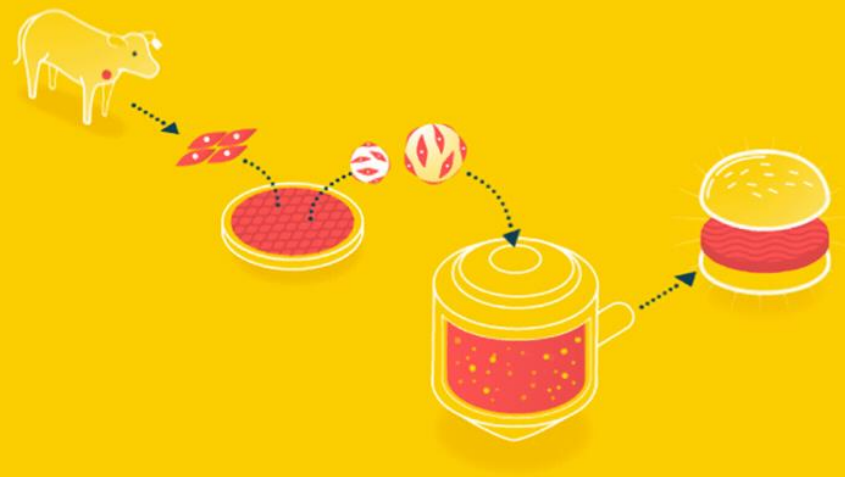
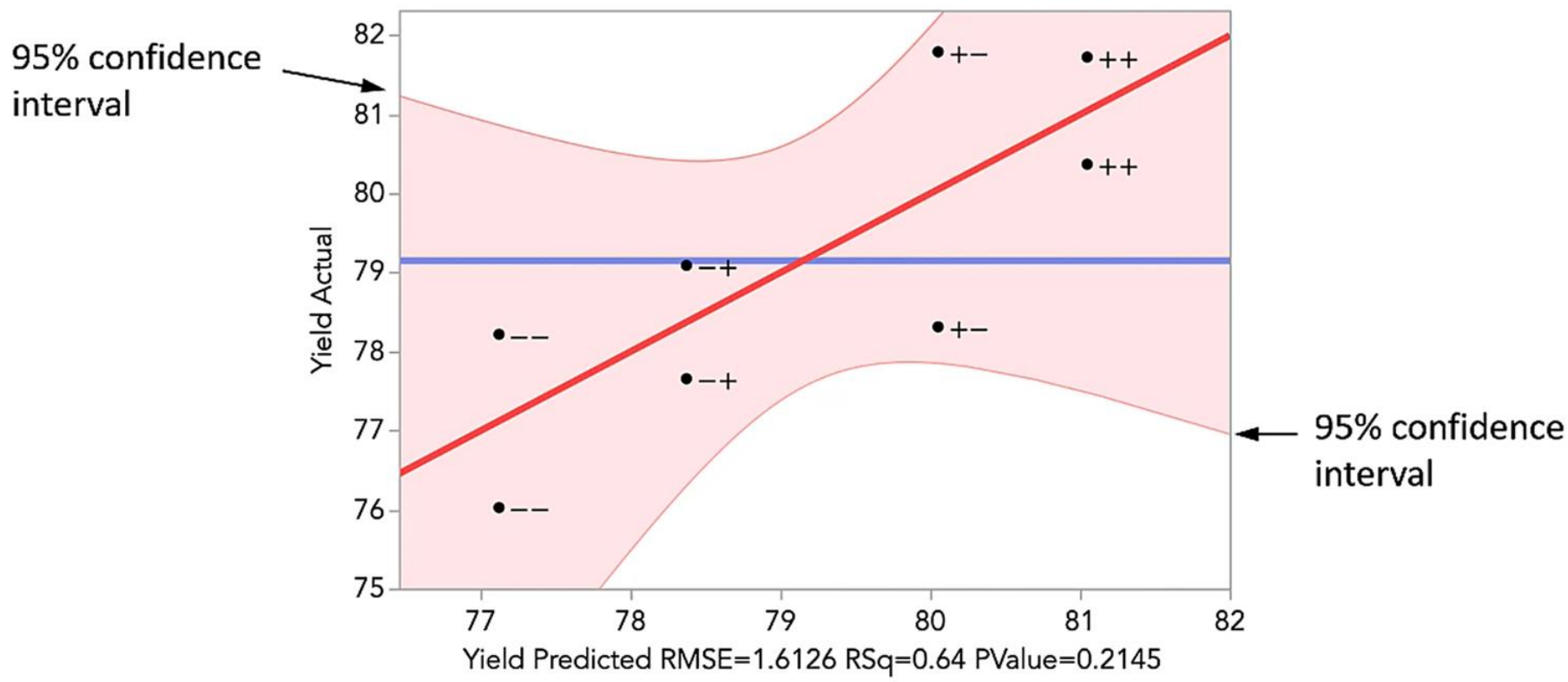
Tufts BME174 – Meat Lab

