=C-CH$_2$-C<u>H$_2$</u>-	0.53	0.53
9	All f.a. except EPA and DHA	-CH$_2$-	0.55	0.54
10	All f.a.	-CH$_3$	0.86	0.88

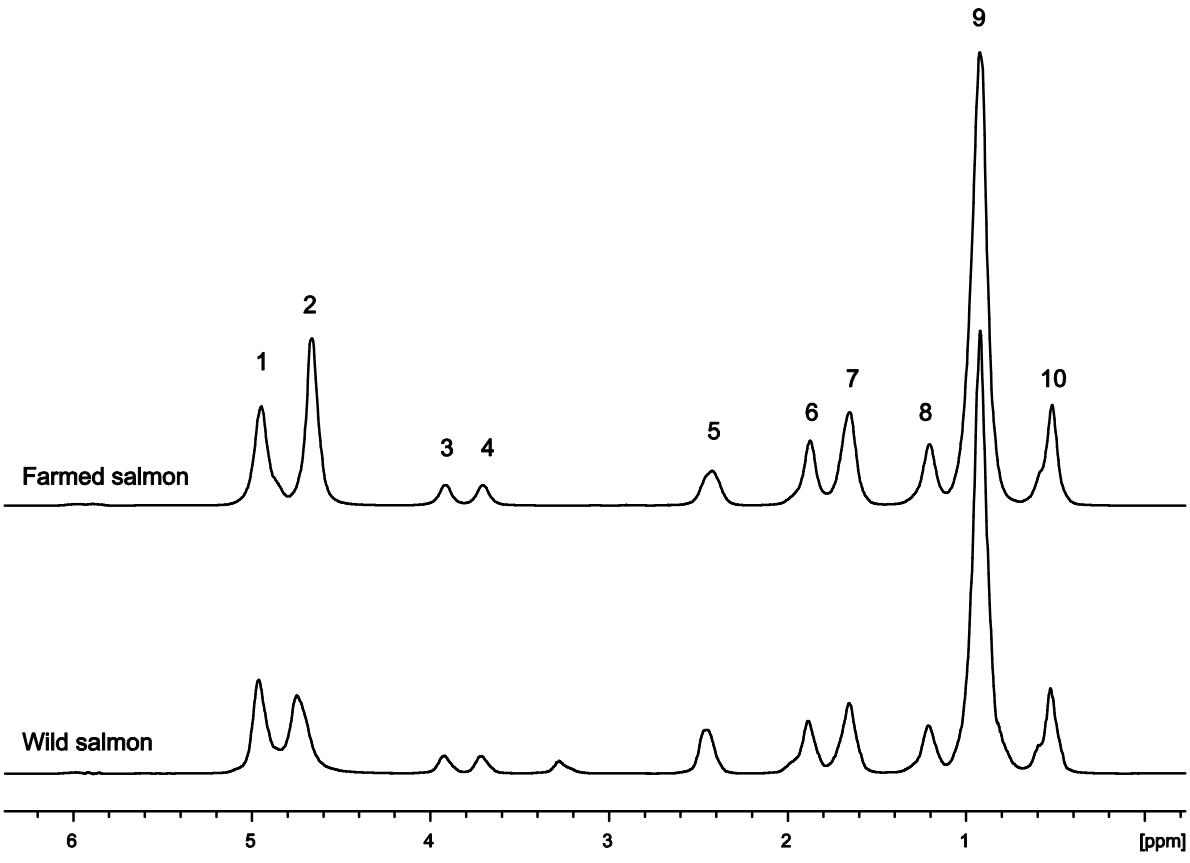


Figure S3. ^{1}H MAS spectra (2.5 kHz) of farmed and wild salmon. Assignments of the numbered peaks are in Table S2.

The similarity of the ^{1}H T_1 values for farmed and wild salmon is due to molecular dynamics in the spin-diffusion regime of lipids in the adipose tissue. Hence, the values are less suited than the ^{13}C T_1 values to evaluate the chemical environment and mobility of the lipids.

F- and T-test

Table S4. All the statistic tests were carried out by comparing the contents of each type of fatty acid in two groups (farmed and wild salmon) at a significance level of 5%. The tests with corresponding p-values are listed in the table.

Fatty acid	F-test	T-test
Total n-3	p=0,15	p=0,38
ALA	*	$p=1,02*10^{-7}$
DHA	p=0,35	$p=2,5*10^{-6}$
EPA	p=0,16	$p=2,1*10^{-6}$
n-6	*	$1,76*10^{-7}$

** It was not possible to perform F-tests for the variances of the fatty acid contents of ALA and n-6, since these fatty acids were not detected in wild salmon. In these t-tests, it was assumed that the variance of the measurements were similar in farmed and wild salmon.*

The F-tests showed that there were no difference in variances of measurements carried out in farmed and in wild salmon, which is indicated by p-values > $\alpha = 0.05$. Since the variances were equal in both groups (farmed and wild salmon) the t-tests with assumed equal variances were carried out. The t-tests were used to test if the differences in the fatty acid contents in farmed and wild salmon are significant.

The results of the t-tests shows that the total content of n-3 is similar in farmed and in wild salmon ($p = 0.38$). The other p-values from the t-tests show that there are significant differences in the fatty acid contents in farmed and wild salmon.

ALA and n-6 are only found in farmed salmon with p-values of $1.02 * 10^{-7}$ and $1.76 * 10^{-7}$, respectively. Furthermore, t-tests on the contents of DHA and EPA differs significantly from farmed and wild salmon with p-values of $2.5 * 10^{-6}$ (DHA) and $2.1 * 10^{-6}$(EPA).