PHYSICOCHEMICAL PROPERTIES OF PEPPER MASH FERMENTED IN WOOD AND PLASTIC

A Thesis

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS……..ii

LISTS OF FIGURES……...……....v

ABSTRACT……...vii

CHAPTER 1. INTRODUCTION……1

CHAPTER 2. LITERATURE REVIEW……..4
2.1 Fermentation ……..4
2.1.1 Vegetable Fermentation……...……4
2.1.2 Lactic Acid Bacteria…….....5
2.1.3 Organic Acids and Reduced pH……...6
2.1.4 Effect of Salt on Lactic Acid Bacteria…….. ……..8
2.1.5 Traditional Pepper Fermentation Process……...……9
2.2 Horticultural Characteristics……...…….……..10
2.2.1 Horticultural History of Tabasco Peppers……..10
2.2.2 Botanical Description……11
2.3 Pepper Physicochemical and Functional Properties……....…….…...…11
2.3.1 Soluble Carbohydrate…….…...11
2.3.1.1 Chemical Structures of Sucrose, Glucose, and Fructose…….……....11
2.3.1.2 Carbohydrate Metabolism in Pepper Fruit……..12
2.3.2 Pectin…….…….13
2.3.2.1 Structures and Characterization of Pectin…….……...13
2.3.2.2 Uronic Acid Quantification Methods……..16
2.3.2.3 Pectin Metabolism during Pepper Maturation…….....…17
2.3.3 Capsaicinoid…….……...18
2.3.3.1 Chemical Characteristics of Capsaicinoid…….……..18
2.3.3.2 Capsaicin Analysis …….....19
2.3.3.3 Capsaicin Metabolism during Pepper Maturation……...……20
2.4 Carbohydrate Changes During Fermentation…….....…20
2.5 Pectin Degradation in Pickled Products…….…...22
2.6 Research Justification…… ……..23
2.6.1 Fermentation in White Oak Barrel vs. Polyethylene Barrel…….…..…….23
2.6.2 Substitution of Wood Barrel with Plastic Barrel……..……. …….24

CHAPTER 3. MATERIALS AND METHODS……..……..25
3.1 Pepper Mash Fermentation……..…….25
3.2 Dry Weight Measurement…….…….……...25
3.3 pH and Titratable Acidity ……....25
3.4 Sugar Analysis by HPLC…….…… 26
3.5 Capsaicin Analysis by HPLC…….……..…27
3.6 Pectin Determination…….……...…….....28

3.6.1 Sample Preparation……..….28
3.6.2 Total Uronide Content Determination……..……28
3.6.3 Chelator-soluble Uronic Acid Determination……...…29
3.6.4 Pectin Depolymerization……...…30
3.7 Statistical Analysis ……..……..…30

CHAPTER 4. RESULTS AND DISCUSSION…….…..….. 32
4.1 Dry Weight……...….32
4.2 pH and Titratable Acidity……..…...34
4.3 Soluble Carbohydrate…….…..….37
4.4 Total Uronide Content……..…41
4.5 Pectin Depolymerization…….…..…42
4.6 Capsaicinoids…….…..…..45

CHAPTER 5. SUMMARY AND CONCLUSION…….……...52

REFERENCES…….….. 55

APPENDIX: DATA OF PHYSICOCHEMICAL ANALYSIS...…..……..61

VITA…….……..78

LIST OF FIGURES

Figure 1. Lactic acid fermentation pathway……..…..……..….7

Figure 2. Chemical structures of disaccharide (a) sucrose and monosaccharides (b) fructose and (c) glucose……..……..…12

Figure 3. Pectin fills intercellular spaces of the middle lamella of plant cell wall.….15

Figure 4. Pectin is a polymer of α-galacturonic acid with a variable number of methyl ester groups……..…… ……..…..16

Figure 5. Chemical structures of capsaicinoids – capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin…….....……21

Figure 6. Polyethylene plastic barrel (left) and oak wood barrel (right) for pepper fermentation…….…….……24

Figure 7. Dry weight matter of pepper mash throughout the 24 months fermentation. For fresh salted pepper mash at time=0; n=7. For fermented pepper mash from 1 months fermentation through 24 months fermentation, n=12…..…33

Figure 8. Average pH of pepper mash (fresh salted, n=7; fermented mash, n=12) fermented in wood and plastic barrels throughout the 24 months time course……..….35

Figure 9. Mean value of titratable acidity (fresh salted, n=7; fermented mash, n=12) of pepper fermented in wood and plastic barrels throughout the 24 months time course……....36

Figure 10a. Average (fresh salted, n=7; fermented mash, n=12) of sucrose concentration (mg/g) based on dry weight, quantified from HPLC chromatogram throughout the 24 month fermentation process……..38

Figure 10b. The mean value (fresh salted, n=7; fermented mash, n=12) of glucose concentration (mg/g) based on dry weight, quantified from HPLC chromatogram throughout the 24 month fermentation process…….…..39

Figure 10c. The mean value (fresh salted, n=7; fermented mash, n=12) of fructose concentration (mg/g) based on dry weight, quantified from HPLC chromatogram throughout the 24 month fermentation process……40

Figure 11. The mean value of total uronide quantification (based on acetone-insoluble dry tissues) for pepper mash fermented in wood and plastic barrel were measured at 520 nm absorbance in Spectrophotometer……..43

Figure 12a. Size-exclusion chromatography profile of CDTA-soluble polyuronide of standard and fresh salted pepper mash. Galacturonic acid (GA) was used as monomer standard. The elution rate was 0.5 ml/min and 4 ml fractions with a total of fifteen fractions were collected……..46

Figure 12b. Size-exclusion chromatography profile of CDTA-soluble polyuronide of One month fermented pepper mash in wood and plastic barrels. Galacturonic acid (GA) was used as monomer standard. The elution rate was 0.5 ml/min and 4 ml fractions with a total of fifteen fractions were collected……47

Figure 13. Standards were prepared using galacturonic acid at concentration of 15-200 ppm. Absorbance measured at 520 nm in a Perkin Elmer Lambda 35 UV/VIS Spectrophotometer. The line graph have formula of y = 99.223x and R^2=0.9975……..…..48

Figure 14a. The mean value of capsaicin (fresh salted, n=7; fermented mash, n=12) on the basis of freeze dried pepper mash quantified from HPLC chromatogram……..……50

Figure 14b. The mean value of dihydrocapsaicin (fresh salted, n=7; fermented mash, n=12) on the basis of freeze dried pepper mash quantified from HPLC chromatogram……..51

ABSTRACT

Red Chile peppers (*Capsicum spp*.) have become one of the fastest growing spices in U.S. market due to the changing American diet, increasing ethnic diversity and the influence of ethnic foods. Louisiana has long been known for its famous hot sauce production especially sauces that are made from Tabasco pepper (*C. frutescens*). The raw peppers are normally ground together with salt and fermented before production. Peppers mash is usually fermented in wood barrel made out of oak. However, a wood barrel is very expensive when used in pepper fermentation. As compared to wood barrel, plastic barrel have longer usage time and better sanitation. Understanding the chemical breakdown process involved in fermentation may assist in the development of better quality sauce, improve production sanitation, and reduce the manufacturing cost.

The main objective of this study was to evaluate physicochemical properties of Tabasco pepper(*C. frutescens*) during a two-year fermentation period as affected by the aging material including wooden barrels and plastic barrels. Physicochemical properties like dry weight, pH, titratable acidity, capsaicin level, and sugar level was investigated in a two-year red Tabasco pepper (*C. frutescens*) fermentation process.

Dry weight, pH, and titratable acidity (TA) were measured using standard AOAC procedure. Capsaicin, dihydrocapsaicin, fructose, sucrose, and glucose were analyzed using HPLC method. The soluble uronide that diffused into solution was determined as uronic acid equivalents by the hydroxybiphenyl method using galacturonic acids as standard. Total uronide content and pectin solubility in chelator was determined in air-dried tissues. The degree of depolymerization of CDTA soluble pectin from air-dried tissues was determined by size exclusion gel chromatography. Dry weight of pepper

mash significantly decreased during fermentation. Titratable acidity increased due to lactic acid production which lead to decreased in pH. After fermentation, pepper mash still contained residual sugar. Fermentation process did not affect the capsaicinoids concentration.

In this study, pepper mash fermented in plastic and oak wood barrel did not show any significant differences in pH, titratable acidity, sugar level, total uronide concentration, pectin degradation, and capsaicin level. Plastic barrels might be an alternative to wood barrels.

CHAPTER 1
INTRODUCTION

Fermentation is one of the oldest and most economical methods of preserving foods. Well known fermented products range from alcoholic beverages, such as beer and wine, to cheeses, sour milk products, various type of breads, yeast products and antibiotics. Collectively fermented food is one of the world's largest industries. In technologically developed regions, these fermented food products have evolved into the large-scale industrial production of consumer goods. Their value, and refined quality guarantee continued and increasing consumption (Deshpande and Salunkhe, 2000). Nearly all vegetable fermentation, including fruit handled like vegetables, such as cucumber, tomatoes, and olives, may be fermented by lactic acid bacteria. These vegetable products contain sugars which are nutritionally adequate as substrate for the growth of the lactic acid bacteria and other microorganisms (Luh and Woodroof, 1975). Vegetable fermentation is normally initiated by the bacterial species *Leuconostoc mesenteroids*, followed by *Lactobacillus brevis*, *Pediococcus cerevisiae*, and *Lactobacillus plantarum* (Pederson and Albury, 1954).

Due to the increasing ethnic diversity and the influence of ethnic foods, the demand for red pepper (*Capsicum spp*.) products has greatly increased. Red peppers are used in pickling, relishes, catsup, sauces, and processed meat and fish in all around the world. The U.S. market for all peppers increased from 95 million pounds dry-weight basis in 1980 to 210 million pounds in 1993 (Buzzanell et al. 1995). The total value of combined capsicum imports averaged $44.6 million in 1990-94, compared with $24.7 million in 1985-89, and $15.5 million in 1980-84 (Buzzanell et al. 1995). Louisiana has

long been known for its famous hot sauce production especially sauce made from Tabasco pepper (*C. frutescens*).

The idea of pepper fermentation was adopted from wine aging process. The raw peppers are normally ground into mash together with salt and fermented before production. Aging helps the mash to develop flavors, bouquet and odor. Pepper mash is usually fermented from 3 months to 3 years depending on the manufacturer's preference. However, pepper mash that is fermented in wood barrels made from oak is often associated with higher quality. Pepper fermentation is a food preservation method to prevent undesirable changes in food and food products. Such changes can be caused by invasion and growth of microorganism or by chemical, physical, and biochemical reactions of compounds present in the food itself (Luh and Woodroof, 1975). Examples of undesirable chemical changes include oxidative rancidity of fats and oils, loss of ascorbic acid and other vitamins through oxidation, degradation of pectin, and discoloration (Luh and Woodroof, 1975). Therefore, the mash fermentation process is a very critical step in final hot sauce quality.

Like wine production, pepper mash may be aged in wood, aluminum, stainless steel or plastic containers, depending on the sauce maker's idea of the style and economic considerations. Due to their relative permeability, the primary disadvantages of wooden containers are leakage and contamination unless they are very carefully built and maintained. Also, wooden barrels are relatively expensive when used in pepper fermentation. In contrast, plastic barrels have longer usage time, lower cost, lower maintenance, less leakage, and are easier to sanitize. If chemical aging processes are the same for pepper fermented in wood barrels and plastic barrels, then plastic barrels could

become an alternative to wooden barrels. However, a comprehensive study to examine the various physicochemical characteristics and functional properties throughout the whole pepper fermentation and aging process has not been reported. Understanding the chemical breakdown process involved in fermentation may assist in the development of better quality sauce, improve production sanitation, and reduce the manufacturing cost.

One of the major concerns of sauce and beverage industries is separation of sediments and layering of the sauce or beverage. Separation is an undesirable production condition because consumers view this as poor quality. Pectin plays an important role in pepper fermentation since it forms gels and influences the final viscosity of the sauce. Pectin integrity during fermentation destined for processing influences the quality of the final product and every effort is made to preserve the desired pectin characteristics. This is especially important for products without added thickeners or stabilizers. Carbohydrates in the pepper serve as substrates for the microorganism to undergo fermentation process. Capsaicin changes in the peppers during the fermentation process in different containers could influence the quality of the final pepper products.

Hence, the purpose of this study was to evaluate physicochemical properties of Tabasco pepper (*C. frutescens*) during a two-year fermentation period as affected by the aging container. Physicochemical properties such as pH, titratable acidity, total and soluble pectin content, capsaicin levels, and sugar levels were investigated in a two-year red Tabasco pepper (*C. frutescens*) fermentation process.

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CHAPTER 2
LITERATURE REVIEW

2.1 Fermentation

The fermented food industry may very well be one of the largest worldwide food process. Fermentation is the "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 contrast to unwanted spoilage or toxin production, fermentation is regarded as a desirable effect of microbial activity in foods. In general, the desirable effects of microbial activity may be caused by the biochemical activity of the microorganisms. Microbial enzymes breaking down carbohydrates, lipids, proteins, and other food components, can improve food digestion in the human gastrointestinal tract and thus increase nutrient uptake (Adams and Nout, 2001). Although modern food technology has contributed to the present day high standard of quality and hygiene of fermented foods, the principles of the age-old processes have hardly changed. In industrialized societies, a variety of fermented foods are very popular with consumers because of their attractive flavor and nutritional value.

2.1.1 Vegetable Fermentation

Fermentation, initiated by the action of microorganisms, occurs naturally and is often part of the process of decay, especially in fruits and vegetables. However, fermentation can be controlled to give beneficial results. Fermentation is a relatively efficient, low energy preservation process, which increases the shelf life and decreases the need for refrigeration or other form of food preservation technology.

A vegetable is "a plant cultivated for food, especially an edible herb or root used for human consumption"(Little et al*,* 1973*).* In general, vegetables tend to be less sweet than fruits and often require some form of processing to increase their edibility. In terms of food processing, vegetables are classified as 'low acid' foods due to their lower levels of acidity. Low acid foods are more prone to deterioration by microorganisms and can in fact provide an ideal substrate for food poisoning organisms when in a moist environment. Low acid foods can be safely preserved by making them more acidic, either through pickling, salting or drying (Anon, 1993).

2.1.2 Lactic Acid Bacteria

The lactic acid bacteria (LAB) are a group of gram-positive bacteria, non-respiring, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. They are the most important bacteria in desirable food fermentations, being responsible for the fermentation of most "pickled" (fermented) vegetables. Historically, bacteria from the genera *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus* are the main species involved. Several more have been identified, but play a minor role in lactic fermentations. Lactic acid bacteria were recently reviewed by Axelsson (1998).