Week 9: Fat Texture Characterization



DOE Analysis: Fit with model

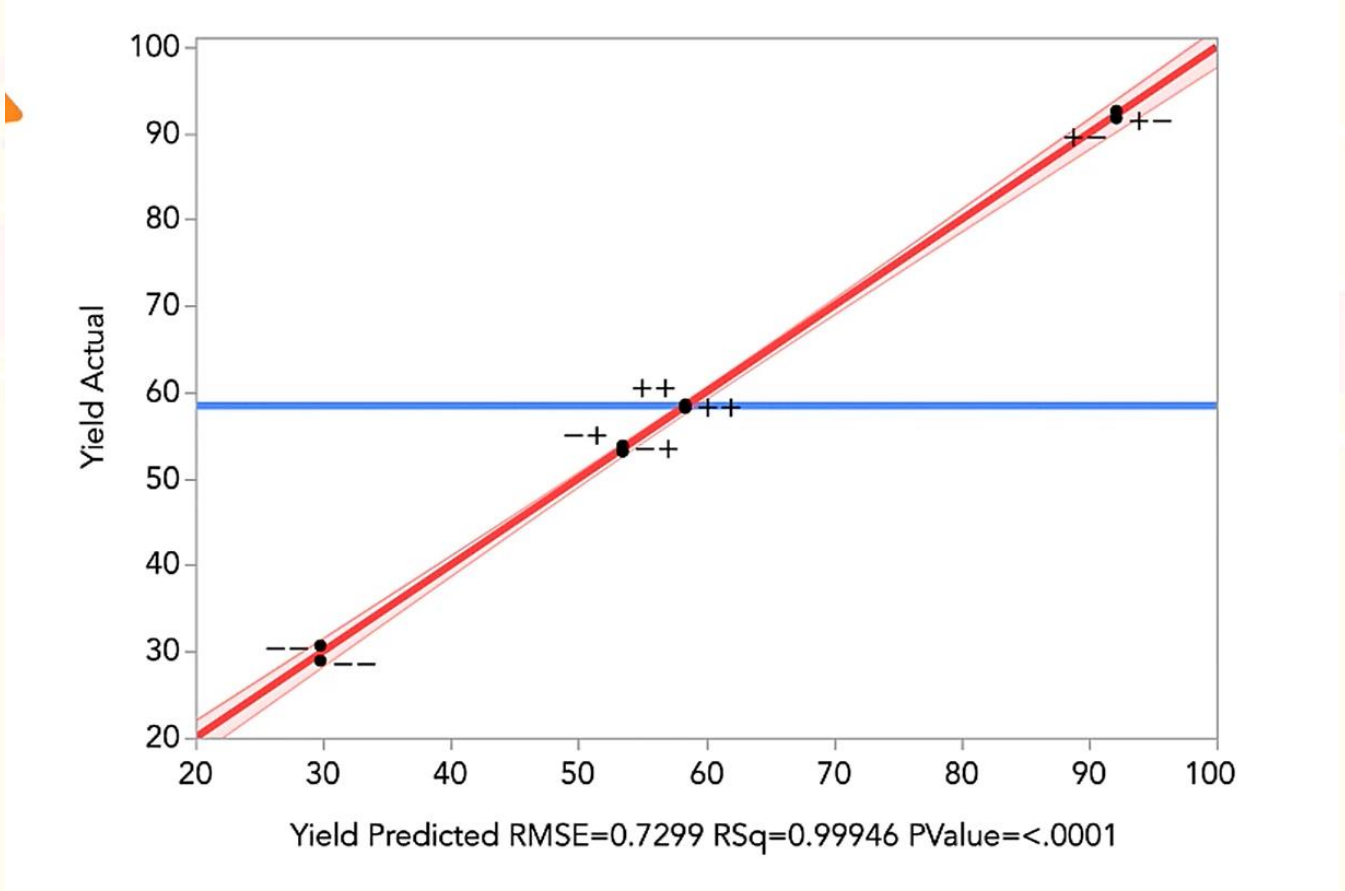
Poor fit



DOE Analysis: Fit with model

Good fit

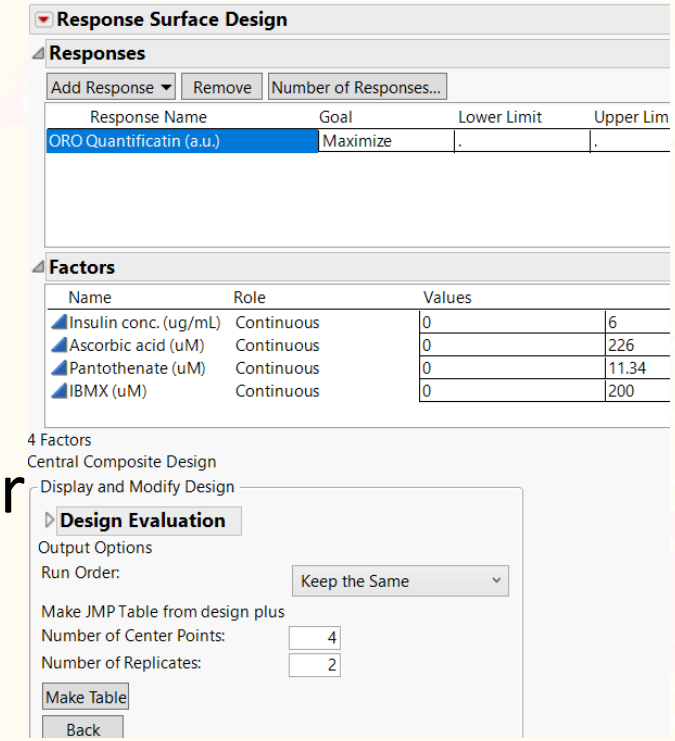
We will be using standard least squares regression for our DOE analysis



Response Surface Design

Made in JMP software

(Available for free through Tufts, but you don't need it for this class)



	Pattern	Insulin conc. (ug/mL)	Ascorbic acid (uM)	Pantothenate (uM)	IBMX (uM)	ORO Quantificatin (a.u.)
1	−−−−	0	0	0	0	•
2	−−−+	0	0	0	200	•
3	−−+−	0	0	11.34	0	•
4	−−++	0	0	11.34	200	•
5	−+−−	0	226	0	0	•
6	−+−+	0	226	0	200	•
7	−++−	0	226	11.34	0	•
8	−+++	0	226	11.34	200	•
9	+−−−	6	0	0	0	•
10	+−−+	6	0	0	200	•
11	+−+−	6	0	11.34	0	•
12	+−++	6	0	11.34	200	•
13	++−−	6	226	0	0	•
14	++−+	6	226	0	200	•
15	+++−	6	226	11.34	0	•
16	++++	6	226	11.34	200	•
17	a000	0	113	5.67	100	•
18	A000	6	113	5.67	100	•
19	0a00	3	0	5.67	100	•
20	0A00	3	226	5.67	100	•
21	00a0	3	113	0	100	•
22	00A0	3	113	11.34	100	•
23	000a	3	113	5.67	0	•
24	000A	3	113	5.67	200	•
25	0000	3	113	5.67	100	•
26	0000	3	113	5.67	100	•
27	0000	3	113	5.67	100	•
28	0000	3	113	5.67	100	•

24 media for corners and sides of tesseract (4D version of cube)

Each group's center point replicate

Data Processing

- Subtracted blanks from each data point
- Normalized data to each group’s center point

Normalization to Center Point

Before normalization

Source	LogWorth	PValue
Group	8.479	0.00000
Ascorbic acid (uM)*Ascorbic acid (uM)	2.108	0.00780
Ascorbic acid (uM)(0,226)	1.291	0.05121 ^
Pantothenate (uM)*Pantothenate (uM)	0.976	0.10566
Ascorbic acid (uM)*IBMX (uM)	0.895	0.12749
Insulin conc. (ug/mL)*Insulin conc. (ug/mL)	0.714	0.19309
IBMX (uM)(0,200)	0.713	0.19384 ^
Insulin conc. (ug/mL)*Ascorbic acid (uM)	0.589	0.25762
Insulin conc. (ug/mL)*Pantothenate (uM)	0.537	0.29071
Insulin conc. (ug/mL)(0,6)	0.432	0.36978 ^
IBMX (uM)*IBMX (uM)	0.399	0.39947
Pantothenate (uM)(0,11.34)	0.196	0.63721 ^
Pantothenate (uM)*IBMX (uM)	0.189	0.64710
Ascorbic acid (uM)*Pantothenate (uM)	0.008	0.98093
Insulin conc. (ug/mL)*IBMX (uM)	0.002	0.99499

