

Supplementary Materials

Lipid Profile of Fresh and Aged *Wollemia nobilis* Seeds: Omega-3 Epoxy lipid in Older Stored Seeds

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Supplementary Figures



Figure S1. Canopy of adult *Wollemia nobilis* trees displaying disintegrating mature seed cones. Image supplied by Botanic Gardens of Sydney.



Figure S2. Seeds of *Wollemia nobilis*, scale – length of each seed approx. 10 mm.

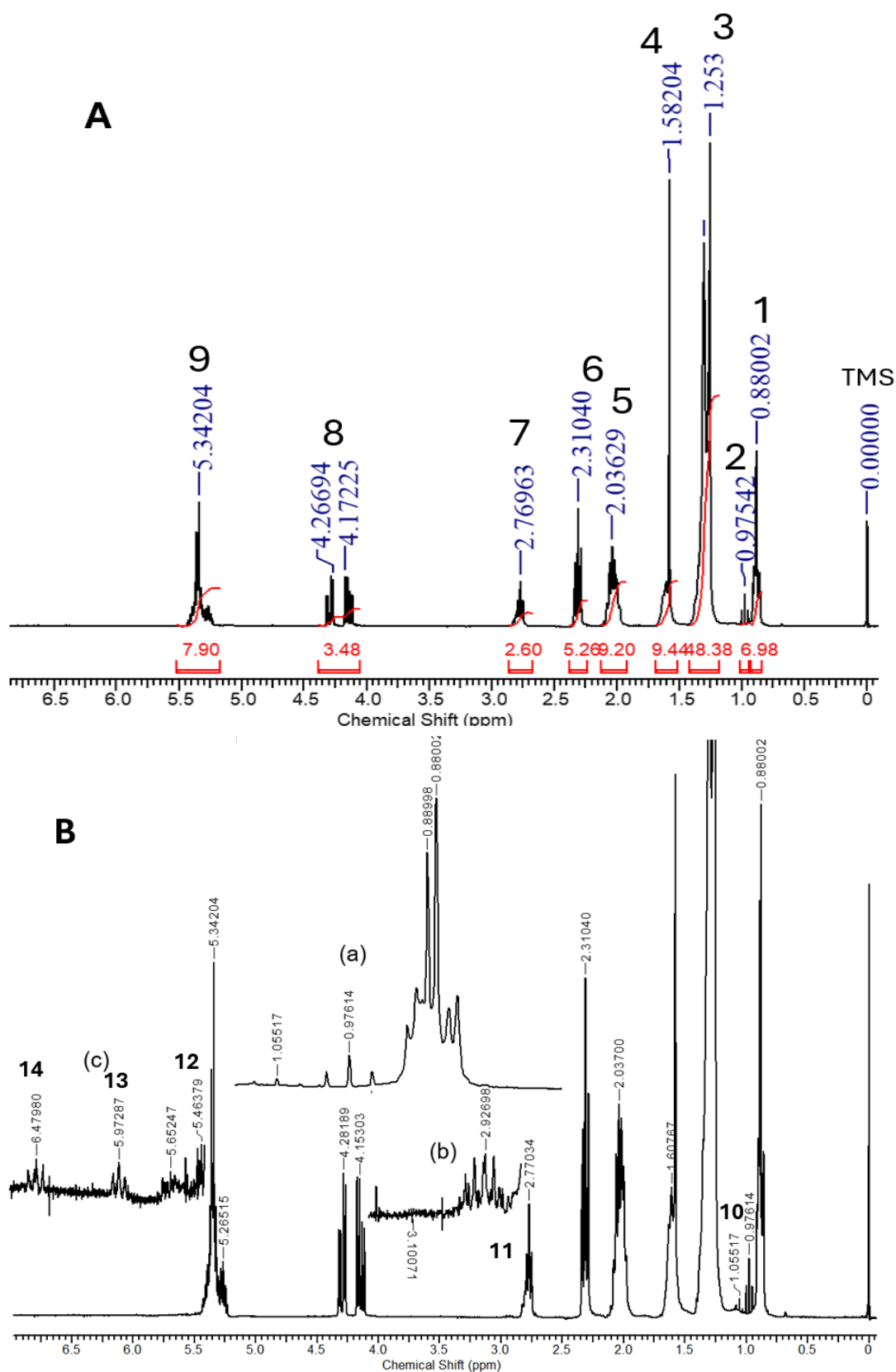


