EFFECTS OF NORMAL FORCE, SAMPLE RELAX-ATION, AND TESTING GEOMETRY ON THE RHEOLOGICAL MEASUREMENTS OF SEMI-SOLID FOOD PRODUCTS

By

MELISSA JEAN DANIELS PEARCE

Bachelor of Science
University of Arkansas
Fayetteville, Arkansas
1996

Master of Science
University of Arkansas
Fayetteville, Arkansas
1998

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2001

EFFECTS OF NORMAL FORCE, SAMPLE RELAX-

ATION, AND TESTING GEOMETRY ON THE

RHEOLOGICAL MEASUREMENTS

OF SEMI-SOLID FOOD

PRODUCTS

Thesis Approved:

Danielle Bellmer
Thesis Advisor

Gerald H. Brusewitz

Carla L. Goad

Christopher A. Miller Dellinger

Timothy J. Pettibone
Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like to thank the Biosystems and Agricultural Engineering Department and the Food and Agricultural Products Research and Technology Center for the continuos support. Both reflected the quality of Oklahoma State University, and it was an honor to be a part of such institutions. I could not have asked for better facilities or examples of researchers and friends. I leave OSU proud to know I learned from the best.

Many thanks and much respect must be extended to my graduate committee. I could not have chosen a better group to guide me through this journey. Each member was an integral factor in the completion of this project. Although each professor offered different expertise, they were all advisors to their fullest capacity and had the common bond of being great listeners. Each gave me what every graduate student hopes for – respect. I hope I returned it ten-fold.

A special thanks must be given to Dr. Carla Goad who could not have been aware of the magnitude of the challenge of helping me understand statistics when she signed on to this project, but met it full force with much patience and determination. Much should also be said about her friendliness and her humor, two elements that always made statistics easier to swallow and trips to her office the most inviting.

Dr. Christina DeWitt gave me two gifts while serving on my committee—both she never knew she gave and both are priceless. First, Dr. DeWitt always sought to fully understand my project to the tiniest detail—never afraid to ask any question in the hopes of learning more. She made me collect and organize my knowledge and formalize answers to questions that I never bothered asking myself or took for granted. I always learned more about my research when answering her questions – she forced me to look at my project from a different perspective and many research notes emerged from our conversations. Second, how fortunate could I be to witness a new professor plant professional roots and begin a career? I lingered a little longer in her office, engaging her in conversations about the transition from student to faculty member. She has done so with such ease and success, I put every effort forward to learn her secrets. My extended stays were also due to her infectious laugh and her positive spirit—again more notes were taken.

Much thanks to Dr. Gerald Brusewitz for showing me why it is all worth it and how not to lose yourself in the chaos of it all. He has displayed the ultimate example of dedication—to both his field and to himself. I have never met a professor who cared so deeply about his profession, but did not define himself fully by his what he does, but rather by who he is—a lesson I really need to learn. Everyday he gives one hundred percent to his research and to his students. I have never seen him engage in anything without full energy and tenacity, thinking of every detail and checking them all twice. Yet, he never seems to be so involved that he cannot see the big picture and remember the things that are truly important, which usually never involve complicated equations or a spreadsheet of any kind. This is why I rarely think about Dr. Brusewitz in the partitioned terms of a great professor or a great researcher, but rather in the whole expression of a great man.

Completely thanking Dr. Danielle Bellmer for all she has given me as my major advisor will certainly be more of a process than simple routine of a few words on a page.

I am quite positive that I am not even aware of all she has done for me yet—only time will introduce me to my full debt to her. However, some things I fully understand and appreciate now. I hope every graduate student is as blessed as I was with such an attentive and caring advisor. I cannot think of a single situation where I was not treated as a top priority when I sought Dani's advice or attention. Regardless if she truly had time to accommodate me or not, she would always act as if I mattered more than any pressing deadline or meeting…and she did so without complaint or even the slightest show of contempt. She takes the commitment of being a graduate advisor seriously, and her open door proves it. I know I walked through that door many times, taking it for granted and I hope to rectify that now by saying thanks.

I would also like to thank Dani for her extraordinary example of balance. I have met only a few others who do so many things well and make it seem so effortless. She is hardworking, yet always seizing every opportunity to have fun. She is detailed oriented, but also always thinking of the bigger picture. She is a working mother who successfully meets the demands of family and work without sacrificing either. She has made me acutely aware that every situation does not have to be black and white and learning to add balance to your approach will help you deal with the gray elements.

What I want to thank Dr. Bellmer for the most is for the freedom she gave me to explore my own project ideas and make my own decisions, and occasionally, my own mistakes. Very few people have ever allowed me so much space to see how far I could make it on my own. She will never know how much it means to me that she trusted me enough to allow me to develop my own project. I am also aware of how much patience this required of her. Instead of prompting me into any specific research direction, she

always waited until I came to her with my ideas and theories. I am proud to leave OSU with the experience of identifying and defining a research problem. I thank Dr. Bellmer for such an opportunity and most of all for just being her.

My lab-mates deserve a few cheers also. Dharmendra, Lynn, Mary, Muluken, Shekhar, and Travis, thanks for keeping the lab fun. I could always count on one of you to put me in my place with a zinger or two or to steal my chair. Take care, all of you and much luck. And noone open that fridge door!

OSU nor Stillwater would have been the same without Jennifer Mitchum. The life of a graduate student always has its highs and lows. I am so glad you were there to share the highs, but I am most thankful and fortunate that you were there to share the lows. Thanks for always telling me that I take life too seriously and for forcing me to have fun even when I wanted to be miserable. Our weekly lunches kept me going and added much comic relief. You were a constant support and I hope I pay you back fully. I am sure one day when you are writing your dissertation (wink, wink), I will have the opportunity.

Michael and Michelle, thanks for proving that this could be done and for going first. Of course, you always got to go first because of that quirky younger sister clause, but this is beside the point. I thank you both for your advice (even though sometimes it did nothing more than irritate me, I appreciate it now). I will even put it in print for you, 95% of it was actually proven to be good suggestions, especially the part about having a little more fun. I thank you both for your countless phone calls of encouragement and therapy. I love you both. I hope you are as proud of me as I am of you.

Mom and Dad, I do not even know where to begin. You have been a constant, living example of kindness, dignity, and grace. The education I have accrued at Universities will never match the lessons you have taught me throughout the years, nor the lessons you still provide each day. Prayers are said each night offering thanks for such wonderful parents and family. Everything that I am, I can attribute back to you. I hope to always make you proud and to show you my love as you have always shown me yours. God bless you and keep you.

Finally, to the one who deserves the most thanks…to the one who has had to live with me as I pursued this degree. Ricky, you have proven over and over again that you are my rock and my anchor. I love you for being you. Thanks for taking the time to learn when I need to be comforted or helped, to be left alone, or to be kicked swiftly in the keester. Is it not always easy to tell when I need which, but you are becoming truly skilled at deciphering the fine line. I hope I always provide you with the same support. I love you…it is as simple and as complicated as that.

TABLE OF CONTENTS

Table of Contents (Continued)

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

List of Figures (Continued)

List of Figures (Continued)

FIGURE PAGE

NOMENCLATURE

cm	centimeters
Delta (δ)	phase angle which relates the input sinusoidal stress and the resulting output sinusoidal response
F_x	vertical force
F_y	horizontal force
G'	storage modulus, calculated by $G' = [\sigma_o/\gamma_o] \cos(\delta)$
G"	loss modulus, calculated by $G'' = [\sigma_o/\gamma_o] \sin(\delta)$
h	height of the sample or the gap between the plates
Hz	Hertz
L	displacement of the upper plate from the center line
N	Newtons
ω	frequency expressed in rad/s
σ_o	amplitude of the shear stress at time t.
mm	millimeters
Pa	Pascals
rad/s	radians per second
s	seconds
t	time
γ	shear strain resulting from the oscillation stress through the equation $\gamma = \gamma_o \sin(\omega t)$
γ_o	amplitude of the strain

CHAPTER 1: INTRODUCTION

Rheology has historically had applications in several industries—concrete and cement, plastics and other polymers, tires and various rubber products, paints, inks, plasma, and cosmetics. Rheological research is becoming increasingly important to the food industry. The investigation of the rheological properties of food materials is essential for food process engineers, quality control supervisors, and food technologists. Rheological data is used in engineering calculations (i.e., pump or pipe sizing or the development of extrusion equipment), in the determination of new product formulations, in defining or testing the quality and stability of products, and to quantify textural characteristics of a product (Steffe, 1996).

Two trends that have corresponded with the growth of rheological research are the use of oscillatory measurements and the study of semi-solid food products. The majority of past rheological research in the food industry has involved the measurement and characterization of flow properties of liquid and semi-liquid materials. Rheological characterization of semi-solid foods has been considered tedious in the past due to limitations arising from time dependency (Kokini and Dickie, 1981), wall effects or slip, and secondary flows resulting from small shear stress rates in narrow gap geometries (Dervisoglu and Kokini, 1986). Until recently, it has been more economical and easier to focus on flow properties of liquid and semi-liquid products.

More sophisticated equipment has allowed the food industry to better characterize semi-solid foods using rheological properties. In particular, the introduction of the controlled stress rheometer has allowed for better oscillatory measurements of rheological parameters. Oscillatory measurements are considered superior to flow measurements due to the non-destructive nature of the test and the ability to relate rheological parameters directly to the structure of the sample. Such improvements have increased demand for oscillatory data to provide needed solutions to food processing problems.

As oscillatory measurements have become more advanced and the demand for oscillatory data has increased, researchers have undertaken many comprehensive projects without considering the basic, fundamental properties of semi-solid foods or of the functions of the equipment. To compensate for the lack of basic research in these areas, the methods in oscillation measurements were founded on ideas borrowed from the literature describing and supporting flow measurements of liquid products. This adaptation of methods from one type of research to another is risky, and its success is dependent on sound assumptions of similarity between the two situations, particularly in this case, the similar behavior of liquid and semi-solid food products.

When reviewing the literature research involving oscillatory measurement of semi-solids, it becomes apparent that there is a problem with data variability. There is no clear understanding or quantification as to precisely what causes the variability and how to eliminate it, but suggestions of possible sources of error include normal force application during loading and sample migration during testing. These are two factors that may not have major influences on liquids, but can potentially have dramatic impacts on semi-solids.

Using sample loading as an example, the mistake of treating liquids and semi-solids equally when making rheological measurements can be fully understood. During rheological testing, a sample is placed between two parallel plates (or sometimes if a liquid is too thin, samples are loaded into a concentric cylinder). As the top plate moves down onto the sample, a force is applied perpendicular to the product. There is little dispute among scientists about whether a normal force is exerted during loading. However, there is much confusion and very little consensus as to how and to what extent this force influences oscillatory property measurements.

These questions were rarely raised when testing liquid products because normal force has little impact on liquids due to their molecular behavior. As the upper plate is applied to liquid samples, instead of resisting the force, the molecules simply slide over one another and allow the sample to flow outwards to relieve pressure. This natural relaxation reaction by liquids allows them to be more uniformly loaded than semi-solids, and much less influenced by normal force.

In contrast, the loading of semi-solid samples is much more complex. First, semi-solid samples are not as uniform as liquid samples. The thickness of semi-solid samples is not guaranteed to be identical, especially when considering food products. This alone presents a problem of loading the sample by setting a specific gap between the plates. If samples are loaded to a specific gap, and if thickness among samples varies, then thicker samples are loaded with more force than other samples. Second, this would not be a problem if the molecules of semi-solids flowed as aptly as molecules of liquids. However, semi-solids will absorb the normal force applied by the upper plate rather than reacting to the force. This eliminates the effectiveness of setting a specific gap to insure identical treatment of samples. Another method of loading might be to adjust the plate manually and eye the placement of the plate on top of the sample. However, the subjectivity of this method leaves much room for operator error. It is easy to understand how sample loading influences liquid and semi-solid materials differently, and how sample loading could prove to be a great source of error for semi-solids.

Because normal force has the potential to introduce error before a sample is ever tested, loading (and the resulting normal force applied during loading) seems to be a critical early opportunity for error introduction when measuring properties of semi-solids. Even if samples are treated identically in all other respects, the introduction of different normal forces during loading can create errors that are magnified throughout testing. In recognition of the potential for error associated with normal force, researchers have generally addressed normal force in one of three ways: (1) they ignore the concept entirely and treat the sample just the same as a liquid product; (2) they recognize that normal force may be a problem but offer no clear evidence of its effects nor any solution as to how to correct or minimize its effects and still treat the sample the same as a liquid; or (3) they suggest that a sufficient relaxation time (about 15 minutes on average) between sample loading and testing will eliminate any effects introduced during loading.

Each of these approaches for dealing with normal force is flawed. The suggestion that normal force impacts are eliminated without providing any evidence as to how or why such a conclusion has been reached is detrimental to future rheological research. First, ignoring normal force altogether or its effects on rheological data relies upon a basic assumption that, if untrue, can introduce and magnify errors throughout testing. If normal force does impact rheological data, and particularly if that impact is not exerted uniformly on all types of samples, particularly as research focuses more on new semi-solid food products, data errors are introduced before testing ever begins. If errors are introduced prior to testing, no solutions offered to correct for other potential errors, such as slip, will increase data repeatability.

Additionally, allowing for relaxation between sample loading and testing relies on basic assumptions for which little support has been provided. Past research that has addressed normal force during loading has often suggested that a relaxation period between loading and testing will minimize or eliminate the impacts of normal force introduced during loading. This method was also developed for liquids, which normal force has minimal effects on in the first place. Research has suggested that relaxation is an "easy solution" to the problem based on very little technical data. The proper relaxation times suggested by these studies have varied from 5 minutes to 2 hours (usually about 15 minutes) with little, if any, justification for the period of time chosen.

Based on the obvious need for basic research involving the validity of these assumptions, this project focused primarily on the effects of normal force during loading on data variability. The need for such investigation was also supported by the preliminary testing of peanut butter slices and provolone cheese, which illustrated the differences in oscillatory parameters of samples loaded to various normal forces. Preliminary testing results (plots of G' or initial G' versus oscillation stress) can be found in Appendix A.

The project design involved three independent experiments analyzing: (1) the impact of normal force during loading; (2) the effects of relaxation time in eliminating or minimizing normal force impacts; and (3) the effects of other potential sources of data variability, such as slip, while strictly controlling normal force during loading. Both semi-solid and liquid food products were analyzed using a controlled-stress rheometer to characterize oscillatory parameters (G' and G"). The oscillatory parameters were analyzed for the impacts of loading normal force and resting, sample composition, and sample migration.

This project was designed to provide basic research that has been primarily bypassed in previous rheological studies. In order to improve data repeatability in future research, the effects of normal force during loading must be identified and accounted for. Fundamental assumptions regarding the impacts of loading normal force and relaxation time have been and continue to be relied upon in rheological research. The validity of these assumptions is essential to data repeatability because loading provides the first critical opportunity for error introduction. If the food industry is to continue its use of rheological measurements and oscillatory data, these basic assumptions must be studied further.

CHAPTER 2: LITERATURE REVIEW

2.1 TESTING EQUIPMENT

The TA1000-N controlled stress/strain rheometer, categorized as a rotational rheometer and characterized by its stabilizing air bearing, can operate in flow, creep, and oscillation modes. This equipment can take measurements using parallel plate, cone and plate, and concentric cylinder apparatuses. The TA1000-N rheometer has the capability of maintaining specific temperatures during testing or subjecting the sample to a range of temperatures during operation. The equipment can also measure the normal force applied to the sample during loading, load to a specific normal force, apply a specific normal force during testing, or allow for the relaxation to a specific normal force before conducting the test after sample loading. Sensitive load cell technology installed in the static lower plate of the rheometer detects and measures normal force in the ranges of 1 to 5000g (TA Instruments TA-1000N Equipment Manual).

2.2 OSCILLATORY MEASUREMENTS

2.2.1 Theory of Oscillatory Measurements

There are three major types of rheological measurements: flow, creep, and oscillation. The majority of past rheological research has involved flow measurements. However, recently there has been a strong emergence of studies using oscillatory measurements to characterize the rheological properties of food products. This project involved the use of oscillatory measurements to examine rheological properties of semi-solid foods.

In oscillatory testing, the sample is subjected to repeated, sinusoidal stress or strain. The application of a sinusoidal stress makes it possible to break the stress into in-phase and out-of-phase components, which are related to the input sinusoidal stress and the resulting output sinusoidal response by the phase angle, delta (δ). From δ, the storage modulus (G') and the loss modulus (G") can be determined by the stress in phase divided by the strain and stress out of phase divided by the strain, respectively (Navickis et al., 1982). G' and G" relate to the viscoelastic behavior of food products: G' is the measure of the energy stored or taken up by the material (the solid or elastic component, affected by the horizontal force F_y), and G" is the measure of the energy lost (the liquid or viscous component, affected by the vertical force F_x), during each cycle of the sinusoidal input.

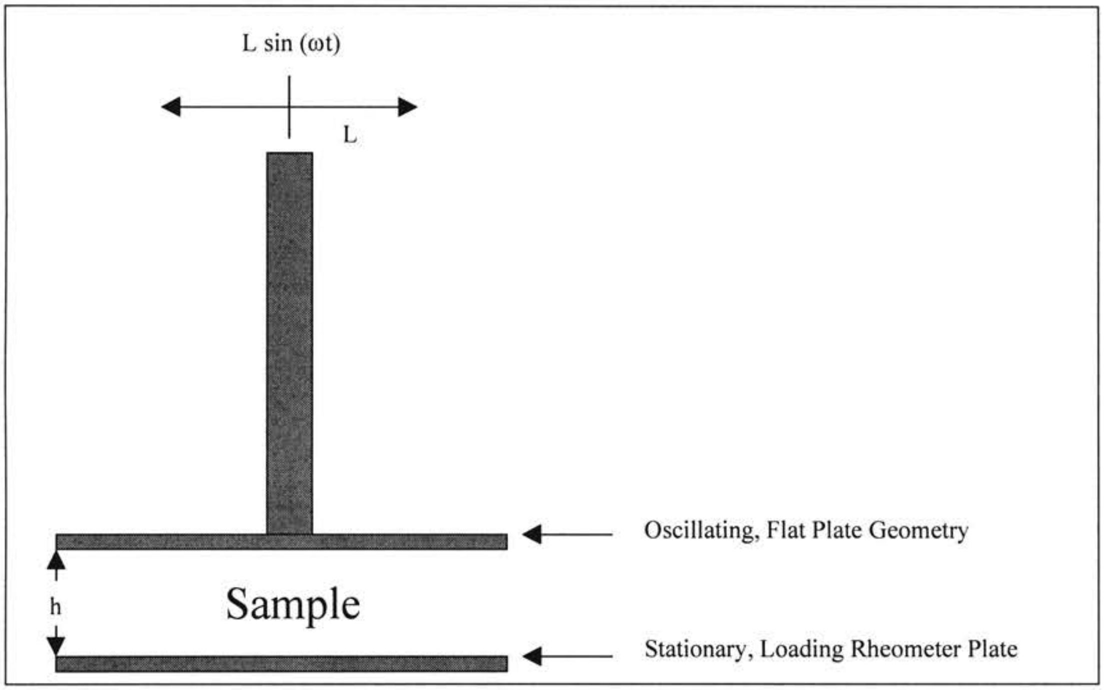

Figure 2.2-1. Sample Loaded Between Parallel Plates of a Controlled-Stress Rheometer (Adapted from Steffe, 1996).

Critical parameters that are needed for the series of calculations in order to determine G' and G" are illustrated in Figure 2.2-1, which depicts a sample loaded between the parallel plates of a controlled stress rheometer. The height of the sample or the gap between the plates (h), the displacement of the upper plate from the center line

(L), and the angular frequency expressed in rad/s (ω) are needed to estimate the shear strain resulting from the oscillation stress through the equation:

$$\gamma = \gamma_o \sin(\omega t)$$

where the amplitude of the strain (γ_o) is expressed as L/h. A second equation is needed to estimate the phase angle or the mechanical loss angle (δ) based on the input shear stress at time t:

$$\sigma(t) = \sigma_o \sin(\omega t + \delta)$$

where σ_o is the amplitude of the shear stress at time t. Finally, G' and G" can be calculated by the following equations:

$$G' = [\sigma_o/\gamma_o] \cos(\delta) \text{ and } G'' = [\sigma_o/\gamma_o] \sin(\delta).$$

2.2.2 Advantages of Oscillatory Measurements

There are two major advantages to using oscillatory measurements to characterize rheological parameters, which have increased their application in rheological research. First, oscillatory measurements are preferable due to the non-destructive nature of the test. The majority of past research, measuring rheological properties with traditional methods such as flow, was based on destructive, single-point testing. Such testing methods are considered destructive because the single set of applied conditions usually exceed the critical strain value of the product; therefore, the results cannot be related to the initial or steady-state properties of the material (Solorza and Bell, 1995).

Second, oscillatory measurements allow the rheological parameters measured to be directly related to the structure of the sample. Flow and creep measurements are made at a single, specific set of conditions of strain or stress rate, and the measured response could be related to properties of the material only if it was consistently subjected to the same set of conditions. In oscillatory measurements, however, the strain can be independently adjusted, which allows for the build-up, breakdown, and recovery of the food structure to be measured as a function of time. Subsequently, the parameters can be directly related to the material structure at rest or under any set of conditions.

2.3 Overview of Research Using Oscillatory Measurements to Define Rheological Parameters

2.3.1 Types of Foods Studied

Due to the development of instrumentation that can more efficiently measure dynamic rheological properties using oscillatory testing and the usefulness of such studies in characterizing texture, many recent studies have been designed to relate dynamic parameters to the structure or composition of food materials. The impetus for several of these research projects has been to determine or revise formulations of food products. One of the important developments in rheological research has been the adaptation of studies to semi-solid foods. Early research focused primarily upon liquids and semi-liquids such as salad dressings (Muñoz and Sherman, 1990; Elliott and Ganz, 1977) and mayonnaise (Peressini et al., 1998). In more recent studies, however, products tested have included a variety of semi-solids, including doughs (Navickis et al., 1982; Létang et al., 1999) and several types of cheese (Ustonol et al., 1995; Subramanian and Gunasekaran, 1997; Solorza and Bell, 1995).

2.3.2 Texture and Composition of Semi-Solid Foods

An important use of oscillatory testing in recent studies has been to correlate rheological properties with the texture and composition of food products. The research indicates several trends. Water content was found to be the most significant variable affecting G' and G" (Navickis et al., 1982). Both Navickis et al. (1982) and Létang et al.

(1999) concluded that G' and G" each decreased as the water content of the dough samples increased. Létang et al. (1999) attempted to study the influence of mixing parameters on the properties of wheat flour-water doughs using a Carri-Med CSL^2 Rheometer by taking a sample from the inside of the dough, placing it on the plates, and reducing the gap to 0.2 mm. Navickis et al. (1982), who measured the G' and G" of five types of wheat flour doughs using an eccentric rotating disc (ERD) rheometer, reported that a 12% change in moisture level (35-47%) resulted in a change in G' and G" by two orders of magnitude. According to this study, the G' and G" levels were also directly related to the protein content of the flours.

Steady shear and dynamic measurements have proven useful to characterize the texture of mayonnaise and salad dressing (Elliott and Ganz, 1977). Research by Peressini et al. (1998) and Muñoz and Sherman (1990) has shown that fat and oil content also impact the G' value of these semi-liquid foods. Peressini et al. (1998) conducted a study to assess the influence of fat content on the emulsion structure in traditional and low-fat mayonnaise. Using a controlled stress rheometer, they performed oscillatory measurements at 25^{o}C. Peressini et al. (1998) reported that G' increased as fat content increased, with light mayonnaise showing the lowest G'.

Muñoz and Sherman (1990) used a controlled stress rheometer to study the rheological characteristics of salad dressings. They ran oscillatory tests at 1 Hz, and a maximum stress amplitude of 14.93 or 8.96 Pa, over a frequency sweep of 600 s. They concluded that regular mayonnaises show a larger linear viscoelastic region than reduced-calorie mayonnaises and salad creams. The G' values were highest for mayonnaise and lowest for salad creams, due to the lower oil content, with reduced-calorie mayonnaise in between. Finally, they reported that mayonnaise showed higher values of maximum stress amplitude, thus creating greater linear viscoelasticity.

Solorza and Bell (1995) found that fat content in milk used to make cheese also affected the storage and loss moduli. They studied the effects of milk composition used to make soft cheese, specifically the levels of calcium, fat, and total solids, on the rheology of cheeses. Using a RTI Controlled Stress Rheometer, frequency sweeps (0.1 to 10 Hz) were conducted under the conditions of 8 mNm torque and 25°C. Samples analyzed included cheese made from whole milk (high-fat) with 12, 15, and 18% solids and from skim milk (low-fat) with 9, 12, and 15% total solids. These series were duplicated with addition of extra calcium, yielding a total of 12 samples. The curves generated by plotting frequency versus the log of the moduli for all samples increased rapidly and then leveled off. The G' and G" were greater for the skim milk samples than for the whole milk samples.

2.3.3 Viscoelasticity of Food Products

Several studies have shown that the elastic component (the storage modulus, G'), not the viscous component (the loss modulus, G"), is the dominant component of viscoelasticity (Ustunol et al., 1995; Subramanian and Gunasekaran, 1997). Peressini et al. (1998) reported that G' was greater than G" for all mayonnaise samples tested. Ustunol et al. (1995) investigated the influence of milk fat reduction on rheological properties of Cheddar cheese. They studied cheddar cheese with varying fat levels (13, 20, 27, and 34%) on a Rheometrics fluid spectrometer. Tests were performed at a constant frequency of 1 rad/s and a constant strain of 0.1% in a parallel plate apparatus (2.5 cm radius). Based upon the data which indicated that G' was greater than G" at all points, Ustunol et al. (1995) concluded that the elastic component contributed more to viscoelasticity than did the viscous component.

Subramanian and Gunasekaran (1997) also found a dominant elastic component of viscoelasticity. Their research studied the influences of refrigerated storage duration (1,4, and 12 weeks) and testing temperature (10, 20, 30, 40, 50, 60, 70 °C) on the storage and loss moduli of low moisture, part-skim milk mozzarella cheese and a low fat, part-skim mozzarella cheese. Using a Bohlin VOR rheometer, frequency sweeps, operated from 0.314 to 124.66 rad/s and at a strain of 0.05%, were conducted, and G' and G'' were recorded. For each cheese and at each temperature, the G' was always greater than G".

2.4 Errors Associated with Rheological Testing

Several factors can introduce error or data variability in rheological testing. Such variables include the history of the sample prior to testing (i.e., processing and storage conditions or steps), the sample preparation, including loading and trimming of the sample for rheological testing, and the conditions of the sample during testing (i.e., environmental conditions such as testing temperature and relative humidity and the behavior of the sample during testing). Many studies have reported a lack of repeatability of measured parameters or behavior, and this variability of measurements can most likely be attributed to one or a combination of these variables. Rheological experiments must be carefully designed to minimize the introduction of these errors; this is usually done by trying to treat all samples the same during the processing and storage of the product, during the loading of the sample in the equipment, and during the test. Two errors that have been explored minimally, but still require much work, are the migration of samples due to slip during testing and the application of normal force during the loading of the samples.

2.4.1 Slip or Migration of Sample During Testing

Previous research has given considerable attention to the phenomenon of slip. In semi-solid foods, as well as most dispersions of solid matter in liquids such as applesauce or mustard, a thin layer of liquid forms at the solid boundaries. This layer results in some deviation from the "no slip" boundary condition which is taken as a constant in equations used for calculating shear rates from rheological data (Qui and Rao, 1989). The slip effect takes place in concentric cylinder (CC), capillary, cone and plate, and parallel plate viscometer arrangements (Grikshatas and Rao, 1993; Kokini and Plutchok, 1987; Yoshimura and Prud'homme, 1988). Slip is difficult to detect and measure, but recognizing and either eliminating or accounting for it are essential to achieving valid and repeatable results in measuring rheological properties of food products.

2.4.1.1 Slip Effect in Flow Measurements

Recent studies have attempted to analyze the slip effect in flow measurements. Qui and Rao (1989) measured the slip effect in applesauce using a Haake RV2 viscometer system using no correction for slip. The team calculated the slip coefficient (defined as slip velocity divided by shearing stress) and the slip velocity ratio. This research found a good correlation between the slip coefficient and the flow behavior index. The slip coefficient was found to decrease with an increase in the pulp content of the sauce, suggesting that dispersions with very high solids content will exhibit less wall slip. By contrast, a low composition of solids will result in significant wall slip. The team concluded that the slip coefficient increased with the magnitude of the torque applied. They emphasized that wall slip depends upon a number of factors attributable to the specific properties of the food studied, and that their equations could not be readily extrapolated beyond the variables of their study.

Grikshtas and Rao (1993) studied slip effects using tomato concentrates and applesauce. Their research concluded that the magnitude of wall slip velocities vary greatly between the cup and the rotating bob. Studies of food dispersions have suggested that mixing in concentric cylinder systems would decrease or avoid errors in measurement due to slip (Rao, 1975; Steffe and Ford, 1985). Other studies have claimed to remedy wall slip using bonding (Lindborg et al., 1997). Lindborg et al. (1997) performed rheological testing on flour doughs using a cone-and-plate system. In addition to mixing the samples, the team bonded the samples to the bottom plate using a drop of cyanoacrylate adhesive. The study reported that the application of the adhesive had entirely eliminated the phenomenon of wall slip. However, this study did not detail how slip was identified or quantified in the preliminary measurements or how it was determined that no slip occurred with bonding.

Navickis and Bagley (1983) studied wheat starch granules on a mechanical parallel plate spectrometer. A major experimental problem faced in the research was wall slip between the gel and the metal plates. This study claimed that large discontinuities in the flow curves illustrated slip. However, since their stress responses were only "intermittently reproducible," they recognized that slip occurs in a highly unpredictable manner. They used two methods for reducing the slip effect. For some samples, they sprayed lacquer on the metal fixture and scattered ordinary sand on the surface. For others, they used a thin layer of cyanoacrylate ester Superglue spread on the gel surface. This research suggested that slip can be avoided by either of these two methods, but again the study gave no indication of how it was determined that slip was eliminated with the solutions.

2.4.1.2 Slip Effect in Compressional Measurements

The slip effect has been discussed frequently in compressional studies. In an early study, Voisey and Reid (1974) concluded that friction is an important factor that must be considered in the operation and standardization of texture test instruments such as the Instron Universial Testing Machine and the TAXT2 Texture Analyzer. According to Goh and Sherman (1987), the presence or absence of friction at both ends of the sample being compressed has been related to changes in stress relaxation. The presence or absence of friction is essentially a slip effect—with no friction, slip would be more readily noticeable.

Research has identified several methods for reducing slip in compressional studies. Many researchers have used a bonding agent (usually a cyanoacrylate resin) to attach the sample to the plate (Nolan et al., 1989; Christianson et al., 1986; Casiraghi et al., 1985). Many have used an adhesive-backed Teflon to attach the sample (Christianson et al., 1986; Casiraghi et al., 1985). Goh and Sherman (1987) also inserted emery paper between the sample and the compressional plates to reduce slip. Many studies have utilized multiple approaches of those discussed above as well as a "normal, non-bonded" test procedure for comparison (Brennan and Bourne, 1994; Goh and Sherman, 1987; Christianson et al., 1986; Casiraghi et al., 1985). Many of the studies claimed to have corrected or eliminated slip by the methods provided. Nolan et al. (1989) concluded that bonding sandpaper and the sample to the plate using a cyanoacrylate resin effectively corrected slip for mozzarella and cheddar cheese. Both Christianson et al. (1986) and

Casiraghi et al., 1985) concluded that bonding is effective in preventing slip and essential to obtaining meaningful compressional data that is uncomplicated by frictional behavior.

