EFFECT OF PRESERVATION TIME on the

DYNAMIC MATERIAL PROPERTIES of SOFT TISSUES

in

LIVER TRANSPLANTATION

by

Sina Ocal

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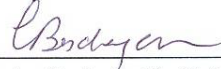
Koç University

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Graduate School of Sciences and Engineering

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Sina Ocal

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

_____
Cagatay Basdogan, Ph. D. (Advisor)

_____
Burak Erman, Ph. D.

_____
Demircan Canadinc, Ph. D.

Date: 20·11·2009

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To them who have a place for me in their hearts

ABSTRACT

We investigate the effect of preservation time on the dynamic material properties of bovine liver using a viscoelastic model derived from the impact and ramp and hold experiments. First, we measure the storage and loss moduli of the bovine liver as a function of frequency using an impact hammer. Second, the time-dependent relaxation modulus of the liver is measured via the ramp and hold experiments performed with a separate compression device. Third, a generalized Maxwell solid model that successfully simulates the frequency and time-dependent dynamic response of the liver is developed to estimate the number of Maxwell arms (N) in the model and the viscoelastic material coefficients by minimizing the error between the experimental data and the corresponding values generated by the model. Finally, the changes in the estimated material coefficients was investigated as a function of preservation time for liver samples tested 1, 2, 4, 8, 12, 18, 24, 36 and 48 hrs after harvesting. The results of the experiments performed with three different animals show that the tissue becomes stiffer and more viscous as the preservation time increases. The results also suggest that a linear model is appropriate for investigating the effect of preservation time on the viscoelastic response of bovine liver.

ÖZET

Bu çalışmamızda, darbe ve sıkıştırma-bekletme (stres gevşemesi) deneyleri kullanılarak viskoelastik bir model yardımı ile büyük baş hayvanların karaciğerlerinde saklama koşullarının dinamik malzeme özellikleri üzerindeki etkisini inceledik. Öncelikle, darbe çekici cihazı kullanarak büyük baş hayvan karaciğerinin frekansa bağlı depo ve kayıp modülü değerlerini buluyoruz. İkinci olarak, karaciğerin zamana bağlı gevşeme modülü değerlerini, önceden tasarladığımız kompresyon aletini kullanarak sıkıştırma-bekletme deneyleri sonucunda buluyoruz. Üçüncü olarak, karaciğerin frekansa ve zamana bağlı dinamik tepkilerini başarılı bir şekilde simüle eden genelleştirilmiş Maxwell modeli kullanıyoruz. Bu modeli kullanmamızın amacı, deneysel sonuçlar ve modelden hesaplanan tepki arasındaki hatayı minimize ederek Maxwell elemanlarının sayısını (N) ve viskoelastik malzeme katsayılarını belirlemektir. Son olarak, deneklerden rezekte edilmiş karaciğerler üzerinde 1, 2, 4, 8, 12, 18, 24, 36 ve 48 saatlik deneyler yapılarak, belirlenen malzeme modeli katsayılarının organ saklama zamanına bağlı değişimleri incelenmiştir. Üç farklı hayvan kullanılarak yapılan deneyler sonucunda dokunun saklama zamanı arttıkça sertleştiği ve viskozitesinin arttığı gözlemlenmiştir. Ayrıca, bu sonuçlar büyük baş hayvan karaciğerinin organ saklama zamanına göre viskoelastik tepkilerinin değişimini doğrusal model ile incelemenin uygun olduğunu göstermiştir.

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Chapter 1

INTRODUCTION

Today, treatment of severe liver failure is not possible unless the diseased organ harvested from the body and replaced with a healthy liver, which is known as liver transplantation. The main reasons for liver failure in adults are chronic viral hepatitis, cirrhosis caused by alcohol abuse, autoimmune hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis, steatohepatitis, liver disorders inherited or present at birth, and drug induced liver damage [10]. The number of liver donors is significantly smaller than the patients who need healthy organs. The sources of liver donors are tried to be increased by using living and deceased donors and the techniques of split and domino transplants. While the success rate with deceased donors is low, they still hold a considerable part in liver transplantation donor sources.

Typically, the donor and the recipient present in different locations which brings up the problem of the preservation. The liver harvested from a donor must be preserved and transported ex vivo with effective, safe and reliable methods and after that transplanted to a suitable recipient immediately. Along this process, tissue damage occurs in harvested liver due to drop in its temperature (hypothermia) and insufficient supply of blood to its vessels (ischemia). The damage occurs during the warm ischemic period (i.e. time period between

harvesting and preservation), the cold ischemic period (preservation) and the reperfusion period [3, 26]. Among these three phases, the preservation period is the longest; hence the most damage occurs during this phase. In the simple hypothermic preservation approach, first, the harvested liver is flushed with an appropriate chemical solution, and then immersed into a plastic bag containing the same solution; finally the bag is covered with ice. The solutions suggested in the literature for preserving a liver slightly differ in components, but they all aim to prevent swelling of liver cells and delay their destruction, which is inevitable. While the effect of preservation time on the cell structure and functionality of animal and human livers have been investigated extensively at the cellular level, the same effect on the gross material properties has been mostly neglected.

Most of the earlier research studies conducted with human and animal liver have focused on the characterization of static material properties. Typically, force versus displacement response of liver has been measured via compression experiments performed at a slow indentation rate and linear elastic modulus at small strains has been estimated. Carter et al. (2001) performed static indentation experiments in vivo with a hand-held mechanical indenter and estimated the linear elastic modulus of human liver as 270 MPa. Based on the results of the more recent studies, we know that this value is much higher than the expected one. Ottensmeyer (2001) designed a robotic indenter to measure mechanical properties of pig liver during a minimally invasive surgery. His probe can apply dynamic stimuli to soft

tissues in the range of ±500 µm. He conducted in vivo experiments with pigs and estimated the elastic modulus as 10–15 kPa. Tay et al. (2006) used a commercial robotic arm to achieve indentation depths up to 8 mm during an open surgery performed on pigs. The force response of pig liver to displacement stimulus was measured invasively using a force sensor attached to the tip of the arm during an open surgery. They estimated the elastic modulus of porcine liver as 13 kPa. Samur et al. (2007) designed a robotic indenter for the minimally invasive measurement of soft tissue properties in abdominal region during a laparoscopic surgery. Using the robotic indenter, the elastic modulus of pig liver was estimated as 10–15 kPa using the small deformation assumption. Nava et al. (2008) performed aspiration experiments on healthy human liver. They estimated the long term and instantaneous linear elastic modulus of human liver as 20 kPa and 60 kPa respectively.

The number of studies investigating the dynamic material properties of animal and human livers are much less than the ones investigating the static material properties. In most of these studies, either time- or frequency-dependent material properties have been measured via stress relaxation and dynamic loading experiments, respectively. Liu and Bilston (2000) investigated the linear viscoelastic properties of bovine liver using a generalized Maxwell model and conducted three types of experiments a) shear strain sweep oscillation, b) shear stress relaxation, and c) shear oscillation. The shear stress and strain were calculated based on the torsional load. In strain sweep oscillation experiments, the liver tissue is subjected to

a sinusoidal angular torsion at a fixed frequency of 1, 5, or 20 Hz using a strain controlled rheometer. The strain amplitudes were gradually increased from 0.06% to 1.5% while the storage and loss moduli of the liver were measured. In stress relaxation, sudden torsional shear strain was applied to liver tissue for 0.02 seconds and the shear relaxation modulus was measured over 3000 seconds. Finally, in shear oscillation experiments performed in the range of 0.006 to 20 Hz, the storage and loss moduli were measured again. The results of relaxation experiments show that the shear modulus reaches to steady state around 0.6 kPa. The results of the oscillatory shear experiments show that the storage modulus increases from 1 kPa to 6 kPa with increasing frequency and the loss modulus is less than 1 kPa, increases to a peak at about 1 Hz and then decreases to 0.4 kPa as the frequency reaches to 20 Hz. Kiss et al. (2004) performed in vitro experiments with canine liver tissue to characterize its dynamic response by applying cyclic stimuli to the tissue. They calculated the storage and the loss moduli of the liver tissue from the frequency-dependent complex elastic modulus for the frequencies ranging from 0.1 to 400 Hz. The resulting moduli spectra were then fitted to a modified Kelvin–Voigt model, which was called as the Kelvin–Voigt fractional derivative model (KVFD) by the authors. They showed that there is an excellent agreement between the experimental data and the KVFD model, particularly at frequencies less than 100 Hz. Valtorta and Mazza (2005) developed a torsional resonator to characterize the dynamic material properties of bovine and porcine liver. By controlling the vibration amplitude, shear strains of less than 0.2% were induced in the tissue so that

the material response can be considered as linear viscoelastic. Experiments were performed at different eigenfrequencies of the torsional oscillator and the complex shear moduli of bovine and porcine were measured in the range 1–10 kHz. The results of the in vitro experiments on porcine liver shows that the magnitude of complex shear modulus varies between 5-50 kPa depending on whether the data collected from the external surface or the internal section of the liver (as reported by the authors, the former leads to a considerably larger shear stiffness due to the presence of the stiff capsula). The shear modulus for bovine liver was shown to vary between 15-30 kPa.