The lactic acid bacteria are a dissimilar group of organisms with diverse metabolic capacity. This diversity makes them very adaptable to a range of conditions and is largely responsible for their success in acid food fermentations (Axelsson, 1998). Lactic acid bacteria carry out their reactions by the conversion of carbohydrate to lactic acid plus carbon dioxide and other organic acids without the need for oxygen. *Lactobacillus acidophilus, L. bulgaricus, L. plantarum, L. caret, L. pentoaceticus, L brevis* and *L.*

thermophilus are examples of lactic acid-producing bacteria involved in food fermentations. All species of lactic acid bacteria have their own particular reactions and niches, but overall, *L. plantarum* (homofermenter) plays the major role and produces high acidity in most vegetable fermentations. The lactic acid they produce is effective in inhibiting the growth of other bacteria that may decompose or spoil the food.

In sauerkraut production, environmental factors such as temperature, anaerobiosis, pH and salt concentration are judiciously adjusted to direct the interactions and optimize LAB growth. After salting, the liquid phase formed by plasmolysis with the intracellular water-carrying vitamins and other growth factors, serves as nutrient medium (Pederson, 1971). The aerobic microflora is repressed while the LAB multiply. Generally *Leuconostoc mesenteroides* initiates the fermentation, then it disappears gradually to be replaced by *Lactobacillus plantarum*. Of the LAB species associated with sauerkraut production, *L. mesenteroides* is the most sensitive to decreasing pH and undissociated forms of lactic and acetic acids. *L. mesenteroides* initiates growth in vegetables more rapidly over a range of temperatures and salt concentrations than any other lactic acid bacteria. The carbon dioxide produced replaces the oxygen, making the environment anaerobic and suitable for the growth of subsequent species of lactobacillus. Removal of oxygen also helps to preserve the color of vegetables and stabilizes any ascorbic acid that is present. In addition to acidification, *L. mesenteroides* is responsible for some flavor compounds but also, in some cases, for spoilage by dextran production (Pederson, 1971).

2.1.3 Organic Acids and Reduced pH

Despite their complexity, the whole basis of lactic acid fermentation centers on the ability of lactic acid bacteria to produce acid, which then inhibits the growth of other non-

desirable organisms. Species of the genera, *Streptococcus* and *Leuconostoc,* produce the least acid. Next are the heterofermentative species of *Lactobacillus* which produce intermediate amounts of acid, followed by the *Pediococcus* and lastly the homofermenters of the *Lactobacillus* species, which produce the most acid (Axelsson, 1998). For most pathogens, growth does not cease until the pH has dropped below 4.5 since bacteria can maintain their internal pH higher than that of their acidic environment (Booth, 1999).

The lactic acid bacteria belong to two main groups – the homofermenters and the heterofermenters. The pathways of lactic acid production differ for the two (Figure 1). Homofermenters produce mainly lactic acid from hexoses via the Embden–Meyerhof pathway. Heterofermenters using the 6-phosphoglucanate/phosphoketolase pathway produce a mixture of lactic acid, ethanol, acetate and carbon dioxide (Axelsson, 1998).

$C_6H_{12}O_6 \longrightarrow 2\ CH_3CHOHCOOH$

Glucose lactic acid

Homolactic fermentation: The fermentation of 1 mole of glucose yields two moles of lactic acid.

$C_6H_{12}O_6 \longrightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$

Glucose lactic acid + Ethanol + carbon dioxide

Heterolactic fermentation: The fermentation of 1 mole of glucose yields 1 mole each of lactic acid, ethanol and carbon dioxide

Figure 1: Lactic Acid Fermentation Pathway

Lactic acid has two important properties:

- It is a weak carboxylic acid that only partially dissociates in aqueous solution

- Its undissociated form carries no net charge and has appreciable lipid solubility, which allows lactic acid to diffuse freely through the cell's plasma membrane down a concentration gradient into the cytoplasm.

In a fermented food, the low pH will increase the proportion of the undissociated form present. When the undissociated acid passes through the cell's cytoplasm membrane into the higher pH environment of the cytoplasm, it will dissociate, thereby acidifying the cytoplasm and releasing the acid anion. The increased leakage of protons into the cytoplasm will place a metabolic burden on the cells, which will divert resources away from growth–related functions, thus slow growing (Adams and Nout, 2001). Lactic acid was the major constituent of the of the three end products determined from all vegetable fermentation which included beans, beets, carrots, cucumbers, peppers (green and red), and tomatoes. Acetic acid was produced in small amounts in all products except carrot. Ethanol was detected only in beans and green and red peppers (Fleming et al. 1983).

2.1.4 Effect of Salt on Lactic Acid Bacteria

Ethchells et al. (1943) conducted extensive bacteriological studies on the fermentation of various vegetables in brines of different concentrations. They stated that the salt concentration used, rather than the kind of vegetable, was the controlling factor on the character of the microbial flora and microbial activity. In fermentation at low salt content, 5 percent or less, large populations of acid forming and other bacteria occurred. At or above 15 percent salt, little or no growth of acid bacteria occurred. Coliform bacteria developed rapidly in brines of 2.5 to 5 percent of salt, with production of much gas. The coliform bacteria rapidly decreased in numbers as soon as an appreciable amount of acid was formed by other bacteria. Yeasts grew and fermented over a wide

range of salt concentration and appeared not to be inhibited by the acidity developed by lactic bacteria.

2.1.5 Traditional Pepper Fermentation Process

Green and red chile peppers are grown for the fresh market and for processing. Processed chile peppers are picked into bins and transported to processing plants for canning, brining, freezing, and drying. Fermented or pickled green jalapeno pepper is a widely consumed product. According to Pederson and Luh (1988), pickled products are those to which edible acids have been added, either lactic or acetic (vinegar); on the other hand, fermented products are such that the acid present was produced from sugars by bacterial action. Jalapeno peppers are sold as intact whole peppers, peppers without seeds, peppers in halves, peppers cut lengthwise, peppers cut in rings, and chopped peppers. Fermentation takes place in 4 to 6 weeks at high salt concentrations 10-15%. It is carried out in closed tanks, with a vent to allow gas formed during the process to dissipate. At the end of the fermentation period, the peppers originally bright green, turn olive green. Red pepper fermentation is usually utilized for sauce production. Whole red peppers are crushed into mash using a hammer mill together with approximately 8% of salt. This mash is placed in suitable containers for aging. Some producers use charred Kentucky white oak barrels from whiskey distillers with salt-sealed wooden lids that have tiny holes which allow the gases of the peppers to escape during fermentation. The wooden tops are secured and placed on the barrels with stainless steel hoops. Each barrel is aged for two to three years. To encourage an anaerobic condition and reduce contamination, salt is added on top of the barrel. The salt topping hardens in atmosphere humidity and naturally seals the barrel after the fermentation process ceases. The barrels are uncovered

and oxidized mash is removed from the top of the barrels. Upon being accepted under certain standards and requirement, the mash is pumped into large blending vats and mixed with distilled, all-natural white vinegar to produce hot pepper sauce. The purposes of fermentation are to increase pepper value, prevent pulp separation after sauce making, and produce a shelf stable sauce.

2.2 Horticultural Characteristics

Red peppers are members of the genus *Capsicum*, a genus that belongs to the Solanaceae family. The name "capsicum" may have been derived from the Latin word "Capsa" meaning box referring to the pod, or from the Greek "Kapso" meaning to bite. The capsicum (red pepper) once discovered was brought back to Europe. In a short time, the Capsicums had spread to many parts of the world. In different areas the pods developed different characteristics in shape, color, size and pungency. These factors make exact classification of the genus difficult. As a result of the peppers' long history, in which a great amount of hybridization has occurred, over ninety species are currently recognized (Wheat, 1987) and includes five domesticated species: *C. annuum*, *C. frutescens, C. baccatum, C. Chinese*, and *C. pubescens*. (Mao, 1999). Red pepper has been used in many food products such as relishes, catsup and as a food ingredient in many sauces. Hot pepper sauce produced in Louisiana is mainly made from pepper varieties of *C. frutescens*.

2.2.1 Horticultural History of Tabasco Peppers

The name of this pepper type is derived from the Mexican city of Tabasco, which had extensive trade with New Orleans during 1850s (DeWitt, and Gerlach, 1990). The Tabasco pepper was first cultivated in Louisiana by Maunsell White, a banker who

introduced the seed from Tabasco, Mexico. White gave pods and a sauce he had made to his friend, Edmund McIlhenny, who began to grow the Tabasco on Avery Island. McIlhenny began marketing a sauce from the pepper in 1869, and in 1870 he patented the now famous brand of Tabasco sauce.

2.2.2 Botanical Description

Botanically, the Tabasco pepper, has an intermediate number of stems, a compact habit, and grows between 1 and 4 feet high, depending upon climate. Generally, the longer the growing season, the larger the plant. The leaves are ovate, smooth, and measure 2½ inches long and 1-¼ inches wide. The flowers have white corollas with no spots. The pods are borne erect, measure 1-½ inches long and 3/8 inch wide (DeWitt, and Gerlach, 1990). Immature pods are yellow, maturing to orange and then turning red. Red color formation during pepper fruit ripening is a result of decreased chlorophyll and increased carotenoids. The major carotenoids are capsanthin and capsorubin (Rylski, 1986). Each plant can easily produce over 100 pods. *C. frutescen* requires a tropical climate, hence is restricted in habitat (Smith et al., 1987). Tabasco is the only member of *C.frutescen* commonly cultivated outside the tropics and in U.S (Mao, 1999). Tabasco chiles measure between 30,000 and 50,000 scoville units, placing them at 8 on the heat scale (DeWitt, and Gerlach, 1990). .

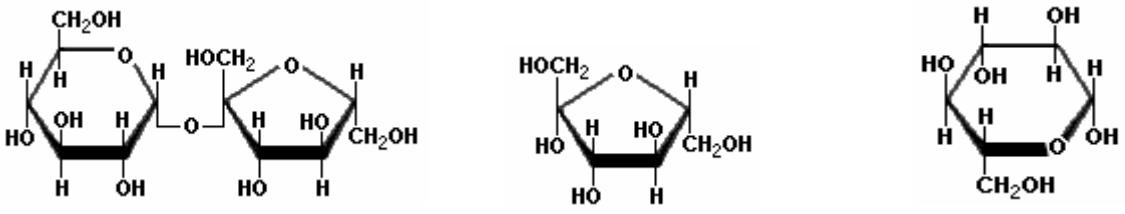
2.3 Pepper Physicochemical and Functional Properties

2.3.1 Soluble Carbohydrate

2.3.1.1 Chemical Structures of Sucrose, Fructose and Glucose

Sucrose, glucose and fructose were the major components of the soluble neutral sugars found in pepper fruit (*C. annuum*) (Nielsen et al., 1991). Fructose and glucose

constituted the known fermentable sugars in beans, cucmbers and peppers. According to Fleming et al., (1983), sucrose, in addition to glucose and fructose was present in carrots and peppers (green and red).



Sucrose Fructose Glucose

Figure 2: Chemical structures of disaccharide (a) sucrose and monosaccharides (b) fructose and (C) glucose commonly found in *C. frutescens*.

The chain form of glucose is a polyhydric aldehyde, meaning that it has multiple hydroxyl groups and an aldehyde group. Fructose, also called levulose or "fruit sugar", is shown here in the ring forms. Although glucose and fructose shared the same molecular formula ($C_6H_{12}O_6$), the arrangement of atoms differs from each others. Glucose, and fructose are "single" sugars or monosaccharides. Two monosaccharides can be linked together to form a "double" sugar or disaccharide (Figure 2). Sucrose is the common table sugar from the combination of glucose and fructose. Although the process of linking the two monomers is rather complex, the end result in each case is the loss of a hydrogen atom (H) from one of the monosaccharides and a hydroxyl group (OH) from the other. The resulting linkage between the sugars is called a glycosidic bond.

2.3.1.2 Carbohydrate Metabolism in Pepper Fruit

Fruit development can be divided into three phases: (1) an initial phase with high relative growth rate and hexose accumulation, (2) a phase with declining growth rate and

accumulation of sucrose and starch, and (3) a ripening phase with no further fresh weight increase and with accumulation of hexoses, while sucrose and starch were degraded (Nielsen et al., 1991). According to Nielsen et al.(1991), the carbohydrate metabolism in the growing fruit tissue is important to the partitioning of photosynthetically fixed carbon in the plant. Furthermore, the content of different sugars is critical to the quality of the fruit for consumption. Pepper fruits are harvested both as unripe and ripe, and the sugar content on the fruit tissue depends strongly on the harvest time. Sucrose is the major product in the source leaves of pepper plants (Nielsen and Veierskov, 1990), and sucrose utilization is expected to be central to sink strength in the fruits, since phloem transport of sucrose is considered to rely upon a downhill gradient of sucrose from source to sink tissues (Eschrich, 1989). According to Nielsen et al. (1991), during maturation of the fruits there was a significant accumulation of hexoses. In the ripe fruits soluble sugars accounted for 4.4% of the fresh weight, which equaled 40% of the dry matter. Starch and sucrose were only transiently accumulated to significant amounts during the period of declining fruit growth rate. Subsequently both of these carbohydrates were broken down, probably leading to the observed increase in reducing sugars 50 days after anthesis. According to Luning et al. (1994), sweetness in bell pepper appeared to be typical for ripe stages and closely related to glucose, fructose, total sugar, and dry matter content. However, sucrose was not related to changes in sweetness during maturation.

2.3.2 Pectin

2.3.2.1 Structures and Characterization of Pectin

Pectin substances are a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plants. They are deposited mainly in the

early stages of growth when the area of wall is increasing. Meristematic and parechymous tissues are therefore particularly high in pectin substances (Northcote, 1986). Pectin is usually used in a generic sense to designate those water-soluble galacturonoglycan preparations of varying methyl ester contents and degrees of neutralization that are capable of forming gels. The specific term protopectin is often used to designate the native pectin fractions in cell walls that cannot be extracted by nondegradative methods (Pilnik, 1990). Postharvest of climacteric fruits involves changes in turgor pressure, anatomical characteristics, and cell wall integrity. It is generally assumed that cell wall disassembly leading to a loss of wall integrity is a critical feature. The most apparent changes, in terms of composition and size, occur in the pectic fraction of the cell wall (Seymour and Gross, 1996), which include increased solubility, depolymerization, deesterification, and a significant net loss of neutral, sugar-containing side chains (Fisher and Bennett, 1991; Seymour and Gross, 1996). The cell wall can be divided into three layers: middle lamella, primary cell wall, and secondary cell wall (Figure 3). The amount of pectin present decreases in this order. In secondary cell walls, pectin may be virtually absent (Northcote, 1986).