Brennan and Bourne (1994), who conducted experiments on provolone cheese and chicken frankfurters, compared the results of lubrication with mineral oil to non-lubricated samples. They concluded that lubrication may not be advisable for compressional research that is designed to simulate what goes on in the mouth, finding that the deformation patterns that result differ greatly from those that occur in the mouth.

2.4.1.3 Slip Effects in Oscillatory Measurements

Few studies have addressed the slip effect in oscillatory measurements. Nolan et al. (1989) analyzed the slip phenomenon using oscillatory measurements. This research described a method for identifying slip, which included keeping strain and frequency constant, and measuring the stress-strain waveforms resulting from the sinusoidal input stress with two different gap separations. If the two waveforms are identical, slip is not occurring. However, if the two waveforms differ, slip has occurred. Studying the waveforms of low moisture, part skim mozzarella cheese, this team discovered slip in the waveforms produced at 10% strain measured with a 4-mm gap and an 8-mm gap. Because the two forms were not identical, they determined that slip had occurred.

In order to avoid slip, Nolan et al. (1989) tried several methods: attaching coarse sandpaper to the plates; using titanium plates instead of aluminum ones; and bonding the cheese directly to the aluminum plates with cyanoacrylate ester adhesive. They discarded the sandpaper because they noticed that it imparted resistance that caused temperature lag. They determined that bonding the cheese to the aluminum plates was most effective in eliminating slip. They concluded that bonding produced repeatable results free of slip for mozzarella cheese slices.

Rosenberg et. al. (1995) looked at reduction of slip in the measurement of cheddar cheese samples using a controlled stress rheometer. The team designed a special serrated plate geometry to help reduce slip. The testing procedure to evaluate the serrated plate involved running frequency sweeps (0.1-10Hz) at 10, 15, 25, and 35°C for cheese discs of varying heights (2, 5, and 10 mm). The team determined that the serrated plate was suitable in eliminating slip due to the fact that regardless of height, a linear (r^2=1) relationship between strain amplitude and applied torque could be obtained for all samples. The study did caution that sample height was a factor to be considered due to slip even when using the serrated plate; this warning was based on the frequency sweep curves of the 2, 5, and 10 mm samples. The curves were identical for the 5 and 10 mm samples, but varied for the 2 mm samples. It was suggested that sample heights below 5 mm would still be influenced by slip even if the serrated plate were used.

Although Rosenberg et al. (1995) concluded that a serrated plate did reduce sample migration during testing, they also noted that the data variability still was not completely eliminated. Reducing slip would only help minimize data repeatability problems that occur during testing; the introduction of error during loading by such means as differing loading normal forces would not be resolved by using a serrated plate. To fully show the impact of the use of a serrated plate as a solution to data repeatability due to sample migration, the study needs to minimize as much data error as possible that does not result from slip. A study which minimizes loading errors along with slip by both controlling normal force during loading and also using a serrated plate would be the most beneficial evaluation of the serrated plate as a solution to slip.

2.4.1.4 Lack of Conclusions on Slip Associated with Any Type of Rheological Testing

After reviewing the literature associated with slip and rheological testing, it is clear that few strong conclusions or decisions about how to define or eliminate slip in rheological testing exist. It is evident that more research is needed to help supplement the current knowledge, especially in oscillatory testing of semi-solid foods. Although there are many problems associated with attempting to isolate and define precisely when slip is occurring, an important objective of this research was to determine whether the use of a serrated plate can minimize or eliminate the effects of slip.

2.4.2 Application of Normal Force During Sample Loading and Relaxation Time of Force Before Testing

Another error associated with oscillatory measurements is the influence of stress applied to the sample during loading. This application of force (i.e. the normal force) is another parameter that should be considered in evaluating the results of rheological testing. Previous research has largely ignored this parameter, most likely due to the inability of past instrumentation to measure the normal force during loading. Little research has focused on determining the effects of normal force during loading on the resulting rheological curve; in fact, little is known about how these forces relate to the repeatability of rheological data. Most investigations concerning normal force during loading have focused on allowing a relaxation time between loading and testing of the sample that would eliminate the problems created during loading, without even determining if these forces are influential factors in the first place. It seems unreasonable to provide a solution for a problem that may or may not exist.

2.4.2.1 Relaxation Time as a Solution to Normal Force Effects

Despite the lack of information supporting the idea that normal force introduction during loading results in inconsistent data, many studies have suggested a relaxation period of anywhere from 5 minutes to 2 hours (about 15 minutes on average) in order for the compressive force to decay so as not to have any influence on the resulting data curves. In oscillatory testing, Nolan et al. (1989) concluded that relaxation to zero force occurred about 5 minutes after the sample came into contact with the plate. This determination was based upon a reading on the instrument being used. Taking the normal force as zero, this study gave no further consideration to this force as an influence on the research.

Research on mayonnaise and salad dressing has also included a relaxation period. Peressini et al. (1998) allowed mayonnaise samples to rest 5 minutes after loading so that induced stress could relax. Muñoz and Sherman (1990) left mayonnaise and salad dressing samples to rest 20 minutes before running any tests to allow for relaxation and temperature equilibration. Hill et al (1995) and Elliott and Ganz (1977) allowed the samples to rest for 15 minutes before taking measurements. None of these studies provided any evidence to suggest that the normal force during loading was a problem or whether this time was sufficient for full relaxation. Nor did they give any justification for the length of time chosen, except that Muñoz and Sherman found that 20 minutes was needed for the sample to reach the desired temperature. Halliday and Smith (1995) also used a 20-minute period for equilibration of high temperature samples, but it appeared that temperature was the only concern, not normal force. Plus, no justification of the chosen equilibration time was given for either variable.

Other studies, while recognizing the possibility that normal force could be a factor, have opted not to examine stress relaxation (Casiraghi et al., 1985). Navickis and Bagley (1983) noted a systematic change in normal force during flow measurements, but concluded that the effects of that force have not been examined in detail and that further investigation of normal stresses is necessary before relaxation time prior to testing could be considered.

Other research, involving compressional studies, has suggested that allowing the sample to rest for a certain period of time would eliminate the effects of work stress. Navickis et al. (1982) compressed prepared dough between the discs to a thickness 1 mm greater than test thickness, and then allowed the sample to rest for 10 to 35 minutes so that the compressive force would reach an essentially constant value. The sample was then reduced to test thickness (usually 3.0 mm) and again allowed to rest for about 5 minutes until compressive stress decayed. However, Létang et al. (1999), also using dough for testing, reported that even after 2 hours rest at room temperature, no real stabilization of the dynamic parameters was obtained for the dough.

2.4.2.2 Lack of Conclusions Concerning Normal Force Introduction During Loading

Just as much confusion surrounds the question of normal force during loading and the relaxation time needed to eliminate this force as with the question of slip and its effects on rheological data, yet much less focus has been given to normal force. The main question has been left unanswered by previous research: does the introduction of normal force during loading influence the data collected in rheological testing? This question has not even been explored. Many researchers have used their own subjective intuition to assume that normal force does affect the resulting rheological data, and then attempted to eliminate the perceived problems associated with normal force. An important focus of this research question was to determine whether normal force applied during loading does affect data repeatability. This research was also designed to determine if any variability introduced by normal force during loading could be eliminated or reduced by allowing the sample to relax before testing.

2.4.3 Need for Basic Research to Help Alleviate Confusion Surrounding Potential Errors Associated with Oscillatory Rheological Research

Although many researchers have focused on oscillatory measurements of semi-solid foods, the validity of these studies could be in question due to the lack of basic research. An over-arching theme of these studies is that they make assumptions about the actual mechanics of oscillatory measurements, and about how semi-solid foods react to oscillatory measurements, that have neither been supported nor negated. These assumptions are claimed to correct for errors (i.e., sample migration and the effect of forces applied during loading, as detailed previously in Section 2.4) that many researchers foresee as potential problems. However, although the researchers acknowledge the potential for errors, many investigators have simply jumped into collecting data without eliminating these pitfalls. Many even believe that they have circumvented these problems by adding regiments to the procedure for collecting data (i.e., using adhesive to eliminate slip and allowing time for stress relaxation between loading and testing). Many of these solution regiments have been chosen at random or by the recommendation of a previously reported study; unfortunately, most often the regiment is lacking true validation or support. Recognizing that potential opportunities for introducing error in rheological measurements is not enough; research must be done to identify and quantify errors in hopes of finding, avoiding, or reducing such errors.

Furthermore, descriptions of how semi-solids with varying compositions react and relate to oscillatory measurements, especially in consideration of how composition interacts with potential errors, would be beneficial information to help rheologists collect more accurate oscillatory measurements. Rheologists have conducted research to compare different textures and compositions using oscillatory parameters (detailed in Section 2.3.2); however, little has been done to actually understand how differing compositions, such as low versus high-fat products, relate to data errors. It is conceivable that different compositions react differently to potential errors (such as slip or loading normal force) and could lead to the masking or enhancing of the effects of these errors. This lack of information based on composition of products prompted the integration of compositional effects into the design of this project.

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 EQUIPMENT DESCRIPTION AND CALIBRATION

A TA1000-N controlled stress/strain rheometer (TA Instruments Ltd., New Castle, DE) was used for all testing. Testing was conducted using two different geometries—a flat, smooth plate (TA #970923, TA Instruments Ltd., England) and a flat, serrated plate (TA #981397, TA Instruments Ltd., England)—depending on both the experiment being conducted and the sample being tested. Both geometries were made of stainless steel and had a 4-cm diameter. The serrated plate had a roughened surface that was created by 40 rows by 40 columns of 1-mm pyramidal teeth. The rheometer was fitted with a Peltier plate, which controlled the temperature of the lower plate and sample during testing. Rheology Advantage (TA Instruments Ltd., New Castle, DE) software was used for both instrument control and data acquisition.

Preparing the rheometer for data collection required many steps. First, because the rheometer is a stabilizing air bearing instrument, it was imperative to turn on and maintain the air supply at 30 psi. Second, once the geometry cap was removed and the rheometer was turned on, the system inertia was calibrated by allowing the geometry spindle (with no attached geometry) to rotate freely and then selecting the "calibrate inertia" function under the instrument menu. Inertia calibration values ranged from 14.83 to 16.82 $\mu Nm.s^2$. Calibration of instrument inertia is important for two reasons; it serves as a system check and also helps to provide better measurement accuracy. The system inertia should not change much with time, so the inertia value calculated during calibration should be compared with past values to assure that the system is not being compromised. Also, the system inertia and geometry inertia (discussed in the next paragraph) provide information about the actual torque application to the sample during testing. Ideally, the entire torque applied by a rheometer to a sample during testing would transfer only to the sample. However, due to the non-zero moments of inertia of both the rheometer spindle shaft and the testing geometry, portions of the applied torque are being used to move or stop both the rheometer spindle and geometry and are not being transferred to the actual sample. In order to accurately represent the actual torque applied to a sample, a correction factor is figured based on both the system and geometry and is applied automatically to the system calculations (TA Instruments, Software Manual, 2000).

Third, the appropriate geometry was attached, selected in the software, and mapped using the mapping function under the instrument menu. Mapping is the process by which the computer makes a digital image of the geometry's placement, storing information such as the exact location and tilt of the geometry for reference. It is important that mapping was done each time a geometry was attached to the rheometer. Mapping is not only important in order to gain parameters that relate to angular positioning of the geometry needed later for rheological calculations but it also helps correct for differences in torque application seen with each revolution of the rheometer's shaft. Baseline corrections of torque can be implemented automatically for the user based on a combination of the absolute angular position of the geometry measured by an optical encoder and the microprocessor control of the motor. After mapping, the geometry inertia was calibrated, with values ranging from 7.23 to 8.61 for the flat, smooth plate and 7.47 to 9.12 for the flat, serrated plate. Calibration of the geometry inertia was performed each time the geometry was attached to the rheometer.

The final step before testing was zeroing the gap, which included positioning the geometry 5-cm above the bottom plate and selecting "zero the gap." Permanent ink was used to trace an outline showing the exact placement of the upper geometry over the bottom plate in order to make sample placement on the lower plate more accurate.

3.2 Samples

3.2.1 Sample Description

To meet the objectives of this study, five products (Table 3.2-1) were selected and tested. These products included four semi-solid samples and one liquid sample. Sample selection was based on four criteria. First, due to the nature of the rheometer and oscillatory testing, it was necessary that the products were uniform. Uniformity included composition homogeneity throughout the product as well as constant physical attributes such as product thickness and surface smoothness. Second, food products with time-dependent rheological properties, such as cornstarch or yogurt, were not considered. Third, since the research objectives included focus on how sample composition related to the potential errors of rheological data, products of varying composition were needed. Compositional diversity was based on the fat content of a product; specifically, similar products with varying fat contents (low v. high) were selected. Finally, products were chosen so that testing parameters, such as the stress range applied during testing, could be identical for each product. For the products selected, torque ramps were conducted using the applied stress range of 0.1 to 1000 Pa.

TABLE 3.2-1. LIST AND DESCRIPTION OF PRODUCTS SELECTED AS TEST SAMPLES.

Product	Description	Fat Composition
Oscar Meyer Thin Sliced Bologna	Semi-Solid	High
Oscar Meyer Fat-Free Bologna	Semi-Solid	Low
Kraft Deli Style Cheddar Cheese	Semi-Solid	High
Sargento Pre-Sliced Mozzarella Cheese	Semi-Solid	Low
French's Yellow Mustard	Liquid	Extremely Low

3.2.2 Sample Acquisition and Storage

All samples were purchased at IGA or Albertson's grocery store in Stillwater, Oklahoma. For statistical reasons, two independent grocery stores were needed to ensure that four different lot numbers (independent preparations) of each product were available for testing. Immediately after purchase, all samples were organized and labeled for storage. Each product was separated into four distinct groups based on lot number. Each lot number was recorded and randomly assigned the number 1, 2, 3, or 4 (Table 3.2-2). Three slices from each individual lot number (1, 2, 3, or 4) of the semi-solid products were removed from the packaging and placed in labeled Zip-Loc® storage bags. Because both the regular and fat-free bologna samples were tested in three different experiments, this step was repeated three times for both bologna products with each bag additionally labeled to indicate whether it was for experiment I, II, or III. All semi-solid samples were stored lying flat in a walk-in cooler until testing took place (average temperature of 4°C). The unopened mustard jars were placed in cabinets at an average room temperature (69°C) until tested.

TABLE 3.2-2. PACKAGE LOT NUMBERS AND ASSIGNED LOT NUMBERS FOR ALL PRODUCTS.

Product	Package Lot #	Assigned Lot #
Oscar Meyer Thin Sliced Bologna	P-2310	1
	P-2312	2
	P-2316	3
	P-2315	4
Oscar Meyer Fat-Free Bologna	P-1446	1
	P-1447	2
	P-1449	3
	P-1450	4
Kraft Deli Style Cheddar Cheese	11May01-C	1
	03June01-C	2
	22June01-C	3
	05May-C	4
Sargento Pre-Sliced Mozzarella Cheese	07July01B	1
	07July01E	2
	08Aug01E	3
	08Aug01C	4
French's Yellow Mustard	M01053 1658	1
	M00315 1924	2
	M01024 2336	3
	M01054 0224	4

3.2.3 Sample Preparation for Testing

All semi-solid samples were removed from the cooler and allowed to equilibrate to room temperature for three hours before testing while remaining sealed within the plastic bags. After temperature equilibration, the slices were removed from each bag and randomly assigned a slice designation of A, B, or C. Using a stainless steel corer (4-cm inside diameter) manufactured at the Biosystems Engineering Laboratory (Oklahoma State University, Stillwater, OK), four 4-cm discs were randomly cut from each slice. Only two discs were needed for testing, but four were cut in case loading or testing errors occurred. All four discs for an individual slice were placed on a plastic plate, which was placed in a labeled bag designating both lot number (1, 2, 3, or 4) and slice letter (A, B, or C) and sealed until testing. Sample discs had to be placed on a plastic plate to ensure the surface of the sample was not disturbed by the plastic bag resting directly on top of the sample; the plastic plate kept the plastic bag elevated above the samples. Mustard samples were opened directly before the start of testing with no additional preparation needed. The mustard samples were not shaken or stirred before testing; however, a plastic spoon was inserted into the middle of the jar and brought to the surface when retrieving samples.

3.3 EXPERIMENTS

3.3.1 Overview

To answer the research objectives, three separate experimental plans were designed and executed. The first experiment (I) studied the influences of loading normal force on oscillatory measurements when testing directly after loading. The second experiment (II) evaluated the effectiveness of sample relaxation between the application of normal force during loading and testing in reducing the error introduced during loading. The third experiment (III) evaluated the advantages of using a serrated plate versus a smooth plate geometry.

3.3.2 Experiment I. Effects of Loading Normal Forces: No Relaxation

This experiment was organized in a split-plot structure in a complete random design with sub-samples at the whole plot level (Table 3.3-1). The treatment structure followed a 5 X 2 (composition X normal force application level) factorial design. All semi-solid products were tested with the application of a normal force of either 5 or 20 N during sample loading. The mustard samples were loaded to 1 or 5 N of normal force.

TABLE 3.3-1. SAMPLING DESIGN FOR TESTING EFFECTS OF NORMAL FORCES DURING LOADING.

Product	Lot ID	Testing Sample ID	Loading Normal Force (N)	Response [G' (Pa)]
Bologna Fat-Free Bologna Cheddar Cheese Mozzarella Cheese OR Mustard	1	A	5	
			20	
		B	5	
			20	
		C	5	
			20	
	2	A	5	
			20	
		B	5	
			20	
		C	5	
			20	
	3	A	5	
			20	
		B	5	
			20	
		C	5	
			20	
	4	A	5	
			20	
		B	5	
			20	
		C	5	
			20	

Sample loading involved three steps. The first step was to place the sample (a semi-solid sample disc or 1 teaspoon of mustard) directly on the rheometer's lower plate within the traced outline of the upper plate geometry. The second step was to move the geometry plate directly above, but not touching, the sample. The final step required turning on the rheometer's normal force control function in order to load the sample to 5.5 or 20.5 N. This function would move the geometry on top of the sample by decreasing the gap, which continued until it compressed the sample to the desired normal force. The rheometer was instructed to load to 0.5 N above the desired normal force in order for the sample to be loaded quickly to the desired normal force. If the sample was loaded directly to 5 or 20 N, the rheometer would compress the sample at a slower pace as it reached its end target normal force, which in return masked some of the effects this research was attempting to study. Instructing the rheometer to load above the actual normal force value allowed for a steady, consistent compression of the sample. Once the actual normal force was achieved, the normal force control function was stopped by selecting run and allowing the test procedure to begin. However, for the liquid mustard sample, loading required one more step. Once the sample had been compressed by the upper plate to the desired normal force, the excess mustard that had migrated from under the upper plate was trimmed with a plastic spatula, and then the test was started.

The testing procedure involved the application of a stress ramp (0.1 to 1000 Pa) with the following testing parameters: plate temperature of 25°C and oscillation frequency of 11 Hz. During this stress ramp the normal force was held constant at 0.5 N below the loading normal force (i.e., at 4.5 or 19.5 N), with a 1 N tolerance. This was accomplished by allowing the gap to increase and/or decrease by 1000 microns to adjust the normal force. Thirty testing points were recorded by the rheometer at an average of every 9 seconds.

The response variable sought was the storage modulus measurement associated with varying stress levels during the testing ramp. In order to calculate it, several parameters had to be measured or calculated and recorded by the rheometer. These parameters included torque (M), angular velocity (ω), angular displacement (L), and gap height (h). During the stress ramp, a varying level of torque is applied to the rheometer spindle shaft, which in return displaces the geometry and causes stress on the sample.

Parameters such as angular velocity, angular displacement, and gap height are detected through the use of sensors and recorded; others have to be calculated. At each torque level, a strain value can be calculated using the following equation:

$$\gamma = \gamma_o \sin(\omega t),$$

where the amplitude of the strain (γ_o) is calculated by L/h, which is the angular displacement of the geometry divided by the gap height between the two parallel plates and t is the time of the displacement. The phase angle (δ) can be also be calculated at each level of torque application through the equation:

$$\sigma (t) = \sigma_o \sin(\omega t + \delta),$$

where σ_o is the amplitude of the shear stress at time t and represents the maximum force applied to the sample. Finally, G' and G" can be calculated by the following equations:

$$G' = [\sigma_o/\gamma_o] \cos(\delta) \text{ and } G'' = [\sigma_o/\gamma_o] \sin(\delta).$$

3.3.3 Experiment II. Effects of Loading Normal Forces: Relaxation

Regular bologna was evaluated during this experiment. This was a one-way treatment structure with three levels (Table 3.3-2). The three treatments were: (1) testing with no relaxation; (2) testing after the sample relaxed to 10 N; and (3) testing after the sample relaxed to 1 N. All samples were loaded in the same manner described in Experiment I, except that all samples were loaded to 20 N. Also, the testing procedures for Experiment I and Experiment II were the same, with the exception that the testing procedure instructed the rheometer to wait until the sample relaxed to a specific normal force (i.e., no relaxation, relax to 10 N, and relax to 1 N) before running the stress ramp. Once the normal force had relaxed to the desired value, the test would hold the normal force at 0.5 N (+/- 0.25 N) below the relaxed force during testing. Twenty data points were collected throughout the stress ramp at an average of every 10 seconds.

TABLE 3.3-2. SAMPLING DESIGN FOR TESTING EFFECTS OF RELAXATION OF LOADING NORMAL FORCES.

Product	Lot ID	Slice ID	Testing Normal Force (N)	Response [G' (Pa)]
Semi-Solid Product Bologna	1	A	20	
			10	
			1	
		B	20	
			10	
			1	
		C	20	
			10	
			1	
	2	A	20	
			10	
			1	
		B	20	
			10	
			1	
		C	20	
			10	
			1	
	3	A	20	
			10	
			1	
		B	20	
			10	
			1	
		C	20	
			10	
			1	
	4	A	20	
			10	
			1	
		B	20	
			10	
			1	
		C	20	
			10	
			1	

3.3.4 Experiment III. Flat, Smooth Plate Versus Flat, Serrated Plate

Bologna and fat-free bologna were tested in Experiment III. The experimental design description in this experiment was a split-plot structure in a complete random design with a 2 X 2 factorial treatment (Table 3.3-3). The wholeplot treatment levels were the two bologna products, and the subplot treatment levels were the two different geometry plates used during testing: (1) the flat, smooth plate; and (2) the flat, serrated plate. Samples were loaded in the same manner as both Experiments I and II, with the exception that all samples were loaded to 15 N. Like Experiments I and II, a stress ramp (0.1 to 1000 Pa) was conducted with the same experimental parameters: plate temperature of 25°C, oscillation frequency of 11 Hz. The response variable measured was for the storage modulus (G') corresponding with the different oscillation stress levels during testing. Twenty data points were collected at an average of every 10 seconds during the stress ramp. It should be pointed out that measurement of G' for the samples tested with the serrated plate might lack the same accuracy of the G' values figured for the smooth plate samples due to the surface area change presented by the roughened surface of the serrated plate. The Rheology Advantage software does not offer any means of recognizing that a serrated plate is being used rather than a smooth plate and proceeds to base all calculations on the smooth plate geometry.

TABLE 3.3-3. SAMPLING DESIGN FOR TESTING EFFECTS OF PLATE TYPE AFTER LOADING TO A SPECIFIC NORMAL FORCE.

Product	Lot ID	Slice ID	Testing Plate Description	Response [G' or G" (Pa)]
Semi-Solid Product	1	A	smooth	
			serrated	
Bologna		B	smooth	
Fat Free Bologna			serrated	
		C	smooth	
			serrated	
	2	A	smooth	
			serrated	
		B	smooth	
			serrated	
		C	smooth	
			serrated	
	3	A	smooth	
			serrated	
		B	smooth	
			serrated	
		C	smooth	
			serrated	
	4	A	smooth	
			serrated	
		B	smooth	
			serrated	
		C	smooth	
			serrated	

3.4 CHEMICAL ANALYSIS

3.4.1 Sample Preparation

To insure a representative sample of each semi-solid product, three slices were used from five different packages of each food type. For each product, the chosen fifteen slices were diced into smaller pieces and combined into a mixing bowl. Portions of the diced pieces were placed into a Black and Decker Handy Chopper Plus® and ground for 45 seconds before being moved to a second mixing bowl. Once all portions of the diced slices were ground and combined together, sub-samples of this material were once again combined into the chopper and ground for another 30 seconds. For each product, all ground material was placed into an air-tight storage container and placed into the refrigerator until chemical analysis could be performed, approximately 12-24 hours later. Triplicates of each sample were analyzed for fat content.

3.4.2 Fat Analysis and Protein Analysis

Fat content was measured in bologna samples by soxhlet (AOAC, 960.39) and in cheese by acid hydrolysis mojonnier (AOAC, 933.05) extraction. For soxhlet analysis, all analysis were conducted using a Soxtec System 1043 Extraction Unit (Tecator, Saskatoon, Saskatchewan, Canada).

Preparation of the samples for protein analysis, specifically the digestion of the sample, was based on AOAC Official Method 928.08. This method was used for both meat and cheese and was followed strictly with the exception of using a selenium-based catalyst over the mercury-based catalyst. After sample digestion, the samples were analyzed using the Kjeltec Analyzer, which calculated all parameters and determined the percent protein based on the equation:

$$\% \text{ N} = [(\text{ml standard acid X normality acid}) - (\text{ml standard NaOH X normality NaOH})] \text{ X } \frac{(1.4007)}{\text{g sample}}$$

Most food proteins contain 16% Nitrogen; therefore, a factor 6.25 (100/16) can be used to convert percent Nitrogen to percent protein (% N X 6.25 = % protein).

3.4.3 Confirmation of Product Labeling

Determination of fat content was conducted for products in order to confirm packaging labels of each product. These products were chosen on the basis of representing high and low-fat products and it was imperative that there was confirmation of the actual fat content. Protein analysis was done to give a better description of the product. Validity of product labels was confirmed and the results of both fat and protein content are shown in Table 3.4-1 and Table 3.4-2.

TABLE 3.4-1. PACKAGE AND LAB ANALYSIS VALUES FOR FAT (GRAMS PER SLICE) OF SEMI-SOLID PRODUCTS.

Product	Package: Fat g / Slice	Lab Analysis: Fat g / Slice
Oscar Meyer Thin Sliced Bologna	8	7
Oscar Meyer Fat-Free Bologna	0	0.016
Kraft Deli Style Cheddar Cheese	10	9.7
Sargento Pre-Sliced Mozzarella Cheese	4.5	4.7

TABLE 3.4-2. LAB ANALYSIS VALUES FOR PERCENT PROTEIN OF SEMI-SOLID PRODUCTS.

Product	Protein (%)
Oscar Meyer Thin Sliced Bologna	11.39
Oscar Meyer Fat-Free Bologna	12.24
Kraft Deli Style Cheddar Cheese	23.17
Sargento Pre-Sliced Mozzarella Cheese	26.04

3.5 STATISTICAL ANALYSIS

Each experiment (I, II, III) was analyzed to answer questions about the effects of the individual treatments on the storage and loss moduli measurements. Three markers of the data curves were pre-selected before testing. These markers were: (1) the initial G' or G" collected; (2) the average G' or G" collected; and (3) the slope value between 370 and 690 Pa (for Experiments I and II) or the slope value between 200 and 500 Pa (for Experiment III). The range of the slopes calculated for each experiment was determined based on the criteria of equilibrium. Most G' data curves stabilized within a range of corresponding oscillation stress during testing, and slope ranges were chosen to include stress values after this equilibration of the G' curves.

The statistical analysis of Experiments I, II, and III was done using the MIXED procedure of SAS® Version 8.1 (SAS Institute, 1999). Specifically, Restricted Maximum Likelihood (REML) methods were used to estimate variances due to lots within products, subsampling (slice within lot), and experimental error of the rheometer operation. Main effects and interaction of product and normal force were evaluated in Experiments I and II, and product effects were evaluated in Experiment I. In Experiment I, meat and cheese effects were compared, and the interaction of normal force with each type of product was tested. In Experiment III, the interaction of plate type with each type of bologna was tested. Appropriate multiple comparisons were made using contrasts or means comparisons without Type I error modification (LSD for pair-wise comparisons). All tests were conducted at the 0.05 level of significance. Examples of SAS codes and examples of SAS output for all three experiments can be found in Appendix B and C, respectively.

CHAPTER 4: PRESENTATION AND DISCUSSION OF RESULTS

4.1 OVERVIEW

The results of each of the three experiments are presented in this chapter. Briefly, it was discovered that: (1) normal force applied during loading does affect oscillatory data; (2) relaxation time between loading and testing neither eliminates nor reduces the effects of normal force introduced during loading; and (3) the serrated plate is effective in minimizing the effects of slip, although normal force still impacts data repeatability.

4.2 EXPERIMENT I. EFFECTS OF LOADING NORMAL FORCE: NO RELAXATION

This experiment addressed the question of whether loading normal force influences oscillatory measurements of G' and G". Samples of five products (bologna, fat-free bologna, cheddar cheese, mozzarella cheese, and mustard) were loaded to either 5 or 20 N and tested at 4.5 or 19.5 N of normal force, respectively. Generally, greater normal force application during loading resulted in greater magnitudes of initial and average G' and G" values throughout testing (Tables 4.2-1 and 4.2-2).

TABLE 4.2-1. MINIMUM, MAXIMUM, AND MEAN INITIAL G' VALUES OF ALL SEMI-SOLID SAMPLES LOADED TO 5 AND 20 N.

	Initial G' (@ 105 Pa)		
	Minimum	**Maximum**	**Mean**
Bologna			
Loaded to 5N	19680	29080	24480
Loaded to 20N	25770	39980	32120
Fat-Free Bologna			
Loaded to 5N	21660	31650	27330
Loaded to 20N	30980	36160	32210
Cheddar Cheese			
Loaded to 5N	47330	66230	57730
Loaded to 20N	52740	67470	61210
Mozzarella Cheese			
Loaded to 5N	47300	58780	51590
Loaded to 20N	51870	68790	61660

TABLE 4.2-2. MINIMUM, MAXIMUM, AND MEAN AVERAGE G' VALUES OF ALL SEMI-SOLID SAMPLES LOADED TO 5 AND 20 N.