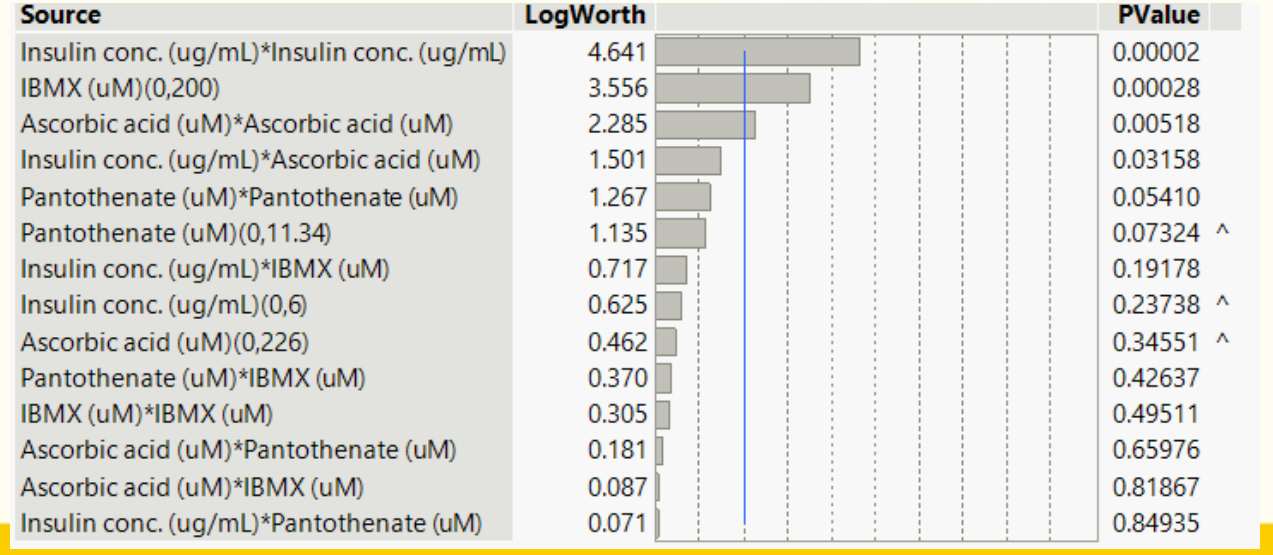
After normalization

Source	LogWorth	PValue
Insulin conc. (ug/mL)*Insulin conc. (ug/mL)	2.951	0.00112
Insulin conc. (ug/mL)*Pantothenate (uM)	2.192	0.00642
Ascorbic acid (uM)*IBMX (uM)	1.125	0.07505
Ascorbic acid (uM)*Pantothenate (uM)	1.019	0.09577
Insulin conc. (ug/mL)*Ascorbic acid (uM)	0.764	0.17209
IBMX (uM)*IBMX (uM)	0.660	0.21900
Ascorbic acid (uM)*Ascorbic acid (uM)	0.644	0.22675
Ascorbic acid (uM)(0,226)	0.429	0.37232 ^
Group	0.382	0.41456
IBMX (uM)(0,200)	0.262	0.54764 ^
Pantothenate (uM)(0,11.34)	0.215	0.60951 ^
Insulin conc. (ug/mL)(0,6)	0.210	0.61673 ^
Pantothenate (uM)*Pantothenate (uM)	0.191	0.64428
Pantothenate (uM)*IBMX (uM)	0.175	0.66899
Insulin conc. (ug/mL)*IBMX (uM)	0.162	0.68931

From Monday Section

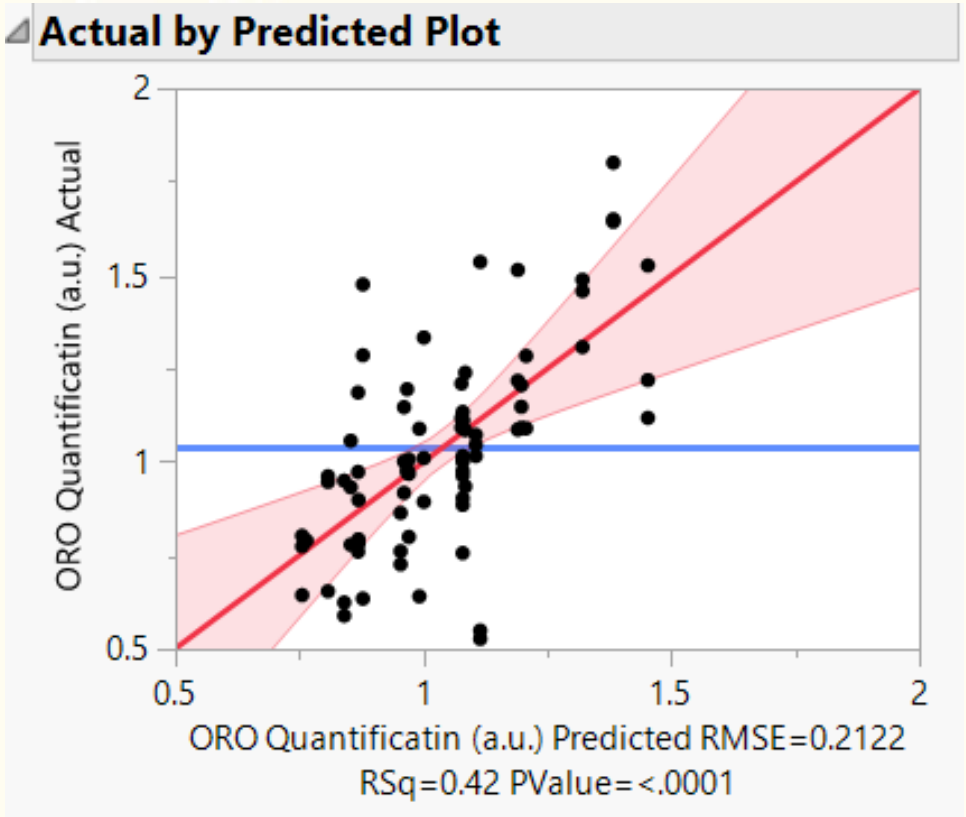
Data Processing

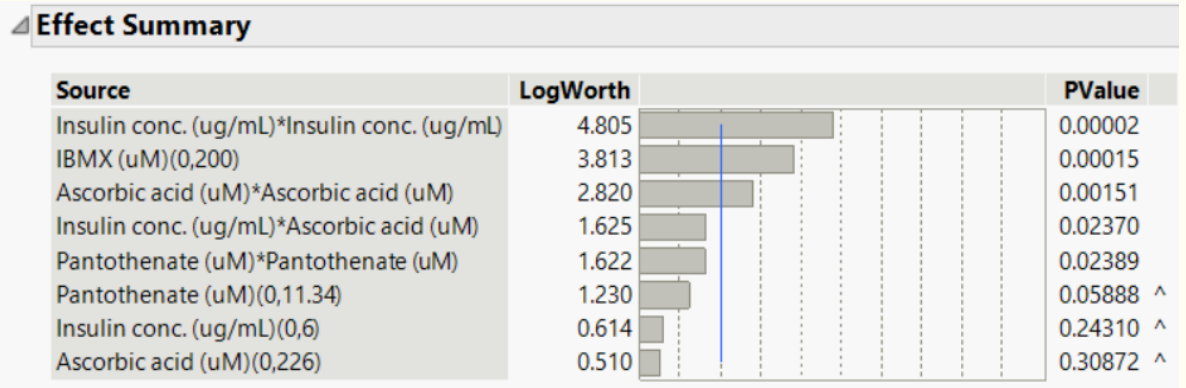
- Subtracted blanks from each data point
- Normalized data to each group's center point
- Run analysis with standard least squares (linear) model
- Remove non-significant terms from the model. Why?
- Maximize desirability to get predicted best medium