Using the material characterization techniques discussed above, the effect of preservation time, method, and environment on the gross material properties of human or animal liver has not been investigated much. Kerdok et al. (2006) investigated the effects of perfusion on the viscoelastic response of pig liver using two indentation devices under four different environmental conditions: in vivo, ex vivo perfused, ex vivo post perfused, and in vitro on an excised section. One device developed by Ottensmeyer (2001) imposed cyclic perturbations on the liver's surface, inducing small strains up to 5% at frequencies ranging from 0.1 to 200 Hz while the other device measured the creep response of the same liver to the compressive loads applied for 300 seconds, inducing large strains up to 50%. The results demonstrated that the unperfused conditions were stiffer and more viscous than the in vivo state and the responses from the ex vivo perfusion condition closely approximated

the in vivo response. Rosen et al. (2008) conducted compression experiments with pig liver using a motorized endoscopic grasper equipped with a force sensor. The results of the experiments show that there are significant differences in material properties of the liver measured in-vivo and postmortem conditions (the excised organ was stored in a solution some time before testing). They found that harvested soft organ tissues get stiffer and more viscous in time.

In this article, we investigate the effect of preservation time on the dynamic (both time and frequency-dependent) material properties of bovine liver. For this purpose, we first measure the frequency-dependent force response of a harvested bovine liver using an impact hammer at progressing time steps upto 48 hours. To our knowledge, this is the first time that the frequency-dependent properties of a soft tissue are characterized using an impact hammer. Second, we measure time-dependent relaxation response of the same liver by conducting ramp and hold experiments using a compression device. Third, we fit the data collected from both characterization experiments (relaxation and impact) to a Generalized Maxwell Solid (GMS) model to obtain the optimum viscoelastic material coefficients. The previous investigators modeling the dynamic response of soft tissues have typically relied on the experimental data collected from one type of experiment only. Hence, our viscoelastic model more accurately mimics the time- and frequency-dependent

responses of bovine liver than the earlier ones. As a final step, we investigate the effect of

preservation time on the response of this model.
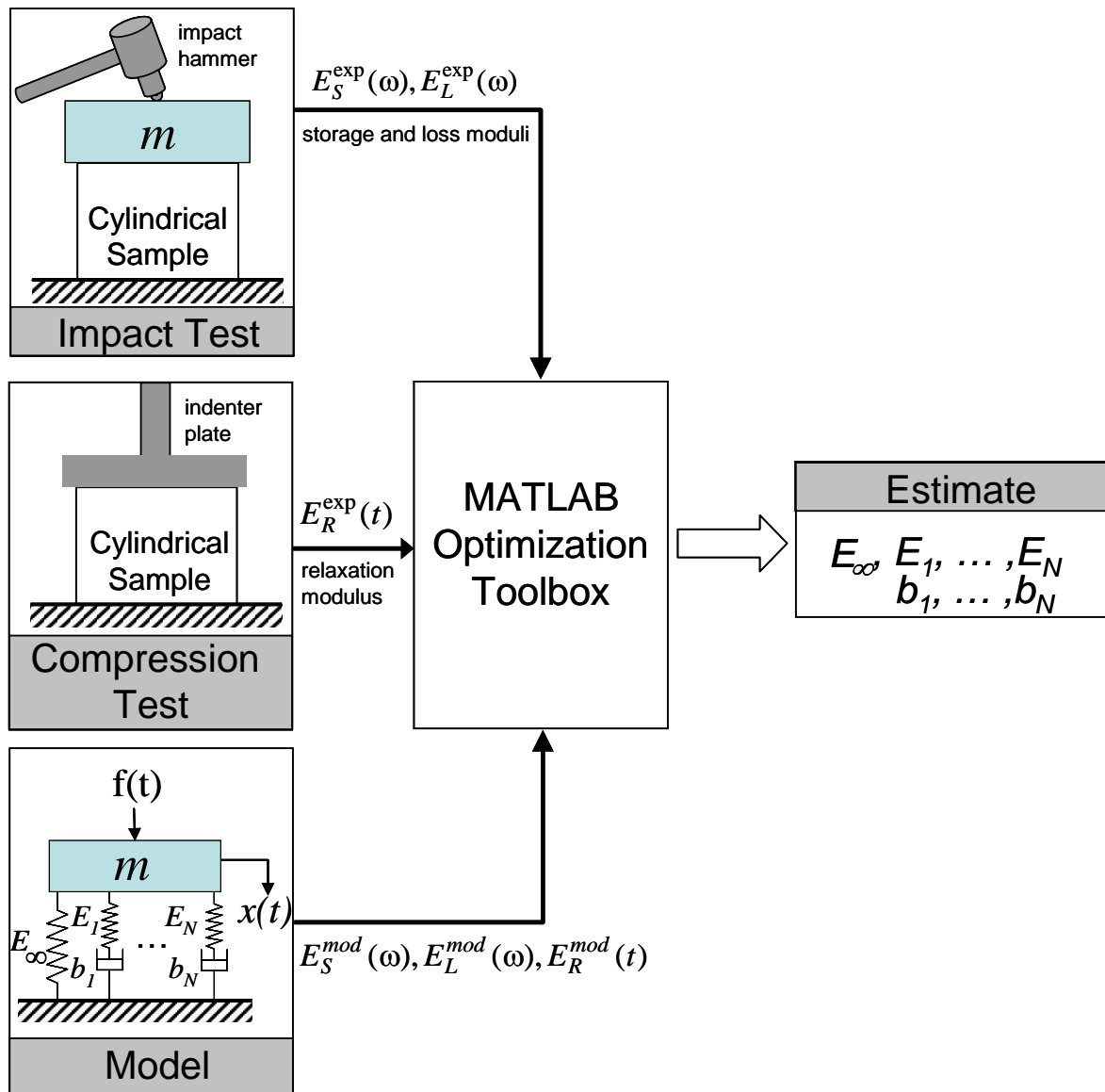
Figure 1. The flow-chart of the optimization process for estimating the viscoelastic material coefficients of soft organ tissues.

Chapter 2

THEORY and METHODOLOGY

2.1 Time-dependent Viscoelastic Material Properties

In biomechanics literature, time-dependent viscoelastic material properties of soft tissues are typically characterized by ramp and hold experiments. When a soft organ tissue is subjected to a ramp and hold strain, the stress response at that strain decreases exponentially with time, reaching to a steady state value. This is explained by the phenomena of stress relaxation under constant strain and can be characterized by a time-dependent relaxation modulus, $E_R(t)$.

2.2 Frequency-dependent Viscoelastic Material Properties

Frequency-dependent viscoelastic material properties of soft tissues are typically characterized by cyclic loading, which can be induced either by a rheometer or a mechanical shaker. An alternative approach for the same purpose is the impulse loading via an impact hammer. The technique involves the use of a hand-held hammer to apply a light impact force on a pre-load mass covering the top surface of a specimen (Nashif, 1985). The

type of material used at the hammer tip adjusts the frequency content of the impact force. This approach is easy to implement and enables to collect data faster than the cyclic loading. The response of the test specimen under the influence of impact loading can be modeled using a simple hysteretic damping model as shown below:

$$m\ddot{x}(t) + k^* x(t) = f(t) \tag{1}$$

where m is the mass of the pre-load placed on the specimen, k^* is the complex stiffness of the specimen, $f(t)$ is the excitation force, which results in a displacement $x(t)$. The same equation can be written in the frequency domain to obtain the following transfer function (also known as the frequency response function, FRF)

$$T(j\omega) = \frac{X(j\omega)}{F(j\omega)} = \frac{1}{-m\omega^2 + k(\omega)(1 + j\eta(\omega))} \tag{2}$$

where, $k(\omega)$ is the dynamic stiffness and $\eta(\omega)$ is defined as the loss factor. Now, if we define r as the ratio of the excitation frequency to the natural frequency, $r = \omega/\omega_n$, then the complex stiffness and the loss factor of the specimen can be calculated from the measured transfer function and the resonance frequency (close to the natural frequency if the loss factor takes small values) as (Lin et al., 2005)

$$k(\omega) = \frac{\mathrm{Re}(T(j\omega))}{|T(j\omega)|(1-r^2)} \tag{3a}$$

$$\eta(\omega) = -\frac{\mathrm{Im}(T(j\omega))}{\mathrm{Re}(T(j\omega))}(1-r^2) \tag{3b}$$

