Electronic Supplementary Information

Deacetylation by mechanochemistry and aging as a pathway to high molecular weight chitosan from chitin

Thomas Di Nardo,[a] Caroline Hadad,[b,c] Albert Nguyen Van Nhien,[b] Audrey Moores*[a]

Table of Contents

Table S1: Comparison of known deacetylation techniques.

Method	Conditions	NaOH equivalents	MW	DDA	Ref
Solvo-Thermal	50% $NaOH_{(aq)}$, RT-140˚C, 3[a]-540 Hrs[b]	42	Varied	70-99%	C. Rong Huei, Carbohydr. Polym., 1996, **29**, 353–358. A. Domard and M. Rinaudo, Int. J. Biol. Macromol., 1983, **5**, 49–52. A. Alimuniar, and R. Zainuddin, In Advances in Chitin and Chitosan, C.J. Beine, P.A. Sanford and J.P. Zikakis (eds.), Elsevier Applied Science, London and New York, pp. 627-632, 1992.

Planetary Milling	1:5 chitin:NaOH, 100 balls ZrO_2^{ZrO2}, 80 mins	5	1-13 kDa	80 %	X. Chen, H. Yang, Z. Zhong and N.Yan, *Green Chem.*, 2017, **19**, 2783–2792.
Microwave	45% $NaOH_{(aq)}$, 5.5 Mins, 900 W	31	85 kDa	80 %	A. Sahu, P. Goswami and U. Bora, *J. Mater. Sci. Mater. Med.*, , DOI:10.1007/s10856-008-3549-4.
Sonication	60 % $NaOH_{(aq)}$, 30 mins, 500 W	>16	Degradation	73 %	M. Anwar, A. S. Anggraeni and M. H. Al Amin, *AIP Conf. Proc.* DOI:10.1063/1.4978144.
High Pressure Run	50% $NaOH_{(aq)}$, 120˚C, 15 PSI	41	1466 kDa	90 %	H. K. No, Y. I. Cho, H. R. Kim and S. P. Meyers, *J. Agric. Food Chem.*, 2000, **48**, 2625–2627.
Maceration	7 days, 80 % $NaOH_{(aq)}$	22	N/A	80 %	M. Anwar, A. S. Anggraeni and M. H. Al Amin, *AIP Conf. Proc.* DOI:10.1063/1.4978144.
Freeze thaw	24% NaOH	>13	180-300 kDa	80-95%	S. V. Nemtsev, A. I. Gamzazade, S. V. Rogozhin, V. M. Bykova and V. P. Bykov, *Appl. Biochem. Microbiol.*, 2002, **38**, 521–526.
This work	Milling for 30 min followed by 6 days of aging	5	N/A	80-95%	

