THE APPLICATION OF EDIBLE BARRIER FILMS TO BEEF IN ORDER TO DELAY LIPID OXIDATION

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By

A. Courtney Hopper

1996-97 University Undergraduate Research Fellow

Texas A&M University

Group: BIOCHEMISTRY/VAPH

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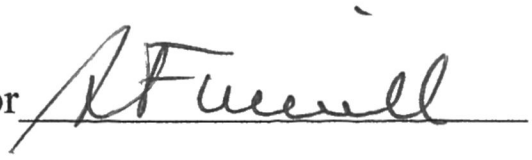
A. Courtney Hopper
University Undergraduate Fellow, 1996-1997
Texas A&M University
Department of Food Science

APPROVED

Fellows Advisor 

Honors Director 

THE APPLICATION OF EDIBLE BARRIER FILMS TO BEEF IN ORDER TO DELAY LIPID OXIDATION. A. Courtney Hopper (Dr. C.W. Dill), Food Science, Texas A&M Univeristy

Americans now live in a world of convenience. The emergence of dual income families has resulted in a population that relies heavily on prepackaged/preprepared foods. The quality of commercially prepared foods is definitely improving. However, commercially prepared foods often lack the fresh flavor, typical texture, and overall quality of foods prepared at home from scratch. The use of meats in commercially prepared foods presents a special challenge. The rapid development of warmed-over flavor (WOF) in cooked refrigerated meat is a major obstacle that seems to become most obvious during reheating. Research indicates that lipid oxidation is what causes WOF.

Many foods have natural films/barriers for protection, ie. peanuts. By the same token, an edible protein film can be directly applied to a meat surface. Consequently, this film should protect against oxygen and moisture loss on the meat surface. Presumably, the inevitable onset of WOF can be greatly slowed through the application of this film.

Much of this research focused on the methodology for applying edible barrier film(s) to beef in order to control moisture and oxygen gas interchanges at the surface. Top round roasts were used, and I was able to successfully develop a zein film that could be sprayed directly on the meat surface. The extent of lipid oxidation was measured chemically by using the TBA test. The majority of lipid oxidation occurred near the meat surface according to TBA values. The values also indicated that lipid oxidation increases with time; moreover, the extent of lipid oxidation is less in meat samples applied with film. The zein film was observed by using scanning electron microscopy. The prevention of oxygen and moisture loss through the application of edible films could have a tremendous effect on extended shelf life.

OBJECTIVES:

1. To develop the technology for controlled addition of edible barrier film-forming materials directly onto the surface of fresh and cooked retail cuts of beef.
2. To determine the ability of applied film materials to stabilize moisture and oxygen activity at the surface of raw and cooked meats.
3. To measure the difference in lipid oxidation levels in raw and cooked beef (with and without film) by using the TBA test.
4. To examine the surface of beef with and without film by using scanning electron microscopy.

LITERATURE REVIEW

Usually, an edible film is defined as a thin layer of edible material produced on a food as a coating or placed (pre-formed) on or between food components. While many foods possess natural films, eg. fats and waxes, to protect them from harmful environmental factors, this protection erodes when surfaces are cut as during peeling and trimming. Edible films will allow cut products this environmental protection.

Successful edible films formed as coatings on foods by dipping or spraying could lessen packaging requirements and waste. According to Kester and Fennema, edible coatings and films are not intended to, nor could they ever, replace nonedible, synthetic packaging methods for extended storage of foods. However, the great advantage of edible films lies in their ability to act as an adjunct for improving overall food quality, extending shelf life, and possibly improving economic efficiency of packaging methods. Moreover, McHugh, Avena-Bustillos, and Krochta acknowledge in their research that consumers require greater quality and longer shelf life in foods while reducing disposable packaging methods and thereby increasing recyclibility. In fact, food packaging has become a primary focus of waste reduction efforts. Hunt et al. observed that packaging accounts for approximately 30 wt % of municipal solid waste (MSW), but seems more significant because it occupies close to two-thirds of trash can volume

due to its bulk. Edible and biodegradable polymer films offer alternate packaging without the environmental damage. While edible coatings are not intended to completely replace synthetic packaging films, they do have the ability to reduce packaging and to limit moisture, aroma, and lipid migration between food components where traditional packaging cannot function. In addition to reducing the amount of packaging, the barrier properties of an edible film coating may also permit conversion from a multilayer, multicomponent plastic package to a single component recyclable package. Edible coatings may also help preserve food quality after packaging is opened by protecting against moisture change, oxygen uptake, and aroma loss. Edible films created or placed between food components can also enhance the quality of multicomponent foods. Such concerns have led to increased interest in edible film research. Edible films, by controlling water, oxygen, carbon dioxide, and lipid movement in food systems, offer possible solutions to such concerns.

Kester and Fennema offer several possible uses of edible films and coatings in Table 1.

Table 1—Possible Functional Properties of
Edible Films and Coatings

- Retards moisture migration
- Slows gas transport (O_2, CO_2)
- Retards oil and fat movement
- Delays solute transport
- Improves mechanical-handling characteristics of foods
- Offers added structural integrity to foods
- Incorporates food additives

It is important to note that a particular film will probably not contain all seven properties identified in Table 1. The food itself will determine what kind of film will be most

effective. It is also important to discuss the significance of each property listed in the table.

First, the possible function of retarding moisture migration in food is of critical importance. Moisture migration in finished food products can seriously compromise quality, stability, and safety. In addition, there is a direct economic or regulatory effect. For example, Hruschka (1977) discovered that weight losses in nectarines and snap beans can be as high as 16 and 32 % respectively, before shriveling is apparent. Since produce is often sold based on weight, significant weight losses can result in a sizeable monetary loss. Moisture levels in foods are crucial for maintaining freshness, restraining microbial growth, and providing mouthfeel and texture. The rate of moisture transfer between a food product and its atmosphere can be decreased by coating the entire product with an edible film.

In addition to water vapor transmission, transport of gases such as oxygen and carbon dioxide can greatly affect storage stability of foods. A principal method of deterioration in many food products involves the oxidation of lipids, vitamins, flavor compounds, or pigments. The coating of especially susceptible food products, such as nutmeats, in an oxygen-impermeable edible film is one method of extending shelf life and possibly reducing the cost of the external, nonedible packaging material. Coating of certain fruits and vegetables to deter aerobic respiration, in a manner comparable to controlled atmosphere storage, would be highly desirable. In fact, the use of edible films to deter aerobic respiration would be economically favorable, given the expense connected with the equipment and operation of controlled atmosphere storage.

Third, some edible films, particularly those based on hydrophilic polymers, are highly impermeable to fats and oils. This is a beneficial functional quality when coated

food products are fried in oil. As a result, the film may slow absorption of oil into the food, thereby yielding more desirable nutritional and organoleptic qualities.

Next, barrier films can slow diffusion of solutes from the surface to the inside of food products. An additional functional property of edible films involves structural support. Covering a food with an edible coating can result in a noticeable improvement in stability during processing, storage, and distribution. Edible coatings are added to the surface of snack foods and crackers to serve as a base or adhesive for seasonings. These coatings are particularly beneficial in low-fat applications where the additional oil of frying might typically serve as a seasoning adhesive. Finally, edible films can act as a vehicle to incorporate certain food additives, such as antimicrobial agents and antioxidants, into foods at specific areas.

Previous research has focused on films composed from a variety of macromolecules: carbohydrates, lipids, and proteins. Each offer different properties of interest. In my project, the film application is intended to delay the onset of warmed-over flavor (WOF). The rapid development of WOF in cooked, refrigerated meat is regarded as a serious flavor defect which becomes most noticeable during reheating (Tims and Watts, 1958; Sata and Hegarty, 1971). Tims and Watts (1958) first detected WOF in cooked meats and attributed lipid oxidation to be the main cause of the off-flavor. This problem has recently become a significant problem due to the appearance of precooked ready-to-eat meat products in the marketplace. The 2-thiobarbituric acid (TBA) test has become the most prominent way to measure the degree of oxidative deterioration in muscle foods (Tarladgis et al., 1960; Gray, 1978; Melton, 1983). Researchers who have connected TBA results with sensory "off-flavor" panel ratings include Tarladgis et al.

(1960) who discovered that TBA numbers were in conjunction with trained sensory panels' scores for rancid odor in ground pork.

LIPID OXIDATION

Fennema (1985) offers much insight into the complicated topic of lipid oxidation. In biological systems, including food, the lipid molecules often exist in a highly ordered state, are somewhat restricted in terms of distance between molecules and mobility, and are closely connected with neighboring nonlipid material, such as proteins, carbohydrates, water, enzymes, salts, vitamins, and pro- and antioxidants. The composition of the lipids, the extent of their molecular order, and their association with non-lipid components deviate considerably depending on the plant or animal species and the location of the lipid within the particular organism.

Lipid oxidation is one of the primary culprits of food spoilage. It is of great economic interest to the food industry because it leads to the production, in edible oils and fat-containing foods, of various off-flavors and off-odors generally called rancid, which deems these foods unacceptable or reduces their shelf life. Moreover, oxidative reactions can reduce the nutritional quality of food, and certain oxidation products are possibly toxic. Conversely, a limited amount of lipid oxidation under controlled conditions is sometimes desirable, as in the production of cheeses or fried-food aromas.

Lipid oxidation results in the production of hydroperoxides, peroxides, and epoxides, which will, in turn, oxidize or alternately react with carotenoids, ascorbic acid, and so on, to bring about the loss of vitamin activity. The fate of other easily oxidizable vitamins, such as folic acid, B_{12}, biotin, and Vitamin D, has not been sufficiently researched, but serious losses are not unexpected. The breakdown of hydroperoxides to

reactive carbonyl compounds could result in losses of other vitamins, specifically thiamine, some forms of B_6, and pantothenic acid.

For these reasons, thorough research has been conducted not only to identify the products of lipid oxidation and the conditions that affect their production, but also to study the mechanisms involved. It is usually agreed that "autoxidation," that is, the reaction with molecular oxygen, is the primary reaction involved in oxidative decomposition of lipids. While photochemical reactions have been studied for a long time, only recently has the role of photosensitized oxidation and its connection with autoxidation begun to be recognized. In foods, the lipids can be oxidized by both enzymic and nonenzymic mechanisms.

Our current knowledge involving the basic mechanisms of lipid oxidation resulted largely from the pioneering work of Farmer and his coworkers, Boll and Gee, and Bateman et al. A great deal of evidence has shown that autoxiation of fats occurs via typical free radical mechanisms as distinguished by a) marked inhibition in rate by chemical species known to interfere with other well-studied free radical reactions; b) catalysis by light and by free-radical yielding substances; c) high yields of hydroperoxide, ROOH; d) quantum yields exceeding unity when the oxidation reactions are started by light; and e) a relatively long induction period detected when beginning with the pure substrate.

Based on experimental data, mostly with ethyl linoleate, the rate of oxygen absorption can be expressed as:

$$\text{Rate} = \frac{-\,d\,[O^2]}{dt} = \frac{K_a\,[RH][ROOH]}{1+\lambda[RH]/p}$$

where RH is the substrate fatty acid (H is an α-methylenic hydrogen atom easily removable due to the activating power of the neighboring double bond or bonds), ROOH is the hydroperoxide produced, p is the pressure of oxygen, and λ and K_a are empirical constants.