Figure S3. 300 MHz ^1H NMR spectra of oil from **A**: fresh seed, WPS VC2005-4A. 9 groups of peaks (1 to 9) were recognised; **B**: stored seed, H0503-3102. An additional 5 groups of peaks were identified (10 to 14).

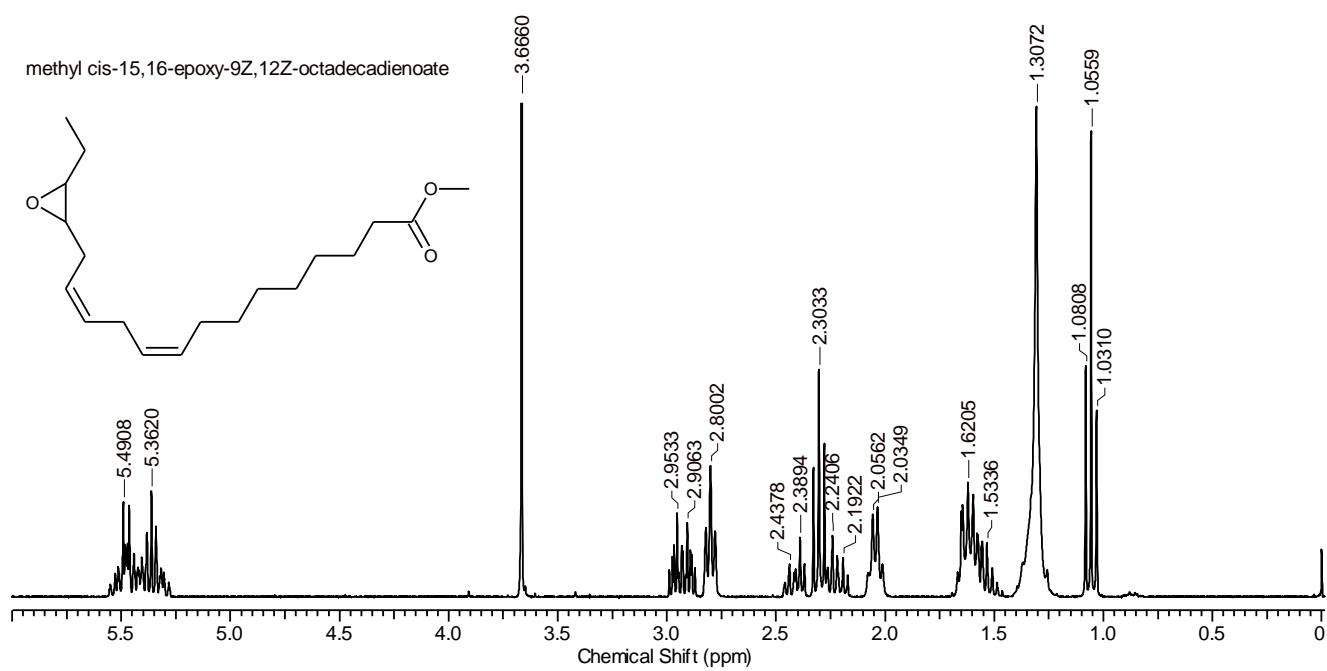


Figure S4. 300 MHz ^1H NMR spectrum of methyl *cis*-15,16-epoxy-9*Z*,12*Z*-octadecadienoate measured in deuteriochloroform relative to tetramethylsilane ([38] Cui et al., 2009).

