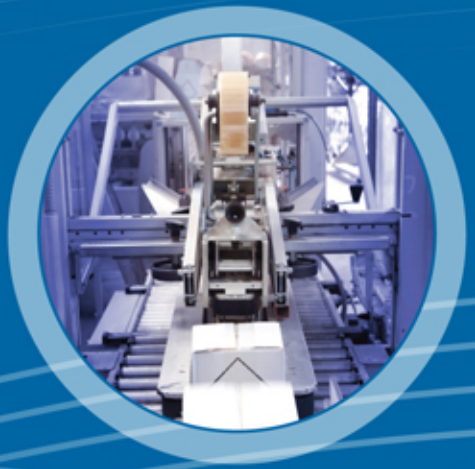
Connecting People, Science and Regulation

Structural and Mechanical Properties of Freeze-Dried Products



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btl
intelligent freeze drying

Contents

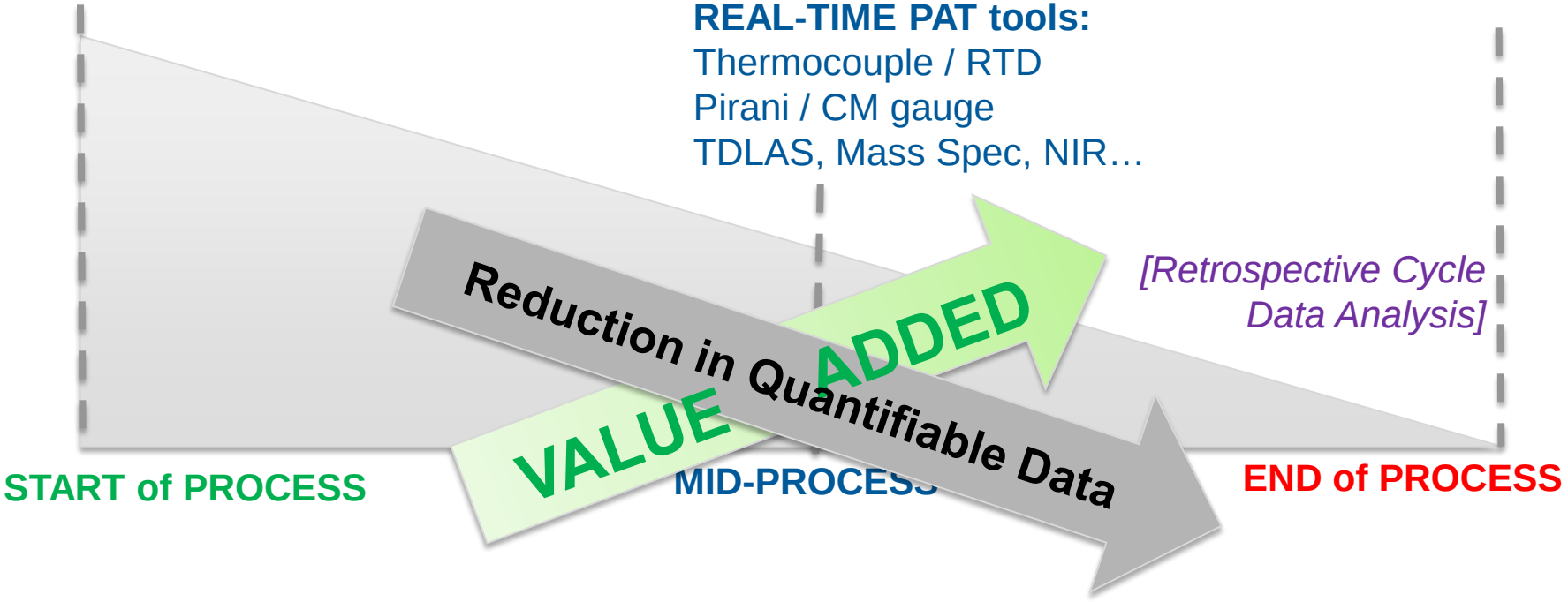
- The current freeze-drying analysis landscape
- What can Mechanical Analysis do for you?
- The best we can do to date – SEM, BET, BJH..
- Mechanical Analysis & case studies for sucrose, mannitol, trehalose, protein formulations
- Summary and Conclusions

The Current Freeze-Drying Analysis Landscape

INITIAL CHARACTERISATION:
Freeze-Drying Microscopy (FDM)
DTA / Impedance Analysis
Differential Scanning Calorimetry (DSC)
pH – stability profiling

POST-PROCESS TESTING:
Karl Fischer / FMS
DSC / stability testing
Activity / potency assay
DVS / GA / XRD / SEM

REAL-TIME PAT tools:
Thermocouple / RTD
Pirani / CM gauge
TDLAS, Mass Spec, NIR…



What can Mechanical Analysis Do for You?

- Inexpensive, rapid, *in situ* (in vial) generation of quantitative data
- Measurement of cake strength, flexibility, cohesiveness

"Is my Good Looking cake <u>really</u> good…?

…or mutton dressed as lamb?!"

Absolute Data:
Young's Modulus
Resistance to transport damage

Development Data:
Concentration studies
Cooling rate studies
Excipient relative mechanical strength

Proxy Data:
Inconsistent Drying
Crust formation
Micro-collapse

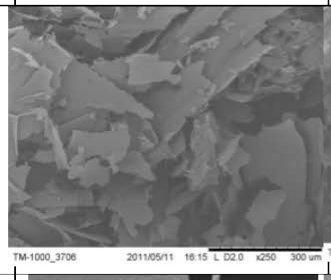
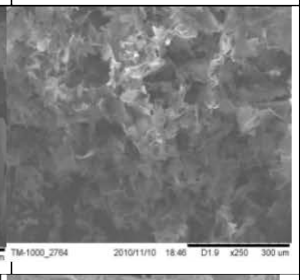
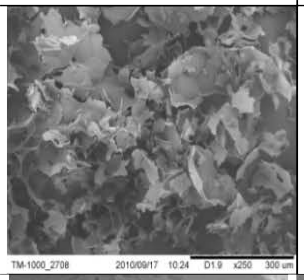
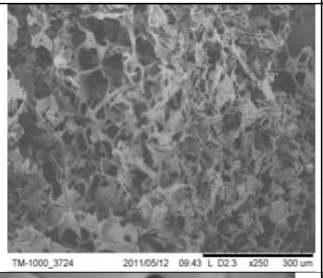
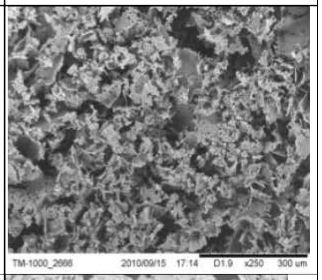
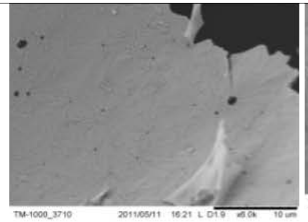
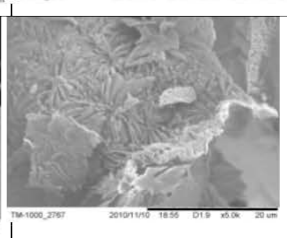
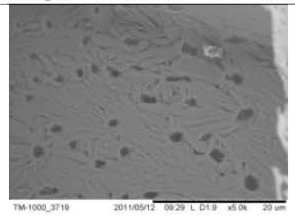
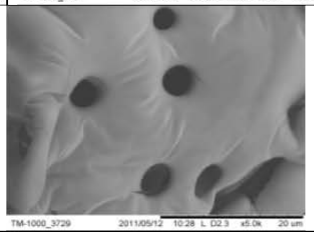
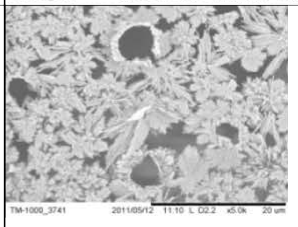
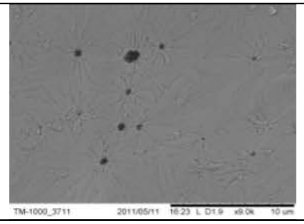
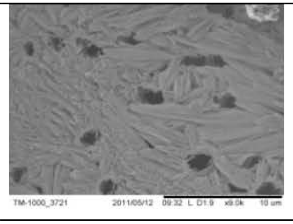
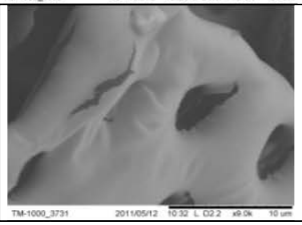
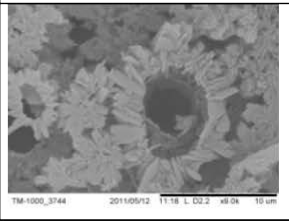
Comparative Data:
For QbD
For Batch comparisons

Current Post-Process Analysis

- "Have I dried it?" [FMS, KF]
- Is it thermally stable? [DSC]
- Does it work? [activity / potency]
- Over-reliance on qualitative visual inspection
  - *"My product is a white-to-off-white cake or powder" (!)*
- SEM is, at best, semi-quantitative (yet expensive)
- Limited quantitative data from gas adsorption

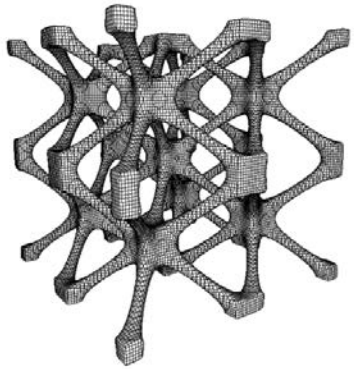
CONCLUSION: Very little absolute quantitative empirical comparison!

SEM (Scanning Electron Microscopy)

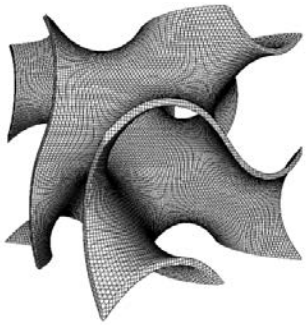
	Quench (LN2) cooled Mannitol	Fast Cooled mannitol	Slow cooled Mannitol	Sucrose	Glucose: Mannitol (2:1)
250x					
5000x					
9000x					

Could we classify microscopic appearance by using categories?