To describe the experimental results, a three-step simplified, free radical scheme has been suggested as follows:

Initiator ------> free radicals ($R^{\cdot}$, $ROO^{\cdot}$)		Initiation	(1)
$R^{\cdot} + O_2$ -------> $ROO^{\cdot}$			(2)
$ROO^{\cdot}$ + RH -------> ROOH + $R^{\cdot}$		Propagation	(3)
$R^{\cdot} + R^{\cdot}$ ---------->			(4)
$R^{\cdot} + ROO^{\cdot}$ ---------->	Nonradical Products	Termination	(5)
$ROO^{\cdot} + ROO^{\cdot}$ ----->			(6)

At high oxygen pressure (λ[RH]/p much less than 1) reactions (4) and (5) can be ignored to give:

$$\text{Rate} = k3 \frac{(k_1)^{1/2}}{K_6} [ROOH][RH]$$

Therefore, the rate of oxygen absorption doesn't depend on oxygen pressure.

At low oxygen pressure (λ[RH]/p larger than 1), steps (5) and (6) can be disregarded to give

$$\text{Rate} = k_2 \frac{(k_1)^{1/2}}{k_4} [ROOH][0_2].$$

Since the reaction RH + O_2 ----> free radicals is thermodynamically difficult (activation energy of approximately 35 kcal/mol), the formation of the first few radicals required to start the propagation reaction normally must occur by some catalytic means. It has been suggested that the initiation step may take place by hydroperoxide decomposition, by metal catalysis, or by exposure to light. More recently, it has been

hypothesized that singlet oxygen is the active species involved, with plant and tissue pigments, such as chlorophyll or myoglobin, serving as sensitizers.

Upon the development of sufficient free radicals, the chain reaction is propagated by the removal of hydrogen atoms at positions α to double bonds. Oxygen addition then takes place at these locations ($P^{\cdot}$), resulting in the generation of peroxy radicals $ROO^{\cdot}$ and these in turn remove hydrogen from α-methylenic groups RH of other molecules to produce hydroperoxides ROOH and $R^{\cdot}$ groups. Then, the new $R^{\cdot}$ groups react with oxygen, and the series of reactions just described is repeated.

Due to the resonance stabilization of the $R^{\cdot}$ species, the reaction series is usually associated with a shift in the position of double bonds, resulting in the production of isomeric hydroperoxides often containing conjugated diene groups. Hydroperoxides, the main initial products of lipid autoxidation, are considerably unstable. They enter into numerous and complicated breakdown and interaction mechanisms responsible for the creation of myriad compounds of assorted molecular weights, flavor thresholds, and biological importance. While the major early oxidation products of unsaturated fatty acids are hydroperoxides, several secondary products of lipid oxidation have been identified. The lipid hydroperoxide may decompose to aldehydes, ketones, and alcohols or in many cases enter into reactions with proteins. There is a growing group of evidence indicating that it is the secondary products that are of the greatest toxicological importance.

Food fats contain groups of fatty acids that differ greatly in their susceptibility to oxidation. Moreover, foods contain many nonlipid components that alter the rate of lipid oxidation. The complex interactions and their influence on the various autoxidation steps make any exact analysis of oxidation kinetics in food almost impossible. The number,

position, and geometry of double bonds change the rate of oxidation. Cis acids oxidize more easily than their trans isomers, and conjugated double bonds are more reactive than nonconjugated. Autoxidation of saturated fatty acids is very slow.

Free Fatty Acids Versus the Corresponding Acylglycerols. Fatty acids oxidize at a slightly faster rate when free than when esterified to glycerol.

Oxygen Concentration. If the supply of oxygen is unlimited, the speed of oxidation is independent of oxygen pressure. However, at very low oxygen pressure the rate is proportional to the pressure of oxygen.

Temperature. Usually the rate of oxidation increases with rising temperature. Temperature is also significant in terms of the effect of oxygen partial pressure on the rate of oxidation. As the temperature rises, the increase in rate with increasing oxygen concentration becomes less apparent, since oxygen becomes less soluble as temperature increases.

Surface Area. The rate of oxidation increases in proportion to the surface area of the lipid exposed to air. However, as the surface-volume ratio becomes larger, reducing the oxygen partial pressure becomes less efficient in reducing the rate of oxidation.

Moisture. In studies of model lipid systems and assorted fat-containing foods, it has been demonstrated that oxidation rate is strongly correlated with water activity. In dried foods with very low moisture contents (a_w values of less than about 0.1) oxidation occurs very quickly. Increasing the water content to an a_w of about 0.3 slows lipid oxidation and often produces a minimum rate. This protective effect of water is thought to occur by lessening the catalytic activity of metal catalysts, by quenching free radicals, by encouraging nonenzymic browning (which creates compounds with antioxidant activities), and/or by delaying the access of oxygen to the food. Interestingly, at

somewhat higher water activities (a_w = 0.55-0.85), the rate of oxidation increases again, probably an effect of greater mobilization of the catalysts present.

Pro-oxidants. The transition metals, especially those possessing two or more valency states with a suitable oxidation-reduction potential between them (eg., cobalt, copper, iron, manganese, and nickel) are major pro-oxidants. If present, even at concentrations as low as 0.1 ppm, they can reduce the length of the induction period and speed the rate of oxidation.

It is obvious that lipid oxidation is an extremely intricate process involving many reactions that yield an assortment of chemical and physical changes. While these reactions appear to follow sequential pathways, they often occur simultaneously and competitively. Since oxidative decomposition is critical in regard to both the acceptability and nutritional adequacy of food products, many methods have been developed for determining the extent of oxidation. No single test, however, can possibly monitor all oxidative events simultaneously; no single test can be equally useful at all stages of the oxidative process. Furthermore, no single test can be applicable to all rats, all foods, or all conditions of processing. At best, a test can measure one or a few physical changes that may furnish important information for specific systems and under specific conditions. It stands to reason that more reliability can be obtained when a combination of tests is used.

TBA TEST

While there are many tests that monitor lipid oxidation, I will only focus on the Thiobarbituric Acid (TBA) Test. This is the test I used to conduct chemical analyses on my meat samples. The TBA test is one of the most widely used tests for measuring the extent of lipid oxidation. Oxidation products of unsaturated systems generate a color

reaction with TBA. Malonaldehyde produces a distinctive red-orange color upon reaction with 2-thiobarbituric acid. It is thought that the color results from the condensation of two molecules of TBA with one molecule of malonaldehyde. This colored chromagen absorbs energy of 530 nm and can be quantitatively measured spectrophotometrically. Usually, TBA-reactive material is produced in significant amounts only from fatty acids containing three or more double bonds. The reaction mechanism was developed by Dahle and coworkers. They postulated that radicals with a double bond β-γ to the carbon bearing the peroxy groups (which bonds) cyclize to make peroxides with five-membered rings, which break down to give malonaldehyde. More recently, however, Pryor et al. determined that malonaldehyde arises, at least in part, from decomposition of prostaglandin-like endoperoxides created during autoxidation of polyunsaturated fatty acids.

Various compounds, other than those present in oxidized systems, have been found to interfere with the TBA test by generating the characteristic red color upon reaction with the reagents. Conversely, abnormally low values may occur if some of the malonaldehyde reacts with proteins in an oxidizing system. Moreover, flavor scores for different systems cannot be consistently gauged from TBA values since the relative level of TBA produced from a given amount of oxidation differs from product to product. In many cases, however, the TBA test is applicable for comparison of samples of a single material at various states of oxidation (Fennema, 1985).

SENSORY EVALUATION

A sensory evaluation is conducted by the senses of taste, smell, touch, and hearing when food is eaten. The complicated sensation that results from the interaction of our senses is used to assess food quality in programs for quality control and new product

development. This assessment may be carried out by one person or by many. The first and simplest method of sensory evaluation is made at the bench by the research worker who creates the new food products. He depends on his own evaluation to identify large differences in products. Sensory evaluation takes place in a more formal manner by laboratory and consumer panels.

When people are used as a measuring instrument, it is essential to tightly control all testing procedures and conditions to overcome errors caused by psychological factors. "Error" is not synonymous with mistakes, but may involve a variety of outside influences. The physical and mental state of the panelist and the influence of the testing environment alter sensory tests. For example, some individuals may possess more flavor sensitivity in the morning; however, others may have more flavor acuity in the afternoon. Even the weather can affect the disposition of the panelists.

Testing Area. For sensory evaluation, a specialized testing area is used so that distractions can be reduced and conditions can be regulated. The testing area should be a quiet, comfortable setting. The preparation area should be different from the testing area. Moreover, the panelists should not enter the preparation room because they might obtain information that would affect their judgment. Foreign odors and odors from food preparation should be blocked from the testing area.

Testing Setup. For the majority of sensory tests, the panelists are required to make independent judgments. In order to discourage communication among the panelists and to reduce distraction, individual booths are utilized. The typical setup is to build booths along the wall that separates the room from the preparation area. This design is desirable because the product to be tested can be passed through from the preparation area to the panelists, and the operators are not required to serve samples in the testing room. It is

critical that some method of communication from panelist to operator be developed. In some laboratories, a switch in each booth is associated with a light in the preparation area so the panelist can indicate when he is ready for another sample. In the laboratory at Texas A&M, a domed hatch is used that the panelist closes toward himself to signal that he is ready for a sample. This method has proven a very effective means of communication, and it is feasible for each booth to be used three or four times during a test without the operator having to enter the testing area. The color of the booths should not affect the appearance of the product being judged. An off-white or light gray color is often suggested.

Lighting. Lighting should be consistent and should not influence the look of the product to be tested. The type of light used should be carefully selected if color and appearance are important factors to be judged, since many fluorescent lights distort color. To eliminate differences in color between samples, colored lights are sometimes utilized. The booths in the laboratory at Texas A&M are furnished with red lights, which are used in a darkened testing area. These lights have not been found to be especially effective because differences in intensity of color are still observable. In some instances, the lights are dimmed so that the panelist is furnished with only enough light to see what he is doing. Amerine et al. (1965) have alluded to the fact that it is not known what effect colored or dim lighting has on judgment. It may have a lesser degree of influence on experienced judges who are familiar with the lighting, but inexperienced judges have expressed a dislike for testing under colored lights.

Testing Schedule. The time of day that the tests are conducted definitely influences results. While this factor cannot be controlled if the number of tests is large, late morning and midafternoon are usually considered the most optimal testing times.

Preparing the Samples

The type of preparation equipment located in the sample preparation area varies with the actual products being tested. A well-equipped kitchen is an excellent start, with specialized equipment added as required. The preparation area should have an adequate ventilation system for the removal of cooking aromas. Ample counter space for serving and assembling samples for presentation is an important requirement.

Preliminary testing is typically needed to determine the method of preparation of the product. All preparation factors must be kept constant throughout tests on the product. The preparation method should not add any odd tastes or odors to the product. Panelists are affected by irrelevant characteristics of the samples. Because of this, every attempt should be made to make the samples from different treatments exactly alike in all characteristics except the one being evaluated. It is sometimes desirable to grind, dice, shop, or puree the samples to establish uniformity. However, Kefford and Christie (1960) observed that judges prefer foods in their normal state.