	Average G' Values		
	Minimum	Maximum	Mean
Bologna			
Loaded to 5N	15126	22924	19124
Loaded to 20N	23293	36410	29153
Fat-Free Bologna			
Loaded to 5N	19359	29657	24926
Loaded to 20N	29773	35574	31920
Cheddar Cheese			
Loaded to 5N	46164	64249	55350
Loaded to 20N	52039	64166	56737
Mozzarella Cheese			
Loaded to 5N	42704	52340	46768
Loaded to 20N	46328	62283	55363

4.2.1 Regular and Fat-Free Bologna

Response Variable Initial G'

The strongest example of higher G' values corresponding to greater loading normal force application was observed for both bologna products. Predominantly for the bologna (Figure 4.2-1) and almost without exception for the fat-free bologna (Figure 4.2-2), the samples loaded to 20 N showed a greater initial G' value. Table 4.2.1 shows the range and mean initial G' values for all semi-solid products. The initial G' values of bologna loaded to 20 N ranged from 25,770 to 39,980 Pa, with a mean initial G' value of 32,120 Pa (Table 4.2-1). By comparison, the range of initial G' values of bologna samples loaded to 5N was 19,680 to 29,080 Pa, with a mean initial G' value of 24,480 Pa. The initial G' value for bologna samples loaded to 20 N exceeded the initial value for samples loaded to 5 N by nearly 8,000 Pa. These differences in initial G' data between the samples loaded to 5 and 20 N were significant (Table 4.2-3).

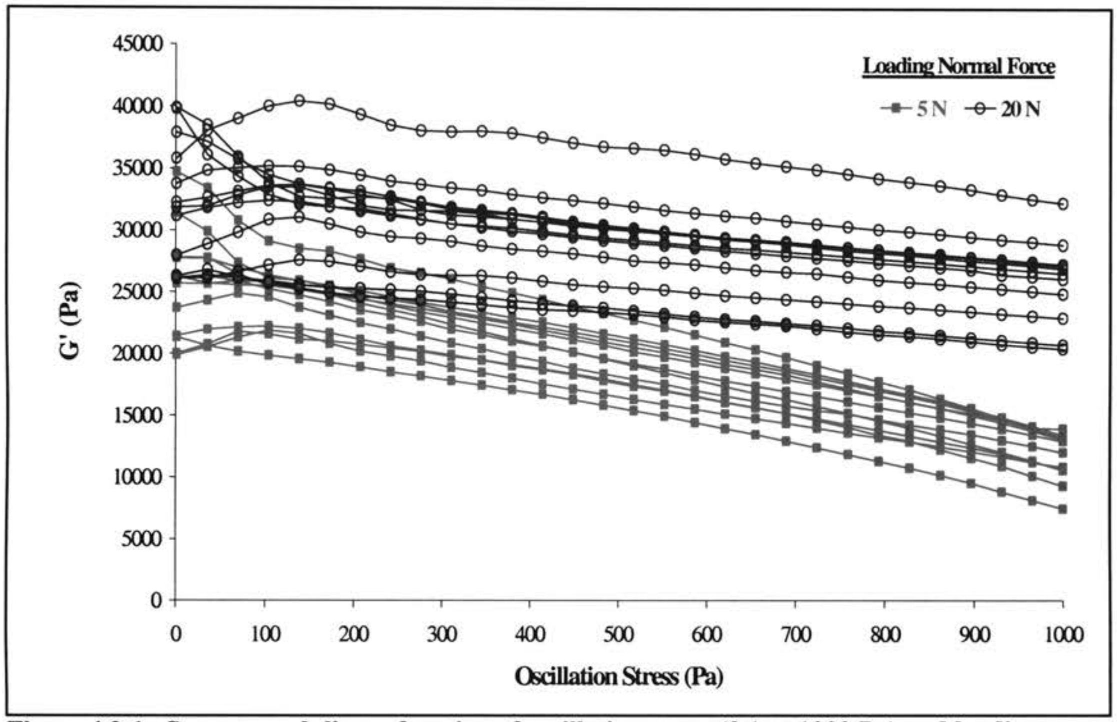

Figure 4.2-1. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and loading normal force (5 or 20 N) of bologna.

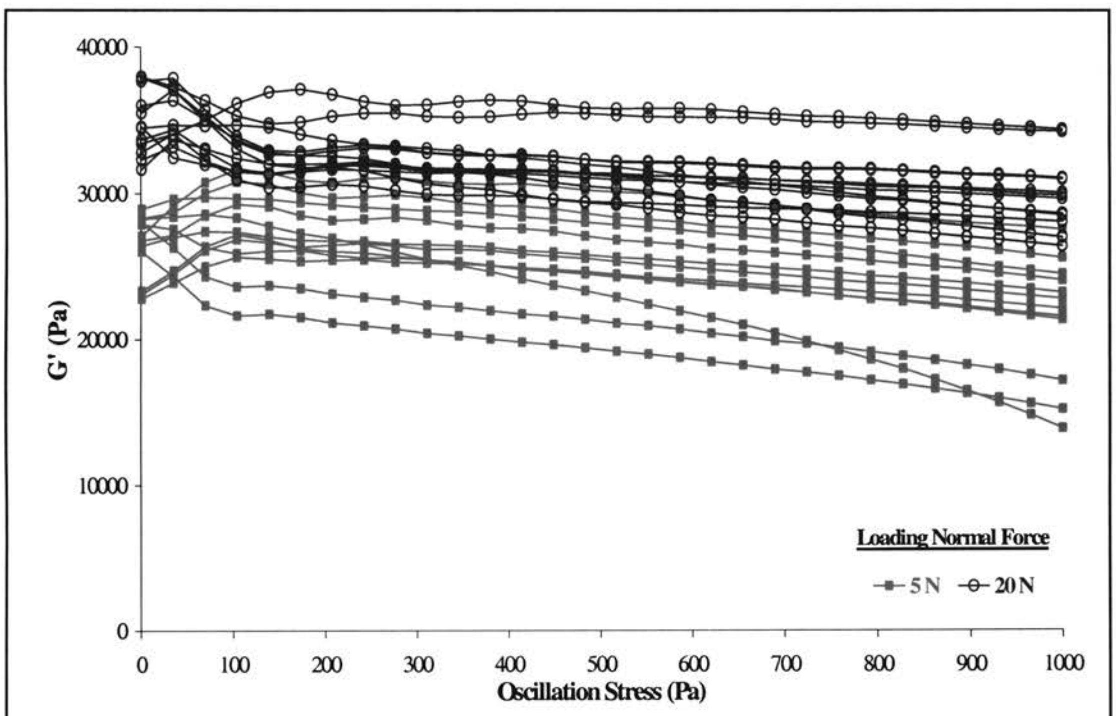

Figure 4.2-2. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and loading normal force (5 or 20 N) of fat-free bologna.

TABLE 4.2-3. STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR INITIAL G' VALUES BASED ON BOLOGNA TYPE AND NORMAL FORCE IN EXPERIMENT I.

Effect	t Value	Pr > |t|	Results of Test
Bologna: 5 vs. 20 N Normal Force	6.26	<0.0001	Significant
Fat-Free Bologna: 5 vs. 20 N Normal Force	4.82	<0.0001	Significant
5 N Normal Force: Bologna vs. Fat-Free Bologna	-1.23	0.2358	Not Significant
20 N Normal Force: Bologna vs. Fat-Free Bologna	-0.47	0.6435	Not Significant

When compared to regular bologna samples, the fat-free bologna followed this trend more consistently, with almost all samples loaded to 20 N corresponding to higher G' values than the samples loaded to 5 N. It can be seen in Figure 4.2-2 that although all the curves have the same basic shape, the curves representing loading to a 5 N normal force (shown in red) fall below the curves created for samples loaded to 20 N before testing (shown in blue). The mean initial G' value of the fat-free bologna samples loaded to 20 N (32,210 Pa) was approximately 5,000 Pa greater than the G' value of samples loaded to 5 N (27,330 Pa) (Table 4.2-1). As with the bologna samples, differences in initial G' data between the fat-free bologna samples loaded to 5 and 20 N were significant (Table 4.2-3).

Response Variable Average G'

Another way of looking at the G' curves is to compare the average G' values throughout the entire stress range. The same trend is true for both bologna products when considering the average G' values: the samples loaded to 20 N remained greater throughout testing, as demonstrated in Figures 4.2-1 and 4.2-2. For both regular and fat-free bologna, the average value of the G' curves for the 20 N samples (blue) are greater than the 5 N (red) curves. Average G' values are therefore higher for the samples loaded to 20 N than those for samples loaded to 5 N. For both bologna products, differences in average G' data based on loading normal force were significant (Table 4.2-4).

TABLE 4.2-4. STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR AVERAGE G' VALUES BASED ON BOLOGNA TYPE AND NORMAL FORCE IN EXPERIMENT I .

Effect	t Value	Pr > |t|	Results of Test
Bologna: 5 vs. 20 N Normal Force	8.49	<0.0001	Significant
Fat-Free Bologna: 5 vs. 20 N Normal Force	5.92	<0.0001	Significant
5 N Normal Force: Bologna vs. Fat-Free Bologna	-2.62	0.0184	Significant
20 N Normal Force: Bologna vs. Fat-Free Bologna	-1.25	0.2293	Not Significant

Response Variables Initial and Average G" (Loss Modulus)

Similar curves for G" data (Figures 4.2-3 and 4.2-4) also depict greater initial G" values for samples loaded to the higher normal force of 20 N. Normal force for all semi-solid samples was significant ($F_{1,88}$ = 117.71, $p < 0.0001$) when considering both initial (Table 4.2-5) and average (Table 4.2-6) G" responses. For all products tested, trends of G' and G" are very similar. Since data are often reported in terms of G' and because the analysis of G' can represent the behavior of both parameters, only G' data are discussed in further sections. However, G" data can be found in Appendix D for consultation.

TABLE 4.2-5 STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR INITIAL G" VALUES BASED ON PRODUCT TYPE AND NORMAL FORCE IN EXPERIMENT I.

Effect	t Value	Pr > |t|	Results of Test
Bologna: 5 vs. 20 N Normal Force	3.07	<0.0037	Significant
Fat-Free Bologna: 5 vs. 20 N Normal Force	3.42	<0.0001	Significant
Cheddar: 5 vs. 20 N Normal Force	3.36	<0.0016	Significant
Mozzarella: 5 vs. 20 N Normal Force	8.75	<0.0001	Significant

TABLE 4.2-6 STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR AVERAGE G" VALUES BASED ON PRODUCT TYPE AND NORMAL FORCE IN EXPERIMENT I.

Effect	t Value	Pr > |t|	Results of Test
Bologna: 5 vs. 20 N Normal Force	8.74	<0.0001	Significant
Fat-Free Bologna: 5 vs. 20 N Normal Force	6.10	<0.0001	Significant
Cheddar: 5 vs. 20 N Normal Force	7.08	<0.0001	Significant
Mozzarella: 5 vs. 20 N Normal Force	6.72	<0.0001	Significant

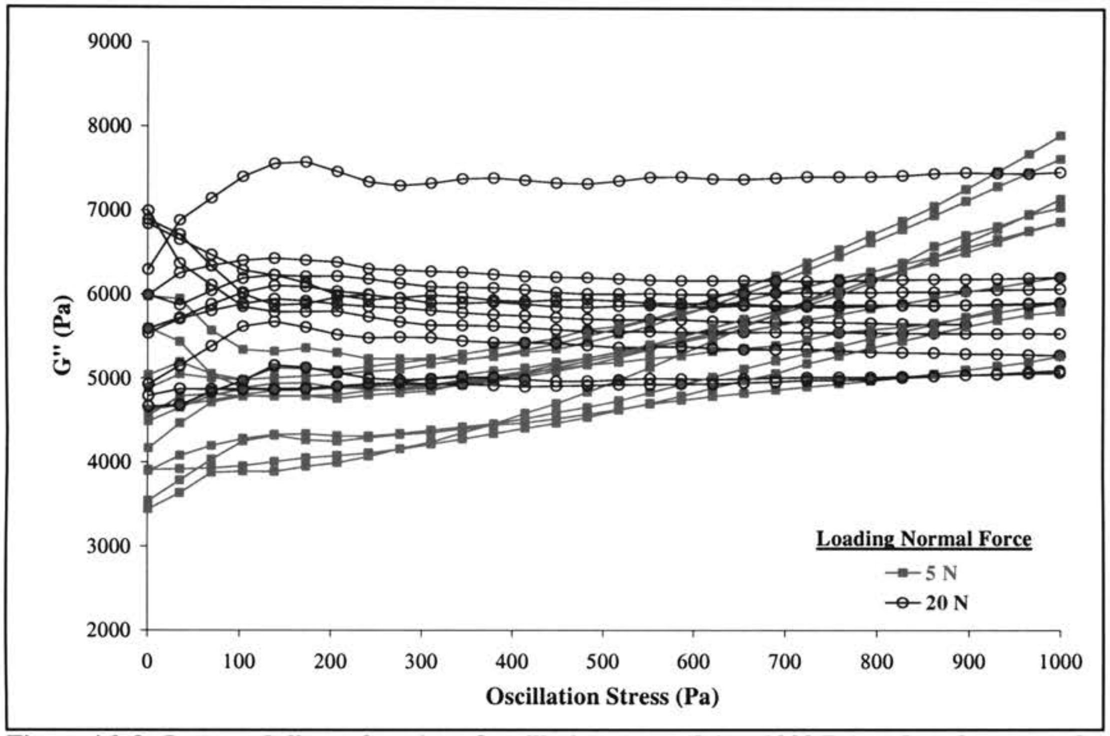

Figure 4.2-3. Loss moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing normal force (5 or 20 N) of regular bologna samples.

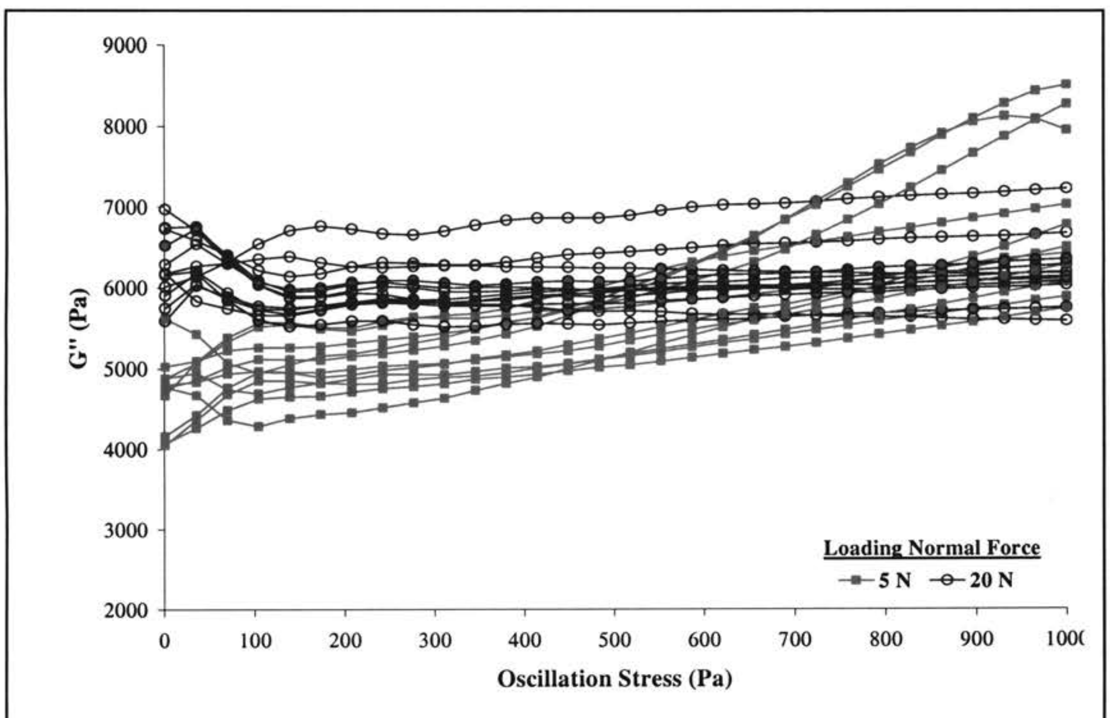

Figure 4.2-4. Loss moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing normal force (5 or 20 N) of fat-free bologna samples.

Response Variable Slope of G'

Another observation can be made when comparing loading normal force of the two bologna products: the slope of the G' curve changed depending on normal force (Figures 4.2-5 and 4.2-6). Specifically, for the regular bologna (Figure 4.2-1), the slopes of G' curves from samples loaded to 20 N were less steep than for those samples loaded to 5 N. The samples loaded to 20 N show an average slope of –6.39 (Table 4.2-7). Whereas, the G' curves of the 5 N samples have an average slope of –13.54, an approximate difference of 53 percent. Slopes for curves of both products were based on oscillation stress values of 350 to 760 Pa. In Figure 4.2-5, the slope of each sample tested is displayed, and it is clear that samples tested at 5 N show a steeper slope than samples tested at 20 N normal force.

TABLE 4.2-7. MINIMUM, MAXIMUM, AND MEAN SLOPE VALUES OF ALL SEMI-SOLID SAMPLES LOADED TO 5 AND 20 N.

	Slope (345-760 Pa)		
	Minimum	Maximum	Mean
Bologna			
Loaded to 5N	-11.69	-16.92	-13.54
Loaded to 20N	-5.09	-8.26	-6.39
Fat-Free Bologna			
Loaded to 5N	-4.37	-13.80	-6.16
Loaded to 20N	-1.09	-6.89	-3.61
Cheddar Cheese			
Loaded to 5N	-5.80	-11.66	-7.76
Loaded to 20N	-5.10	-9.40	-6.76
Mozzarella Cheese			
Loaded to 5N	-6.43	-10.32	-8.19
Loaded to 20N	-4.93	-14.40	-8.16

There was a significant interaction between loading normal force and product ($F_{1,88} = 30.66$, $p < 0.0001$) when considering the slope of G' curves. Comparisons among products of the same type or among the same level of normal force were

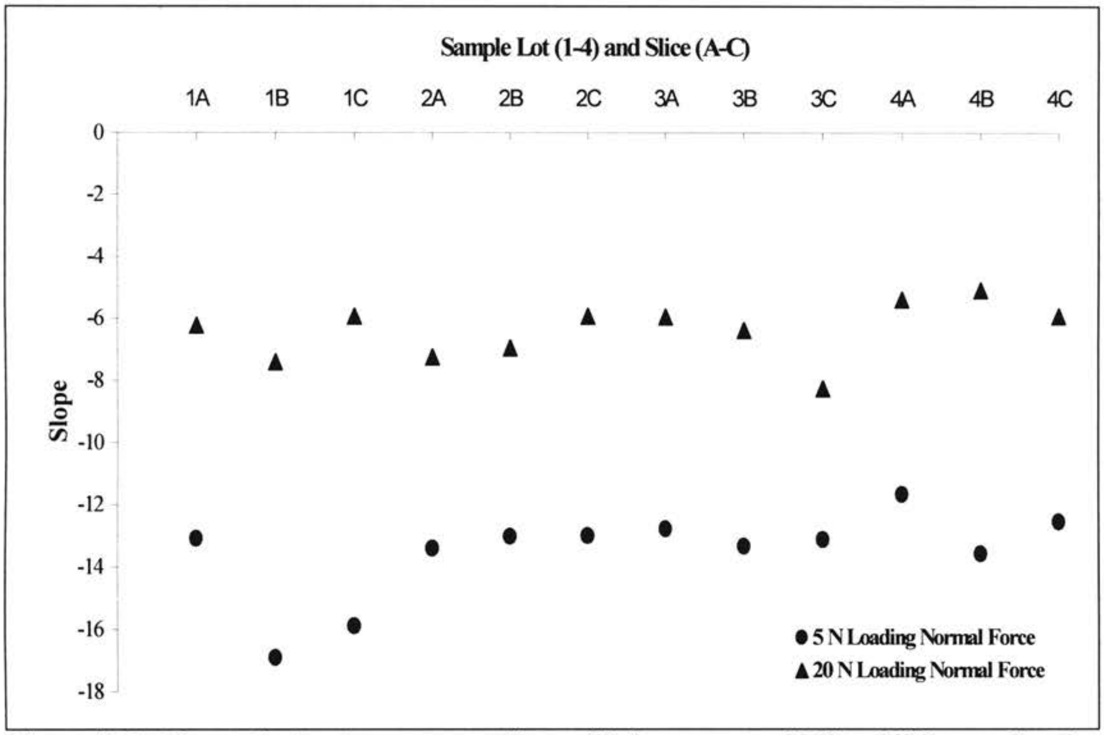

Figure 4.2-5. Slope calculated at corresponding oscillation stresses of 350 to 760 Pa as a function of sample lot (1-4) and slice (A-C) and loading normal force (5 or 20N) for bologna.

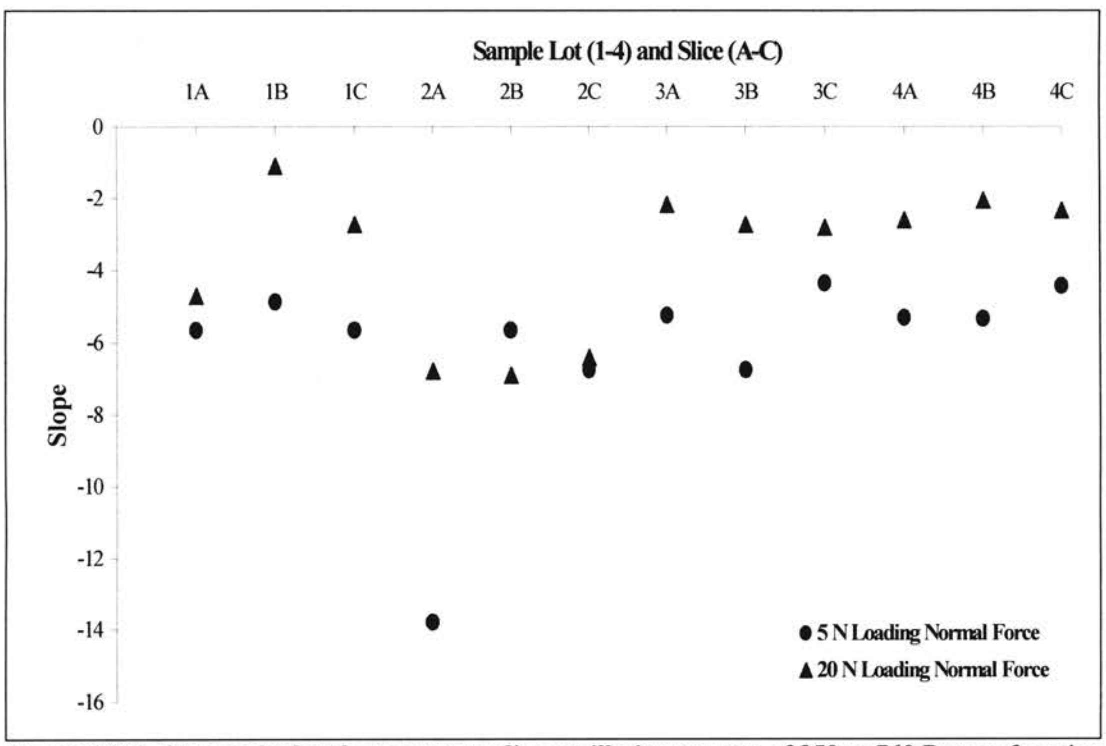

Figure 4.2-6. Slope calculated at corresponding oscillation stresses of 350 to 760 Pa as a function of sample lot (1-4) and slice (A-C) and loading normal force (5 or 20N) for fat-free bologna.

performed. There is a significant difference in slopes of G' curves based on whether the bologna samples ($t_{44} = 12.55$, $p < 0.0001$) are loaded to 5 or 20 N.

Similar trends in slope of G' are also apparent, but less dramatic, for the fat-free bologna curves (Figure 4.2-6). There is marginal slope difference, approximately 41 percent, between the samples loaded to 5 and 20 N for these samples, with the averages reported as –6.16 and –3.61, respectively (Table 4.2-7). There was a significant difference in slopes of fat-free bologna G' curves when loaded to 5 or 20 N ($t_{44} = 4.48$, $p < 0.0001$). Also, there is more variability (less consistent curve pattern) in the slopes of the curves for both loading normal forces when compared to the regular bologna samples, especially for the samples loaded to 20 N.

Linear Regression

In addition to recognizing that loading normal force significantly influenced storage and loss moduli behavior, it would be helpful if the G' response to various loading normal forces could be predicted through mathematical equations. Being able to predict how G' reacts to loading forces could prompt correction factors to remove error from measurements mathematically. Examples of such equations have been developed for the response variable of average G' versus loading. Plots of average G' values versus loading normal force are shown for bologna and fat-free bologna in Figures 4.2-7 and 4.2-8, respectively. A linear regression line has been fitted for these plots for both products. The regression equations for bologna and fat-free bologna are:

Bologna: average G' = 688(normal force) + 15,781
Fat-Free Bologna: average G' = 467(normal force) + 22,595

The R^2 value for bologna was 0.74 and the R^2 value for fat-free bologna was 0.69. This project only evaluated the effects of two loading normal forces. Consequently, the

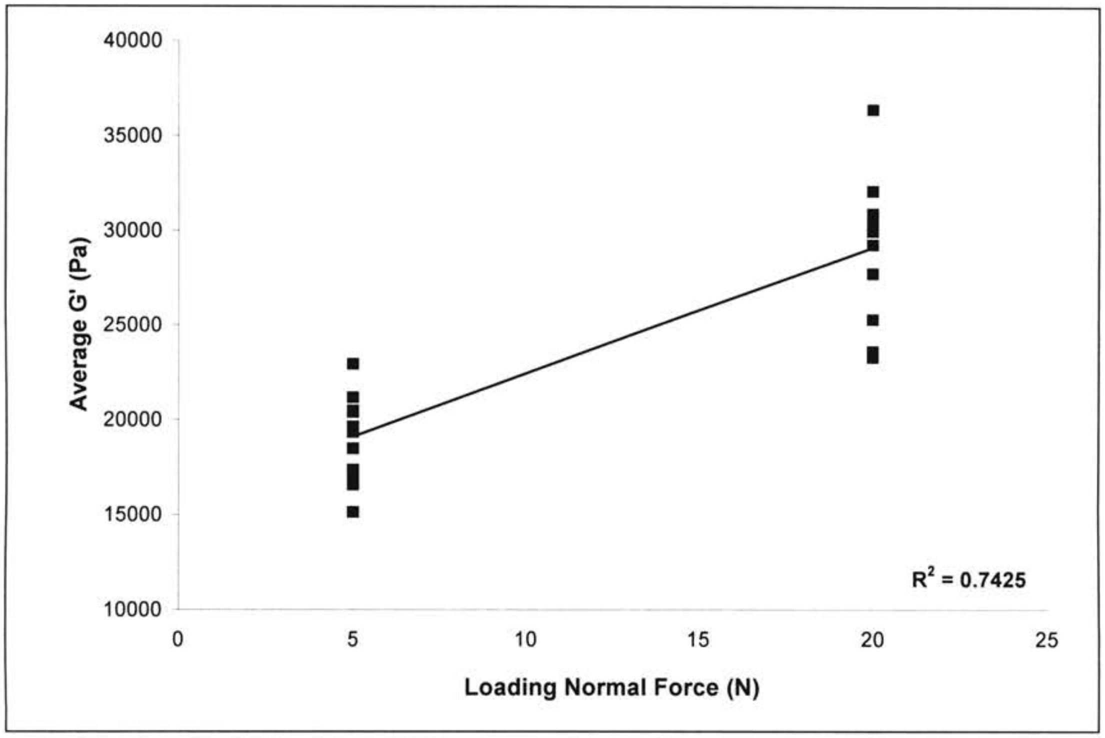

Figure 4.2-7. Regression modeling of average G' plotted as a function of loading normal force (5 vs. 20 N) for regular bologna.

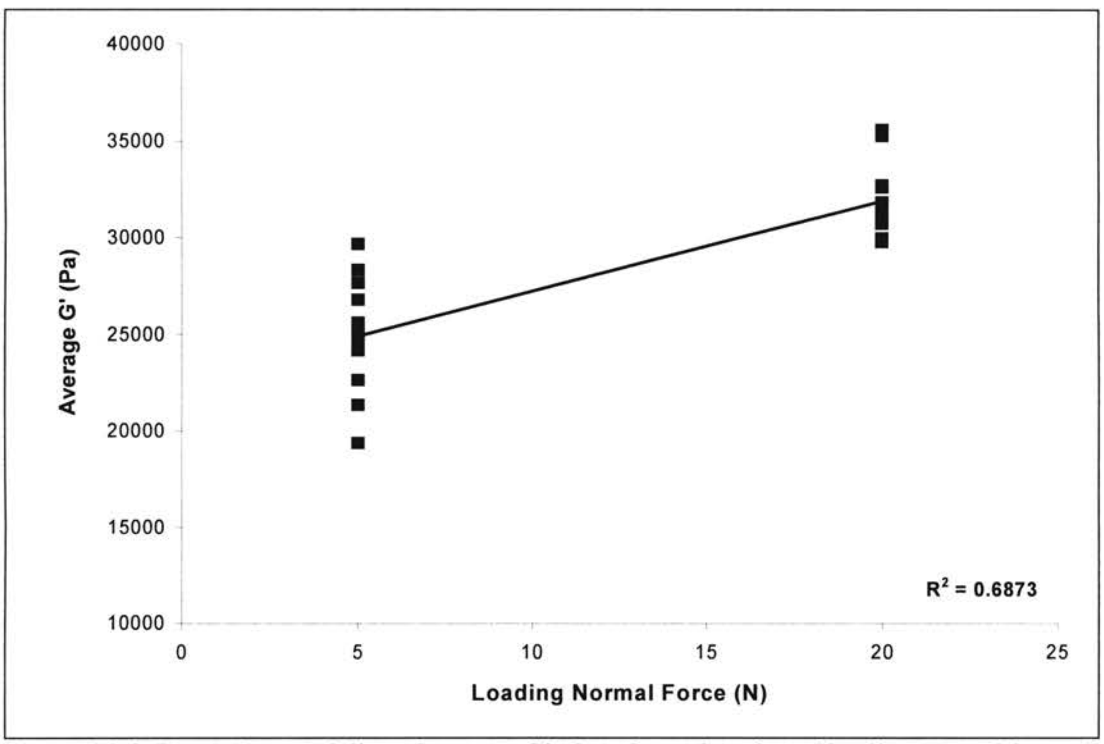

Figure 4.2-8. Regression modeling of average G' plotted as a function of loading normal force (5 vs. 20 N) for fat-free bologna.

regression equations for these plots are based solely on these two points, and hence, the nature of the trend cannot be described. It was only assumed that the relationship between loading normal force and resulting G' values was linear. In order to confirm the linearity, other loading normal forces would need to be evaluated and plotted.

Although limited, examples of linear regression were discussed here to introduce the possibility of predicting G' response to normal force. If a linear response of G' values to loading normal force were confirmed, it would also be possible to predict the G' value of samples if testing could be conducted at 0 N loading normal force (in essence, yielding a "true" or "apparent" G' value independent of the effects of loading normal force).

4.2.2 Cheddar and Mozzarella Cheese

Response Variable Initial G'

The response trends for initial G' of mozzarella and cheddar cheese were very similar to those exhibited for bologna (Figures 4.2-9 and 4.2-10). Mozzarella samples loaded to 20 N showed greater G' values than samples loaded to 5 N normal force (Figure 4.2-9), which was also depicted for the bologna and fat-free bologna samples in Figures 4.2-1 and 4.2-2. The initial G' values for mozzarella loaded to 20 N ranged from 51,870 to 68,790 Pa compared to the initial G' values for the samples loaded to 5 N, which ranged from 47,300 to 58,780 Pa (Table 4.2-1). The mean initial G' value for the samples loaded to 20 N (61,660 Pa) was more than 10,000 Pa greater than the mean initial G' value for the samples loaded to 5 N (51,590 Pa). For initial G', there were significant differences between mozzarella samples loaded to either 5 or 20 N (Table 4.2-8).