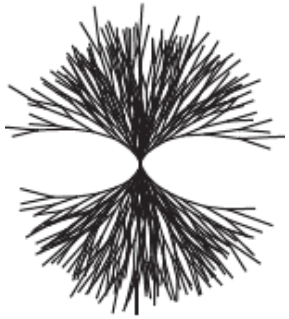
Possible discrete classification



Fibrous

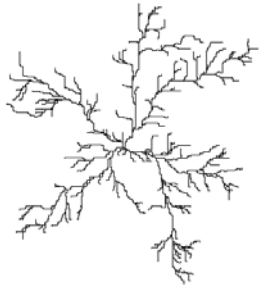


Lamellar-like



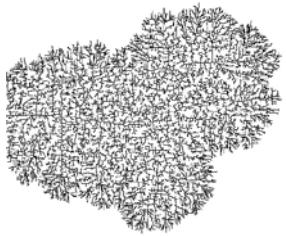
Spherulites

Definitions used to describe cellular materials



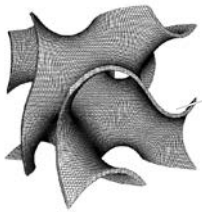
Branched

Dendritic

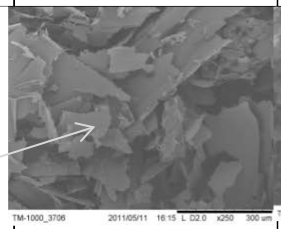
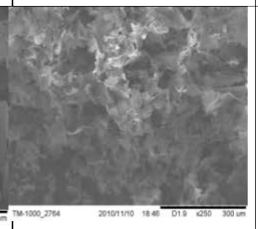
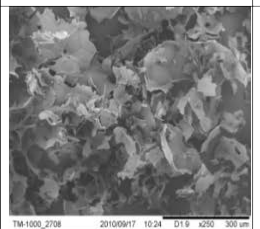
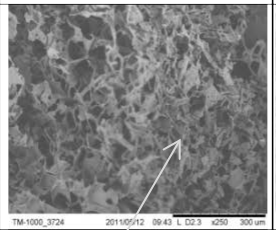
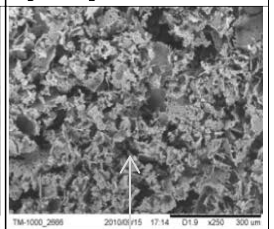
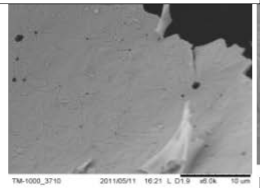
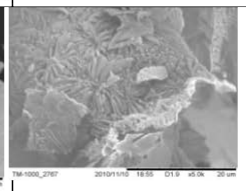
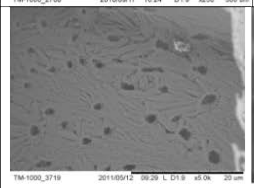
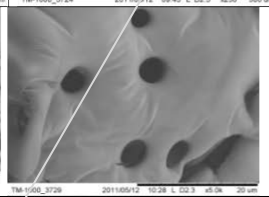
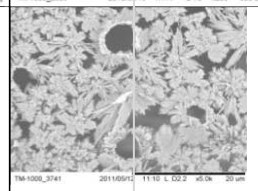
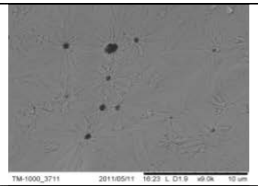
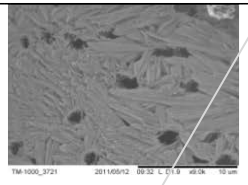
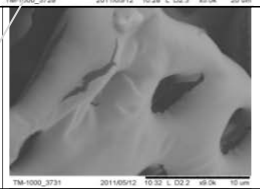
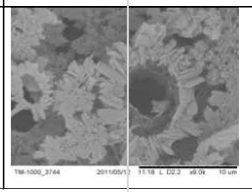


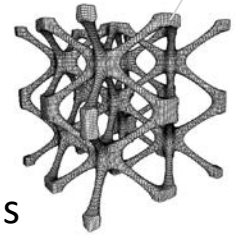
same volume fraction but different spatial arrangement, yet preserving interconnectivity of the solid phase.

Application of Classifications

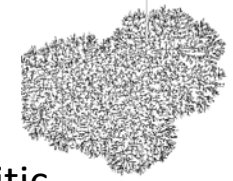


Lamellar-like

	Quench (LN2) cooled Mannitol	Fast Cooled mannitol	Slow cooled Mannitol	Sucrose	Glucose: Mannitol (2:1)
250x					
5000x					
9000x					



Fibrous

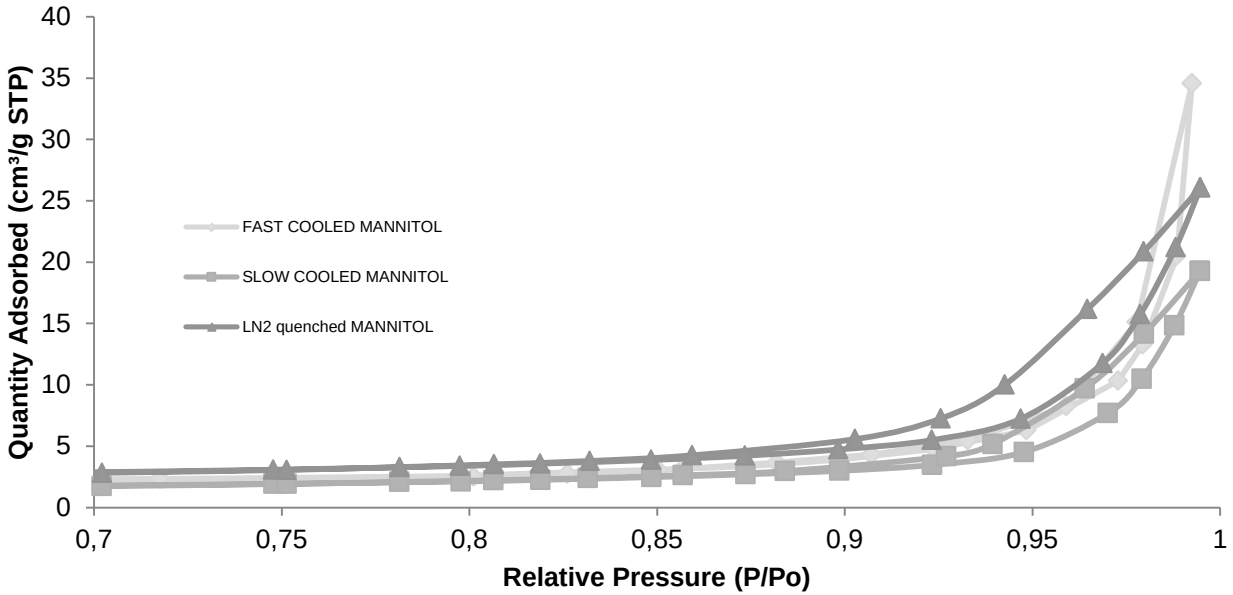


Dendritic

Surface area and porosity (BET, BJH)

- N_2 sorption based Brumauer-Emmett-Teller (BET) and Barrett- Joyner-Halenda (BJH)/ Kelvin equation
- Determine specific surface area and pore size distribution
- In the following example, nitrogen adsorption and desorption isotherms were measured at -195.8° C for lyophilised mannitol that had been frozen in 3 ways:
  - LN_2 immersion [“QUENCH cooled”]
  - Loading onto cold shelves (below 0° C) [“FAST cooled”]
  - Gradual ramp down from RT° [“SLOW cooled”]
- ASAP tristar 3000 (Micromeritics Instrument Corporation, USA) volumetric adsorption system was used

Isotherm Plot from BET analysis



Similar isotherms but hysteresis wider for the LN_2 quenched mannitol samples

This indicates that the evaporation from the pores is distinctly different from condensation within it. The condensate build up is generally from the wall to inwards to the centre of decreasing diameter, but evaporation requires a different curvature there by inhibiting evaporation causing a decreasing portion of the loop to lag behind

Pore diameters (BJH)

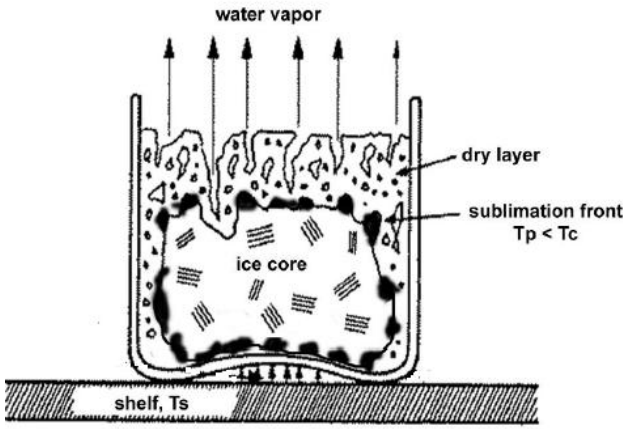
Summary Report	SLOW COOLED MANNITOL	FAST COOLED MANNITOL	LN2 quench cooled MANNITOL
BJH Adsorption cumulative surface area of pores between 17.000 Å and 3000.000 Å diameter:	1.8450 m^2/g	2.2449 m^2/g	4.0122 m^2/g
BJH Desorption cumulative surface area of pores between 17.000 Å and 3000.000 Å diameter:	1.9405 m^2/g	3.4072 m^2/g	4.7217 m^2/g
Pore Size			
BJH Adsorption average pore diameter (4V/A):	529.026 Å	472.534 Å	390.293 Å
BJH Desorption average pore diameter (4V/A):	525.916 Å	321.386 Å	336.040 Å

