2008

A determination of the physical, chemical, and biological features of suspended dark flecks in hot sauce

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A DETERMINATION OF THE PHYSICAL, CHEMICAL, AND BIOLOGICAL FEATURES OF SUSPENDED DARK FLECKS IN HOT SAUCE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

Horticulture

by
André Brock
B.S., Louisiana State University, 2000
December 2008

ACKNOWLEDGEMENTS

I owe a debt of gratitude to Dr. Paul Wilson, whose support made this project possible. His expertise and patience will always be appreciated. Thanks also to the rest of my committee. Dr. Carl Motsenbocker, Dr. Marlene Janes, and Dr. Jack Losso were all instrumental in the completion of the project. Their guidance in content and in scientific techniques in various fields was important in forming a well-rounded education. Ms. Gloria McClure was a huge help as well, providing much-needed assistance in the Horticulture laboratories. Thanks also to Dr. Ed Bush, Dr. Joan King, and Ms. Cindy Henk for the use of their laboratories and for help with using their equipment.

My parents, Jeanette and Larry Brock cannot be thanked enough for their love and support my entire life. They have always encouraged me to do my best and to conquer higher academic challenges. My wife Tracey Brock has been an indispensable emotional crutch in my life. She has listened to my troubles more than anyone else has. Thank you all for your help and understanding in accomplishing this latest challenging endeavor.

Thanks to the McIlhenny Company and all the staff at Tabasco. Their financial support that made this project possible is greatly appreciated. Thanks especially to the research and development team, led by Mr. Charlie Cheng and Ms. Ming Koh. Your ideas and suggestions were always helpful, as well as your willingness to work with me extensively.

I will always appreciate the knowledge imparted to me by our esteemed emeritus faculty, Dr. Leon Standifer and Dr. Ed O'Rourke. They taught me more before eight in the morning than most people learn all day. Dr. Charlie Johnson and Dr. Jimmy Boudreaux have also taught me more in the field than can be taught in a classroom. Their encouragement and help in finishing the project is appreciated immensely.

Finally, thanks to my coworkers. Ms. Pam Myers and Dr. Pam Hodson who have let me put a high priority on my writing of this thesis. It could never have been finished otherwise. Thanks also to Mrs. Faye Ritchie for helping me with the details I would never have been able to handle on my own. Thank you, Ms. Amy Blanchard for the incredible help with editing. Education is an ongoing journey, and I cannot thank enough the people who have helped me get this far.

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ABSTRACT

Hot pepper and hot sauce production and consumption in the United States are on the rise. The demands of consumers need to be met by high-quality products in an increasingly competitive market. Increasingly knowledgeable and discerning consumers notice any problems in hot sauce, and problems with appearance are often the first noticed. A well-known and successful hot sauce company has found an occasional problem with discoloration in their flagship product.

Small dark specks have been found in the sauce, and the company in question is interested in elucidating their makeup and cause. This is of especially pertinent scientific interest because the specks themselves may be products of lipid oxidation, a constant concern in any food products containing lipids of any kind. In hot sauce in particular, one of its most important components is capsaicin, a lipid-like molecule that imparts its characteristic pungency. Capsaicin is also an antioxidant, and peppers and hot sauce are also high in carotenoids, another antioxidant. Antioxidants are becoming increasingly interesting to the public and to scientists because of their purported health benefits.

In this study, centrifugation was used in an attempt to separate out the specks in the hot sauce, and to compare it to oils found in normal hot sauce. Samples were taken to perform microbiological tests to see if the specks were microbiological colonies. It was shown that they were not. Light microscopy and scanning electron microscopy were used to view samples and to compare them to known pepper parts visually. They were found to resemble microscopic pieces of pepper skin.

Finally, pepper mash was made by grinding pepper samples with varying amounts of salt and, in some treatments, adding oxygen. The peppers were grown in two different locations and all samples were made in duplicate. This was an attempt to re-create a black oil that is sometimes

found on the top of barrels of mash in commercial production and which may be the cause of the specks in question. A single sample was found to produce the dark oil, although its appearance was apparently random with regard to treatment.

CHAPTER 1
INTRODUCTION

1.1 Economic Importance / Explanation of Problem

Pepper sauce in the United States is a major part of the food industry, contributing significantly to the U. S. economy. The consumption of hot sauce and peppers is increasing, partly because of the increase in populations of Latin American and Asian immigrants in the U.S., two ethnic groups that use peppers and hot sauce in their traditional cooking. Furthermore, the overall popularity of these ethnic foods has increased, which drives consumption of hot peppers and hot pepper sauce. Peppers are a valuable horticultural crop, grown in the United States and worldwide (Al-Khatib, 2006). In the United States, Louisiana is especially well known as a bastion of hot sauce production. Hot sauces and hot peppers have been shown to be of significant economic importance.

Most hot sauces in Louisiana are made from peppers of the *Capsicum* genus. These include tabasco, Cayenne, chili, and jalapeño peppers. Tabasco peppers (*C. frutescens*) in Louisiana sold at wholesale prices of $70 and $60 per hundredweight, respectively, for red and green peppers in 2005. Cayennes (*C. annuum*) sold for $25 per hundredweight, and Anaheim chilies (*C. annuum*) sold for $15 per 25 pound bushel (LSU AgCenter, 2005). Information on jalapeños (*C. annuum*) was not available on LSU's agricultural website. These varieties constitute the majority of peppers used for hot sauce production in Louisiana.

Black specks have been observed occasionally and randomly in a particular popular hot sauce. The main issues of these black specks are product returns and potential loss of sales, both of which are significant economic problems. The particles are typically discovered after a bottle of hot sauce is purchased and the consumer finds the problem. If the product is returned to the company, the customer is refunded the cost of the sale via issuance of a coupon. Furthermore,

this problem generates a negative image for the company. Customers typically prefer a homogenous sauce that is consistently the same in every bottle (Cheng, 2006). There has been no evidence which suggests these specks are toxic or hazardous, at least not in the quantities typically consumed.

1.2 Research Problem

Hot sauces are those sauces with added spice from peppers. Hot sauce research and development personnel and quality control personnel have found small, separate specks or particles of discoloration in a hot sauce. The specks are about one millimeter in diameter, seemingly floating in the sauce. However, the particles do not float to the top of the bottle, but rather stay evenly dispersed throughout the product. These specks do not dissipate nor conglomerate out of suspension over time, nor do they seem to fade. They also stay in suspension when the bottle is shaken.

1.3 Possible Sources of Specks

Though the source of the specks of discoloration is currently unknown, this study proposes to elucidate their composition and origin. A number of possible explanations exist. The specks could be caused by enzymatic browning. This type of browning can occur in foods, and is caused by enzymatic oxidation of phenolics. Non-enzymatic browning, another possible cause, also occurs in foods. Different food constituents are discolored in this type of browning as well, but it is not catalyzed by enzymes.

Lipid oxidation is another possible source of the specks. The total lipid content of peppers is only about 4.26%, including mostly linoleic, palmitic, and oleic acids (Guil-Guerrero and others, 2006). But oxidation of even this small amount of lipids could result in visible localized discoloration. The idea that the specks are lipophilic is consistent with the fact that they do not dissolve in the mostly hydrophilic hot sauce.

The specks in the hot sauce could also be microbial colonies. Microscopic organisms are widely known to sometimes conglomerate into visible, often stable colonies. If this happens in hot sauce, they could be seen as small, separate specks of a different color than the rest of the sauce.

If any of the aforementioned possibilities are not found to be the likely culprit, other ideas may be considered. The existence of diseases, for instance, in tabasco peppers could have led to the dark specks in the hot sauce they are used to produce. Likewise, some sort of yet unknown chemical or physical adulteration could occur during processing. The following sections address the more tangible of these possibilities.

1.3.1 Browning

Browning in foods may be desirable in some cases, while undesirable in others. In hot sauces, the latter is the case. Browning of foods is generally divided into two categories: enzymatic and non-enzymatic. Enzymatic browning, as the name implies, refers to a group of biological reactions in which enzymes are involved as catalysts. This type of browning results from an enzymatic oxidation reaction of phenolics in the presence of oxygen. The phenolic substrates are converted to quinones, which in turn can be turned to dark melanin pigments (Werij and others, 2007). Non-enzymatic browning is the result of chemical reactions that do not include enzymes.

The three main types of non-enzymatic browning are 1) sugar amine browning, or Maillard reaction browning, 2) sugar browning, or caramelization, and 3) ascorbic acid browning. Maillard browning involves the formation of a sugar amine from a reducing sugar reacting with an amine group. It can happen at relatively low temperatures, but is inhibited by low pH such as in hot sauce (Luh and Woodroof, 1975). Hot sauce is also low in both sugars and proteins, two necessary constituents for the Maillard reaction. Sugars are consumed during lactic

acid fermentation, and proteins are not usually major components of plant materials. Caramelization can occur at low pH, but high temperatures are needed for this type of browning. Hot sauce is seldom produced or stored above room temperature. Ascorbic acid browning occurs only in products with a high amount of the acid, and low pH, such as citrus fruits (Luh and Woodroof, 1975). This type of browning is unlikely to occur in hot sauce as well.

The most common enzymatic browning is catalyzed by the enzyme polyphenol oxidase (PPO). This enzyme is commonly found in many fruits and vegetables (such as peppers), and its substrates are phenols found in the same fruits and vegetables. These substrates include monohydroxyphenols and ortho dihydroxy phenols, which can be converted to quinones then to dark melanin pigments. The resulting dark pigments created may be gray, black, or brown (Werij and others, 2007). These colors roughly describe the specks in the hot sauce being studied. Mateos and others reported a number of oxidative enzymes to be present in *Capsicum annum* pepper fruits in a study published in 2003. Schweiggert and others (2005) reported that polyphenol oxidase specifically can be found in pepper fruits.

Polyphenol oxidase, like other enzymes, is sensitive to temperature. Cooler temperatures will slow the reaction, while high temperatures can destroy the enzyme altogether. Hot sauce is neither produced nor typically stored at low temperatures. Nor is it brought to temperatures high enough to destroy the enzymes during processing. In fact, the mash from which hot sauce is produced is often stored in non-air-conditioned warehouses in the southern part of the United States. This environment provides an almost ideal temperature for enzymatic activity. These temperature factors could allow enzymes potentially present in hot sauce to catalyze oxidation reactions efficiently and quickly. No efforts are made to remove oxygen from hot sauce mash during production. Some sauce products such as ketchup are heat-treated. This not only destroys enzymes but also removes oxygen, preventing oxidation reactions while in storage. Hot sauce

does not undergo such preventive measures, leaving abundant oxygen available to react with other substrates.

Addition of sulfite, sulfur dioxide, proteases, and ascorbic acid can all slow or prevent enzymatic browning, (Chaisakdanugull and others, 2007) but the hot sauce in question does not contain any such additives. The two notable chemical factors the hot sauce has to prevent enzymatic browning are a low pH and the salt content. Therefore, enzymatic browning is not a likely possibility for the cause of the unknown specks of discoloration.

1.3.2 Capsaicin and Other Lipids That May Be Oxidized in Hot Peppers / Hot Sauce

Another possible source of discoloration is lipid oxidation. Capsaicin is a major active ingredient found in hot peppers of the genus *Capsicum*. It is a homovanillic lipid-like acid derivative, shown to act on nerve receptors in humans, causing an inflammatory response similar to responses to chemical or heat burns (Mori and others, 2006).

Capsaicin is a member of a larger group of similar compounds called capsaicinoids, which also includes dihydrocapsaicin, an analog of capsaicin with a different degree of saturation in a single side chain. All capsaicinoids share a common aromatic moiety, vanyllamine (Mori and others, 2006). Capsaicin is a waxy, crystalline substance found in concentration in peppers on the scale of mg/g. These lipophilic, acidic compounds are phenolic alkaloids with pharmacological properties (Materska and others, 2005). Much of the relevant research regarding hot sauces and hot peppers has centered on chemical and physical properties of capsaicin and its analogs.

Much current research in food products focuses on antioxidant properties of food components such as capsaicin and other phenols found in peppers (Materska and others, 2005). Tsuji and others showed in 2005 that liquid chromatography/mass spectrophotometry (LC/MS) and gas chromatography/mass spectrophotometry (GC/MS) are effective in qualitatively

identifying these oxidized components. However, they did not work with finished hot sauce, much less with the black specks described in the proposed study here.

