Instron Tensile Testing: The Effect of Heat on the Material Properties of Chicken Skin

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Background

In a biomedical setting it is important to understand the significance of environmental stresses on organs, such as skin. In humans, skin has multiple roles: it acts as a protective barrier defending against bacterial infections and a regulator in maintaining body temperature and hydration. In cases of burn victims, damage to the skin can lead to alterations of inherent properties and functionality of the organ. These stresses may cause scar tissue to form and may lead to detrimental effects in the growth and elasticity of skin and the propensity of infection in the skin.

This lab simulates the aftermath of the effects of burns on the tensile properties of skin by affecting chicken skin with high temperatures. Tensile and material properties are commonly associated with the intrinsic physical properties of tissue. Since burn victims experience a change in the composition and make up of the skin, it was chosen to further investigate the consequences of burns on the material properties, rather than structural properties, of chicken skin. This experiment expands on the Instron Uniaxial Tensile Testing lab by using the formulas and concepts from that lab (force-displacement curves, stress-strain curves, and Hooke's Law) as a basis for further analyses in this experiment. The Instron 4444 machine will provide a digital signal that will determine the force-displacement curve of the force acting on the sample. It will be critical to take this information and determine the stress-strain graph of both "burned" and "unburned" skin samples; the corresponding points of elastic limit, ultimate stress, and failure stress, among others, will have to be compared to determine any statistical difference between the material properties of the skin specimens.

The results of a laboratory testing of temperature on material properties of skin can be applied to a clinical setting. In a clinical setting, these results can be used to determine applications for skin regeneration via tissue engineering. Through an understanding of the damage to the skin caused by burns, it is possible to reconstruct the dermal layer of skin (*Reference 1)*.

Hypothesis/Aims/Objective

The purpose of this lab is to compare the mechanical properties of chicken skin under two heating conditions: 25ºC to simulate an "unburned" sample at room temperature and 85ºC to simulate a "burned" sample. Since burning an object changes the inherent properties of the material under investigation, it is hypothesized that the material properties will be statistically different for the two conditions of the skin sample. In particular, the aim of the lab is to focus on one of the material properties, based on past in-class experiments; thus it is hypothesized that the elastic modulus of chicken skin will be statistically significantly different under the two conditions. Moreover, the elastic modulus of the samples at 25ºC will be higher than the elastic modulus of the samples at 85ºC.

The samples will be split into two groups; the first group of chicken skin samples will be placed in an oven at 25ºC (room temperature) for thirty minutes, the second group will be placed in an oven at 85ºC for thirty minutes (*Reference 3*). To test the data, a paired two-tailed t-test at the 95% confidence interval will be performed to determine support or rejection of the null hypothesis.

Equipment

Major Equipment

Instron 4444 Universal Testing Machine: The machine applies a force to the sample under investigation, while generating a digital signal of the applied force, and sending this signal to the PC. The Instron 4444 Machine will allow us to measure and record the material properties of the chicken skin sample.

GPIB Controller, LabView™, PC: Load force and Force-Displacement Data signals from the Instron 4444 are sent to the PC via the GPIB connection. LabView^{TIM} is the software used to analyze the data signal from the Instron 4444.

Lab Equipment

Weight Set (500g, 1kg, 2kg): Used during calibration and placement in "SI" mode of the Instron machine. See lab manual for specific use and instructions.

Supplies

Scalpel, Scissors, Cutting Board, Forceps: Instruments used to cut and handle the chicken skin specimen. Forceps should be used during the heating process of the skin.

Caliper, Ruler: Instruments used to measure the dimensions of the chicken skin into 3" x 1" rectangles.

Wooden Surrogate Popsicle Sticks: Surrogate sticks will serve as experimental samples used by group to familiarize themselves with the capabilities of the Instron machine.

Safety Goggles: Should be worn during use of the Instron machine in case specimen fails and skin and germs scatter.

Newly Purchased Equipment

Laboratory Oven: The oven is necessary to heat the chicken skin sample to synthesize a burn in an enclosed oven area; chicken skin sample will be placed in oven for 30 minutes and set at two temperatures 25ºC and 85ºC.

Thermometer: Oven-safe instrument placed within laboratory oven to maintain and record temperature across trials.

Chicken Skin Samples: Chicken specimens will be used as samples under investigation.