Supplementary Tables

**Table S1 : Fresh WPS seed, kernel and oil weights, and whole seed and kernel oil yields:
(n = 27)**

Date Collection	Seed ID	Seed wt (mg)	Kernel wt (mg)	Oil wt (mg)	% oil (in whole seed)	% oil (in Kernel)
27/3/05	0503-2901	40.2	31.9	11.20	27.9	35.1
27/3/05	0503-2902	38.7	31.1	12.90	33.3	41.5
27/3/05	0503-2903	44.4	37.2	12.90	29.1	34.7
27/3/05	0503-2904	32.2	25	9.40	29.2	37.6
27/3/05	0503-2905	29.3	23.4	8.80	30.0	37.6
25/2/05	0503-3001	12.9	9.4	3.50	27.1	37.2
25/2/05	0503-3002	18.4	14.0	6.00	32.6	42.9
25/2/05	0503-3003	15.9	12.5	5.00	31.4	40.0
25/2/05	0503-3004	10.1	6.5	3.00	29.7	46.2
25/2/05	0503-3005	13.9	10.9	4.50	32.4	41.3
25/2/05	0503-3006	7.9	5.8	2.20	27.8	37.9
12/2/2007	WPS-VC 2005-16A	24.9	20.8	8.6	34.5	41.3
12/2/2007	WPS-VC 2005-16B	23.6	19.6	7.1	30.1	36.2
12/2/2007	WPS-VC 2005-4A	22.3	18.5	8.7	39.0	47.0
12/2/2007	WPS-VC 2005-4B	22.8	18.7	8.3	36.4	44.4
12/2/2007	WPS-VC 2005-7	21.4	18.1	8.8	41.1	48.6
12/2/2007	WPS-VC-A	24.5	20.0	8.2	33.5	41.0
12/2/2007	WPS-VC-B	21.8	17.6	6.3	28.9	35.8
12/2/2007	WPS-VC-C	25.5	20.2	7.6	29.8	37.6
12/2/2007	WPS-VC-D	25.0	19.9	8.9	35.6	44.7
12/2/2007	WPS-VC-E	27.2	23.0	9.5	34.9	41.3
12/2/2007	WPS-VC-F	22.0	17.1	7.2	32.7	42.1
5/2/2008	WPS-1	6.7	1.1	NA	NA	NA
5/2/2008	WPS-2	31.1	25.3	11.2	36.0	44.3
5/2/2008	WPS-3	27.4	22.4	9.1	33.2	40.6
5/2/2008	WPS-4	36	30	14.4	40.0	48.0
5/2/2008	WPS-5	17.2	11.3	4.8	27.9	42.5
Avg		23.8	18.9	7.5	33.4	42.2
SD		9.5	8.4	2.9	3.9	4.0

NA not available

Table S2 : Stored WPS seed, kernel and oil weights, and whole seed and kernel oil yields: (n = 34)