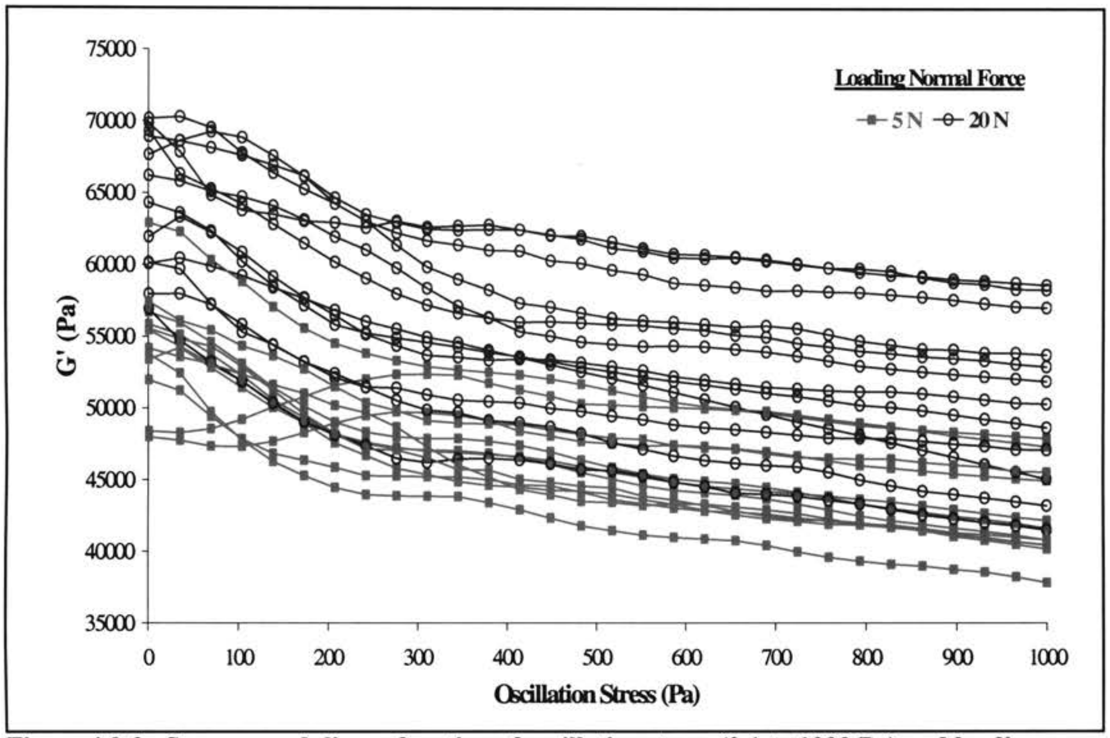

Figure 4.2-9. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and loading normal force (5 or 20 N) of mozzarella cheese samples.

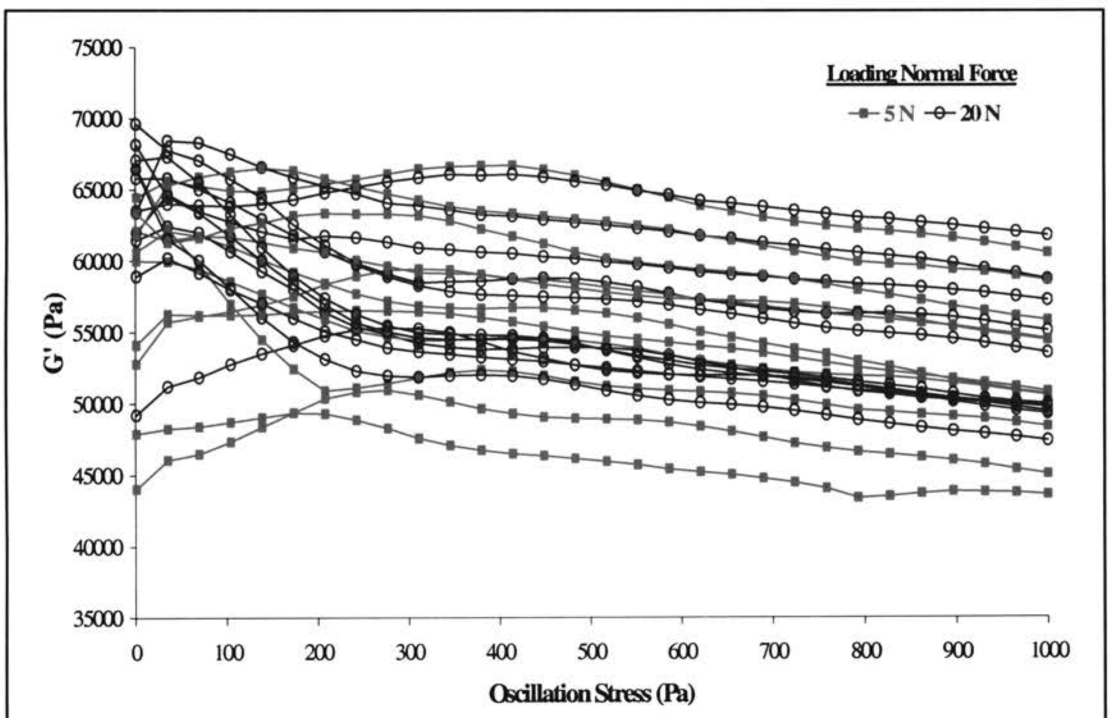

Figure 4.2-10. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and loading normal force (5 or 20 N) of cheddar cheese samples.

TABLE 4.2-8. STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR INITIAL G' VALUES BASED ON CHEESE TYPE AND NORMAL FORCE IN EXPERIMENT I.

Effect	t Value	Pr > |t|	Results of Test
Cheddar: 5 vs. 20 N Normal Force	2.86	0.0053	Significant
Mozzarella: 5 vs. 20 N Normal Force	8.26	<0.0001	Significant
5 N Normal Force: Cheddar vs. Mozzarella	2.66	0.0171	Significant
20 N Normal Force: Cheddar vs. Mozzarella	-0.19	0.8492	Not Significant

Loading normal force appeared to affect the cheddar cheese samples less predictably than the other products (Figure 4.2-10). There is no clear distinction between the samples based on loading normal force as seen in the mozzarella and bologna samples. However, there was a significant difference in initial G' values based on loading normal force (Table 4-2.8). Initial G' ranges also suggest that loading normal force influences the G' curves of the cheddar samples. The mean initial G' value for cheddar samples loaded to 20 N (61,210 Pa) was approximately 3500 Pa greater than for samples loaded to only 5 N (57,730 Pa), a trend observed for all products (Table 4.2-1).

Response Variable Average G'

The average G' values for mozzarella generally remained higher throughout testing for samples loaded to 20 N than for those loaded to 5 N. As seen in Figure 4.2-9, the 20 N curves (blue) are generally seen above the 5 N curves (red), although this trend is not as evident as for the bologna and fat-free bologna (Section 4.2.1). For the mozzarella, there were significant differences in average G' between samples loaded to either 5 or 20 N (Table 4.2-9). As suggested by the curves for the cheddar samples (Figure 4.2-10), average G' values of cheddar were not significantly affected by normal force during loading (Table 4.2-9). For the cheddar, the 20 N (blue) and 5 N (red) curves generally cross each other throughout testing.

TABLE 4.2-9. STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR AVERAGE G' VALUES BASED ON CHEESE TYPE AND NORMAL FORCE IN EXPERIMENT I.

Effect	t Value	Pr > |t|	Results of Test
Cheddar: 5 vs. 20 N Normal Force	1.17	0.2436	Not Significant
Mozzarella: 5 vs. 20 N Normal Force	7.28	<0.0001	Significant
5 N Normal Force: Cheddar vs. Mozzarella	3.87	0.0013	Significant
20 N Normal Force: Cheddar vs. Mozzarella	0.62	0.5438	Not Significant

Response Variable Slope of G'

Mozzarella samples could not be separated based on normal force and slope; there was no coherent pattern in behavior of the curves when comparing the slopes of the 5 N and 20 N curves (Figure 4.2-11). Likewise for the cheddar, no clear pattern was observed comparing the slope values of the 5 and 20 N curves (Figure 4.2-12).

4.2.3 Mustard

Mustard samples (Figure 4.2-13) showed greater G' values for samples loaded to greater normal force, but this trend is less dramatic for the mustard samples than the semi-solid samples. Since mustard is a liquid product, it was not possible to apply the same forces as applied to the other product. The molecular structure of a liquid allows for the particles of the product to slide over one another when pressed upon, which ultimately allowed the sample to flow outside the perimeter of the testing plates. It was thus expected that mustard would show less response to normal force application than the semi-solid products.

Statistical analysis verified that the liquid product, mustard, responded to normal force quite differently than the semi-solid products. There was a significant difference when comparing mustard against all the semi-solid products with respect to initial G' values ($F_{1,20.2} = 538.71$, $p < 0.0001$). However, there was not a significant difference in G' value based on different loading normal forces ($t_{95} = -0.05$, $p=0.9640$).

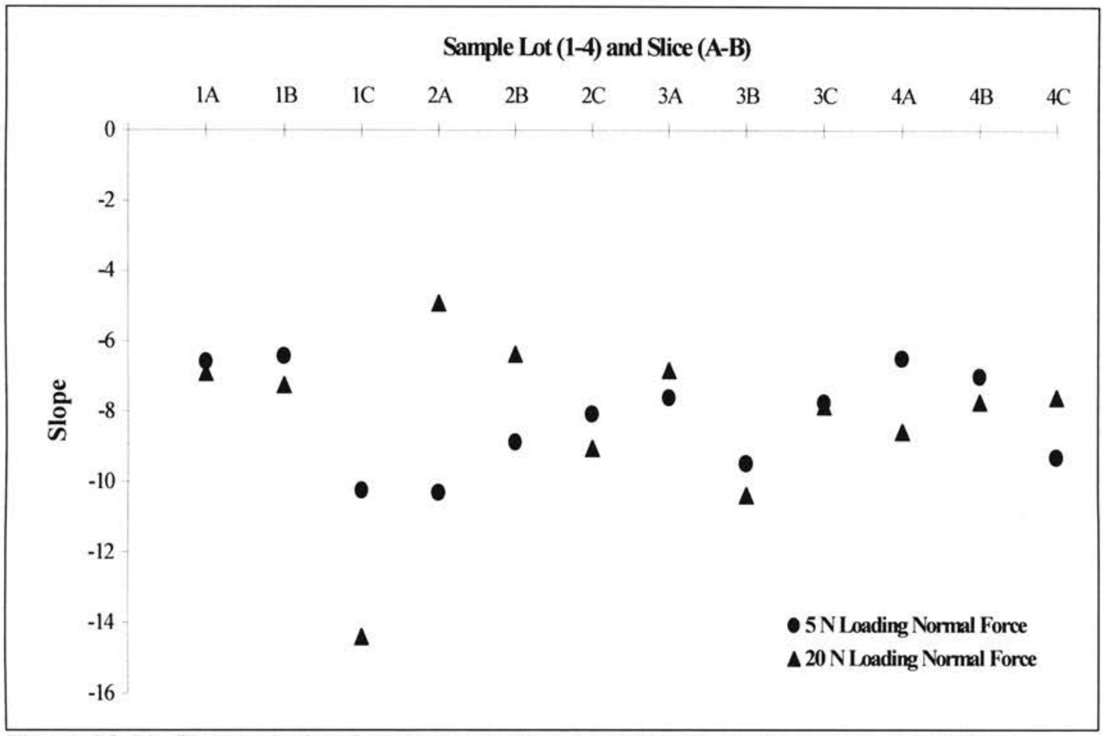

Figure 4.2-11. Slope calculated at corresponding oscillation stresses of 350 to 760 Pa as a function of sample lot (1-4) and slice (A-C) and loading normal force (5 or 20N) of mozzarella cheese samples.

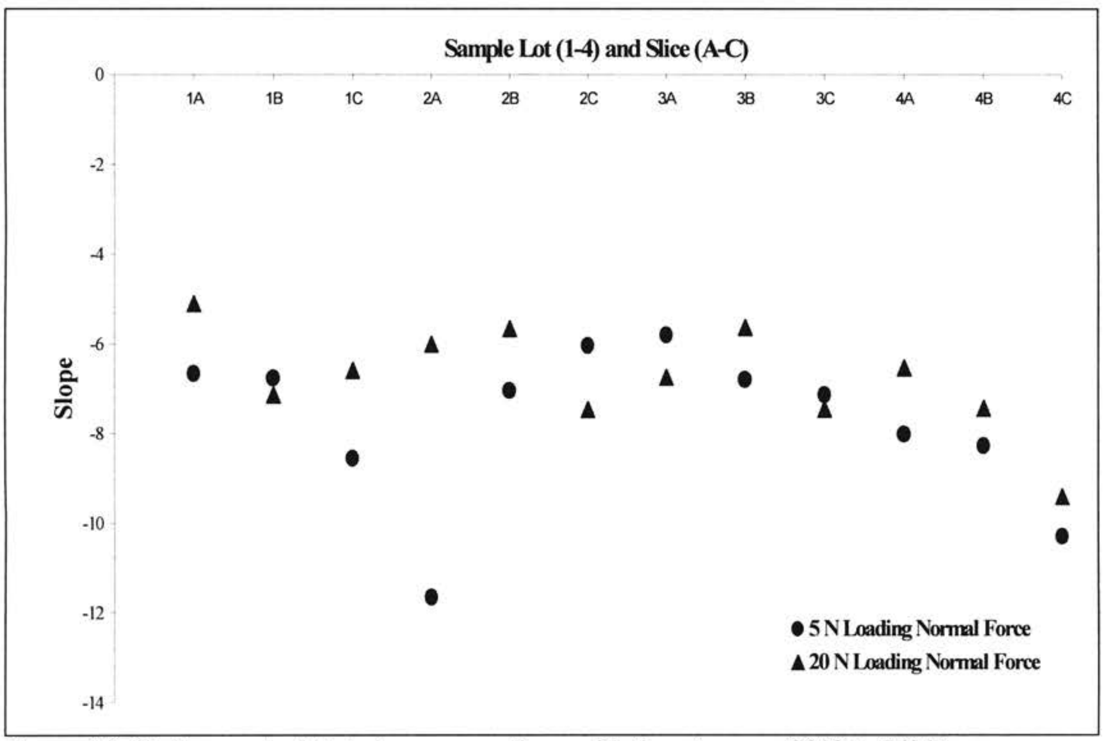

Figure 4.2-12. Slope calculated at corresponding oscillation stresses of 350 to 760 Pa as a function of sample lot (1-4) and slice (A-C) and loading normal force (5 or 20N) for cheddar cheese samples.

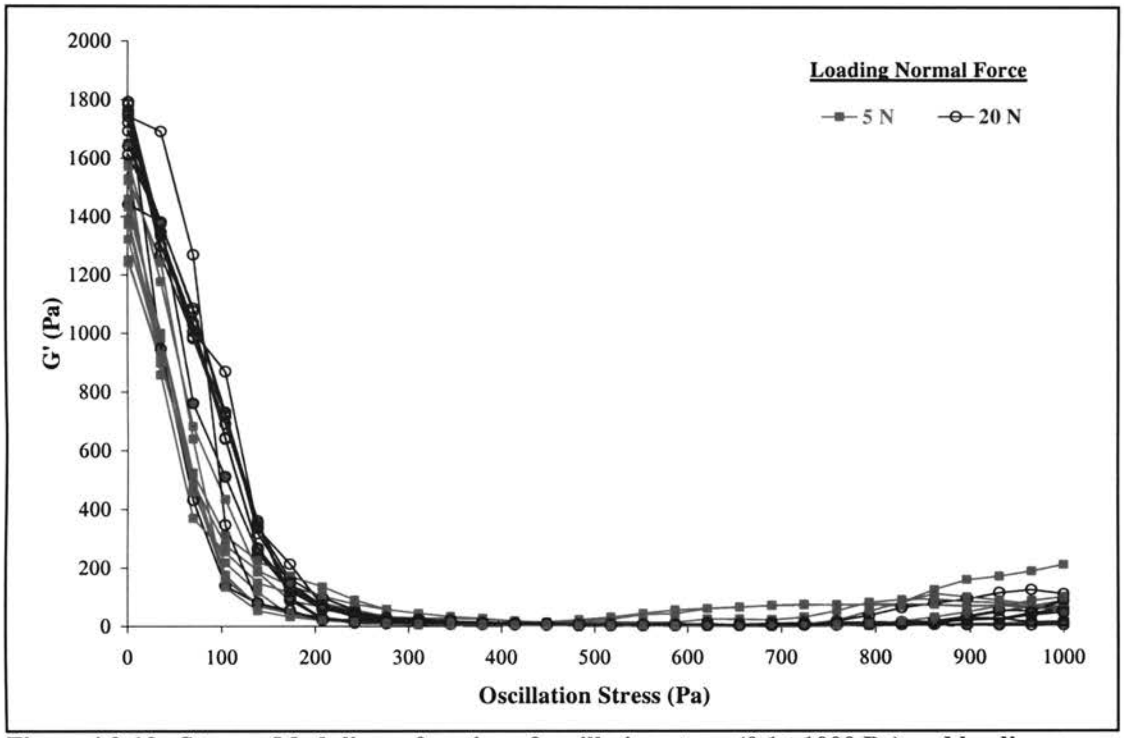

Figure 4.2-13. Storage Moduli as a function of oscillation stress (0.1 t 1000 Pa) and loading normal force (5 or 20 N) of mustard.

4.2.4 Compositional Effects

Similar trends were observed for both the bologna and the fat-free bologna products. This suggests that composition (or percent fat) affects G' data less dramatically than does loading normal force. This conclusion is supported by the statistical findings. When considering initial G' values, there is no significant difference between bologna and fat-free bologna loaded to either 5 or 20 N (Table 4.2-3). There was no significant difference in average G' values between the bologna and fat-free bologna loaded to 20 N (Table 4.2-4). The only significant difference between bologna and fat-free bologna was observed for average G' values of samples loaded to 5 N (Table 4.2-4) and for G' slopes loaded to both 5 and 20N ($t_{18.8} = -8.36$, $p < 0.0001$; $t_{18.8} = -3.15$, $p = 0.0053$). Generally, this demonstrates that both high and low-fat samples respond similarly to the application of different normal forces during loading, with the exception of the slopes of the curves.

The trends were not as consistent for the cheese samples. The mozzarella and cheddar samples did not respond in the same manner to greater normal force during loading, with the mozzarella behaving more like the bologna products. By contrast, the cheddar samples were similar to the other semi-solid products only in that slightly higher initial G' values were seen for samples loaded to 20 N. There were significant differences between the mozzarella and cheddar samples loaded to 5 N for both initial and average G' (Tables 4.2-8 and 4.2-9), but not for either initial or average G' of the mozzarella and cheddar samples loaded to 20 N.

It should be noted that making comparisons based on fat content is more appropriate for the regular and fat-free bologna because they are essentially the same products with two different levels of fat. However, the cheddar and mozzarella samples are two entirely different types of cheese products, which also contain different levels of fat, and direct comparisons of these products could be based on many factors rather than fat content only.

Additionally, the varying rheological behaviors and responses to normal force during loading are probably more related to textural differences among the products, rather than being due to varying compositional properties. To support this idea, a texture profile analysis (TPA) was conducted on the cheese samples thought the means of a Texture Analyzer. Using a 1.7 cm corer, three samples (1.27 cm in height) of each cheese type were cut and tested using an acrylic cylinder probe (2.5 cm) by compressing the cylindrical samples to 75% of their height. It was found through TPA of the cheese samples using a texture analyzer that mozzarella is substantially more springy, cohesive, and harder than the cheddar samples (Table 4.2-10). These differences in texture are probably stronger evidence of why cheddar and mozzarella react to loading normal force in different manners than the fat content of cheddar being greater than mozzarella.

TABLE 4.2-10. TEXTURE PROFILE ANALYSIS FOR CHEDDAR AND MOZZARELLA CHEESE.

	Springiness	Cohesiveness	Hardness
Cheddar	0.440	0.204	6,126
Mozzarella	0.694	0.323	13,635

4.2.5 Summary of General Observed Trends

Among all semi-solid products, normal force significantly affected both initial values ($F_{1,88}$ = 123.24, $p < 0.0001$) and average G' values ($F_{1,88}$ = 130.64, $p < 0.0001$), thus supporting the observed trend of higher initial and average G' values with greater loading normal force. This trend was seen, to varying degrees, for all products, with cheddar cheese showing the least dramatic adherence to the pattern.

4.2.6 Explanation of Trends

Explanation for higher loading normal forces corresponding to greater storage moduli must be provided on a molecular level. Considering what a greater normal force during loading means to a semi-solid food helps justify this trend. The greater the normal force during loading, the more compact the sample becomes due to the properties of a semi-solid food. When subjected to a 90-degree force, the sample will respond in two directions: both pushing its molecules outwards to relieve pressure and also forcing the molecules closer together. Due to the nature of a 90-degree force, the molecules will move closer together to a greater extent than they will move outward, thus creating a compact sample. The more compact the sample becomes, the more it will mimic solid-like behavior. Since the storage modulus (G') is the measure of the solid component of a product, it is logical that G' will increase with greater applied force. This also explains why mustard showed much less response to normal force application; the molecules of the liquid product flow over one another to a higher degree instead of being moved closer together, yielding only minimal differences in compactness.

Explanation of why G" also followed the trend of higher G" values associated with greater normal force during loading must also be addressed on a molecular perspective. Since G' is the measure of solid-like behavior and G" is the measure of liquid-like behavior, it seems easy to view G' and G" as opposite measures that should respond in opposite manners to the same stimulus. For example, in this experiment, it would seem that G" values should decrease with greater application of normal force during loading since G' increases with the same applied force. However, these two parameters cannot be explained as opposite measures of one another just because one describes the solid nature of a product and the other the liquid behavior of a product. G" is actually the measurement of the product's ability to lose energy rather than to store energy. By this definition, it is logical that G" values increase as greater normal force is applied during loading. It was explained that G' values were greater for samples loaded to higher normal forces and that this is due to the compacting of molecules into a more dense, solid sample as more energy was transferred through normal force application. G" values also increase due to the increased transfer of energy into the food product simply due to the idea that more energy transfer means there is more energy to be lost also, thus increasing G" values. It is not necessarily that the sample could be considered more liquid-like with the application of greater normal force. It is that more energy is transferred to the product, which in return allows for more loss of energy to be measured in terms of G".

Slope differences, particularly between the bologna and fat-free bologna, were also important trends observed during Experiment I. Textural differences between bologna and fat-free bologna, due to differences in fat content, are most likely the basis for this diverse slope. As discussed earlier, bologna samples exhibit a greater slope, suggesting that bologna samples respond to changes in stress more readily than fat-free bologna samples. Fat-free samples exhibit more solid-like characteristics. Because the molecules move more freely in the bologna, due to the additional fat content, the bologna is more susceptible to changes in G' values throughout testing.

There were also slope differences observed for all the products between the samples loaded to 5 N and those loaded to 20 N. Samples loaded to 5 N showed greater slopes, suggesting that these samples were more sensitive to changes in shear stress than samples loaded to 20 N. It would seem that the increased force of 20 N applied to a sample would create greater slope differences. When considered on a molecular level, this trend is more easily explained. It could be that samples loaded to 20 N have experienced such a dramatic change due to stress that they are no longer susceptible to great slope changes as increased stress is applied throughout testing. Applying a 20 N force could compact the molecules of the sample so intensely that the additional applied shear stress has only a limited impact. In contrast, the 5 N samples were subjected to a force which is not nearly as dramatic as the 20 N force. Therefore, the 5 N samples have not been compacted as densely as had the 20 N samples. This could allow for more changes throughout testing as the less-severe stress continues to be applied.

4.3 EXPERIMENT II. EFFECTS OF LOADING NORMAL FORCE: RELAXATION

Regular bologna samples were loaded to an initial normal force of 20 N before testing. The samples were subsequently either tested immediately (i.e., no relaxation) or allowed to relax until a desired normal force was achieved (i.e., relaxation to 10 N and relaxation to 1 N). Samples were allowed to relax in order to address the research question of whether relaxation of the sample prior to testing eliminates the impact of initial loading normal force. Only one semi-solid product was analyzed in Experiment II; it was not anticipated that normal force or relaxation would play an important role for liquid samples. As discussed in Section 4.2.4 above, the mustard samples did not show effects based upon normal force during loading; therefore, relaxation would not be a relevant consideration.

Previous rheology literature confirms that many researchers have suspected variability could be introduced if measures were not taken to control normal force during loading. Based on this theory, the methodology for rheological testing often included relaxation of the sample before testing (usually for 15 minutes) to correct for any variability caused by normal force during loading. However, the data collected in this experiment for bologna samples indicated that relaxation is not a complete remedy for variability caused during loading.

Response Variables Initial and Average G'

The first observation from the data collected is that relaxation of normal force before testing did not eliminate the effects of 20 N normal force on bologna samples as seen in Experiment I (Section 4.2) (Figures 4.3-1 and 4.3-2). The plot of G' versus the oscillation stress of samples tested at 10 N and 20 N (Figure 4.3-1) illustrates this finding. Samples tested immediately at 20 N and those tested after relaxation to 10 N of normal force showed similar curves, with all the initial G' values ranging between 28,160 and 44,950 Pa (Table 4.3-1) and all average G' values ranging from 23,390 to 39,990 (Table 4.3-2). Relaxation to 10 N occurred on average 15 minutes after loading (Table 4.3-3). Results for samples allowed to relax for 15 minutes showed little difference in initial G' values (35,245 Pa on average for samples tested immediately; 34,525 Pa for those allowed to rest to 10 N) from samples loaded and tested immediately.

TABLE 4.3-1. MINIMUM, MAXIMUM, AND MEAN INITIAL G' VALUES FOR EXPERIMENTS I AND II.

Experiment Number	Description of Testing	Minimum Initial G' (Pa)	Maximum Initial G' (Pa)	Mean Initial G' (Pa)
I	Loaded to 5N; No Relaxation	19,890	34,620	24,480
I	Loaded to 20N; No Relaxation	26,120	39,880	32,120
II	Loaded to 20N; No Relaxation	28,340	40,690	35,245
II	Loaded to 20N; Relaxed to 10N	28,160	44,950	34,525
II	Loaded to 20N; Relaxed to 1N	28,470	53,500	37,260

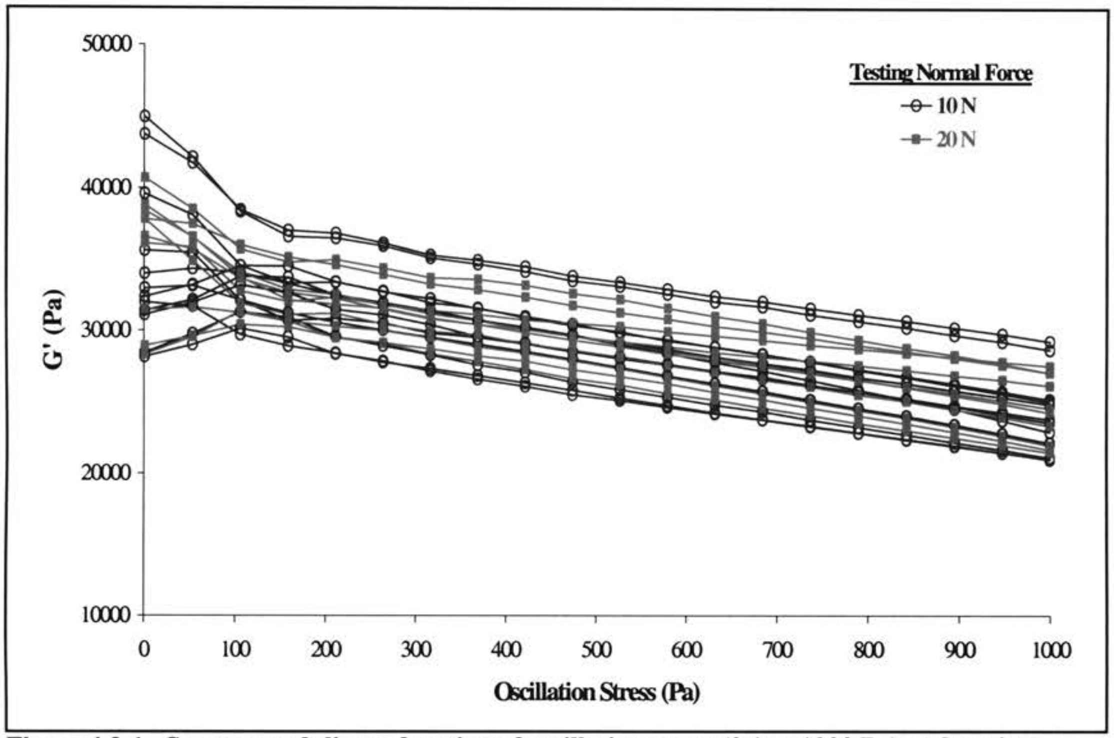

Figure 4.3-1. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing normal force (10 or 20 N) of regular bologna samples loaded to 20N and tested immediately or allowed to relax.

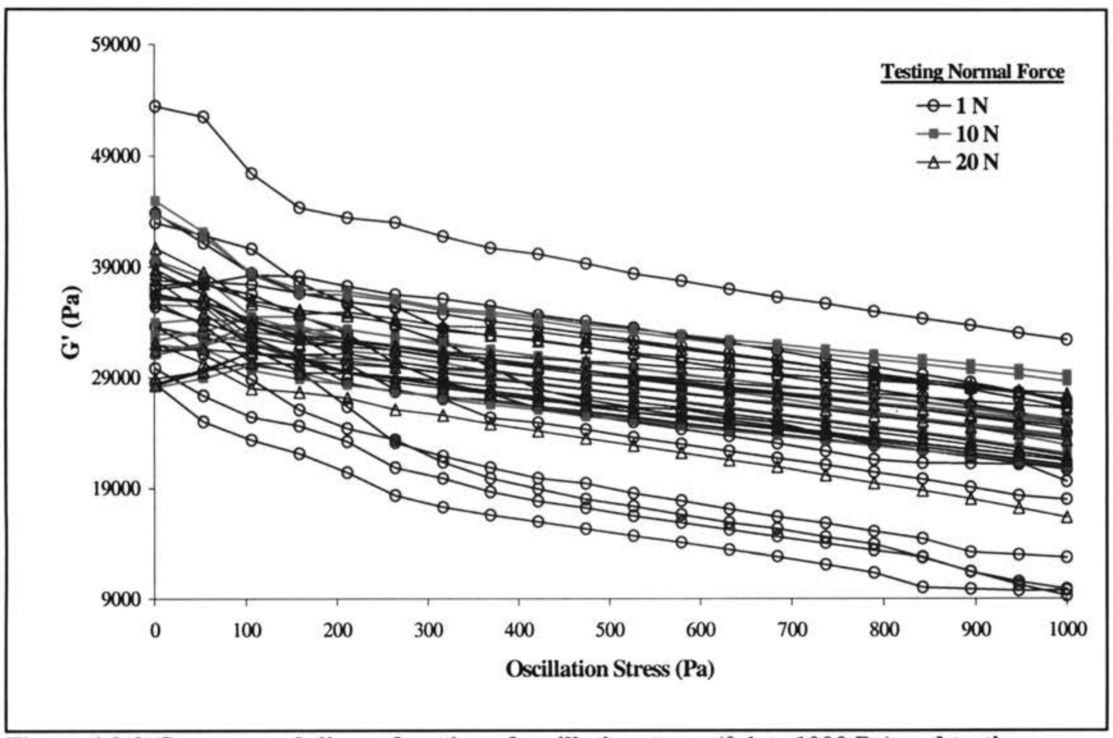

Figure 4.3-2. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing normal force (1, 10 or 20 N) of regular bologna samples loaded to 20N and tested immediately or allowed to relax.