After obtaining the dynamic stiffness, the dynamic modulus, $E(\omega)$, can be calculated using the following relation derived from Hooke's Law

$$E(\omega) = \frac{k(\omega)L}{A} \tag{4}$$

where, L is the length of the specimen in the direction of the loading and A is the cross sectional area of the sample. Now, similar to the complex stiffness term in Eq. 1, the complex elastic modulus can be written as

$$E^*(\omega) = E(\omega)(1 + \eta(\omega)j) \tag{5}$$

Alternatively, it can be written in terms of real and imaginary parts as

$$E^*(\omega) = E_S(\omega) + jE_L(\omega) \tag{6}$$

The real part, $E_S(\omega)$, is known as the storage modulus and it is an indicator of energy storage capacity of the viscoelastic material. The imaginary part, $E_L(\omega)$, is known as the loss modulus and it is related the energy dissipation capacity of the material. The ratio of $E_2(\omega)/E_1(\omega)$ is the loss factor $\eta(\omega)$ given in Eq. 3b, and equal to the tangent of the phase angle, $\delta(\omega)$, between strain and stress (or between displacement and force in the actual measurements).

2.3 Viscoelastic Model

In earlier studies, the dynamic response of soft tissues has been investigated by either a ramp and hold experiment to determine the stress relaxation modulus, $E_R(t)$, or by a cyclic loading experiment to determine the complex modulus, $E^*(\omega)$. In developing a viscoelastic soft tissue model, these studies have relied on the data collected from one type of experiment only. However, due to the nature of the loading, the information that can be extracted from each experiment is different. Hence, a more accurate viscoelastic tissue model can be developed if the results of both experiments are taken into account. If a GMS is used for modeling the viscoelastic behavior of a soft tissue (see the model in Figure 1), then the time-dependent relaxation modulus can be calculated from its stress relaxation response to a constant strain input as

$$E_R(t) = E_0\left[1 - \sum_{j=1}^{N} \alpha_j\right] + E_0 \sum_{j=1}^{N} \alpha_j e^{-t/\tau_j} \tag{7}$$

This representation is also known as the Prony series. The response of the same viscoelastic model to an impact loading (or equivalently cyclic loading) enables us to calculate the storage and loss moduli as

$$E_S(\omega) = E_0\left[1 - \sum_{j=1}^{N} \alpha_j\right] + E_0 \sum_{j=1}^{N} \frac{\alpha_j \tau_j^2 \omega^2}{(1 + \tau_j^2 \omega^2)} \tag{8}$$

$$E_L(\omega) = E_0 \sum_{j=1}^{N} \frac{\alpha_j \tau_j \omega}{(1 + \tau_j^2 \omega^2)} \tag{9}$$

Here, E_0 is the short-term elastic modulus, $\alpha_j = E_j/E_0$ is the relative modulus and $\tau_j = b_j/E_j$ is the time constant where b_j respresents the damping coefficient and N is the number of terms (i.e. Maxwell arms) used in the GMS model. Note that the long term modulus (steady state response) is related to the short term modulus through the relative moduli,

$$E_\infty = E_0(1 - \sum_{j=1}^{N} \alpha_j).$$

The goal of the optimization is to estimate the number of Maxwell arms (N) and the material coefficients E_0, α_j, and τ_j in the GMS model by minimizing the error between the experimental data and the corresponding values generated by the GMS model (see Figure 1). This error function, F_{min}, can be defined as

$$F_{\min} = \sum_{i=1}^{M} \left\{ \left[E_R^{\exp}(t) - E_R^{\mathrm{mod}}(t) \right]^2 + \left[E_S^{\exp}(\omega) - E_S^{\mathrm{mod}}(\omega) \right]^2 + \left[E_L^{\exp}(\omega) - E_L^{\mathrm{mod}}(\omega) \right]^2 \right\} \qquad (10)$$

where, $E^{\exp}$ and E^{mod} represent the moduli obtained from the experimental data and calculated from the model, respectively, and M is the number of data points used for the optimization.

Chapter 3

EXPERIMENTS

3.1 Sample Preparation

The bovine liver was harvested from veal and flushed and preserved with Lactated Ringer's (LR) solution at 4^0C. During the preservation period, the liver is kept in a commercial cooler and the temperature is controlled by a digital thermometer. Cylindrical samples were obtained from the liver at different time periods: 1, 2, 4, 8, 12, 24, 36 and 48 hrs after harvesting. All the samples were taken from the right lobe of the liver for consistency. The diameter and the length of the samples were 50 mm and 25 mm, respectively. Before the experiments, the samples were covered by Vaseline to prevent fluid loss and dehydration. First the impact and then the ramp and hold tests were performed on each sample to conserve physical texture.

3.2. Ramp and Hold Experiments

In ramp and hold experiments, stress relaxation responses of the liver samples were measured at different preservation times. For this purpose, an experimental set-up was developed to apply compressive strains to the liver samples and measure their force response through a force sensor (see Figure 2). The major components of this set-up include a high torque step motor guiding a compression plate attached to a power screw via a moving shuttle and a force sensor attached to the shaft of the indenter plate. The step motor (Intelligent Motion Systems Inc., model MDrive23Plus, 51200 steps/rev) was programmed to compress the liver samples in vertical direction at a specified rate using the indenter plate. As the sample was compressed, the force response was measured using a force transducer (ATI Industrial Automation Inc., model Nano 17) having a force range of ± 70 N in the normal direction, ± 50 N in other principal directions and a resolution of 1/1280 N along each of the three orthogonal axes when attached to a 16-bit A/D converter. The force data was acquired using a 16-bit analog input card NI PCI-6034E (National Instruments Inc.) with a maximum sampling rate of 200 kS/s.
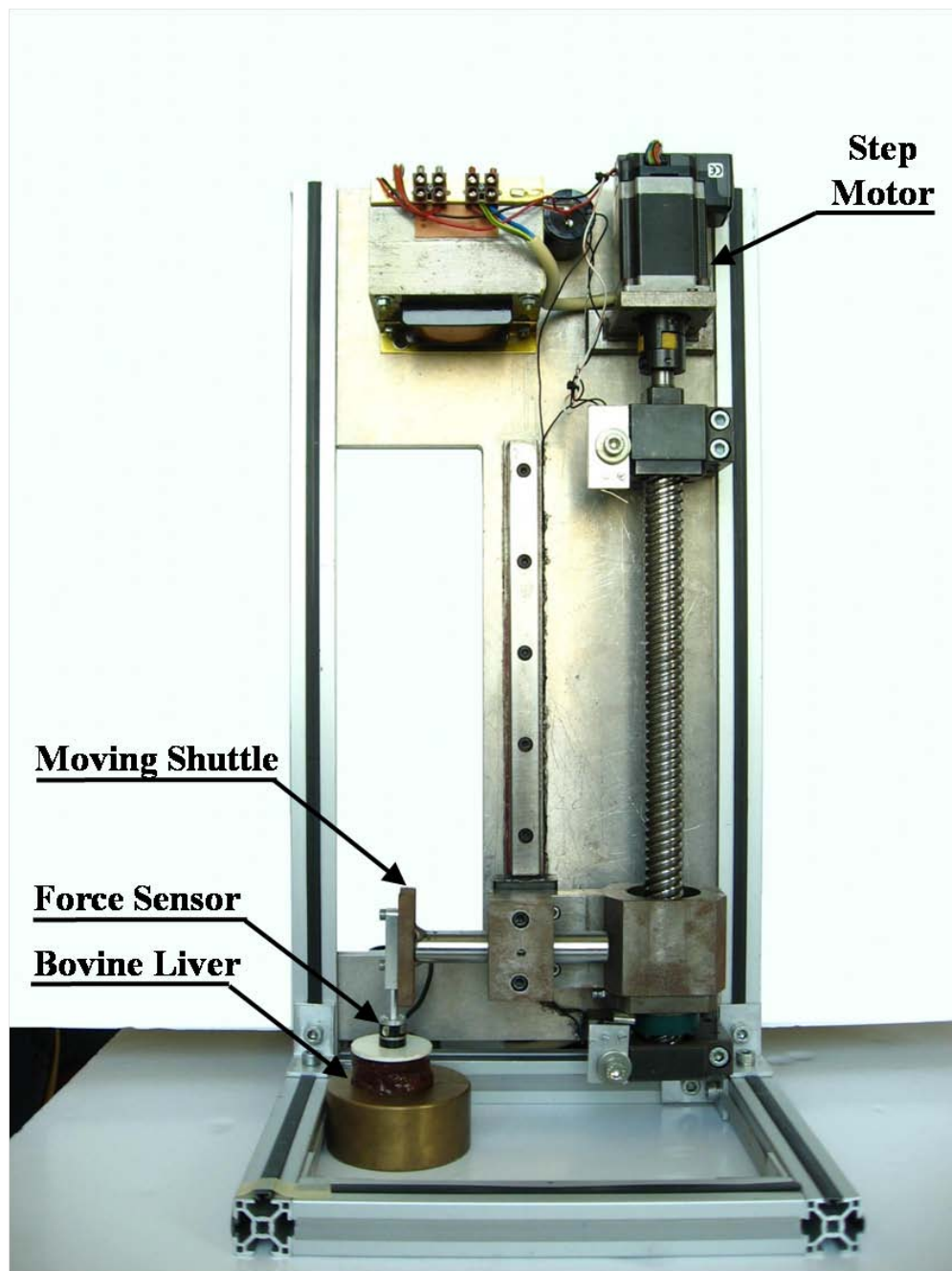
Figure 2. The set-up for conducting ramp and hold experiments to determine the stress relaxation modulus of bovine liver.