Pectin has a chain structure of α-(1, 4)-linked D-galacturonic acid units interrupted by the insertion of (1, 2)-linked L-rhamnopyranosyl residues in adjacent or alternate positions. Other constituent sugars are attached in side chains, the most common being D-galactose, L-arabinose, and D-xylose. D-glucose, D-mannose, L-fucose, and D-glucuronic acid are found less frequently. Pectins also carry nonsugar substituents, essentially methanol, acetic acid, phenolic acids, and in some commercial samples, amide group. The esterification of galacturonic acid residues with methanol and/or acetic acid is

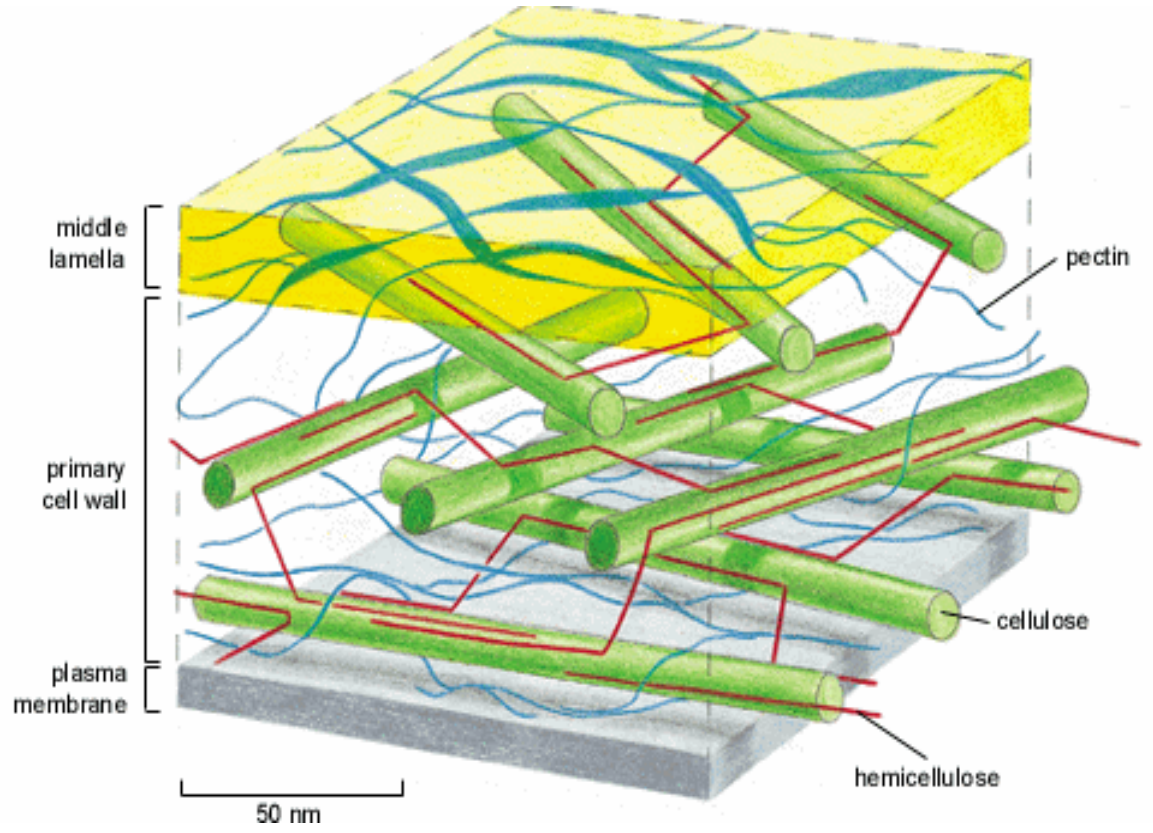


Figure 3: Pectin fills intercellular spaces of the middle lamella of a plant cell wall.

a very important structural characteristic of pectin substances. The unbranched blocks aggregate through Ca^{2+} bridges between de-esterified carboxylic groups of adjacent polymers keeping the cell wall matrix coherent and maintaining cell to cell adhesion. As the calcium concentration increases, the number of polyuronides that aggregate increases which is directly related to gel stiffness (Tibbits et al., 1998). The degree of methylation (DM) is defined as the percentage of carboxyl groups esterified with methanol (Figure 4). If more than 50% of the carboxyl groups are methylated, the pectins are called high methoxyl (HM) pectin; if fewer than 50% methylated, they are called low methoxyl (LM) pectins.

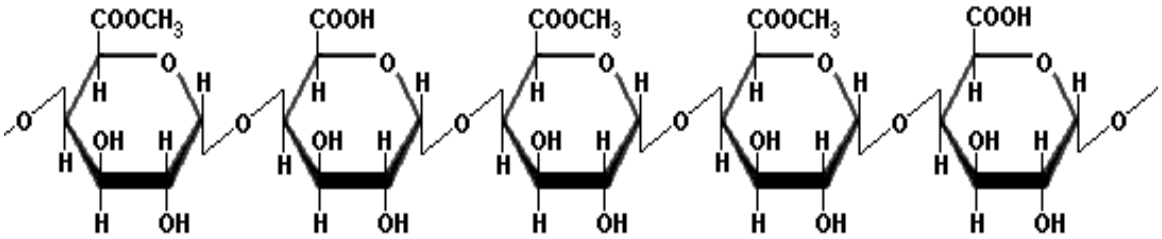


Figure 4: Pectin is a polymer of α-Galacturonic acid with a variable number of methyl ester groups

2.3.2.2 Uronic Acids Quantification Methods

Analysis for pectin carbohydrates (polyuronides) in plant materials is difficult because of the varied and complex matrix of nonuronide carbohydrates associated with the samples. Galacturonic acid residues are the fundamental units of pectin chains and quantification of this acid is a primary method used to determine the amounts of pectin material present in a sample (Kintner and Van Buren, 1982). Previous researches have shown that pectin fractions can be obtained by sequeantial extraction of the purified cell wall materials with cold and/or hot water or buffer solution, cold and /or hot solutions of chelating agents [ammonium oxalate, sodium hexametaphosphate, ethylene diamine tetraacetate (EDTA), cyclohexane diamine tetraacetate (CDTA)], hot diluted acids and finally cold, diluted sodium hydroxide (Massiot et al, 1988; Rombouts and Thibault, 1986; Chambat et al., 1984). Uronic acids quantification was first determined by using colorimetric carbazole sulfuric reaction (Dische, 1947). However, this method was not fully accurate because the interference of nonuronide compounds such as sugars, starches and cellulose (Selvendran, 1975). In 1973, Blumenkrantz and Asboe-Hansen developed the m-hydroxydiphenyl method which significantly reduced the interference of neutral sugars by adding m-hydroxydiphenyl as chromogen to heated solution of uronides in a sulfuric acid-boric acid mixture. Ahmed and Labavitch (1977) modified and tested this

procedure for native and extracted pectins. Kintner and Van Buren (1982) reported that the m-hydroxydiphenyl method would result in significant errors if the final sample dilutions contain more than 200ug/ml of nonuronide carbohydrate material. Therefore, a correction of the sample blank absorbance is suggested to compensate for this interference.

2.3.2.3 Pectin Metabolism during Pepper Maturation

Arancibia (2003) suggested that disruption of the cell wall structure and separation of cellular components by grinding with 100% alcohol and facilitated the release of soluble pectin. Chelator soluble uronide extracted throughout fruit ripening followed the same sigmoidal pattern of water soluble uronide for each genotype of Tabasco pepper. Total uronide was analyzed for differences between two Tabasco genotypes. Arancibia (2003) concluded that pectin content remained the same throughout the ripening process which ranged between 78 μg/mg to 104μg/mg with an average of 88μg/mg. Pectin content extracted by 100% alcohol for both genotypes was the same, but it increased slightly as fruit ripened. Metabolism and dissolution during ripening of alcohol insoluble carbohydrates may account for this slight increase of the uronide proportion. According to the studies of Arancibia (2003), Pectin content in Tabasco peppers slightly increased as the fruit ripened from green to red. Pectin metabolism during fruit ripening in Tabasco pepper was characterized by an increase in soluble uronide and a decrease in polyuronide molecular size.

The effect of polygalacturanase (PG) activity *in vivo* can be evaluated by the degree of pectin degradation determined by molecular-size exclusion chromatography. Uronide polymers of large molecular size constituted the larger proportion of EDTA-

soluble pectin that eluted first in the void volume followed by a tail of polyuronide of decreasing molecular size that extended up to the end of the separation range (DellaPenna et al., 1990). According to the research conducted by Arancibia (2003), the elution profile of premature green Tabasco pepper was the same for two genotypes; however, red-mature tissue was different between the genotypes. The elution profile of uronide from ripe (hue 40) EZ (easy pick) genotype showed an almost complete downshift of the uronide molecular size to oligomers of a few galacturonic acid residues as a consequence of extensive depolymerization. In the case of ripe (hue 44) genotype HP (hard pick), the elution profile showed that uronide was distributed throughout a wide range of large to medium size UA polymers.

2.3.3 Capsaicinoid

2.3.3.1 Chemical Characteristics of Capsaicinoids

Capsaicinoids are alkaloid compounds that produce the hot flavor or pungency associated with eating chiles (Collins et al., 1995). Pungency is the most outstanding property of capsicums, resulting from the direct effect of capsaicin and its analogues on the pain receptors in the mouth and throat (Krajewska and Powers, 1988). Pungency in pepper is due to the amount of capsaicinoids, including capsaicin and four structurally related compounds, namely nordihydrocapsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin (Hoffman et al., 1983). Capsaicin, which is the most abundant capsaicinoids, contributes most to pungency. Quantification of these pungent compounds is therefore an important index of pepper quality (Gibbs and O'Garro, 2004).

Capsaicin and its analogues are the pungent principles of *Capsicum* fruits. All of the identified capsaicinoids are vanillylamides of fatty acids. These compounds differ in the

length of the aliphatic side chain, the presence or absence of a double bond, the branching point, and by their relative pungency (Figure 5) (Krajewska and Powers, 1988). Most capsicum products such as red pepper, chili pepper, and oleoresin of red pepper are traded on the basis of their pungency or the level of capsaicinoids they contain (Parrish, 1996). Capsaicin itself is practically devoid of odor and flavor. Capsaicinoid contents typically range from 100ug/g in chili pepper to 2.5mg/g in red pepper and 0.06g/g in oleoresin red pepper (Parrish, 1996).

2.3.3.2 Capsaicin Analysis

The first reported reliable measurement of chile pungency is the Scoville Organoleptic Test. This test used a taste panel of five individuals who evaluate a chile sample and then record the hot flavor level. A sample was then diluted until pungency could no longer be detected (Scoville, 1912). This dilution is referred to as the Scoville Heat Unit. This test is subjective, and members of the taste panel cannot determine the amount of each of the capsaicinoids present in the sample. Food industry researchers need reliable, safe, and standard analytical procedures that are useful for comparing pungency levels among different samples (Collins et al., 1995). Therefore, the Scoville Organoleptic Test has since been replaced with instrumental methods. In 1977, the spectrophotometric vanadium oxytrichloride method was developed, measuring capsaicinoids as a group, not taking into account the difference in pungencies of the major capsaicinoids (Todd et al., 1977). Hot peppers contain other strongly UV-absorbing compounds that eluate with the void volume and shortly thereafter. Fluorescense detection lessens the interference of these compounds (Woodburry, 1980). Since 1980, newer columns (5 um particle size) have reduced this interference by separating the interfering compounds more efficiently.

Detectors have been improved as well. Hoffman et al. (1983) successfully separated and quantified five major heat principles (capsaicin, nordihydrocapsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsacin) in red pepper using reverse-phase high-pressure liquid chromatography. Currently, analysis of capsaicinoids is conducted by using spectrophotometric (Bajaj, 1980), gas chromatography (Krajewska and Powers, 1988), and high-performance liquid chromatography (HPLC) procedures (Attuquayafio and Buckle, 1987; Cooper et al., 1991; Collins et al., 1995). Techniques using HPLC provide accurate and efficient analysis of content and type of capsaicinoids present in a sample (Collins et al., 1995).

2.3.3.3 Capsaicin Metabolism during Pepper Maturation

Capsaicin content, as determined by the method outlined by Bajaj (1980), was generally higher in ripe pepper fruit than green pepper fruit. The capsaicin content range from 37.6 to 497.0mg/100g in ripe fruits and 278 to 404.5mg/100g in green pepper fruit. In contrast, Wheat (1987) found Tabasco peppers at the mature green stage had a significantly higher capsaicin concentration than the orange and red stages of maturation. This is in agreement with Mathew et. al. (1971) study that reported capsaicin concentration was lowest in the young green immature fruit. However, once this fruit is fully mature. It had the highest capsaicin content just prior to beginning the ripening stage. After the ripening stages begin, as seen by a change in color from green to red, the capsaicin concentration decreased (Mathew et al., 1971).