Date Collection	Seed ID	Seed wt (mg)	Kernel wt (mg)	Oil wt (mg)	% oil (in whole seed)	% oil (in Kernel)
18/2/1999	S 1, 23/04/04	34.4	24.4	8.0	23.3	32.8
18/2/1999	S 2, 29/04/04	27.7	16.6	7.1	25.6	42.8
18/2/1999	S 3, 29/04/04	38.6	28.1	9.2	23.8	32.7
18/2/1999	S 4, 29/04/04	38.0	27.7	13.7	36.1	49.5
23/5/1998	0503-3101	30.3	25.2	10.8	35.6	42.9
12/5/1998	0503-3102	29.2	23.8	11.1	38.0	46.6
12/6/1998	0503-3103	20.4	14.3	5.1	25.0	35.7
18/2/1999	0503-3104	40.8	33.4	16.4	40.2	49.1
18/2/1999	0503-3105	39.0	31.9	15.2	39.0	47.6
29/1/1999	0503-3106	37.2	30.0	13.6	36.6	45.3
29/1/1999	0503-3107	36.4	29.7	13.9	38.2	46.8
29/1/1999	0503-3108	38.8	31.3	14.8	38.1	47.3
18/2/1999	0503-3109	36.3	28.5	12.7	35.0	44.6
18/2/1999	0503-3110	42.8	32.1	14.9	34.8	46.4
27/3/2008	WPS-1-270308	33	27.2	12.6	38.2	46.3
27/3/2008	WPS-2-270308	35.3	29.3	14.3	40.5	48.8
27/3/2008	WPS-3-270308	13	6.2	2.7	20.8	43.5
27/3/2008	WPS-4-270308	32.8	26.4	12.2	37.2	46.2
27/3/2008	WPS-5-270308	33.6	27.5	14	41.7	50.9
27/3/2008	WPS-6-270308	25.9	20.2	9.4	36.3	46.5
27/3/2008	WPS-7-270308	9.1	5.2	2.0	22.0	38.5
27/3/2008	WPS-8-270308	21.4	16.7	7.4	34.6	44.3
27/3/2008	WPS-9-270308	34.4	28.2	13.8	40.1	48.9
27/3/2008	WPS-10-270308	4.7	0	NA	NA	NA
21/3/2011	WPS-1	5.2	0.3	NA	NA	NA
21/3/2011	WPS-2	25.8	9.4	32.6	89.4	32.6
21/3/2011	WPS-3	27.1	11.6	38.0	88.8	38.0
21/3/2011	WPS-4	7	2.4	NA	NA	NA
21/3/2011	WPS-5	8.7	3.1	NA	NA	NA
21/3/2011	WPS-6	18.7	14	38.5	51.4	38.5
21/3/2011	WPS-7	24.8	18.7	32.7	43.3	32.7
21/3/2011	WPS-8	5	0.8	NA	NA	NA
21/3/2011	WPS-9	22.8	16.1	35.1	49.7	35.1
21/3/2011	WPS-10	24.5	18	34.3	46.7	34.3

NA not available

Table S3 : Fatty acid profiles determined by GC-MS analysis of fatty acid methyl esters prepared from WPS oil samples (n=11). The method used to calculate the % of each FAMES is 100 X the area of the particular FAME / the sum of the total area of all KNOWN FAMES. Three common unknown peaks (RT: 19.7, 19.8, 20.4) are present in all the samples at around 1 – 3%.

RT	FAME	FA	% Area										Ave.	SD
			1	2	3	5	6	7	8	9	10	11		
			WPS-VC 2005-16A	WPS-VC 2005-16B	WPS-VC 2005-4A	WPS-VC 2005-7	WPS- VC-A	WPS- VC-B	WPS- VC-C	WPS- VC-D	WPS- VC-E	WPS- VC-F		
14.4	C16:0	palmitic	6.68	6.62	7.45	7.10	7.12	7.02	6.74	6.30	6.47	6.67	6.82	0.35
16.7	C18:0	stearic	11.5	9.41	8.92	8.92	8.59	7.85	7.35	6.93	6.55	6.86	8.29	1.5
16.9	C18:1	oleic	35.4	30.3	35.0	34.5	33.7	34.8	32.5	30.1	30.8	30.0	32.7	2.2
17.4	C18:2	linoleic	22.8	23.0	25.0	24.6	26.8	25.3	25.5	26.1	25.4	25.3	25.0	1.3
18.1	C18:3	α -linolenic	3.31	3.08	2.9	3.33	3.38	3.31	2.87	3.17	3.04	2.91	3.13	0.20
18.8	C20:0	arachidic	8.05	8.43	8.44	8.35	7.24	7.82	7.62	7.32	7.32	6.59	7.72	0.61
19.0	C20:1	gondoic*	2.26	1.97	2.18	1.97	2.3	1.97	2.24	2.25	1.84	1.75	2.07	0.20
19.5	C20:3	mead#	1.12	1.72	1.25	0.97	1.23	1.07	1.49	1.45	1.1	1.55	1.30	0.24
20.8	C22:0	behenic	5.24	8.68	7.18	8.37	7.58	6.78	7.51	8.07	8.3	7.95	7.57	1.0
23.2	C24:0	lignoceric	2.84	6.79	1.71	1.9	2.06	1.57	1.97	1.99	1.96	2.08	2.49	1.55
	Sum		99.2	100	100	100	100	97.4	95.8	93.7	92.7	91.7	97.1	

