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Optimised Solid State 13C CP-MAS NMR for Accurate Determination of %Degree of Acetylation of Extracted Cancer Pagurus Crab Shell Chitin

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Optimised solid state ^{13}C CP-MAS NMR for accurate determination of %Degree of Acetylation of extracted *Cancer pagurus* crab shell chitin

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Introduction

- 70% annual global shellfish production (1.5Mt) ends up in waste streams in landfill, incineration or dumped at sea.
- Push for valorisation of waste streams for Chitin (β-(1,4)-N-acetyl-D-glucosamine) bio-polymer due to anti-inflammatory/anti-bacterial applications and potential for use in value added products in food and nutraceuticals.
- Optimisation of extraction and detailed characterisation required for valorisation - green chemistry and upscaling principles applied.
- The percentage degree of acetylation (%DA) of chitin dictates properties such as solubility, particle size and thermal stability - processing techniques and different potential uses dependent on %DA.
- ^{1}H NMR established in literature for accurate determination of low %DA of chitosan - solution based technique.
- Major challenge in characterising chitin is its poor solubility in any polar or organic solvent due to dense hydrogen bonding between polymer chains - thus solid state techniques are explored for analysis.

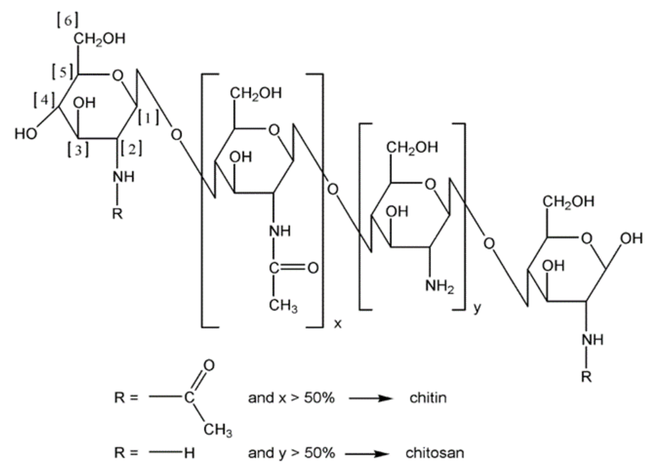

Fig 1 - Monomer Structure of chitin and chitosan

Experimental

- ^{13}C Cross Polarization - Magic Angle Spinning (CP-MAS) NMR was performed using a Bruker 400MHz Ultrashield NMR with solid state CP-MAS probe. Optimised parameters are 128 scans, spin rate of 10kHz, 60kHz carbon polarisation with contact time of 1ms at 25˚C. %DA determined by relative comparison of integrations of C-H3 and C-1 peaks.
- FTIR, Raman spectroscopy and DSC are used as supplemental solid state techniques for qualitative analysis of thermal and molecular properties of the raw shells and extracted bio-polymer.
- Four commercially produced chitin/chitosan standards, extracted from prawn and mushroom sources, with known %DA are used to determine accuracy.
- North Atlantic *Cancer pagurus* crab shell samples sourced from commercial waste stream.

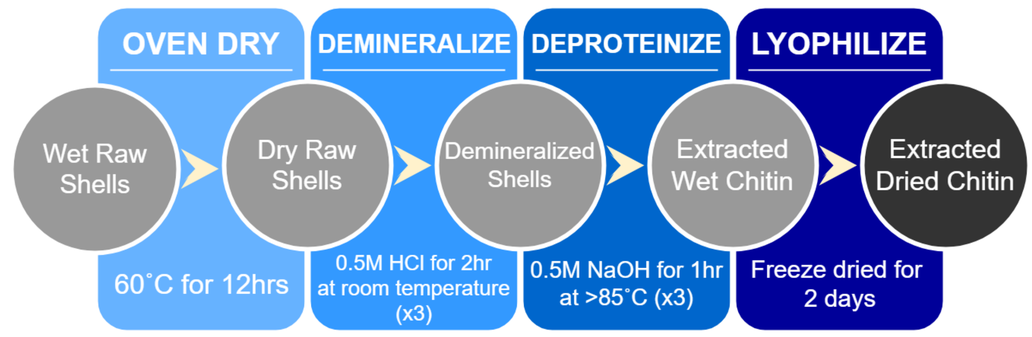

Fig 2 - Extraction Procedure

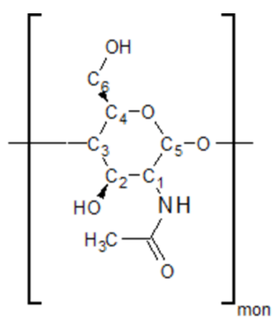

Fig 3 - CP-MAS labelled carbons

Table 1 – CP-MAS determined %DA of samples.

Extracted Sample	Calculated %DA
Fine Ground Shell and Tissue	93.57
Fine Ground Shell	97.09
Crude Ground Shell	94.90

Results

- Under optimised parameters the acceptance criteria for accuracy is met for all standards.
- %DA for all extracted samples calculated as > 90% - high purity chitin.
- Use of FTIR and Raman spectroscopy for rapid qualitative analysis of sample purity and for indication of %DA of extracts is demonstrated well.
- DSC indicates polymorphic stability of extracts - transition from anti-parallel α-chitin to greater spaced parallel β-chitin not observed in crab shell chitin as it is in mushroom chitosan standard.
- By comparison of the spectra/profiles produced, extraction is deemed necessary for accurate characterisation but the de-pigmentation step is determined to be unnecessary - reduction in materials and time needed for extraction and analysis.