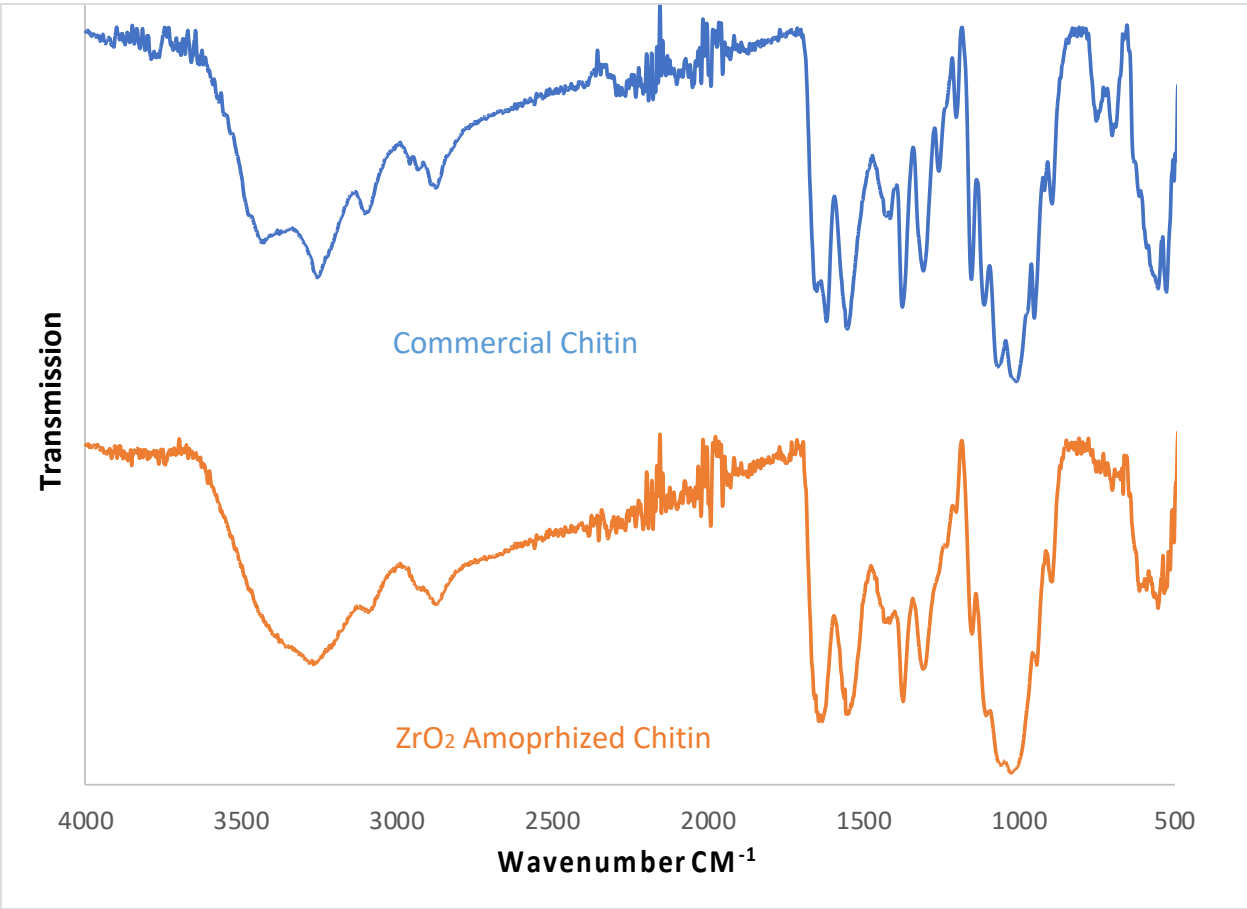
Transmission
Commercial Chitin
ZrO2 Amoprhized Chitin
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber CM-1

Figure S1: FTIR spectra of commercial chitin pre and post amorphization in ZrO_2 jar with ZrO_2 ball for 30 minutes milling.