TABLE 4.3-2. MINIMUM AND MAXIMUM AVERAGE G' VALUES FOR EXPERIMENTS I AND II.

Experiment Number	Description of Testing	Minimum Average G' (Pa)	Maximum Average G' (Pa)
I	Loaded to 5N; No Relaxation	15,130	22,920
I	Loaded to 20N; No Relaxation	22,160	35,030
II	Loaded to 20N; No Relaxation	23,390	39,990
II	Loaded to 20N; Relaxed to 10N	25,460	34,300
II	Loaded to 20N; Relaxed to 1N	16,080	32,430

TABLE 4.3-3. AVERAGE TIME REQUIRED FOR SAMPLES TO RELAX.

Relaxation Treatment Applied	Average Relaxation Time
No Relaxation	None
Relaxation to 10N Before Testing	15 minutes*
Relaxation to 1N Before Testing	3.5 hours**

* Note: Average relaxation time based on only 10 samples.

** Note: Average relaxation time based on only 7 samples.

Comparing G' curves of samples allowed to relax to 1 N to samples relaxed less or not at all also confirms the observation that relaxation did not eliminate the effects of loading normal force. Figure 4.3-2 compares the G' values versus the oscillation stress for the samples tested at all three testing normal forces (1, 10, and 20 N). Again, when looking at initial and average G' values for all the curves, most are similar, even with three different relaxation treatments represented.

Relationship of Experiment I and Experiment II Data

Further support that relaxation does not reverse or eliminate the effects of loading normal force can be seen when comparing data collected for bologna samples from Experiment I. Table 4.3-1 shows the initial G' ranges for samples in Experiment I tested at 5 and 20 N and for all those in Experiment II.

All samples loaded to 20 N showed similar minimum, maximum, and mean initial and average G' values, regardless of relaxation history. In contrast, the range and mean of Experiment I samples loaded to 5 N (from 19,890 to 34,620 Pa; 24,480 Pa mean) were considerably lower than corresponding ranges and averages of all samples loaded to 20 N (from 26,120 to 39,880 Pa; 32,120 Pa mean). Samples loaded to 20 N and tested at relaxed forces of 1 or 10 N had significantly higher initial G' ranges than the samples loaded to 5 N in Experiment I. The mean initial G' value was 37,620 Pa for samples allowed to relax to 1 N in Experiment II and 34,525 Pa for samples relaxing to 10 N. Samples loaded and tested at 5 N in Experiment I had a mean initial G' value of 24,480 Pa (more than 10,000 Pa less than those in Experiment II). If relaxation truly acts as a counter balance to the effects of normal force during loading, there should be a clear distinction between initial G' values for the samples loaded to 20 N and allowed to relax and the samples loaded to 20 N and tested immediately.

Specifically, it would be expected that initial G' values of samples allowed to relax from the 20 N introduced during loading to 10 or 1 N would approach the lower G' values seen for the samples loaded to 5 N in Experiment I. Because the samples allowed to relax maintained initial G' values much closer to the 20 N samples, it could be interpreted that sample relaxation does not reduce or mask influences of 20 N loading normal force on regular bologna samples. On a molecular level, this interpretation demonstrates that the changes to the sample introduced by loading normal force are of some permanence; time is not altering these effects, nor is the alleviation of nearly all the force introduced during loading. The finding that the bologna samples undergo a permanent change during loading is of great significance because it shows that solutions previously offered to eliminate or minimize data variability, such as relaxation, may not be effective. It also stresses the need to treat samples identically during loading, including controlling the normal force associated with loading. Otherwise, individual samples might be permanently altered before testing can occur.

Results of this experiment should be kept within the context of the product tested (regular bologna) and the choice of loading normal force (20 N). Both of the factors are important and different products or loading normal forces might produce different relaxation behavior.

Effect on Variability

A second observation of the data further discourages the use of relaxation as a solution to data variability when testing regular bologna. Greater relaxation time (i.e., 3.5 hours on average for the sample to relax to 1 N) may increase the variability of G' curves. Figure 4.3-2, discussed above, shows the G' values versus the oscillation stress of all the samples in Experiment II. Comparison of the curves shows that the samples allowed to relax to 1 N appear to have a greater variance of G' values; these curves, although having G' values in the same range of the other samples, are more spread out and varied than other samples. Specifically, the range of initial G' values for samples relaxed to 1 N was 28,470 to 53,500 Pa, nearly 10,000 Pa greater than the range for samples relaxed to 10 N (28,160 to 44,950 Pa).

A plot of initial G' values (@ 1.0 Pa oscillation stress) versus the three different testing normal forces also illustrates that greater relaxation time may introduce greater variability (Figure 4.3-3). The range of the initial G' values for each testing normal force was similar, but the samples tested at 1 N showed the greatest range and most variability, whereas the samples tested immediately without relaxation have the smallest range and the least variability. It should be noted that each testing normal force is represented by the same number of samples (12), even though some forces appear to have fewer samples in Figure 4.3-3. For example, there seems to be fewer samples tested at 20 N, but in reality all 12 samples are plotted, on top of each other in some cases. The actual ranges of G' also support this observation (Table 4.3-1). The minimum initial G' values for samples at all three testing normal forces are similar, but the maximum value increases with greater relaxation time.

Response Variable Slope of G'

Comparing the G' curves for all samples in Experiment II provides further support for this observation. The slopes of the G' curves (Figure 4.3-2) are different based upon relaxation time. Samples tested at 10 and 20 N have similar slopes, but steeper slopes are shown for the samples relaxed to 1 N of normal force before testing. Plots of the testing normal force versus the slope comparing each of the relaxation treatments (Figure 4.3-4, 1 N v. 20 N; Figure 4.3-5, 1 N v. 10 N; Figure 4.3-6, 10 N v. 20 N) show that the slopes are steeper at lower testing normal forces.

Explanation of Findings

A plausible reason why relaxation leads to greater variation of the data involves the type of geometry (serrated plate) used during testing. The serrated plate has metal cleats that bear into the semi-solid food samples. Once the bologna samples were loaded to 20 N, the gap of the geometry did not change. As the sample relaxed, if the geometry did not relax uniformly across its surface, the cleats of the plate could be pressing deeper into some parts of the sample and less into other parts. If this occurred, it would mimic the testing of samples without a smooth surface and hence could offer variable results. The texture of bologna is softer than some other semi-solid products such as cheese; these

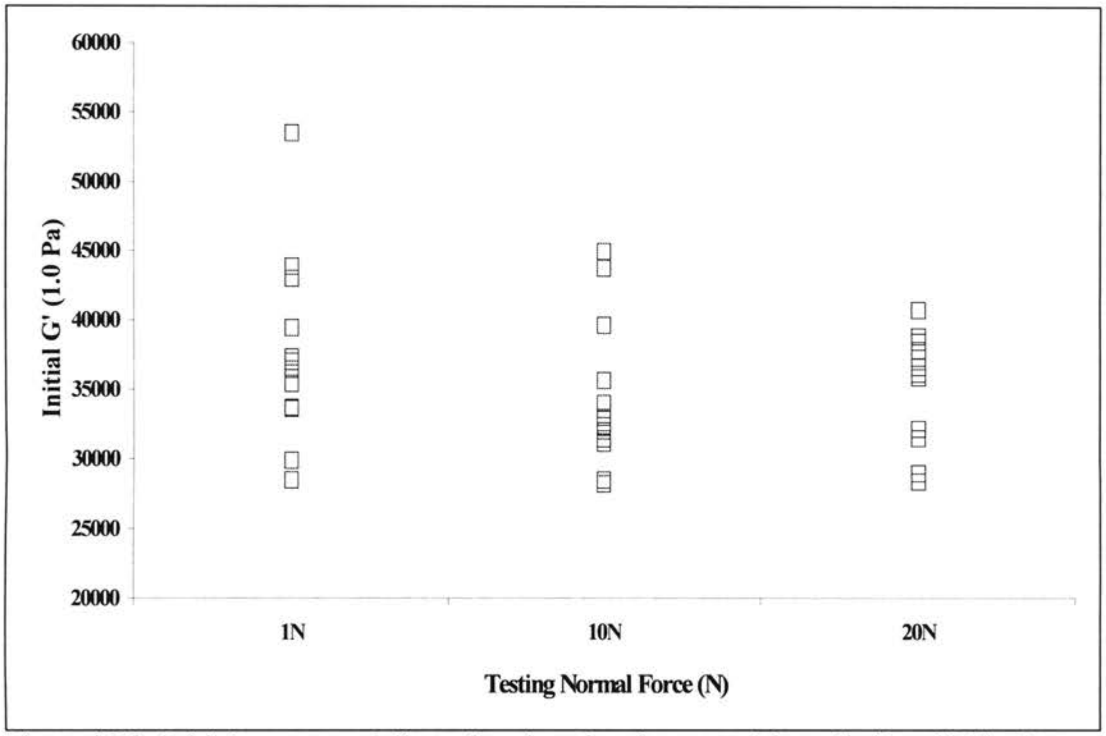

Figure 4.3-3. Initial storage moduli as a function of testing normal force (1, 10 or 20 N) of regular bologna samples loaded to 20N and allowed to relax.

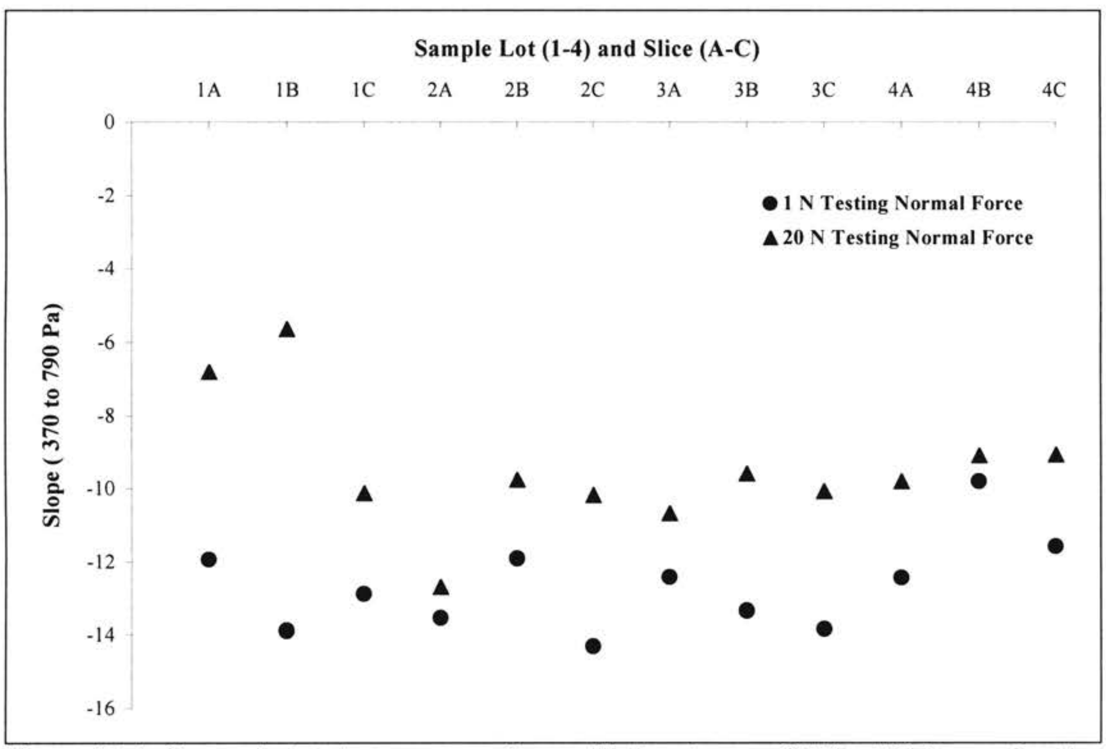

Figure 4.3-4. Slope calculated at corresponding oscillation stresses of 370 to 790 Pa as a function of sample lot (1-4) and slice (A-C) and testing normal force (1 or 20N) for regular bologna after loaded to 20N and allowed to relax.

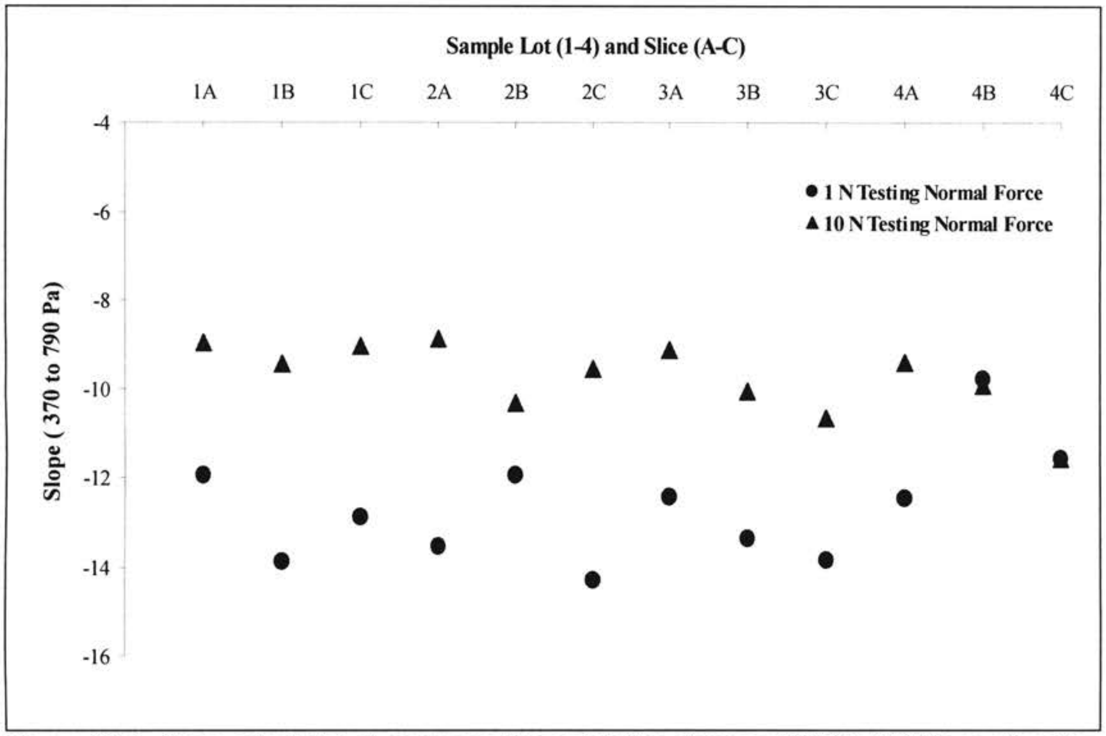

Figure 4.3-5. Slope calculated at corresponding oscillation stresses of 370 to 790 Pa as a function of sample lot (1-4) and slice (A-C) and testing normal force (1 or 10N) for regular bologna after loaded to 20N and allowed to relax.

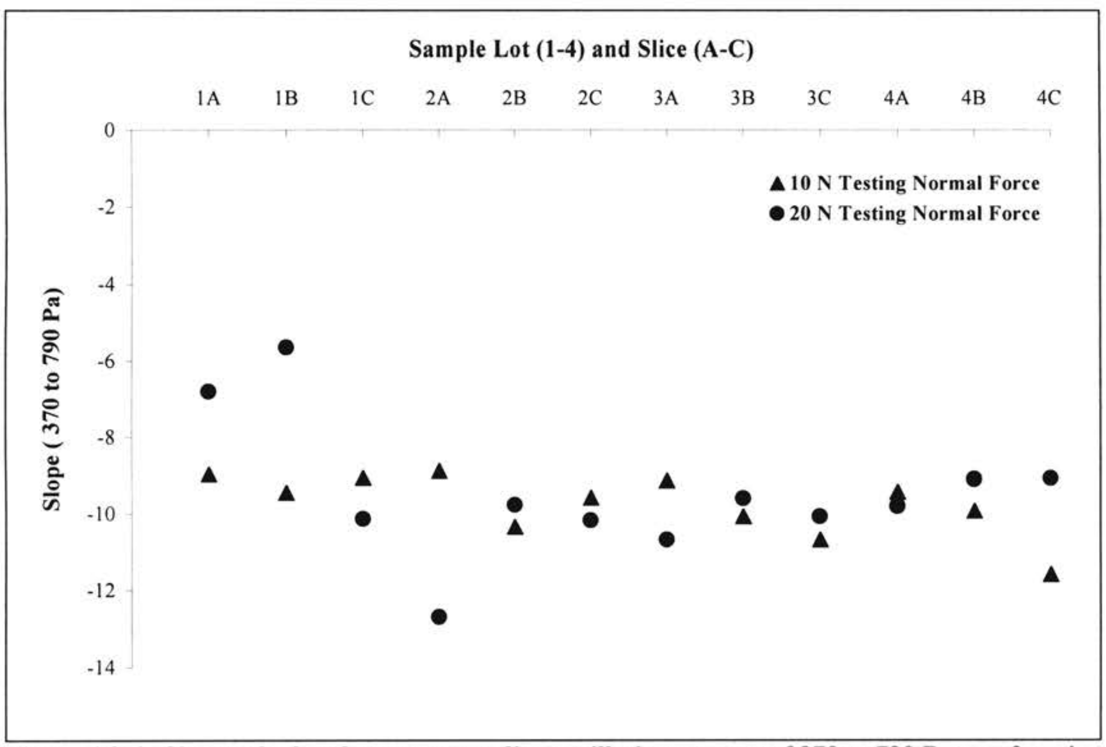

Figure 4.3-6. Slope calculated at corresponding oscillation stresses of 370 to 790 Pa as a function of sample lot (1-4) and slice (A-C) and testing normal force (10 or 20N) for regular bologna after loaded to 20N and allowed to relax.

textural differences might lead to a higher likelihood for the serrated plate to behave in this manner.

Statistical Analysis Supporting Findings

Testing normal force was not a significant effect with respect to initial or average G' values ($F_{2,30}$ = 1.66, p =0.4810; $F_{2,30}$ = 1.59, p = 0.4896). Also, there was a significant difference among testing normal forces when considering slope ($F_{2,30}$ = 23.38, $p < 0.0001$). Slopes of G' curves for the samples tested at 10 N and 20 N were significantly different than slopes of G' curves for the samples tested at 1N (Table 4.3-4).

TABLE 4.3-4. STATISTICAL ANALYSIS: SUMMARY OF TEST RESULTS FOR SLOPE OF G' BASED ON TESTING NORMAL FORCE IN EXPERIMENT II.

Effect	t Value	P Value	Results of Test
Testing Normal Force: 1 vs. 10 N	-5.62	< 0.0001	Significant
Testing Normal Force: 1 vs. 20 N	-6.18	< 0.0001	Significant
Testing Normal Force: 10 vs. 20 N	-0.57	0.5756	Not Significant

This research has shown that sample relaxation before testing does not eliminate or lessen the effects of normal force introduced during loading in this particular case. In fact, greater data variability is introduced as relaxation time for bologna loaded to 20 N increases. These findings seem to contradict previous literature that has suggested that allowing samples of various products to relax for a set time (normally 15 minutes) will eliminate data variability introduced by normal force during loading. These results indicated that relaxation of bologna samples loaded to 20N was ineffective in eliminating the effects of loading normal force. Future research is needed to determine if the same is true for other products and for other loading normal forces. However, it is important that this data does show the potential flaws of previous assumptions in the literature that relaxation can be used as a solution to normal force effects without regard to the specific normal force applied or product tested.

4.4 EXPERIMENT III. FLAT, SMOOTH PLATE VERSUS FLAT, SERRATED PLATE

4.4.1. Observed General Trends

Regular and fat-free bologna samples were loaded to a constant normal force (15 N) and subjected to a stress ramp (0 to 1000 Pa) using either a smooth or serrated plate during testing. Plotting the testing oscillation stress (Pa) against the storage modulus of the regular and fat free samples (Figures 4.4-1 and 4.4-2, respectively) revealed two major trends.

The G' values of the samples tested with either the smooth or serrated plate is the focus of the first trend. From Figures 4.4-1 and 4.4-2, it is quite clear that the G' values collected are different, with the samples tested using the smooth plate representing greater initial G' values.

Second, there is a noticeable difference in the behavior of the storage moduli data of both bologna products when comparing the curves collected using the different geometries. The curves of the samples tested with the serrated plate are relatively flat and smooth, whereas the curves of the samples tested with the smooth plate show greater variability over the testing stress range.

Additionally, another observed trend can been seen for some curves: if monitoring normal force control during the stress ramp, it is obvious that the rheometer loses control of normal force momentarily, which might be an indication of slip. Figure 4.4-3 plots the normal force measurement against testing time for a bologna samples being tested with a smooth or serrated plate. Protocol for this stress ramp includes the sample being loaded to 15 N and this force being held within +/- 1N of this throughout the test. Looking at the normal force behavior for the flat, serrated plate in Figure 4.4-3, it is apparent that normal force stayed within the allowed limits of +/- 1 N of 15 N and a data point is available for

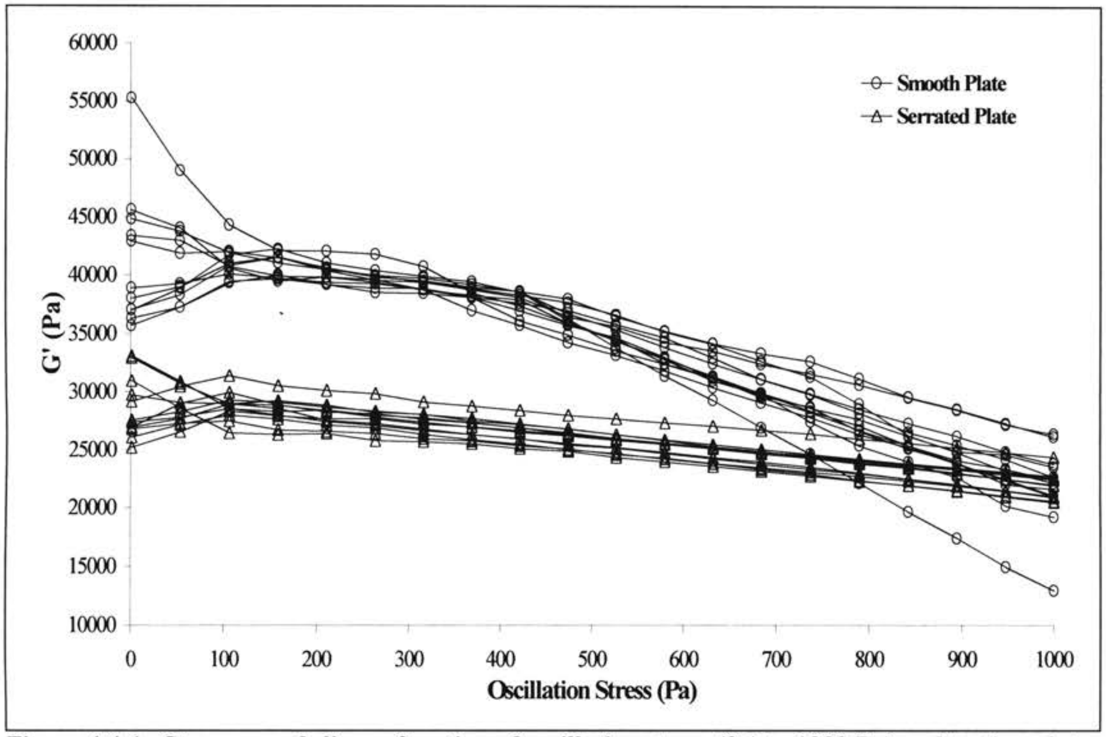

Figure 4.4-1. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing plate (smooth or serrated) of regular bologna samples loaded and tested at 15N.

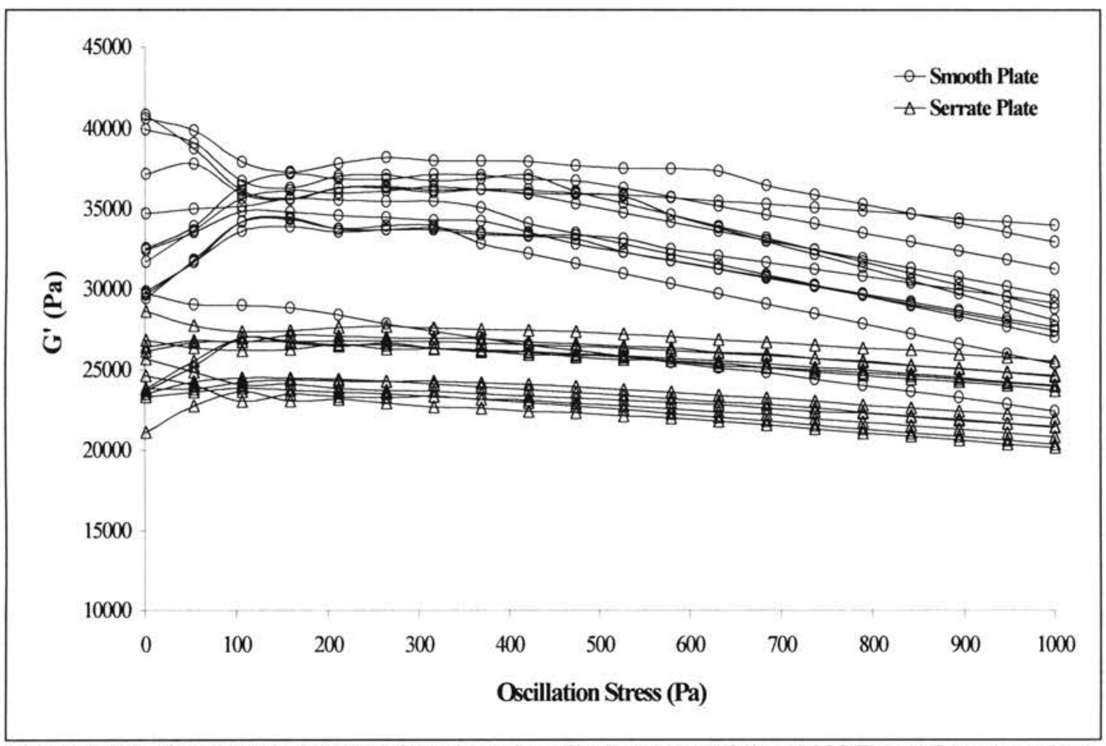

Figure 4.4-2. Storage moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing plate (smooth or serrated) of fat-free bologna samples loaded and tested at 15N.

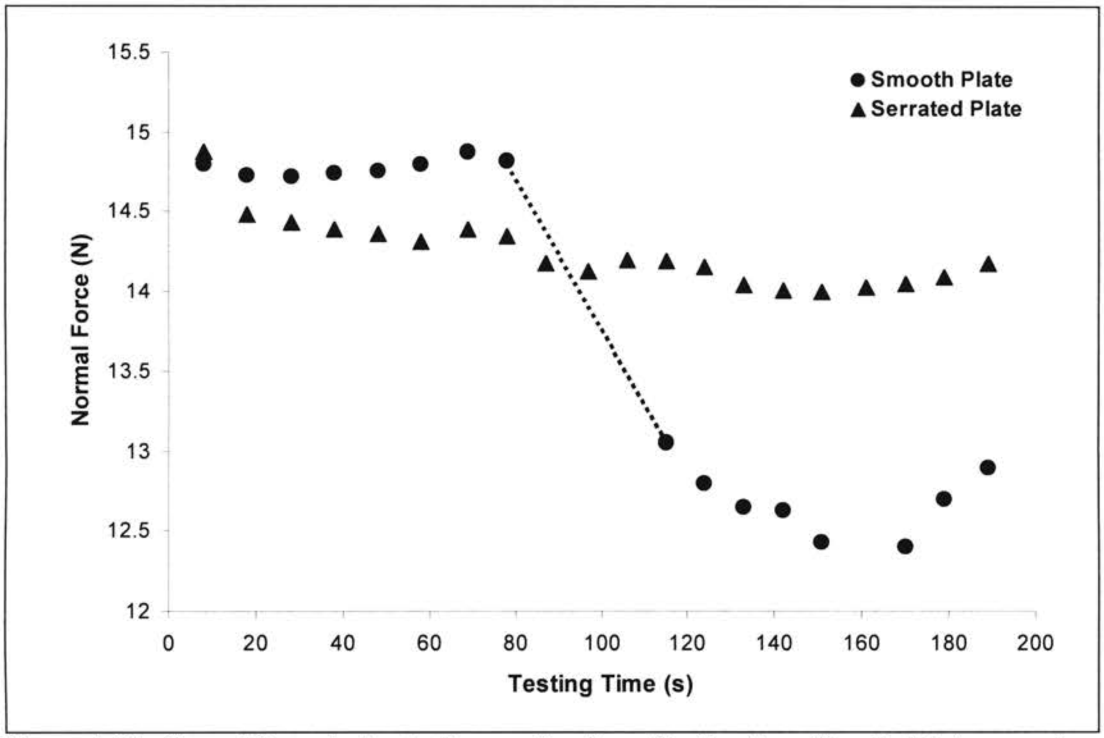

Figure 4.4-3. Normal force during testing as a function of testing time (s) and plate type used during testing (smooth vs. serrated) for bologna samples.

each point throughout the test. In contrast, normal force control for the flat, smooth curve proved more difficult for the rheometer. Not only does the normal force for the sample tested with the smooth plate not stay within the limits set for normal force, the rheometer does not record any measurements for the sample during the middle of the test (dashed line). Between the break in measurement, normal force drops from just below 15 N to under 13 N. This behavior might be indicative of slip; there is the possibility that slip coincides with the loss in normal force measurement. It is thought that the slip occurs, which impairs the rheometer ability to control or measure normal force, justifying the missing data points for normal force and for the jump from 15 N to 12 N. This pattern was not seen for every sample tested with the smooth plate, but it did occur more than once.

4.4.2 Observed Trends and Compositional Effects

For samples tested using the smooth plate, the trend of greater G' values was true for nearly all fat-free bologna samples during the entire range of oscillation stress. However, it was only true for all of the regular bologna samples in the stress range of 1 to 600 Pa; after 600 Pa, many of the smooth plate storage moduli curves began to decrease and cross over the serrated plate curves for the regular bologna samples.