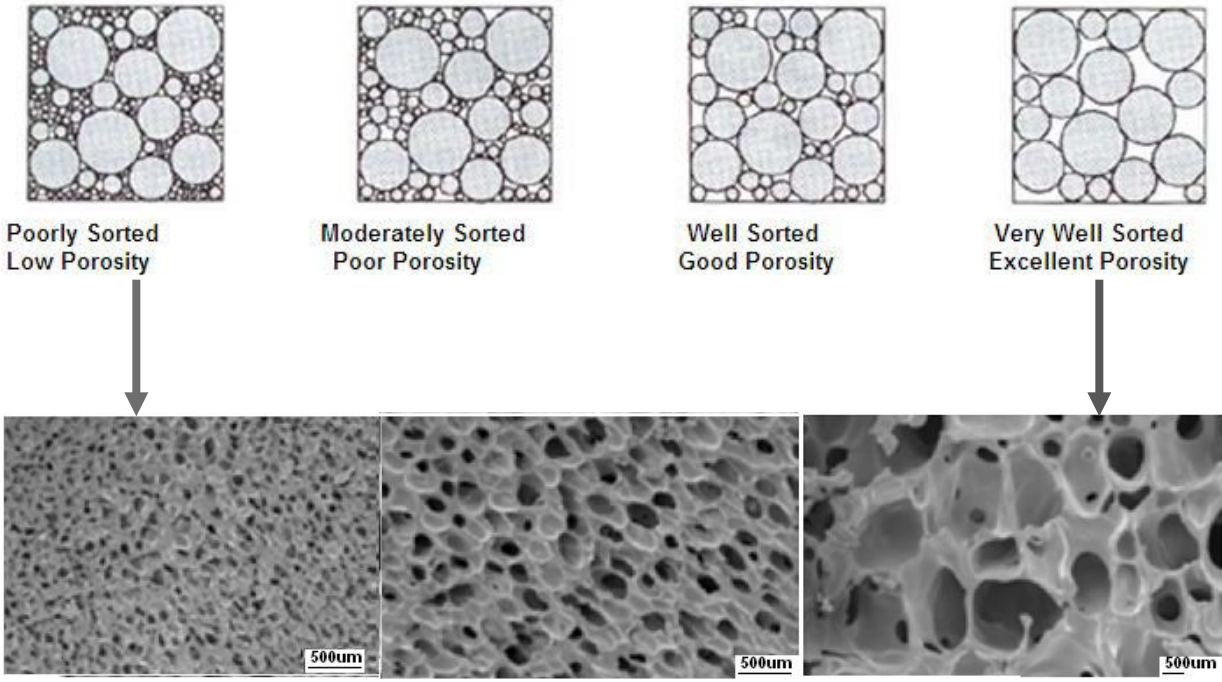
- The BJH theory for open ended pores and condensation shows the trend expected of the different freezing rates (hence ice-crystal size)
- Slow cooling forms larger ice crystals, and leaves larger pores
- Quench cooling forms smaller crystals form leaving a smaller pores but larger surface area of the pores overall

Porous structure of a lyophile

Structure depends on:

- *Freezing temperature* → *Routes of vapour escape*
- *Freezing rate*
- *Formulation, concentration*

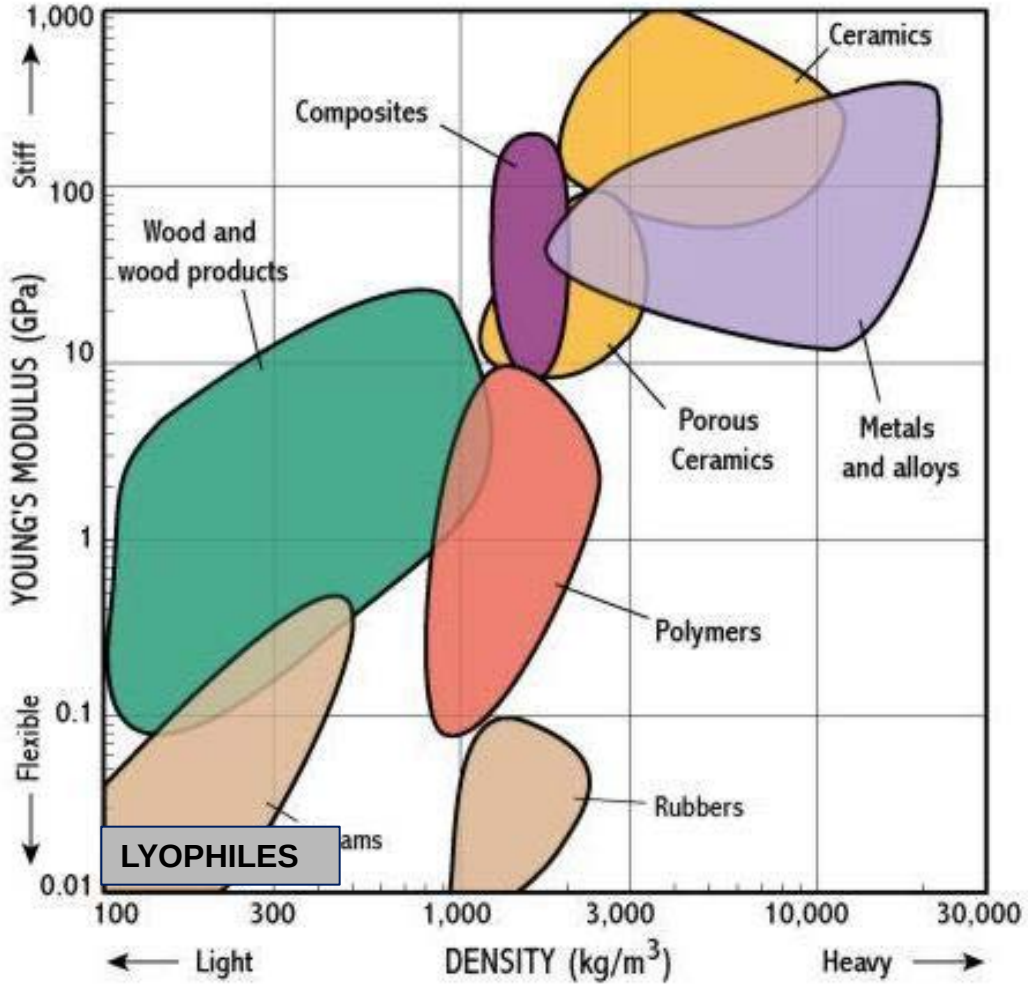




Note that porosity and surface area are typically ***inversely*** proportional!

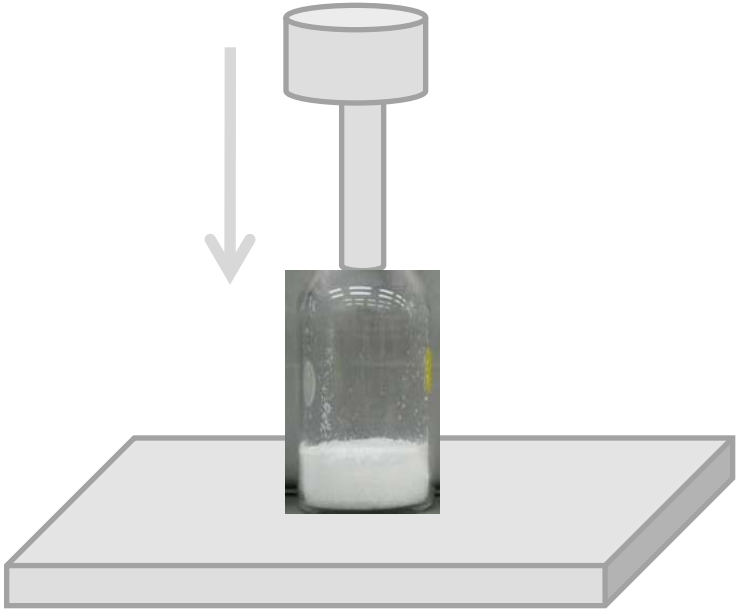
Cellular Solids: Map of Young's Modulus versus Density

- Looking at the mechanical properties of lyophiles is a challenge
- Compared with most materials that are tested for Young's Modulus (stress-strain), they are:
  - VERY light
  - VERY flexible



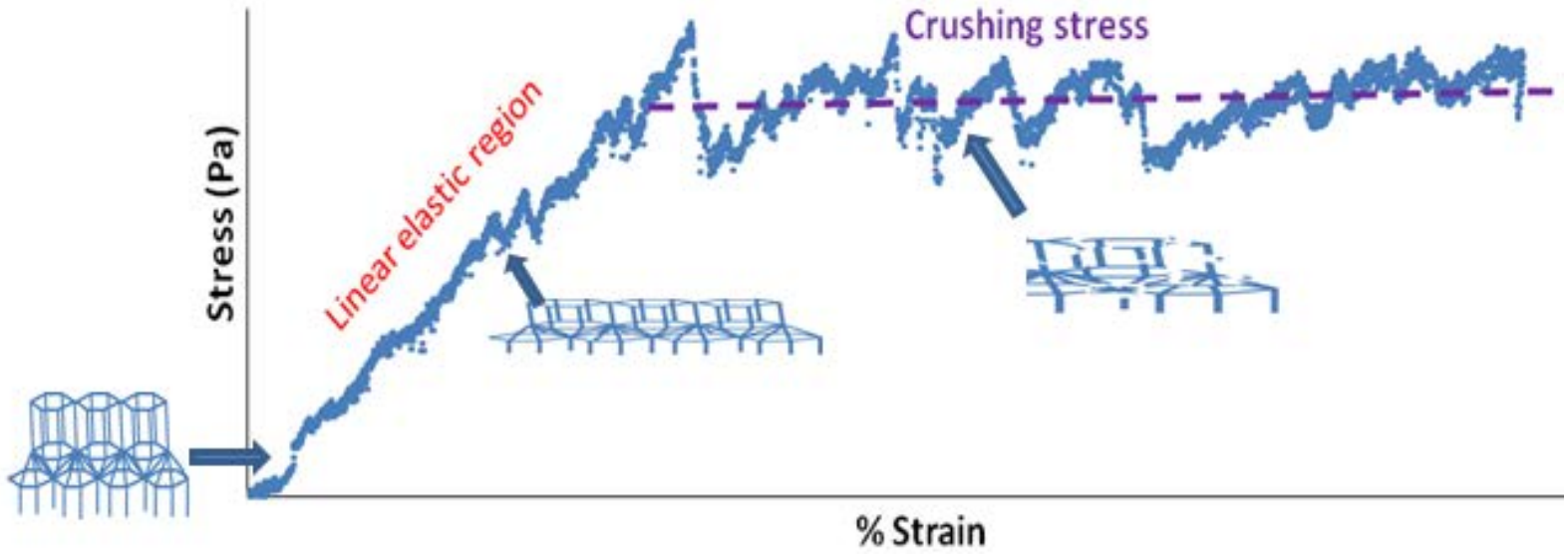
Adapted Compression Testing
(in collaboration with Imperial College London)

- Standard compression testers typically have a minimum force of *100gr – 250gr*
- However, we needed to use ***much smaller*** forces than this