Oxidation is a chemical reaction that results in a gain in the number of oxygen atoms or a loss of the number of hydrogen atoms in a molecule (Holum, 1978). Oxidation of lipids and lipid-like compounds in foods is commonplace. It involves hydrolysis into glycerol and fatty acids first, then a separate oxidation of the resulting substances (Jacobs, 1951). Phenolics have also shown to be easily oxidized, often resulting in drastic color changes. In this case, the phenolic ring is attacked by oxidizing agents, causing a loss of electrons (Holum, 1978).

Mori and others (2006) showed that capsaicin can evoke physiological responses in humans. This shows that it is a reactive oil component in foods, and that nutraceutical properties may exist in foods that contain capsaicin, such as hot sauces. This underscores the importance of research to further explain chemical properties of capsaicin and similar lipid components of foods. Osaka and others (2000) suggested the use of capsaicin to study pain neuropathways and biological temperature control. Ohnuki and others, (2001) reported that a capsaicin analog can be used to increase thermogenesis and combat body fat accumulation. This suggests that further research is possible to understand potential health benefits of capsaicin. All this underscores the importance of research to understand the chemistry of capsaicin-containing foods such as hot sauce.

Other lipids, of course, have been shown to exist in peppers. It has been shown that linoleic acid, palmitic acid, and oleic acid together make up the great majority (80%) of the lipid content in peppers. The total lipid content of peppers is no more than 4.26% of total dry weight (Guil-Guerrero and others, 2006). Any of the lipids that exist in peppers may be considered potential sources of lipid oxidation compounds that may be responsible for causing the black specks.

1.3.3 Pigments in Hot Peppers / Hot Sauce

In making hot sauce, the defining ingredient is ground hot peppers. Though much of the solids in the peppers are strained out (mostly seeds) during processing, the hot sauce does get its color from the peppers. Hot sauce is commonly red or reddish, but may also be yellowish or even green depending on fruit maturity. Although sauce color is sometimes controlled by addition of artificially produced food dyes, naturally colored sauces are dependent on the pigments in the peppers from which the sauces are made. The sauce being studied is primarily red, from mature pepper fruit. Red coloration in peppers, as well as orange or yellow, comes mainly from carotenoids in the pepper fruit, including beta-carotene (Wall and others, 2001). A number of other compounds found in peppers, such as phenolics, carotenoids, flavonoids, vitamin A, ascorbic acid, tocopherols have also shown antioxidant properties (Rosa and others, 2002). It has been shown that processing at elevated temperatures can have significant effects on the red carotenoid and xanthophyll pigments in chili and paprika peppers (Schweiggert and others, 2007). The effects of salt and vinegar on the pigments have been less studied.

1.3.4 Pepper Diseases

Like almost any agricultural product, peppers are subject to a number of diseases. If diseased peppers are used to produce pepper sauce, the diseased portion may survive the production process intact. Parts of the peppers that were discolored when harvested, or in storage, could impart discoloration to the finished hot sauce. Diseases that cause dark discoloration in peppers include bacterial spot, anthracnose (ripe rot), pepper mild mottle, tomato spotted wilt, and blossom end rot (Black and others, 1991).

1.4 Problem Statement

The mash used in the fermented hot sauce to be studied undergoes anywhere from two months to six years of fermentation with 6-15% salt in oak barrels in non-air-conditioned

warehouses. All production of the sauce in question is done in Louisiana, in a hot and humid climate. After fermentation, the barrels are opened, the mash is drained, and the more solid material is further processed to make the sauce. A dark layer is sometimes found as a top layer in these barrels after extended fermentation. Much of the mash used for production is shipped in barrels to Louisiana from Central and South America. The same discoloration is sometimes found as a top layer in these barrels as well, after only partial fermentation has occurred in transit. Research and development personnel believe this layer may be a product of fermentation. They also suspect that the layer may be the source of the black specks in question. Production personnel suspect this source is oil related to the specks in the final product because the layer is similar in color to the specks, and because lipids may behave as the discolored layers and the specks do, regarding separation in a polar solution.

Research on the subject of hot sauces has been limited to peppers and to hot sauces in general. The specific problem of discoloration in fermented pepper mash and sauce has not yet been addressed.

1.5 Need for Research

This research will make a significant contribution to current research, as it will investigate lipid, pigment, and darkening properties in hot sauces. Hot sauces are typically preserved only by pH reduction and salting, not by heating, chilled storage, or vacuum packaging. Other sauces and condiments, such as ketchup, are heat-treated prior to aseptic packaging. This destroys spoilage microorganisms and removes much of the gas in the product, including oxygen. Oxygen in a product helps retain potential for oxidation and enzymatic degradation, as discussed earlier. Heat can also destroy enzymes naturally found in a product, but this particular hot sauce is not heated. Hot sauce does not have the benefits of heating during processing, and is therefore more susceptible to oxidation and enzymatic degradation. Microbes

exist in this type of hot sauce as well, further contributing to oxidation and degradation of the product. Canned products are also preserved by heat, and have the added advantage of a vacuum to limit aerobic microbial growth. Hot sauces such as this one are preserved neither by heat nor by oxygen exclusion. This makes the category of sauces a unique product with unusual properties that invite interesting and significant opportunities for scientific investigation. This study proposes to discover the composition and possibly the source of the dark specks sometimes found suspended in hot sauce.

CHAPTER 2
LITERATURE REVIEW

2.1 Color in Foods

In making a purchase decision, one of the first things a consumer notices is the color of the food or food product. Color of the product can infer freshness and relative quality of the product in question. When a given product, including hot sauce, is faded or oxidized, there exists an implication that the product is stale or less fresh than a comparable brightly colored product. If a product is discolored, or has areas or specks that are discolored, the quality of the product is similarly brought into question. If a consumer has purchased a product in the past, he or she expects it to look the same every time it is purchased. Furthermore, the discolored or problematic food reflects poorly upon the producer or manufacturer and can adversely affect future sales.

As food producers and manufacturers readily understand the importance of color in their products, they seek to understand the complex issues involved in food color. Various chemicals, both natural and artificial, can be the source of distinguishing colors in various foods. Hot sauces in particular are most often red, but can often be found in orange, yellow, or green. Though artificial coloring can be used to produce or enhance the appearance of these colors, some manufacturers prefer not to use added coloring agents (Cheng and Koh, 2006). In this case, the only coloring in the sauce is from the peppers themselves, as the two other ingredients (salt and vinegar) are practically colorless. In green hot sauce, the peppers are picked relatively early, so that the main color component in the peppers is chlorophyll. But as peppers ripen, most change color to yellow, orange, or red because of the ripening development of carotenoids of the same colors. The color is also affected by the simultaneous degradation of green chloroplasts (Almela and others, 1996).

2.2 Discoloration in Foods

2.2.1 Polyphenol Oxidase / Enzymatic Browning

Though hot sauce enjoys vibrant coloring, mostly because of the carotenoid content, it is still subject to some of the same threats of discoloration as other foods. Enzymatic browning is a common cause of discoloration among foods. In this type of browning, it is the action of particular enzymes breaking down their specific substrates that causes the discoloration. A common enzymatic browning reaction in plant foods involves the enzyme polyphenol oxidase (PPO). As the name implies, polyphenolic substances act as the substrates for this enzyme. The enzyme / substrate system is nearly ubiquitous in some form in a plethora of fruits and vegetables, including peppers. As with some other enzyme / substrate systems, the effects are often seen after plant parts and cell walls are broken. In preparation of mashes for hot sauces, the plant parts (pepper pods) are thoroughly broken apart, so this type of browning must be considered a serious candidate for an explanation of the discoloration seen in the hot sauce in question.

Oxidative enzymatic reactions occur exclusively in the presence of oxygen. In making mash, ample oxygen is incorporated into the mix by the mechanical action of grinding and crushing the peppers. No effort is then made to remove the oxygen before fermenting and aging the mash. In enzymatic discoloration, PPO oxidizes phenolic compounds such as monohydroxyphenols and ortho dihydroxy phenols to intermediate compounds called quinines. The quinines are then further oxidized to form melanins, which are dark gray, brown, or black colored pigments (Friedman, 1997).

These reactions have been observed in a number of vegetable crops. Mateos and others, reported in 2003 that oxidative compounds can be found in peppers. PPO specifically was reportedly present in peppers, according to Schweiggert and others in a 2005 study.

Polyphenolics including chlorogenic acid have been found in plants of the *Solanaceae* family, of which tabasco peppers (*Capsicum frutescens*) are a member.

The polyphenol oxidase compounds are involved in plants' defending themselves against certain pathogens, including viruses, bacteria, and fungi. They may also be useful in protecting the plant against adverse effects of mechanical and light injury. The role of PPO in plant defense is evident in the discoloration following bruising in many plants and plant foods, such as potatoes. Wounding of plant tissues, in fact, can lead to increased production of PPO in the surrounding area. PPO gene manipulation has been suggested as a possible preventative measure against browning in food products. For PPO gene expression to lead to development of PPO, it follows the biosynthetic pathway of forming chlorogenic acid from phenylalanine (Friedman, 1997).

Gene manipulation is not, at this point, a practical course in eschewing browning in peppers, or in most food products. However, a number of anti-browning treatments exist. Avoiding mechanical injury in processing is impossible in hot sauce production, so other options are more viable in this situation. Chemical additives have been shown to be effective in ameliorating enzymatic discoloration in vegetables. Sulfur-containing compounds in particular have been shown to inhibit PPO. Sulfites act as reducing agents, and they can also form complexes with intermediates in the discoloration chain reactions. Effective sulfhydryl (SH or thiol) compounds include cysteine, N-acetyl-L-cysteine, and reduced glutathione. SH-containing amino acids and peptides have been shown to inhibit not only enzymatic browning by PPO, but also non-enzymatic browning in some foods (Friedman, 1997). Cysteine in particular has been shown to inhibit browning by forming colorless addition compounds with quinones (Chitsuda and others, 2007). Sodium sulfite was once used as a discoloration preventive, but it is no longer

used because of health concerns. Also, ascorbic and citric acids have been shown to inhibit the PPO enzyme (Friedman, 1997). Citric acid does so by chelating copper at the active site of PPO.

None of the aforementioned chemical preventatives, however, are realistic options to prevent discoloration in this particular instance. The hot sauce company that produces the sauce in question is not willing to include such additives to their flagship product. The company follows a traditional recipe and procedure, and bases much of its product promotion on this concept and the public perception it creates.

Polyphenol oxidase, like other enzymes, is temperature-sensitive. The proteins involved can be denatured not only by chemical means, but also by high temperatures. At no point in hot sauce processing does the mash or sauce undergo extremely high temperatures that would be sufficient to destroy the activity of these enzymes. Similarly, extremely cold temperatures can inhibit or even halt enzyme activity. A decrease in kinetic energy in a chemical reaction can be expected to slow its progression. Some enzymes are biologically adapted to colder temperatures, especially in plants that are native to colder climates. But peppers are native to tropical regions and cannot be expected to hold cold-active enzymes. Therefore the temperatures at which the mash is stored and aged in South Louisiana provide an ideal temperature band for enzyme activity in peppers and in the mash containing crushed peppers.

2.2.2 Non-Enzymatic Browning

Non-enzymatic browning in foods is a general term to describe all those chemical reactions that cause discoloration but are not enzyme-mediated. Since enzymes lower the activation energy of chemical reactions, the non-enzymatic reactions typically involve more energy input into the reaction and therefore occur less quickly and commonly than enzymatic browning. Three categories of non-enzymatic browning are 1) sugar amine browning, or

Maillard reaction browning, 2) sugar browning, or caramelization, and 3) ascorbic acid browning.

In Maillard browning, the carbonyl group of a reducing carbohydrate links with the amino group of a free amino acid or to lysyl residues in proteins (Ajandouz and others, 2001). This results in the formation of brown coloration in a food product, which may be beneficial in some products, but is detrimental in hot sauce. Compared to other types of non-enzymatic browning, this can happen at relatively low temperatures, but this requires the presence of sugar as well as protein as a source of amine groups (Luh and Woodroof, 1975). Peppers have not been shown to contain high amounts of sugars or proteins. Furthermore, nearly all sugar present in peppers would be consumed by lactic acid bacteria during the fermentation process in this particular hot sauce. Finally, Maillard browning does not tend to occur as readily at low pH compared to neutral or high pH (Ajandouz and others, 2001). Fermented pepper sauce, because of the presence of lactic and other acids, does have a low pH that would likely inhibit this type of browning.

Caramelization is another form of non-enzymatic browning that occurs in foods. In this process, low molecular weight carbohydrates are heated to produce a wide array of brown polymers. The products formed are dependent on the reactants used (which sugars) and the conditions of the reaction, such as presence or absence of acid. But in general, thermal caramelization will produce brown pigments in a food by way of intramolecular re-arrangements of the typically monosaccharide sugar or sugars involved (Fadel and Farouk 2001). Higher temperatures are needed for this type of reaction to take place compared to Maillard browning. Therefore, temperature-induced caramelization is not a likely cause for the discoloration found in the hot sauce.