2.4 Carbohydrate Changes during Fermentation

Before pasteurization was introduced into the United States pickle industries, commercial preservation of many pickle products relied upon conversion of fermentable

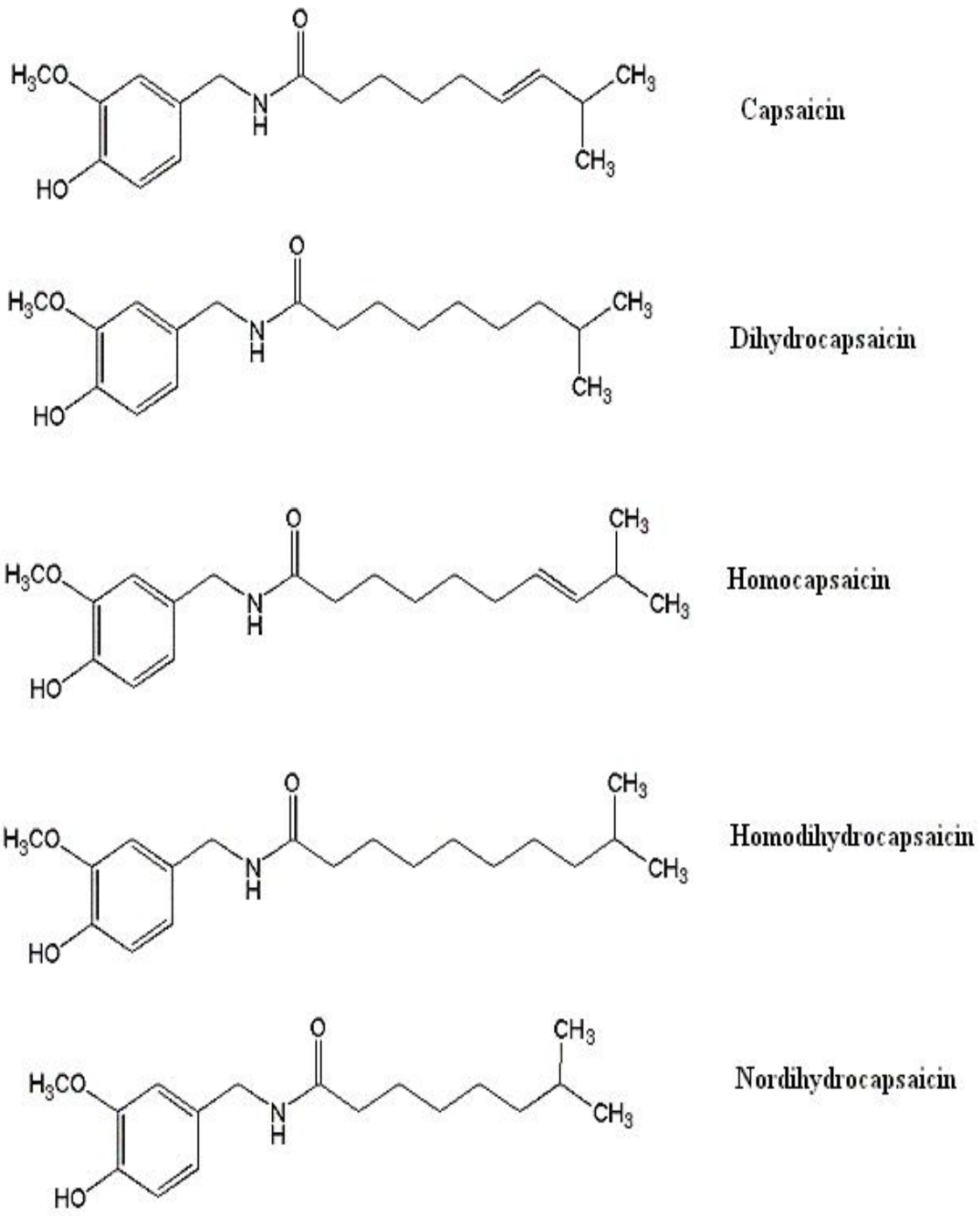


Figure 5: Chemical Structures of capsaicinoids–capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin.

carbohydrate to organic acids during bulk storage and/or the addition of sufficient amounts of vinegar, sugar, and other ingredient to fully cured and packed product to preclude microbial growth (Bell et al., 1951). In the case of cucumber products, "fully cured" refers to the complete removal of fermentable sugars and changes in the flesh from an opaque to a translucent appearance. Successful fermentation of brined vegetables is influenced by numerous chemical and physical factors including the concentration and type(s) of fermentable carbohydrate of the raw product, and the buffering capacity of the vegetables (Fleminget al., 1983). During the fermentation, carbohydrates are converted to acids and other end products by lactic acid bacteria and yeast. If sugars are incompletely fermented during primary fermentation, the product will be susceptible to secondary fermentation by yeasts (Fleming et al., 1983). In Fleming et al. (1983) studies, bell peppers, both green and red, underwent secondary fermentation when stored at pH 4.3 as evidenced by gas pressure in some jars and by a slight increase in acidity. However, peppers did not contain measurable fermentable sugars after fermentation.

2.5 Pectin Degradation in Pickled Products

Pectin degradation attributed to the action of PG has been studied extensively in tomato and has been associated with fruit softening (Brummell and Harpter, 2001). Pectin degradation is characterized by uronide depolymerization and dissolution during fruit ripening (DellaPenna et al., 1990). DellaPenna et al., (1990) concluded that the site of action of polygalacturonase is the cell wall, where it hydrolyzes polyuronides, or pectin. The increase in the level of chelator-soluble polyuronides during ripening and their corresponding decrease in molecular size have been well documented and are attributed to the action of polygalacturonase. Howard et al. (1994) study showed that softening of

jalapeno pepper ring was accompanied by increased level of water soluble pectin, and decreased levels of chelator-soluble pectin and sodium hydroxide soluble pectin. Howard stated that glycosidic and ionic linkages of pectin molecules were cleaved under high acid environments, resulting in greater water solubility of pectin substances. The capacity of pectin to form gels and influence the viscosity of solutions depends on the integrity of pectin polymers.

2.6 Research Justification

Collective evidence indicates that fermentation process increases the quality of the product, but the effect of the fermenting vessel is unknown. No systematic investigation has been made to understand the process of Tabasco pepper mash fermentation. The demand for red pepper products has been increased dramatically in recent years (Buzzanell et al. 1995). Hot sauce, pickles, and relishes play an important role in the food market. In this health conscious society, consumers prefer premium food quality and demand for healthy foods. Understanding pepper fermentation could potentially increases food qualities and food production to meet the consumption worldwide.

2.6.1 Fermentation in White Oak Barrel vs. Polyethylene Barrel

Many previous researches have suggested that wine fermented in oak barrels showed an increased in pleasant aroma or bouquet, and the addition of a soft oak wood flavor or vanillin flavor. However, there have been no reports on pepper mash fermented neither in oak barrel nor in plastic barrel. Yokotsuka et. al (1994) have compared the composition of koshu white wines fermented in oak barrel and plastic tanks. The conclusions of the studies are slower fermentation rate occurs in a wood barrel than in a

plastic tank might be due to smaller amount of suspended solids, less yeast growth as a result of polyphenols extracted from wood in spite of the warmer temperature of white wines fermented in wooden barrels.

2.6.2 Substitution of Wood Barrel with Plastic Barrel

The primary disadvantages of wooden containers are, due to their relative permeability, they are subject to leakage and contamination unless very carefully made and maintained. Also, wood barrels are relatively expensive when used in pepper fermentation. As compared to wood, plastic barrels have a longer usage time, lower cost, lower maintenance, and better sanitation. If chemical characteristics processes are the same for pepper fermented in wood barrels and plastic barrels, then plastic barrels could be an alternative to replace wooden barrel (Figure 6). A comprehensive study to examine various physiochemical characteristics and functional properties throughout the pepper fermentation process has not yet been reported. Understanding the chemical process involved in fermentation may assist in the development of better quality sauce, improvement of factory sanitation, and reduction of the cost of pepper manufacturing.

Figure 6: Polyethylene plastic barrel (left) and oak wood barrel (right) for pepper fermentation

CHAPTER 3
MATERIALS AND METHODS

3.1 Pepper Mash Fermentation

Fresh Tabasco peppers were ground and 8% salt was added. Samples of freshly ground peppers before and after salt was added were taken for analysis. Pepper mash was fermented in twelve wooden and twelve plastic barrels. The wood barrels have a volume of 55 gallon and the plastic barrels are 50 gallon. For easy sampling, a hole was drilled on top of the wooden barrel's cover and closed with a rubber stopper. Plastic barrel had an easily removable gasketed cover for sampling. Fermented samples were taken at 1 month, 2 months, 3 months, 6 months, 10 months, 12 months, 17 months, and 24 months. Samples were taken by using a 42 inch drum sampler (Conbar, NJ) with minimum disruption to the anaerobic pepper fermentation process. This avoided also taking the top layer of oxidized pepper mash or mixing the layers. The pepper mash was collected from the barrels into a 500 ml sampling cup (Fisher, TX).

3.2 Dry Weight Measurement

Moisture determination was performed in duplicate on pepper mash samples by drying 48 hr in a 60°C convection oven (VWR Scientific Product, OR) (AOAC, 1990). The samples were weighed into an aluminum pan, and recorded as weight of dry sample. Calculated dry weight content as:

$$\frac{\text{dry weight, g}}{\text{wet weight, g}} \times 100 = \% \text{ of dry weight content}$$

3.3 pH and Titratable Acidity

Titratable acidity and pH were measured using an Orion EA920 pH meter (Orion, MA). Titratable acidity (TA) was determined as ml of 0.1N NaOH used to obtain a pH

8.1 endpoint (AOAC, 1990). Lactic acid serves as a major organic acid in fermented pepper mash due to lactic acid fermentation. The formula to calculate %TA is as below:

$$\%TA = \frac{(\text{ml of NaOH}) \times (\text{N of NaOH}) \times (\text{Equivalent Weight})}{10 \times \text{Sample Weight}}$$

Equivalent Weight = 90.08

3.4 Sugar Analysis by HPLC

Ten grams of pepper mash was homogenized (Brinkman PT 110/35, Westbury, NY) with 10 ml of distilled water and frozen at -5°C until extracted. Samples were then sonicated for 20 minutes at 40°C and centrifuged (International Equipment, MA) for 20 minutes. The supernatant was decanted and filtered through Whatman No.4 filter paper (Whatman Int. Ltd., Maidstone) into 20 ml scintillation vials. A 2 ml aliquot of the filtered supernatant was again filtered using a 0.45 μm Millex®-HN filter unit on a 5 ml disposable syringe (Millipore, Bedford, MA) into a HPLC sample vial (Waters Corp., MA), which was capped, and stored at -5°C freezer until analyzed. The HPLC method was adopted from Nielsen et al. (1991) with some modifications. The sugars in the eluate were analyzed using a Waters (Waters Corp., MA) HPLC equipped with a 600E multisolvent delivery system, a 410 differential refractometer, and a 717 autosampler. Sugars were separated with an Aminex® HPX-87N 300 x 7.8 mm column (Bio-Rad 125-0143, Hercules, CA) fitted with a precolumn guard (Bio-Rad Micro-Guard). This column accommodates a salt matrix which will tolerate up to 15% of salt. The 8% salt soluble in water will interfere the accuracy of the results in regular column. The mobile phase was degassed HPLC grade water (Fisher, TX) having a flow rate at 0.6 ml min^{-1} at 85°C with 15 min run time. During HPLC sample analysis, a standard solution was injected every

15 samples in order to evaluate the retention time verification and instrument calibration. Standards consisted of fructose (80µg), glucose (80µg), and sucrose (20µg) and were obtained from Sigma Chemical, St. Louis, MO.

3.5 Capsaicin Analysis by HPLC

Samples were extracted according to Collins et al. (1995) with some modifications. Pepper mash was lyophilized in a freeze drier (The Virtis Company, MA) and stored in sealed plastic bags at 0°C in the refrigerator until processed. A 1:10 (w/v) ratio of freeze dried pepper mash to acetonitrile was placed in 120 ml glass bottles with teflon–lined lids. Bottle were capped and placed in an 80°C water bath (Blue M., IL) for 4 hours with occasional agitation. Samples were removed from the water bath and cooled to room temperature. A 2 ml aliquot of supernatant was extracted and filtered using a 0.45 µm Millex®-HN filter unit on a 5 ml disposable syringe (Millipore, Bedford, MA) into a sample vial which was capped, and stored at 5°C in the refrigerator until analyzed. A 1 µl aliquot was used for each HPLC injection.

A Waters (Waters Corp., Milford, Mass) HPLC equipped with a 600E multisolvent delivery system, a 474 fluorescence detector with excitation at 280 nm and emission at 338 nm, and a 717 autosampler was used. HPLC operating conditions included ambient temperature, a flow rate of 1 $ml.min^{-1}$, and a run duration of 7 min. The mobile phase was isocratic using degassed HPLC grade 73% methanol and 27% water (Fisher, TX). The reverse-phase chromatographic column was a Nova-Pak C18 3.9 x 150-mm packed with silica (Waters Corp., Milford, Mass). A precolumn guard cartridge, Nova-Pak C18 (Waters Corp., Milford, Mass) was also used. During HPLC sample analysis, a standard solution was injected every 15 samples in order to evaluate the

retention time verification and instrument calibration. These capsaicinoid standards consisted of 20µg 8-methyl-n-vanillyl-6-nonenamide (capsaicin) and 20µg 8-mrthyl-n-vanillylnonanamide (dihydrocapsaicin) and were obtained from Sigma Chemical Co, St. Louis, MO.

3.6 Pectin Determination

3.6.1 Sample Preparation

Ten grams pepper mash samples were homogenized (Brinkman PT 110/35) for 1 min in 10 ml 100% EtOH using a Waring blender (Scientific Industries, NY) to inactivate any endogenous enzyme present in the pepper mash and remove alcohol-soluble solids. Homogenates were then placed in a hot water bath at 80°C for 20 minutes, cooled to room temperature, centrifuged at 8000 rpm for 20 minutes, and the supernatant discarded (Ahmed and Labavitch, 1977). To conduct structural studies on pectins from plant cell walls, a clean-up of the pectin source to remove interfering compounds is necessary. The pellet residue was washed 3 times with 10 ml acetone (Fisher, TX), centrifuged for 20 min, and the supernatant discarded (Aracibia, 2003). The resulting pellet was air dried for 48 hours. The sample of air dried material was stored in 20 ml scintillation vials until analyzed. Pectin was measured according to Blumenkrantz and Asboe-Hansen (1973) with modification.

3.6.2 Total Uronide Content Determination

Approximately 5 mg of the air dried sample was weighted into a 20 ml scintillation vial. The vial was placed in a water-ice bath and 2 ml of chilled concentrated sulfuric acid (Fisher, TX) was added and the mixture swirled gently. The mixture was agitated gently as 0.5 ml distilled water was added to the vial. Another 0.5 ml water was added in

dropwise fashion and the sample stirred for 1 hour. Aliquots of this digestion were assayed for uronic acids quantification. For each preparation, a sample and "blank" test tube were prepared, with 0.2 ml of the dissolved material placed in each. To each tube, 1.2 ml of chilled tetraborate reagent (0.0125M sodium tetraborate in concentrated sulfuric acid) ((Fisher, TX) was added and the mixture vortexed for 10 seconds. The tubes were heated in a dry bath at 100°C for 5 min then cooled in a water-ice bath. Total pectin content was determined by the hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using galacturonic acid as a standard. Twenty ul of m-hydroxydiphenyl reagent (0.15% solution of m-hydroxydiphenyl in 0.5%NaOH) (Sigma Chemical Co, St. Louis, MO) was added to the sample tube. Carbohydrates produce a pinkish chromogen with sulfuric acid/tetraborate at 100°C. The blank sample was run in place of the m-hydroxydiphenyl using 20 ul of 0.5% NaOH. The tubes were vortex for 10 seconds and absorbance measured at 520 nm in a Perkin Elmer Lambda 35 UV/VIS Spectrophotometer (Norwalk, CT). Absorbance for the sample was adjusted by subtracting the absorbance for the blank. Blanks were prepared to ensure that substances other than pectin that might react with the chromogen will not be quantifiedas pectin. Standards were prepared using galacturonic acid (Sigma Chemical Co., St Louis, MO) at concentration of 15-200 ppm. Standard curves were developed manually.