- Stable Microsystems Texture analyser and Lloyds EZ50.
- Loaded at speed of 1mm/sec, tested at 0.01mm/sec to a depth of 3mm ***(which we had adapted to be able to start at 1g force)***
- Samples tested ***in situ*** in the vial
- Samples: SLOW, FAST and LN_2 QUENCH- cooled mannitol

Compressive Indentation of a Lyophile



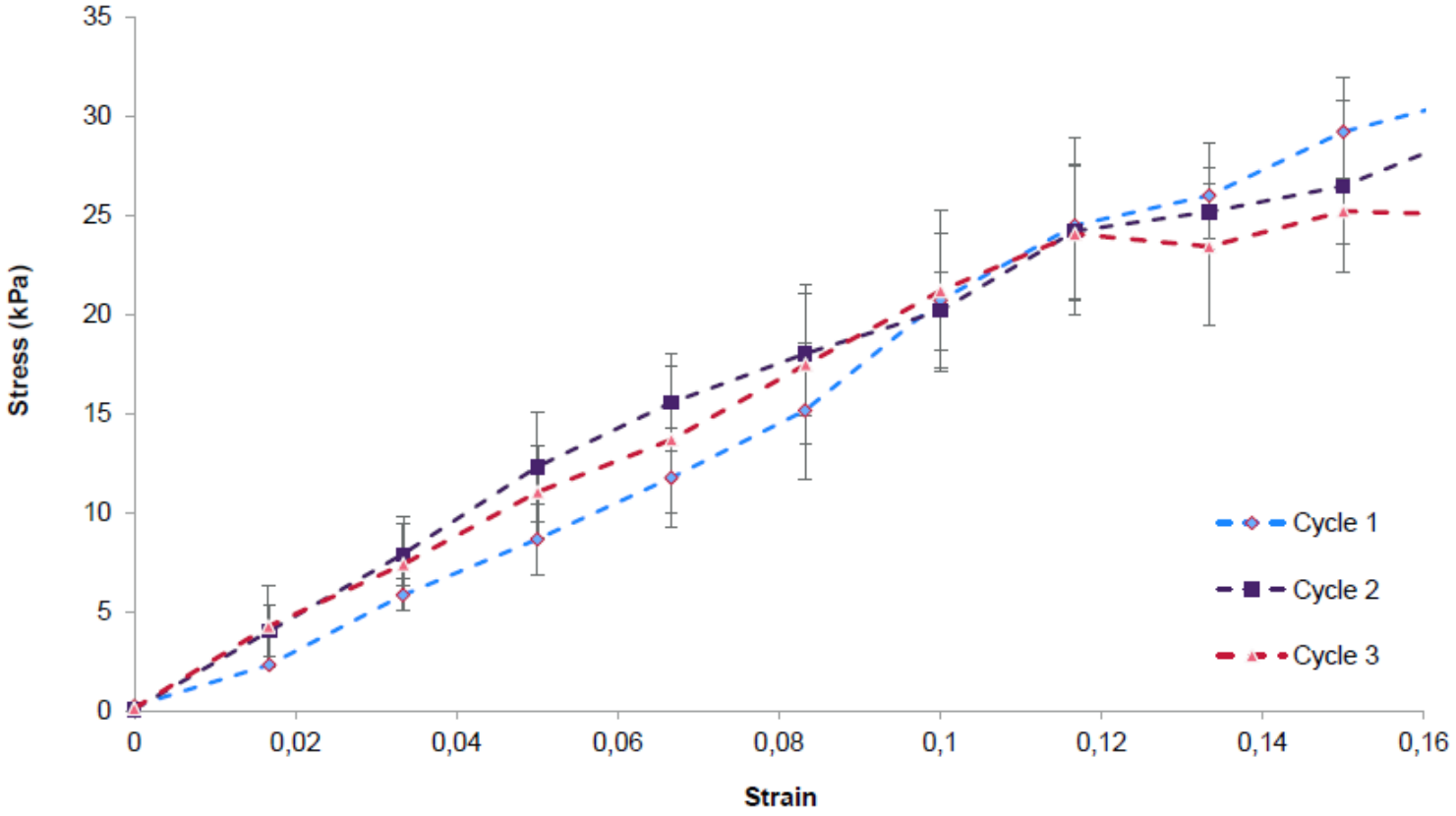
$$E = \frac{\sigma}{\varepsilon}$$

•where σ is the stress and ε is the strain, and E is the Young's/Elastic modulus

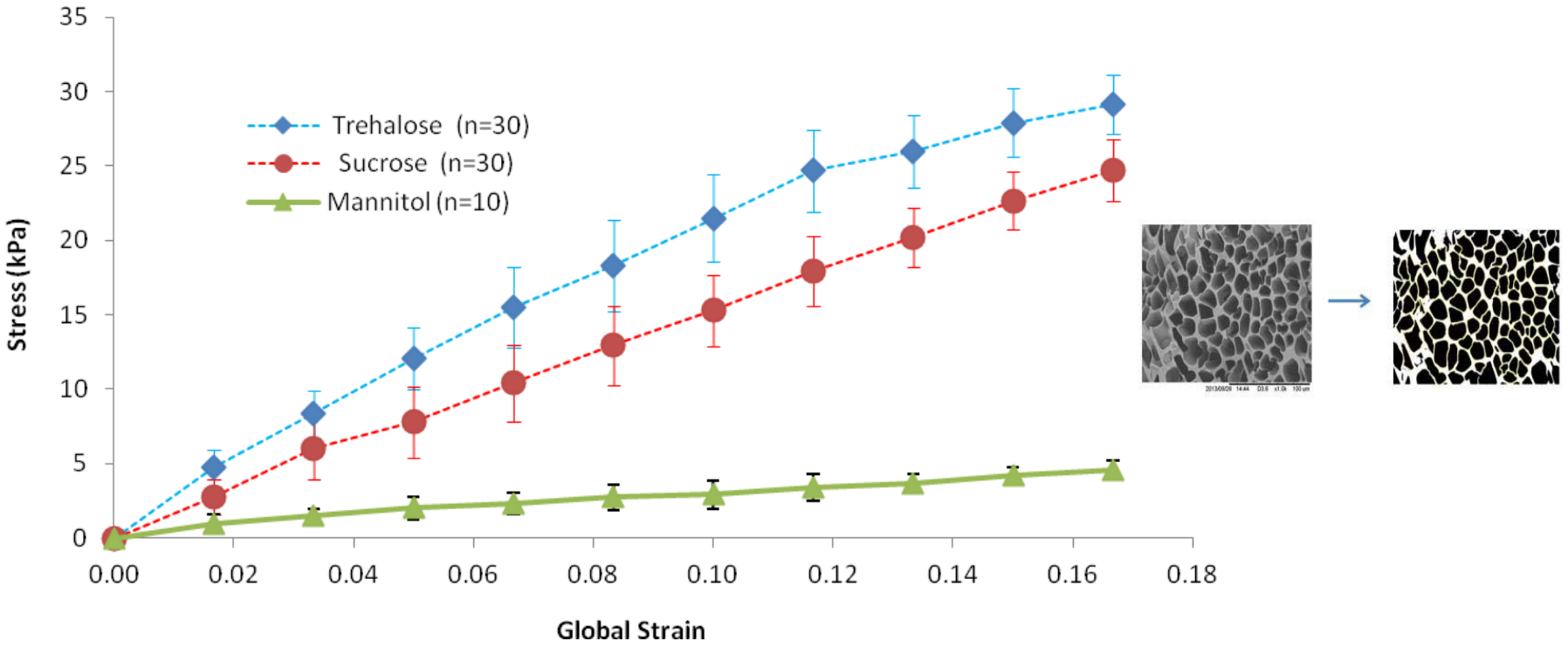
- *Test takes less than 1 minute*
- *Cake stays within vial - in situ test*
- *No moisture effect if test time less than 3 minutes*

Trehalose cake reproducibility

- n=10 for each run (+/- 1σ)
- Same FD cycle but on 3 different runs



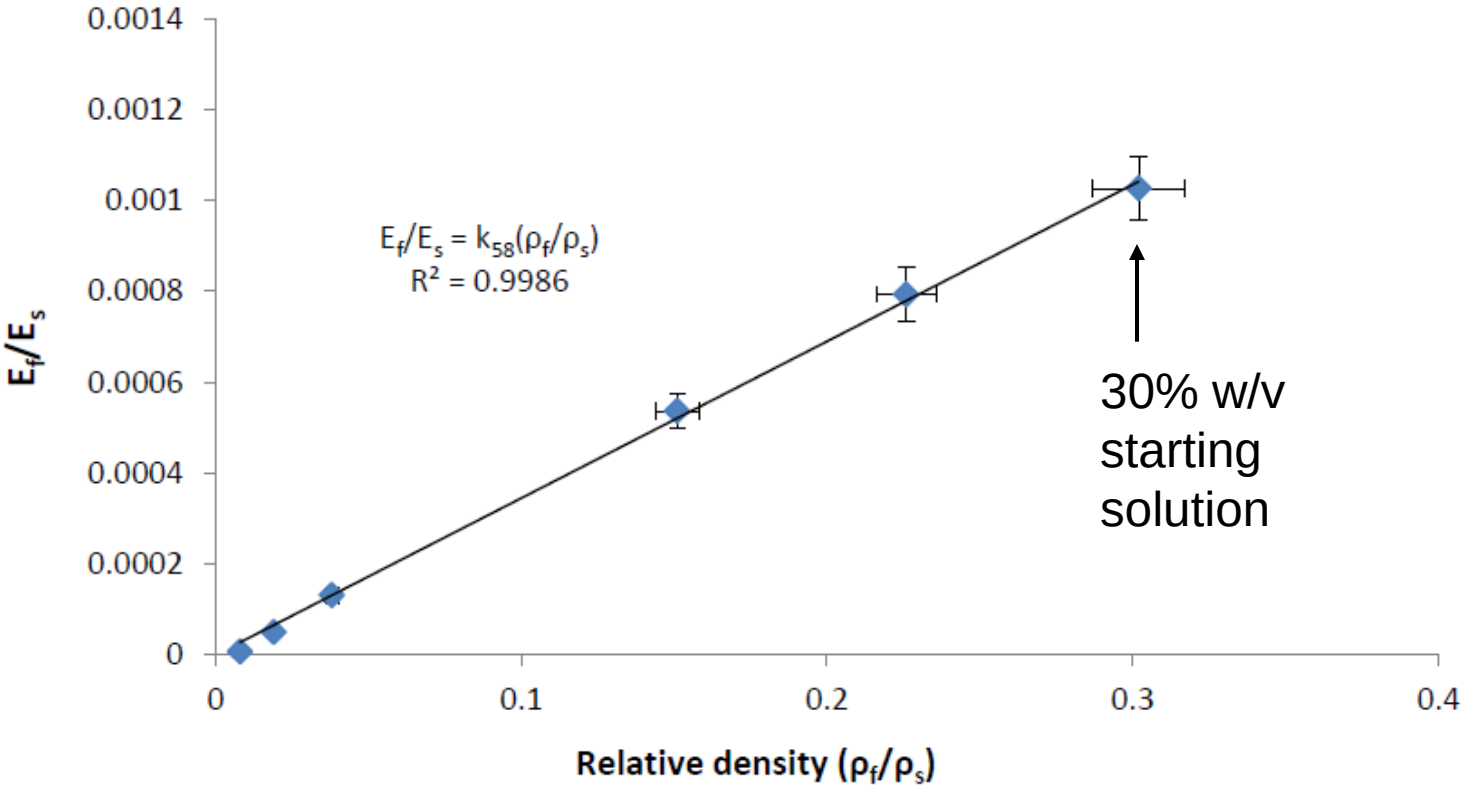
Compressive Mechanics:
Trehalose, Sucrose and Mannitol Cakes