Ascorbic acid browning is another type of non-enzymatic browning known to occur in low pH foods with a high amount of the acid, such as in citrus fruits (Luh and Woodroof, 1975). It is caused by the degradation of ascorbic acid into other colored chemicals over time, without the need for other reactants or catalysts. Nonetheless, it is unlikely to occur in hot sauce. Though the sauce does have a low pH, it does not contain a significant amount of ascorbic acid.

2.3 Pepper Chemistry

2.3.1 Proximate Analysis

The small specks of interest are only a miniscule part of the hot sauce compared to the total volume or weight of the product. Though vinegar makes up a large part of the sauce, it can be safely assumed that the ultimate source of the specks is a small part of the peppers from which the sauce is made. Therefore, it may be helpful to know the constituent chemicals of peppers.

Many species and varieties of peppers are cultivated worldwide. The peppers can vary in size, color, and nutrient content. A particular study in 2006 by Guil-Guerro and others investigated the composition of *Capsicum annuum* peppers. These are closely related to *Capsicum frutescens*, the tabasco pepper that is the focus of the current study. It may be assumed that the two species at least fall within the same approximate range of composition of various constituent chemicals.

The above mentioned study found the moisture content of the peppers to range from 89.4% to 94.7%. Crude protein was measured at 1.20% to 0.70%. Available carbohydrates were 1.34% to 4.82%. Total lipids were 0.19% to 0.95%, ash was 1.02% to 2.07%, and vitamin C was 380 to 102 milligrams per 100 g fresh weight of peppers (Guil-Guerro and others, 2006). Table 2.3.1 from the same study outlines the proximate analysis of the ten pepper varieties.

Table 2.3.1 Proximate composition and Vitamin C content of 10 pepper varieties on 100 g of fresh weight.

Variety	Moisture (g)	Crude protein (g)	Available carbohydrates (g)	Lipids (g)	Neutral detergent fiber (g)	Ash (g)	*E* (kcal)	£(kJ)	Vitamin C (mg)
Red Lamuyo	92.5 ±1.0	0.81 ±0.02	2.78 ±0.32	0.60 ±0.06	1.22 ±0.10	1.42±0.14	19.0±1.1	79.8 ±5.2	293 ± 12
Yellow Lamuyo	92.8 ±1.2	0.96 ±0.02	2.63 ±0.51	0.50 ±0.04	1.09±0.11	1.34±0.l0	18.2 ±1.6	76.5 ±4.9	251±20
Green Lamuyo	94.7 ±1.3	0.70 ±0.06	1.83 ±0.29	0.19 ±0.06	1.04±0.11	1.02 ±0.09	11.3 ±0.9	47.5 ±3.8	119±12
Red California	92.7 ±0.8	0.99 ±0.08	2.96 ±0.23	0.37 ±0.04	1.09 ±0.09	1.41 ±0.11	18.3 ±1.7	76.7 ±4.9	348±25
Yellow California	92.3 ±1.5	0.95 ±0.07	3.08 ±0.68	0.46 ±0.06	1.22±0.11	1.49 ±0.14	19.5 ±1.8	81.7±7.6	380±0.10
Orange California	90.8 ±0.8	0.96 ±0.08	3.17 ±0.61	0,60 ±0.03	1.09 ±0.12	1.76±0.18	21.1±2.1	88.5 ±7.0	378±22
Green California	93.9 ±1.4	0.63 ±0.03	1.34 ±0.09	0.31 ±0.04	2.05 ±0.15	1.17±0.13	10.3 ±1.6	43.2 ±3.2	268±22
Red Italian	89.4 ±1.4	1.20 ±0.10	4.82 ±0.44	0.53 ±0.04	1.55 ±1.14	2.07 ±0.16	27.6 ±2.0	115.8±11.1	287 ±21
Green Italian	93.7 ±1.7	0.71 ±0.01	2.11 ±0.24	0.38 ±0.02	1.43 ±0.09	1.21 ±0.12	14.2 ±0.8	59.3 ±4.2	201 ± 19
Green Pricking	92.7 ±0.5	1.12 ±0.03	1.57 ±0.30	0.95 ±0.06	1.93 ±0.22	1.42 ±1.10	18.9 ±1.0	79.3 ±6.8	102 ±14

2.3.2 Capsaicin and Lipids in Peppers

The most important "active ingredient" in *Capsicum* hot peppers is capsaicin. This hydrophobic chemical is what gives hot peppers their "heat" in food, or the burning sensation one experiences when consuming these peppers and products such as hot sauce made from them. Capsaicin is a waxy, crystalline, homovanillic lipid-like acid derivative that acts on nerve receptors in humans and other mammals, causing an inflammatory response similar to responses to chemical or heat burns (Mori and others, 2006). This reaction is typically more pronounced in the tissues of the mouth, but capsaicin can evoke this response anywhere it contacts skin.

Figure 1 shows the approximate makeup and structure of capsaicin:

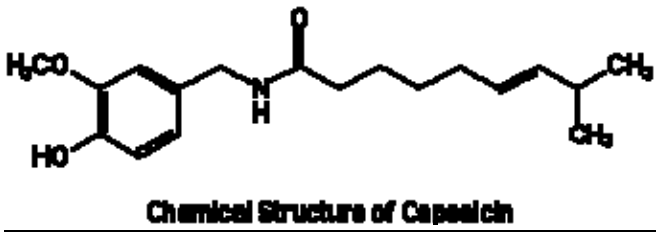

Figure 2.3.2. Chemical structure of capsaicin.

Most research on pepper chemistry focuses on capsaicin in particular. In fact, capsaicin is part of a larger group of compounds called capsaicinoids, many of which are found in peppers. Dihydrocapsaicin is another capsaicinoid which is an analog of capsaicin and is commonly found in peppers. All capsaicinoids are lipophilic, acidic, phenolic alkaloid compounds (Materska and others, 2005). Phenolics have been found to react in oxidation reactions. The phenolic ring of these molecules can be attacked by oxidizing agents, resulting in a loss of electrons. This can result in noticeable color changes (Holum, 1978).

Capsaicinoids have been found to hold antioxidant properties (Rosa and others, 2002), further contributing to their study in recent research. Their antioxidant quality also means that the compounds may be oxidized. Since oxidation is often a source of discoloration in foods, this

implies that capsaicinoid compounds may be the substrate for chemical reactions leading to discoloration in hot sauce.

In oxidation reactions, a molecule gains oxygen atoms or loses hydrogen atoms (Holum, 1978). In lipid oxidation, a lipid molecule is first hydrolyzed into glycerol and fatty acids. The resulting substrates are then oxidized in a separate reaction (Jacobs, 1951).

Lipids constitute only 0.19% to 0.95% of peppers by total weight, or 4.26% by dry weight (Guil-Guerro and others, 2006), but their presence in peppers is important partly because capsaicin is a lipid. Furthermore, lipids are known to oxidize in food products and cause discoloration. Other lipids exist in peppers, 80% of which can be attributed to linoleic acid, palmitic acid, and oleic acid (Guil-Guerro and others, 2006). The source of the discoloration in the hot sauce in question may be linked to its constituent lipids and lipophilic compounds.

2.3.3 Carotenoids

Pigments such as carotenoids in peppers are often lipophilic compounds, and they are found especially in later stages of development in ripe peppers (Minguez-Mosquera and Hornero-MBndez, 1993). Carotenoids have also been studied recently as antioxidants in food, indicating that they may also readily react with oxygen, possibly causing color changes. They are known to be chemically unstable pigments. Carotenoids are especially of interest in this study because they are known to be commonly found in peppers and pepper products. They are pigment compounds, meaning that they can give color to foods that contain them. Most of the color associated with peppers, including yellow, orange, and red, can be attributed to carotenoid content.

Ripe peppers tend to have higher concentrations of carotenoids than less mature peppers (Almela and others, 1996), and the hot sauce in question uses only completely ripe peppers. The final red color found in ripe peppers of certain varieties (including Tabasco) can be attributed

mainly to the carotenoids: capsanthin, capsorubin, and capsanthin 5,6-epoxide (Guil-Guerrero and others, 2006). It has been theorized that the bright color attracts birds to the ripe fruit, the seeds of which are then well-dispersed. It is the carotenoids that give the peppers their red color, and the same is true for the hot sauce. Beta-carotene is one carotenoid in particular that has been identified in peppers (Wall and others, 2001). Food processing at elevated temperatures has been shown to have significant effects on the red carotenoid and xanthophyll pigments in chili and paprika peppers (Schweiggert and others, 2007). However, the hot sauce studied here is not thermally processed. It may be assumed that carotenoids are still unchanged even after the sauce has been bottled, and it may continue to be active as a reactant antioxidant.

2.3.4 Nutritive Properties of Peppers

Peppers have been shown to contain capsaicin and carotenoids, which have both displayed antioxidant properties. They contain other antioxidants as well, including ascorbic acid, flavonoids, phenolic acids, tocopherol, and provitamin A (Rosa and others, 2002; Guil-Guerro and others, 2006). Carotenoids, ascorbic acid, and provitamin A have been shown to increase along with increasing red color development as peppers ripen (Guil-Guerrero and others, 2006). These antioxidants (along with many others) have been studied as nutraceuticals. They have been linked to reduction of incidence and severity of cancer, cardiovascular disease, diabetes, macular degeneration, and other degenerative diseases. This is because they readily react with free radicals in the body, binding them so that they cannot easily damage living cells.

2.4 Pepper Horticulture and History

Tabasco peppers are members of the genus *Capsicum*, within the family *Solanaceae* (Wheat, 1987). This genus includes a large and widely varied number of species often collectively referred to as “chilies” or “peppers.” More than 90 species of peppers exist (Wheat, 1978). Five of these species and countless cultivars are cultivated by man, and many (including

Tabasco) contain capsaicin, leading to their common use as a spice in foods. Those that lack capsaicin may be eaten as vegetables, but these are not used in hot sauces. Peppers have also been used in medicines historically, and they have been studied in modern times for medicinal properties (Eastwood, 1999). Their pungent ingredient, capsaicin, is used in topical pain relievers and circulatory stimulants. It can also be used to deter attackers as in pepper spray, or in animal deterrents for landscapes and gardens. Mammals may feel a burning sensation from ingesting or contacting capsaicin, but birds do not appear to suffer this reaction. Much of the renewed research interest is because of peppers' high content of carotenoids, nearly ubiquitous in ripe peppers and now studied as antioxidants.

The *Capsicum* genus is native to the Americas, where five species were cultivated by natives before the arrival of Europeans to the New World (Guil-Guerrero and others, 2006). The cultivated species are *Capsicum annuum*, *C. frutescens*, *C. Chinense*, and *C. pubescens* (Koh, 2005). As soon as Europeans made contact with the New World, they learned of the uses of peppers in food. Peppers then quickly enjoyed more widespread distribution thanks to European ships and trade alliances. They were traded along with other spices and quickly enjoyed worldwide distribution. It was also found that these peppers could be readily cultivated in some areas of Europe. This was especially true in warmer regions near the Mediterranean, and most notably in Spain (Guil-Guerrero and others, 2006). Since many *Capsicum* species are easily cross-pollinated, many new cultivars were developed. Modern pepper cultivars range from bell peppers, which have no pungency, to the 'Bhut Jolokia' with over a million Scoville units. The Scoville scale is used to objectively compare relative pungency of peppers. Even within a species, different cultivars can vary widely in appearance and flavor.

Of these innumerable cultivars of peppers, tabasco (*Capsicum frutescens*) and Cayenne, (*Capsicum annuum*) are used as the bases of the majority of pepper sauces made in Louisiana

today. The Cayenne is not native to Louisiana, but it grows there quite readily. This obviously lends it to local value-added processing. The focus of this paper is hot sauce made from tabasco peppers, native to their namesake state of Tabasco in Mexico.