Serving Temperature. The temperature at which samples are served is often a dilemma. For acceptance/preference tests, the samples would be served at the temperature at which they are typically consumed. Usually, this rule of thumb applies to discrimination tests as well. However, it is sometimes the case that panelists are able to differentiate better when the samples are slightly warmer or slightly cooler than normal. To compare results, the same temperature should be used during all individual tests with the food samples.

Once the serving temperature is decided, some way of keeping the samples at that temperature must be found. Warming ovens with controlled temperature and humidity are an option. If the samples are to be held for any period of time, precautions must be taken to prevent the samples from drying out or changing in quality during holding.

Utensils. Serving utensils should not add any taste or odor to the product. Identical containers should be utilized for each sample so that no bias will be caused from this source. Unless differences in color are being hidden, it is prudent to use colorless or white containers. Disposable dishes made from plastic, paper, or styrofoam are appropriate when large numbers are to be served, as in consumer tests, but it must be decided beforehand so that no taste is transferred to the product.

Quantity of Sample. The amount of sample served to each panelist is often defined by the quantity of experimental material available. The Sensory Evaluation Committee of ASTM (1968) suggests that in discrimination tests, each panelist should receive at least 16 ml (0.5 oz) of a liquid and 28 grams (1 oz) of a solid, and the portions should be doubled for preference tests. The sample sizes presented should be consistent throughout testing.

Number of Samples. The number of samples that can be fairly judged in one session should be decided during preliminary testing. The type of product being evaluated and the experience of the judges must be considered when deciding the number of samples to test in one session. Motivation is a critical factor in this area. Panelists commonly lose their desire to discriminate before they lose their ability.

Coding and Order of Presentation. The effect of order of presentation of samples to the judges has been studied by several researchers. Klemmer (1968) examined sequential effects in his paper on psychological principles of sensory evaluation. The presentation of a sample of high quality just prior to one of lower quality results in the rating of the second being lower than it would normally be. In a like manner, if a good sample succeeds a poor one, it will be given a higher score. This phenomenon is termed contrast effect.

Due to convergence effect, which also occurs when two or more samples are judged at the same time, a sample tends to be judged as similar to the samples it is being evaluated against, in spite of the quality. In some tests, especially the triangle test, a positional bias has been shown. When very subtle differences are present, there is a tendency to choose the middle sample as odd. Because of these and other psychological and physiological effects, the order of presentation of the samples to each judge is randomized or balanced. With a small number of samples and judges, the order can be balanced so that every feasible combination occurs an equal number of times. In larger experiments, the order can be determined from tables of random numbers.

The code allotted to the samples should not give the panelists any clue of the identity of the treatments, and the actual code itself should not present any bias. Three-digit random numbers gathered from tables of random numbers are suggested for coding the samples.

Rinsing. The judges are provided with an agent for oral rinsing between samples. Taste-neutral water at room temperature is favored by many researchers. When fatty foods are being tested, warm water is a more desirable rinsing tool. Crackers, apples, celery, and bread have all been utilized for removing flavor from the mouth. Whatever the method, the panelists should routinely follow the same process after each sample.

Information About Samples. As little information as possible about the test should be given to the judges, because this information may affect results. When a panel was told that tomato juice was prepared from high-quality raw products, the ratings were much higher than when the samples were served with the information that low-quality raw products had been used (Pettit, 1958). This preconceived impression is termed expectation error. Panelists often find what they expect to find. Due to expectation error,

persons who are directly associated with the experiment should not serve on the taste panel.

Choosing and Training Panelists. The value of a taste panel depends on the objectivity, precision, and reproducibility of the judgment of the panelists. Before a panel can be used with confidence, the ability of the panelists to duplicate judgments must be evaluated. Interest, motivation, general attitude, and emotional condition of the panelists may contribute to inconsistent judgments. Panelists are typically office, plant, or research staff. Full cooperation of panelists is necessary, and no one person should be forced to evaluate foods to which he objects. The greater the number of individuals on a panel, the more likely it is that individual variations will even out. However, a small highly trained panel typically yields more reliable results than a large untrained panel. The minimum number of panelists should be no smaller than four or five. A laboratory panel is often make up of 10 to 20 persons with three or four replications by each judge for each treatment.

Persons who serve as panelists should be in optimal health and should remove themselves when suffering from conditions that might affect the normal functions of taste and smell. Emotional factors, desire, and interest seem to be more important than the age or sex of a panelist. It is usually suggested that panelists abstain from smoking, chewing gum, eating, or drinking for at least 30 minutes prior to testing.

Designing Experiments and Choosing Methods of Analyzing Data. All experiments should be developed in advance so that a simple mathematical model can be applied to the data. Because of the fluctuation in results of sensory tests, interpretation cannot be made by direct examination. The results must be tested by statistical methods. These methods contrast the results actually obtained with those that would occur by chance

alone. Results are generally reported in degrees of significance, which is the probability that the results are caused by chance.

Methods for Sensory Testing. Several different sensory evaluation methods have been devised. The experimenter should be completely aware of the advantages and disadvantages of each method. The most sensible and efficient method should be selected for each situation. No one test can be used exclusively. The experimenter must accurately define the purpose of the test and information he wishes to obtain.

There are three basic types of sensory tests: preference/acceptance tests, discriminatory tests, and descriptive tests. Preference/acceptance tests are tests based on a degree of preference or a measure from which a relative preference can be evaluated. Discriminatory tests are used to evaluate whether a difference exists among samples. The panelist should not allow his personal likes and dislikes to affect his response. Descriptive tests are utilized to measure the nature and intensity of the differences. It should be known that there are a variety of each type of test available (Manual on Sensory Testing Methods, 1968).

ZEIN FILMS

While the development of edible films is not a totally new concept, there is still much work to be done. Since the films can be composed of proteins, carbohydrates, or fat, I needed to decide what macromolecule would make the most effective film for beef. I decided to focus my research on the use of corn protein called zein. Zein is a commercially produced corn protein that is noted for its ability to form tough, glossy, hard, grease-proof coatings after evaporation of aqueous alcoholic solution. Zein is a combination of proteins with an average molecular weight of 45,000 in the native state. However, during commercial extraction some disulfide bonds among polypeptide chains

break, resulting in a product with a molecular weight of 25,000-35,000. In regard to the amino acid content, zein has a high number of nonpolar hydrophobic amino acids, such as leucine, alanine, and proline. This fact accounts for the insolubility of zein in water, the insolubility in anhydrous alcohol, and the solubility in a mixture of the two. In addition, zein is composed of a high level of glutamic acid, approximately 20-22%, which exists primarily as glutamine. Glutamine adds to the insolubility of zein in water through hydrogen bonding (Reiners et al., 1973). Zein films are advantageous because they yield very low water and oxygen permeability; still, tensile strength is low. Interestingly, a zein film has been added to nutmeats reducing their affinity for oxidation and lengthening their shelf-life 5-fold.

At present, the primary use of zein is the creation of edible coatings for pharmaceutical tablets and confectionery products (nuts, dried fruits, and jelly beans). It is chiefly used as an alternative to shellac (confectioner's glaze). The benefit of using zein-based coating rather than shellac include faster drying rate and greater stability under long-term storage and under high temperature and humidity conditions. Zein has been generally recognized as safe (GRAS) in any quantity by the U.S. Food and Drug Administration (FDA) since March 1985.

Meyer and Spencer (1973) placed zein films on shell eggs and evaluated the effects on shell strength and on the quality of the eggs. Both characteristics were enhanced by the zein film. A study by Tryhnew et al. (1973) suggested that zein films on eggs offered a noticeable reduction in microbial penetration.

MATERIALS AND METHODS

While films may be developed from a variety of different macromolecules, my research focuses on the use of zein proteins. In this project, I developed the technology for adding edible barrier films to meat for the intention of regulating moisture losses and gas interchanges at the meat surface. Spraying techniques were created for the addition of the zein solution to the meat surface. Furthermore, rapid-drying techniques for optimizing film deposition on lean and fatty tissue surfaces were also designed.

Zein is purchased in a powdered form. To prepare a 90% zein solution, I measure 10 grams of zein powder. Next, I use a graduated cylinder to measure 90 ml of 100% ethanol and 10 ml of double-distilled water. Then, I combine 90 ml of this 90% ethanol solution to the 10 grams of zein powder. Finally, I swirl the mixture until the zein dissolves to make a solution. Ethanol is used as the solvent because it is easy to remove by evaporation. In addition, traces of ethanol left behind will only add to the minute levels of ethanol present in many food systems.

I chose to use top round roasts in my experiments. I chose this cut of meat because it is composed of one large muscle; moreover, it has a large surface area. Zein films can be applied through a variety of methods, ie. spraying, dipping, and brushing. Spraying is the application technique of choice because it lessens the possibility of cross-contamination. Nitrogen gas provides the pressure for the spray gun containing the zein solution. Much time was spent developing an exact spraying technique. It is critical to spray the film in even coats. Rapid-drying procedures were developed to promote consistent film formation. I used a steady stream of hot air to dry the protein solution. Basically, the film starts forming almost immediately after application of the zein solution. The film was always applied to the roasts prior to cooking.

All roasts were cooked in exactly the same way. I preheated the oven to 350 degrees Fahrenheit. I used a special thermometer to monitor the exact temperature of the oven. All roasts were cooked to an internal temperature of 165 degrees Fahrenheit. The internal temperature of each roast was determined by the insertion of a thermocouple prior to cooking. The thermocouple is attached to a thermometer that reports the meat's exact internal temperature.

I had several problems with the tensile strength of the film. Since meat is very flexible, the film would commonly crack in areas where the meat position changed. Since the film is meant to serve as a barrier to oxygen, the cracks are undesirable because oxygen can enter through the cracks. I did find that the film with a few cracks was more resistant to oxygen than no film at all. If time would have allowed, I would have worked on ways to improve the film's tensile strength.

It is important to note what the film actually looks like. When sprayed on a raw piece of meat, the film dries a light yellow color. Consequently, one can see the film on the raw meat surface. However, after the meat is cooked, one cannot see the film. The meat surface appears very clean. When meat without film is cooked, it has a crusty appearance where moisture has seeped from the meat. The cooked meat with film is very clean looking because the film prevents the moisture from escaping from the meat itself. Figure 1 demonstrates the difference between a raw piece of meat with and without film. Figure 2 represents cooked pieces of meat with and without film.