Proposed Methods & Analysis

Specimen Harvest and Preparation: *Time ~2 hours*

- Remove skin from the chicken legs provided to each group as directed in lab manual (*References 5*).
- Cut skin pieces into samples of equal length and width; based on the size of the chicken skin available to each group, the dimensions should be ~3" x 1". Measure samples with calipers and ruler (*Proposed Pitfalls*[1]). Note the exact dimensions of each sample.
  - Sample dimensions were chosen to obtain at least 5 samples for each of the trial conditions.
  - Sample dimensions accounted for potential experimental errors; extra chicken should be available to ensure for backup samples if necessary.
- Take one group of skin samples (n=5) and prepare as the 25ºC trial group.
  - Familiarize yourself with the oven settings and temperature adjustment.
  - Place the oven-safe thermometer within the oven and preheat the oven to 25ºC using the dial setting on the oven. Do this prior to placing samples within oven.
  - Place the five skin samples in the oven for thirty minutes. After fifteen minutes flip the skin specimens over with the forceps to ensure proper heating on both sides.
  - While the samples are in the oven monitor time and temperature, record any deviations from the assigned settings.
    - The time was chosen based on past literature experiments and in-class time constraints of the lab (*Proposed Pitfalls*).
  - Remove the samples from the oven and place on tray for further analyses. Let the samples cool down before further analysis.
- Take the remaining skin samples (n=5) and prepare as the 85ºC trial group.
  - Repeat the aforementioned steps in the 25ºC trial group, but now for the 85ºC trial group. Do this by adjusting the oven temperature to 85ºC.

Instron Set-Up and Specimen Tensile Testing: *Time ~1.5 hours*

- Refer to Instron Set-Up section in the laboratory manual to calibrate Instron with free weights, place in SI mode, and save files of data signals using the GPIB, and LabView™.
- Set the speed and direction of movement on Instron at 25 mm/min
  - Speed is determined based on past in-class experiments, to determine the optimal balance between time to failure and data points received from signals (*Appendix 1*).
- To familiarize the group with the Instron apparatus perform surrogate tests with wood samples as described in lab manual.
- Follow the no-load instruction in the lab manual to ensure that all initial geometries are at zero. When loading the samples be sure to adjust the clamps to similar heights, so each sample is loaded in a similar fashion.
  - When loading samples define a specific clamp height to ensure a consistent, minimal-slack initial position that is consistent across all samples. Record the height measurements across all samples.
- Repeat Instron methodology for all samples in the 25ºC trial group and 85ºC trial group.

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[1] See section of 'Proposed Pitfalls & Alternative Methods' for elaboration on possible errors associated with this part of the methodology.

Analysis

- From the data obtained produce force-displacement graphs for each of the sample conditions. Additionally calculate the stress-strain graphs from the force-displacement graphs.
  - Use data to determine the stress-strain curve (*Appendix 2*).
- Calculate the material properties of the skin samples from the stress-strain graph. These include, but are not limited too, elastic modulus, ultimate strength, and failure strength. Be consistent in determining the exact location of these points on the graph (*Potential Pitfalls*).
  - Elastic Modulus is the slope of the stress-strain line. In order to account for initial loading error and the assumption that the relationship is linear, it may be necessary to extrapolate a portion of the graph that is visibly the most linear to determine a value for Elastic Modulus. If extrapolating, be consistent in using the same linear range of strain across all samples. (*Appendix 2*)
  - Ultimate Strength is the largest value of stress on the stress-strain curve. It is defined as the peak of the stress-strain graph. (*Appendix 2*)
  - Failure Strength is the stress off the stress-strain graph before the point at which the stress falls to zero. Again, be certain to use consistency in determine this point on all sample graphs. (*Appendix 2*)
  - Determine and record any other useful points of comparison from the stress-strain graph.
- Perform data analysis of the material property figures obtained through the two conditions of the experiment. The analysis should include the dimensions of the samples, variation in loading rates, and material properties (elastic modulus, ultimate strength, and failure strength). Calculate averages and standard deviation for data. (*Appendix 3*)
- Run a paired two-tailed t-test at the 95% confidence interval for the data obtained for the elastic modulus in the two groups. This test will provide a p-value that will support (p-value > 0.05) or reject (p-value < 0.05) the null hypothesis. (*Appendix 4*)

Proposed Pitfalls & Alternative Methods

A few aspects of the experiment may give rise to experimental or calculation pitfalls and error; these can be grouped into two main areas of the experiment: preparation of sample, and analysis of the data.