Despite structural similarity, the cakes display different mechanical properties, which demonstrates the choice of excipient is important

Freeze-Dried Sucrose:

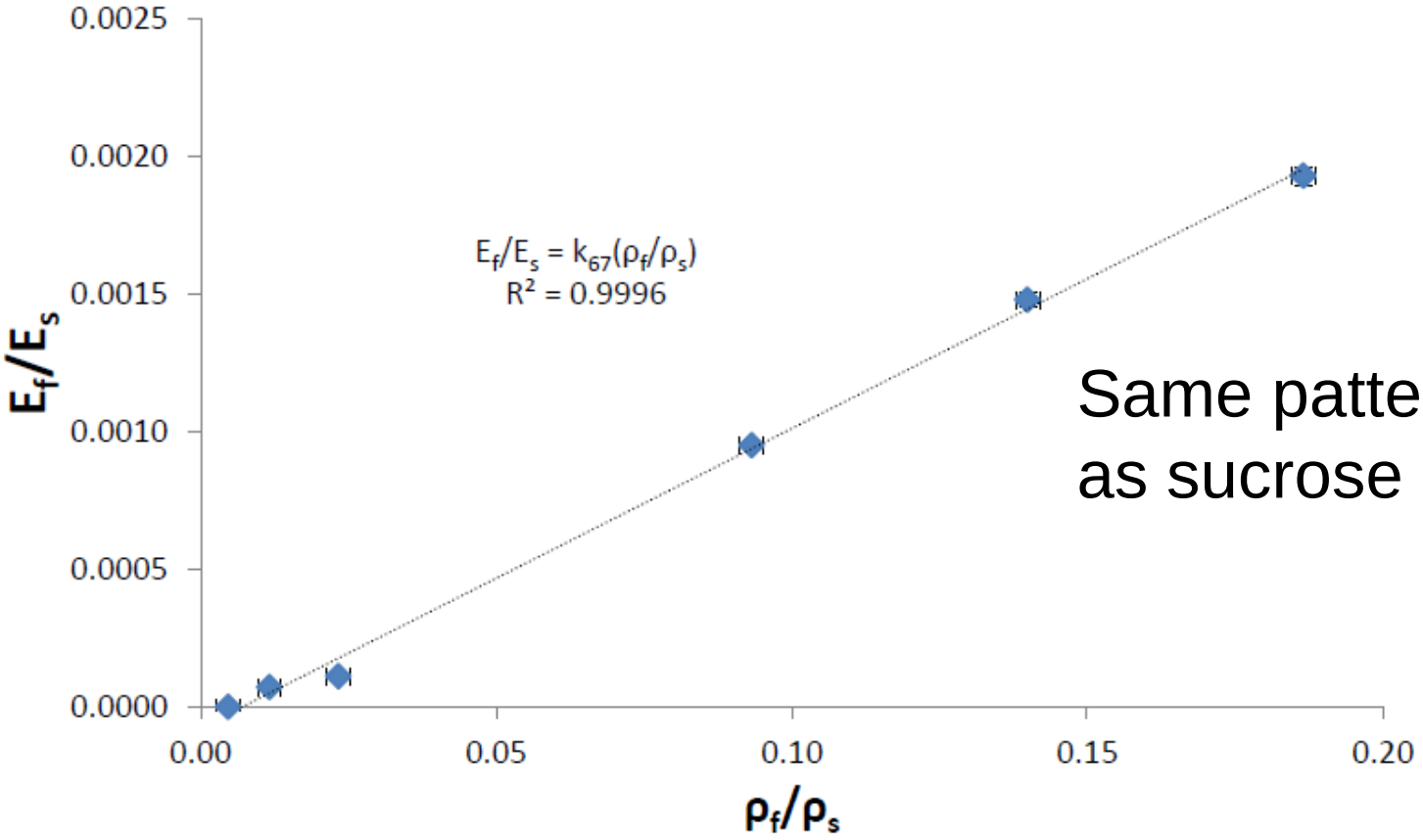
Modulus vs. Density Linear over wide range



Graph of Relative Young's modulus as a function of relative density of freeze dried cakes where k_{58} is 0.0034 ± 0.0001

Freeze-Dried Trehalose:

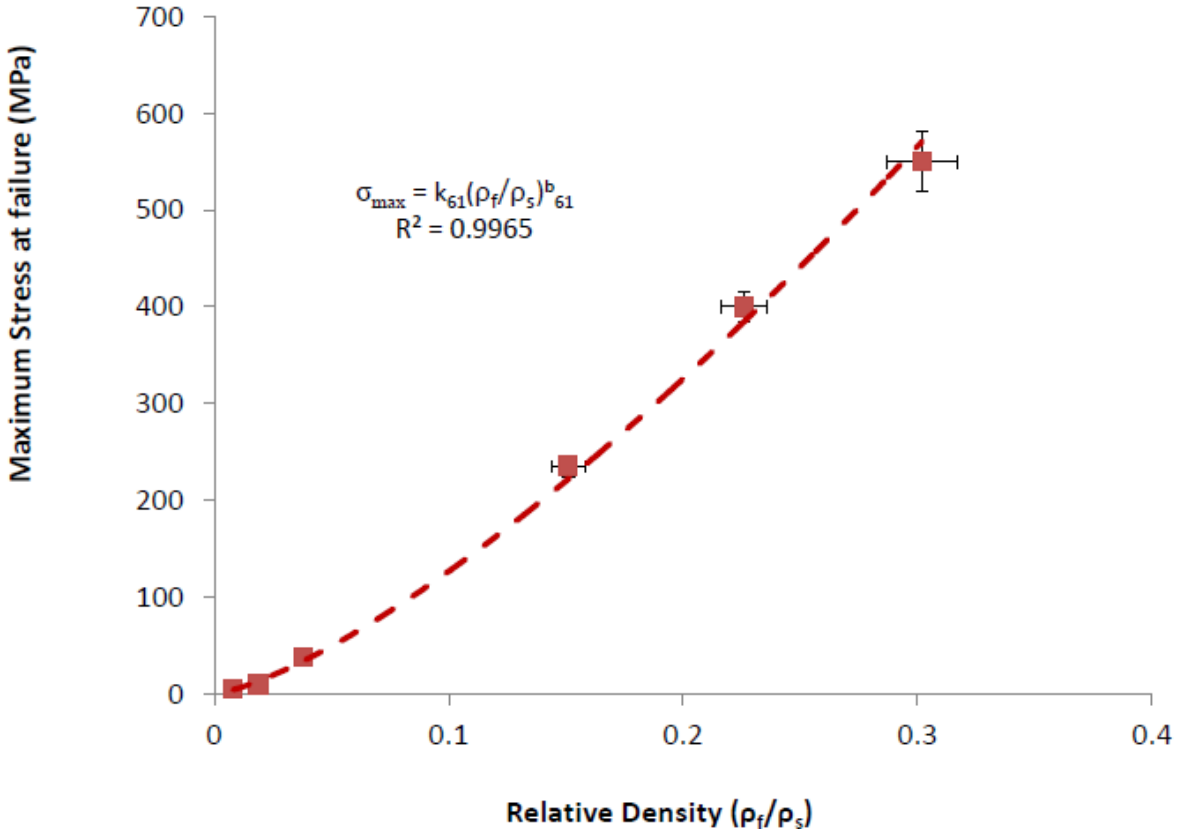
Modulus vs. Density Linear over wide range