3.6.3 Chelator-soluble Uronic Acid Determination

Approximately 50 mg of the air dried sample was weighted into a vial. Two ml of extraction buffer (50 mM Na acetate, 40 mM EDTA, pH 4.5) (Sigma Chemical Co, St. Louis, MO) was added and vortex for 10 seconds. The sample was stored for 24 hours at 5°C in refrigerator. After 24 hours in suspension, the sample was centrifuged and an

aliquot was taken to determine the chelator UA content by the hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) using galacturonic acid as a standard (Sigma Chemical Co, St. Louis, MO).

3.6.4 Pectin Depolymerization

The degree of depolymerization of chelator-soluble pectin extracted was determined by size-exclusion chromatography using a Sepharose CL-4B column (30 x 1.5cm) (Amersham Bioscience, NJ) following the method of DellaPenna et al. (1990) with some modification. The air-dried alcohol acetone-insoluble cell wall material was suspended in EDTA extraction buffer (50mM Na acetate, 40mM EDTA, pH4.5) for 24 hours at 5°C in the refrigerator. The sample was centrifuged and a 5 ul aliquot was analyzed for uronide concentration using a Perkin Elmer spectrophotometer (Norwalk, CT) at 520nm. Based on this analysis the sample was diluted to give a uronide concentration of 0.5mg/ml. A 1-ml of this adjusted sample was passed through the (Sepharose CL-4B, Sigma-Aldrich, St. Louis, MO) column equilibrated with an elution buffer (100 mM Na acetate, 20 mM EDTA, pH 6.5). The elution rate was 0.5 ml/min and 4 ml fractions with a total of 15 fractions were collected by Bio-Rad Fraction Collector (Hercules, CA) and analyzed for uronic acid content. The UA content of each fraction was determined by the hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). A 0.5 mg/ml solution of galacturonic acid (Sigma Chemical Co, St. Louis, MO) was used as the monomer standard.

3.7 Statistical Analysis

All experiments were carried out in 12 times (n=12) the twelve wood and twelve plastic barrels. Fresh pepper mash experiments were carried out triplicate (n=3) from

three different batches and fresh salted pepper mash were carried out in 7 times (n=7) with seven different batches. Average values (means) and standard deviations were reported. Mean separations were analyzed using the ANOVA (SAS) and Tukey's studentized range tests at $\alpha = 0.05$.

CHAPTER 4
RESULTS AND DISCUSSION

Results of the proximate analysis and physiocochemical properties throughout the pepper fermentation process are presented in Figures 7-15.

4.1 Dry Weight

The average dry weight of Tabasco peppers immediately following grinding was 38% and moisture was 62% with a range of 61.7% to 62.9%. After salt added to the pepper mash, moisture was 54% with a range of 52.7% to 55.8%. These results are consistent with Mao (1999) who found that pod moisture content of red *Capsicum annuum* was between 65% and 80%, depending on whether the pods were partially dried on the plant or harvested while still succulent.

Within 1 month fermentation there was a significant decrease in dry matter for both wooden and plastic containers from the original salted mash (Figure 7). This may have due to an initial separation of liquid from pepper solids immediately after salting and filling barrels with an average dry weight of 38.2% $\pm$ 0.326 (plastic) and 39% $\pm$ 0.248 (wood). No significant changes in dry matter were observed after 1 month. The early water loss may have occurred between grinding and filling of the barrels as leakage from machinery. Though not significant, the dry matter of pepper mash fermented in wooden barrels was greater than in the plastic barrels. This may have been due to the porous nature of the wooden barrels that allowed water to evaporate or leak during the 24 months of fermentation.

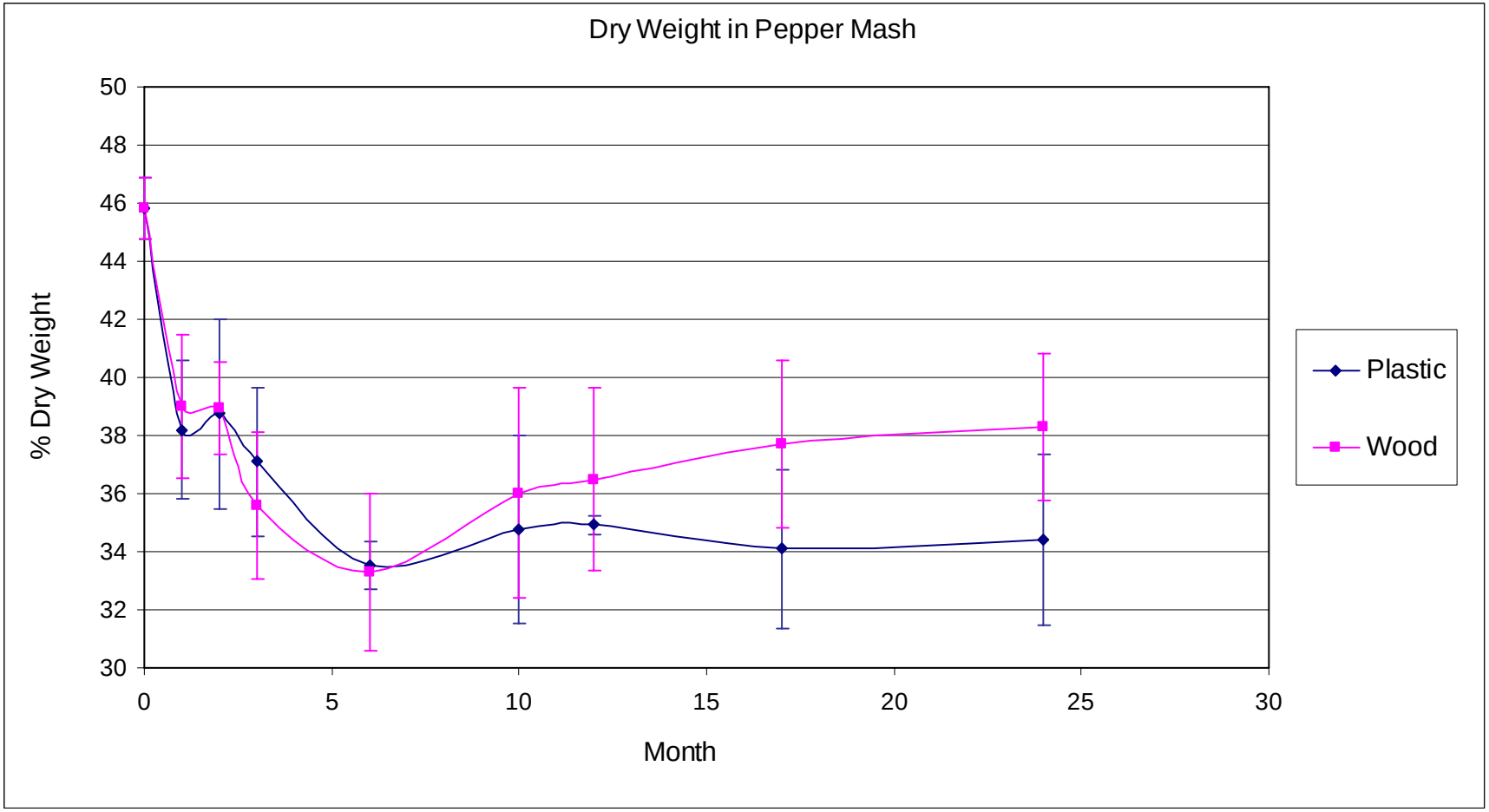


Figure 7: Dry weight matter of pepper mash throughout the 24 months fermentation. For fresh salted pepper mash at time=0; n=7. For fermented pepper mash from 1 months fermentation through 24 months fermentation, n=12.

4.2 pH and Titratable Acidity

The average pH of ground fresh peppers before salting was 4.98with a range of 4.97 to 4.99. Following the addition of 8% salt, the average pH was 4.7 with a range of 4.59 to 4.80 (Figure 8). Within the first month of fermentation, the pH significantly decreased to 3.9 (range to 3.07 to 4.41) and 3.7 (range to 3.17 to 4.6) for plastic and wooden barrels, respectively. After 1 month there were no significant changes in pH. The initial decrease in pH was likely due as the result of lactic acid bacteria growing in the salted mash (Pederson and Luh, 1988) and producing lactic acid. The general specification project for pickled products established by the processed fruit and vegetable committee of the Codex Alimentarius Commision FAO/OMS, notes a maximum pH specification of 4.6 for pickled products (FDA, 2003). During 24 months fermentation, salted pepper mash in both wooden and plastic barrels was below this maximum. Therefore, the pepper mash pH meets the specification standard.

The average titratable acidity (TA) of pepper mash before salting was 0.58% of acidity (expressed as lactic acid) with a range of 0.57% to 0.58%. Immediately following salting the average TA was 0.54% (Figure 9) with a range of 0.43 to 0.62%. The acidity significantly increased to about 1.6% after 1 month of pepper fermentation for both wood (range 1.04% to 2.10%) and plastic (range 0.97% to 2.52%) barrels. Our study agrees with work reported by Fleming et al. (1983) where fermenting red bell pepper reached 1.53% of lactic acid after two weeks fermentation. Galicia et al. (1996) found that during jalapeno pepper fermentation TA increased from 0.8% to 1.5%, which promoted a decreased in pH. Statistically there are no significant differences ($p>0.05$) in TA between plastic or wood barrel during the fermentation time course.

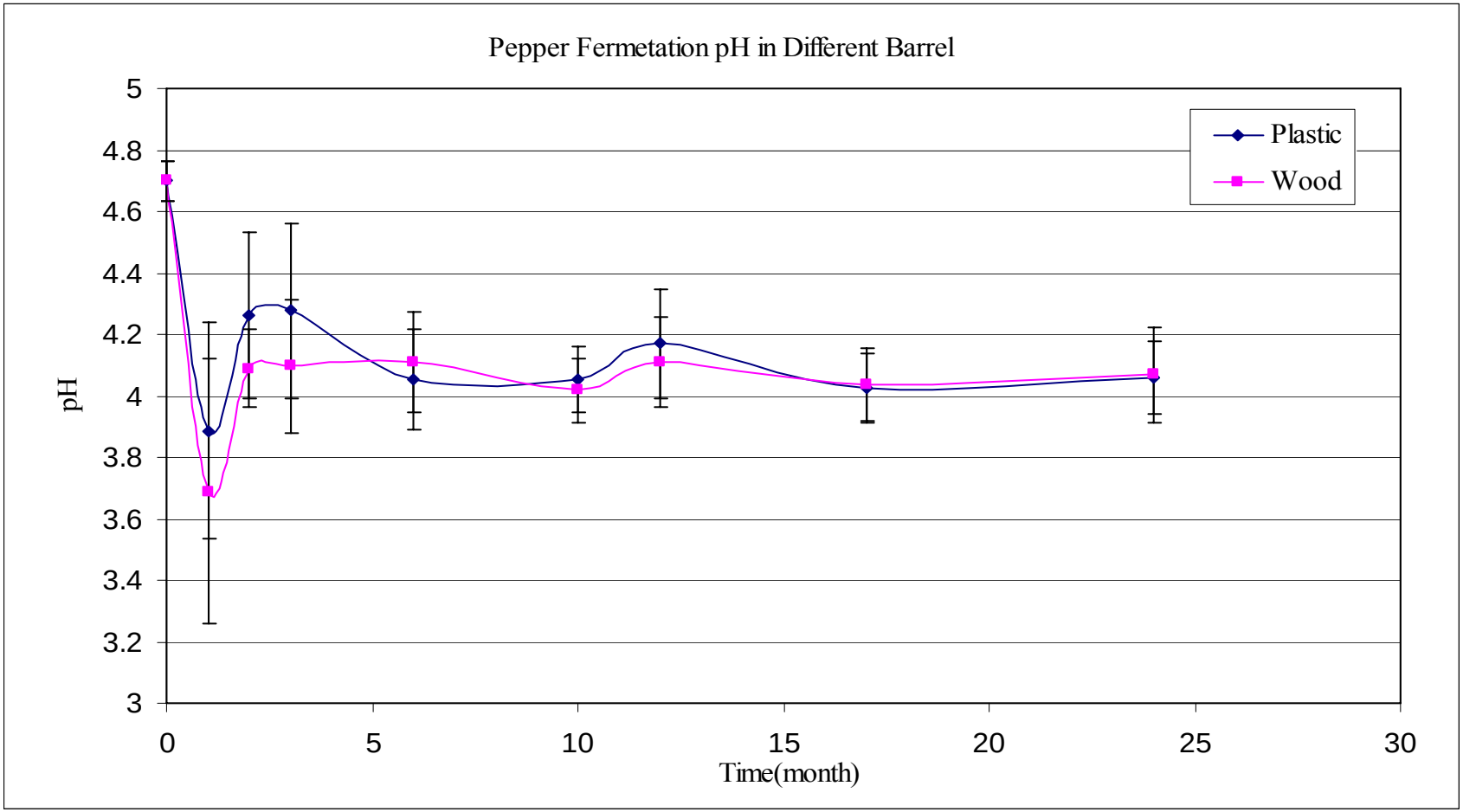


Figure 8: Average pH of pepper mash (fresh salted, n=7; fermented mash, n=12) fermented in wood and plastic barrels throughout the 24 months time course.