FIGURE 1

a) Raw Meat, Film Added

b) Raw Meat, No Film

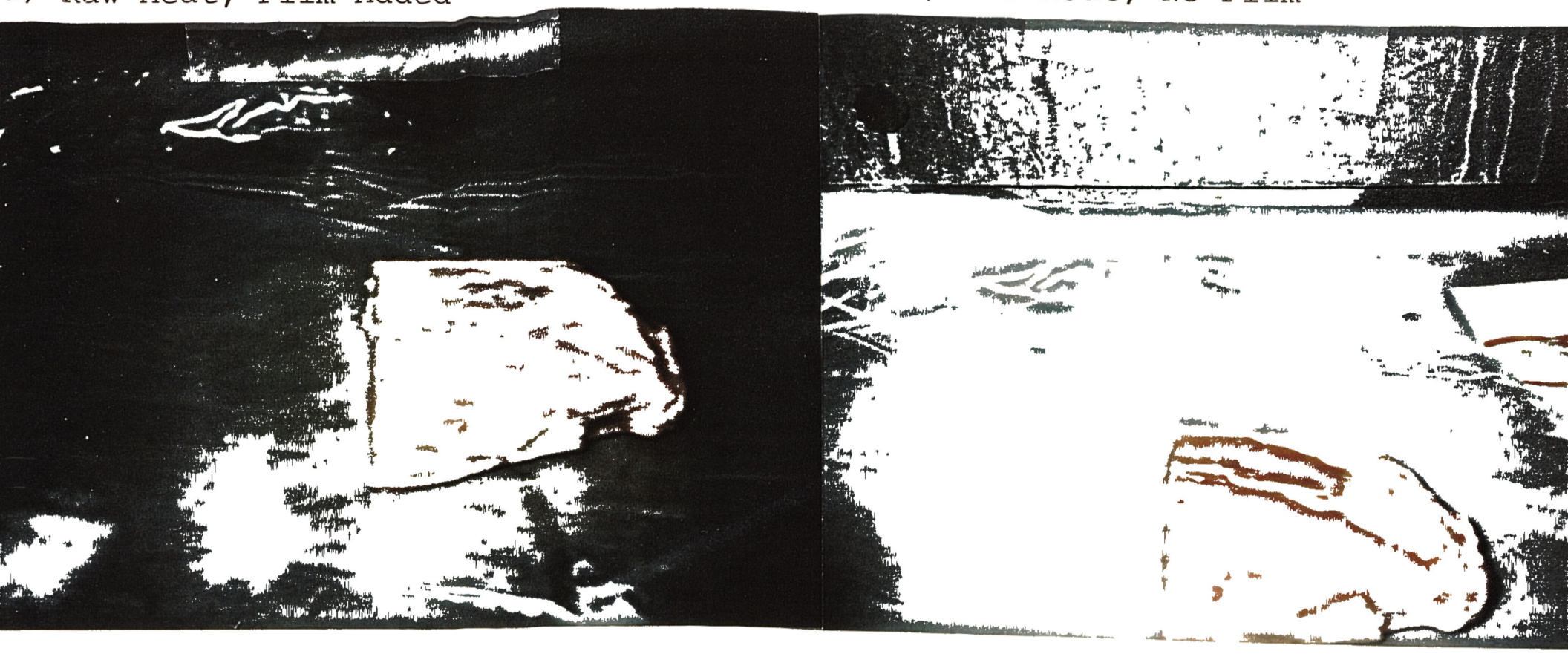
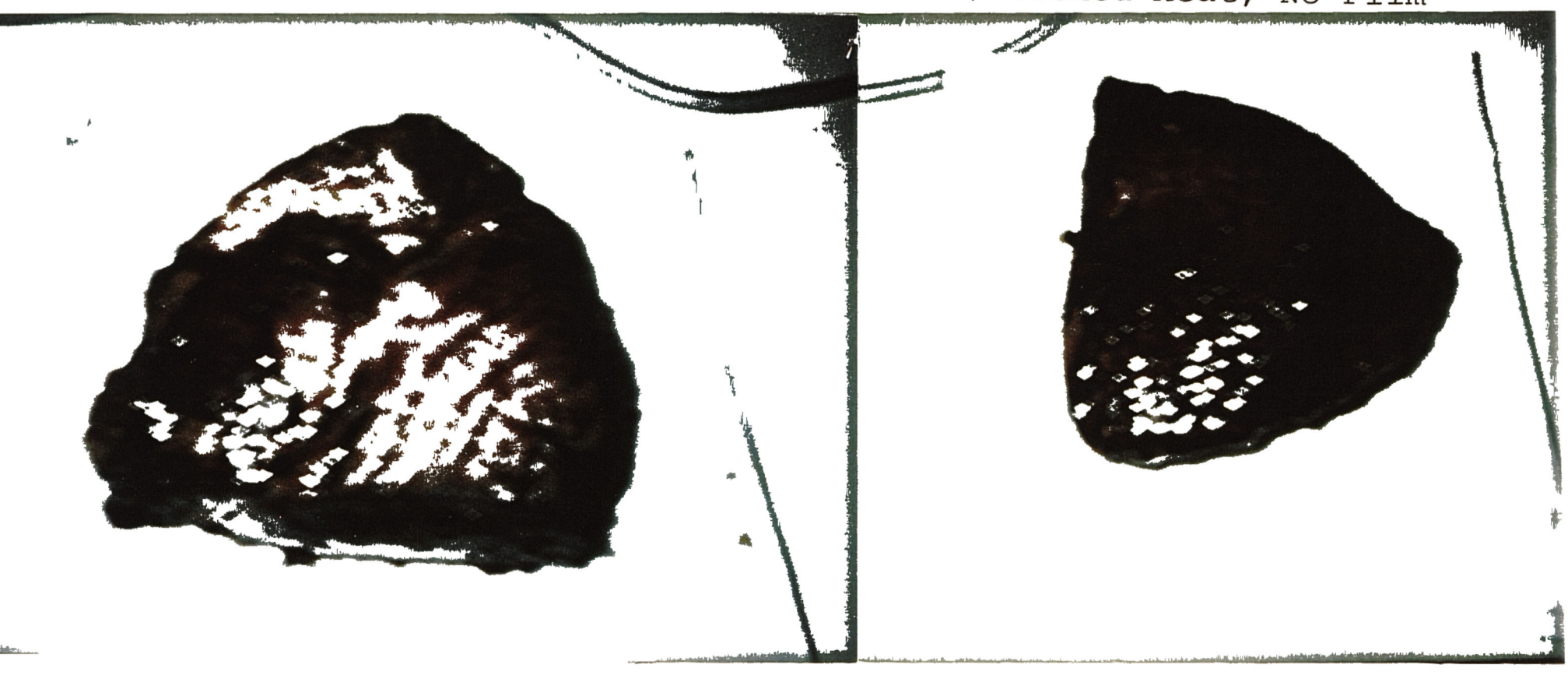


FIGURE 2

a) Cooked Meat, Film Added

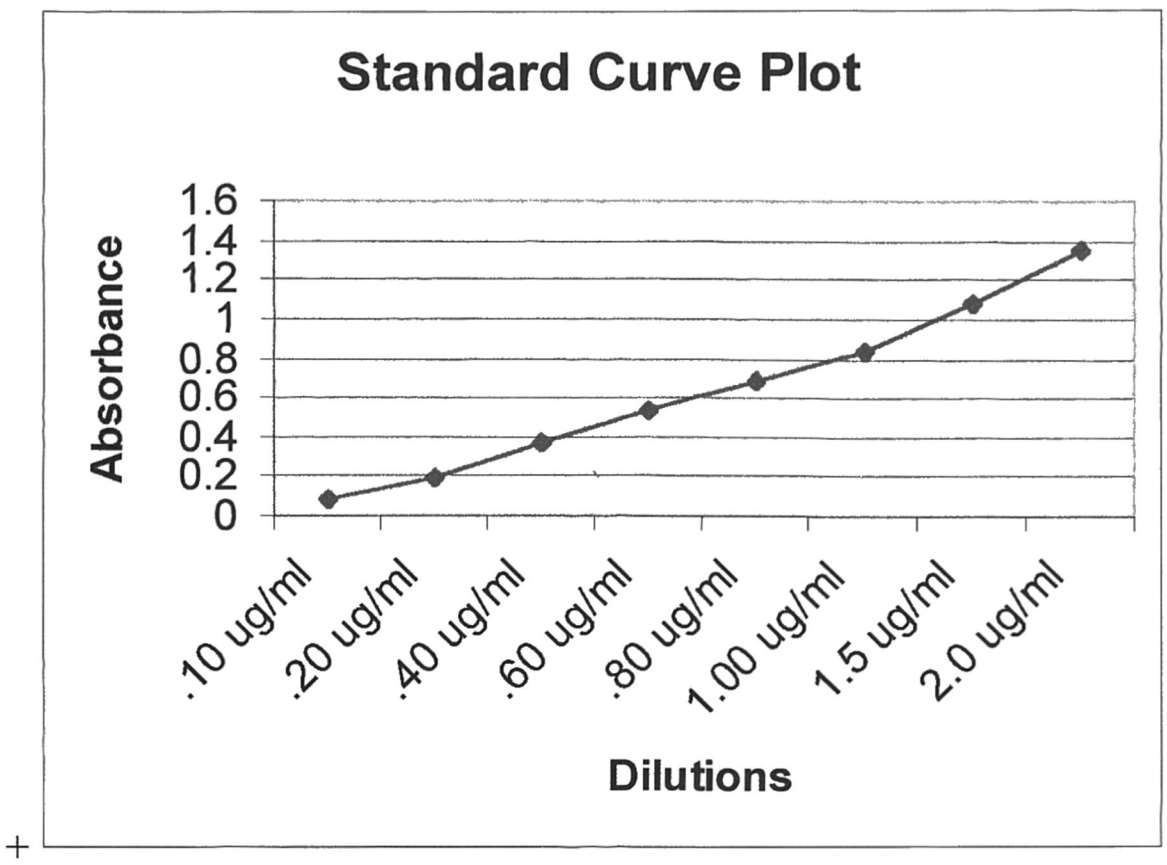
b) Cooked Meat, No Film



The TBA test is the only chemical analysis I conducted on the top round roasts in my project. In order to conduct the TBA test, I needed to first cook twelve top-round roasts to an internal temperature of 165 degrees Fahrenheit. I obtained TBA measurements for days 0,1,2,3,4, and 5 of refrigerated storage. For each of these days I evaluated two roasts: one with film and one without film. Furthermore, I acquired TBA readings for layers 1,2,3, and 4 for each roast.

The first step in the TBA test is to obtain a standard curve. A stock solution of malonaldehyde bis(dimethyl acetal) contains 2.0 μg/ml malonaldehyde. The next step is to dilute the stock solution with redistilled water to concentrations of .10, .20, .40, .60, .80, 1.00, 1.50, and 2.00 μg/ml using 100 ml volumetric flasks and volumetric pipettes. I ran the standard curve at these concentrations and performed determinations in triplicate.