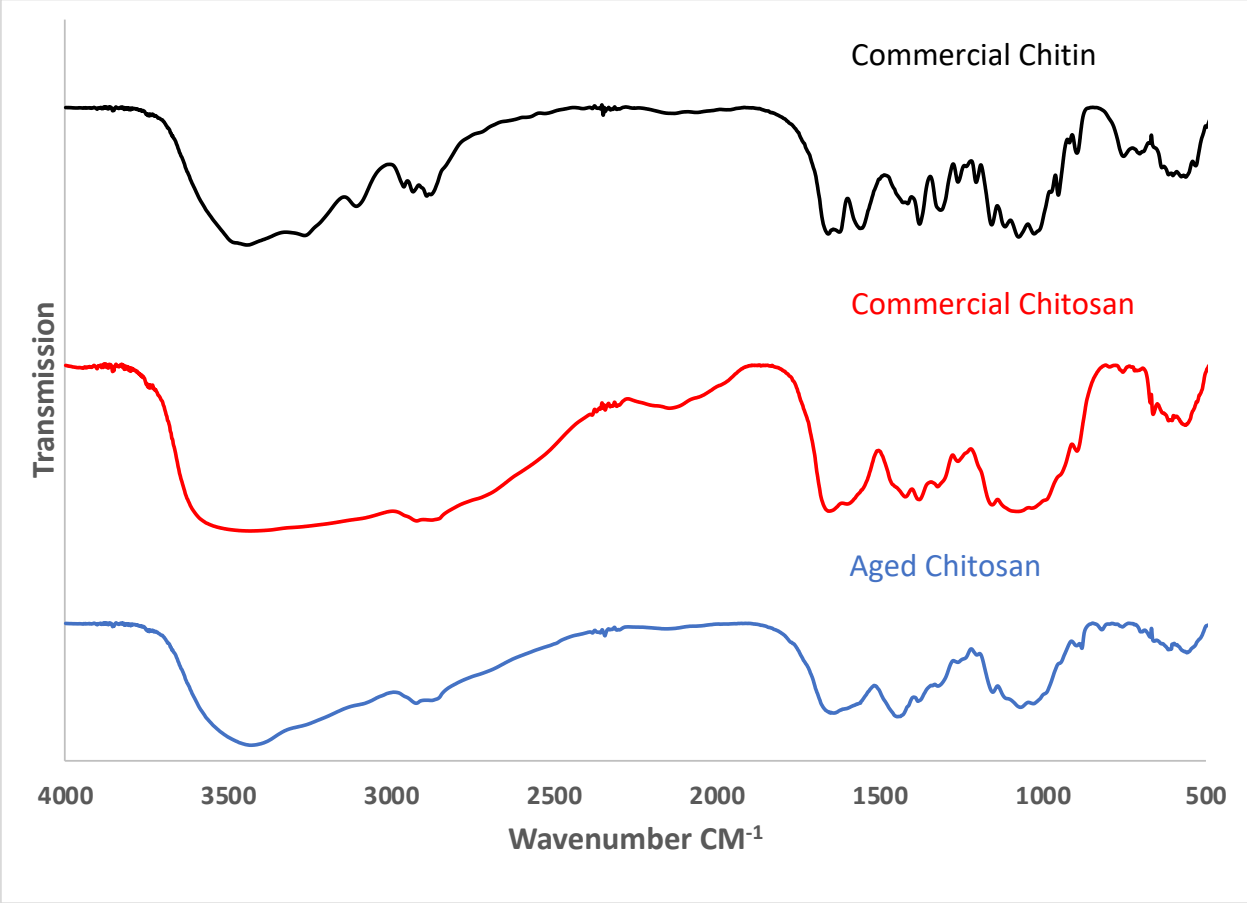

Figure S2: IR spectra of commercial chitin, commercial chitosan, and chitosan produced with the aging process at room temperature, 98% RH 1:5 chitin:NaOH. The amide peak at 1655 cm^{-1} is measured in reference to the hydroxyl peak at 3450 cm^{-1}.