Tabasco plants have indeterminate stems, a compact growth habit, and grow up to four feet high in tropical zones. It often grows as a perennial in tropical zones. However, in temperate zones the plants may grow only one foot high. It often does not survive the winters of temperate climates, or is at least damaged by winter's cold, moderating its size. The 2.5 by 1.25 inch leaves are ovate and smooth. Flowers have white corollas with no spots. Pods are erect, measuring 1.5 inches long by 3/8 of an inch wide. An individual plant can produce over 100 of the small pods (DeWitt and Gerlach, 1990). The immature pods start off yellow, turning orange, then finally red when fully mature. It has been found that pods detach from the calyx more easily when fruit is fully mature, facilitating easier picking (Arancibia and Motsenbocker. 2006). It is at this stage that peppers are picked for hot sauce production. The change is color is because of the breakdown of chlorophyll and the increase in red carotenoids. This happens by way of internal enzyme-mediated metabolic activities of the plant that convert green chloroplasts to carotenoid-rich chromoplasts (Almela and others, 1996). Carotenoids are typically red or reddish. Tabasco peppers measure from 30,000 to 50,000 Scoville heat units, or an eight out of ten on the heat scale (DeWitt and Gerlach, 1990).

The region of Tabasco, Mexico traded heavily with New Orleans in the 1850s, and it was during this period that tabasco seed first made its way to Louisiana (DeWitt and Gerlach, 1990). Maunsell White, a New Orleans banker at the time, is believed to have been the first person to cultivate tabasco peppers in Louisiana. He gave some pods and a hot sauce he made from the peppers to a friend named Edmund McIlhenny, who began growing tabasco peppers on Avery Island (Koh, 2005). Avery Island provided a hot, humid climate favorable for the pepper plants,

and had the added advantage that it is situated atop a salt dome. The abundant supply of salt lent a hand in making hot sauce, which McIlhenny began producing in 1869. He patented the sauce in 1870 as Tabasco® sauce, and it soon gained international popularity. Today it is one of the most recognized and widespread hot sauces worldwide. Other flavors of Tabasco® sauce have since been developed, but the Original Tabasco® Sauce has not deviated from Edmund McIlhenny's original recipe.

2.5 Description of Sauce Making, Including Fermentation

2.5.1 Fermentation

Original Flavor Tabasco® Sauce is one of the few commercially produced hot sauces made from fermented and aged mash. A form of food preservation, fermentation is "slow decomposition process of organic substances induced by micro-organisms, or induced by complex nitrogenous substances (enzymes) of plant or animal origin (Walker, 1988). In the case of the hot sauce in question, bacteria, especially *Lactobacillus spp.*, are the microorganisms responsible for the fermentation. Lactic acid-producing bacteria, such as *Lactobacillus spp.*, are used in many sour fermented foods such as sausages, sauerkraut, and pickles. Their production of acid lowers the pH of a food, which inhibits growth of competing microbes that may otherwise spoil the food. Some also produce bacteriocins, small proteins that inhibit microbes that share their environment. Both of these factors were apparently evolved in the bacteria to shun competitors for resources such as nutrients, water, and space. But these traits have also been exploited by man for his own benefit to preserve foods.

Consumption of bacteria in our foods is actually not a new concept at all. Fermented products such as cheese and beer have been parts of the human diet for thousands of years. The fermented products, due to controlled breakdown by microorganisms, are more resistant to spoilage than their unfermented raw counterparts. The fermenting microbes have commonly

been consumed along with the fermented product, even though the science behind fermentation was not truly understood until after Louis Pasteur's experiments in the late 1800s. Our understanding of the microbial action of fermentation has improved much since it was first used. Modern technology has streamlined mass-quantity fermentation and perhaps led to more consistent quality control in hot sauce production. But the microbiological processes involved have, like the McIlhenny's recipe, remained unchanged.

2.5.2 Hot Sauce Production

Different hot sauces are made in a wide variety of ways, each imparting its own flavor. Generally, hot sauce production involves the combination of peppers and vinegar brine, giving the finished product the tastes of salty, sour, and hot. The hot sauce that is the focus of this study also ferments the peppers to impart a unique flavor of its own. Due partly to lactic acid-producing bacteria involved in fermentation, this process adds some acid itself.

Peppers are picked at their ripest red stage. Some of these are grown on Avery Island, but the majority of the peppers are grown on contract farms in South and Central America. In the case of the overseas peppers, they are ground with eight percent salt by weight and mixed into large vats. Once crushed and mixed with salt, the substance is called "mash." These vats of mash then are shipped to the processing facility at Avery Island, Louisiana. The shipping itself takes from weeks up to a month (Cheng and Koh, 2006). Upon arrival, the vats are emptied into wooden or plastic barrels and sealed for about three years' fermentation and aging in an ambient-temperature warehouse. In the case of peppers grown on-site, they are simply crushed in a hammer mill, and then poured into barrels with eight percent salt. The salt is scooped into barrels, and it is not necessarily homogenous throughout the mash mixture. Some barrels used are now made of plastic with threaded plastic lids. However, charred white oak barrels from whiskey distillers in Kentucky are more traditionally used (Koh, 2005). Lids are secured onto the

tops of the barrels with stainless steel hoops. The lids on the barrels have small holes to allow the escape of gas (mostly carbon dioxide) produced during fermentation. To reduce contamination from unwanted microorganisms, the tops of the barrels are layered with salt. This layer is semi-permeable, allowing minimal gas exchange and near exclusion of microbiological contaminants. Wet bubbles from the fermenting mash escape fairly easily. But fermentation eventually slows, and gas no longer rapidly escapes. The salt then forms a hard, packed layer in the high-humidity environment. Once fermentation slows and the layer dries hard, permeability decreases.

The salt layer is not a perfect barrier, especially to gas exchange. After the roughly three years of mash fermentation and aging, there is considerable oxidation on the top of the barrel of mash. When the tops are removed, this top layer is discarded. Rarely, and apparently randomly, a layer of dark oil is found on top of the mash in the barrel. This oil is discarded as well. However, it is theorized that some of this oil may remain in the rest of the mash, and may be a source of discolored specks found post-production. The rest of the mash is inspected and, pending acceptance, pumped into large vats. In the vats, the mash is blended with distilled white vinegar for 24 hours. Though this is not the final product, it is referred to as “hot sauce” at this point. Solids and seeds are strained from the hot sauce, and it is bottled, shipped, and sold worldwide. Because of the fermentation and the addition of vinegar, it is considered a shelf-stable high acid food with a pH below 4.6. Its pH typically ranges from 2.70 to 3.10.

CHAPTER 3
MATERIALS AND METHODS

3.1 Processing and Preparation of Mash Samples

All samples of hot sauce were provided by McIlhenny Co., Avery Island, Louisiana. Fresh peppers were also provided by McIlhenny, and other fresh peppers were grown at LSU's Burden Center, Baton Rouge, Louisiana. Pepper processing included grinding the peppers in a 120 volt one-quart capacity blender (Waring model 51BL32, Torrington, CT 06790) for approximately five minutes per sample. Ground peppers were then stirred with Cargill 50 CMF (Calcium-Magnesium Free) Evaporated Salt (Cargill, Inc., Minneapolis, MN 55440) using a clean glass rod.

A batch of peppers grown at Avery Island was processed as described above, with varying amounts of salt added. Salt contents were 4%, 8%, and 12% salt by weight. Sets of peppers from Burden Center ("LSU peppers") were ground, then mixed with varying amounts of salt as described above. One batch of LSU peppers was deliberately picked and processed with excess stems and leaves, but salt contents were the same as the Avery Island peppers. Another set of LSU peppers were left whole at room temperature (averaging roughly 37° C) without salt.

A third set of LSU peppers had their seeds and placental tissue removed. A knife was used to split the individual peppers vertically. Then the same knife was used to gently scrape the placental tissue and seeds out, leaving the rest of the pepper. This material (seeds and placental tissue together) was ground in a 120 volt one-quart capacity blender (Waring model 51BL32, Torrington, CT 06790) with salt and water for approximately one minute. All samples were then left to ferment at room temperature (averaging roughly 37° C) for approximately three months. These scenarios attempted to simulate an identified quality defect found in hot sauce resulting in dark specks within the final product.

3.1.1 Fresh Mash Samples' Preparation and Measurements

Objective and subjective comparisons of color were made between fresh mash and fermented mash and between different treatments. For these comparisons, mash samples were freshly made, then allowed to ferment. Fresh mash samples (ground peppers with salt concentrations at, above, and below normal levels) were produced from both pepper sources (Avery Island and Baton Rouge), then later compared to fermented mash, both with and without added oxygen. Oxygen was added to half the samples. Oxidation may be a source of the specks in question, and the addition of oxygen may affect formation of specks, or general changes in color. Oxygen-added samples included those from Avery Island and from Baton Rouge. They also included all variations of salt content. Oxygen was added by attaching ¼" tubes to a pressurized oxygen tank, then placing the tubes in samples of mash. The tank was opened, pressure was adjusted to 80 kPa (11.6 psi) using a pressure regulator, and the tubes were stirred within the containers of mash for approximately one minute each. The mash was then flattened on top with a metal spoon. All products described were compared to one another for organoleptic and visual appearance properties, as well as pH, since pH can potentially affect the color of a food product.

Samples of mash were prepared using roughly 300 g of tabasco peppers each. The peppers were blended in a Waring model 51BL32 food-grade blender (Waring Products, Inc., Torrington, CT 06790) for approximately five minutes per sample. Various concentrations of Cargill 50 CMF (Calcium-Magnesium Free) Evaporated Salt (Cargill, Inc., Minneapolis, MN 55440) were added and stirred in with a glass rod immediately after blending. Commercial production often does not use accurate measurements, nor are barrels of mash very homogenous in their mixtures of salt. Therefore, although the average salt content is supposed to be eight percent, other levels were used in these experiments. Salt concentrations were four percent, eight

percent, and 12 percent by weight. The salt was added to the ground peppers at these levels in polypropylene 500 ml screw-top containers (model H5002, Starplex Scientific, Inc., Etobicoke, Ontario, Canada) after grinding the peppers. Each salt level was represented in four containers per pepper source.

Figure 3.1.1. The apparatus for adding oxygen to mash samples before fermentation.

After the salt was mixed in with a glass rod, caps were closed and the mash was left at room temperature for 24 hrs. This waiting period allowed salt to draw any juices out of the ground peppers, and to allow pH to stabilize in the product. The following tables show the different treatments and initial weights of peppers and salt in each sample.

Table 3.1.1. Mash treatments.

Avery Island Peppers date: 9/4/2007			Baton Rouge Peppers date: 10/30/2007		
Treatment	pepper wt. (g)	salt (g)	Treatment	pepper wt. (g)	salt (g)
4% salt	299.3	12.0	4% salt	300.2	12.6
4% salt	298.5	11.9	4% salt	301.0	12.6
8% salt	299.3	24.0	8% salt	300.5	24.0
8% salt	297.5	23.8	8% salt	300.5	24.0
12% salt	300.0	36.0	12% salt	299.9	36.0
12% salt	299.2	35.9	12% salt	300.1	36.0
4% salt O_2	300.2	12.0	4% salt O_2	299.3	12.0
4% salt O_2	299.9	12.0	4% salt O_2	300.3	12.0
8% salt O_2	300.0	24.0	8% salt O_2	300.0	24.0
8% salt O_2	299.9	24.0	8% salt O_2	300.2	24.0
12% salt O_2	300.0	36.0	12% salt O_2	299.8	36.0
12% salt O_2	300.8	36.1	12% salt O_2	300.2	36.0

After 24 hrs, the mash was stirred again with a glass rod and measurements were taken. Each sample's pH using an Orion model EA 920 bench top pH meter (Thermo Scientific, Waltham, MA) was taken for later comparison. Subjective appearance was recorded on a scale of 1-5. A score of "1" was given for the worst looking sauce, and a "5" was assigned for sauce of ideal appearance. A Minolta spectrophotometer (model CM-3500d, Konica Minolta, Ramsey, New Jersey) was used to take objective color measurements. Mash samples were placed into a liquid sample petri dish and each color measurement was taken six times per sample, turning the sample dish approximately 60 degrees between measurements. The instrument's computer program was used to automatically average these six measurements per sample, giving average L, a*, and b* color measurements. After initial color measurements were taken, oxygen was added as previously described. At the end of approximately three months' fermentation, roughly an inch of mash was removed from the tops of samples before subjective color measurements and objective L, a*, and b* measurements were taken of the mash below this top layer. However, L, a*, and b* values were not measured for any mash samples with added oxygen.

Two more mash samples were prepared using peppers from Burden Center, Baton Rouge, that had been deliberately picked with leaves and stem parts from the plants included. Since parts of the plant can sometimes be picked with peppers in commercial production, this test was made to simulate this scenario. The mash was made with eight percent salt and observed for the appearance of black specks after 24 hrs. It was then observed again after roughly three months fermentation. One last sample from peppers was made using only seeds and eight percent salt in 200 g water. This sample was blended for five minutes and observed for oil formation or black specks, after 24 hrs and after roughly three months' fermentation. Water was used in this sample to increase volume, approximating the normal proportion of seeds. Forty grams of seeds were used with 184 grams of water and 16 grams of salt. Raw fresh peppers were also left without salt in one of two containers. One was a 150 g sample in a plastic 5.5" x 5.5" x 1" polystyrene (VWR International, West Chester, PA 19380) weigh boat left to open air. The other was a 150 g sample in another 500 ml plastic container similar to those used in the salted samples. The lid was closed slightly on the latter sample, but ambient gas exchange was not prevented around the sample.