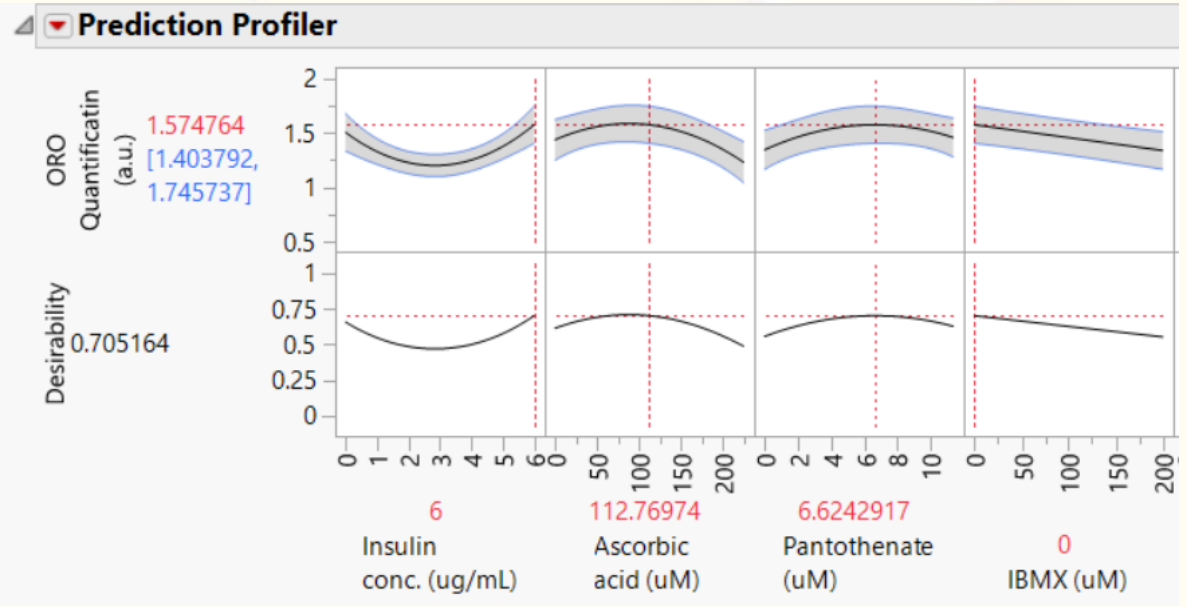


Source	LogWorth	PValue	
Insulin conc. (ug/mL)*Insulin conc. (ug/mL)	4.641	0.00002	
IBMX (uM)(0,200)	3.556	0.00028	
Ascorbic acid (uM)*Ascorbic acid (uM)	2.285	0.00518	
Insulin conc. (ug/mL)*Ascorbic acid (uM)	1.501	0.03158	
Pantothenate (uM)*Pantothenate (uM)	1.267	0.05410	
Pantothenate (uM)(0,11.34)	1.135	0.07324	^
Insulin conc. (ug/mL)*IBMX (uM)	0.717	0.19178	
Insulin conc. (ug/mL)(0,6)	0.625	0.23738	^
Ascorbic acid (uM)(0,226)	0.462	0.34551	^
Pantothenate (uM)*IBMX (uM)	0.370	0.42637	
IBMX (uM)*IBMX (uM)	0.305	0.49511	
Ascorbic acid (uM)*Pantothenate (uM)	0.181	0.65976	
Ascorbic acid (uM)*IBMX (uM)	0.087	0.81867	
Insulin conc. (ug/mL)*Pantothenate (uM)	0.071	0.84935	

Final results



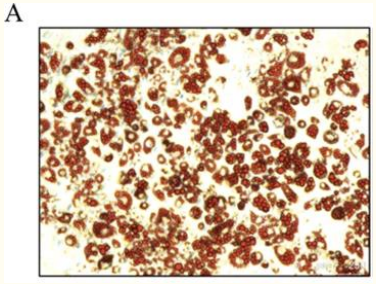




Recap of Week 8…

Part 1

- Lipid accumulation in differentiated fat cells was quantified through Oil Red O staining.
- Oil Red O is a red stain for neutral lipids that can be visualized with light microscopy and quantified using a plate reader by isopropanol elution.



Part 2

- Differentiated fat cells were seeded into a scaffolding material of choice.
- Scaffolds were stored at 4C over 2 weeks.

Hypothesis: Fat cell addition will change the mechanical properties of the material.

Zhang et al., 2022

Week 9

Part 1

- Overview and importance of dynamic mechanical analysis (DMA) (SciTech Room 124)

Part 2

- DMA testing of fat cell-seeded scaffolds (SciTech Room 225)

Fat Texture Characterization

Why is it important to characterize the properties of seeded scaffolds?
(5 min discussion in groups!)

Fat Texture Characterization

Why is it important to characterize the properties of seeded scaffolds?

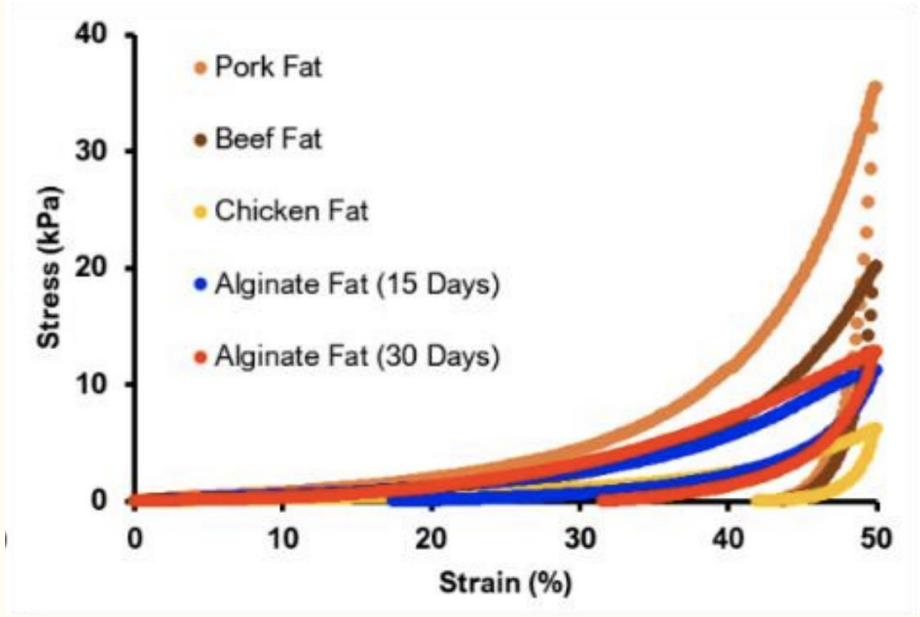
- Alterations to the mechanical properties of scaffolds affect…
  - cellular responses and cell-surface interactions[1]
    - as substrate stiffness increases, cell adhesion to surfaces increases[1]
  - cell growth and viability[1]
- Fat affects texture!
  - To replicate mechanical properties of traditional fat, which increases tenderness and juiciness of real meat[2,3]

Yuen et al., 2022 (to be published)

Fat Texture Characterization

Mechanical Testing

- Dynamic Mechanical Analysis (DMA) is a technique used to characterize a material's properties as a function of temperature, time, frequency, stress, atmosphere, or a combination of these parameters through small deformations in a cyclic manner.