Stock Solution	Flask Volume	Concentration
5 ml	100 ml	0.10 μg/ml
10 ml	100 ml	0.20 μg/ml
20 ml	100 ml	0.40 μg/ml
30 ml	100 ml	0.60 μg/ml
40 ml	100 ml	0.80 μg/ml
50 ml	100 ml	1.00 μg/ml
75 ml	100 ml	1.50 μg/ml
100 ml	100 ml	2.00 μg/ml



After determining the standard curve, I performed the actual TBA test. The steps are not difficult, but this test takes a long time to perform. I performed distillations over a series of several days. For example, I performed the TBA test for roast samples with and without zein film for days 0,1,2,3,4, and 5 of refrigerated storage. The basic procedure can be described as follows:

1. Combine 15 grams of meat, 22.5 ml of redistilled water, and 7.5 ml of heated PG/EDTA solution in a glass blending container. Blend for 2 minutes.
2. Weigh 30.0 grams of the meat slurry into a 250 ml beaker.
3. Spray Slipicone into the beaker for 5 seconds.
4. Transfer the weighed meat slurry to the distillation flask. Add 77.5 ml of redistilled water (rinse the beaker with this water), 2.5 ml 4N HCl, and 5-6 boiling chips.
5. Assemble distillation apparatus and begin distillation.
6. Collect 50 ml of distillate in a 50 ml graduated cylinder. Transfer all of the distillate to a 50 ml screw top test tube and close. Invert the test tube several times to make the distillate homogenous.
7. Transfer 5 ml of the homogenous distillate to a 20 ml screw top test tube containing 5 ml of TBA reagent. For comparison, prepare a blank with 5 ml redistilled water and 5 ml TBA reagent.
8. Vortex and heat each tube in boiling water for 35 minutes. Let the test tubes cool to room temperature.

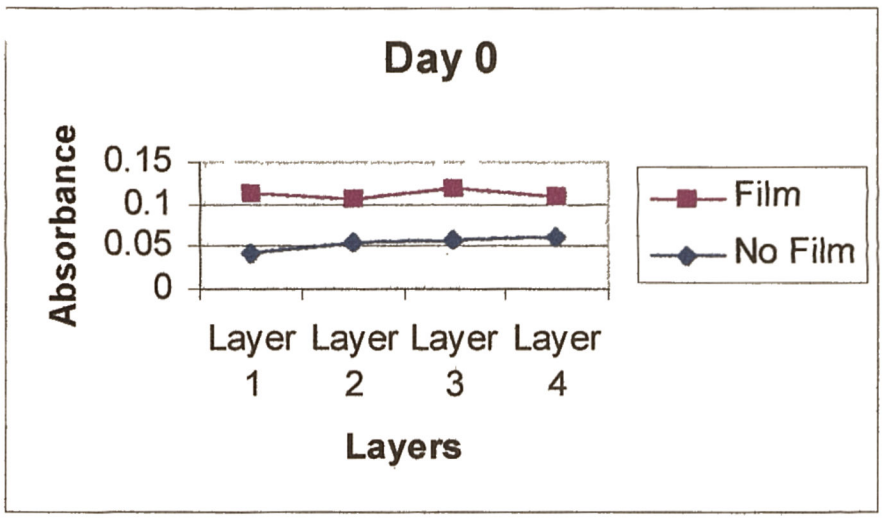
9. Read the percent transmittance using the spectrophotometer at 530 nm against the blank.
10. Use the Standard curve to estimate the concentration of malonaldehyde in the samples.

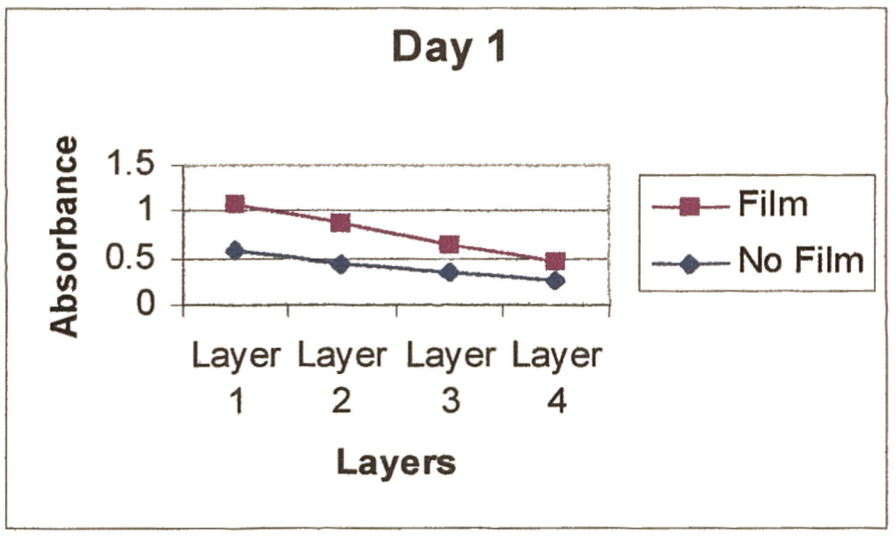
I conducted this TBA test for 48 samples. There were eight total samples for each of the following days of storage: Days 0,1,2,3,4, and 5. Four of the samples represented the top round roast without film and four of the samples represented the top round roast with zein film. The four samples for each roast were taken from four consecutive layers of the roast. I used a meat slicer to shave four 1 millimeter layers.

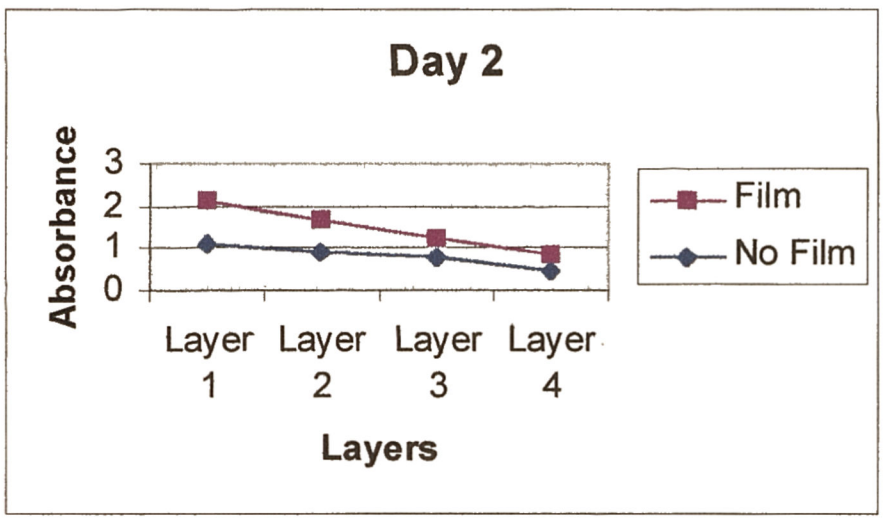
I chose to test the level of lipid oxidation in the different layers of meat because I wanted to see if the lipid oxidation decreased with the increasing layers. I expected the majority of the lipid oxidation to occur on the meat surface since this is the meat area most exposed to oxygen. I tested samples with and without film because I hoped that the samples with film would display a lesser level of lipid oxidation due to the film's oxygen barrier properties. I also looked at the development of lipid oxidation over time. I expected the level of lipid oxidation to increase with increasing days of storage. Moreover, I anticipated that the development of lipid oxidation would be significantly delayed in the roasts with zein film.

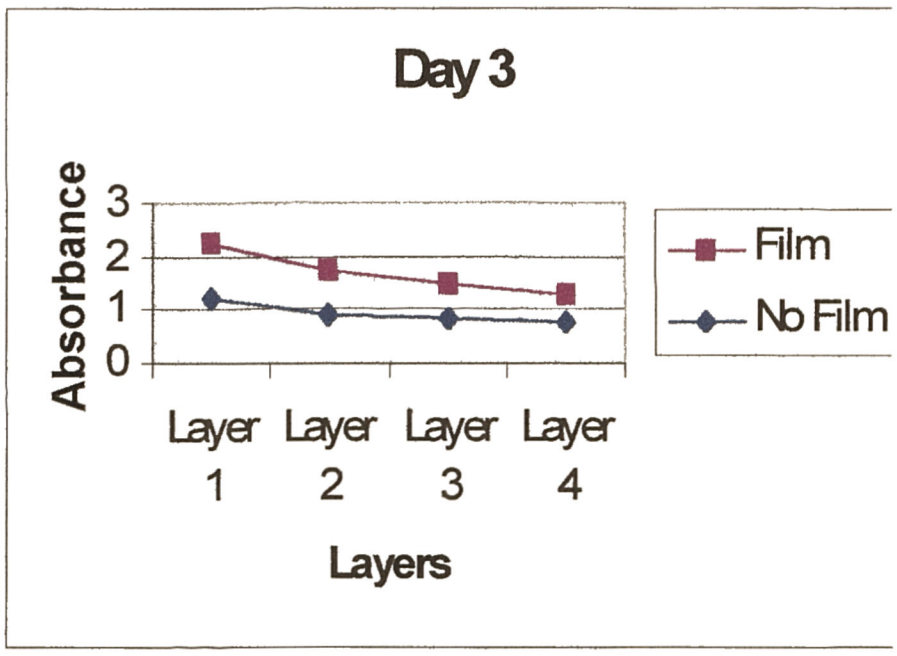
RESULTS AND CONCLUSIONS

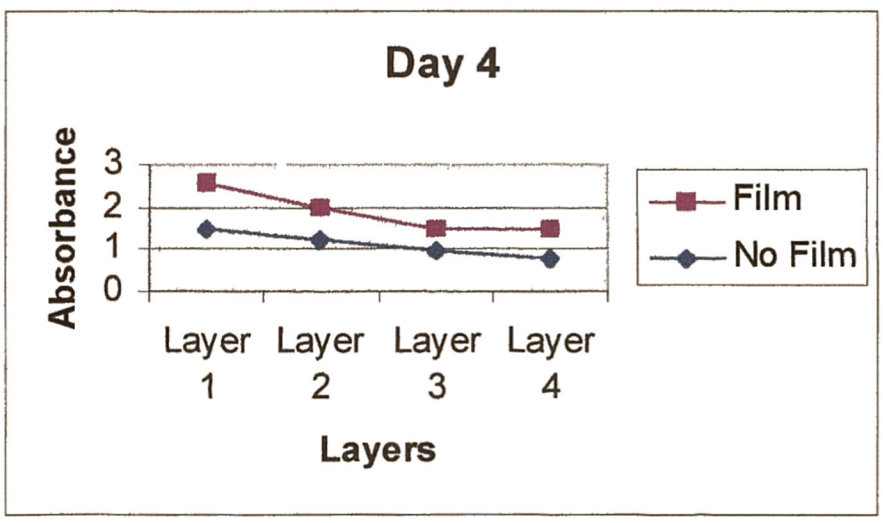
I ran TBA distillations for days 0,1,2,3,4, and 5 of refrigerated storage. The results are plotted as follows:

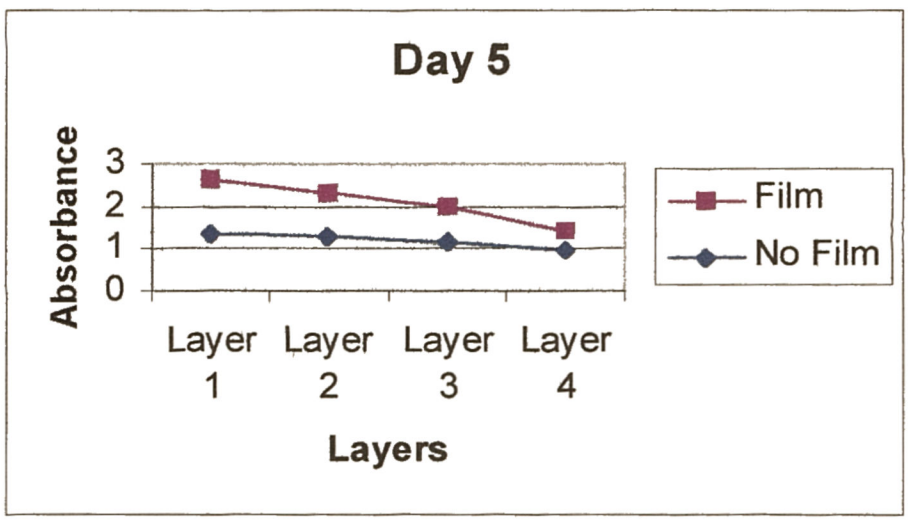

Day 0
Absorbance
0.15
0.1
0.05
0
Layer 1
Layer 2
Layer 3
Layer 4
Layers
Film
No Film


Day 1
Absorbance
1.5
1
0.5
0
Layer 1
Layer 2
Layer 3
Layer 4
Layers
Film
No Film

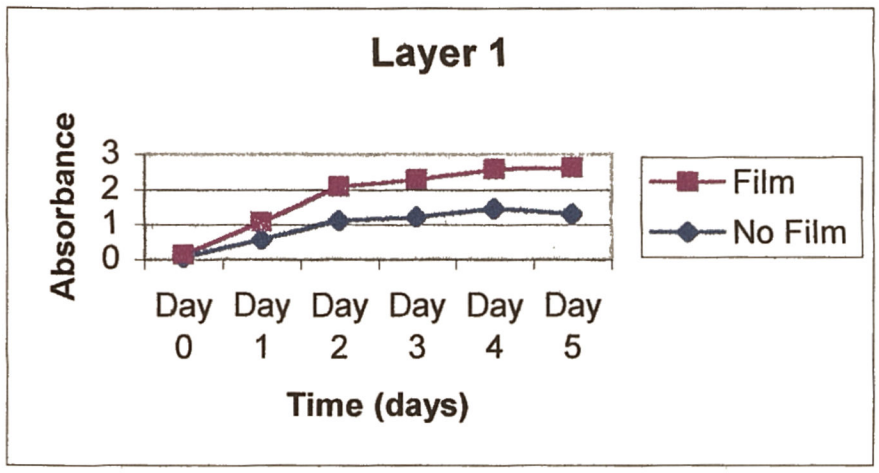

Day 2
Absorbance
3
2
1
0
Layer 1
Layer 2
Layer 3
Layer 4
Layers
Film
No Film

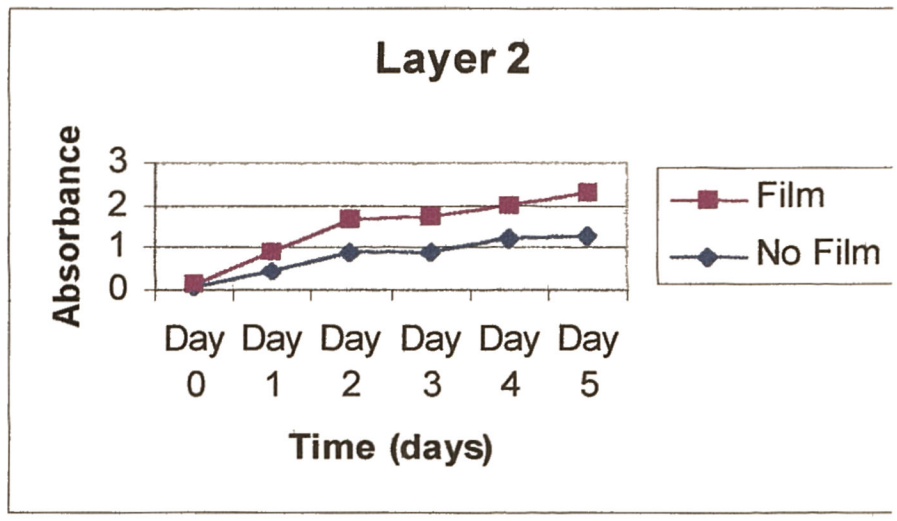

Day 3
Absorbance
3
2
1
0
Layer 1
Layer 2
Layer 3
Layer 4
Layers
Film
No Film

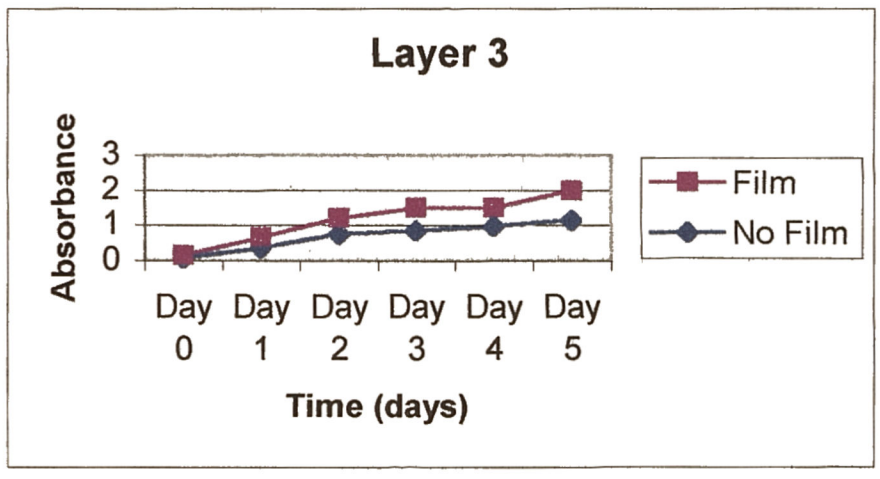

Day 4
Absorbance
3
2
1
0
Layer 1
Layer 2
Layer 3
Layer 4
Layers
Film
No Film

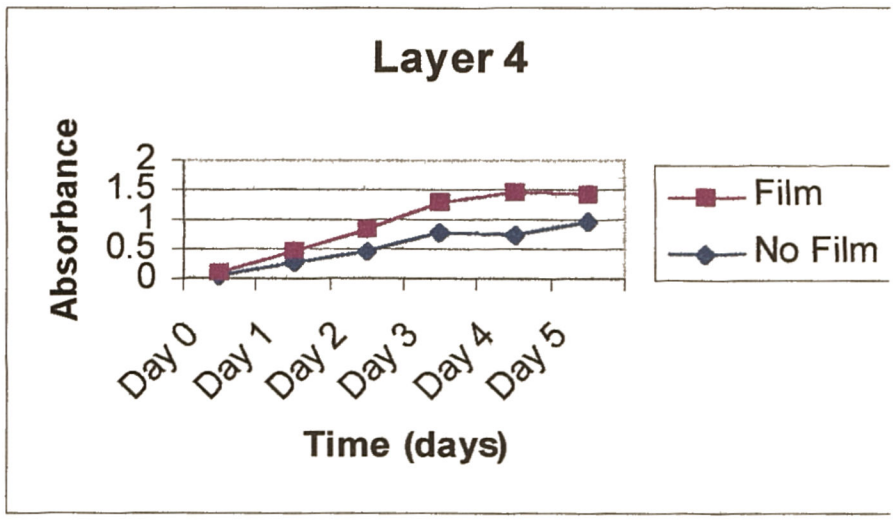

Day 5
Absorbance
3
2
1
0
Layer 1
Layer 2
Layer 3
Layer 4
Layers
Film
No Film

In addition, I ran TBA tests for each of the layers. The results are plotted as follows:









The higher the absorbance value, the less the extent of lipid oxidation. Therefore, TBA values show that the extent of lipid oxidation is less in the samples with film. While lipid oxidation is inevitable, the film definitely delays the onset. As predicted, the extent of lipid oxidation decreases with increasing meat layers. Therefore, most of the lipid oxidation occurs on the meat surface.

SCANNING ELECTRON MICROSCOPY

Meat samples were taken to the Electron Microscopy Center on campus. Pictures of the roasts involved both raw and cooked samples. The raw sample was divided into three sections: no film, one layer of film, and two layers of film. The cooked sample was treated in the same manner. The purpose of the pictures is basically to give the reader an idea of what the film actually looks like.

The surface of the meat becomes smoother with each film layer application. After two layers of film have been added, the meat surface looks almost like an ice rink.

SENSORY EVALUATION OF TOP ROUND ROASTS

I chose the triangle test, which is a popular type of difference test, to evaluate the lipid oxidation in the top round roasts. Originally, I planned two phases of research. First, I planned to use a triangle test to evaluate whether or not the panelists could taste warmed-over flavor (WOF) caused from lipid oxidation. The second set of triangle tests would observe whether or not the panelists could taste a difference between samples with and without film. However, time only allowed for the first set of tests. In the preliminary phases of testing, I determined exact cooking, cooling, reheating, and serving procedures. As mentioned above, all roast were cooked in a 350 degrees Fahrenheit oven to an internal temperature of 165 degrees Fahrenheit. All roasts were cooled for thirty minutes on the counter and then placed in the refrigerator. The roasts were reheated in the microwave.

My taste panel included seven people. The panel was essentially untrained; however, the panelists were all trained in the disciplines of either food or meat science. Each panelist received three coded samples. The panelists were told that two of the samples were identical, and one was different. Then, the panelists were asked to pick the

odd sample. My triangle test included both a freshly cooked roast and a roast that had been cooked three days beforehand. I expected that three days would be ample time for sufficient WOF to develop. As mentioned above, two samples were the same and one was different. This means that by randomization, the two alike samples could either be a fresh roast or a three-day old roast. I flipped a coin to assign which samples would be which.

Each triangle test was performed in triplicate in order to be statistically significant. All roasts were prepared and served consistently. The samples were cut into 1-inch cubes and served in individual plastic cups. All samples were reheated in the microwave for 90 seconds prior to serving. Analysis of the results of triangle tests is based on the probability that if there is no observable difference, the odd sample will be selected by chance one-third of the time. Figure 3 is a copy of the questionnaire given to the panelists in the triangle test.

Figure 4 QUESTIONNAIRE FOR TRIANGLE TEST

NAME_____DATE_____

Two of these samples are identical. One is different.

1. Smell and taste the samples in the order indicated and identify the sample that is different.

Code	Check different sample
149	_____
582	_____
241	_____

2. Indicate the degree of difference between the duplicate samples and the different sample.

Slight	_____
Moderate	_____
Much	_____
Extreme	_____

3. Comments:

Unfortunately, the results of the triangle test were not statistically significant. The panelists could definitely identify WOF, but they couldn't consistently identify it three times in a row. I believe that there are several reasons why the triangle test didn't achieve the expected results. First, the series of tests was conducted during cold season. As a result, many of the panelists' senses of taste and smell were altered. These symptoms made the evaluation much more difficult. Next, WOF is very characteristic. It almost tastes like old cardboard. However, once the taste is encountered, it is difficult to eliminate. Even though the panelists were given room-temperature water and crackers in between samples, I believe that the taste could not be escaped from. As a result, my panelists couldn't differentiate between samples once the original taste of WOF was encountered. Another explanation as to why my panelists could not consistently identify WOF is that many Americans are accustomed to the warmed-over flavor of leftovers. Consequently, the WOF may have tasted "normal". I was really disappointed in the results of the triangle test because the general consensus was that the WOF could be detected. Unfortunately, the panelists couldn't identify the flavor in triplicate.