3.2 Initial Sample Preparations from Hot Sauce

Samples were prepared in the Horticulture Department, Julian C Miller Hall, Louisiana State University, Baton Rouge, Louisiana. The first step in analyzing the black specks in the hot sauce was to isolate them by centrifuging sauce with the defect in a model IEC HN-SII centrifuge for approximately ten minutes of high-speed (5,000 rpm) at room temperature (25° C) (International Equipment Company, Needham Heights, MA 02494.) Samples of "normal" sauce were similarly centrifuged for comparison. Individual plastic 80 ml test tubes contained roughly 20 ml of sauce each. Disposable plastic pipettes were then used to draw droplets from the tops of centrifuged hot sauce sample tubes. The droplets were placed on slides, covered with slide

covers and then observed under a light microscope (AO Series Fifty, American Optical Corporation, Buffalo, New York) at up to 100X magnification. Photographs of these slides were taken at the same magnifications using an Intel Blue digital camera (Prime Entertainment, Inc., Marietta, Georgia 30062). This method was sufficient to observe visual differences between droplets of "normal" and "specked" hot sauce and to perform microbiological tests.

A thin layer of pepper skin was peeled from freshly harvested tabasco peppers using tweezers and a scalpel. The skins were then observed under the same light microscope. This was intended to determine if morphology and structure of pepper skin were similar to that of the solid part of specks observed under light microscopy.

3.3 Scanning Electron Microscopy (SEM)

For further analysis, larger samples of the specks of unknown origin were needed than those provided by initial centrifugation. Sample size was increased to 500 g per centrifuge tube. Each tube was an 800 ml capacity plastic tube. Four tubes at a time were spun at 4,000 rpm at 15° C for one hour. The longer centrifuge time was needed because of the larger sample size. The centrifuge machine used was a Sorvall® RC6 Plus, by Thermo Electron Corporation of Asheville, North Carolina.

Scanning electron microscopy is a tool frequently used to obtain useful information about the microscopic structure and texture of a wide variety of subjects. Only a minute amount of sample is needed for this technique.

Samples of the speck defects from commercial sauce, skin samples from fresh tabasco peppers, and samples of ground dried fruit, stems and leaves were examined using SEM. Since presence of oil may interfere with SEM, oil was removed from samples with chemical solvents in 80 ml test tubes. Five grams of sample were placed at the bottom of an 80 ml test tube. Then 50 ml of hexane (Fisher, Fair Lawn, New Jersey) were added to the tube, and the mixture was

vigorously shaken by hand. The tube was then set on a motionless rack for an hour. After letting particulates settle, the supernatant was pipetted off and the remaining solids were dried under a fume hood in the same test tube. Fifty ml of ethanol (Pharmco, Shelbyville, Kentucky) were then added, and the procedure was repeated. Finally, 50 ml of a 2:1 chloroform (Fisher, Fair Lawn, New Jersey): methanol (Fisher, Fair Lawn, New Jersey) solution was added, and the procedure was repeated. After excess liquid was pipetted off and the sample was dried under a fume hood, the solids left over were used as the control sample in the SEM procedure.

Fresh pepper skins were divided into two samples. One of these samples was 1) air-dried and the other 2) chemically treated, then air-dried. The “air-dried” pepper skins were desiccated in an open weigh boat under a fume hood at room temperature, then observed under SEM. Others (“treated”) were treated with the same chemical treatment (solvent rinsing) followed by desiccation in the same manner by which specks were previously prepared.

Since the source of the specks may have come from some other part of the pepper, whole peppers were also ground. Then the ground samples were similarly divided into two samples. One sample was air-dried, while the other was chemically treated (as previously described), then air dried. Since other parts of the tabasco plant could possibly be the source of the specks, leaf and stem samples from tabasco plants were also ground and divided into two samples. One sample each was 1) air dried or 2) chemically treated, then air dried.

Dried samples were taken to the Socolofsky Microscopy Center, Department of Biological Sciences, LSU, which is specifically dedicated to SEM. The dried samples were attached to aluminum SEM tabs with conductive tape. They were then coated with a 60:40 ratio of gold: palladium in an Edwards S-150 Sputter Coater (Edwards, Ltd., Crawley, England). The samples were imaged with a Cambridge S-260 Stereoscan SEM (Carl Zeiss SMT, Inc., Peabody,

MA). Digital images were produced and examined to observe similarities and differences among the different treatments.

3.4 Microbial Activity

One theory of the source of the specks is that they may be visible colonies of unknown microorganisms. To test for microbial activity, samples of the specked sauce and the "normal" sauce were plated separately onto Petrifilm™ aerobic bacteria culture medium (3M, St. Paul, MN 55144) and incubated in an incubator (Hotpack model #352700, Phila, Pennsylvania) at 37° C for total plate count. Plate counts were taken at 48 hours post-inoculation. Most bacteria can grow at this temperature and will show visible colonies within 48 hours on appropriate media. Broad spectrum aerobic culture media allow for a broad range of microbes to grow. Aforementioned tests were performed in duplicate of dilutions 10^{0} through 10^{-3}.

Viable microbes in the sauce would most likely consist of molds and yeasts, if anything, because of the low pH of the product. Petrifilm™ Yeast and Mold Count Plates (3M, St. Paul, MN 55144) were inoculated and tested as described above, except that this set of plates was allowed to grow at room temperature for one week before observation, since most yeasts and molds grow well at room temperature. One week was enough time for any sufficient colony growth to be observed. Dilutions of 10^{-1} through 10^{-3} were used for growth of yeasts and molds.

The aforementioned tests were repeated on samples of 1) specked sauce, 2) specks separated from hot sauce by centrifugation, and 3) oil separated out by centrifugation of "normal" sauce. All samples were plated using sterile pipettes and sterile media under a positive air flow fume hood.

3.5 Solubility and Chemical Tests

Solubility tests are commonly used to determine the degree of polarity of unknown substances. Craft and Soares showed in 1992 that carotenoids can be dissolved in a number of

organic solvents, and to varying degrees. Therefore, similar tests were performed on substances removed from hot sauce samples by centrifugation. Initial sample size used to be spun down was 500 g per centrifuge tube. Each tube was an 800 ml capacity plastic tube. Four tubes at a time were spun at 4,000 rpm at 15° C for one hour. The longer centrifuge time was needed because of the larger sample size. The centrifuge machine used was a Sorvall® RC6 Plus, by Thermo Electron Corporation of Asheville, North Carolina. Each 500 g initial sample was spun to produce unknown supernatant for use as a refined sample.

One mg at a time of the unknown substance was mixed with 10 ml of solvent. The solvents used separately were as follows: water, ethanol, methanol, chloroform, and hexane. Also, mixtures combining more than one of the preceding solvents were used. The mixtures used were 2:1 chloroform: methanol; 3:1 hexane: ethanol; and 2:1 chloroform: ethanol. Ten mg ethanol was also used with 1 ml substrate for another sample. Yet another 1 ml sample was treated with the same amount (ten mg) of chloroform, and then the liquid portion was pipetted off. Then the solid part of this sample was treated with the same amount of ethanol.

Of these solutes, water is the most polar, ethanol is the next most polar, methanol is less polar, chloroform is non-polar, and hexane is the most non-polar. The mixtures were intended to remove any dissolvable products that were of similar polarity to those of the individual constituents of the mixtures. Any given substance will dissolve best in a solute that matches it best in terms of polarity. The samples were observed to see which solvents dissolved them best.

Matrix-assisted laser desorption / ionization (MALDI) is a qualitative test for analyzing unknown chemicals. Used in conjunction with time-of-flight mass spectrometry, it has been shown (Naumann, 2007) to be an effective technique in qualifying unknown lipophilic compounds. Since the compounds involved in the sauce defects were unknown, MALDI was suggested as a productive analytical method for discovering their chemical makeup. However,

samples were lost before the test could be completed, partly due to mechanical failure of chilled storage facilities.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Initial Sample Preparation

One of the first steps in sample preparation was to centrifuge samples. “Normal sauce” and “specked sauce” were centrifuged in separate tubes. It was found that centrifugation over 5,000 rpm for roughly ten minutes was sufficient to separate some components of the samples. This suggested early on that the heterogeneous mixture contained substances of differing densities.

The black specks in the “specked sauce” samples separated out as a supernatant on top of the fluid, but they still did not aggregate appreciably. Some of the specks did stick to the sides of the plastic tubes, however, and to the sides of plastic pipette tubes used to attempt to further isolate the specks. These facts suggest that the unknown substance is lipophilic or at least displays some lipophilic properties in sticking to plastic. It seems that the majority of the black specks did separate out, although some did stay suspended in the sauce. Centrifugation separated out a significant portion of the specks to suggest that this method of separation is effective to obtain significant quantities of samples to run the various tests suggested.

Samples of “normal” hot sauce underwent some separation as well. However, the supernatant was simply an oil layer of a red color lighter than that of the specks in the affected samples but lighter than the rest of the hot sauce in the tube. This oil layer also tends to stick to the sides of pipette tubes. Despite this fact, both the oil layer centrifuged from normal sauce and the specks from specked sauce were successfully pipetted into dilution tubes for microbiological sample preparation.

4.2 Microbiological Tests

Results as read on 3M Petrifilm™ showed few microbes present on a significant level. More importantly, the number and quality of microbes did not differ significantly between samples of normal sauce and specked sauce, nor between oil and specks centrifuged out from normal and specked sauces, respectively. Both normal and specked sauce showed some aerobic bacterial colonies on a scale of 10^4 colony forming units per milliliter (cfu's / ml). Normal hot sauce averaged 4638 colony forming units per milliliter (cfu's / ml). Specked hot sauce averaged 1903 cfu's / ml. Both types of hot sauce varied greatly between individual samples. Neither coliform nor *E. coli* colonies were observed in any samples. These raw data are summarized in the appendix table A.1.

Yeast and mold plate counts were first conducted on specked and non-specked sauce. Again, sample dilutions plated were from 10^{-1} to 10^{-3}. Of eight total samples, only one showed any yeast or mold growth. This single sample of specked sauce showed yeasts and molds were unreadable. Therefore, a second set of yeast and mold count samples were tried. The second trial consisted of samples of specked sauce, specks, and of oil centrifuged from normal sauce. The sample dilutions plated were from 10^{-1} to 10^{-3}once again. After incubation, none of these samples showed any colonies of yeasts or molds.

There were no appreciable differences in the number or quantity of microbes in samples whether the samples came from "normal" sauce or "specked" sauce. This indicates that the specks are not likely to be of microbial origin.

4.3 Light Microscopy

Some of the supernatant particles and oil separated by centrifugation at 5,000 rpm for ten minutes were observed with light microscopy on slides. Samples isolated from "specked" hot sauce were compared to samples from "normal" hot sauce. Specks isolated from hot sauce could

be observed at 10X magnification to resemble pepper skin and oil. The lighter colored, more solid part resembled pepper skin, or plant skin in general. The darker part appeared to be a dark globule of oil attached to the skin. Magnification at 100X confirmed this observation.

The oil layer from normal sauce appeared to have less distinct pieces of skin. These pieces appeared more disjoined and random. Spots of oil slightly darker than the solid pieces were also present, but smaller and randomly scattered.

Photographs of these samples made observation easier. Two examples are pictured in figures 4.3.1 and 4.3.2 below.