* or gadoleic

or dihomo-gamma-linolenic

Table S4 : Fresh WPS: ¹H NMR Analysis for Double Bond Equivalents, Omega-3, Omega-6, Omega-9 (plus saturated) Fatty Acid Content (n = 26)

Date Collected	Seed ID	DB equiv	CH ₃ /glyc	%omega- 3	%omega- 6	%omega-9 + sat.	% omega-3 epoxylipid
27/3/05	0503-2901	1.4	2.7	7.9	44.7	47.5	<0.1
27/3/05	0503-2902	1.4	2.8	7.9	45.9	46.2	<0.1
27/3/05	0503-2903	1.4	2.7	6.6	46.1	47.2	<0.1
27/3/05	0503-2904	1.5	2.6	8.8	45.1	46.2	<0.1
27/3/05	0503-2905	1.5	2.6	9.4	47	43.6	<0.1
25/2/05	0503-3001	1.5	2.6	7.8	46.3	45.9	<0.1
25/2/05	0503-3002	1.5	2.7	7.7	45.5	46.8	<0.1
25/2/05	0503-3003	1.5	2.7	7.7	45.5	46.8	<0.1
25/2/05	0503-3004	1.4	2.8	7.2	44	48.7	<0.1
25/2/05	0503-3005	1.4	2.8	7	46.1	46.8	<0.1
25/2/05	0503-3006	1.4	2.5	5.8	43	51.1	<0.1
12/2/2007	WPS-VC 2005-16A	1.5	2.8	6.1	40.3	53.6	<0.1
12/2/2007	WPS-VC 2005-16B	1.5	2.8	6.1	45.5	48.4	<0.1
12/2/2007	WPS-VC 2005-4A	1.4	2.9	6.9	41.4	51.7	<0.1
12/2/2007	WPS-VC 2005-4B	1.5	2.8	6.2	40.6	53.2	<0.1
12/2/2007	WPS-VC 2005-7	1.5	2.9	7.3	41.1	51.6	<0.1
12/2/2007	WPS-VC-A	1.6	2.8	7	45.1	47.9	<0.1
12/2/2007	WPS-VC-B	1.5	2.8	5.7	43.6	50.7	<0.1
12/2/2007	WPS-VC-C	1.6	2.8	6.7	43	50.3	<0.1
12/2/2007	WPS-VC-D	1.5	2.8	6.9	46.6	46.6	<0.1
12/2/2007	WPS-VC-E	1.5	2.9	7	44.3	48.6	<0.1
12/2/2007	WPS-VC-F	1.4	3	7.8	43.9	48.3	<0.1
8/2/2008	WPS-2	1.5	2.9	6.2	44.1	49.7	<0.1
8/2/2008	WPS-3	1.4	3	6.6	43.4	50.1	<0.1
8/2/2008	WPS-4	1.3	3.1	6.2	42.4	51.4	<0.1
8/2/2008	WPS-5	1.4	2.6	9.5	40	50.4	<0.1
	AVERAGE	1.5	2.8	7.2	44.0	48.8	
	STDev	0.1	0.1	1.0	2.0	2.5	

Table S5 : Stored WPS: ¹H NMR Analysis for Double Bond Equivalents, Omega-3, Omega-6, Omega-9 (plus saturated) Fatty Acid Content. (n = 31)