In ramp and hold experiments, the liver specimens were compressed to 4.8 mm in 0.1 s and the indenter plate held there for 500s to record force versus time response for the characterization of relaxation response. A total of 9 measurements were taken at 1, 2, 4, 8, 12, 18, 24, 36 and 48 hours for each liver. The stress relaxation modulus calculated from experimental data for different preservation times are shown in Figure 3.
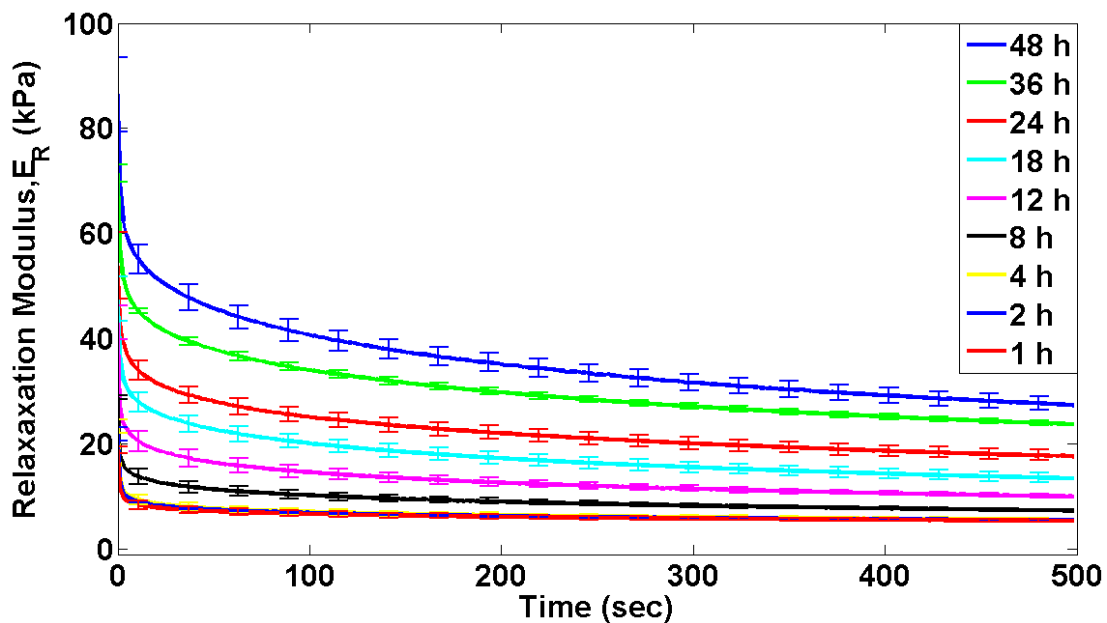

Figure 3. Filtered stress relaxation modulus of bovine livers measured at 1, 2, 4, 8, 12, 18, 24, 36 and 48 hours after harvesting.

3.3. Impact Experiments

In impact experiments, the dynamic moduli of the liver samples are measured as a function

of frequency using an impact hammer. An impact hammer is a specialized instrument that

produces short duration vibrations if the specimen being tested is hit by it. Compared to the

dynamic loading test, the measurement time in impact test is much shorter. The hammer

incorporates a sensor that produces a signal proportional to the force of impact. This

enables precise measurement of the excitation force. Different impact tip materials allow

tailoring of the frequency content of the impact force. For low frequency measurements as

in our case, a soft rubber tip concentrates the excitation energy in a narrow frequency

range. The frequency response function (FRF) is obtained by taking the Fast Fourier

transform of the impulse response. In cyclic loading test via electromagnetic shaker, the

same FRF is obtained by applying small periodic strains to the specimen and measuring its

force response at different frequencies for a range of frequencies.

In impact experiments, an impulse excitation force was applied to a pre-load mass (400

gram) placed on top of the sample using an impact hammer (PCB Piezotronics Inc., Model

086C03, sensitivity is 2.1 mV/N) equipped with a force sensor (see Figure 4). Note that the

weights of the all liver samples (40 ± 3 grams) were significantly smaller than the weight

of the preload to eliminate the influence of the sample mass on the results (see Eq. 1) and