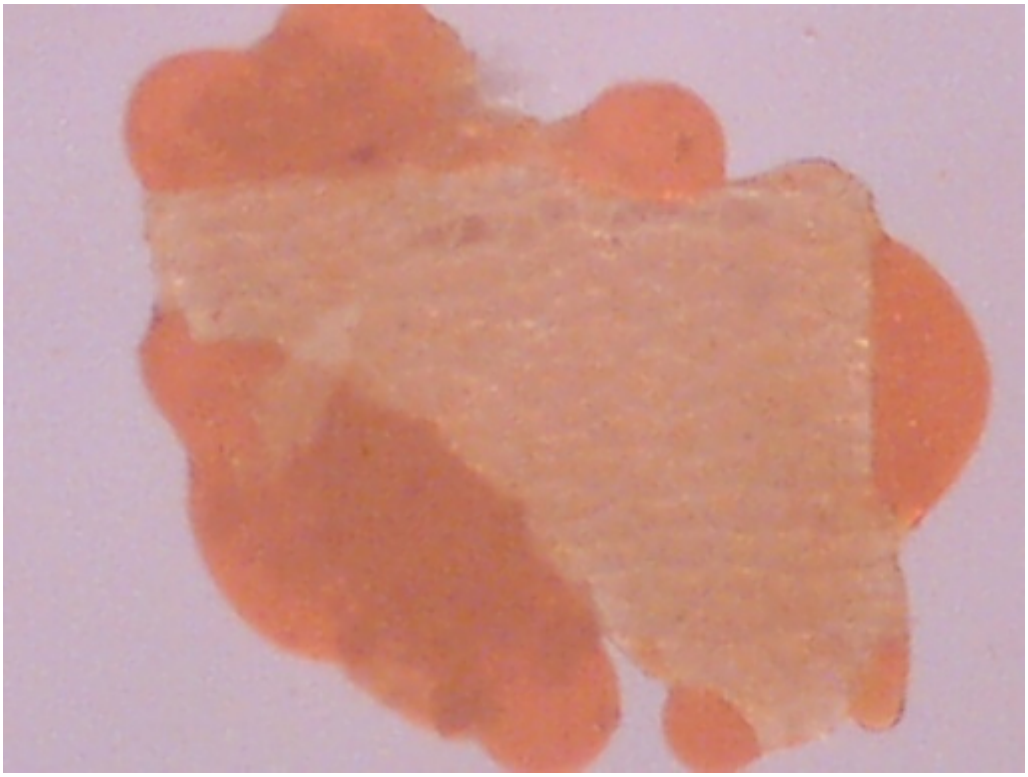

Figure 4.3.1 100X Magnification of isolate from specked hot sauce.

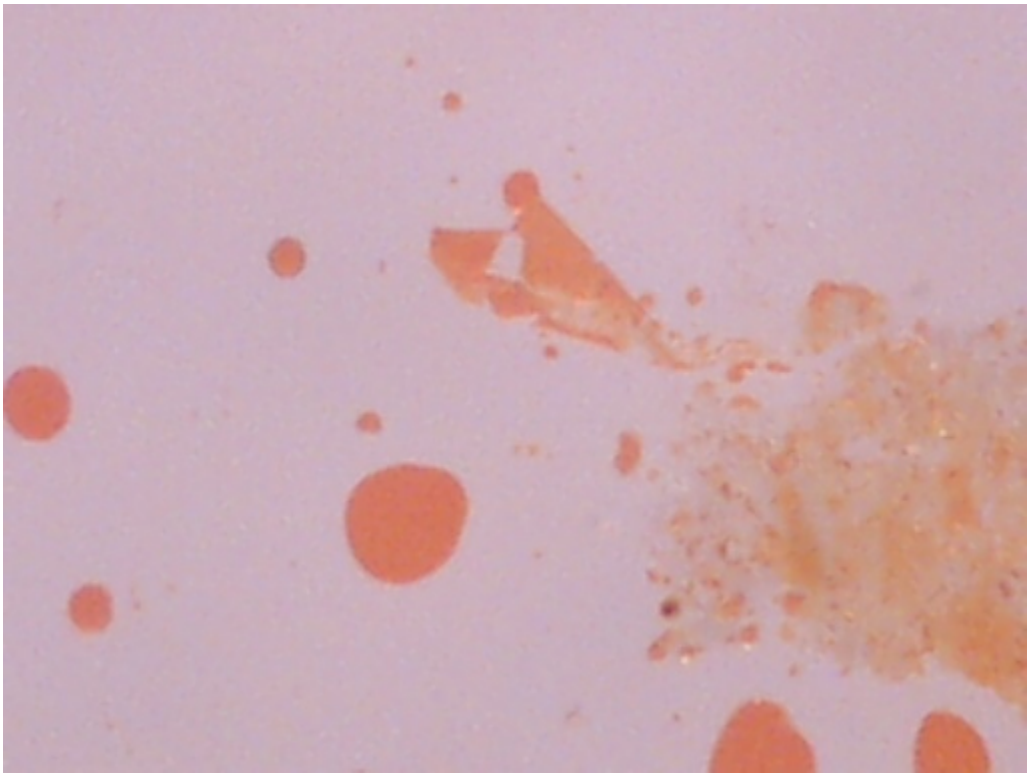

Figure 4.3.2 100X Magnification of isolate from normal hot sauce.

4.4 Scanning Electron Microscopy (SEM)

Due to the success of the light microscopy, it was decided to use scanning electron microscopy to further study the specks. Samples were again obtained by one-hour centrifugation in a Sorvall centrifuge at 4,000 rpm. After centrifugation of the hot sauce sample containing dark specks, a dark substance resembling the specks was removed from the top of the centrifuge tubes by using a metal spatula to scoop the substance into a plastic tube for storage. The same technique was applied to samples of normal sauce, though they only had a slight amount of lighter oil on top of the containers. To prepare samples for electron microscopy, oil had to be removed. Hexane, ethanol, and a 2:1 chloroform: methanol solution were used to accomplish this. The samples were then desiccated. Electron micrographs were made from isolates of normal and specked hot sauce. It was observed that some microstructures from specked hot sauce

isolates resembled pepper skin as well. Structures in micrographs from normal sauce were less organized entirely.

Upon observation that samples resembled peppers skins, tabasco pepper skins were taken as another SEM sample for comparison. Half of these samples were air-dried to prepare for SEM, while the other half were subjected first to the same chemical treatment that had been applied to hot sauce isolates. The procedure was repeated with ground whole peppers and with plant parts (leaves and stems) from tabasco peppers. It was found that the isolates from specked hot sauce most closely resembled pepper skin samples, especially after the latter had been treated with chemicals and desiccated.

Figures 4.4.1 through 4.4.5 show scanning electron micrographs of various samples, with the scale marked at the top of each micrograph to demonstrate degree of magnification.

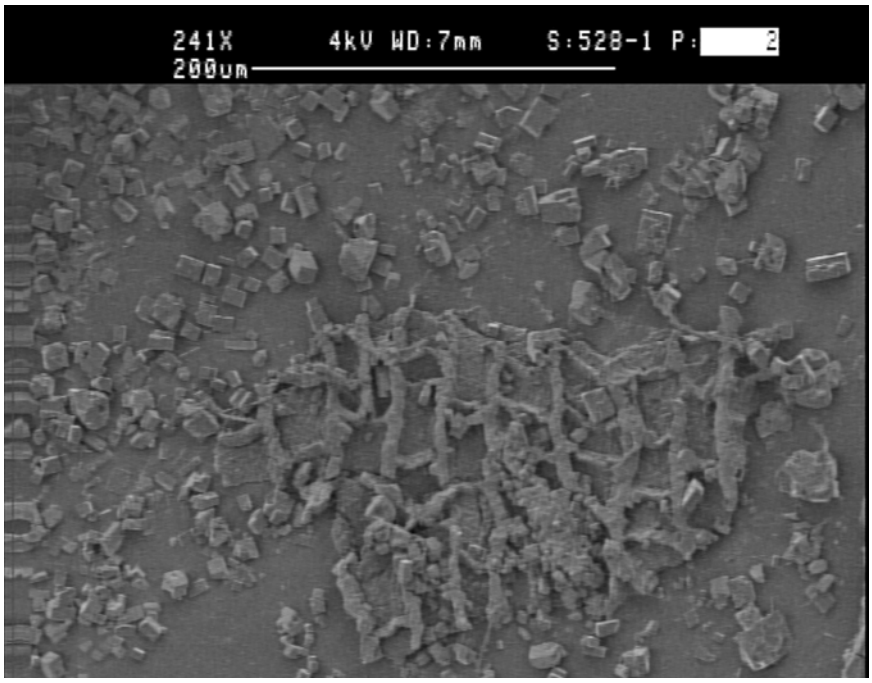

Figure 4.4.1. Isolate from normal hot sauce.

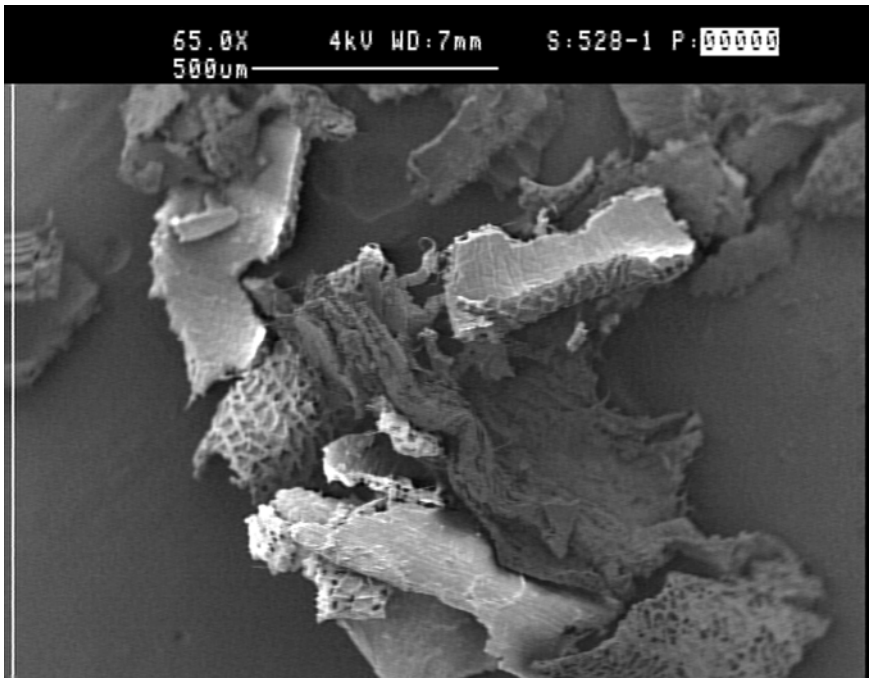

Figure 4.4.2. Isolates from hot sauce with black specks.

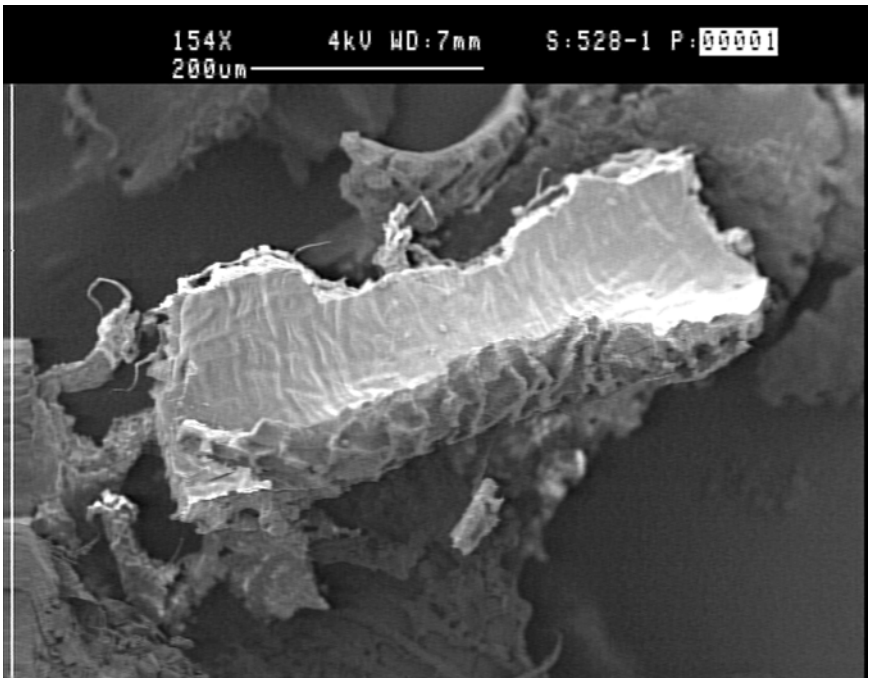

Figure 4.4.3 200 µm scale showing suspected skin piece in specks.