Date Collected	Seed ID	Storage Conditions	Storage Time (months)	DB equiv.	CH ₃ /glyc	% omega-3	% omega-6	% omega-9 + sat.	% omega-3 epoxy lipid
18/2/1999	S 1, 23/04/04	dry 5°C, n=4	84	1.4	2.9	8.2	43.0	46.0	3.39
18/2/1999	S 2, 29/04/04	dry 5°C, n=4	84	1.6	2.7	7.5	42.5	49.0	1.49
23/5/1998	0503-3101	dry 5°C, n=4	84	1.2	2.9	5.2	42.6	50.0	2.42
12/5/1998	0503-3102	dry 5°C, n=4	84	1.2	2.7	6.0	40.6	52.0	1.32
			Average	1.4	2.8	6.7	42.2	49.3	2.2
			STDev	0.2	0.1	1.4	1.1	2.5	1.0
18/2/1999	S 3, 29/04/04	dry -18°C, n=10	84	1.6	3.1	8.3	42.3	49.0	0.1
18/2/1999	S 4, 29/04/04	dry -18°C, n=10	84	1.6	2.6	7.1	43.6	49.0	0.43
12/6/1998	0503-3103	dry -18°C, n=10	84	1.3	3.0	7.0	35.3	58.0	0.19
18/2/1999	0503-3104	dry -18°C, n=10	84	1.4	3.0	7.1	41.7	51.0	0.17
18/2/1999	0503-3105	dry -18°C, n=10	84	1.4	2.8	7.6	46.4	46.0	0.16
29/1/1999	0503-3106	dry -18°C, n=10	84	1.2	3.8	5.3	36.1	57.0	1.23
29/1/1999	0503-3107	dry -18°C, n=10	84	1.4	2.8	6.1	46.3	48.0	0.1
29/1/1999	0503-3108	dry -18°C, n=10	84	1.4	2.9	5.9	41.5	52.0	0.15
18/2/1999	0503-3109	dry -18°C, n=10	84	1.4	3.0	6.5	42.9	50.0	0.22
18/2/1999	0503-3110	dry -18°C, n=10	84	1.4	6.8	6.7	44.4	49.0	0.33
			Average	1.4	3.4	6.8	42.1	50.9	0.3
			STDev	0.1	1.2	0.9	3.8	3.8	0.3
8/2/2008	WPS-2-080208	60% RH, 45°C, n=11	12	1.5	2.9	6.2	44.1	49.7	0
8/2/2008	WPS-3-080208	60% RH, 45°C, n=11	12	1.4	3.0	6.6	43.4	50.1	0
8/2/2008	WPS-4-080208	60% RH, 45°C, n=11	12	1.3	3.1	6.2	42.4	51.4	0
8/2/2008	WPS-5-080208	60% RH, 45°C, n=11	12	1.4	2.6	9.5	40.0	50.4	0
27/3/2008	WPS-1-270308	60% RH, 45°C, n=11	12	1.6	2.3	7.4	39.3	53.3	0.20
27/3/2008	WPS-2-270308	60% RH, 45°C, n=11	12	1.6	2.4	6.5	40.4	53.1	0.16
27/3/2008	WPS-4-270308	60% RH, 45°C, n=11	12	1.7	2.2	9.8	42.5	47.7	0.23
27/3/2008	WPS-5-270308	60% RH, 45°C, n=11	12	1.7	2.2	7.0	41.8	51.2	0.23
27/3/2008	WPS-6-270308	60% RH, 45°C, n=11	12	1.6	2.3	6.5	38.2	55.3	0.19
27/3/2008	WPS-8-270308	60% RH, 45°C, n=11	12	1.7	2.4	7.6	40.7	51.6	0
27/3/2008	WPS-9-270308	60% RH, 45°C, n=11	12	1.7	2.1	6.1	41.6	52.3	0.31
			Average	1.6	2.5	7.2	41.3	51.5	0.1
			STDev	0.1	0.4	1.3	1.8	2.0	0.1
21/03/2011	WPS-2	60% RH, 45°C, n=6	49	1.4	1.6	8.2	33.0	58.5	0.27
21/03/2011	WPS-3	60% RH, 45°C, n=6	49	1.3	1.6	7.7	25.3	66.6	0.4
21/03/2011	WPS-6	60% RH, 45°C, n=6	49	1.4	1.4	9.3	31.6	58.8	0.3
21/03/2011	WPS-7	60% RH, 45°C, n=6	49	1.3	1.5	8.5	30.6	60.6	0.29
21/03/2011	WPS-9	60% RH, 45°C, n=6	49	1.4	1.8	8.3	32.3	59.0	0.39
21/03/2011	WPS-10	60% RH, 45°C, n=6	49	1.3	1.5	7.8	29.3	62.6	0.27

Average	1.4	1.6	8.3	30.4	61.0	0.3
STDev	0.1	0.1	0.6	2.8	3.1	0.1