the cross-sectional area of the preload was larger than that of the samples, covering their surface. For better response at low frequencies, a soft tip and an extender mass were utilized as suggested by the manufacturer. The impulse response of the specimen was measured by a piezoelectric accelerometer (PCB Piezotronics Inc., Model 333B30, sensitivity is 101.2 mV/g, where is the gravitational acceleration, range is 0.5-3000 Hz). The accelerometer was attached to the pre-load mass using a thin film of adhesive wax. As suggested by the manufacturer, five measurements were taken from each specimen and the average values were used in the analysis. The accelerometer and the force sensor were connected to a dynamic signal analyzer (Data Physics Corporation, type SignalCalc Mobilyzer) to calculate the frequency response function (FRF). Figure 5 shows the variation in storage and loss moduli of the liver samples as a function of frequency (note that due to the singularities at r =1 in Eq. 3, large variations occur around the resonance frequency.
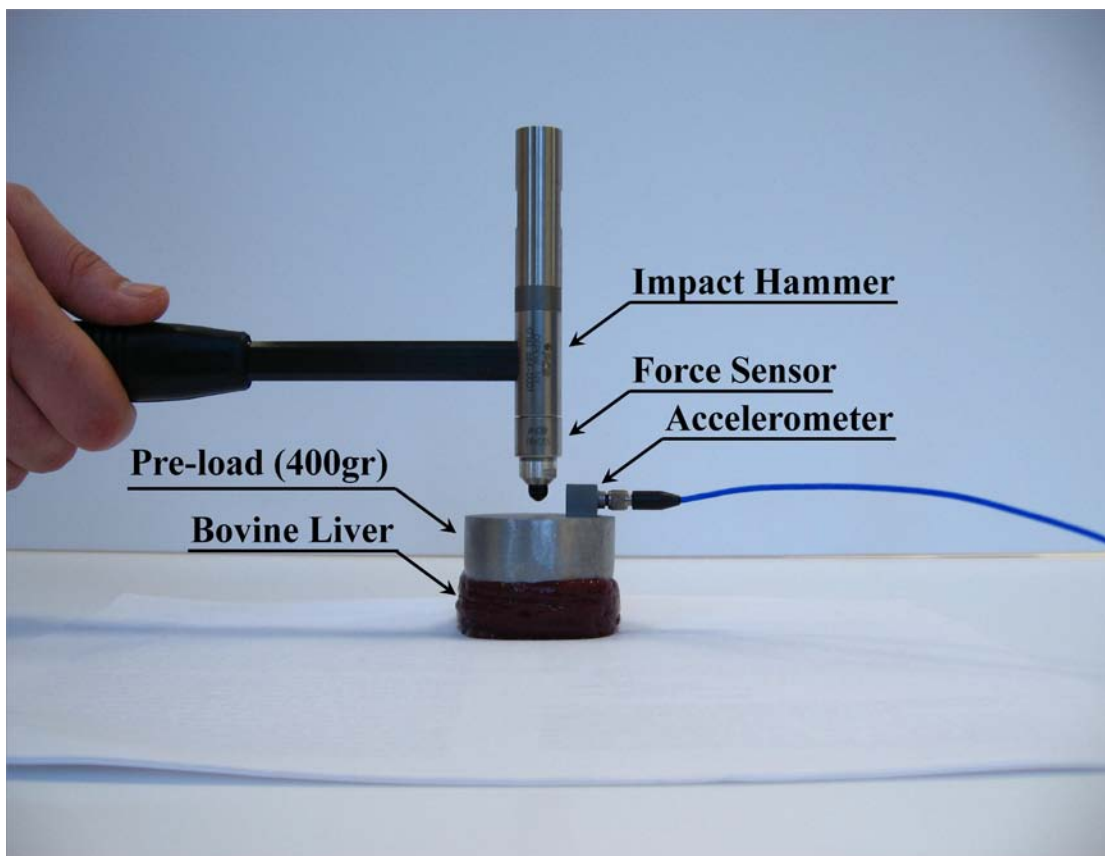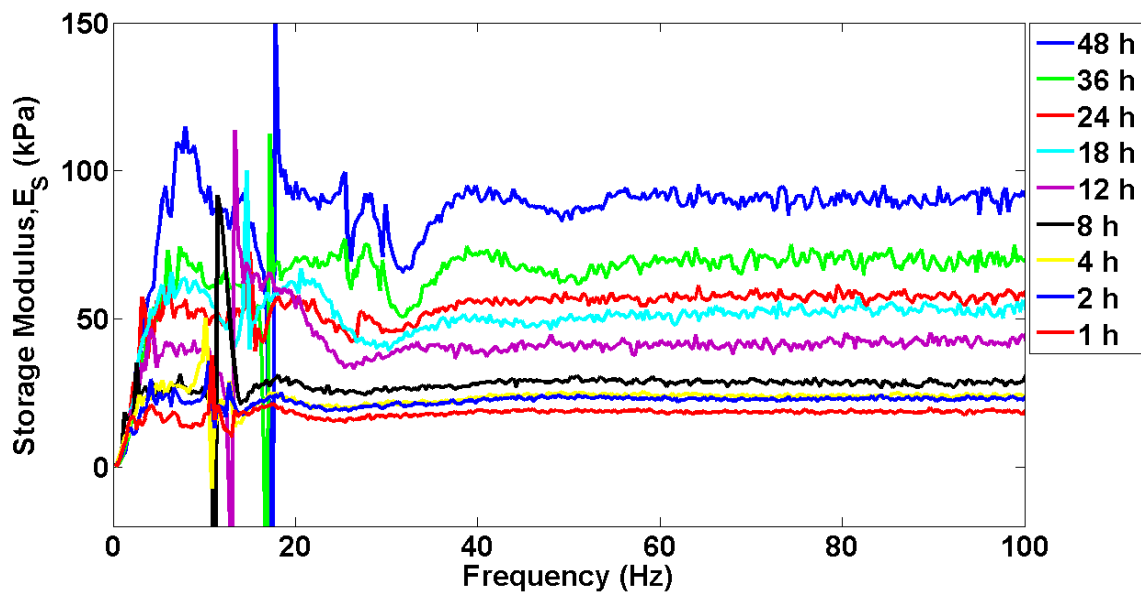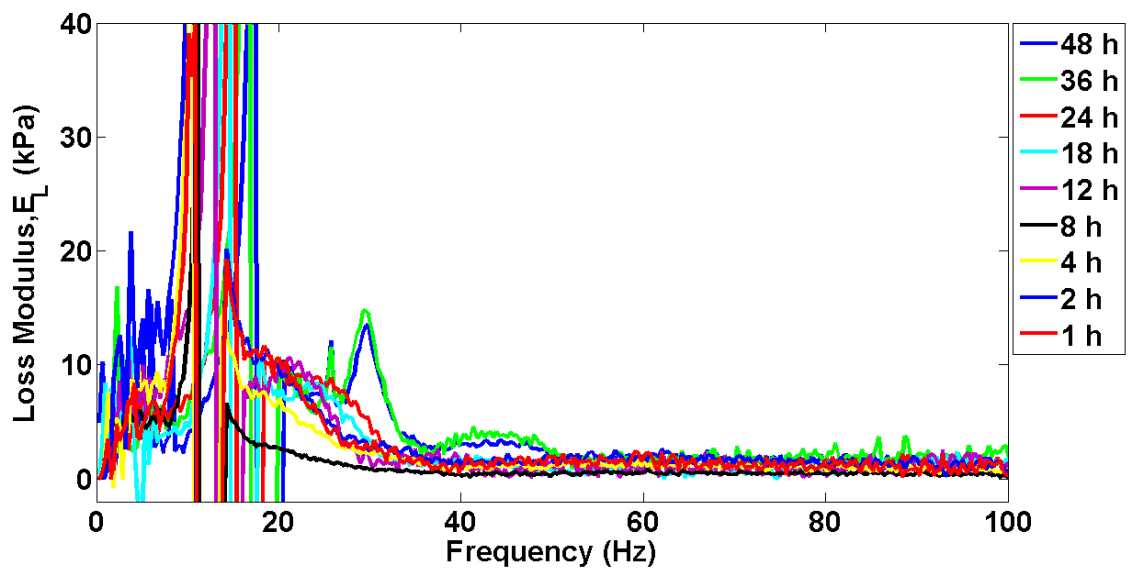
Figure 4. The set-up for conducting impact experiments to determine the storage and loss moduli of bovine liver.

(a)

(b)

Figure 5. Storage (a) and loss (b) moduli of bovine liver measured at 1, 2, 4, 8, 12, 18, 24, 36 and 48 hours after harvesting.

Chapter 4

CHARACTERIZATION of MATERIAL PROPERTIES

In order to estimate the material coefficients of the GMS model via the nonlinear optimization approach, one needs good initial guesses for their initial values. This was achieved by curve fitting a Prony series to the experimental relaxation modulus obtained from the ramp and hold experiments. The coefficients of the Prony series are the desired initial guesses for the optimization procedure. The formulation given in Eq. 7 was used with $N = 2$ and $N = 3$ for curve fitting to the experimental relaxation modulus measured at different preservation times using the LSQNONLIN function of MATLAB (Table 1).

Table 1. The Prony series was fitted to the experimental relaxation data for N = 2 and N = 3. The residual values (R^2) reported in the second and third columns show the quality of fit.

| Time (hrs)	N = 2	N = 3
1	0.97	0.99
2	0.97	0.99
4	0.97	0.99
8	0.98	0.99
12	0.98	0.99
18	0.98	0.99
24	0.98	0.99
36	0.98	0.99
48	0.98	0.99

Following the estimation of initial values, the viscoelastic material coefficients were determined using FMINCON function of MATLAB that minimizes the error between the experimental and simulated data. FMINCON attempts to find a constrained minimum of the error function F_{min} (see Eq. 10) of desired material coefficients starting at some initial estimates. Instead of initializing the optimization process with some random values, the Prony series coefficients estimated from the stress relaxation experiment for N = 3 were used as the starting values. A lower boundary was defined so that the optimization function was prevented to return negative values of the parameters. As a solver, "Line Search" algorithm was selected to obtain feasible results. The results of the optimization process for

the experimental data collected one hour after harvesting are shown in Figure 6. Figure 7

shows the optimum storage and loss moduli of bovine liver as a function of frequency for

different preservations times. The optimum viscoelastic material coefficients for all

preservation times are tabulated in Table 2.