The first major area of pitfalls crops up during the preparation of the sample. Past in-class experiments using chicken-skin specimens indicate it may be difficult to cut the sample into exact 3" x 1" dimensions. The skin is flimsy and must be held taut to guarantee precision in cutting. Although stretching and holding the skin rigid is difficult, in order to minimize the problem, the experimenter must determine a regular methodology to maintain consistency in this process. The error using the ruler and caliper to measure dimensions with an uncertainty of ±0.01 cm and ±0.001 cm, respectively. A second potential pitfall is due to the time of the sample placed in the oven. Based on literature sources it was determined that the ideal time to heat the 85ºC skin sample would be approximately 3 hours for each skin sample (*References 3*). Due to the restrictions of the time in lab and the extrapolation of specimens in literature sources to the grocery-store bought chicken skin, a time of 30 minutes in the oven was chosen instead. In order to use the ideal time settings for the experiment, a longer lab period is needed. If time permits the lab group can extend and record the effect of the time span in the oven. Two pitfalls that may occur during the preparation of the sample relates to the manner of heating the specimen. The first pitfall occurs when recreating the flame; an open flame would be more accurate in creating the effects of burns in victims, however an open flame runs the risk of burning holes in the skin specimen. The second pitfall occurs in the heating of skin samples. There is no guarantee that equal heating will occur since multiple samples are placed in the oven at a given time, in future experiments this problem may be minimized by placing individual samples in the oven at a given time. Additionally, there is no assurance that all pieces of skin will react the same way to heat. Experimentally this problem is difficult to eliminate since it depends on the inherent properties of the skin, this problem may be minimized increasing the number of skin specimens for each trial. Lastly, maintaining temperatures at a stable 25ºC and 85ºC while incubating the samples in the oven may be not viable. The oven adjusts to temperature zones that cover a small range of temperatures, exact temperatures must be determined and set with the aid of a thermometer. In order to maintain the oven temperature the heating dial must be continually adjusted and the thermometer checked for stability. Between the use of the oven and stabilizing the thermometer at a given temperature the temperature will be accurate with an uncertainty of ±5ºC.

The second area of pitfalls arises during the analysis of the data. First, a major assumption in the data is that the elastic modulus curve is linear. As mentioned in the protocol, this is not always the case in experiments, and a linear relationship must be extrapolated from the graph. In order to minimize the error in estimation, the same range of strain must be determined from a graph and applied to all subsequent graphs during calculation of the elastic modulus. Also, increasing the number of signal points delivered by the Instron machine, will allow for more points to analyze and extrapolate from the graph. Lastly, all readings of material properties from the stress-strain graph (ultimate strength and failure strength) must be determined at the peaks of the graphs, looking at the graphs and data and determining the highest stress point given the corresponding strain point (*Appendix 2*).

Budget

Purchase:	Fisher Isotemp 500 Series Economy Lab Oven *(References 6)*
Cost:	$861.70
Supplier:	Thermo Fisher Scientific, Inc.
Specifications:	Aluminum Chamber. Temperature Range - 5ºC to 210ºC
Purpose:	The laboratory oven is necessary to heat the chicken skin sample to synthesize a burn in an enclosed oven area; it will be used as an alternative to an open flame from a Bunsen burner.

Purchase:	Fisher Isotemp 500 Series Oven Thermometer *(References 7)*
Cost:	$27.30
Supplier:	Thermo Fisher Scientific, Inc.
Specifications:	Thermometer for 500 Series Isotemp Ovens 35ºC to 230ºC.
Purpose:	Record and ascertain stability in temperature across trials.

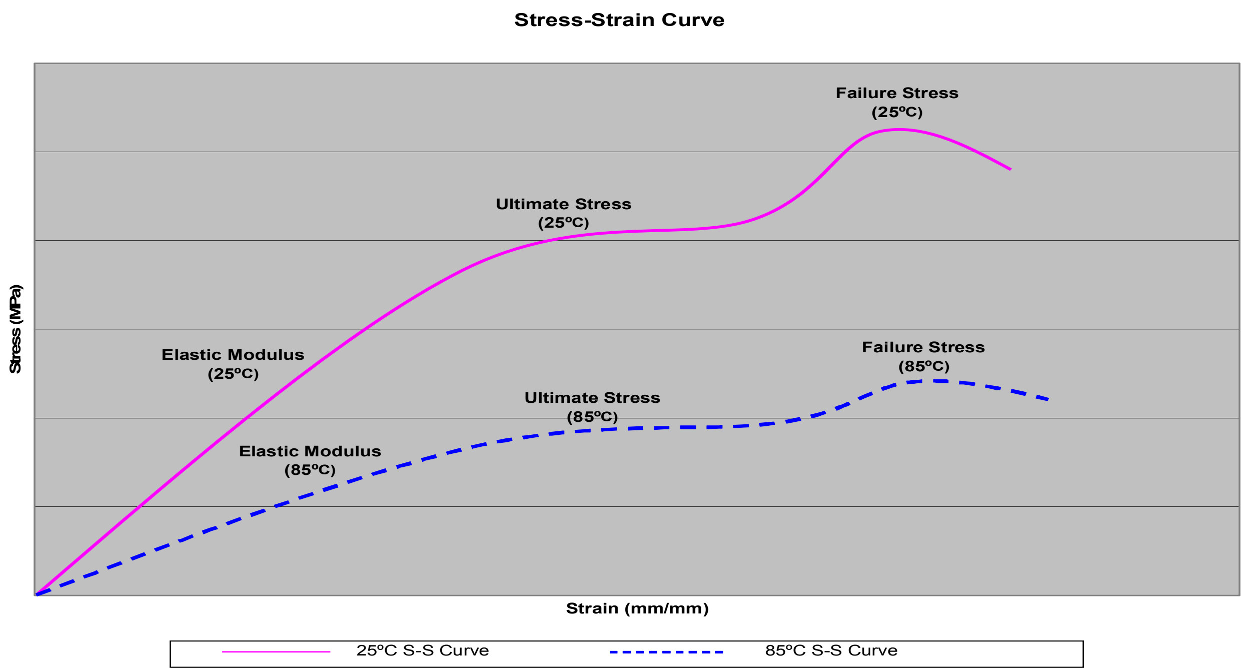
Purchase:	Frozen Chicken thighs to obtain Chicken Skin Samples *(References 4)*
Cost:	$42.00 ($1.00/lb or $0.30/Leg)
Supplier:	Chicken Manufacturer and Local Grocery Store
Specifications:	Must contain chicken with skin. Five chicken legs for each group and account for 20 groups.
Purpose:	Used as specimen under investigation during the experiment.