Same pattern as sucrose

Testing cakes until they crack…

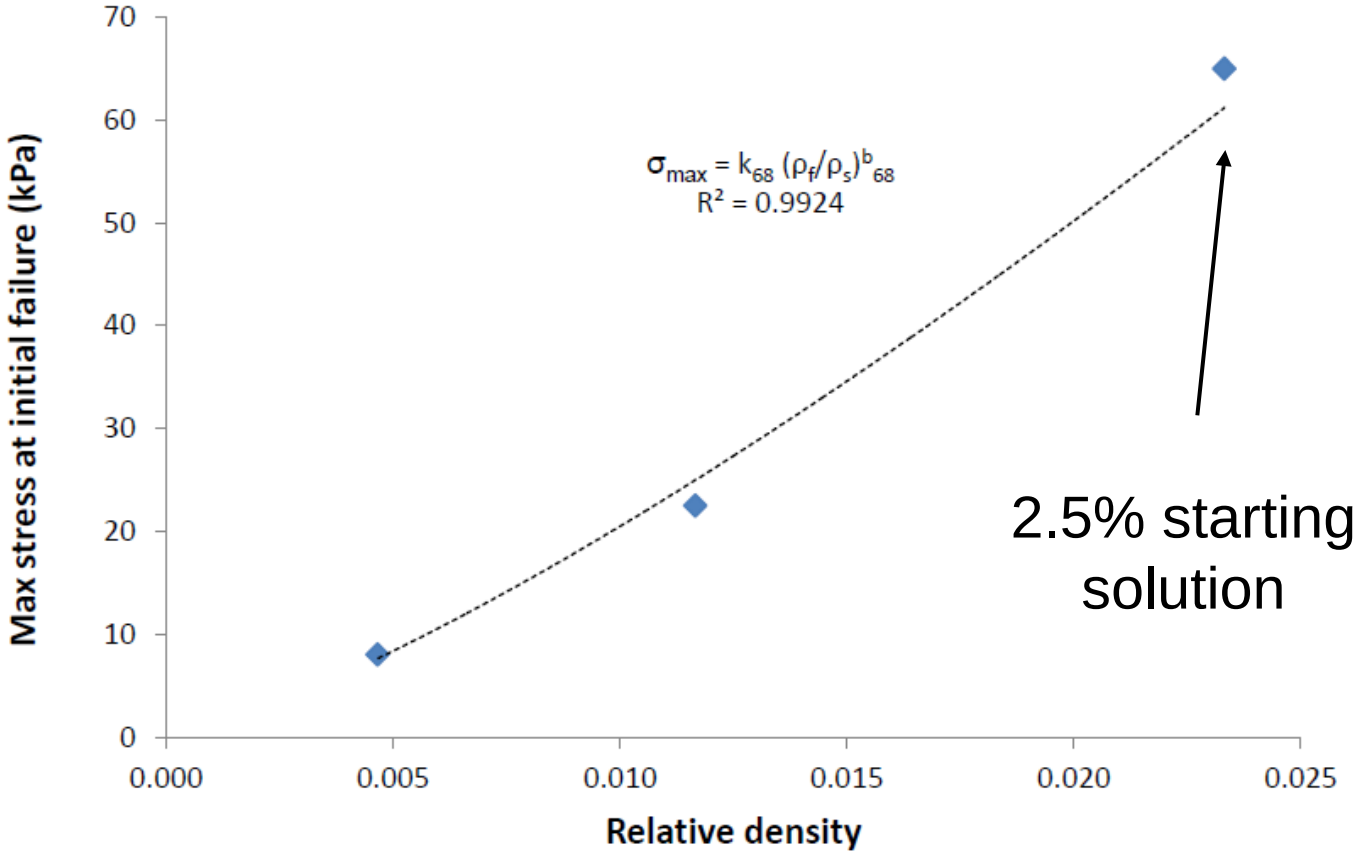
Sucrose: *higher concentrations can take more stress*



Graph of maximum stress at failure of freeze dried sucrose cakes using indentation compression where k_{61} is 3800 ± 220 and b_{61} is 1.48 ± 0.04

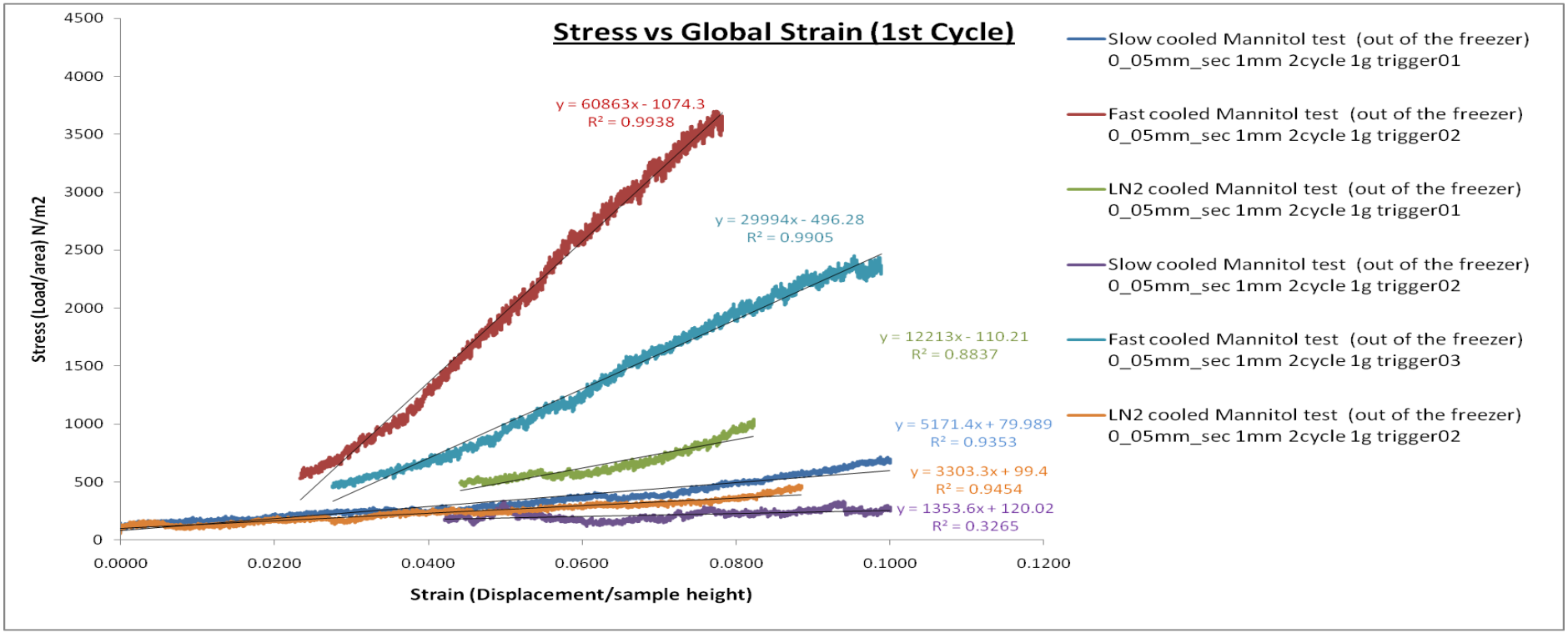
Looking at failure stress at low densities:

Trehalose: *still gives* ***near****-linearity*



Graph showing maximum stress at failure of freeze dried trehalose cakes using indentation compression; k_{68} is 7700 ± 350 and b_{68} is 1.46 ± 0.12

Stress / Strain data for Mannitol



$$E = \frac{\sigma}{\varepsilon}$$

•where σ is the stress and ε is the strain, and E is the Young's/Elastic modulus

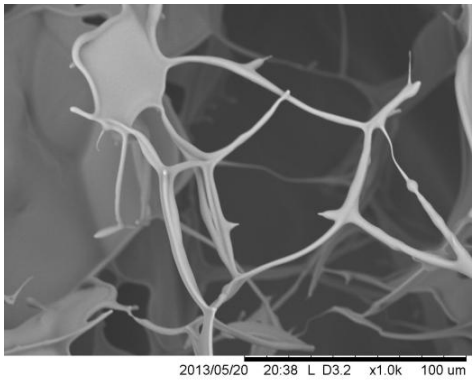
Mannitol Data Summary

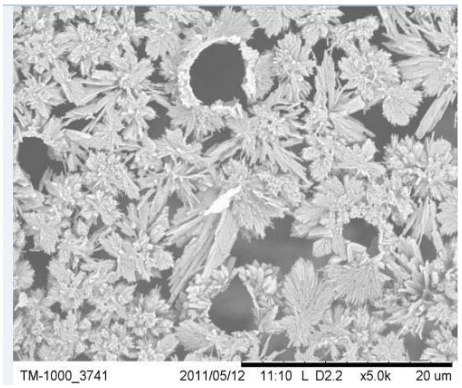
E modulus data	Individual Results	MEAN (N/m^2)
Slow Cooled	3303	2328
	1353	*Weakest*
'Fast' Cooled	12213	8692
	5171	
LN_2 Quench Cooled	60683	45428
	29994	*Strongest*

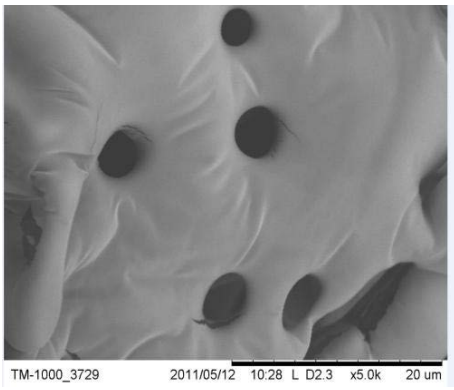
- Due to morphology or fundamental material properties (or both)?
- Slow cooled has larger pores, therefore the support may not be strong
- SEM pictures show small holes are visible in the LN2 and slow cooled samples; holes are the weakest points for the cakes to crack / break
- Is there an **inherent strength** / physical stability of the mannitol (or any of its polymorphic forms / hydrates / amorphous form)?
- More work needed to figure these issues out…

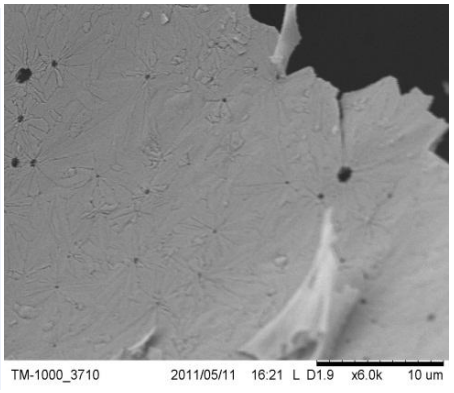
Contributing Factors to Mechanical Properties

- Flaws such as cracks and splits
- Solid distribution in the cell walls and struts
- Brittleness and ductility of material
- Elastic modulus of the solid of construction
- Wide distribution in cell sizes
- Mixture of open and closed cells









What about more complex (realistic) formulations?