WEIGHT STUDIES

In order to determine the extent to which the film prevented water loss, I weighed a roast with and without film both before and after cooking.

Roast With Film		Roast Without Film
Before cooking	1099.9 g	965.5 g
After cooking	812.3 g	103.0 g

$$\% \text{ water loss} = \frac{\text{Roast Weight (before cooking)} - \text{Roast Weight (after cooking)}}{\text{Roast Weight (Before)}} \times 100$$

The sample without film experienced a 27.2% weight loss, and the sample with film only experienced a 26.1% weight loss. This difference may not seem all that great, but it can be significant to the restaurant industry.

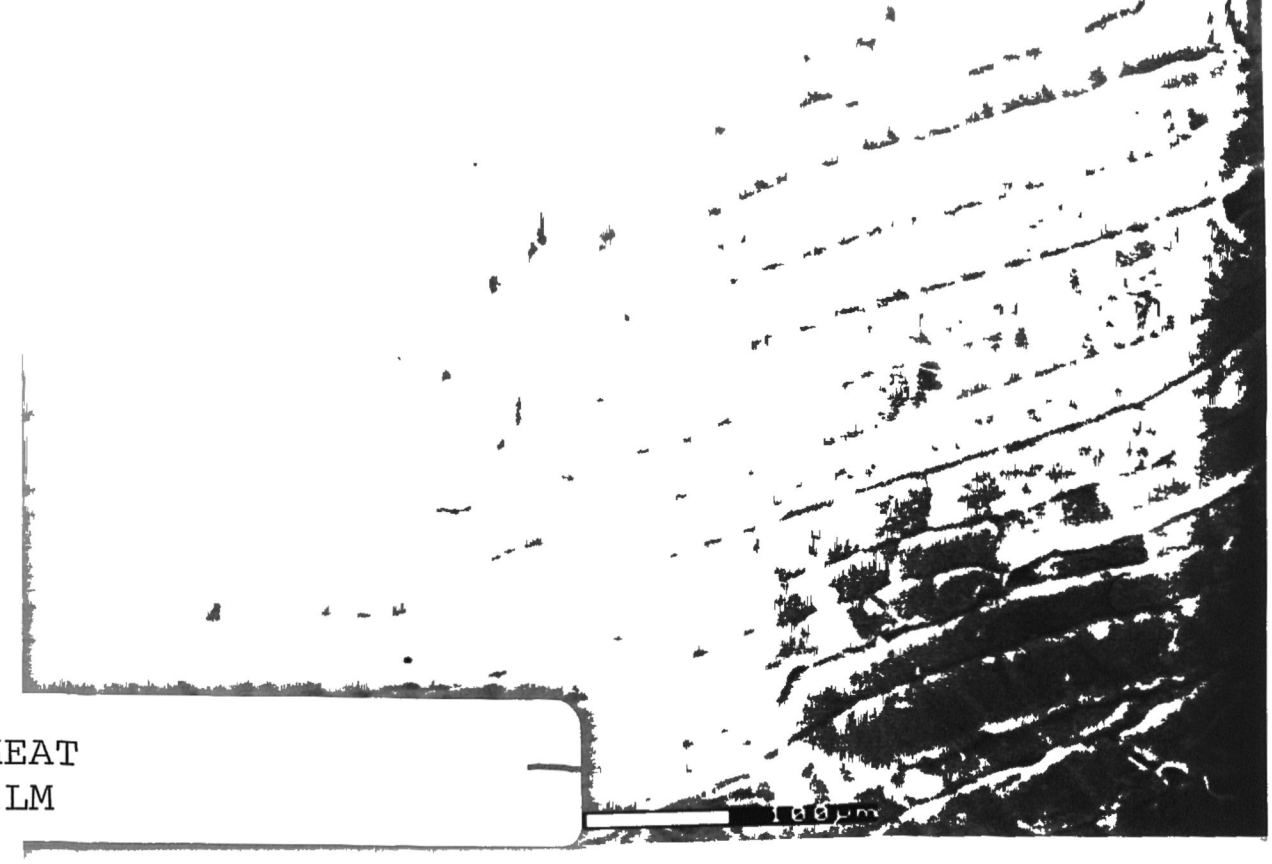
FUTURE WORK

This project still leaves much to be done. The application of edible barrier films has tremendous potential; the possibilities are endless. One area of future research would experiment with the use of more than one type of film on the top round roast. The zein film I used has excellent moisture barrier properties; however, the tensile strength of the film is very low. As a result, I had much trouble with the film breaking whenever I touched the meat. A possible solution would be to spray one coat of zein film followed by one coat of a film made from gluten. Gluten's moisture barrier properties are very low, but its tensile strength is high. By combining film types, both moisture barrier and tensile strength could be high. It is also an option to make a heterogeneous film, consisting of a blend of polysaccharides, proteins, and/or lipids. This approach allows one to advantageously optimize the specific functional characteristics of each class of film.

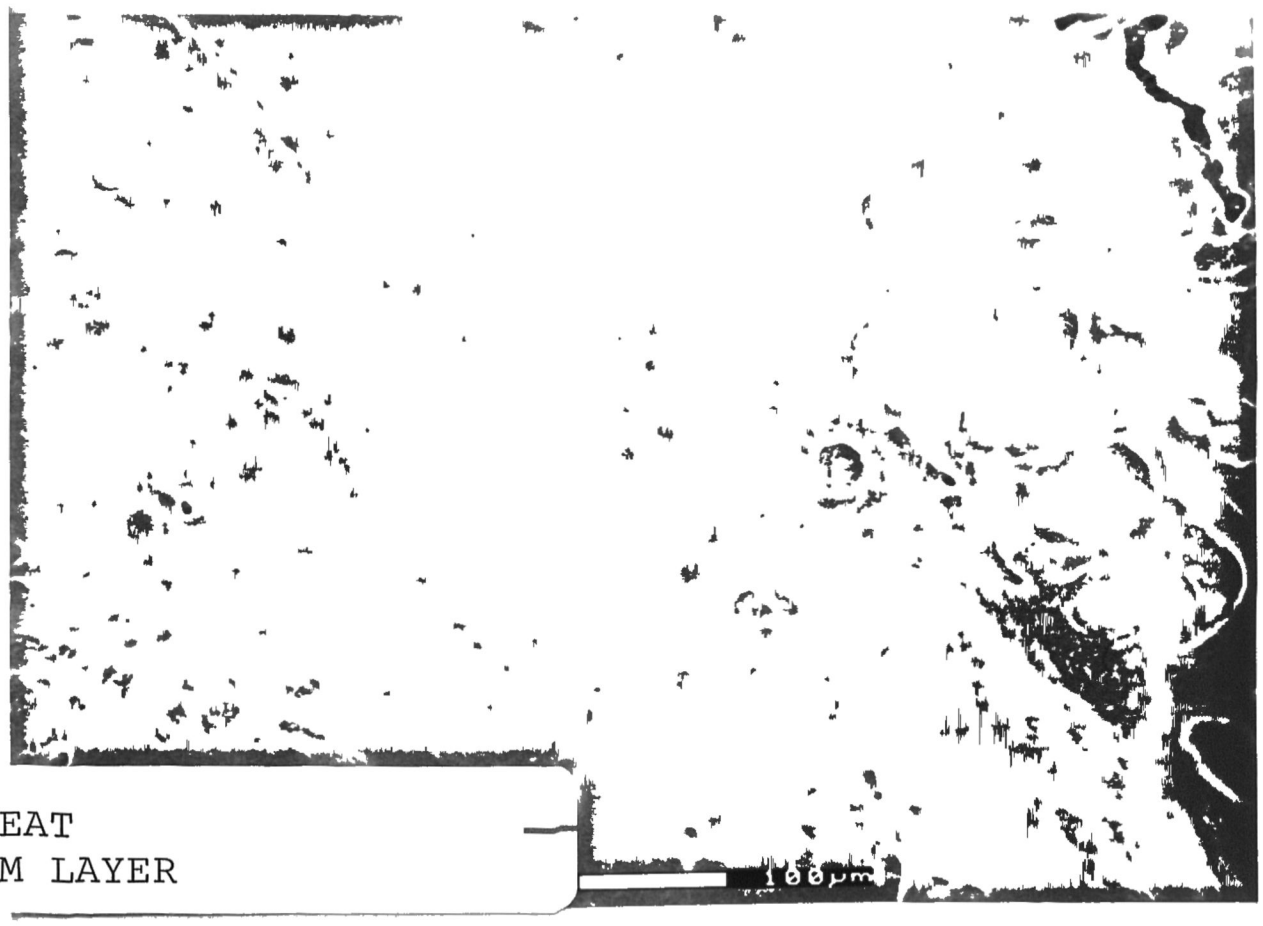
Assorted materials can be added to edible films to affect mechanical, protective, sensory, or nutritional characteristics. Kester and Fennema (1986) suggest that an edible film or coating may be utilized as a method for incorporating food additives such as antioxidants and antimicrobial agents onto the surface of the food, where breakdown of many solid foodstuffs by microbial growth or oxidation starts. Many products, such as enriched bread products, are supplemented with additional nutrients that may be deficient in the average American's diet. In today's fast-paced world, it seems to be increasingly difficult to obtain adequate nutrients in our diets. Fortified films could be the answer. Enhanced organoleptic or nutritional characteristics of a food product can be attained by adding flavoring agents or enhancers, pigments, or nutritional additives in an edible film or coating. For example, the lack of Vitamin B_{12} in pregnant womens' diets has been

linked to the occurrence of neural tube defects (NTD's) in babies. The incorporation of B_{12} into a film could be beneficial.

In conclusion, the research possibilities for edible barrier films are phenomenal. Much needs to be done in determining the actual mechanisms by which the film works. The possibility for improvement in cooked and stored beef products in regard to regulating gas and water interchanges at meat surfaces are astronomical. For instance, the occurrence of warmed-over flavor linked to lipid oxidation is a primary concern of the beef industry. The anaerobic environment created by edible films could possibly eliminate this defect regardless of heating by conventional or microwave methods. Moisture regulation during frozen storage of precooked items and regulation of antimicrobials,which tend to dilute in surface moisture and dissolve into meats, both are areas of extreme future value. This edible film technology promises to improve the quality of, and lead to greater consumer satisfaction with beef and beef products.