Total Budget: $931.00

Appendix

$$\sigma = F / A \qquad\qquad \varepsilon = \Delta l / l$$

Appendix 1: The above equation on the left is the equation to determine stress; this calculation will be determined from the Force-Displacement Graph and the cross-sectional area calculated from the dimensions determined during the experiment. The above equation on the right is the equation used to calculate strain, based on the length of the skin specimen.



Appendix 2: The above graph is the stress-strain curve determined by the Force-Displacement graph and the above equations. The material properties of Elastic Modulus, Ultimate Stress, and Failure Stress, are determined from this graph. The approximate stress-strain curves for 25ºC and 85ºC are based on the hypothesis that the material properties will be statistically different.

Chicken Skin Specimen

	Geometric Measurements				*Material Properties*		
	$L_{initial}$ (mm)	$W_{initial}$ (mm)	$Thickness_{initial}$ (mm)	C-Sec Area (mm^2)	E (MPa)	Ultimate Strength (MPa)	Failure Strength (MPa)
25ºC - 1	16.00	24.00	1.40	33.60	0.86	1.81	0.51
25ºC - 2	18.00	25.00	1.20	30.00	0.91	1.52	0.44
25ºC - 3	18.00	23.00	1.80	41.40	0.79	1.81	0.53
25ºC - 4	18.00	22.50	1.50	33.75	1.03	1.93	0.52
25ºC - 5	16.00	24.20	1.60	38.72	0.50	1.22	0.23
AVG	**17.20**	**23.74**	**1.50**	**35.49**	**0.82**	**1.66**	**0.45**
ST DEV	**1.10**	**0.99**	**0.22**	**4.53**	**0.20**	**0.29**	**0.13**
85ºC - 1	45.00	24.50	1.80	44.10	0.93	0.91	0.37
85ºC - 2	44.50	25.20	1.50	37.80	1.88	1.60	0.56
85ºC - 3	42.50	25.00	1.50	36.00	1.17	0.99	0.45
85ºC - 4	47.80	24.00	1.50	36.00	1.83	1.38	0.41
85ºC - 5	41.80	24.00	1.70	40.80	1.58	1.54	0.25
AVG	**44.32**	**24.54**	**1.60**	**38.94**	**1.48**	**1.28**	**0.41**
ST DEV	**2.36**	**0.55**	**0.14**	**3.49**	**0.42**	**0.32**	**0.12**

Appendix 3: The above table represents the geometric measurements and material properties that should be tabulated during the lab.

t-test: Elastic Modulus (25ºC vs 85ºC)
Two-Sample Assuming Unequal Variances

	25ºC	*85ºC*
Mean	0.164317088	1.283560586
Variance	0.000656718	0.100423198
Observations	5	5
Hypothesized Mean Difference	0	
df	4	
t Stat	-7.833776455	
P(T<=t) one-tail	0.00071692	
t Critical one-tail	2.131846782	
P(T<=t) two-tail	0.001433841	
t Critical two-tail	2.776445105	

Appendix 4: The table to the left represents the results of a paired two-tailed t-test at the 95% confidence interval comparing the data from the two groups. The test will provide a p-value (in this case, 0.001433841) that will either support (p-value > 0.05) or reject (p-value < 0.05) the null hypothesis.

References

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