Table 2. The optimum viscoelastic material model coefficients of bovine livers for different

preservation times.

time	α_1	α_2	α_3	τ_1	τ_2	τ_3	E_0	E_∞
1	0.09±0.01	0.53±0.05	0.12±0.03	36.86±13.18	0.44±0.32	225.67±19.14	18674±769	4853±484
2	0.11±0.01	0.54±0.01	0.11±0.00	27.24±8.53	0.39±0.22	241.00±1.73	21805±1602	5275±100
4	0.12±0.03	0.53±0.04	0.11±0.01	23.93±5.30	0.31±0.05	251.33±8.08	23210±1170	5461±31
8	0.12±0.04	0.46±0.09	0.18±0.05	29.45±13.58	0.47±0.11	267.33±15.53	27082±1231	6562±369
12	0.13±0.03	0.46±0.03	0.19±0.04	24.95±4.89	0.29±0.03	271.22±12.69	42544±743	9405±495
18	0.08±0.06	0.42±0.04	0.25±0.02	38.77±10.21	0.43±0.07	261.80±15.76	49471±2147	12142±197
24	0.11±0.06	0.34±0.06	0.26±0.02	40.38±11.53	0.24±0.12	257.23±21.49	55628±1298	16032±431
36	0.09±0.04	0.35±0.05	0.27±0.01	40.01±4.66	0.18±0.08	262.40±17.90	69909±1017	20228±1245
48	0.12±0.07	0.31±0.02	0.28±0.03	40.38±8.41	0.23±0.06	279.24±5.19	88005±2654	25143±1342

| time	α_{total}	τ_{total}
1	0.74±0.02	262.97±6.25
2	0.76±0.01	268.64±7.36
4	0.76±0.01	275.57±10.07
8	0.76±0.02	297.26±2.91
12	0.78±0.01	296.46±13.10
18	0.75±0.01	301.00±16.98
24	0.71±0.00	297.86±10.06
36	0.71±0.02	302.59±15.20
48	0.71±0.01	319.85±12.61
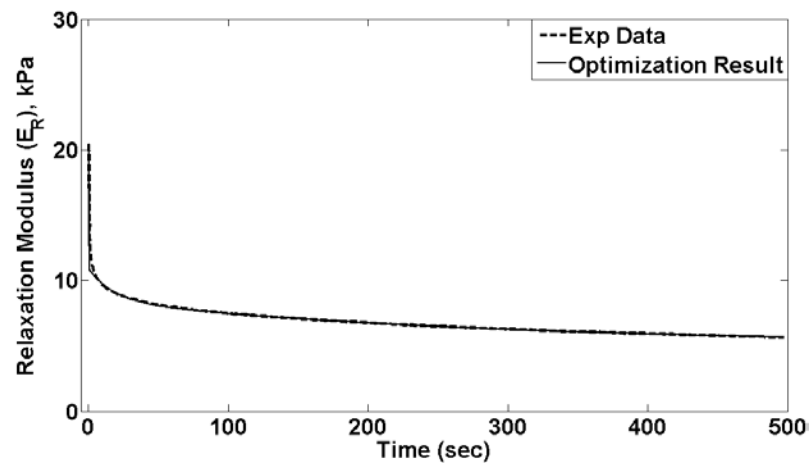
(a)
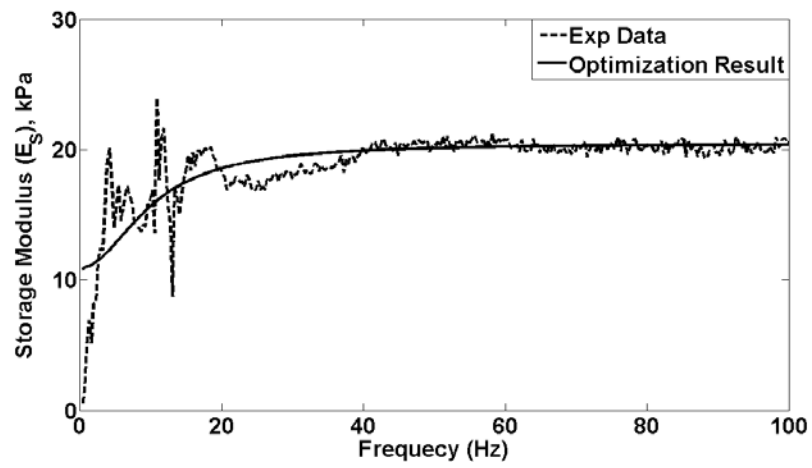
(b)
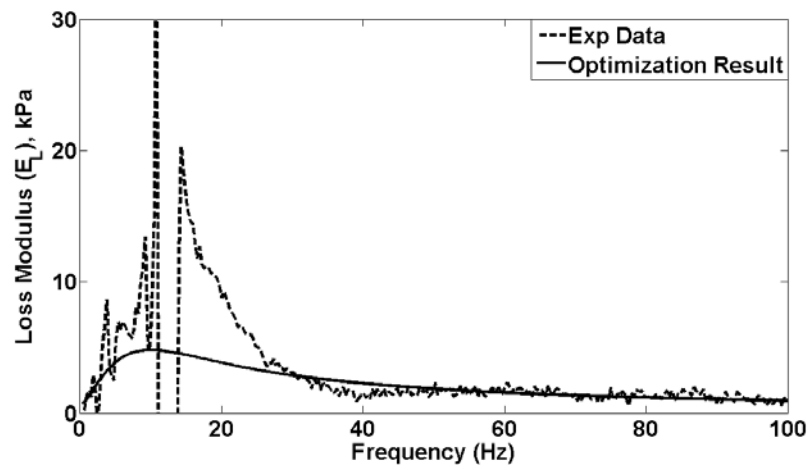
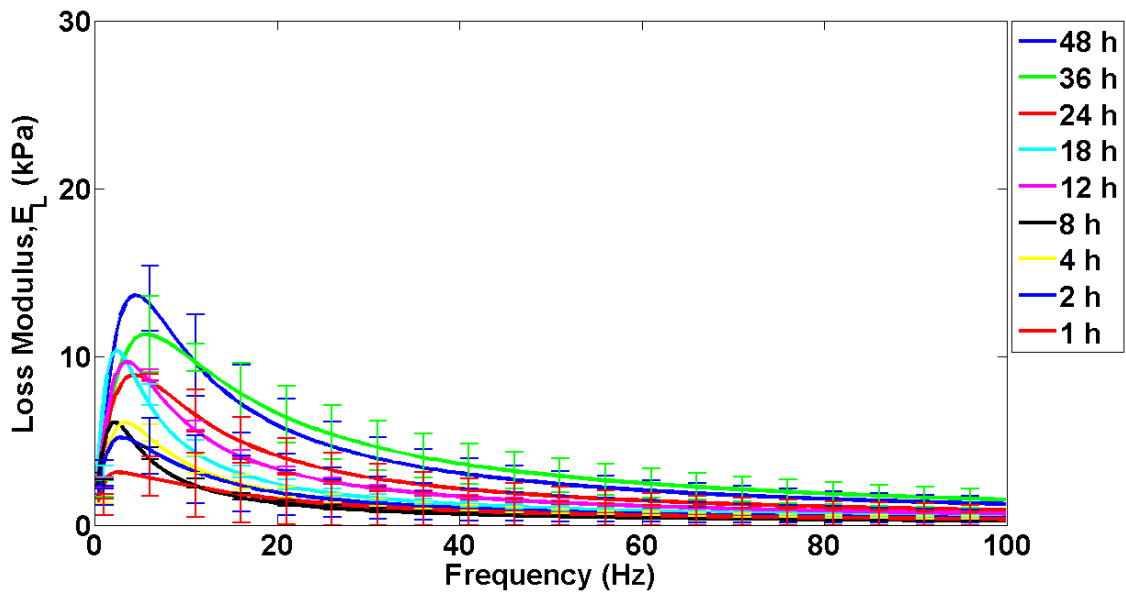(c)

Figure 6. The relaxation modulus (a), storage modulus (b), and loss modulus (c) obtained through the optimization process (solid lines) for the experimental data collected one hour after harvesting (dashed lines).
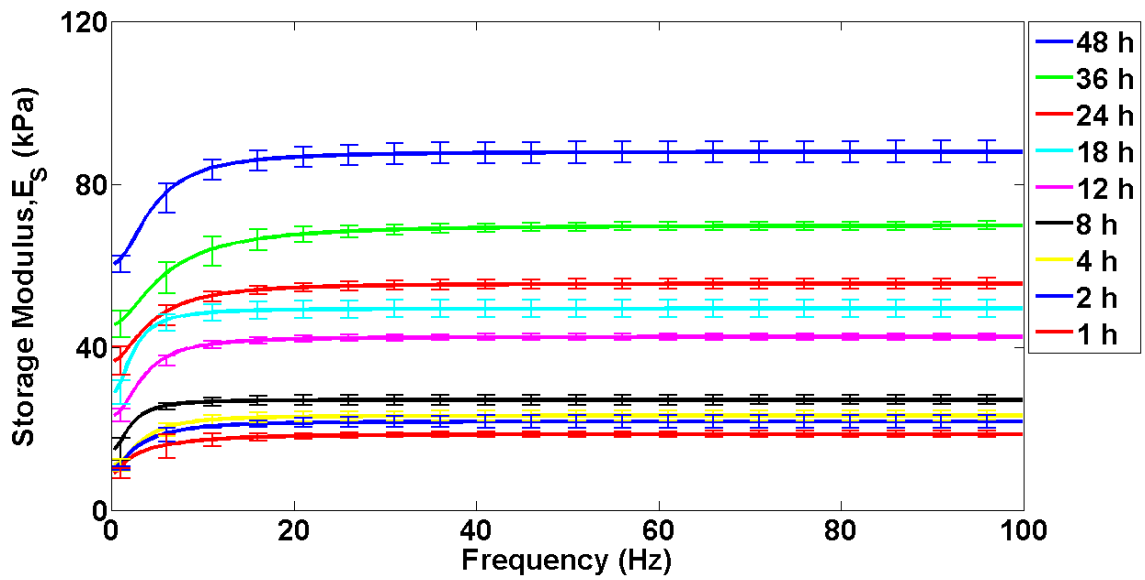
(a)

(b)

Figure 7. Storage (a) and loss (b) moduli of bovine livers estimated by the optimization process for the preservation time of 1, 2, 4, 8, 12, 18, 24, 36 and 48 hours.