The discussion of slip and composition (Section 4.2.6) plus a discussion of plate geometry type could help explain this observation. Again, dramatic changes in slope occurred for the regular bologna samples but the same observation was not seen for the fat-free samples. As described earlier, the regular bologna samples would be more susceptible to slip due to the presence of more oil within the sample than the fat-free samples. It is most likely that slip is occurring after 600 Pa during the test ramp. Just as compositional properties may influence slip, geometry type could also contribute to a greater risk of slip. After 600 Pa, the G' curves for the smooth plate bologna samples are decreasing to the point of crossing over the curves of the serrated sample. This could be explained by slip because the samples would be more stable due to the anchoring of the sample through the insertion of metal cleats from the serrated plate being used. This roughened surface does not allow the oil to form a consistent layer between the sample surface and the geometry plate. Thus, the smooth plate would not be able to combat slip as well as the serrated plate.

Further evidence that the serrated plate could reduce slip and show more stable G' curves came from visual observations of the samples during and after testing. Smooth plate samples could actually be seen migrating during some tests and many were no longer centered under the testing plates. Also, several of the smooth plate samples had markings left by the upper geometry plate after testing that indicated movement during testing. These markings were characterized by outlined grooves that fanned outwards, which were made by the geometry's edge as it oscillated.

Table 4.4-1 summarizes the range of initial G' values for all samples with respect to testing plate geometry and composition and helps show the difference in G' curves based on these factors. For the regular and fat-free bologna samples, the ranges of the serrated plate initial G' values varied similarly, with a range spanning 7900 and 7480 Pa, respectively. However, there is a marked difference when comparing the ranges of the smooth plate initial G' values of the regular and fat-free bologna, with the smooth plate regular bologna samples showing the greatest difference of 19,580 Pa, compared to the smooth plate fat-free bologna having a difference of 11,440 Pa. If it is suggested that the smooth plate introduces a greater probability of unstable contact between the sample and the testing geometry, the greater variance of the initial G' for the regular bologna samples can be explained by composition, specifically by the percent fat of the sample. The higher fat content of the regular bologna allows for a more slippery testing surface. This increased slipperiness, combined with the lack of ability of the smooth plate to adhere to the surface, could lead to increased variability of the starting curve.

TABLE 4.4-1. RANGES FOR INITIAL STORAGE MODULI CURVES FOR BOTH BOLOGNA TYPES.

	Minimum G' (Pa)	Maximum G' (Pa)	Range (Pa)
Regular Bologna			
Smooth Plate	35710	55290	**19850**
Serrated Plate	25190	33090	**7900**
Fat Free Bologna			
Smooth Plate	29410	40850	**11440**
Serrated Plate	21140	28620	**7480**

ANOVA tests were generated using SAS® to analyze the effects of product type and plate for initial G', average G', and slope. The interaction between product and plate type is significant for initial G' values; therefore, plate type comparisons must be made for each product (Table 4.4-2). For the average G' values, the interaction was not significant. Looking at the main effects, plate type was significant. Therefore, use of a serrated or flat plate significantly affected average G' values.

TABLE 4.4-2. STATISTICAL ANALYSIS: SUMMARY OF ANOVA RESULTS FOR INITIAL G', AVERAGE G', AND SLOPE IN EXPERIMENT III.

Variable Analyzed	F Value	P Value	Results of Test
Initial G' Value			
Product v. Plate	29.11	< 0.0001	Significant
Product	N/A*	N/A	N/A
Plate	N/A	N/A	N/A
Average G' Value			
Product v. Plate	0.24	0.6298	Not Significant
Product	1.10	0.3345	Not Significant
Plate	676.97	< 0.0001	Significant
Slope			
Product v. Plate	43.01	< 0.0001	Significant
Product	N/A	N/A	N/A
Plate	N/A	N/A	N/A

* Note: Interaction was found to be significant, so the main effects were not listed here.

The differences in the slopes of the curves using the two different geometries for both the regular bologna samples and fat free samples are seen in Figures 4.4-4 and 4.4-5, respectively. The slope values seen in these figures were calculated for the stress range of 370 to 790 Pa. Both of these figures show a greater slope variation for the smooth plate when compared to the slope variation of the curves representing the serrated plate samples. The range of slopes, when considering all samples from all lots and slices of each bologna type (for a total of 12 samples for each plate type), are listed in Table 4.4-3. The regular bologna shows a greater variation in slopes (with a range of –19.76 to –44.62) than the smooth plate fat-free bologna samples (with a range of –6.50 to –9.13). This suggests that higher fat composition coupled with testing with a smooth plate could make the sample more susceptible to slip during testing, which in turn would result in more varied data.

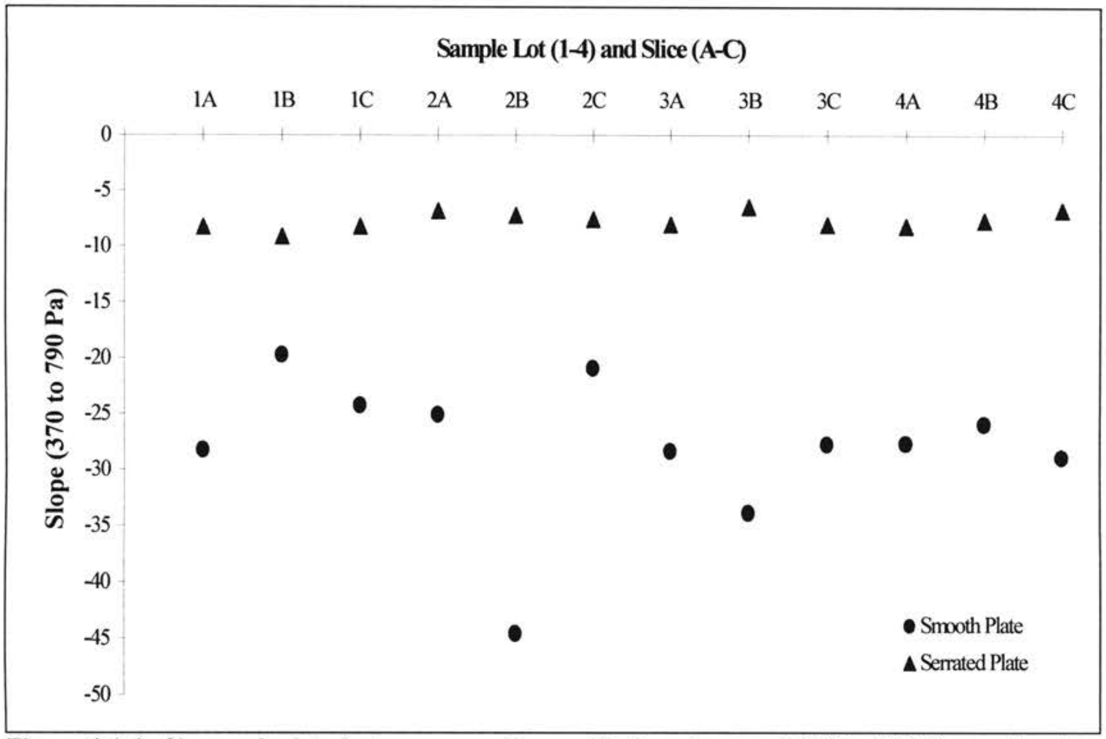

Figure 4.4-4. Slope calculated at corresponding oscillation stresses of 350 to 760 Pa as a function of sample lot (1-4) and slice (A-C) and testing plate (smooth or serrated) of regular bologna samples loaded and tested at 15N.

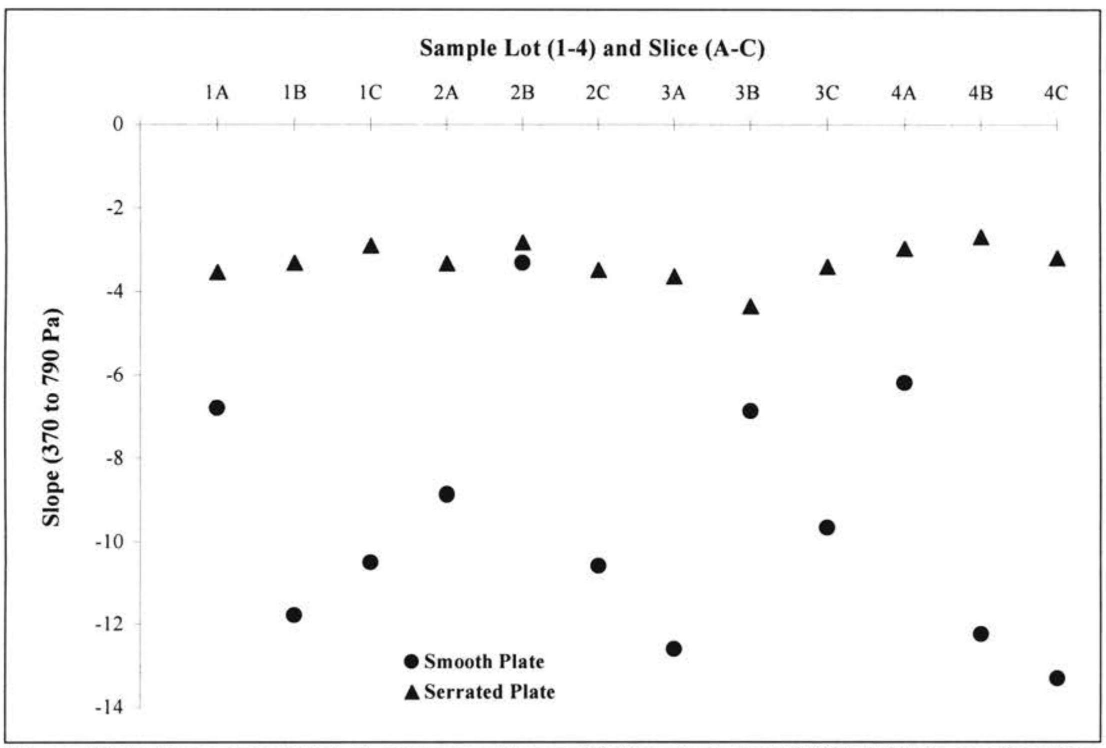

Figure 4.4-5. Slope calculated at corresponding oscillation stresses of 350 to 760 Pa as a function of sample lot (1-4) and slice (A-C) and testing plate (smooth or serrated) of fat-free bologna samples loaded and tested at 15N.

TABLE 4.4-3. SLOPE RANGES FOR STORAGE MODULI CURVES FOR BOTH BOLOGNA TYPES.

	Slope Range	
	Regular Bologna	**Fat Free Bologna**
Smooth Plate	-19.76 to –44.62	-3.31 to –13.32
Serrated Plate	-6.50 to –9.13	-2.68 to –4.36

4.5 DISCUSSION OF SOURCES OF VARIABILITY

Any research project is not free from inherent error, which exists no matter how many precautions are taken to eliminate the introduction of error during data collection. This is especially true for testing samples of a biological nature such as food products. Variability during these tests can be attributed to three main influences: different processing lots, differences due to product homogeneity within a package, and differences due to the rheometer testing procedure.

The variable situations and environments during processing lead to variability in food products, which cannot be avoided and can lead to variable responses when testing. Based on the statistical design of each experiment, the variances of products in this project were estimated based on the differences at the level of lot and packages using Restricted Maximum Likelihood (REML) in SAS®. Error is also associated with testing equipment. Specifically for this project, the inherent error associated with the operation of the rheometer was taken as the Residual variance in the REML output. The following equations were used to break down the variance into percentages based on lot, package, and rheometer (residual):

VAR (response) = Variance of Lot + Variance of Package + Residual

% Variance of Lot = Variance of Lot / VAR (response)

% Variance of Package = Variance of Package / VAR (response)

% Variance of Rheometer = Variance of Residual / VAR (response)

VAR(response) is based on the statistical analysis of each response variable of initial G', average G' or slope G' data.

Summaries of the percentage of variance attributed to each of the influences for all three response variables for experiments I, II, and III are shown in Table 4.5-1. From this table, it is clear that the majority of error is contributed by lot difference and rheometer operation, with lot difference contributing around 40% and rheometer operation contributing approximately 50% or more error. For initial G' and average G' response variables, when compared for each experiment separately, similar percentages of error for lot, package, and rheometer operation were shown. Error percentages based on the response of slope of G' were not similar, suggesting that slope might not be the most effective choice as a response variable. It should be noted that values for slope of G' for experiment III suggest that the operation of the rheometer was responsible for 100% of the variability. Reported values of zero for lot and package variability are most likely a reflection of REML not being able to detect a pattern in variability rather than no error associated at all with these factors. Differences in error allocation among different experiments might be explained by the actual products used for each particular experiment. For example, experiment I used five different products and experiment II only evaluated two products. It is interesting to note that nearly as much variability occurs due to lot product differences as attributed to the actual measurement of rheological parameters.

TABLE 4.5-1. VARIANCES AND PERCENTAGE OF VARIANCES ASSOCIATED WITH FOOD PRODUCT LOT, PACKAGE, AND EXPERIMENTAL ERROR FOR ALL RESPONSE VARIABLES FOR EXPERIMENTS I, II, AND III.

	Response Variable					
	Initial G'		Average G'		Slope G'	
	Variance	%	Variance	%	Variance	%
Error for Experiment I						
Based on Lot	1945	42.21	1854	45.61	0.72	20.40
Based on Packages	1181	6.49	0	0	0.68	20.87
Experimental Error	0	51.29	2894	54.32	0.64	56.72
Error for Experiment II						
Based on Lot	3623	40.94	3089	39.76	0.60	13.47
Based on Packages	1880	2.21	1763	8.36	0	0
Experimental Error	4352	55.72	3554	52.62	0.65	86.59
Error for Experiment III						
Based on Lot	946	43.56	1199	55.16	0	0
Based on Packages	633	23.65	668	11.77	0	0
Experimental Error	510	31.90	610	32.39	1.60	100

CHAPTER 5: FUTURE RESEARCH

Expanded research is needed to fully characterize the errors associated with loading normal force. This project identified that loading normal force does significantly influence oscillatory data and that relaxation of the sample between loading and testing may not effectively eliminate or reduce these effects. However, this project was not a complete examination of this problem, but rather an introductory framework for more in-depth investigations. Only a limited number of products and normal forces were used (especially true of the examination of relaxation times), and this limits the application of the conclusions to these and similar products. Broader selection of products and normal forces would offer a more developed understanding of the relationship between normal force and oscillatory data.

Other factors that are commonly varied in rheological research were also held constant in this project in order to isolate the effects of normal force. Such factors include test type (i.e., oscillatory frequency sweeps or creep testing), test temperature, and sample thickness. Only torque ramps were conducted in this project, and it is assumed that loading normal force would influence the G' and G" data collected in frequency sweeps or creep testing in the same manner, but a formal investigation should be conducted to confirm this assumption.

Testing temperature could have dramatic effects on the relationship between loading and testing normal force and oscillatory data. Many products soften with the application of heat. If a product is loaded at different temperatures, the transfer of energy due to normal force application during loading might be different for a softer product; thus, the compactness of the sample might also be different, consequently leading to varying moduli data. If samples are loaded at the same temperature and to the same normal force, what impact does heating during testing have on the data collected? In this project, samples were kept at a constant temperature and tested while normal force was being controlled. If a temperature ramp was applied in the same manner with normal force control during testing, the rheometer might have a difficult time trying to maintain the testing normal force with the temperature changing the texture of the product constantly, which could lead to data variability. Temperature ramps are common rheological tests and have many applications in the food industry. Because of this, the relationship between temperature and loading or testing errors due to normal force needs to be addressed.

Another objective of this project was to describe the effects of composition on the response of samples to normal force application during loading. For the samples tested, it appeared that composition did not have a dramatic influence when considering product reaction to normal force. Further investigation of a broader range of products might produce more definite correlations between composition and product response to normal force during loading. Perhaps more important, a new question was raised about the effects of textural properties, rather than composition, on such product behavior. A formal investigation of products with varying textural properties (which could be tested with a texture analyzer and described by the resulting texture profile analysis) should be conducted. In this study, an explanation for the different behavior of the oscillatory data for the cheddar cheese samples (when compared to the meat and mozzarella) was provided based upon the diverse textural properties of cheddar in comparison to the other products. In fact, cheddar seemed more sensitive to the application of normal force, with the lower loading normal force appearing to influence the data as strongly as the higher normal force. This type of information could be beneficial for researchers beginning research on a new product or trying to improve on data repeatability.

Beyond future research that continues to characterize and describe the errors related to normal force, the scope of other research problems should turn to solutions or corrections for the effects of normal force. Since relaxation time may not be effective in eliminating errors associated with normal force, test protocols must be written which attempt to control normal force in other ways. Additionally, future research identifying a target loading normal force that creates the most repeatable results would be of great use to the food industry. Short of such a target normal force, a correction factor based on the loading normal force would also be useful. In some manner, rheological researchers must find a way to predict or eliminate the errors associated with loading normal force.

This study was made possible, at least in part, by rheometer advancements which allow samples to be loaded to a specific normal force and for normal force to be controlled and measured during testing. However, many researchers still lack the ability to measure or control normal force. Due to these constraints, a simple solution to data variability cannot be provided by suggesting that samples should always be loaded to a specific target normal force. Investigations into solutions not requiring normal force measurements should be conducted. One possible solution relates to the compaction of the sample. Throughout this study, the data error introduced by normal force has been related to compaction of the sample by the testing plates. As a sample is subjected to a greater normal force, that sample responds by forcing molecules together and becoming more compact (i.e., solid) and therefore showing higher G' values. Finding an accurate method of measuring sample thickness and relating this measurement to the gap between the plates after loading could provide an alternative to strictly controlling normal force during loading as was done in this study.

Even when normal force cannot be measured, researchers could have the capability of calculating the deformation of the testing sample. This would involve measuring the sample before testing and comparing that measurement to the gap width after loading. The difference between the two, or the percent deformation, could then be determined. Because normal force is essentially a measurement of how the sample is compacted by the plates, loading to a consistent percent deformation could be equally as useful in increasing data repeatability as strictly controlling normal force. Research could focus on trying to relate loading normal force to percent deformation by describing and investigating the physical changes (e.g., the density change) in samples when loaded to different normal forces. If percent deformation could be directly related to the levels of normal force applied, this could provide a common measurement that correction factors or equations could be based on so more consistent data can be taken using all rheometers.

Of course, the possibility of using sample deformation to describe normal force during loading is restricted by the ability to measure sample thickness to a high degree of accuracy. During this study, the gap measurement of samples loaded to 5 or 20 N differed on average by only 300 microns. Measurement instruments and methods have not proven to be this accurate and would have to be developed or modified before sample deformation could be a realistic approach.

In this study, it was mentioned that a regression model could be valuable in predicting the moduli values, especially when using such an equation to predict the G' or G" data of a sample if no normal force was being applied. It might be possible to use sample deformation in the same way. Collecting preliminary data by loading samples to several levels of percent deformation, and then trying to fit a model to this data, could lead to a predicting equation of G' and G" that does not involve the measurement of normal force. These steps could be investigated, and if proven possible, could be suggested to researchers as a preliminary means of preparing for better data collection. Additionally, this simple step would be possible even without the most advanced rheological equipment and could be conducted by individual researchers for any product. Research is needed to prove the viability of using percent deformation to predict and control moduli responses.

Further research should also be conducted to correlate normal force during testing with sample migration (or slip). During tests of bologna using a smooth, flat plate, it was observed that the rheometer did not record normal force at several points during testing and that the rheometer could not maintain normal force within the proper ranges once it began recording again. This suggests that the sample may have slipped within the testing geometry and was not making contact with the plates during at least some part of the test. In the past, researchers have had much difficulty identifying the occurrence of slip. Research in this area could provide useful guidance as to when slip has taken place by analyzing normal force, which in turn might ultimately lead to a model of the slip behavior of products.

As rheological measurements of semi-solid food products become more complex, researchers will often be dealing with specific products and analyzing very specific properties of those products. As a foundation for any work on a specific product, researchers should carefully analyze how that product responds to normal force application during loading. In addition to the information that may be learned about that specific product though this exercise (i.e., density, uniformity, response to compaction), a knowledge of the normal force impacts will lead to more reliable and repeatable results. Additionally, when repeatedly dealing with a single product, it may be possible for the researcher to determine an ideal target loading or testing normal force for that product. Regardless, understanding the impacts of normal force should provide a deeper understanding of the meaning of rheological data.

CHAPTER 6: CONCLUSIONS

There has been a dramatic growth of interest in using oscillatory measurements of rheological data in recent years in the food industry. The application of rheological data for regular use in quality control and product development has created a greater need for repeatability of the data. As demand for rheological data continues to grow and become more specific, repeatability of results will be the determining factor in the usefulness of this data.

The most significant finding of this study is that fundamental assumptions relied upon in previous rheological research are flawed and represent a major source of data variability. It is not surprising that numerous past rheological studies of food products have encountered difficulties with the repeatability of results. For the most part, these studies have relied upon the assumption that normal force exerted upon the sample during loading did not impact rheological data. Stemming from that first assumption, many studies have likewise presumed that, even if normal force during loading could impact G' and G" data, such effects were greatly minimized or eliminated by allowing the sample to relax for some period of time before testing. Relying on these basic assumptions, previous researchers have created methods and testing procedures which ignore normal force completely or utilize a relaxation period (approximately 15 minutes) to eliminate the effects of normal force.

The specific findings of this study are related to sources of data variability and errors associated with rheological testing. First, this study demonstrated that normal force during loading does affect G' and G" data. Significantly, it was observed that initial G' values and average G' values throughout testing generally increase for most products with greater normal force applied during loading. However, although all products followed this general pattern to some degree, this trend was most significant for semi-solid products, particularly bologna. For more liquid-like products such as mustard, normal force did not have such a significant impact. Since practical application of rheological data has begun to include semi-solid products, this is an important finding for the use of oscillatory measurements to predict or determine functional characteristics of food products. Specific conclusions are:

- Significant differences were seen among initial G' and G" and average G' and G" values for all semi-solid products loaded to either 5 or 20 N normal force (with the exception that no significant differences, based on loading normal force, were found for cheddar cheese for average G' or G").
- Significant differences for slope of G' based on loading normal force were found for bologna products only.
- No significant difference for any response variable based on loading normal force was seen for mustard.
- Compositional effects influenced G' data less dramatically than loading normal force.

Second, it was discovered that sample relaxation prior to testing does not eliminate or minimize the effects introduced to bologna samples by normal force application during loading. Samples that were allowed to relax for either minutes or hours after being loaded to a high normal force still exhibited very different G' values than samples that were loaded to low normal forces from the start. Relaxation to 1 N of normal force from an initial force of 20 N often occurred 3.5 hours after loading. This suggests that the relaxation times provided for in previous studies are not sufficient to allow samples to fully relax. In addition, the range of initial G' values was largest for samples relaxed the most (1 N), suggesting that increased relaxation may only increase the variability of rheological data rather than minimizing or eliminating it.

- No significant difference was found between initial and average G' values for samples allowed to relax to various testing normal forces.
- Slopes of G' curves tested at 10 and 20 N (i.e., minimal or no relaxation) were significantly different from slopes of G' curves tested at 1 N (i.e., long relaxation period).
- There was an increase in data variability associated with longer relaxation times.

Third, this research revealed that while sample migration (slip) can be minimized through the use of a serrated plate, data repeatability is not assured by correcting slip alone. The effects of normal force are introduced before testing begins. Thus, variability in results can often be seen due to handling and loading of the sample prior to testing, regardless of whether other sources of variability are minimized or eliminated.

- There was a significant difference between average G' values for samples tested with a flat, smooth plate or a flat, serrated plate.
- G' curves generated with the serrated plate are less variable than the curves generated with the smooth plate, indicating that the serrated plate does help prevent slip.

The findings of this study indicate that the basic assumptions regarding relaxation time are not accurate. This has significant real-world implications. For food industry professionals using rheological data in quality control or product development applications, data variability causes great concern. In fact, rheological data that varies based on effects that are generally not tested or considered, such as loading normal force, is for the most part useless in these applications.

REFERENCES

Association of Official Analytical Chemists. 1997. The Official Methods of Analysis of AOAC International. 16th ed., Vol. 2. AOAC International: Gaithsburg, MD.

Brennan, J.G. and M.C. Bourne. 1994. Effect of lubrication on the compression behaviour of cheese and frankfurters. J. Texture Studies 25: 139-150.

Casiraghi, E.M., E.B. Bagley and D.D. Christianson. 1985. Behavior of mozzarella, cheddar and processed cheese spread in lubricated and bonded uniaxial compression. J. Texture Studies 16: 281-301.

Christianson, D.D., E.M. Casiraghi and E.B. Bagley. 1986. Deformation and fracture of wheat, corn and rice starch gels lubricated and bonded uniaxial compression. Carbohydrate Polymers 6: 335-348.

Dervisoglu, M. and J.L. Kokini. 1986. Steady shear rheology and fluid mechanics of four semi-solid foods. J. Food Science 51(3): 541-546.

Elliott, J.H. and A.J. Ganz. 1977. Salad dressings—preliminary rheological characterization. J. Texture Studies 8: 359-371.

Goh, H.C. and P. Sherman. 1987. Influence of surface friction on the stress relaxation of gouda cheese. J. Texture Studies 18: 389-404.

Grikshtas, R. and M.A. Rao. 1993. Determination of slip velocities in a concentric cylinder viscometer with Mooney and Kiljanski methods. J. Texture Studies 24: 173-184.

Halliday, P.J. and A.C. Smith. 1995. Estimation of wall slip velocity in a capillary flow of potato granule paste. J. Rheology 39(1): 139-149.

Hill, M.A., J.R. Mitchell and P.A. Sherman. 1995. The relationship between the rheological and sensory properties of a lemon pie filling. J. Texture Studies 26: 457-470.

Kokini, J.L. and A. Dickie. 1981. An attempt to identify and model transient viscoelastic flow in foods. J. Texture Studies 12: 539-557.

Kokini, J.L. and G.J. Plutchok. 1987. Viscoelastic properties of semisolid foods and their biopolmeric components. Food Technology 41(3): 89-95.

Létang, C., M. Piau and C. Verdier. 1999. Characterization of wheat flour-water doughs. Part I: Rheometery and microstructure. J. Food Engineering 41:121-132.

Lindborg, K.M., C. Trägårdh, A-C. Eliasson and P. Dejmek. 1997. Time-resolved shear viscosity of wheat flour doughs—effect of mixing, shear rate, and resting on the viscosity of doughs of different flours. Cereal Chem. 74(1): 49-55.

Muñoz, J. and P. Sherman. 1990. Dynamic viscoelastic properties of some commercial salad dressings. J. Texture Studies 21: 411-426.

Navickis, L.L., R.A. Anderson, E.B. Bagley and B.K Jasberg. 1982. Viscoelastic properties of wheat flour doughs: variation of dynamic moduli with water and protein content. J. Texture Studies 13: 249-264.

Navickis, L.L. and E.B. Bagley. 1983. Yield stresses in concentrated dispersions of closely packed, deformable gel particles. J. Rheology 27(6): 519-536.

Nolan, E.J., V.H. Holsinger and J.J. Shieh. 1989. Dynamic rheological properties of natural and imitation mozzarella cheese. J. Texture Studies 20: 179-189.

Peressini, D., A. Sensidoni and B. de Cindio. 1998. Rheological characterization of traditional and light mayonnaises. J. Food Engineering 35: 409-417.

Qui, C-G. and M.A. Rao. 1989. Effect of dispersed phase on the slip coefficient of apple sauce in a concentric cylinder viscometer. J. Texture Studies 20: 57-70.

Rao, M.A. 1975. Measurement of flow properties of food suspensions with a mixer. J. Texture Studies 6: 533-539.

Rosenberg, M., Z. Wang, S. Chuang and C. Shoemaker. 1995. Viscoelastic property changes in cheddar cheese during ripening. J. Food Science 60(3): 640-644.

SAS Institute. 1999. SAS User's Guide: Statistics. SAS Institute: Cary, N.C.

Solorza, F.J. and A.E. Bell. 1995. Effect of calcium, fat and total solids on the rheology of a model soft cheese system. J. Society of Dairy Technology 48(4): 133-139.

Steffe, J.F. 1996. Rheological Methods in Food Process Engineering. 2nd Ed. Freeman Press: East Lansing, MI.

Steffe, J.F. and E.W. Ford. 1985. Rheological techniques to evaluate the shelf-stability of starch-thickened, strained apricots. J. Texture Studies 16: 179-192.

Subramanian, R. and S. Gunasekaran 1997. Small amplitude oscillatory shear studies on mozzarella cheese. Part II. Relaxation spectrum. J. Texture Studies 28: 643-656.

Ustunol, Z., K. Kawachi, and J. Steffe. 1995. Rheological properties of cheddar cheese as influenced by fat reduction and ripening time. J. Food Science 60(6): 1208-1210.

Voisey, P.W. and W.S. Reid. 1974. Effect of friction on the performance of texture test cells. J. Texture Studies 5: 239-248.

Yoshimura, A.S. and R.K. Prud'homme. 1988. Wall slip corrections for couette and parallel disk viscometers. J. Rheology 32: 53-67.

APPENDIX A

PRELIMINARY STORAGE MODULI DATA FOR PEANUT BUTTER SLICES AND PROVOLONE CHEESE

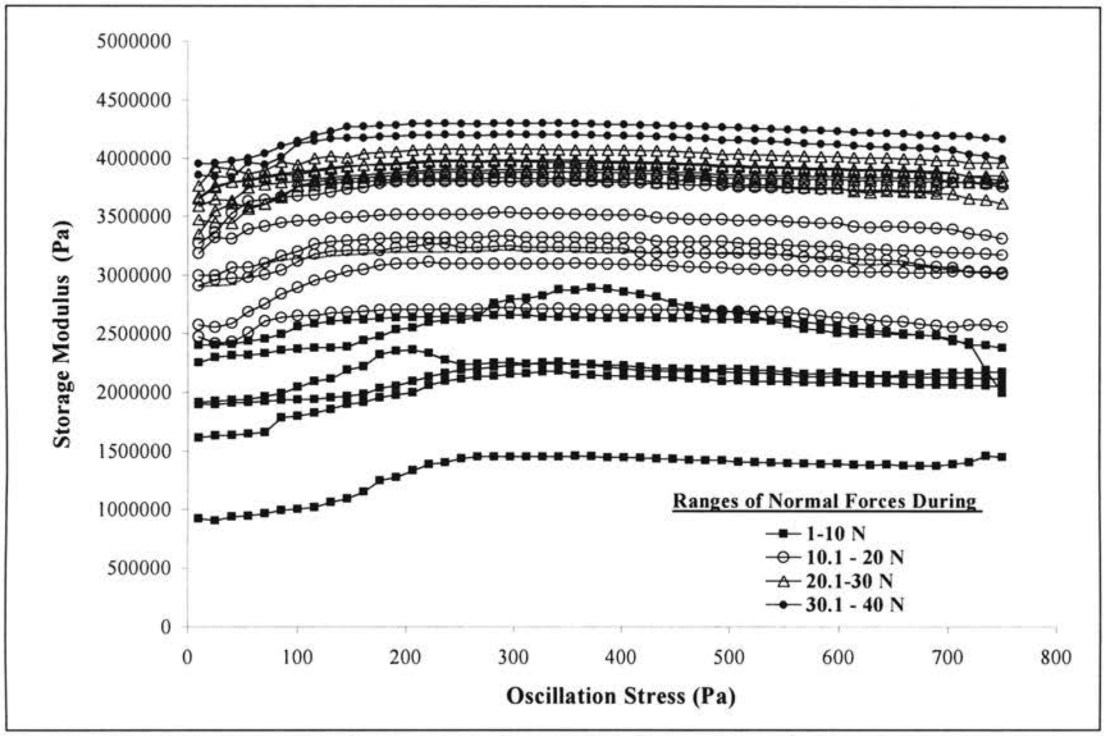

Figure A-1. Storage moduli as a function of oscillation Stress (0.1 to 800 Pa) versus various ranges of loading normal force for peanut butter slices (Preliminary Data).