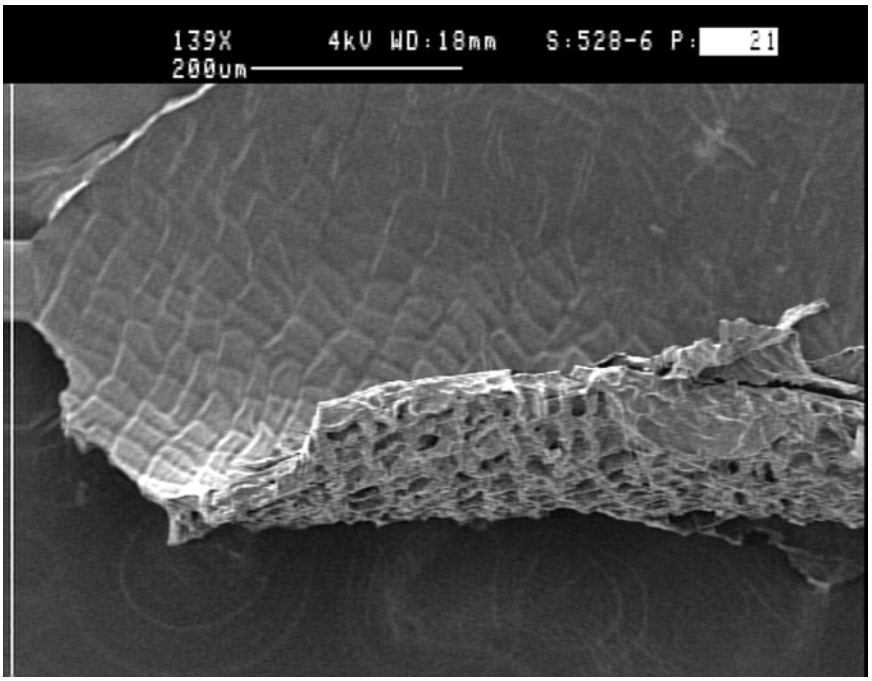

Figure 4.4.4 200 µm scale showing treated skin piece taken from pepper.

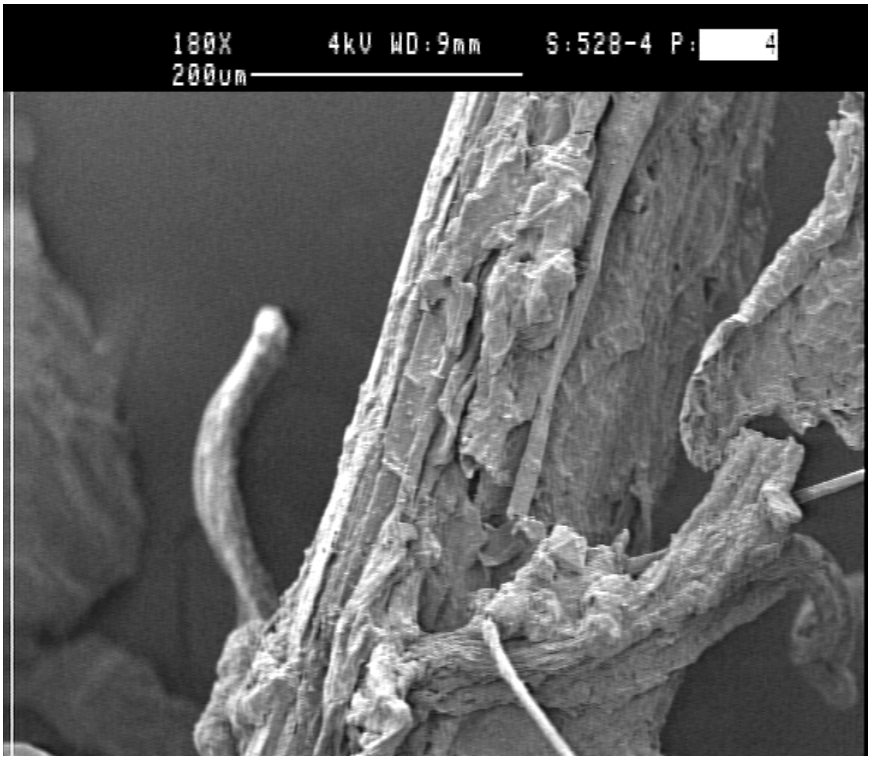

Figure 4.4.5 200 µm scale SEM image of treated leaves and stems sample.

4.5 Solubility Tests

Centrifugation at 4,000 rpm for one hour in a Sorvall centrifuge again produced isolates from normal and specked hot sauces. These isolates were treated separately with solutes of differing degrees of polarity. Solutes included water, ethanol, methanol, chloroform, and hexane separately. Of these solutes, water is the most polar, ethanol is the next most polar, methanol is less polar, chloroform is non-polar, and hexane is the most non-polar. Mixtures combining these solutes were 2:1 chloroform: methanol; 3:1 hexane: ethanol; and 2:1 chloroform: ethanol.

Polar solvents will dissolve polar substances; non-polar solvents dissolve non-polar substances. A given substance will dissolve best in a solvent that is closest to it in polarity. Therefore, this test was conducted to determine how polar the unknown substance is that seems to be the source of the specks. The isolated specks did not dissolve in water, the most polar substance. However, they did dissolve better in ethanol than in any other solute alone. This indicates that, regarding degree of polarity, the majority of soluble oil in the specks most closely resembles ethanol. A mixture of chloroform and ethanol in a 2:1 ratio dissolved nearly all oil from the specks, leaving only minute solid particles which were bleached nearly clear. This indicates that multiple lipophilic substances probably exist in the specks. Some that were not dissolved by ethanol alone were dissolved by chloroform, which is less polar than ethanol.

4.6 “Fresh” Mash

Samples used included peppers grown at Avery Island and at Burden Center in Baton Rouge. Weights of peppers and salt for mash were recorded just before grinding samples. Treatment categories for which these measurements were taken included salt percentage and whether or not the samples were exposed to additional oxygen. Initial measurements of L, a*, and b* color and pH were recorded 24 hours after grinding these samples. Objective color and pH were not recorded for samples with extraneous plant material or added water. Subjective

measurements of mash appearance were recorded for all samples, including those with extraneous plant parts included in the mash, and "mash" made from seeds and salt water.

On a scale of one to five, with five as the best, all mash samples were given an initial score of five. A score of four was assigned to each of the two samples with extraneous plant material ground in. The latter two showed small, dark green specks which were apparently the aforementioned leaves and stems. The leaves and stems did not at this point resemble the dark specks found in the finished product.

Though subjective color measurements were recorded immediately after grinding, the other measurements were taken 24 hours post-grinding. At this time, it was observed that all samples had a small amount of a light oil on top, though in slightly differing amounts. It was also observed at this time that some of the mash samples had apparently begun producing some gas. This was evident in the fact that the given samples' containers bulged, and the hissing sound of gas escaping could be heard when the caps of the containers were unscrewed.

Table 4.6 outline the averages of initial measurements (including 24 hours post-grinding) recorded on the first two categories of samples, as well as final pH of those samples for comparison. Actual raw data can be found in the appendix tables A.4 and A.5.

Table 4.6 Averaged measurements on mash made from peppers grown at Avery Island and Baton Rouge.

Avery Island Peppers Dates: 9/4/2007 and 12/11/2007					
Treatment	1-5 score	pepper wt. (g)	salt (g)	Initial pH	Final pH
4% salt	5	298.9	12.0	5.25	3.90
8% salt	5	298.4	23.9	5.09	4.47
12% salt	5	299.6	36.0	5.03	4.68
4% salt O_2	5	300.1	12.0	5.33	3.92
8% salt O_2	5	299.8	24.0	5.11	4.45
12% salt O_2	5	300.4	36.1	5.08	4.67

(table con'd.)

Baton Rouge Peppers
Dates: 10/30/2007 and 2/22/2008

Treatment	1-5 score	pepper wt. (g)	salt (g)	Initial pH	Final pH
4% salt	5	300.6	12.6	4.98	4.29
8% salt	5	300.5	24.0	4.97	4.57
12% salt	5	300.0	36.0	4.87	4.59
4% salt O_2	5	299.8	12.0	5.01	4.31
8% salt O_2	5	300.1	24.0	4.97	4.66
12% salt O_2	5	300.0	36.0	4.87	4.56

4.7 L, a*, and b* Measurements

Subjective color measurements were taken at 24 hrs ("initial") and at approximately three months aging ("final.") Initial subjective color measurements all were "5" on a "1-5" scale, with "5" being best. Table 4.7 shows the L, a*, and b* values related to various treatments.

Table 4.7.1. Average initial and final L, a*, and b* measurements on mash from Avery Island and Baton Rouge peppers.

Avery Island peppers
Dates: 9/4/2007 and 12/11/2007.

	Initial			**Final**		
Treatment	**L**	**a***	**b***	**L**	**a***	**b***
4% salt	28.1353	35.5076	17.9296	25.3163	31.1991	15.5392
8% salt	28.5412	36.3361	18.2117	25.3869	31.2247	15.6763
12% salt	28.7523	36.1140	18.3165	24.9500	32.3019	15.7328
4% salt O_2	27.8856	35.3208	17.8934	24.5412	31.5444	15.2354
8% salt O_2	27.8544	35.9957	18.0124	24.5959	30.0355	14.8705
12% salt O_2	27.8771	35.5982	17.9142	25.1504	30.7726	15.4172

Baton Rouge Peppers
Dates: 10/30/2007 and 2/22/2008.

	Initial			**Final**		
Treatment	**L**	**a***	**b***	**L**	**a***	**b***
4% salt	31.0655	43.0520	20.4356	24.4987	33.1927	15.3660
8% salt	30.1867	42.3471	19.8594	24.8490	32.8685	15.6412
12% salt	29.0373	41.4898	19.1174	24.4941	35.6376	15.9408
4% salt O_2	31.0445	42.4737	20.4521	24.1574	31.4863	14.9279
8% salt O_2	29.9654	42.2347	19.6791	23.3312	31.7860	14.9306
12% salt O_2	29.6976	41.3878	19.3369	23.4406	32.8201	15.2345

Initial samples were nearly identical except that two of the Burden Center samples had apparently started producing gas within 24 hrs. Radicle emergence from the seeds was observed in many aged samples. The seeds apparently had enough available moisture to begin

germination. Some standing liquid was observed in some samples. Oil was produced in many samples, though most of it seemed light and innocuous. However, one sample (Burden Center, 8% salt, with added oxygen) produced dark oil that was of interest to the study. It closely resembled the oil sometimes found in shipping containers and in fermented mash in hot sauce production. It is this oil that is suspected of being the root of the dark specks in the hot sauce. Its presence in the container indicates it may be produced at average salt levels with added oxygen. However, its single appearance does not imply reliable or predicable repeatability in fermented mash.

Many samples grew mold on the surface, which is commonly observed in hot sauce production. Spots resembling bacterial colonies were also observed. These are both common occurrences on the layer that is typically scraped off and discarded in hot sauce production. The layer was discarded in the present study as well. Initial observations on mash made from Avery Island peppers showed that they were apparently all identical. Initial observations on mash made from Baton Rouge peppers showed that they were identical with the following exceptions: One sample each of 4% salt and 8% salt let out a slight “hiss” after 24 hours, indicating some gas was being produced early on. Also, one 12% salt sample with added oxygen had a slight amount of oil on the surface.

Observations were made on the fermented containers of mash before the surface layers were scraped off. One inch below the surface, nearly all mash was identical. The surface observations were particularly valuable because subjective measurements showed some noticeable differences between individual containers. However, significant differences between treatments were not observed. Tables 4.7.2 show comments on final observations of mash from peppers grown at Avery Island and Baton Rouge.

Table 4.7.2. Subjective observations on mash made from peppers grown at Avery Island and Baton Rouge, post-fermentation.

Final Observations on Avery Island Mash		Final Observations on Baton Rouge Mash	
Treatment	**Comments**	**Treatment**	**Comments**
4% salt	radicle emergence; some clear oil	4% salt	cap sucked in; radicles
4% salt	radicles	4% salt	radicles
8% salt	radicle emergence; some clear oil	8% salt	radicles
8% salt	copious mold on top; some oil	8% salt	radicles; light film on top
12% salt	radicles; spots; little mold; little oil	12% salt	oily liquid; radicles
12% salt	radicles; spots	12% salt	oily liquid; few radicles
4% salt O_2	radicles	4% salt O_2	radicles
4% salt O_2	radicles	4% salt O_2	n/a
8% salt O_2	few radicles; little oil	8% salt O_2	spots of white mold; spots (bacterial colonies)
8% salt O_2	little oil	8% salt O_2	radicles; liquid and dark oil resembling specks
12% salt O_2	spots (bacterial colonies); clear oil	12% salt O_2	large bacterial colonies; radicles; liquid
12% salt O_2	spots (bacterial colonies); radicles	12% salt O_2	radicles; bacterial colonies; liquid

On a “1-5” scale, all samples were judged “5” at approximately one inch below the surface after aging. L, a*, and b* values were also approximately the same.