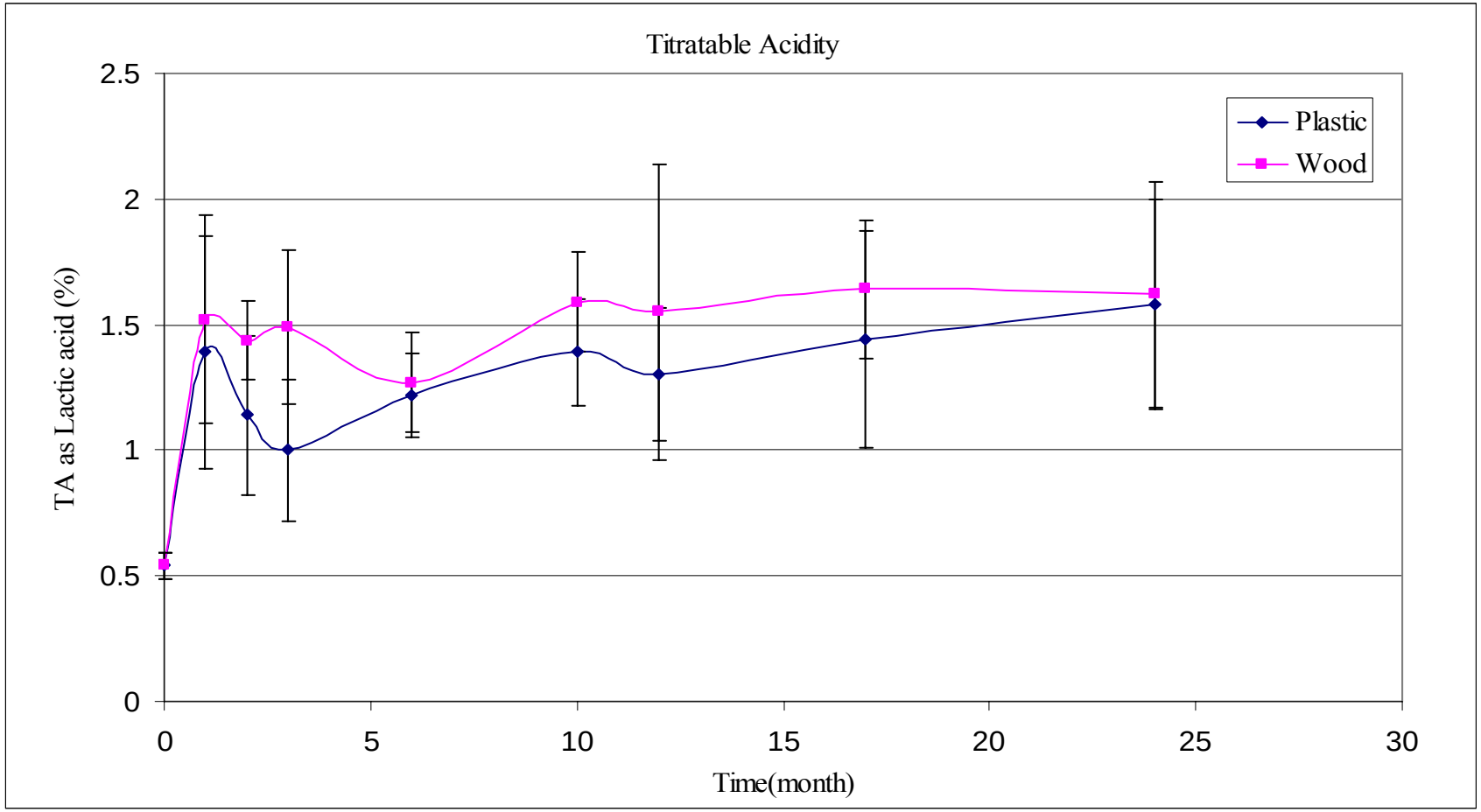


Figure 9: Mean value of titratable acidity (fresh salted, n=7; fermented mash, n=12) of pepper fermented in wood and plastic barrels throughout the 24 months time course.

4.3 Soluble Carbohydrate

High performance liquid chromatography was used to quantify soluble sugars based on dry weight particularly for samples containing high salt. Fresh ground peppers soluble sugars are fructose (20.26 mg/g of dry weight), glucose (15.83 mg/g of dry weight) and sucrose (0.07 mg/g of dry weight). No sucrose was detected in the mash following the addition of 8% salt, and the concentration

of glucose and fructose decreased to 7.57 mg/g and 9.80 mg/g of dry weight respectively (Figure 10a, b, and c). The mean values of glucose and fructose had decreased 50% during the grinding and salting process.

Irrespective of fermentation container, sucrose was difficult to quantify. Although HPLC method is relatively fast method in sugar analysis, it is less sensitive for low sugar concentrations especially when the refractive index detector was used (Agblevor, 2004). The sucrose concentration may have been at the limit of detector sensitivity. Glucose and fructose did not significantly change during the fermentation process for either wood or plastic barrel (Figure 10B, C). Concentration of glucose remained at about 12mg/g while fructose decreased to a concentration that was difficult to quantify with refractive index.

In many fermented products, lactic acid bacteria utilize sugars as a carbon source (Fleming et al., 1983). In our study, glucose remained at a constant concentration during fermentation process (Figure 10). The lactic acid bacteria may not have fully utilized glucose as a carbon source. Hughes and Lindsay (1985) reported that glucose persisted longer than sucrose or fructose in sauerkraut fermentation, but was absent except in very late stages of sampling (18 weeks). Peterson and Viljoen's (1925) work showed that about 0.4% residual reducing sugars (glucose) remain in sauerkraut after fermentation

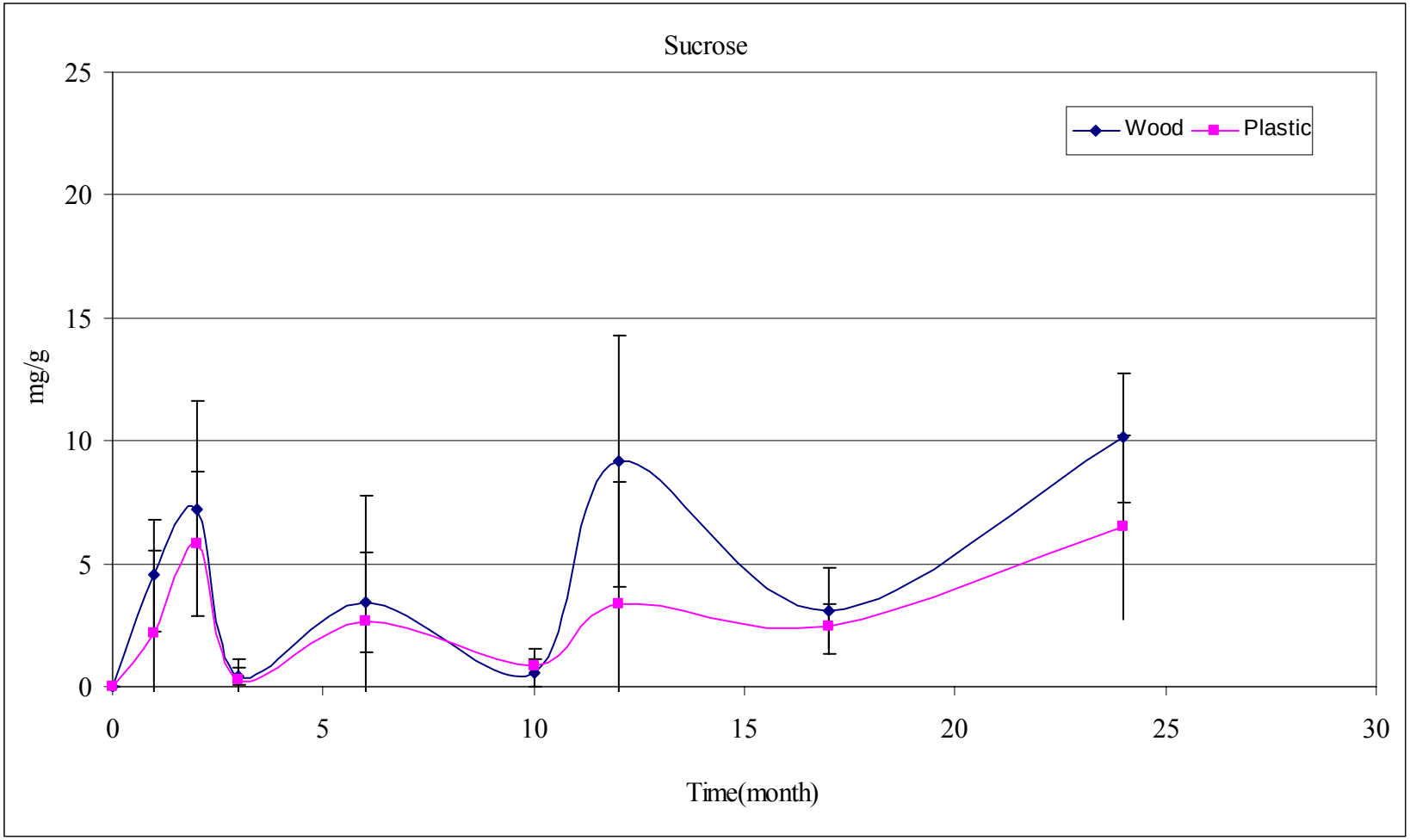


Figure 10a: Average (fresh salted, n=7; fermented mash, n=12) of sucrose concentration (mg/g) based on dry weight, quantified from HPLC chromatogram throughout the 24 month fermentation process.

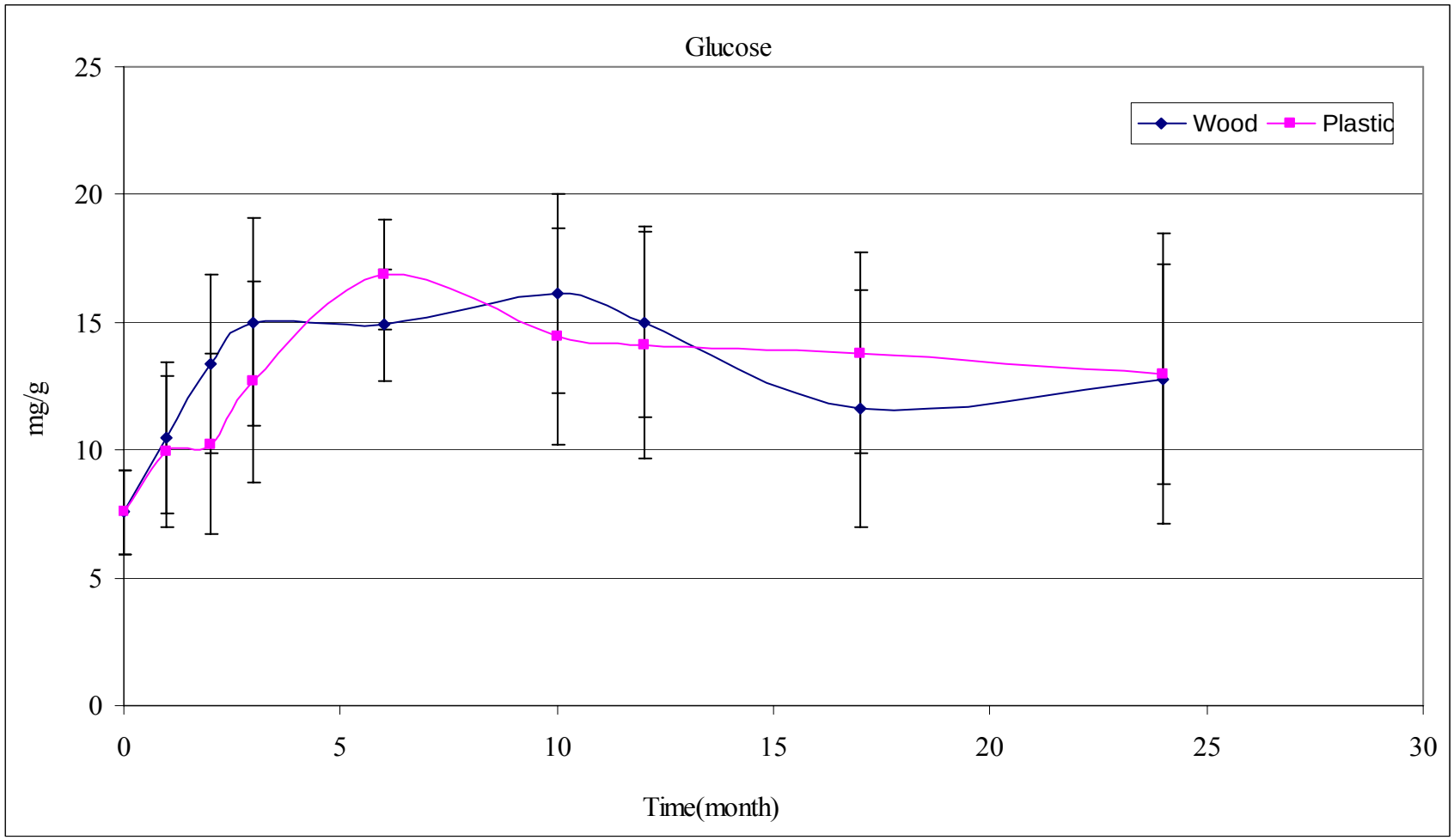


Figure 10b: The mean value (fresh salted, n=7; fermented mash, n=12) of glucose concentration (mg/g) based on dry weight, quantified from HPLC chromatogram throughout the 24 month fermentation process.

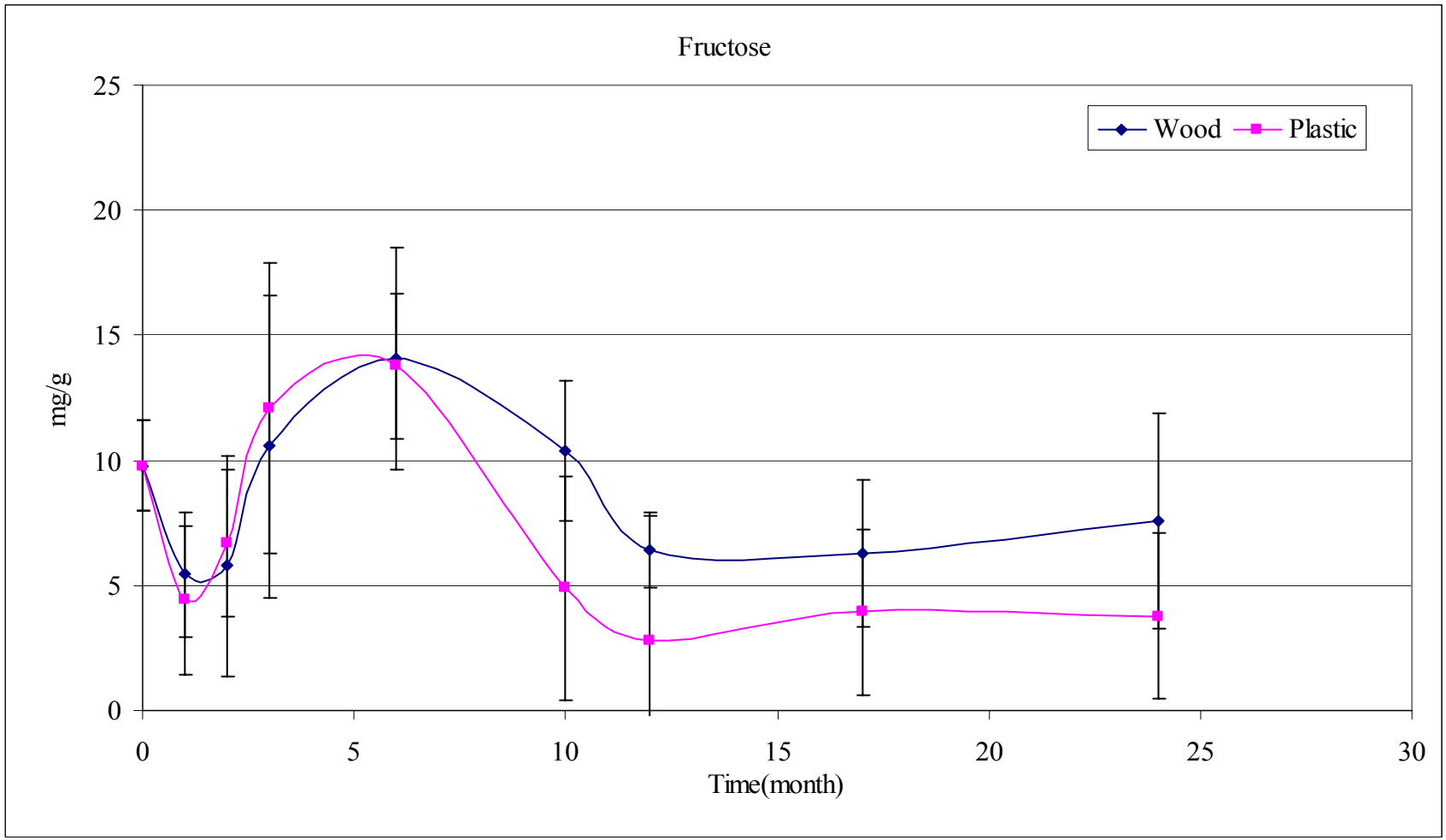


Figure 10c: The mean value (fresh salted, n=7; fermented mash, n=12) of fructose concentration (mg/g) based on dry weight, quantified from HPLC chromatogram throughout the 24 month fermentation process.

was complete. It has been hypothesized that an increase in glucose may have been some type of secondary metabolic activity. Fleming et al., (1983) suggested that dextran accumulations often disappeared upon holding of sauerkraut, and hydrolysis of the dextran could account for the unexpected appearance of glucose in the late stages of fermentation. Another possibility is pepper fruit pectin might contain constituent sugars in the side chains. During fermentation, enzymes break down the side chain and release the residual sugar after fermentation has ceased.