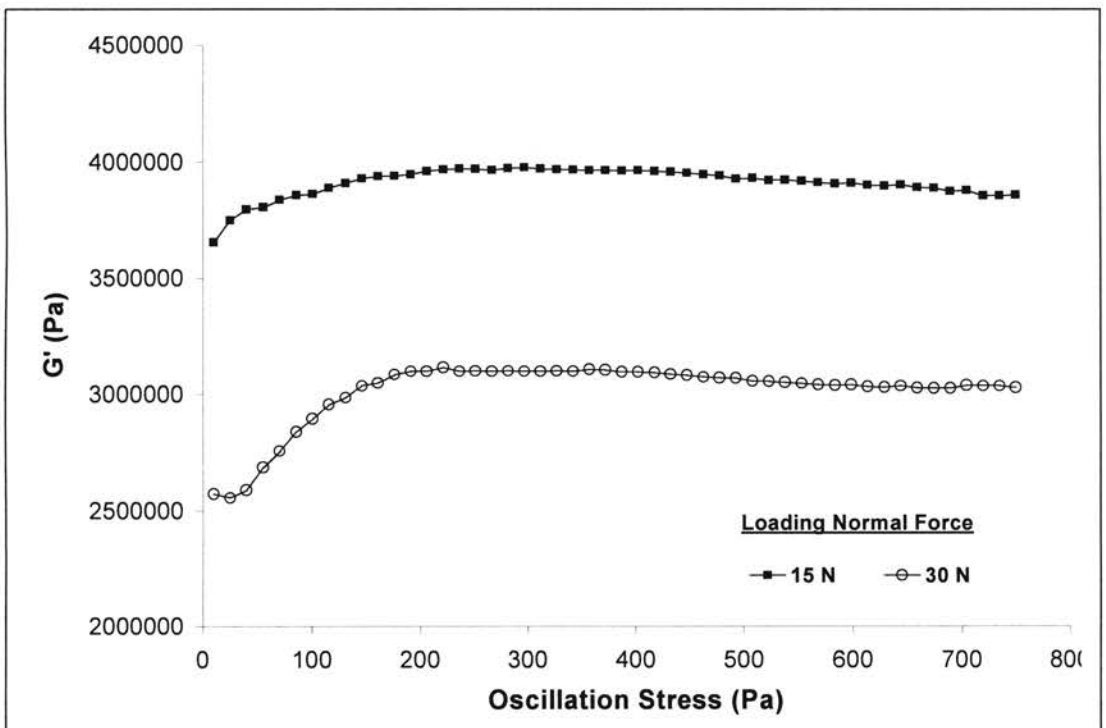

Figure A-2. Storage moduli as a function of oscillation Stress (0.1 to 800 Pa) versus loading normal force (15 or 30 N) for peanut butter slices (Preliminary Data).

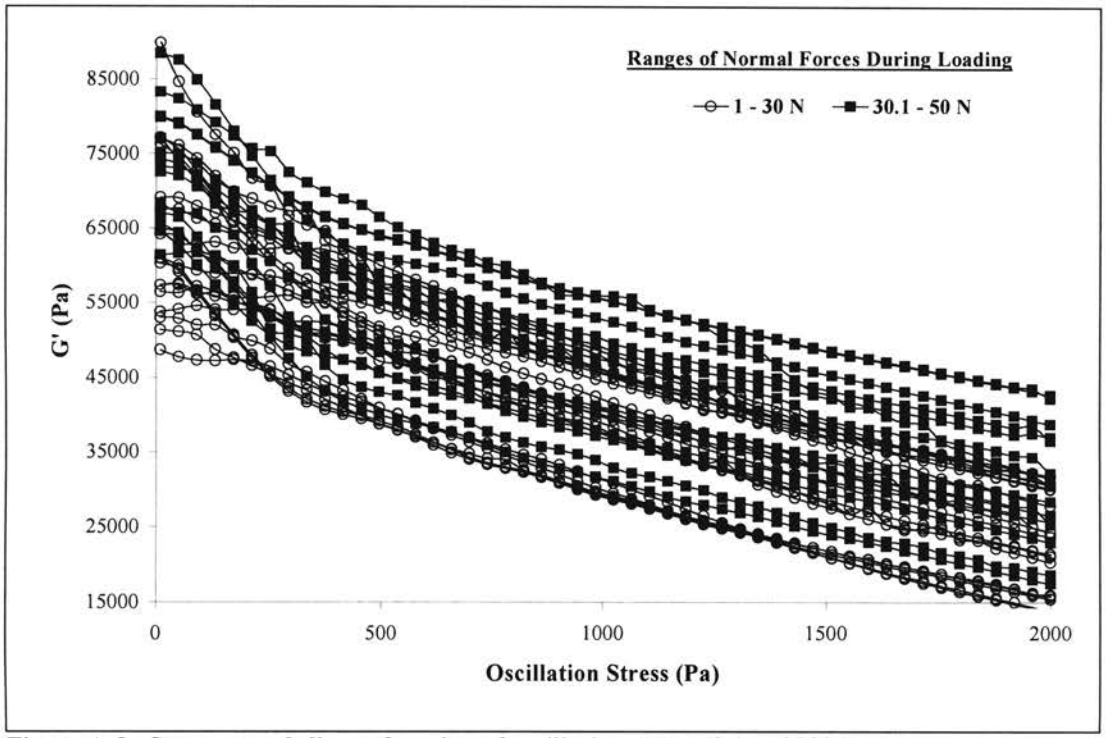

Figure A-3. Storage moduli as a function of oscillation stress (0.1 to 2000 Pa) and various ranges of loading normal force for provolone cheese (Preliminary Data).

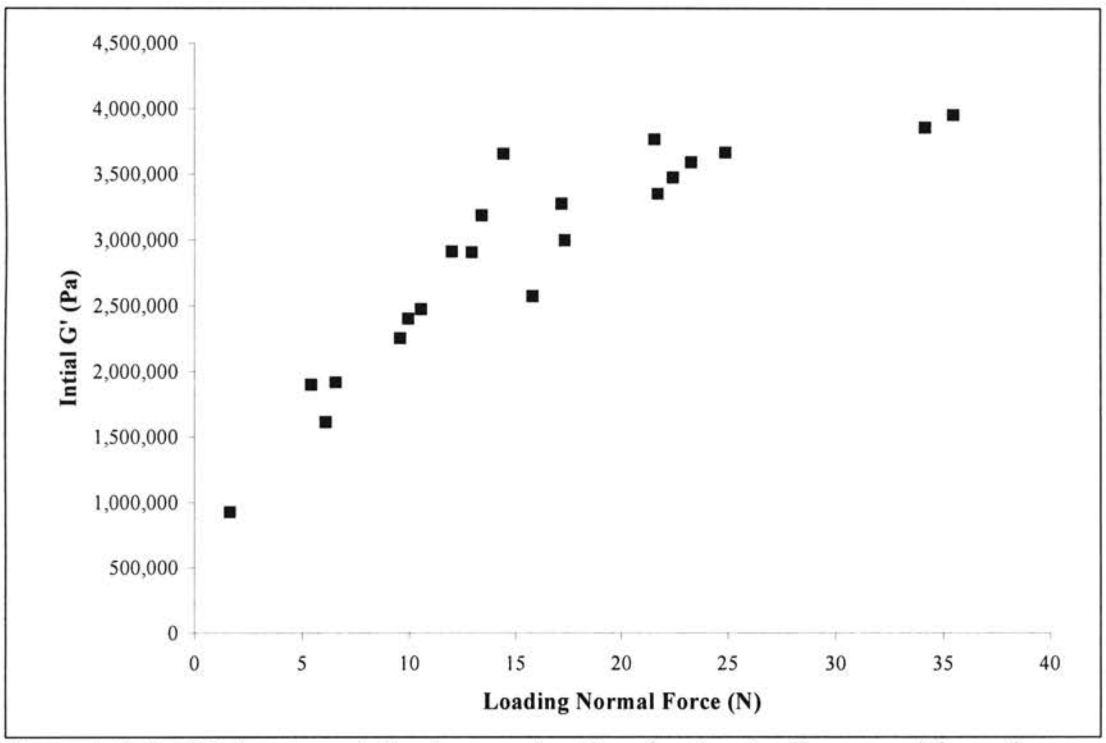

Figure A-4. Initial storage moduli values as a function of various loading normal forces for peanut butter slices (Preliminary Data).

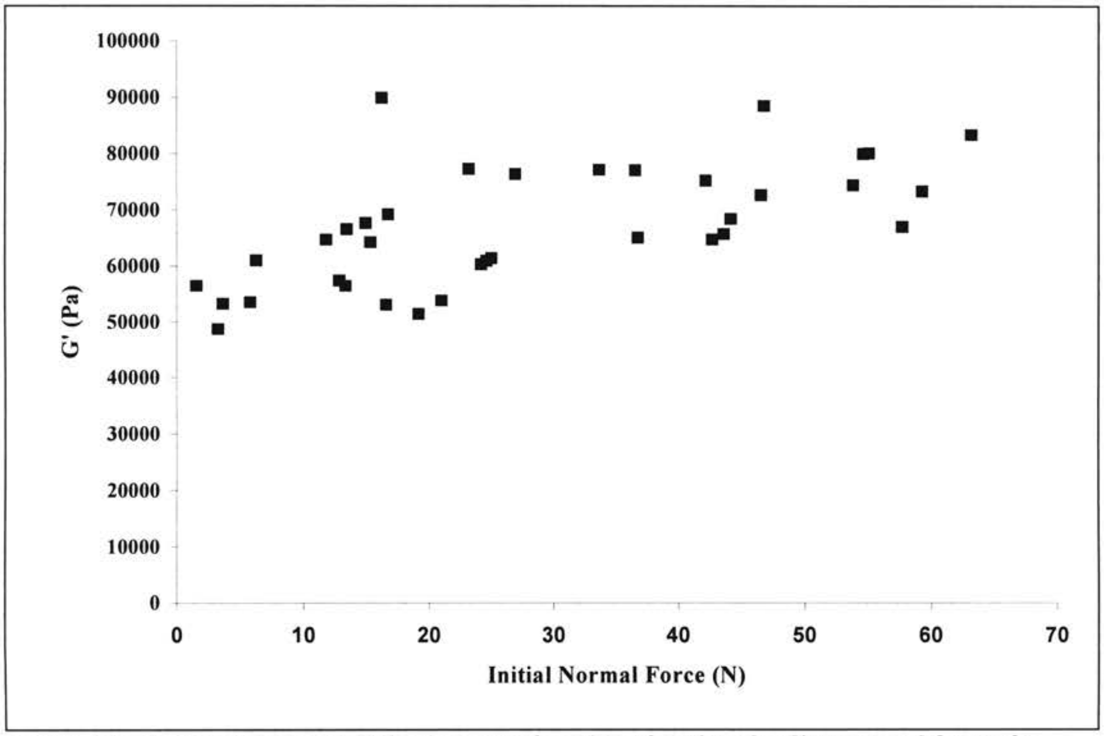

Figure A-5. Initial storage moduli values as a function of various loading normal forces for provolone cheese (Preliminary Data).

APPENDIX B

SAS PROGRAM CODE FOR EXPERIMENTS I, II, AND III

EXPERIMENT 1: SAS ANALYSIS CODE

```
dm 'log; clear; output; clear; ';
*** filename:  exp1.sas ***;
data exp1;
infile 'c:\stats\ exp1.txt' dlm='09'x dsd missover firstobs=6 obs=53;
input blank product $ lot slice $ sample $ initialg avgg slopeg ;
drop blank ;
if sample='L' then nforce=1;
if sample='H' then nforce=2;

proc format;
value nforcefmt 0=' ' 1='L' 2='H' 3=' ' ;
*proc print data=exp1;
*title2 'Experiment I Data';
run;
%macro missy3(title, data=, y= , plotdata= );
title;
proc mixed data=&data covtest cl ;
&title ;
title3 "Analysis Variable = " &y;
     Class  product lot slice nforce;
     model &y = product nforce product*nforce / ddfm=satterth;
     random lot(product) slice(lot product);
                 lsmeans nforce*product / slice=(nforce product) ;
     ods output lsmeans=&plotdata;
     format nforce nforcefmt. ;
run;
title;
symbol1 value=circle i=join cv=red l=1 w=2;
symbol2 value=dot i=join cv=red l=1 w=2;
proc gplot data=&plotdata;
plot estimate*nforce = product / haxis= 1 to 2 by 1;
format nforce nforcefmt. ;
&title ;
title2 "Response versus Normal Force for Each Product";
title3 "Analysis Variable = " &y;
run;
%mend;
********* Experiment I Analysis ***********************;
%missy3(title= title1 "Experiment I Analysis"; , data=exp3, y=initialg, plotdata=exp3intg ) ;
%missy3(title= title1 "Experiment I Analysis"; , data=exp3, y=avgg, plotdata=exp3avgg) ;
%missy3(title= title1 "Experiment I Analysis"; , data=exp3, y=slopeg, plotdata=exp3slopeg ) ;
run;
quit;
```

EXPERIMENT 2: SAS ANALYSIS CODE

```
dm 'log; clear; output; clear; ';
*** filename:  exp2.sas ***;
data exp2;
infile 'h:\temp\clients\pearce, melissa\exp2.txt' dlm='09'x dsd missover firstobs=6 obs=41;
input blank product$ lot slice$ nforce initialg avgg slopeg;
*relax='yes';
drop blank;
*proc print data=exp2;
*title2 'Experiment 2 Data';
%macro missy2(title, data=, y= , plotdata= );
title;
proc mixed data=&data covtest cl ;
&title ;
title3 "Analysis Variable = " &y;
     Class  product lot slice nforce;
     model &y = nforce / ddfm=satterth;
     random lot(product) slice(lot);
                lsmeans nforce / pdiff ;
     ods output lsmeans=&plotdata;
run;
title;
proc gchart data=&plotdata;
vbar nforce / type=mean sumvar=estimate
          discrete;
&title ;
title2 "Response versus Normal Force";
title3 "Analysis Variable = " &y;
run;
%mend;
********* Experiment 2 Analysis ***********************;
%missy2(title= title1 "Experiment 2 Analysis"; , data=exp2, y=initialg, plotdata=exp2intg ) ;
%missy2(title= title1 "Experiment 2 Analysis"; , data=exp2, y=avgg, plotdata=exp2avgg) ;
%missy2(title= title1 "Experiment 2 Analysis"; , data=exp2, y=slopeg, plotdata=exp2slopeg ) ;
run;
quit;
```

EXPERIMENT 3: SAS ANALYSIS CODE

```
dm 'log; clear; output; clear; ';
*** filename:  exp3.sas ***;
data exp3;
infile 'h:\temp\clients\pearce, melissa\exp3.txt' dlm='09'x dsd missover firstobs=6 obs=53;
input blank product $ lot slice $ sample $ initialg avgg slopeg ;
drop blank ;
if sample='SM' then plate=1;
if sample='SR' then plate=2;

proc format;
value platefmt 0=' ' 1='SM' 2='SR' 3=' ' ;
*proc print data=exp3;
*title2 'Experiment 3 Data';
run;
%macro missy3(title, data=, y= , plotdata= );
title;
proc mixed data=&data covtest cl ;
&title ;
title3 "Analysis Variable = " &y;
     Class  product lot slice plate;
     model &y = product plate product*plate / ddfm=satterth;
     random lot(product) slice(lot product);
               lsmeans plate*product / slice=(plate product) ;
     ods output lsmeans=&plotdata;
     format plate platefmt. ;
run;
title;
symbol1 value=circle i=join cv=red l=1 w=2;
symbol2 value=dot i=join cv=red l=1 w=2;
proc gplot data=&plotdata;
plot estimate*plate = product / haxis= 1 to 2 by 1;
format plate platefmt. ;
&title ;
title2 "Response versus Plate for Each Product";
title3 "Analysis Variable = " &y;
run;
%mend;
********* Experiment 3 Analysis ***********************;
%missy3(title= title1 "Experiment 3 Analysis"; , data=exp3, y=initialg, plotdata=exp3intg ) ;
%missy3(title= title1 "Experiment 3 Analysis"; , data=exp3, y=avgg, plotdata=exp3avgg) ;
%missy3(title= title1 "Experiment 3 Analysis"; , data=exp3, y=slopeg, plotdata=exp3slopeg ) ;
run;
quit;
```

APPENDIX C

EXAMPLE OF SAS OUTPUT FOR EXPERIMENTS I, II, AND III

Experiment 1 Analysis
Analysis Variable = initialg

The Mixed Procedure

Model Information

Data Set	WORK.EXP1
Dependent Variable	initialg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	4	B C FFB Mz
lot	4	1 2 3 4
slice	3	A B C
nforce	2	H L

Dimensions

Covariance Parameters	3
Columns in X	15
Columns in Z	64
Subjects	1
Max Obs Per Subject	96
Observations Used	96
Observations Not Used	0
Total Observations	96

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1729.62981288	
1	1	1707.00629027	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	7337656	3784601	1.94	0.0263	0.05	3282499	28443738
slice(product*lot)	1128990	1396705	0.81	0.2095	0.05	253697	2.4466E8
Residual	8915662	0	.	.	.	.	.

Fit Statistics

-2 Res Log Likelihood	1707.0
AIC (smaller is better)	1713.0
AICC (smaller is better)	1713.3
BIC (smaller is better)	1715.3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
product	3	12	120.75	<.0001
nforce	1	88	123.24	<.0001
product*nforce	3	1	5.22	0.3088

Least Squares Means

Effect	product	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
product*nforce	B	H	32118	1634.46	16.2	19.65	<.0001
product*nforce	B	L	24482	1634.46	16.2	14.98	<.0001
product*nforce	C	H	61212	1634.46	16.2	37.45	<.0001
product*nforce	C	L	57729	1634.46	16.2	35.32	<.0001
product*nforce	FFB	H	33208	1634.46	16.2	20.32	<.0001
product*nforce	FFB	L	27334	1634.46	16.2	16.72	<.0001
product*nforce	Mz	H	61658	1634.46	16.2	37.72	<.0001
product*nforce	Mz	L	51585	1634.46	16.2	31.56	<.0001
product*nforce	B	H	32118	1634.46	16.2	19.65	<.0001
product*nforce	B	L	24482	1634.46	16.2	14.98	<.0001
product*nforce	C	H	61212	1634.46	16.2	37.45	<.0001
product*nforce	C	L	57729	1634.46	16.2	35.32	<.0001
product*nforce	FFB	H	33208	1634.46	16.2	20.32	<.0001
product*nforce	FFB	L	27334	1634.46	16.2	16.72	<.0001
product*nforce	Mz	H	61658	1634.46	16.2	37.72	<.0001
product*nforce	Mz	L	51585	1634.46	16.2	31.56	<.0001

Least Squares Means

Effect	product	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce		H	47049	817.23	16.2	57.57	<.0001
nforce		L	40283	817.23	16.2	49.29	<.0001
product	B		28300	1516.57	12	18.66	<.0001
product	C		59470	1516.57	12	39.21	<.0001
product	FFB		30271	1516.57	12	19.96	<.0001
product	Mz		56622	1516.57	12	37.34	<.0001

Differences of Least Squares Means

Effect	product	nforce	_product	_nforce	Standard Error	DF	t Value	Pr > |t|
product*nforce	B	H	B	L	1218.99	88	6.26	<.0001
product*nforce	B	H	C	H	2311.48	16.2	-12.59	<.0001
product*nforce	B	H	C	L	2311.48	16.2	-11.08	<.0001
product*nforce	B	H	FFB	H	2311.48	16.2	-0.47	0.6435
product*nforce	B	H	FFB	L	2311.48	16.2	2.07	0.0549
product*nforce	B	H	Mz	H	2311.48	16.2	-12.78	<.0001
product*nforce	B	H	Mz	L	2311.48	16.2	-8.42	<.0001
product*nforce	B	L	C	H	2311.48	16.2	-15.89	<.0001
product*nforce	B	L	C	L	2311.48	16.2	-14.38	<.0001
product*nforce	B	L	FFB	H	2311.48	16.2	-3.77	0.0016
product*nforce	B	L	FFB	L	2311.48	16.2	-1.23	0.2348
product*nforce	B	L	Mz	H	2311.48	16.2	-16.08	<.0001
product*nforce	B	L	Mz	L	2311.48	16.2	-11.73	<.0001
product*nforce	C	H	C	L	1218.99	88	2.86	0.0053
product*nforce	C	H	FFB	H	2311.48	16.2	12.12	<.0001
product*nforce	C	H	FFB	L	2311.48	16.2	14.66	<.0001
product*nforce	C	H	Mz	H	2311.48	16.2	-0.19	0.8492
product*nforce	C	H	Mz	L	2311.48	16.2	4.16	0.0007

product*nforce	C	L	FFB	H	2311.48	16.2	10.61	<.0001
product*nforce	C	L	FFB	L	2311.48	16.2	13.15	<.0001
product*nforce	C	L	Mz	H	2311.48	16.2	-1.70	0.1083
product*nforce	C	L	Mz	L	2311.48	16.2	2.66	0.0171
product*nforce	FFB	H	FFB	L	1218.99	88	4.82	<.0001
product*nforce	FFB	H	Mz	H	2311.48	16.2	-12.31	<.0001
product*nforce	FFB	H	Mz	L	2311.48	16.2	-7.95	<.0001
product*nforce	FFB	L	Mz	H	2311.48	16.2	-14.85	<.0001
product*nforce	FFB	L	Mz	L	2311.48	16.2	-10.49	<.0001
product*nforce	Mz	H	Mz	L	1218.99	88	8.26	<.0001
nforce		H		L	609.50	88	11.10	<.0001
product	B		C		2144.75	12	-14.53	<.0001
product	B		FFB		2144.75	12	-0.92	0.3761
product	B		Mz		2144.75	12	-13.21	<.0001
product	C		FFB		2144.75	12	13.61	<.0001
product	C		Mz		2144.75	12	1.33	0.2088
product	FFB		Mz		2144.75	12	-12.29	<.0001

Tests of Effect Slices

Effect	product	nforce	Num DF	Den DF	F Value	Pr > F
product*nforce		H	3	16.2	103.38	<.0001
product*nforce		L	3	16.2	105.99	<.0001
product*nforce	B		1	88	39.24	<.0001
product*nforce	C		1	88	8.16	0.0053
product*nforce	FFB		1	88	23.21	<.0001
product*nforce	Mz		1	88	68.28	<.0001

Experiment 1 Analysis
Analysis Variable = avgg

The Mixed Procedure

Model Information

Data Set	WORK.EXP1
Dependent Variable	avgg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	4	B C FFB Mz
lot	4	1 2 3 4
slice	3	A B C
nforce	2	H L

Dimensions

Covariance Parameters	3
Columns in X	15
Columns in Z	64
Subjects	1
Max Obs Per Subject	96
Observations Used	96
Observations Not Used	0
Total Observations	96

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	1718.36216772	
1	2	1693.94439246	0.00000002
2	1	1693.94437489	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	7023555	3437039	2.04	0.0205	0.05	3247726	24859398
slice(product*lot)	0	.	.	.	.	.	.
Residual	8372892	0	.	.	.	.	.

Fit Statistics

-2 Res Log Likelihood	1693.9
AIC (smaller is better)	1697.9
AICC (smaller is better)	1698.1
BIC (smaller is better)	1699.5

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
product	3	12	121.22	<.0001
nforce	1	88	130.64	<.0001
product*nforce	3	1	10.27	0.2246

Least Squares Means

Effect	product	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
product*nforce	B	H	29153	1566.41	16.3	18.61	<.0001
product*nforce	B	L	19124	1566.41	16.3	12.21	<.0001
product*nforce	C	H	56737	1566.41	16.3	36.22	<.0001
product*nforce	C	L	55350	1566.41	16.3	35.34	<.0001
product*nforce	FFB	H	31920	1566.41	16.3	20.38	<.0001
product*nforce	FFB	L	24926	1566.41	16.3	15.91	<.0001
product*nforce	Mz	H	55363	1566.41	16.3	35.34	<.0001
product*nforce	Mz	L	46768	1566.41	16.3	29.86	<.0001
product*nforce	B	H	29153	1566.41	16.3	18.61	<.0001
product*nforce	B	L	19124	1566.41	16.3	12.21	<.0001
product*nforce	C	H	56737	1566.41	16.3	36.22	<.0001
product*nforce	C	L	55350	1566.41	16.3	35.34	<.0001
product*nforce	FFB	H	31920	1566.41	16.3	20.38	<.0001
product*nforce	FFB	L	24926	1566.41	16.3	15.91	<.0001
product*nforce	Mz	H	55363	1566.41	16.3	35.34	<.0001
product*nforce	Mz	L	46768	1566.41	16.3	29.86	<.0001

Least Squares Means

Effect	product	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce		H	43293	783.20	16.3	55.28	<.0001
nforce		L	36542	783.20	16.3	46.66	<.0001
product	B		24138	1450.78	12	16.64	<.0001
product	C		56043	1450.78	12	38.63	<.0001
product	FFB		28423	1450.78	12	19.59	<.0001
product	Mz		51066	1450.78	12	35.20	<.0001

Differences of Least Squares Means

Effect	product	nforce	_product	_nforce	Standard Error	DF	t Value	Pr > |t|
product*nforce	B	H	B	L	1181.31	88	8.49	<.0001
product*nforce	B	H	C	H	2215.23	16.3	-12.45	<.0001
product*nforce	B	H	C	L	2215.23	16.3	-11.83	<.0001
product*nforce	B	H	FFB	H	2215.23	16.3	-1.25	0.2293
product*nforce	B	H	FFB	L	2215.23	16.3	1.91	0.0742
product*nforce	B	H	Mz	H	2215.23	16.3	-11.83	<.0001
product*nforce	B	H	Mz	L	2215.23	16.3	-7.95	<.0001
product*nforce	B	L	C	H	2215.23	16.3	-16.98	<.0001
product*nforce	B	L	C	L	2215.23	16.3	-16.35	<.0001
product*nforce	B	L	FFB	H	2215.23	16.3	-5.78	<.0001
product*nforce	B	L	FFB	L	2215.23	16.3	-2.62	0.0184
product*nforce	B	L	Mz	H	2215.23	16.3	-16.36	<.0001
product*nforce	B	L	Mz	L	2215.23	16.3	-12.48	<.0001
product*nforce	C	H	C	L	1181.31	88	1.17	0.2436
product*nforce	C	H	FFB	H	2215.23	16.3	11.20	<.0001
product*nforce	C	H	FFB	L	2215.23	16.3	14.36	<.0001
product*nforce	C	H	Mz	H	2215.23	16.3	0.62	0.5438
product*nforce	C	H	Mz	L	2215.23	16.3	4.50	0.0003
product*nforce	C	L	FFB	H	2215.23	16.3	10.58	<.0001

product*nforce	C	L	FFB	L	2215.23	16.3	13.73	<.0001
product*nforce	C	L	Mz	H	2215.23	16.3	-0.01	0.9953
product*nforce	C	L	Mz	L	2215.23	16.3	3.87	0.0013
product*nforce	FFB	H	FFB	L	1181.31	88	5.92	<.0001
product*nforce	FFB	H	Mz	H	2215.23	16.3	-10.58	<.0001
product*nforce	FFB	H	Mz	L	2215.23	16.3	-6.70	<.0001
product*nforce	FFB	L	Mz	H	2215.23	16.3	-13.74	<.0001
product*nforce	FFB	L	Mz	L	2215.23	16.3	-9.86	<.0001
product*nforce	Mz	H	Mz	L	1181.31	88	7.28	<.0001
nforce		H		L	590.65	88	11.43	<.0001
product	B		C		2051.71	12	-15.55	<.0001
product	B		FFB		2051.71	12	-2.09	0.0587
product	B		Mz		2051.71	12	-13.12	<.0001
product	C		FFB		2051.71	12	13.46	<.0001
product	C		Mz		2051.71	12	2.43	0.0320
product	FFB		Mz		2051.71	12	-11.04	<.0001

Tests of Effect Slices

Effect	product	nforce	Num DF	Den DF	F Value	Pr > F
product*nforce		H	3	16.3	89.08	<.0001
product*nforce		L	3	16.3	121.81	<.0001
product*nforce	B		1	88	72.08	<.0001
product*nforce	C		1	88	1.38	0.2436
product*nforce	FFB		1	88	35.05	<.0001
product*nforce	Mz		1	88	52.94	<.0001

Experiment 1 Analysis
Analysis Variable = slopeg

The Mixed Procedure

Model Information

Data Set	WORK.EXP1
Dependent Variable	slopeg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	4	B C FFB Mz
lot	4	1 2 3 4
slice	3	A B C
nforce	2	H L

Dimensions

Covariance Parameters	3
Columns in X	15
Columns in Z	64
Subjects	1
Max Obs Per Subject	96
Observations Used	96
Observations Not Used	0
Total Observations	96

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	371.79683610	
1	1	361.46830850	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	0.6767	0.5218	1.30	0.0973	0.05	0.2273	7.5138
slice(product*lot)	0.6924	0.4654	1.49	0.0684	0.05	0.2581	4.9123
Residual	1.9477	0.4152	4.69	<.0001	0.05	1.3348	3.1078

Fit Statistics

-2 Res Log Likelihood	361.5
AIC (smaller is better)	367.5
AICC (smaller is better)	367.8
BIC (smaller is better)	369.8

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
product	3	12	14.51	0.0003
nforce	1	44	88.52	<.0001
product*nforce	3	44	30.66	<.0001

Least Squares Means

Effect	product	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
product*nforce	B	H	-6.3933	0.6238	18.8	-10.25	<.0001
product*nforce	B	L	-13.5408	0.6238	18.8	-21.71	<.0001
product*nforce	C	H	-6.7650	0.6238	18.8	-10.84	<.0001
product*nforce	C	L	-7.7567	0.6238	18.8	-12.43	<.0001
product*nforce	FFB	H	-3.6100	0.6238	18.8	-5.79	<.0001
product*nforce	FFB	L	-6.1633	0.6238	18.8	-9.88	<.0001
product*nforce	Mz	H	-8.1617	0.6238	18.8	-13.08	<.0001
product*nforce	Mz	L	-8.1900	0.6238	18.8	-13.13	<.0001
product*nforce	B	H	-6.3933	0.6238	18.8	-10.25	<.0001
product*nforce	B	L	-13.5408	0.6238	18.8	-21.71	<.0001
product*nforce	C	H	-6.7650	0.6238	18.8	-10.84	<.0001
product*nforce	C	L	-7.7567	0.6238	18.8	-12.43	<.0001
product*nforce	FFB	H	-3.6100	0.6238	18.8	-5.79	<.0001
product*nforce	FFB	L	-6.1633	0.6238	18.8	-9.88	<.0001
product*nforce	Mz	H	-8.1617	0.6238	18.8	-13.08	<.0001
product*nforce	Mz	L	-8.1900	0.6238	18.8	-13.13	<.0001

Least Squares Means

Effect	product	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce		H	-6.2325	0.3119	18.8	-19.98	<.0001
nforce		L	-8.9127	0.3119	18.8	-28.57	<.0001
product	B		-9.9671	0.5550	12	-17.96	<.0001
product	C		-7.2608	0.5550	12	-13.08	<.0001
product	FFB		-4.8867	0.5550	12	-8.80	<.0001
product	Mz		-8.1758	0.5550	12	-14.73	<.0001

Differences of Least Squares Means

Effect	product	nforce	_product	_nforce	Standard Error	DF	t Value	Pr > |t|
product*nforce	B	H	B	L	0.5697	44	12.55	<.0001
product*nforce	B	H	C	H	0.8823	18.8	0.42	0.6783
product*nforce	B	H	C	L	0.8823	18.8	1.55	0.1389
product*nforce	B	H	FFB	H	0.8823	18.8	-3.15	0.0053
product*nforce	B	H	FFB	L	0.8823	18.8	-0.26	0.7972
product*nforce	B	H	Mz	H	0.8823	18.8	2.00	0.0597
product*nforce	B	H	Mz	L	0.8823	18.8	2.04	0.0560
product*nforce	B	L	C	H	0.8823	18.8	-7.68	<.0001
product*nforce	B	L	C	L	0.8823	18.8	-6.56	<.0001
product*nforce	B	L	FFB	H	0.8823	18.8	-11.26	<.0001
product*nforce	B	L	FFB	L	0.8823	18.8	-8.36	<.0001
product*nforce	B	L	Mz	H	0.8823	18.8	-6.10	<.0001
product*nforce	B	L	Mz	L	0.8823	18.8	-6.06	<.0001
product*nforce	C	H	C	L	0.5697	44	1.74	0.0888
product*nforce	C	H	FFB	H	0.8823	18.8	-3.58	0.0020
product*nforce	C	H	FFB	L	0.8823	18.8	-0.68	0.5036
product*nforce	C	H	Mz	H	0.8823	18.8	1.58	0.1301
product*nforce	C	H	Mz	L	0.8823	18.8	1.62	0.1229
product*nforce	C	L	FFB	H	0.8823	18.8	-4.70	0.0002
product*nforce	C	L	FFB	L	0.8823	18.8	-1.81	0.0870
product*nforce	C	L	Mz	H	0.8823	18.8	0.46	0.6515

product*nforce	C	L	Mz	L	0.8823	18.8	0.49	0.6290
product*nforce	FFB	H	FFB	L	0.5697	44	4.48	<.0001
product*nforce	FFB	H	Mz	H	0.8823	18.8	5.16	<.0001
product*nforce	FFB	H	Mz	L	0.8823	18.8	5.19	<.0001
product*nforce	FFB	L	Mz	H	0.8823	18.8	2.27	0.0355
product*nforce	FFB	L	Mz	L	0.8823	18.8	2.30	0.0333
product*nforce	Mz	H	Mz	L	0.5697	44	0.05	0.9606
nforce		H		L	0.2849	44	9.41	<.0001
product	B		C		0.7849	12	-3.45	0.0048
product	B		FFB		0.7849	12	-6.47	<.0001
product	B		Mz		0.7849	12	-2.28	0.0415
product	C		FFB		0.7849	12	-3.02	0.0106
product	C		Mz		0.7849	12	1.17	0.2664
product	FFB		Mz		0.7849	12	4.19	0.0013

Tests of Effect Slices

Effect	product	nforce	Num DF	Den DF	F Value	Pr > F
product*nforce		H	3	18.8	9.34	0.0005
product*nforce		L	3	18.8	26.41	<.0001
product*nforce	B		1	44	157.38	<.0001
product*nforce	C		1	44	3.03	0.0888
product*nforce	FFB		1	44	20.08	<.0001
product*nforce	Mz		1	44	0.00	0.9606

Experiment 2 Analysis
Analysis Variable = initialg

The Mixed Procedure

Model Information

Data Set	WORK.EXP2
Dependent Variable	initialg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	1	B
lot	4	1 2 3 4
slice	3	A B C
nforce	3	1 10 20

Dimensions

Covariance Parameters	3
Columns in X	4
Columns in Z	16
Subjects	1
Max Obs Per Subject	36
Observations Used	36
Observations Not Used	0
Total Observations	36

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	670.19826689	
1	1	661.07321519	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	13655997	13125986	1.04	0.1491	0.05	3829323	4.2436E8
slice(lot)	750307	3532743	0.21	0.4159	0.05	65668	1.907E40
Residual	8945533	0	.	.	.	.	.