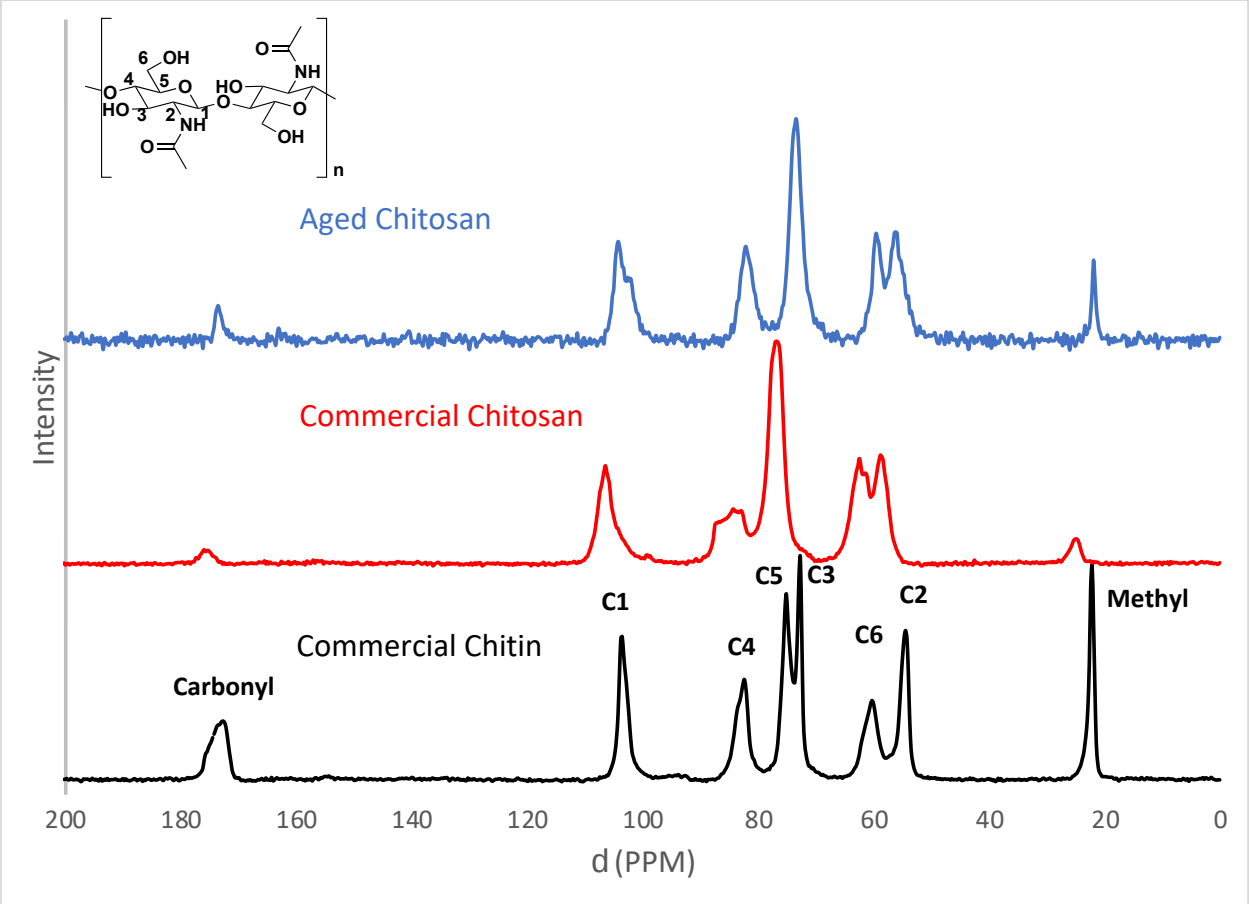

Figure S3: Solid-State ^{13}C NMR spectra of commercial chitin, commercial chitosan, and chitosan produced with the aging process at room temperature. DDA is calculated by comparing the methyl peak at 22 ppm to a reference C1 carbon peak at 104 ppm.