Table 2 – %DA of standards and %Recovery

Standard	Known %DA	Experimental %DA	%Recovery
Mushroom chitosan	16.00	15.81	98.81
Prawn chitin	81.00	80.39	99.25
Prawn chitosan	18.40	19.07	103.56

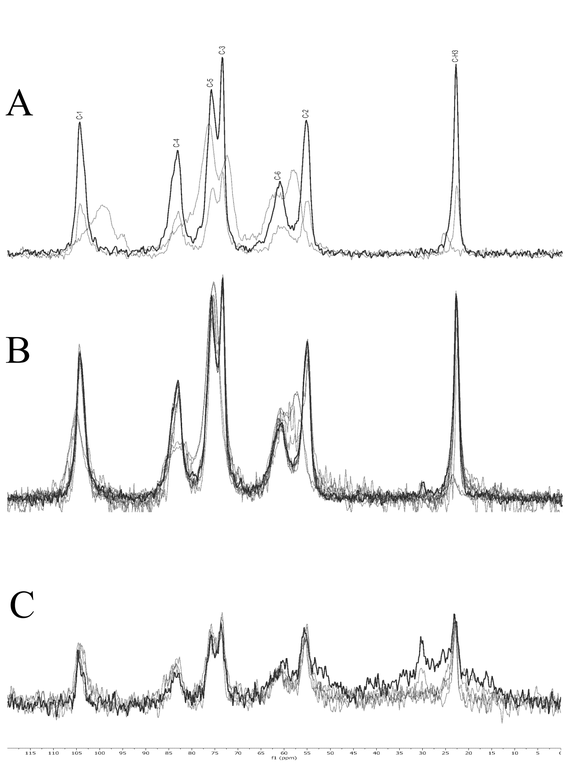

Fig 4 - Overlayed CP-MAS spectra of standards (A), Overlayed CP-MAS spectra of extracted samples (B), Overlayed CP-MAS spectra of raw samples (C).

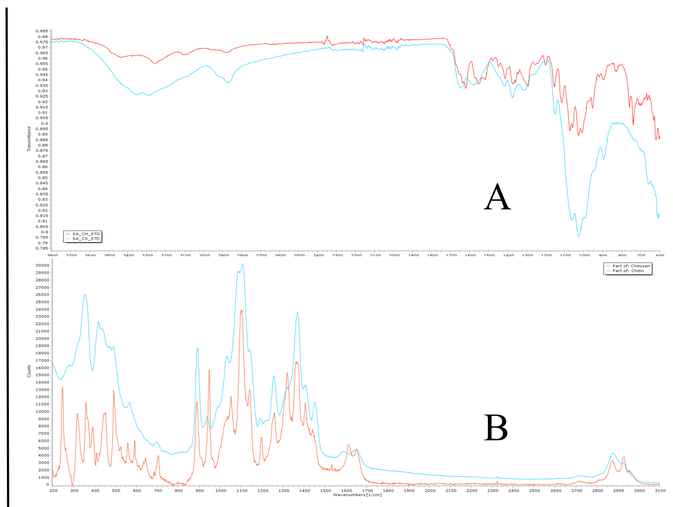

Fig 5 - Overlayed FTIR spectra of crab shell chitin and prawn shell chitosan standard (A), Overlayed Raman spectra of crab shell chitin and prawn shell chitosan standard (B).

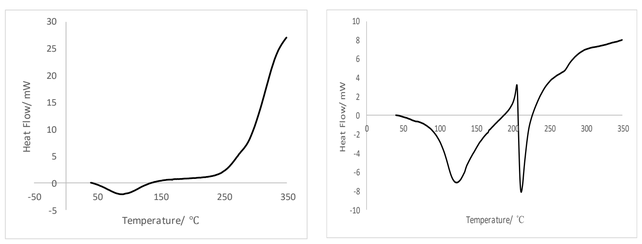

Fig 6 - DSC profile of crab shell chitin (left) and mushroom chitosan standard (right).

Conclusion

- Optimised extraction allows for accurate characterisation via solid state techniques.
- Optimised ^{13}C CP-MAS NMR used in tandem with established ^{1}H NMR technique allows for accurate %DA determination of any crustacean sample from 0-100%.
- Chitin extracted from *Cancer pagurus* waste stream shown to be of > 90% DA, high purity, with relative polymorphic stability and a minimum water content due to hygroscopic nature.
- Analytical suite for rapid and scalable analysis of crustacean sourced chitin recommended in Fig 7.
- Analytical suite to be applied to shrimp and mussel waste streams allowing for building of library of spectra and datasets for reference.

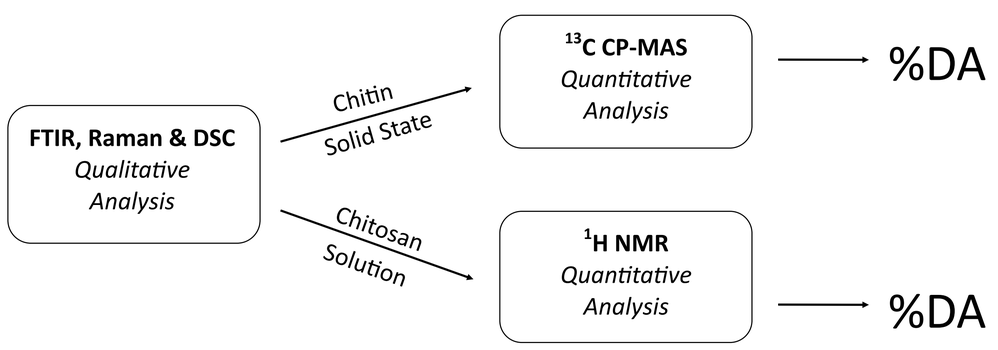

Fig 7 - Recommended analytical suite for characterisation of extracted crustacean sourced chitin and chitosan.

Acknowledgements

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