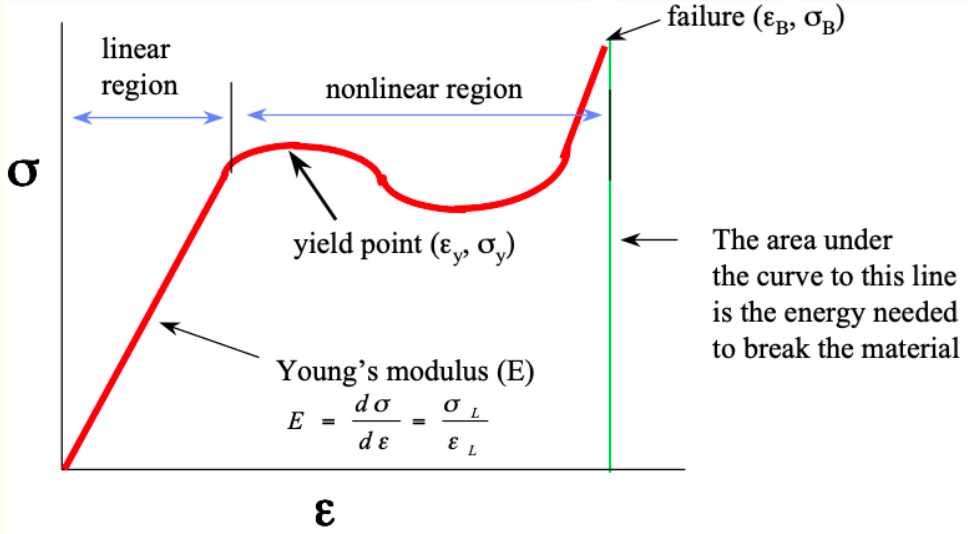


Yuen et al., 2022 (to be published)

Fat Texture Characterization

Stress-Strain Curves

A stress-strain curve is a graphical representation of the strength and elasticity of the material after a load is applied.



- Stress (σ): Force (F) applied per unit area (A). The unit of stress is N/m^2

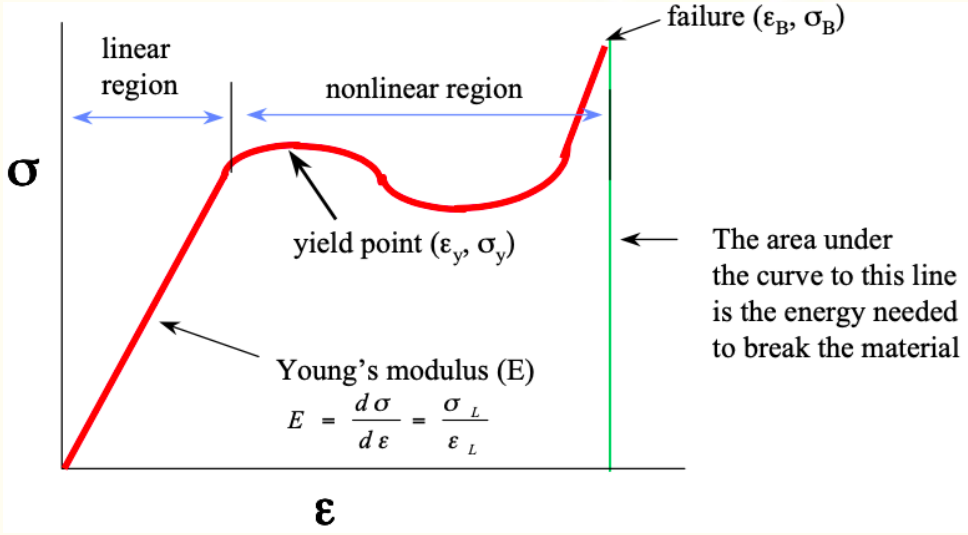
$$\sigma = \frac{F}{A}$$

- Stress applied to a material can be of two types: tensile and compressive
  - Tensile Stress: The external force per unit area of the material resulting in the stretch of the material.
  - **Compressive Stress: The force that is responsible for the deformation of the material, such that the volume of the material reduces.**

Fat Texture Characterization

Stress-Strain Curves

A stress-strain curve is a graphical representation of the strength and elasticity of the material after a load is applied.



- Strain (ε): Amount of deformation of the material in the direction of the force applied. The strain is a dimensionless quantity as it just defines the relative change in shape.