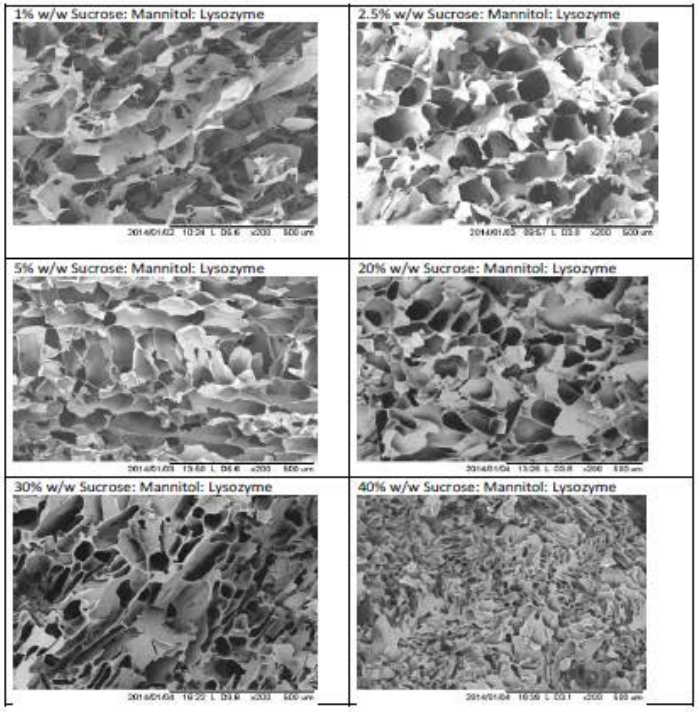
- Studies were carried out using lysozyme as a model protein, formulated with sucrose + mannitol
- SEM of lyophiles dried from different total starting concentrations demonstrated that the concentration had a far greater effect on pore size than for sucrose alone
- This suggests that other factors can be important, although more work needs to be done to establish why some factors outweigh others in different examples…

Viscosity Heat Transfer **Container Type** Fill Depth…

Microscopic structure is related to formulation, not just total concentration… SEM evidence

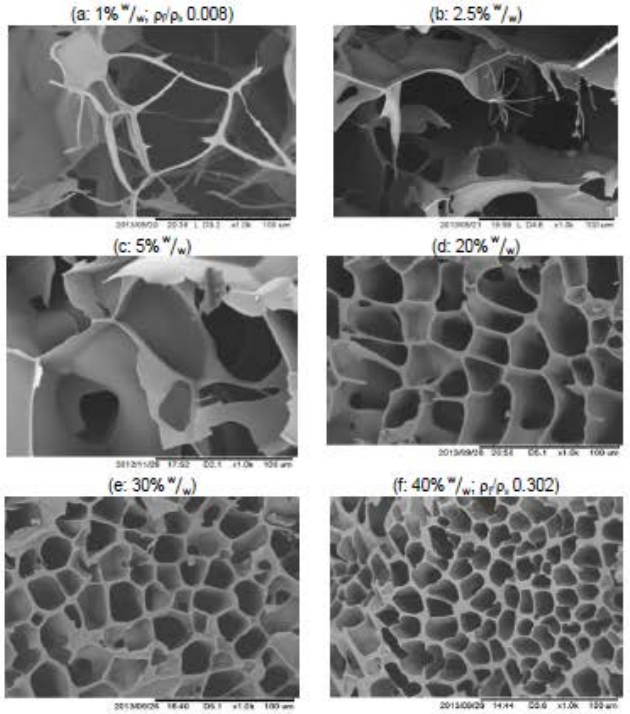
(the samples below were freeze-dried at the same time)

X200, Lysozyme formulation



Wide distribution in cell size

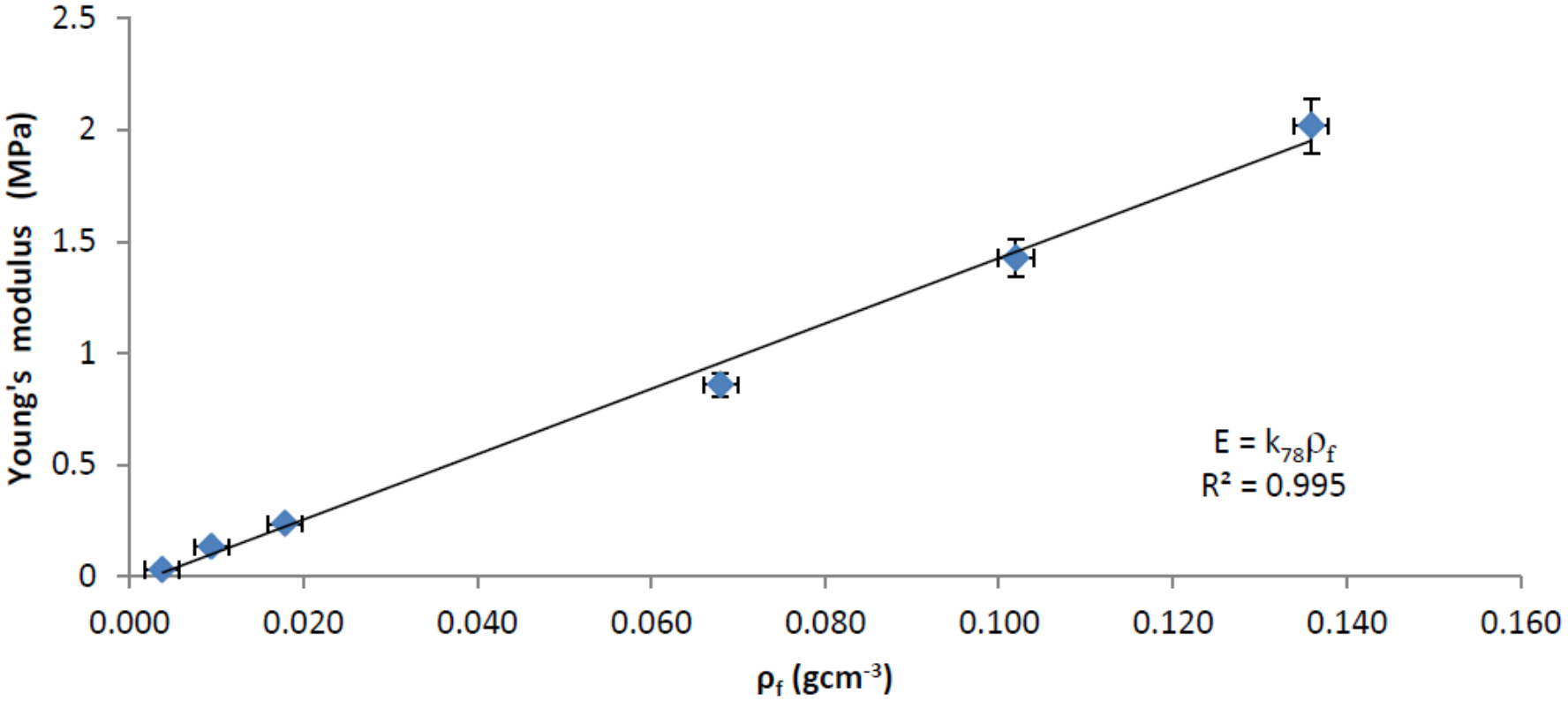
X1000, Sucrose



Cells are comparatively more uniform

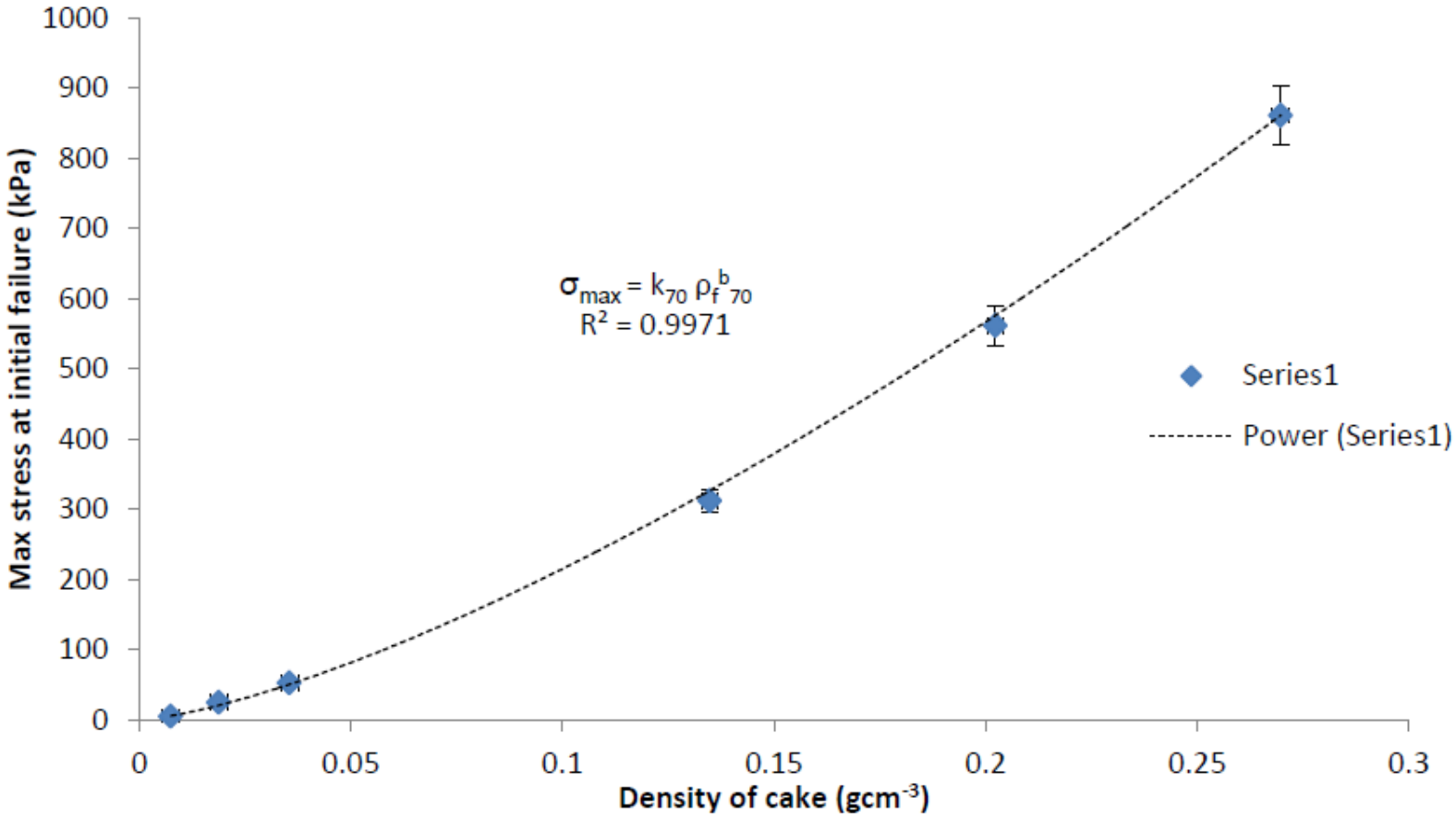
Modulus of Lysozyme Formulations

Still linear with density… despite there having been apparently more variability in pore size in SEM