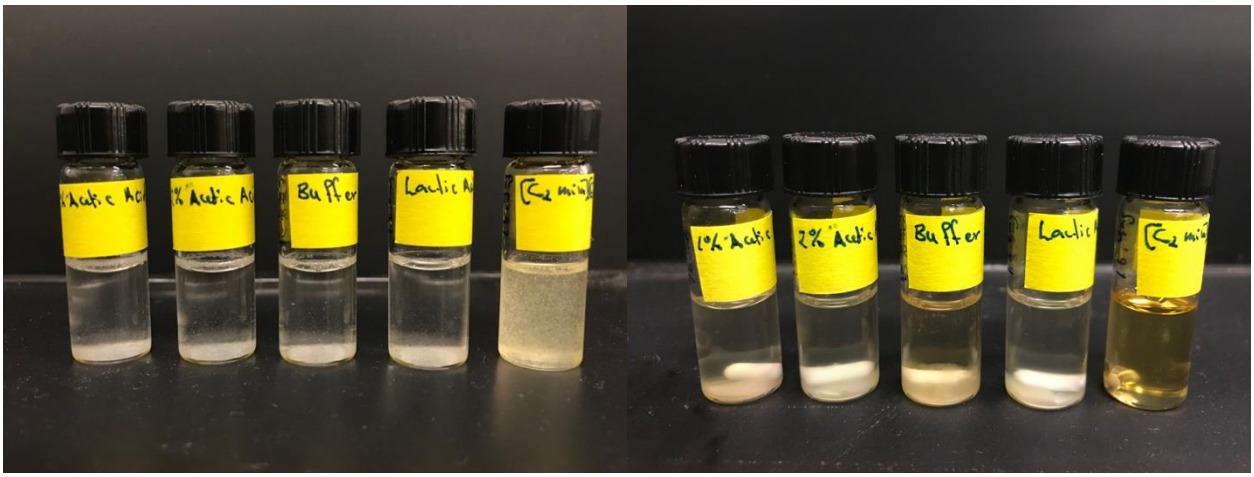

Figure S4: Pictures of aged chitosan (from left to right) in 1% acetic acid, 2% acetic acid, buffer solution (0.3M acetic acid, 0.25M sodium acetate, 0.8mM sodium azide), 0.8% lactic acid and [C_2mim][OAc] pre (left) and post (right) heating (80˚C. Images show that the age based chitosan only dissolves in [C_2mim][OAc].

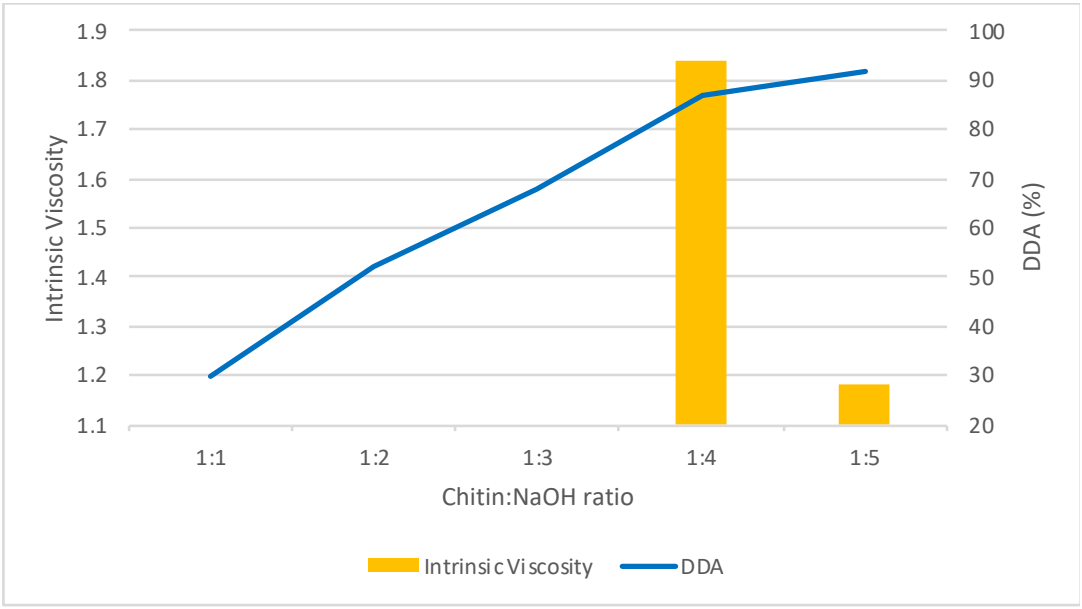

Figure S5: Effect of the chitin:NaOH ratio on [η] and DDA in the amorphization/aging based chitin deacetylation experiments. Conditions of scheme 2 with aging at 50°C for 6 days. [η] not measured for 1:1, 1:2 and 1:3 chitin:NaOH ratios.