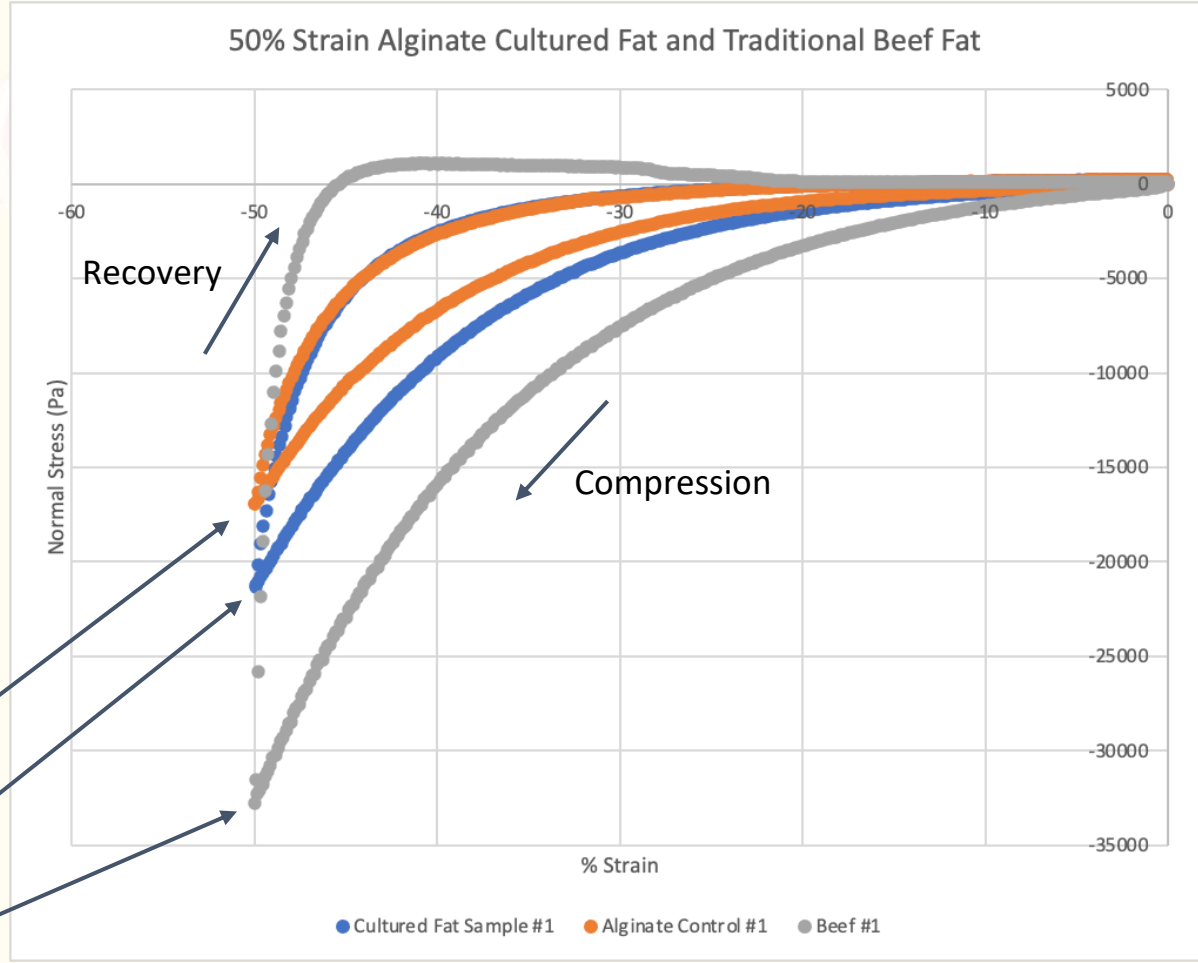
$$\epsilon = \frac{\delta l}{L}$$

- Young's modulus (E)
  - Measure of a material's elasticity/ stiffness.
  - High young's modulus = higher tensile strength = more stiff material

Fat Texture Characterization

Analysis of Data

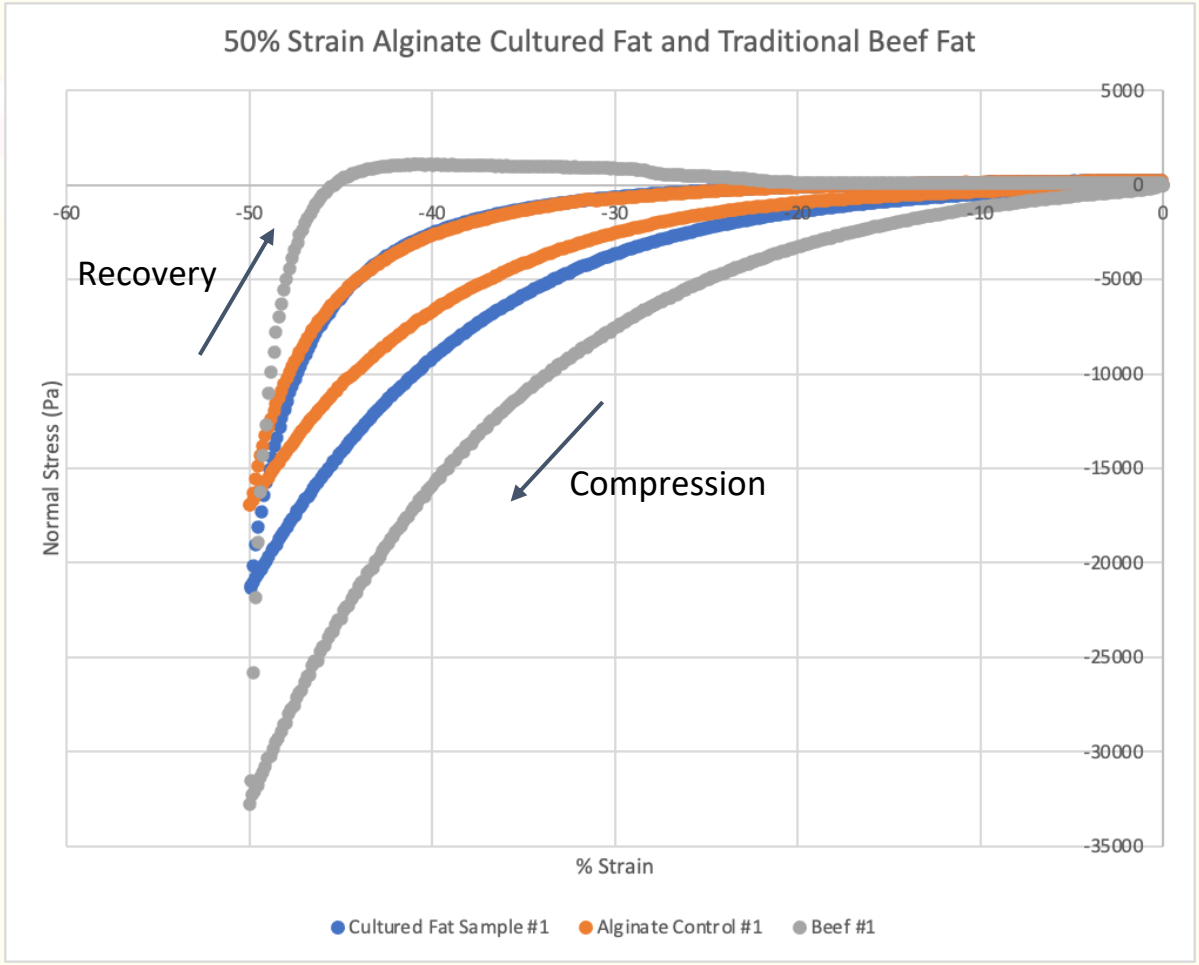
- Normal stress is measured both during compression and elastic recovery
- Young's modulus can be analyzed by determining the slope of compressive stress/strain curve
- Observe relative stress of samples at 50% strain