4.4 Total Uronide Content

Figure 11 illustrates the total uronide quantified based on acetone-insoluble dry tissues in wood and plastic barrel throughout the 24 months of fermentation. After the air-dried tissues were digested, various types of glycosidic linkages can be degraded (BeMiller, 1967). All the different pectin backbone structures break down into uronic acid monomer. The total uronide content quantified uronic acid present in the alcohol acetone insoluble air-dried tissues. Fresh ground peppers had a mean total uronide content of 128.14 mg/g with a range of 86.14 mg/g to 173 mg/g and fresh salted pepper mash had a mean total uronide content of 123.61 mg/g with a range of 83.25 mg/g to 167.93mg/g. Though not significant, after two months fermentation, total uronide content gradually increased to average of 333 mg/g for plastic (range to 92.34 mg/g to 472.16 mg/g) and 338 mg/g for wood (range to 134.88 mg/g to 444.68 mg/g) at six month fermentation. The total uronide content then decreased to 189 mg/g for plastic (range to 23.06 mg/g to 348.11 mg/g) and 212mg/g for wood (range to 20.20 mg/g to 251.53 mg/g). The total uronide concentration remained stable from 10 month to 24 month of fermentation.

Previous researches have shown that pectin fractions can be obtained by sequeantial extraction of the purified cell wall materials with cold and/or hot water or buffer solution, cold and /or hot solutions of chelating agents [ammonium oxalate, sodium hexametaphosphate, ethylene diamine tetraacetate (EDTA), cyclohexane diamine tetraacetate (CDTA)], hot diluted acids and finally cold, diluted sodium hydroxide (Massiot et al, 1988; Rombouts and Thibault, 1986; Chambat et al., 1984). In our study, only CDTA-soluble pectin was extracted followed by depolymerization study using size exclusion chromatography to determine degradation of CDTA-soluble pectin.

4.5 Pectin Depolymerization

Separation in red pepper mashes is thought to be caused by degradation of pectin molecules because polygalacturonase activity results in the depolymerization of polyuronide polymers (Huber, 1983) and polyuronide solubilization, the size of chelator soluble polyuronides was investigated in fermented pepper mash using size exclusion chromatography (Figure 10A, B). IN fresh Tabasco peppers, uronide polymers of large molecular size constituted the largest proportion of CDTA-soluble pectin that eluted first in the void volume followed by a tail of polyuronides of decreasing molecular size that extended up to the end of the separation range (Arancibia, 2003). A 0.5mg/ml solution of galacturonic acid was used as the monomer standard. Since it is the end point of pectin degradation, galacturonic acid has the smallest molecular unit and is the last one to be eluted. The molecular size profile of CDTA-soluble polyuronide extracted from fresh unfermented and fermented pepper samples were compared to galacturonic acid standard (Figure12a). In our study, galacturonic acid (standard) eluted at fraction #11 (Figure 12a); while the fresh pepper sample eluted at fraction #9.

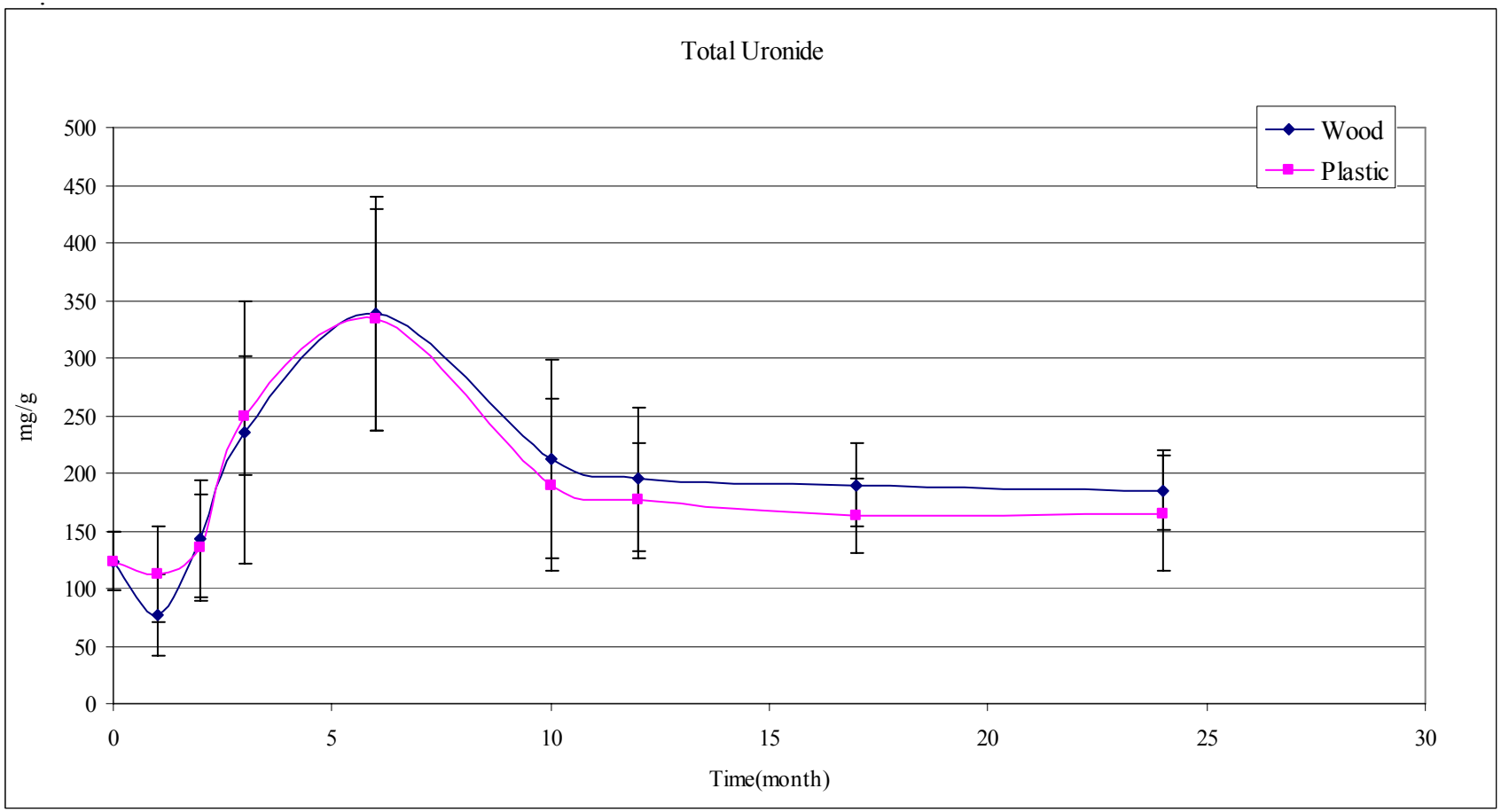


Figure 11: The mean value of total uronide quantification (based on acetone-insoluble dry tissues) for pepper mash fermented in wood and plastic barrel were measured at 520 nm absorbance in Spectrophotometer.

This indicated that the mature fresh red pepper sample was already extensively but not fully degraded to galacturonic acid residues. At this stage, pectin polymers were tightly bound so they were not released from fresh tissue into water, unless the cell wall structure was disrupted allowing some dissolution. Yield from size exclusion column was always more than 85%.

Pectin metabolism during fruit ripening in Tabasco pepper was characterized by an increase in soluble uronide and a decrease in polyuronide molecular size (Arancibia, 2003). Pectin dissolution depends on the size of the uronide polymer. In contrast to galacturonic acid and uronic acid oligomers, dissolution of larger pectin polymers is difficult because they are ionically bound and form part of the cell wall structure (Jarvis 1984). Arancibia's study stated that red-ripe hard-pick Tabasco fruit has a limited degree of pectin depolymerization comparable to levels found in ripe wild-type tomatoes. However, Arancibia's studies used whole Tabasco fruit for research; our study used fresh pepper that was coarsely ground, salted and fermented. Comparatively, fresh salted pepper sample eluted at fraction #10 (Figure 12a) which has more degraded chelator-soluble pectin than the fresh pepper mash. The cell wall structure was disrupted allowing dissolution of pectin prior to the fermentation process. After one month fermentation, chelator soluble pectin fully degraded to monomer which eluted at fraction #11 (Figure12b). Highly degraded oligouronide could no longer hold the cell wall structure leading to its disintegration. Consequently, oligouronide easily diffused out from fresh tissue. Results were consistent with pepper fermented in wood and plastic barrel after one month fermentation.

Glycosidic and ionic linkages of pectin molecules may have been cleaved during the acidic environment of the pepper mash, resulting in greater water solubility of pectin substances (Figure 8, 9, and 12b). A study of pasteurized jalapeno rings stored in acetic acid above 1.5 % for 1 month showed an increased in water soluble pectin and a significant decreased in chelator soluble pectin (Howard et al, 1994). In Araujo et al. (1994) study on olive processing showed that the sum of the water and CDTA-soluble uronic acids in unprocessed and processed olives represents 42-44% of the wall uronic acid. The pectin that remains behind the olive fruit is less soluble. Chelator-soluble pectin quantification was declared by using serial dilutions of galacturonic acid solution which is 50, 100, 150, and 200 ug/ml (Figure 13).

4.6 Capsaicinoid

In the present study, fresh ground pepper had a mean value of 0.30 mg/g (range 0.29 mg/g to 0.32 mg/g) capsaicin; while fresh salted pepper mash had a greater capsaicin concentration than fermented pepper mash as seen in Figure 14A which has a mean value of 0.32 mg/g. The capsaicin concentration decreased significantly when the fermentation process started. Generally, the capsaicin concentration remained stable throughout the twenty four months of fermentation process. Pepper mash fermented in either wood or plastic did not significantly affect ($p<0.05$) the capsaicin concentration.

Dihydrocapsaicin concentration followed the same trend in fermentation as did capsaicin concentration in regard to the fermentation process during the twenty four months. Dihydrocapsaicin has a mean value of 0.12 mg/g for fresh (range 0.11 mg/g to 0.12 mg/g) and fresh salted pepper mash 0.12mg/g (range to 0.09 mg/g to 0.14 mg/g).

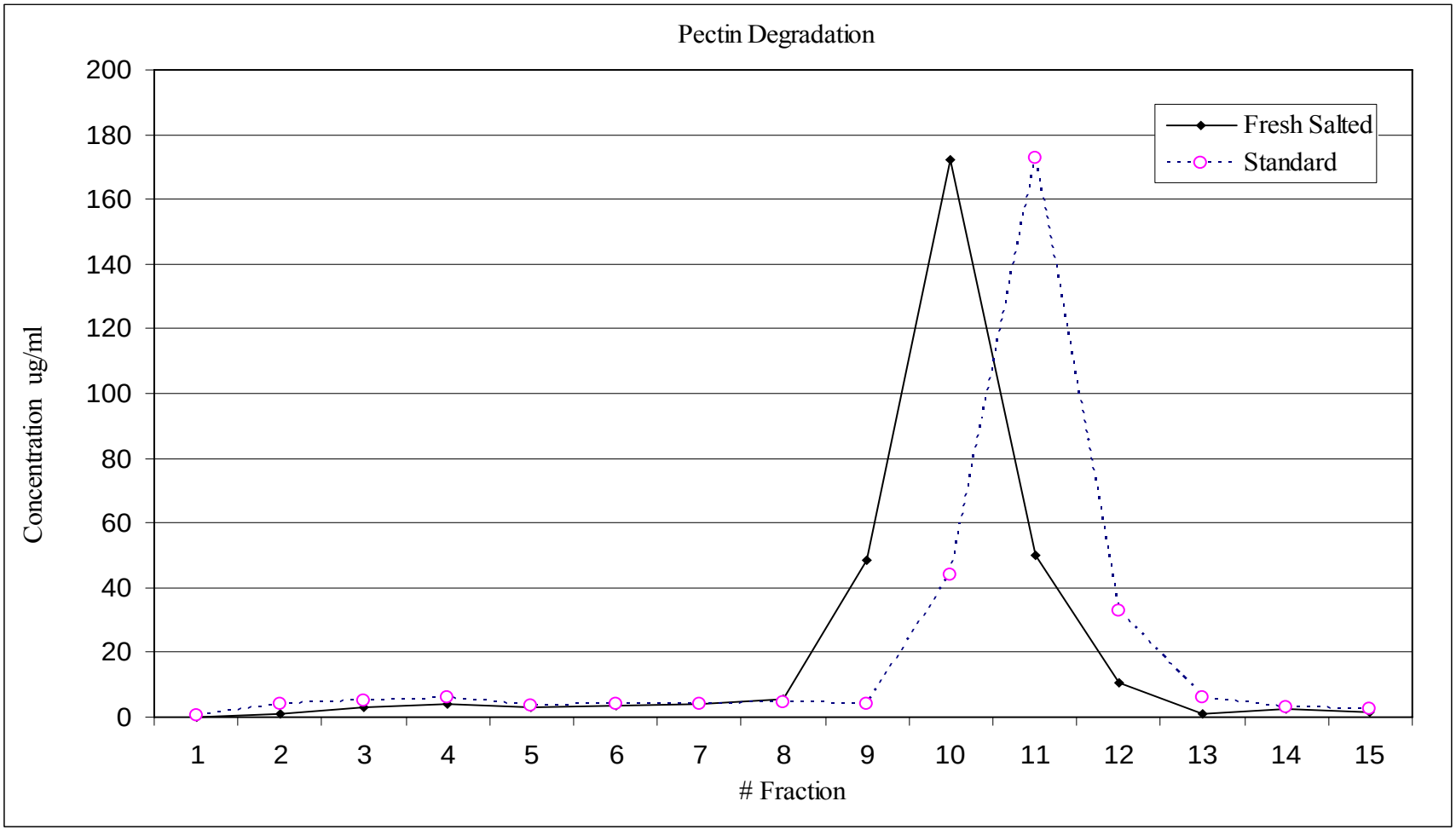


Figure 12a: Size-exclusion chromatography profile of CDTA-soluble polyuronide of standard and fresh salted pepper mash. Galacturonic acid (GA) was used as monomer standard. The elution rate was 0.5 ml/min and 4 ml fractions with a total of fifteen fractions were collected.