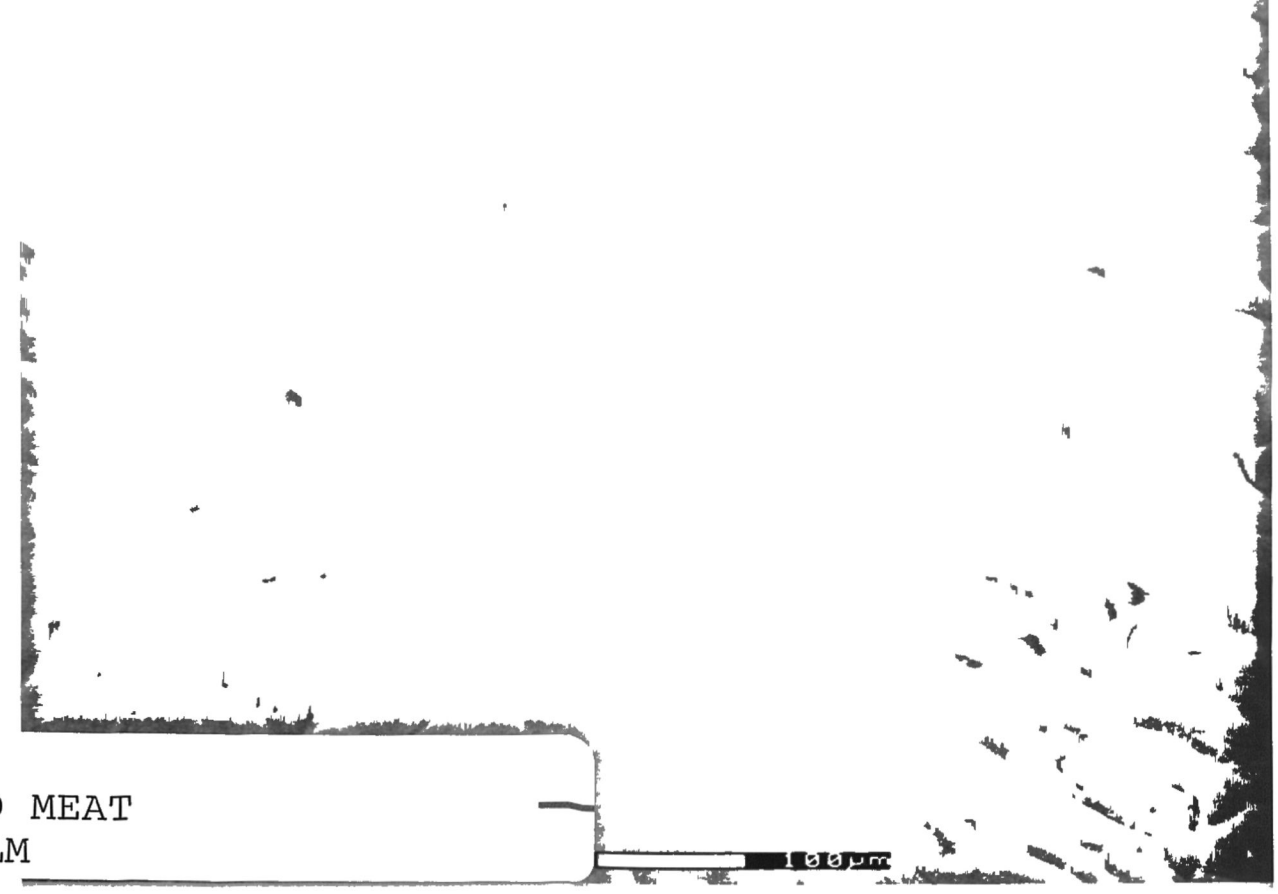


RAW MEAT
NO FILM

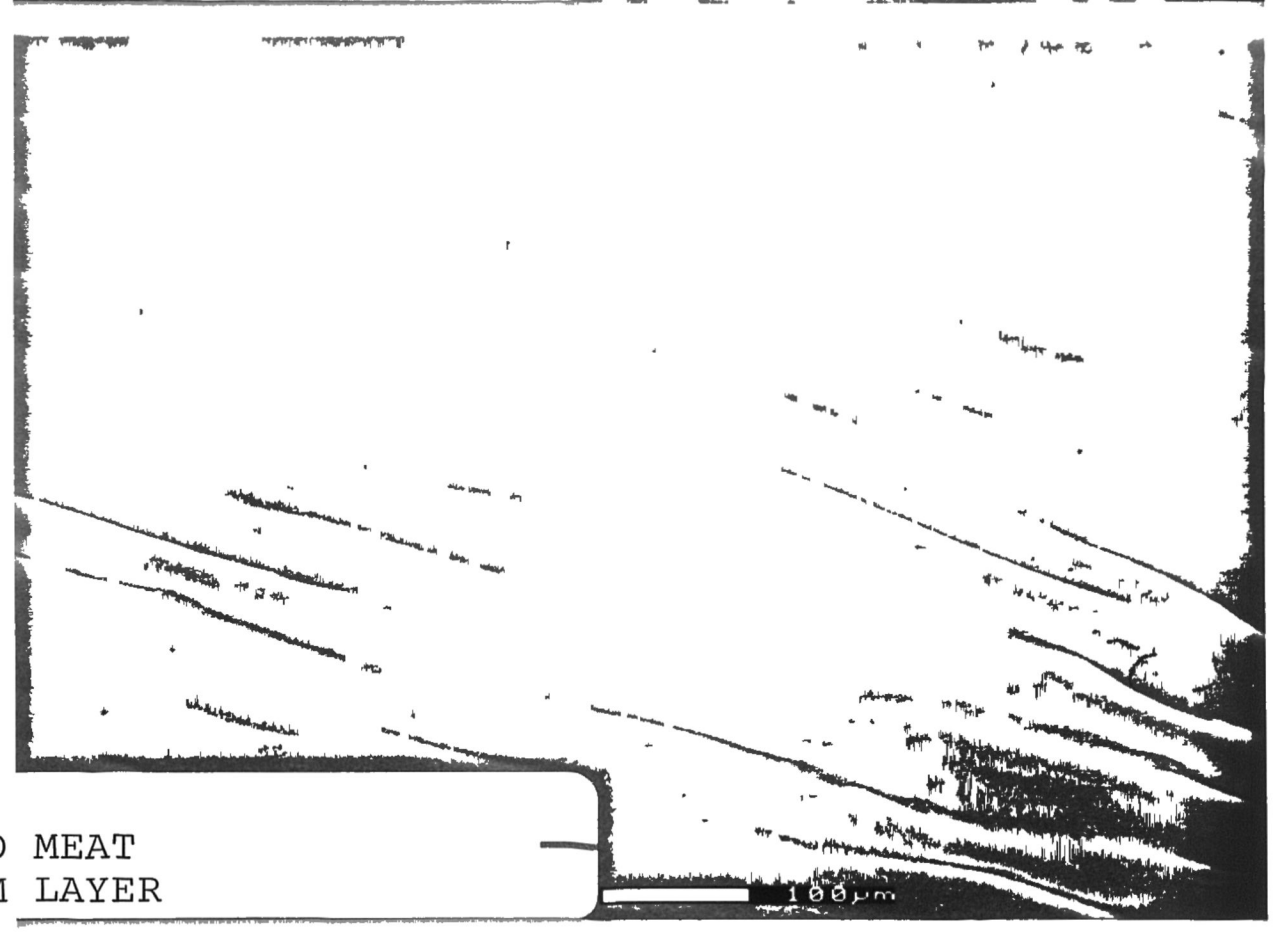


RAW MEAT
1 FILM LAYER

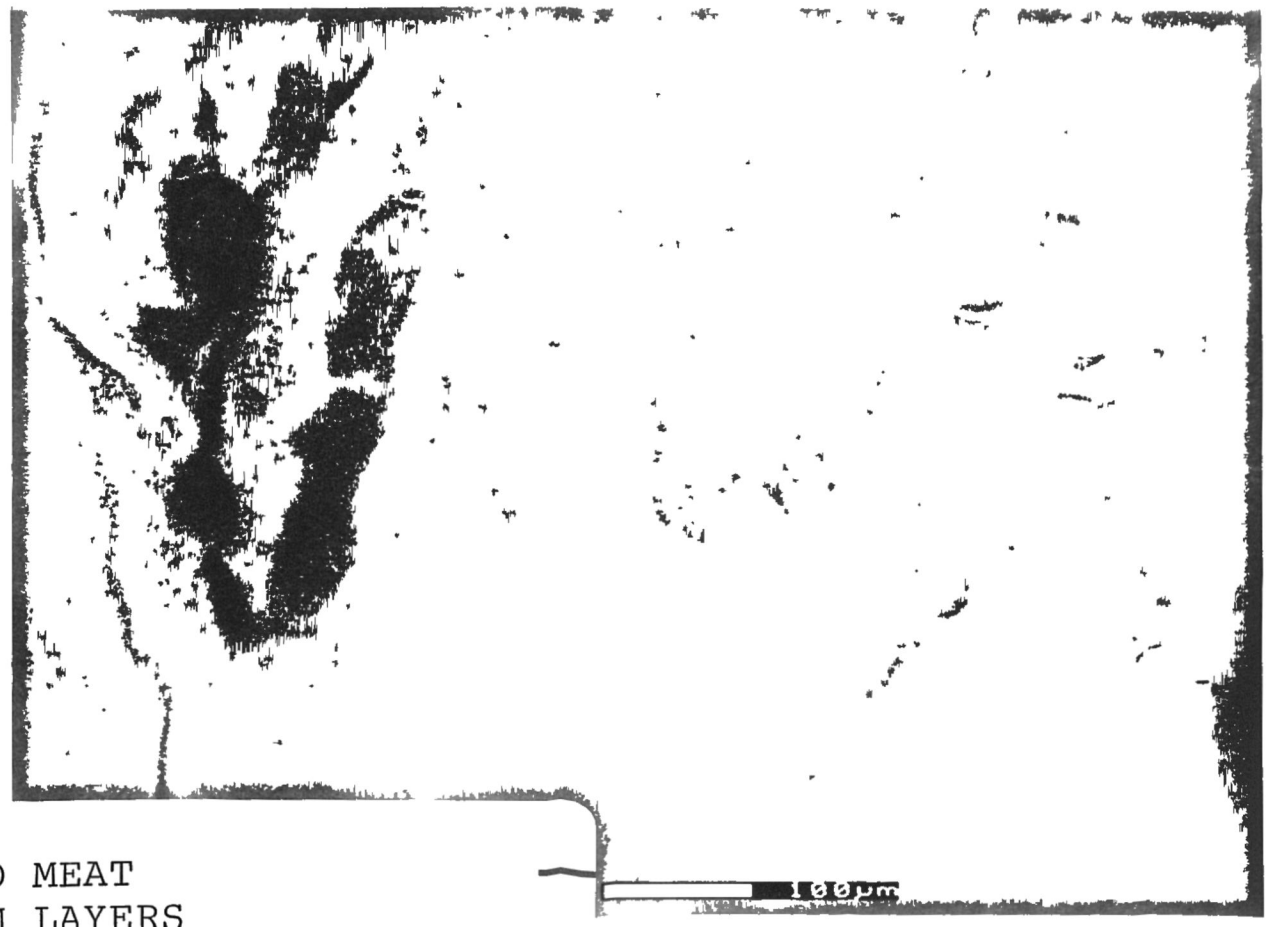
RAW MEAT
2 FILM LAYERS



COOKED MEAT
NO FILM



COOKED MEAT
1 FILM LAYER



COOKED MEAT
2 FILM LAYERS

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