Fat Texture Characterization

Analysis of Data

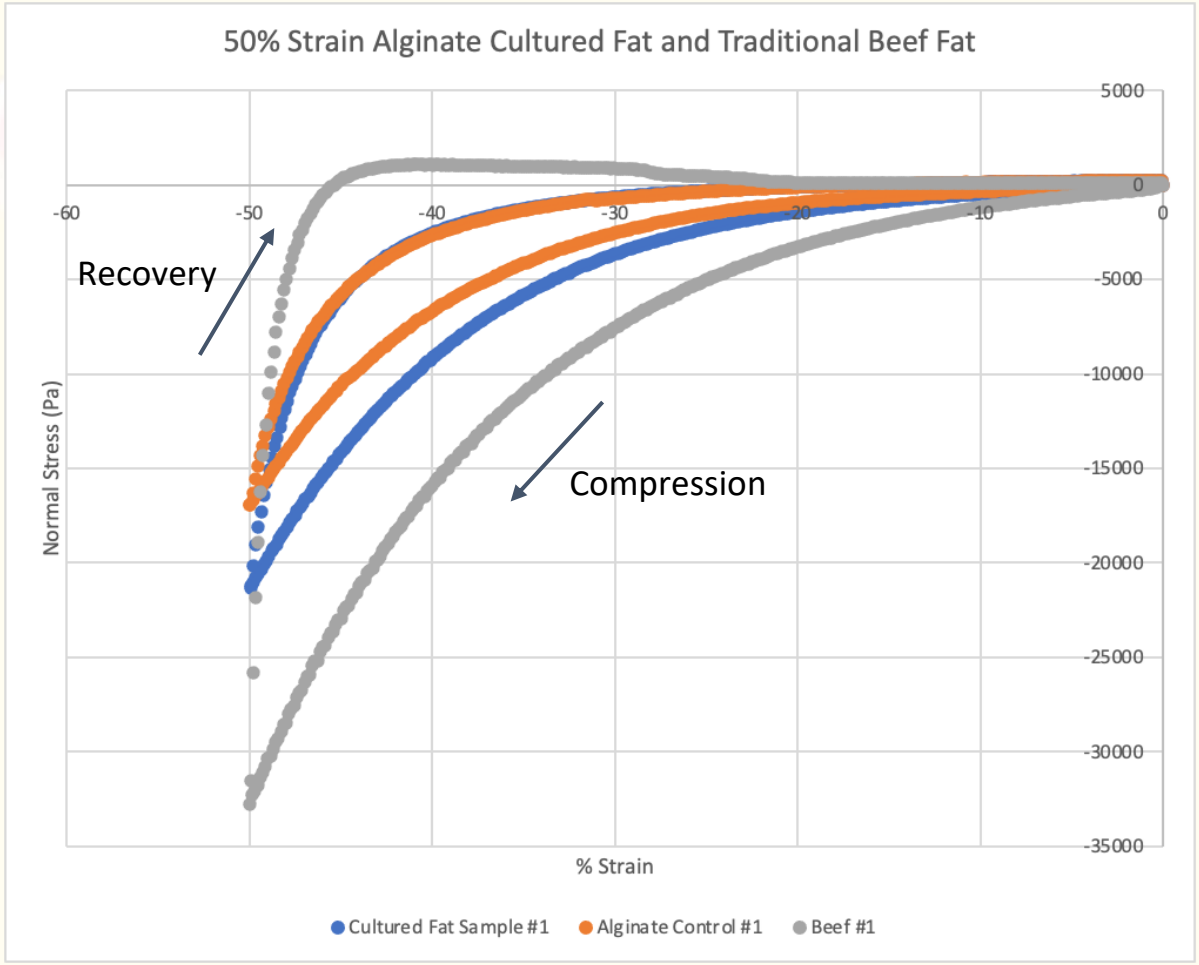
- What is the stress-strain curve telling us?



Fat Texture Characterization

Analysis of Data

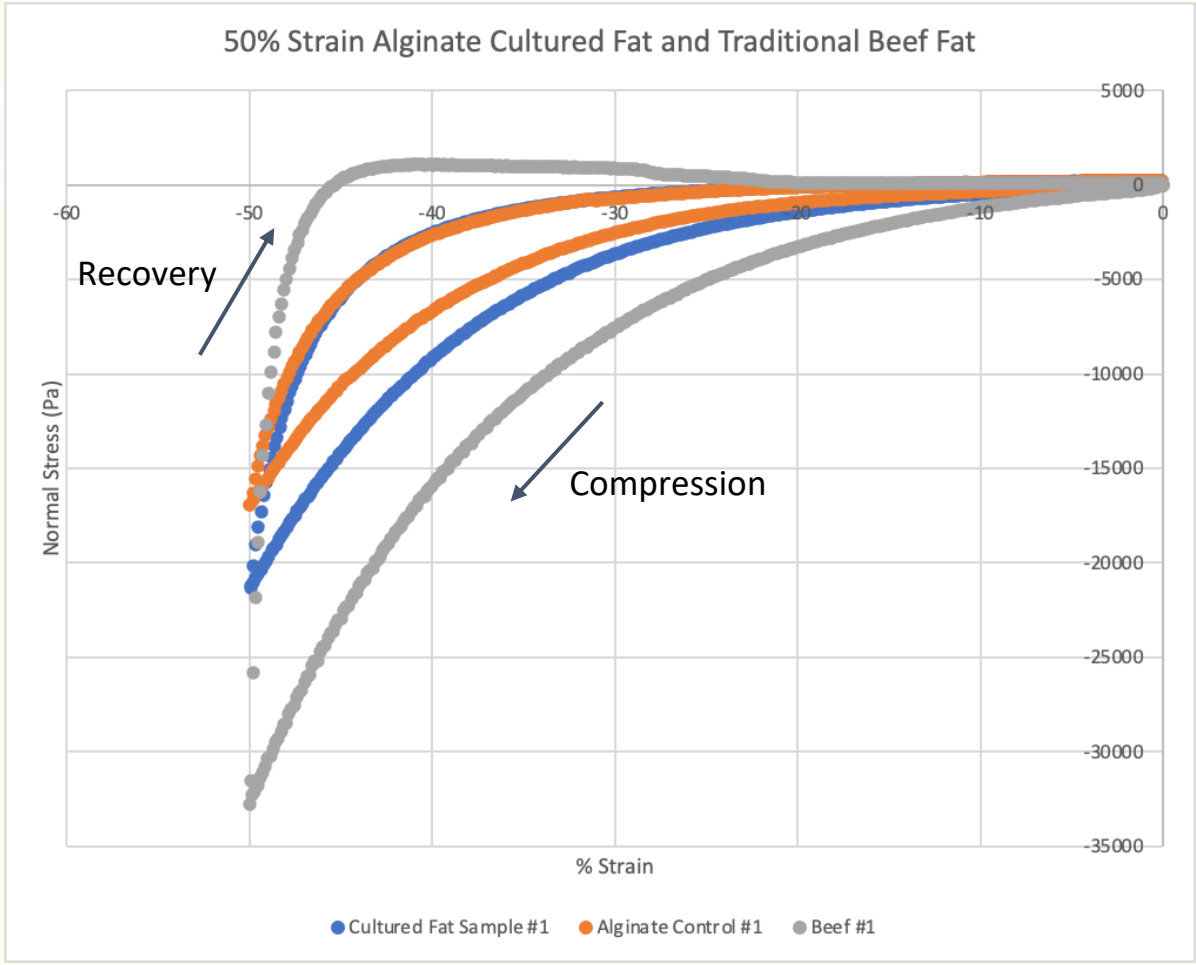
- What is the stress-strain curve telling us?



Fat Texture Characterization

Analysis of Data

- What is the stress-strain curve telling us?
  - Material is not perfectly elastic → plastic deformation occurs



Fat Texture Characterization

Analysis of Data

- Why do you think this is occurring?

Hint: Think about alginate.