In order to further investigate the effect of preservation time on the viscoelastic material

properties of bovine livers, we plotted the material model coefficients $\alpha_{total} = \sum\limits_{j=1}^{N} \alpha_j$ and

$E_\infty = E_0(1 - \sum\limits_{j=1}^{N} \alpha_j)$ as a function of preservation time (see Figure 8).
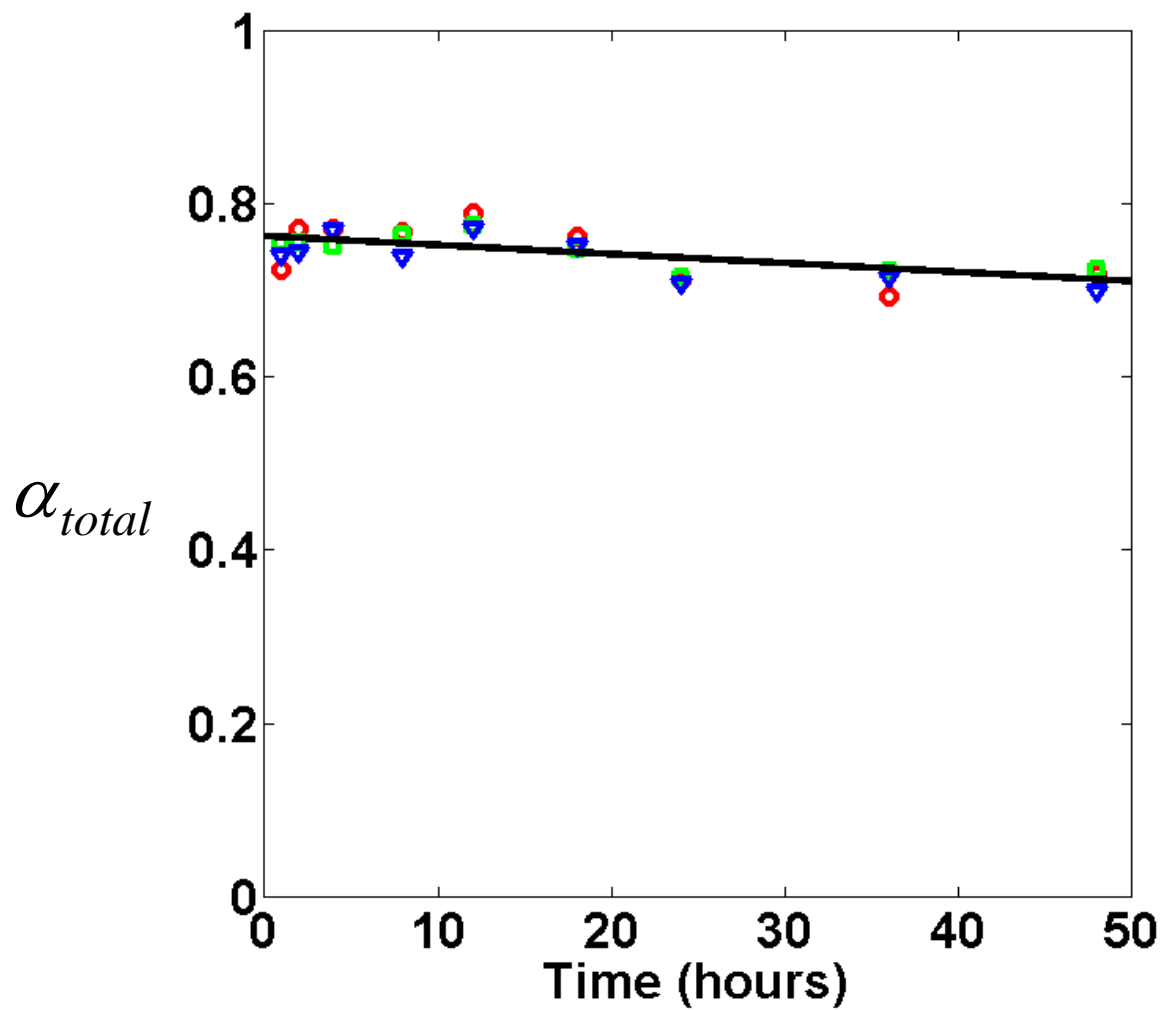
(a)
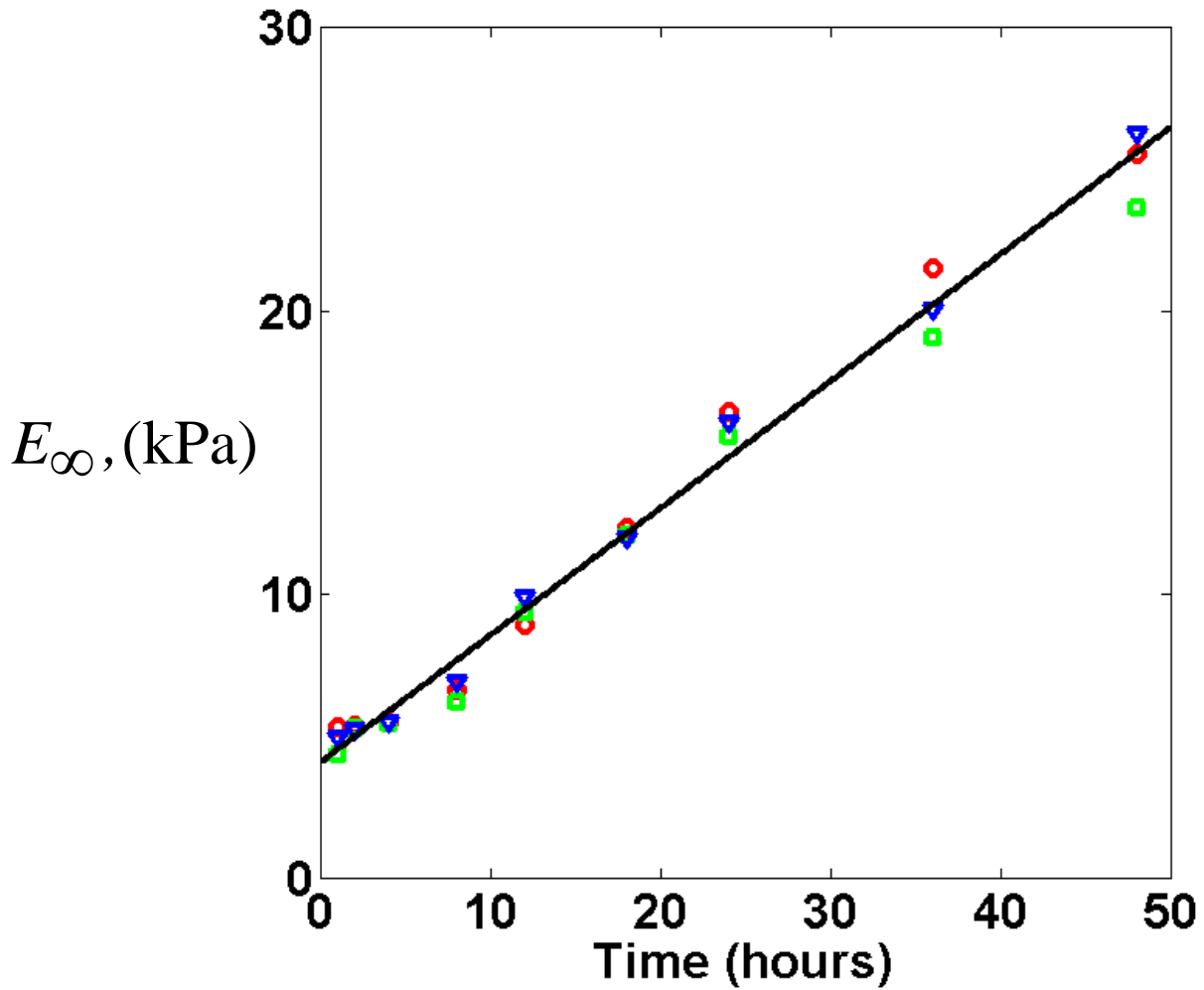
(b)

Figure 8. a) The total relative modulus as a function of preservation time ($\alpha_{total} = -0.0010T + 0.763$, where T is the preservation time in hours). b) Long term modulus as a function of preservation time ($E_\infty = 448.44T + 4054.5$ where T is the preservation time in hours).