Max Stress of Lysozyme Formulations

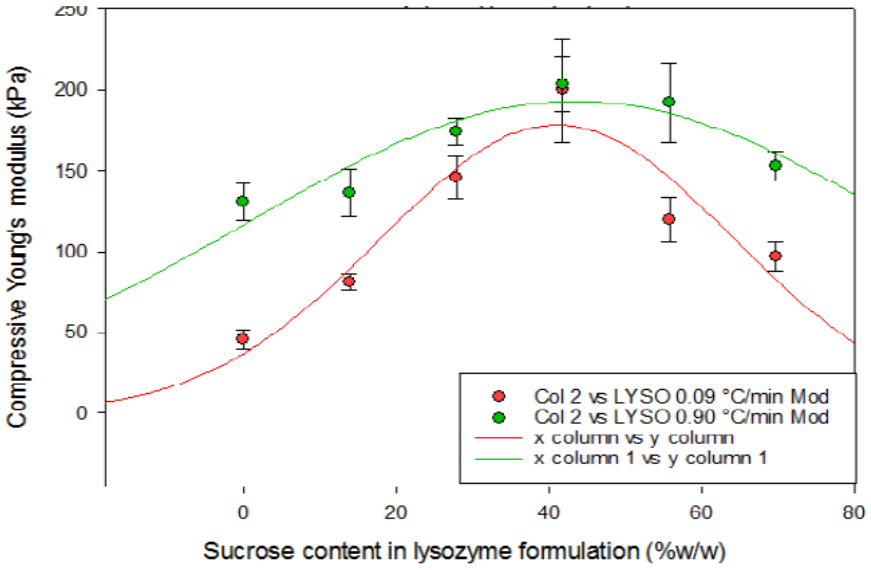
Exactly the same relationship with density as the individual samples of sucrose and trehalose



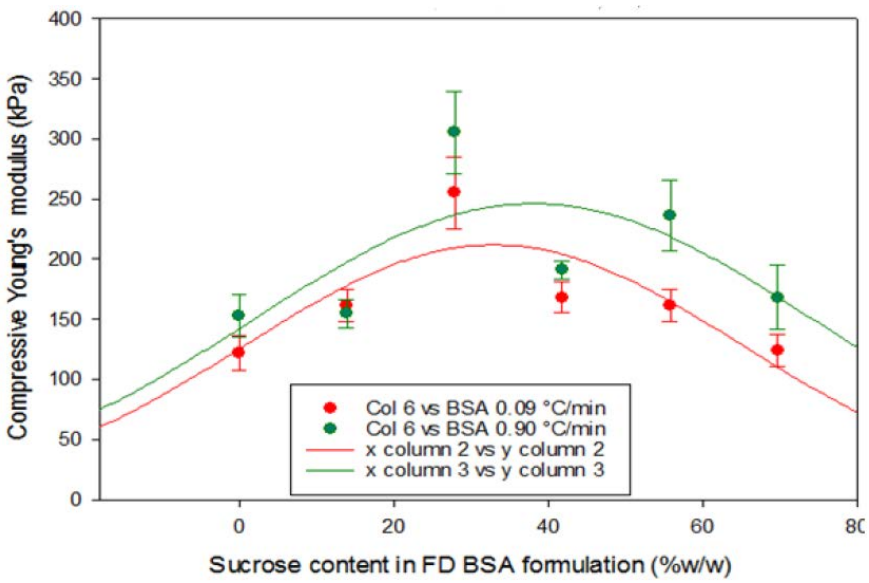
BSA as gives similar results to lysozyme:

Young's Modulus vs. relative sucrose content

Lysozyme formulations



BSA formulations



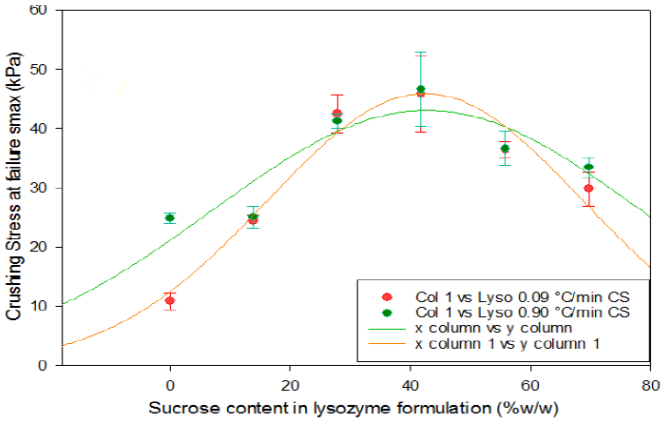
Same solid content, protein and buffer concentration at 2 freezing rates

Red data points = slow freezing (0.09 degC/min)
Green data points = fast freezing (0.9 degC/min)

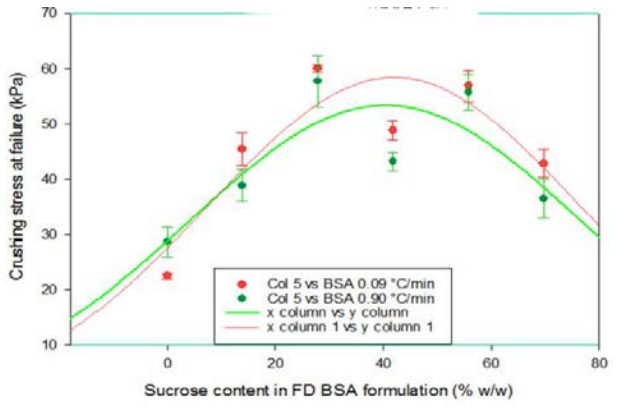
BSA as gives similar results to lysozyme:
Young's Modulus vs. relative sucrose content

OBSERVATIONS:

- Strongest cakes at around 50% Sucrose making up the total excipients *i.e.* 1:1 ratio sucrose:mannitol as total excipient content
- Faster cooling rate (0.9˚ C/min) produces slightly more elastic/durable cakes than 0.09˚ C/min
- There observations were supported by Maximum Stress data:



Graph showing a Gaussian 3 parameter curve fitted to maximum stress at failure of FD lysozyme cakes as a function sucrose content in the FD lysozyme cakes.



Graph showing a Gaussian 3 parameter curve fitted to maximum stress at failure data of FD BSA formulation cakes as a function of freezing rate and sucrose contribution to total excipient used

Summary of Mechanical Performance

	Pore size	Wall thickness
Excipient		
Trehalose Sucrose Mannitol	similar	similar
Freezing rate		
slow fast		similar
Primary drying shelf temperature		
-40°C -50°C	similar	similar
Solution Concentration (%w/w)		
1 2.5 5 20 30 40		
Nucleation		
Controlled	89+/-12	8+/-4
Uncontrolled	100+/-36	10+/-5
Excipient composition in Lysozyme/BSA formulation		
Mannitol : Sucrose (10:0) Mannitol : Sucrose (8:2) Mannitol : Sucrose (6:4) Mannitol : Sucrose (4:6) Mannitol : Sucrose (2:8) Mannitol : Sucrose (0:10)	similar	similar

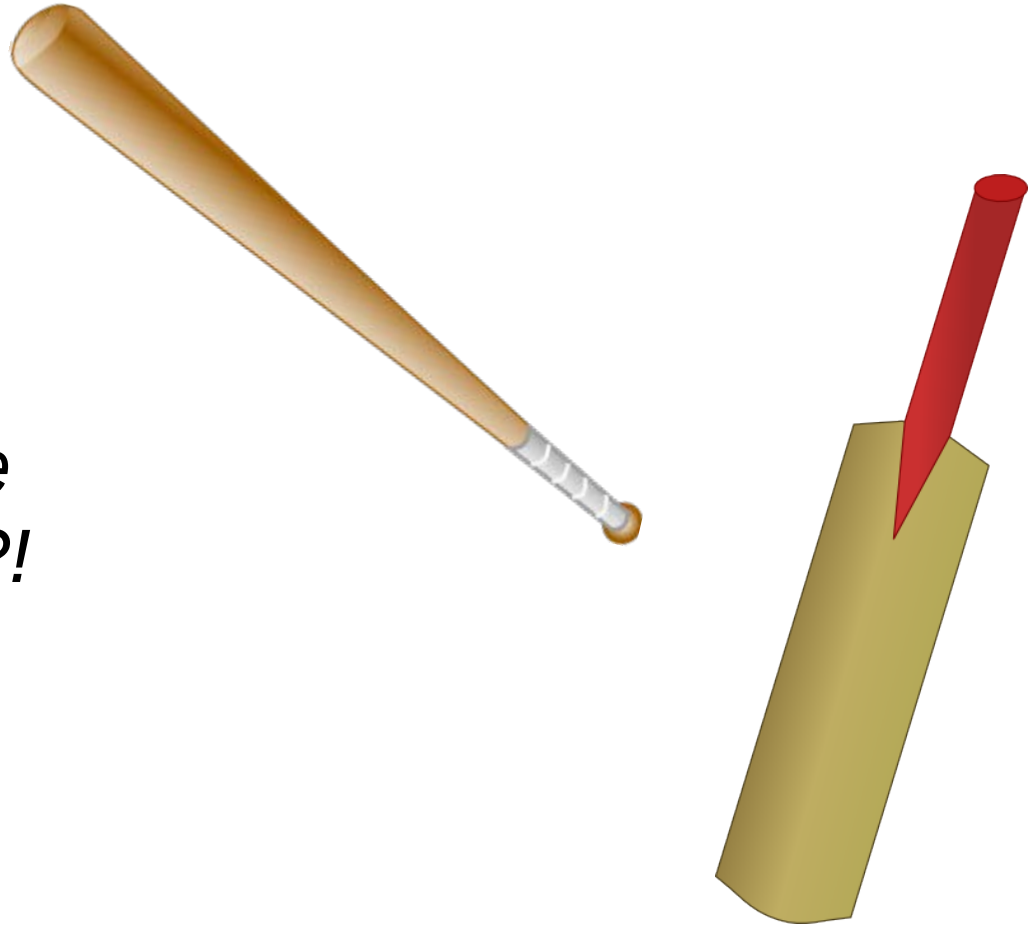
SUMMARY & CONCLUSIONS (1)

- Mechanical Analysis offers quick, cheap, *in situ*, comparative post-process data - a partial end to reliance on qualitative visual / SEM data
- As a Development Tool
  - Excipient selection: don't just choose on collapse temperature!
  - Quantitative comparison of effect of cooling rates
  - Quantitative comparison of effect of concentration change
- As a QbD Tool
  - Fill in further blanks in your Prior Knowledge!
  - Fits in with Risk Reduction as part of QbD initiative
  - Contribution to Regulatory Filing
- As a Process Development / Control Tool:
  - To demonstrate Success of Scale-Up / Technology Transfer
  - Batch Reproducibility / Comparability testing

SUMMARY & CONCLUSIONS (2)

BUT……

Do the advantages outweigh developing another regulatory yardstick that might be used to beat you later?!



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…and Thank You for your attention!