Fit Statistics

-2 Res Log Likelihood	661.1
AIC (smaller is better)	667.1
AICC (smaller is better)	667.9
BIC (smaller is better)	665.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
nforce	2	1	1.66	0.4810

Least Squares Means

Effect	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce	1	37621	2248.40	4.79	16.73	<.0001
nforce	10	34526	2248.40	4.79	15.36	<.0001
nforce	20	35246	2248.40	4.79	15.68	<.0001

Differences of Least Squares Means

Effect	nforce	_nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce	1	10	3095.00	1776.96	1	1.74	0.3318
nforce	1	20	2375.00	1776.96	33	1.34	0.1905
nforce	10	20	-720.00	1776.96	33	-0.41	0.6880

Experiment 2 Analysis
Analysis Variable = avgg

The Mixed Procedure

Model Information

Data Set	WORK.EXP2
Dependent Variable	avgg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	1	B
lot	4	1 2 3 4
slice	3	A B C
nforce	3	1 10 20

Dimensions

Covariance Parameters	3
Columns in X	4
Columns in Z	16
Subjects	1
Max Obs Per Subject	36
Observations Used	36
Observations Not Used	0
Total Observations	36

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	659.67743894	
1	1	650.16260081	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	9541687	9538889	1.00	0.1586	0.05	2587261	3.7618E8
slice(lot)	2006011	3107876	0.65	0.2593	0.05	367428	7.8168E9
Residual	12629222	0	.	.	.	.	.

Fit Statistics

-2 Res Log Likelihood	650.2
AIC (smaller is better)	656.2
AICC (smaller is better)	657.0
BIC (smaller is better)	654.3

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
nforce	2	1	1.59	0.4896

Least Squares Means

Effect	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce	1	26691	1898.69	4.63	14.06	<.0001
nforce	10	29142	1898.69	4.63	15.35	<.0001
nforce	20	28625	1898.69	4.63	15.08	<.0001

Differences of Least Squares Means

Effect	nforce	_nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce	1	10	-2450.79	1450.82	1	-1.69	0.3403
nforce	1	20	-1933.79	1450.82	33	-1.33	0.1917
nforce	10	20	517.00	1450.82	33	0.36	0.7238

Experiment 2 Analysis
Analysis Variable = slopeg

The Mixed Procedure

Model Information

Data Set	WORK.EXP2
Dependent Variable	slopeg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	1	B
lot	4	1 2 3 4
slice	3	A B C
nforce	3	1 10 20

Dimensions

Covariance Parameters	3
Columns in X	4
Columns in Z	16
Subjects	1
Max Obs Per Subject	36
Observations Used	36
Observations Not Used	0
Total Observations	36

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	120.80118523	
1	3	119.47246999	0.00000363
2	1	119.47236211	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	0.2508	0.3539	0.71	0.2393	0.05	0.05002	248.28
slice(lot)	0	.	.	.	.	.	.
Residual	1.6112	0.4160	3.87	<.0001	0.05	1.0289	2.8787

Fit Statistics

-2 Res Log Likelihood	119.5
AIC (smaller is better)	123.5
AICC (smaller is better)	123.9
BIC (smaller is better)	122.2

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
nforce	2	30	23.38	<.0001

Least Squares Means

Effect	nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce	1	-12.6600	0.4438	9.43	-28.53	<.0001
nforce	10	-9.7483	0.4438	9.43	-21.97	<.0001
nforce	20	-9.4550	0.4438	9.43	-21.30	<.0001

Differences of Least Squares Means

Effect	nforce	_nforce	Estimate	Standard Error	DF	t Value	Pr > |t|
nforce	1	10	-2.9117	0.5182	30	-5.62	<.0001
nforce	1	20	-3.2050	0.5182	30	-6.18	<.0001
nforce	10	20	-0.2933	0.5182	30	-0.57	0.5756

Experiment 3 Analysis
Analysis Variable = initialg

The Mixed Procedure

Model Information

Data Set	WORK.EXP3
Dependent Variable	initialg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	2	B FFB
lot	4	1 2 3 4
slice	3	A B C
plate	2	SM SR

Dimensions

Covariance Parameters	3
Columns in X	9
Columns in Z	32
Subjects	1
Max Obs Per Subject	48
Observations Used	48
Observations Not Used	0
Total Observations	48

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	782.33096726	
1	1	764.87441469	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	1175964	893639	1.32	0.0941	0.05	399434	12377558
slice(product*lot)	638584	399695	1.60	0.0551	0.05	250668	3741217
Residual	861232	259671	3.32	0.0005	0.05	515137	1725236

Fit Statistics

-2 Res Log Likelihood	764.9
AIC (smaller is better)	770.9
AICC (smaller is better)	771.5
BIC (smaller is better)	771.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
product	1	6	25.55	0.0023
plate	1	22	1716.62	<.0001
product*plate	1	22	29.11	<.0001

Least Squares Means

Effect	product	plate	Estimate	Standard Error	DF	t Value	Pr > |t|
product*plate	B	SM	40890	647.28	7.16	63.17	<.0001
product*plate	B	SR	28345	647.28	7.16	43.79	<.0001
product*plate	FFB	SM	35020	647.28	7.16	54.10	<.0001
product*plate	FFB	SR	25366	647.28	7.16	39.19	<.0001

Tests of Effect Slices

Effect	product	plate	Num DF	Den DF	F Value	Pr > F
product*plate		SM	1	7.16	41.12	0.0003
product*plate		SR	1	7.16	10.59	0.0135
product*plate	B		1	22	1096.41	<.0001
product*plate	FFB		1	22	649.32	<.0001

Experiment 3 Analysis
Analysis Variable = avgg

The Mixed Procedure

Model Information

Data Set	WORK.EXP3
Dependent Variable	avgg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	2	B FFB
lot	4	1 2 3 4
slice	3	A B C
plate	2	SM SR

Dimensions

Covariance Parameters	3
Columns in X	9
Columns in Z	32
Subjects	1
Max Obs Per Subject	48
Observations Used	48
Observations Not Used	0
Total Observations	48

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	797.58196820	
1	1	776.80645431	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	2096049	1438172	1.46	0.0725	0.05	769568	15786252
slice(product*lot)	536009	447406	1.20	0.1155	0.05	168981	8183663
Residual	1230930	371139	3.32	0.0005	0.05	736268	2465822

Fit Statistics

-2 Res Log Likelihood	776.8
AIC (smaller is better)	782.8
AICC (smaller is better)	783.4
BIC (smaller is better)	783.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
product	1	6	1.10	0.3345
plate	1	22	676.97	<.0001
product*plate	1	22	0.24	0.6298

Least Squares Means

Effect	product	plate	Estimate	Standard Error	DF	t Value	Pr > |t|
product*plate	B	SM	33923	819.30	7.02	41.40	<.0001
product*plate	B	SR	25746	819.30	7.02	31.42	<.0001
product*plate	FFB	SM	32911	819.30	7.02	40.17	<.0001
product*plate	FFB	SR	24421	819.30	7.02	29.81	<.0001

Tests of Effect Slices

Effect	product	plate	Num DF	Den DF	F Value	Pr > F
product*plate		SM	1	7.02	0.76	0.4115
product*plate		SR	1	7.02	1.31	0.2904
product*plate	B		1	22	325.89	<.0001
product*plate	FFB		1	22	351.32	<.0001

Experiment 3 Analysis
Analysis Variable = slopeg

The Mixed Procedure

Model Information

Data Set	WORK.EXP3
Dependent Variable	slopeg
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
product	2	B FFB
lot	4	1 2 3 4
slice	3	A B C
plate	2	SM SR

Dimensions

Covariance Parameters	3
Columns in X	9
Columns in Z	32
Subjects	1
Max Obs Per Subject	48
Observations Used	48
Observations Not Used	0
Total Observations	48

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	243.81169132	
1	1	243.81169132	0.00000000

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
lot(product)	0	.	.	.	.	.	.
slice(product*lot)	0	.	.	.	.	.	.
Residual	11.9102	2.5393	4.69	<.0001	0.05	8.1626	19.0048

Fit Statistics

-2 Res Log Likelihood	243.8
AIC (smaller is better)	245.8
AICC (smaller is better)	245.9
BIC (smaller is better)	245.9

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
product	1	44	120.19	<.0001
plate	1	44	160.67	<.0001
product*plate	1	44	43.01	<.0001

Least Squares Means

Effect	product	plate	Estimate	Standard Error	DF	t Value	Pr > |t|
product*plate	B	SM	-26.8517	0.9963	44	-26.95	<.0001
product*plate	B	SR	-7.6900	0.9963	44	-7.72	<.0001
product*plate	FFB	SM	-9.3958	0.9963	44	-9.43	<.0001
product*plate	FFB	SR	-3.3017	0.9963	44	-3.31	0.0018

Tests of Effect Slices

Effect	product	plate	Num DF	Den DF	F Value	Pr > F
product*plate		SM	1	44	153.50	<.0001
product*plate		SR	1	44	9.70	0.0032
product*plate	B		1	44	184.97	<.0001
product*plate	FFB		1	44	18.71	<.0001

APPENDIX D

G" DATA FOR EXPERIMENTS I, II, AND III: PLOTS OF OSCILLATION STRESS VS. TESTING VARIABLES

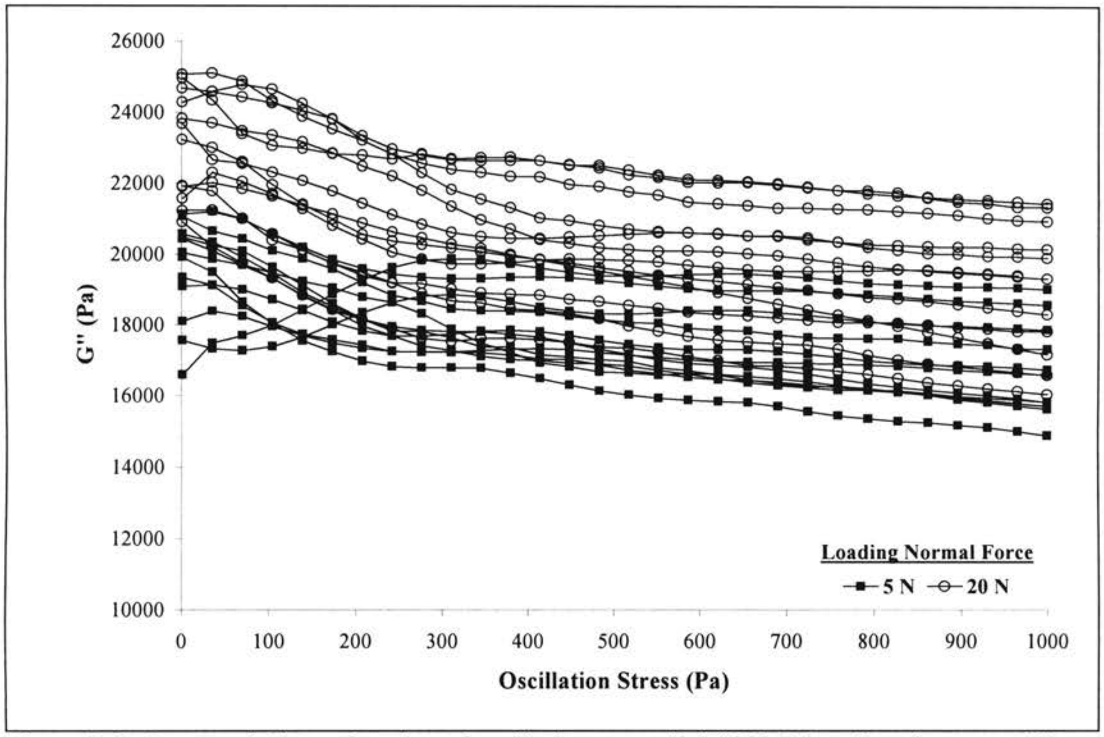

Figure D-1. Loss moduli as a function of oscillation stress (0.1-1000 Pa) and testing normal force (5 or 20 N) of mozzarella samples.

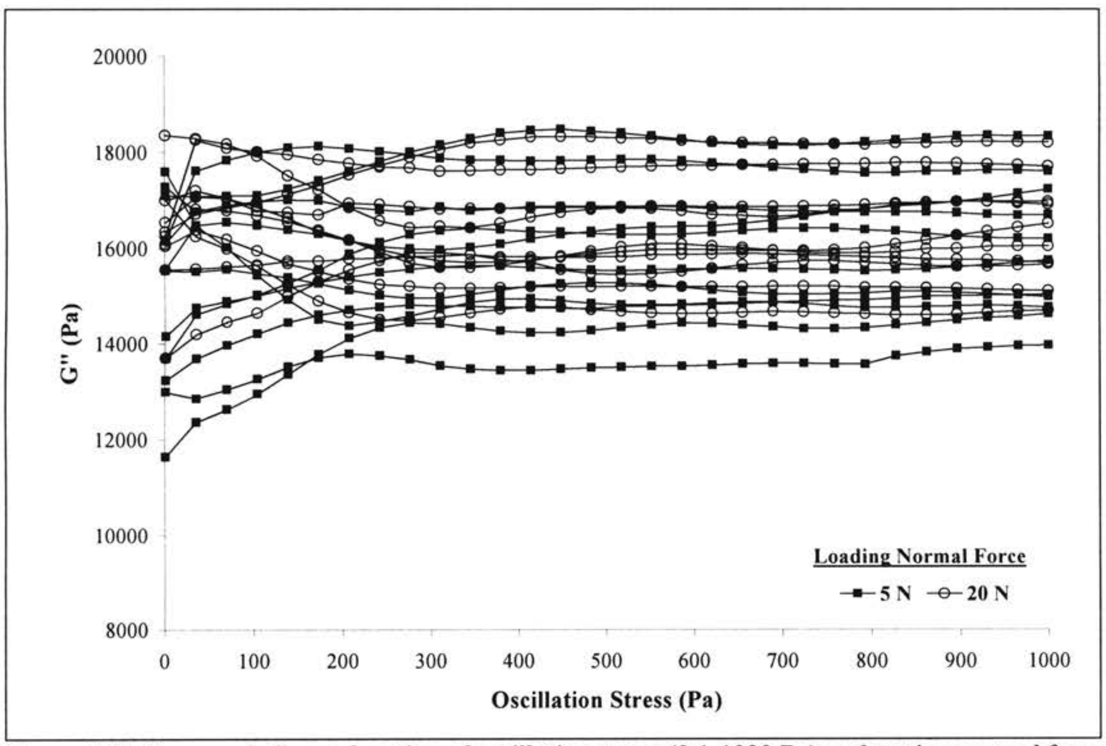

Figure D-2. Loss moduli as a function of oscillation stress (0.1-1000 Pa) and testing normal force (5 or 20 N) of cheddar samples.

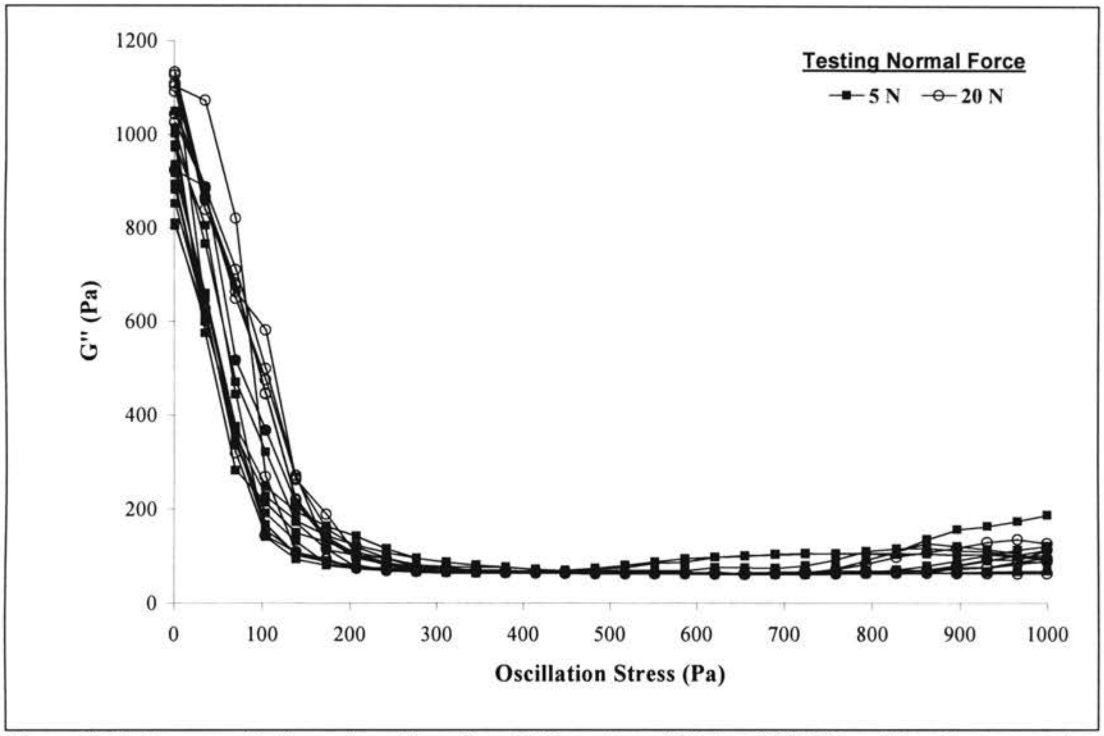

Figure D-3. Loss moduli as a function of oscillation stress (0.1 to 1000 Pa) and loading normal force (5 or 20N) of mustard samples (Experiment I).

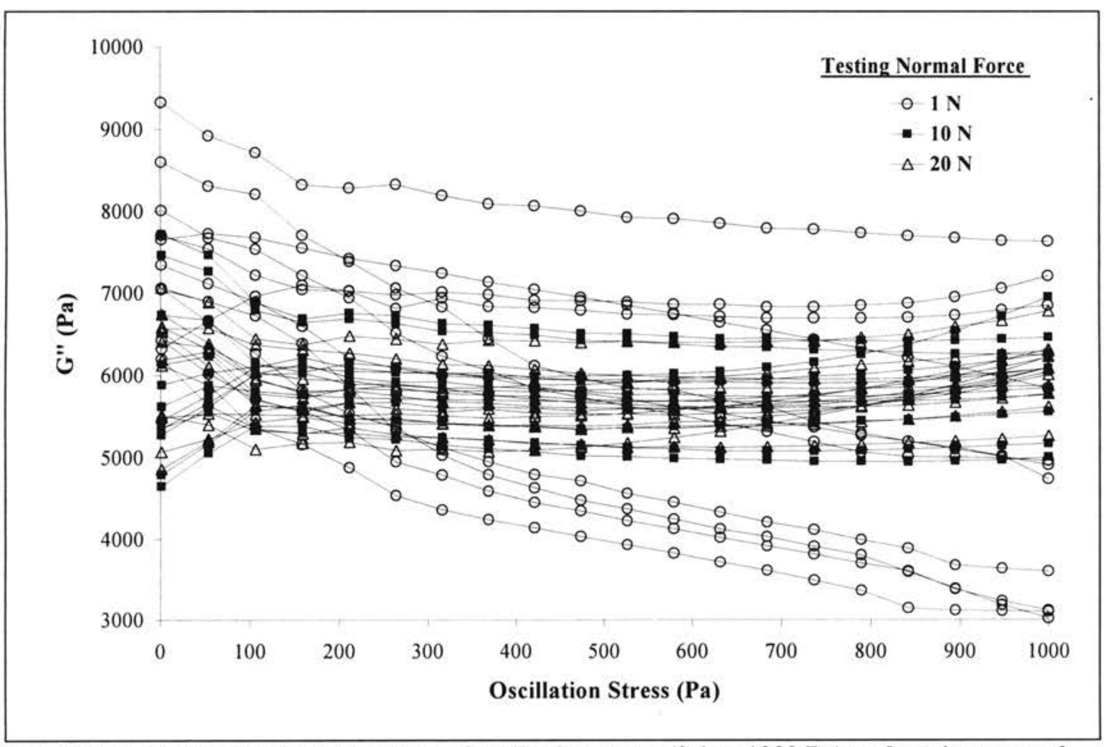

Figure D-4. Loss moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing normal force (1, 10, or 20N) of bologna samples (Experiment II).

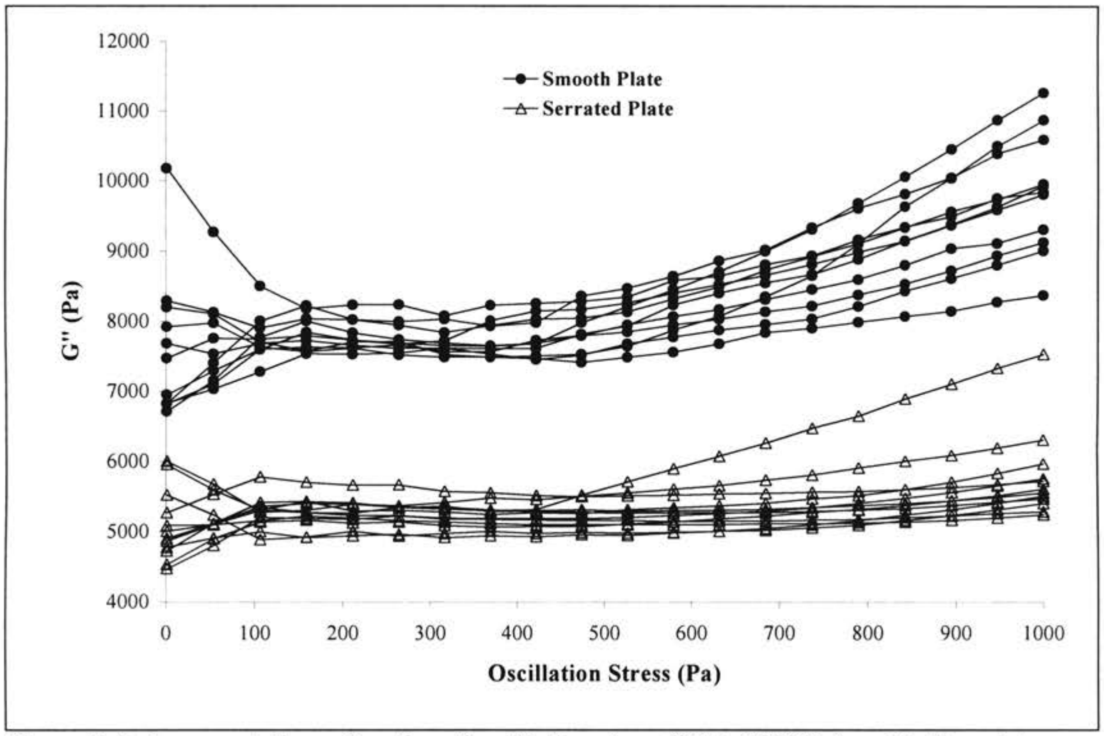

Figure D-5. Loss moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing plate (smooth or serrated) of regular bologna samples loaded and tested at 15N (Experiment III).

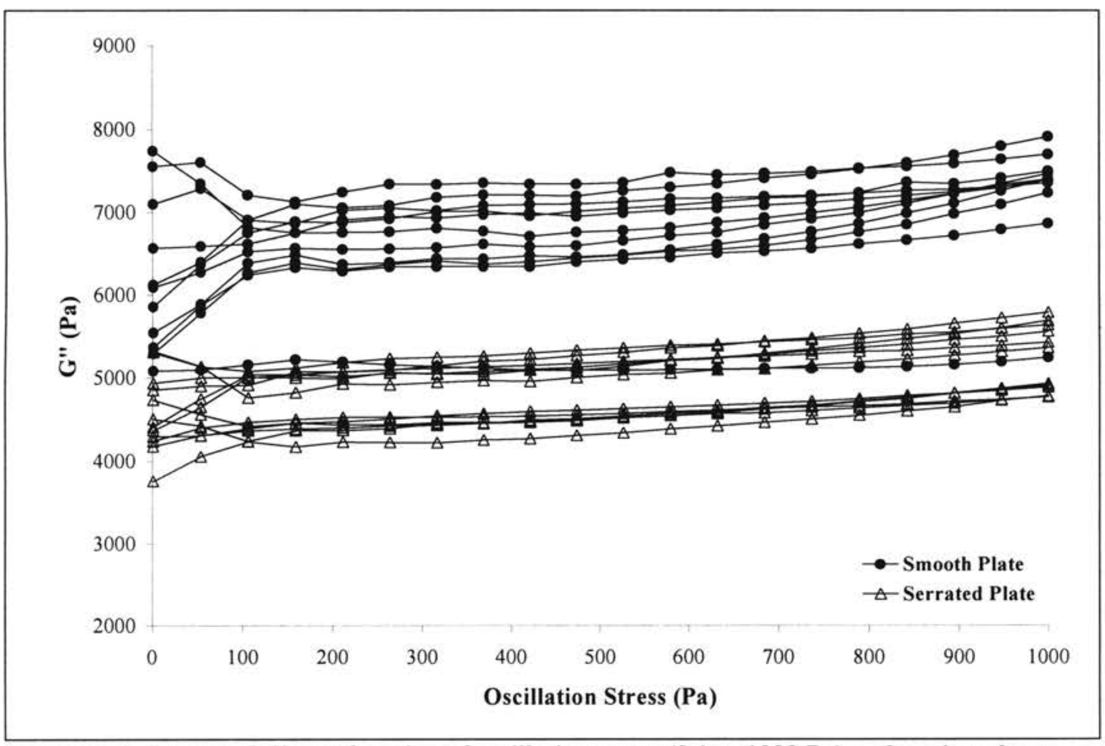

Figure D-6. Loss moduli as a function of oscillation stress (0.1 to 1000 Pa) and testing plate (smooth or serrated) of fat-free bologna samples loaded and tested at 15N (Experiment III).

ə

VITA

Melissa Jean Daniels Pearce

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF NORMAL FORCE, SAMPLE RELAXATION, AND TESTING GEOMETRY ON THE RHEOLOGICAL MEASUREMENTS OF SEMI-SOLID FOOD PRODUCTS

Major Field: Biosystems Engineering

Biographical:

Education: Graduated from Fayetteville High School, Fayetteville, Arkansas in 1992. Received Bachelor of Science and Master of Science degrees in Biological and Agricultural Engineering from the University of Arkansas in August of 1996 and May of 1998, respectively. Completed the requirements for the degree of Doctor Of Philosophy with a major in Biosystems and Agricultural Engineering at Oklahoma State University in December, 2001.

Experience: Conducted research in the areas of sensory analysis of food products, rough rice storage, and oscillatory measurements of semi-solid foods.

Professional Memberships: American Society of Agricultural Engineers; Institute of Food Technologists