In order to get a better idea about the relaxation responses for different preservation times, we inspect the slopes of the curves at the beginning (t= 0 sec) and end (t= 500 sec) of their relaxation period. The slopes at the beginning and end of the relaxation period are

$$slope_{t=0} = \frac{dE_R(t)}{dt}\bigg|_{t=0} = -\sum_{j=1}^{N} \frac{E_j}{\tau_j}$$

$$slope_{t=500} = \frac{dE_R(t)}{dt}\bigg|_{t=500} = -\sum_{j=1}^{N} \frac{E_j}{\tau_j} e^{-500/\tau_j}$$

Based on the estimated material coefficients given in Table 2, we observed that the slopes at the beginning and end of the relaxation period are approximately equal to $slope_{t=0} \approx -E_2/\tau_2$ and $slope_{t=500} \approx -E_3/\tau_3 \, e^{-500/\tau_j}$ respectively. If these slopes are plotted as a function of preservation time, a linear increase is observed in both, which also indicates an increase in the tangent angles with the vertical and horizontal lines for the slopes at the beginning and end of the curves respectively. For a fast relaxation response, smaller slopes at the beginning and end of the curves are desired. As can be seen from the Figure 9, the relaxation response for T=48 hours is much slower than that of the T=1 hour.
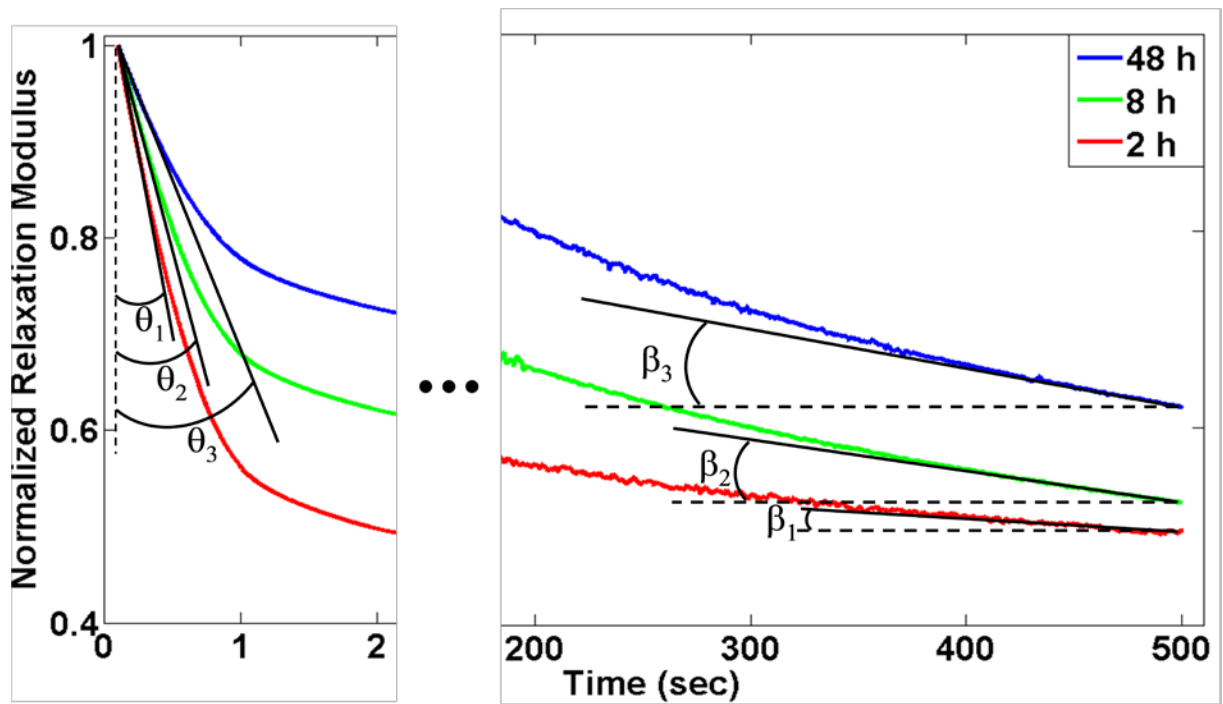
Figure 9: Change in tangent angle both at the beginning and end of the stress relaxation curves at different preservation time periods

Chapter 5

DISCUSSION of RESULTS

The results of the ramp and hold experiments show that both short (E_0) and long-term (E_∞) elastic moduli of bovine liver increase with an increase in preservation time (see Figure 3). This result supports the earlier findings suggesting that excised liver tissue becomes stiffer in time (Kerdok et al., 2006 and Rosen et al., 2008). For example, the steady state (i.e. long-term) elastic modulus of the liver sample collected at T = 48 hours is more than 4 times stiffer than that of the one collected at T = 1 hours. It is interesting to note that the ratio of long-term modulus to the short-term one, $E_\infty / E_0 = 1 - \alpha_{total}$, is almost constant and independent of the preservation time (see Figures 8a). This result suggests that a linear model is appropriate for investigating the effect of preservation time on the viscoelastic relaxation response of bovine liver. The results of the ramp and hold experiments also suggest that liver tissue becomes more viscous in time (see the length of relaxation in Figure 3). This result is also supported by the plot shown in Figure 8b. The time constants (τ_{total}) obtained from the GMS model increases linearly with the preservation time. Hence, the relaxation response of the liver tissue slows down as it spends more time in the preservation cycle. For example, the relaxation response of the

liver sample collected at T = 48 hours is approximately 1.3 times slower than that of one the collected at T = 1 hours.

The results of the impact experiments show that the storage and loss moduli increase with an increase in preservation time (see Figure 7). The storage modulus increases with frequency up to the resonance frequency and then stays almost constant after that (see Figure 7a). The loss modulus also increases with frequency, reaching to a peak value at resonance frequency (maximum energy dissipation occurs at resonance), but then decreases to zero as the frequency is further increased. Since the storage and loss moduli are related to the energy storage and dissipation capacities of the tissue respectively, the results of the impact experiments are aligned with the ramp and hold experiments. For example, the storage modulus of the liver sample collected at T = 48 hours is more than 4 times higher than that of the one collected at T = 1 hours. This is due to the fact that the former is more than 4 times stiffer than the latter. For the same amount of compression, a stiffer material obviously stores more energy than the softer one. The similar argument can be made for the loss modulus. The increase in the loss modulus of bovine liver as a function of preservation time is an indication of more energy dissipation, which is related to the viscosity of the material. As the viscosity increases, the time constant of the liver increases and the liver responds more slowly to the external loading.

Chapter 6

CONCLUSION

A viscoelastic model that can simulate the time- and frequency-dependent characteristics of soft tissues was developed. The model was tested on a bovine liver to investigate the effect of preservation time on the viscoelastic material properties. For this purpose, first, time-dependent relaxation modulus and frequency-dependent complex modulus of the bovine liver were measured experimentally. In order to measure the frequency-dependent dynamic modulus of bovine liver, an impact hammer equipped with a force sensor was used. Using the hammer, a light impact was applied to a pre-load placed on top of the cylindrical liver samples and the vibrations of the pre-load were measured using an accelerometer. The FRF was developed from the measured force and acceleration data. The dynamic stiffness and loss factor of each sample were obtained from the FRF using Eq. 3. Finally, the dynamic modulus was obtained from dynamic stiffness using the cross-sectional area and the length of the samples. In order to measure the time-dependent relaxation modulus of bovine liver, an experimental set-up was developed and ramp and hold experiments were conducted with the same cylindrical samples. Each sample was compressed to a fixed depth using a compression plate moving with a constant velocity and then the plate was held there for 500 seconds to measure its force relaxation response using a force sensor attached to the

plate. The stress relaxation response was obtained by dividing the force response to the original cross-sectional area of the samples. Second, a lumped GMS was selected to model the dynamic response (both time- and frequency dependent) of the bovine liver and then the equations representing its time-dependent stress relaxation modulus and frequency-dependent complex modulus were developed. Third, a MATLAB-based optimization subroutine that minimizes the residual error between the data collected from the experiments and artificially generated by the GMS model was developed to estimate the optimum viscoelastic material coefficients. Finally, the variations in these coefficients were investigated as a function of preservation time.

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