Table S2: Initial DDA of commercial chitin, commercial chitosan and solution deacetylated chitosan acquired by ^{13}C CP MAS NMR

Sample	**DDA (%)**
PG-Chitin	4
Commercial Chitosan LMW	89
Commercial Chitosan MMW	96

Commercial Chitosan HMW	85
Solution Deacetylated Chitosan	76

Table S3: Mixer-mill based commercial chitin deacetylation experiments in PTFE jar with a ZrO_2 ball. DDA = [H]/([COMe]+[H])x100, determined by ^{13}C CP MAS NMR. LAG solvent 10 wt%: none, dichloromethane, acetonitrile, ethyl acetate, methanol, ethanol and deionized water.

Milling time (min)	NaOH:chitin ratio	DDA (%)	LAG solvent
30	1	5	none
30	2	6	none
30	3	6	none
30	4	7	none
30	5	7	none
60	5	6	none
90	5	7	none
30	1	7	Water
30	1	7	Ethanol
30	1	6	Methanol
30	1	6	Dichloromethane
30	1	6	Ethyl Acetate
30	1	4	Acetonitrile
30	1	9.9	Water (10%)
30	1	14	Water (20%)
30	1	11.76	Water (30%)
30	1	11.65	Water (50%)
30	5	12.4	Water (10%)
30	5	23.8	Water (20%)
30	5	20.56	Water (30%)
30	5	19	Water (50%)

Table S4: Table of the relative humidity (RH) study of aging of crystalline chitin for 6 days at 22˚C with a chitin to NaOH ratio of 1:5.

RH(%)	DDA(%)
43	40
75	43
98	60

Table S5: DDA and [η] of commercial chitin treated with or without amorphization pre-treatment (30 min milling in ZrO_2 apparatus) followed by 3 to 6 days aging at 22 to 50°C, at 98% humidity and with a chitin to NaOH ratio of 1:5.

Amorphization pre treatment	Temp. (°C)	Aging time (days)	DDA(%)	Intrinsic Viscosity [η]
Yes	22	6	73	2.52
	30	6	90	1.41
	40	6	90	1.27

	50	6	92	1.18
No	50	3	83	3.12
		4	84	3.04
		5	84	3.04
		6	87	3.01
	22	3	50	3.23
		4	51	3.22
		5	65	3.12
		6	60	3.19
yes	50	3	95	1.27
		4	93	1.26
		5	94	1.22
		6	92	1.18
	22	3	53	2.64
		4	76	2.76
		5	69	2.69
		6	73	2.52

Table S6: DDA and [η] of commercial chitin treated with amorphization pre-treatment (30 min milling in ZrO_2 apparatus) followed by 6 days aging at 50°C, at 98% humidity and with a chitin to NaOH ratio ranging from 1:1 to 1:5.

Chitin:NaOH ratio	DDA(%)	Intrinsic Viscosity [η]
1:1	30	Not measured
1:2	52	Not measured
1:3	68	Not measured
1:4	87	1.84
1:5	92	1.18

Table S7: Energy consumption comparison of solvo-thermal and aging methods for deacetylation of 1 Kg of chitin into chitosan. Solvothermal was calculated for heating 1 Kg of chitin, 18.5 Kg of water, and 18.5 Kg of NaOH to 133 ˚C. Aging was calculated for heating 1 Kg of chitin, 2 Kg of water, and 0.9 Kg of NaOH. Amorphization includes 30 mins of milling in ZrO_2 jar with ZrO_2 ball. Mixing requires 5 mins milling in PTFE jar with one ZrO_2 ball.

Method	Solvo-thermal	Amorphization	Mixing	Aging
Energy (KJ/Kg)	11,467	3,330	555	269