No L, a*, and b* observations nor pH measurements were recorded for peppers ground with leaves and stems. The same is true for the sample mixed with water. These samples were taken solely to attempt to recreate the dark oil or black speck phenomenon. None of these samples showed black specks after fermentation, nor did they show dark oil. Mash samples with leaves and stems had visible specks of leaves and stems in them both before and after aging. After aging, radicles were observed in both samples with extraneous plant material, and bacterial colonies were observed in one of these samples.

The sample with added water appeared orange before aging. After aging, it still looked orange, but it also had a whitish haze. A foul, rotten smell was also observed with this sample.

The whole peppers left to ferment in open air were found to be missing after about two weeks. Presumably, rodents prevalent in the laboratory took the samples. The whole peppers in a

plastic screw-top container had a thick mold on them and a small amount of liquid. They were otherwise mostly unchanged.

4.8 Conclusions

The source of the dark specks in some hot sauce did not appear to be microbial colonies. No microbial differences, either in quantity or quality were found between normal hot sauce and specked hot sauce. Under light microscopy, the specks looked like bits of pepper skin with oil globules attached. Though oil had to be removed chemically to conduct scanning electron microscopy (SEM), the solid parts could be observed with this technique. Under SEM, specks clearly appeared to be pieces of pepper skin. Solids from normal hot sauce were of much smaller volume than solids of specked hot sauce. The solids from normal sauce occasionally showed what seemed to be pieces of skin as well. However, the solids from normal sauce were in smaller, less distinct pieces that appeared more disjoined.

A dark oil seems to be attached to bits of pepper skin in the sauce with specks. The reason for this attachment is still unknown. A similar oil seems to exist in normal sauce as well, although it does not attach to solid particles nearly as much. This attachment to pepper skins seems to enhance the oil's tendency to aggregate. The aggregation likely leads to higher visibility of these particles, compared to oil in normal sauce.

The appearance of dark oil in mash appears to be nearly random with regards to salt content, oxygen addition, or where the peppers were grown. Though the experiments were successful in producing a small amount of oil, no particular treatment seems to reproduce it consistently.

The possibility still exists that the specks could be caused by pepper disease or diseases, since this possibility was not thoroughly examined through experiments in this study. Anthracnose is a common fungal disease in peppers that affects the skin of pods. It is caused by

the fungus *Colletotrichum orbiculare*, and can be quickly spread, especially in rainy periods. It has been found in large outbreaks in some pepper-growing regions, and it may be specific to a given region. Other diseases that cause discoloration in pepper pods include bacterial spot, pepper mild mottle, tomato spotted wilt, and blossom end rot. Any of these may be possible sources of the specks as well. The source of the specks may be limited to a given country, an idea that was not thoroughly investigated. The fact that pepper diseases can be unique to a given region would fit in with this theory.

Since there was no appreciable difference in microbial content between specked sauce and normal sauce, it can be concluded that the specks are not of microbial origin. Salt treatments, place peppers were grown, and addition of oxygen failed to yield consistent results in the mash trials. We cannot conclude that any of these treatments affect the specks or the dark oil the specks may be attributed to. Centrifugation was an effective method of separating the specks out of the hot sauce. Light microscopy and scanning electron microscopy showed that the specks closely resemble pepper skin, and they do not noticeably resemble other parts of peppers or pepper plants. We can confidently conclude that the specks are made of small bits of pepper skin with small droplets of oil attached. A similar oil does exist in normal hot sauce, although in much smaller quantities when separated by centrifuge. Very small solid bits exist in normal oil as well, though they are less distinctly formed.

Further research into this subject would need a larger amount of specked hot sauce. Larger batches of mash could possibly produce another sample of dark oil, which could have been the source of the specks. More numerous repetitions could also possibly produce more oil. It would also be interesting to leave the caps of some of the plastic containers slightly open to allow more gas exchange with the surrounding environment.

Had more oil been available, it could have been chemically compared to the specks found in sauce. Matrix-assisted laser desorption/ ionization (MALDI) could have been useful for this purpose. The degree of polarity of specks and oil could also have been compared using the same organic solvents used on the specks. If the two were related, the oil should also have dissolved in ethanol and chloroform. Other experiments could include the addition of vinegar to the dark oil to reproduce a hot sauce with a high amount of the suspect oil. This would, in theory, reproduce actual specked sauce.

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APPENDIX
SUPPLEMENTARY DATA

Table A.1. Microbial counts for total aerobic count and coliform count.

Total aerobic counts					
"Normal" sauce			"Specked" sauce		
dilution	colonies / plate 1	colonies / plate 2	dilution	colonies / plate 1	colonies / plate 2
10^{-1}	6	7	10^{-1}	10	2
10^{-2}	14	13	10^{-2}	7	6
10^{-3}	15	10	10^{-3}	5	5
Coliform (EC) counts (2 day)					
"Normal" sauce			"Specked" sauce		
dilution	colonies / plate 1	colonies / plate 2	dilution	colonies / plate 1	colonies / plate 2
10^{0}	non-detectable	non-detectable	10^{0}	non-detectable	non-detectable
10^{-1}	non-detectable	non-detectable	10^{-1}	non-detectable	non-detectable
10^{-2}	non-detectable	non-detectable	10^{-2}	non-detectable	non-detectable
10^{-3}	non-detectable	non-detectable	10^{-3}	non-detectable	non-detectable

Table A.2. Yeast and mold counts.

Yeasts and molds trial 1 (6 day)					
"Normal" sauce			"Specked" sauce		
dilution	colonies / plate 1	colonies / plate 2	dilution	colonies / plate 1	colonies / plate 2
10^{0}	non-detectable	non-detectable	10^{0}	non-detectable	non-detectable
10^{-1}	non-detectable	non-detectable	10^{-1}	non-detectable	non-detectable
10^{-2}	non-detectable	non-detectable	10^{-2}	unclear	non-detectable
10^{-3}	non-detectable	non-detectable	10^{-3}	non-detectable	non-detectable

Table A.3. Yeast and mold counts trial 2.

Yeasts and molds trial 2 (6 day)					
"Specked sauce"			"Specks"		
dilution	colonies / plate 1	colonies / plate 2	dilution	colonies / plate 1	colonies / plate 2
10^{-1}	non-detectable	non-detectable	10^{-1}	non-detectable	non-detectable
10^{-2}	non-detectable	non-detectable	10^{-2}	non-detectable	non-detectable
10^{-3}	non-detectable	non-detectable	10^{-3}	non-detectable	non-detectable

"Good oil"		
dilution	colonies / plate 1	colonies / plate 2
10^{-1}	non-detectable	non-detectable
10^{-2}	non-detectable	non-detectable
10^{-3}	non-detectable	non-detectable

Table A.4. Actual measurements taken on mash made from peppers grown on Avery Island.

Mash Measurements Avery Island Peppers dates: 9/4/2007 and 12/11/2007					
Treatment	**1-5 score**	**pepper wt. (g)**	**salt (g)**	**Initial pH**	**Final pH**
4% salt	5	299.3	12.0	5.25	3.88
4% salt	5	298.5	11.9	5.24	3.91
8% salt	5	299.3	24.0	5.08	4.63
8% salt	5	297.5	23.8	5.10	4.30
12% salt	5	300.0	36.0	5.06	4.67
12% salt	5	299.2	35.9	4.99	4.68
4% salt O_2	5	300.2	12.0	5.29	3.97
4% salt O_2	5	299.9	12.0	5.36	3.87
8% salt O_2	5	299.7	24.0	5.10	4.40
8% salt O_2	5	299.9	24.0	5.11	4.49
12% salt O_2	5	300.0	36.0	5.06	4.63
12% salt O_2	5	300.8	36.1	5.10	4.71

Table A.5. Measurements on mash made from peppers grown in Baton Rouge.

Mash Measurements Baton Rouge Peppers dates: 10/30/2007 and 2/22/2008					
Treatment	**1-5 score**	**pepper wt. (g)**	**salt (g)**	**Initial pH**	**Final pH**
4% salt	5	300.2	12.6	4.98	4.22
4% salt	5	301.0	12.6	4.98	4.35
8% salt	5	300.5	24.0	4.97	4.59
8% salt	5	300.5	24.0	4.96	4.55
12% salt	5	299.9	36.0	4.89	4.62
12% salt	5	300.1	36.0	4.85	4.55
4% salt O_2	5	299.3	12.0	5.00	4.31
4% salt O_2	5	300.3	12.0	5.02	4.31
8% salt O_2	5	300.0	24.0	4.93	4.63
8% salt O_2	5	300.2	24.0	5.01	4.68
12% salt O_2	5	299.8	36.0	4.84	4.61
12% salt O_2	5	300.2	36.0	4.90	4.50

Table A.6. Initial L, a*, and b* values of mash made from peppers grown at Avery Island.

Initial Mash Measurements Avery Island Peppers 9/4/2007.			
Treatment	**L**	**a***	**b***
4% salt	27.7987	35.3075	17.6693
4% salt	28.4718	35.7077	18.1899
8% salt	28.6538	36.1826	18.2882
8% salt	28.4286	36.4895	18.1352
12% salt	28.7652	36.3731	18.3690
12% salt	28.7394	35.8549	18.2639
4% salt O_2	27.7370	35.0268	17.7819
4% salt O_2	28.0342	35.6148	18.0049
8% salt O_2	28.2393	36.5104	18.2961
8% salt O_2	27.4694	35.4810	17.7286
12% salt O_2	28.2692	35.3573	18.0288
12% salt O_2	27.4849	35.8390	17.7996

Table A.7. Final L, a*, and b* values of mash made from peppers grown at Avery Island.

Final Mash Measurements Avery Island Peppers 12/11/2007.			
Treatment	**L**	**a***	**b***
4% salt	25.8893	30.9967	15.7846
4% salt	24.7432	31.4015	15.2938
8% salt	25.0911	31.9209	15.7563
8% salt	25.6827	30.5284	15.5962
12% salt	25.0708	32.4710	15.8270
12% salt	24.8291	32.1328	15.6385
4% salt O_2	24.5467	31.2797	15.2022
4% salt O_2	24.5356	31.8090	15.2685
8% salt O_2	24.5363	29.7736	14.7138
8% salt O_2	24.6555	30.2974	15.0271
12% salt O_2	24.5704	30.9473	15.1020
12% salt O_2	25.7304	30.5978	15.7324

Table A.8. Initial L, a*, and b* measurements on mash made from Baton Rouge peppers.

Initial Mash Measurements Baton Rouge Peppers 10/30/2007.			
Treatment	**L**	**a***	**b***
4% salt	30.9760	43.0475	20.4037
4% salt	31.1549	43.0564	20.4675
8% salt	29.1360	42.0983	19.1717
8% salt	31.2373	42.5959	20.5471
12% salt	29.8657	42.1390	19.6528
12% salt	28.2089	40.8405	18.5819
4% salt O_2	30.9428	42.0898	20.3691
4% salt O_2	31.1462	42.8576	20.5351
8% salt O_2	30.1093	42.4031	19.7811
8% salt O_2	29.8215	42.0662	19.5771
12% salt O_2	29.9598	41.4556	19.4932
12% salt O_2	29.4353	41.3200	19.1806

Table A.9. Final L, a*, and b* measurements on mash made from Baton Rouge peppers.

Final Mash Measurements Baton Rouge Peppers 2/22/2008.			
Treatment	**L**	**a***	**b***
4% salt	24.2003	33.3552	15.2351
4% salt	24.7970	33.0301	15.4969
8% salt	23.6607	32.5409	15.0474
8% salt	26.0373	33.1960	16.2349
12% salt	24.9298	36.2583	16.2494
12% salt	24.0584	35.0169	15.6322
4% salt O_2	23.9323	30.2610	14.8591
4% salt O_2	24.3824	32.7116	14.9967
8% salt O_2	23.5247	33.5920	15.1565
8% salt O_2	23.1377	29.9799	14.7046
12% salt O_2	22.9024	32.6267	14.9755
12% salt O_2	23.9787	33.0135	15.4934

VITA

The author was born in May of 1978, in Metairie, Louisiana. He lived his formative years in Reserve, Louisiana, and in Gramercy, Louisiana. He graduated from St. Charles Catholic High School in LaPlace, Louisiana, in May 1996. He began studies at Louisiana State University Agricultural and Mechanical College the following autumn. Graduating with a Bachelor of Science in animal, dairy, and poultry science in May of 2000, he then worked nearly a year in private industry. In March 2001, he returned to Louisiana State University Agricultural and Mechanical College to work as a research associate under the supervision of Dr. Kenneth McMillin for approximately four years.

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