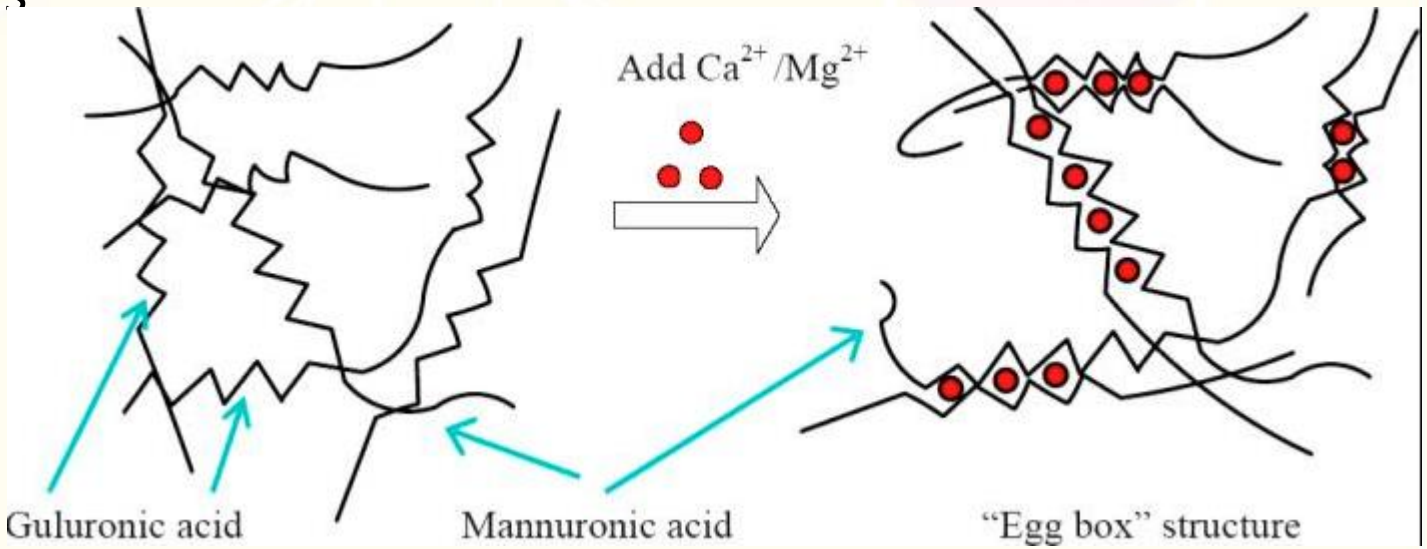
Fat Texture Characterization

Analysis of Data

- Why do you think this is occurring?

Hint: Think about alginate.

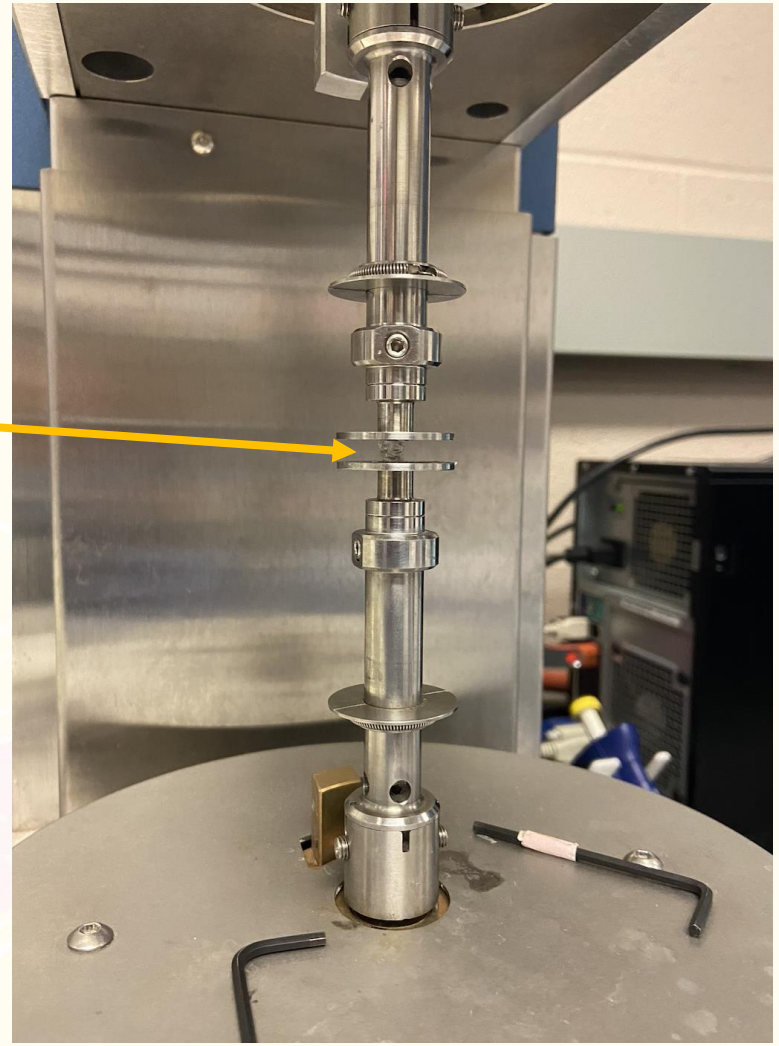
- Egg-box structure changes



L. Q. Wan, J. Jiang, D. E. Arnold, X. E. Guo, H. H. Lu, and V. C. Mow, "Calcium Concentration Effects on the Mechanical and Biochemical Properties of Chondrocyte-Alginate Constructs," *Cell Mol Bioeng*, vol. 1, no. 1, pp. 93–102, Mar. 2008, doi: 10.1007/s12195-008-0014-x.

Today's plan

1. Take samples for mechanical testing to Sci Tech room 225
2. Use 6mm diameter biopsy punch to create sample tissues
   - Control (scaffold with no cells)
   - Scaffold of choice with fat cells
3. Analyze results of different samples



Notes on Lab Reports

- **Main advice: Look at how published manuscripts write/organize as examples**
- Methods: Be general in describing volumes. Better to say concentration/percentage (e.g., 10% FBS) rather than writing what volumes you used (e.g., 10 mL FBS in 90 mL GM)
- Figures with plate reader data: Make your own graph, in a program such as excel. Values should be average and figure captions show note sample size (e.g., n = 3)
- Take care to make figures look nice and professional

Notes on Lab Reports

- Results: Be selective in what figures/data you discuss. We do not need to see images of the cells every day. Figure captions do not count towards final word count. The main body of results is the text, figures accompany your explanations.
- Discussion: Discuss what worked, what didn't work, and state hypotheses for why. Use outside literature to support ideas for future work or ideas for why things worked/didn't. What are the implications of these results?

Notes on Lab Reports

- References: Important in introduction and discussion to provide context and explanations. Need references beyond those discussed in class. As a guideline, please include at least 3 external references for each the introduction and discussion.
- Come to office hours or email us if you have questions!

Module 2 Lab Report

Expectations

1. Introduction (*include references)*
2. Methods (*can reference lab notebook entries*)
3. Results (*include figure captions, state observations*)
4. Discussion (*include references, interpret data*)
5. References (*any format*)

Due date: ***April 6th 5:59pm EST***

Module 2 Lab Report Suggestions

- Introduction
  - Do not explain cultured meat. Instead, focus on the goal and methods used in Module 2
  - Examples:
    - Why fat accumulation media need to be optimized?
    - Why use DOE?
    - What texture means for meat and how is it measured?

Module 2 Lab Report Suggestions

- Figures/Results: Include only important figures. Below are suggestions of what you can include. Aim to make figures for the below, with maybe a couple additional ones.
  - Class 6: Table for methods section showing the different media tested and highlighting which your group made.
  - Class 7: Table describing different alginate/CaCl2 conditions tested and your qualitative observations.
  - Class 8: Figures from the DOE results (in these lecture slides).
  - Class 9: Figure with stress/strain curves. Figure or table comparing Young's moduli (or other quantitative metric) between the with and without cells.

Questions?