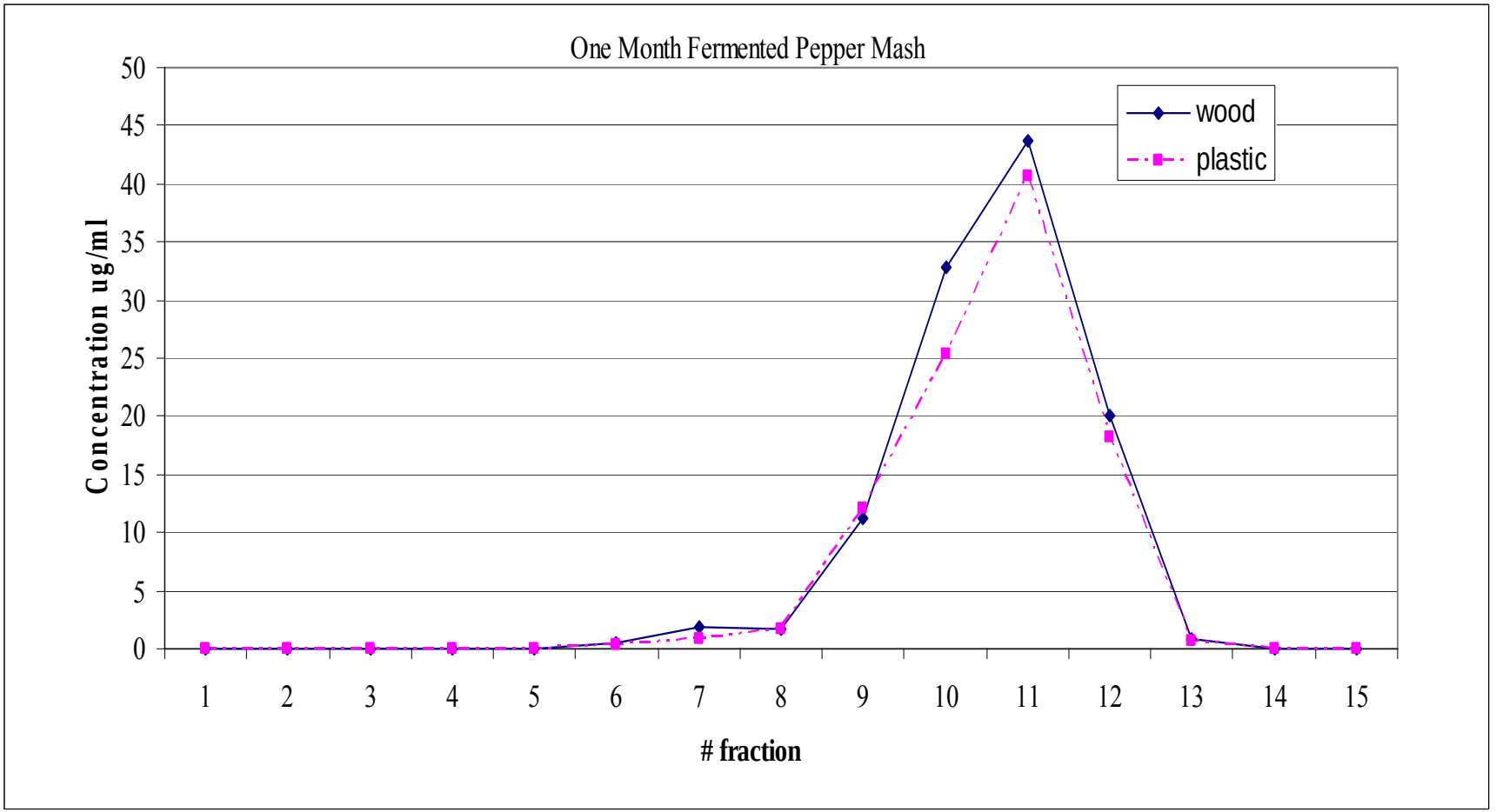


Figure 12b: Size-exclusion chromatography profile of CDTA-soluble polyuronide of one month fermented pepper mash in wood and plastic barrels. Galacturonic acid (GA) was used as monomer standard. The elution rate was 0.5 ml/min and 4 ml fractions with a total of fifteen fractions were collected.

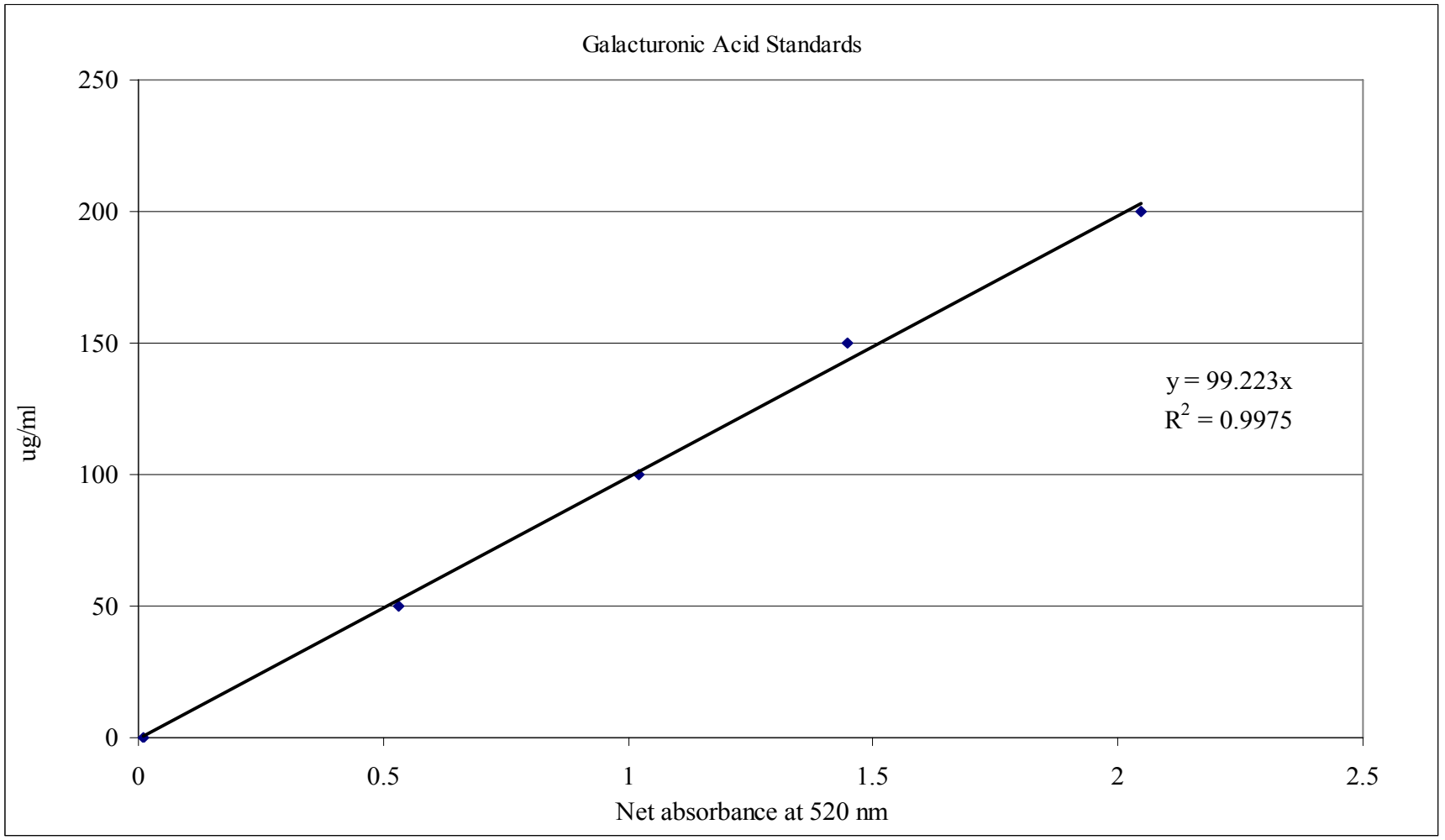


Figure 13: Standards were prepared using galacturonic acid at concentration of 15-200 ppm. Absorbance measured at 520 nm in a Perkin Elmer Lambda 35 UV/VIS Spectrophotometer. The line graph have formula of y = 99.223x and R^2=0.9975.

The mean value of dihydrocapsaicin decreased significantly after 1 month fermentation to 0.09 mg/g for plastic (range 0.08 to 0.12 mg/g) and 0.1 mg/g for wood (range 0.08 to 0.12 mg/g). Dihydrocapsacin concentration did not affect by barrel material or the fermentation process because statistically dihydrocapsaicin concentration is not significantly different.

In this study only capsaicin and dihydrocapsaicin were quantified. Generally capsaicin and dihydrocapsaicin concentration is stable throughout the fermentation process. Pepper mash fermented in wood or plastic did not affect concentration significantly ($p<0.05$). Capsaicin concentration was about twice those of dihydrocapsaicin. Wheat (1987) stated that approximately 95% of the pungency is contributed by these two compounds.

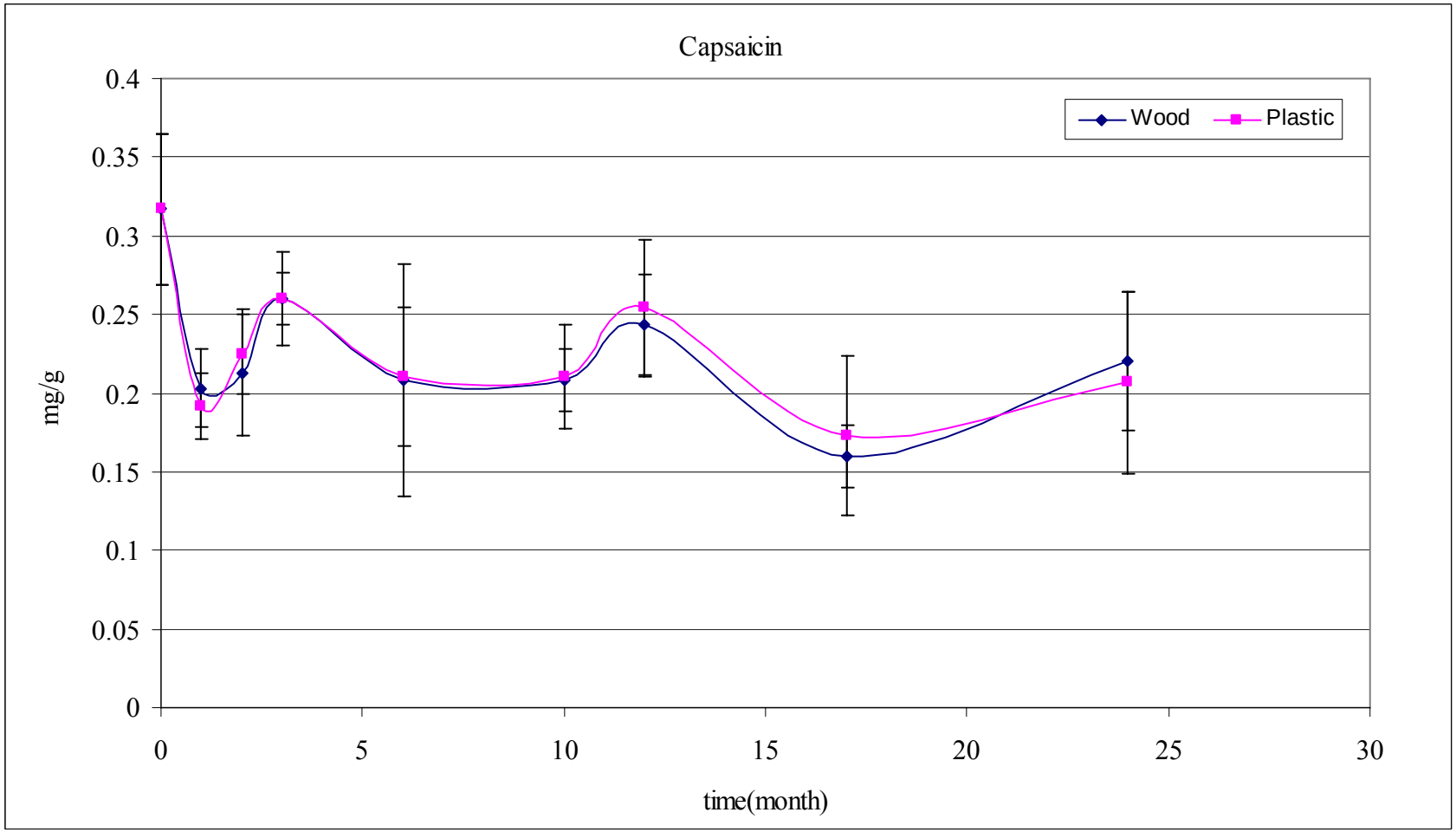


Figure 14a: The mean value of capsaicin (fresh salted, n=7; fermented mash, n=12) on the basis of freeze dried pepper mash quantified from HPLC chromatogram.

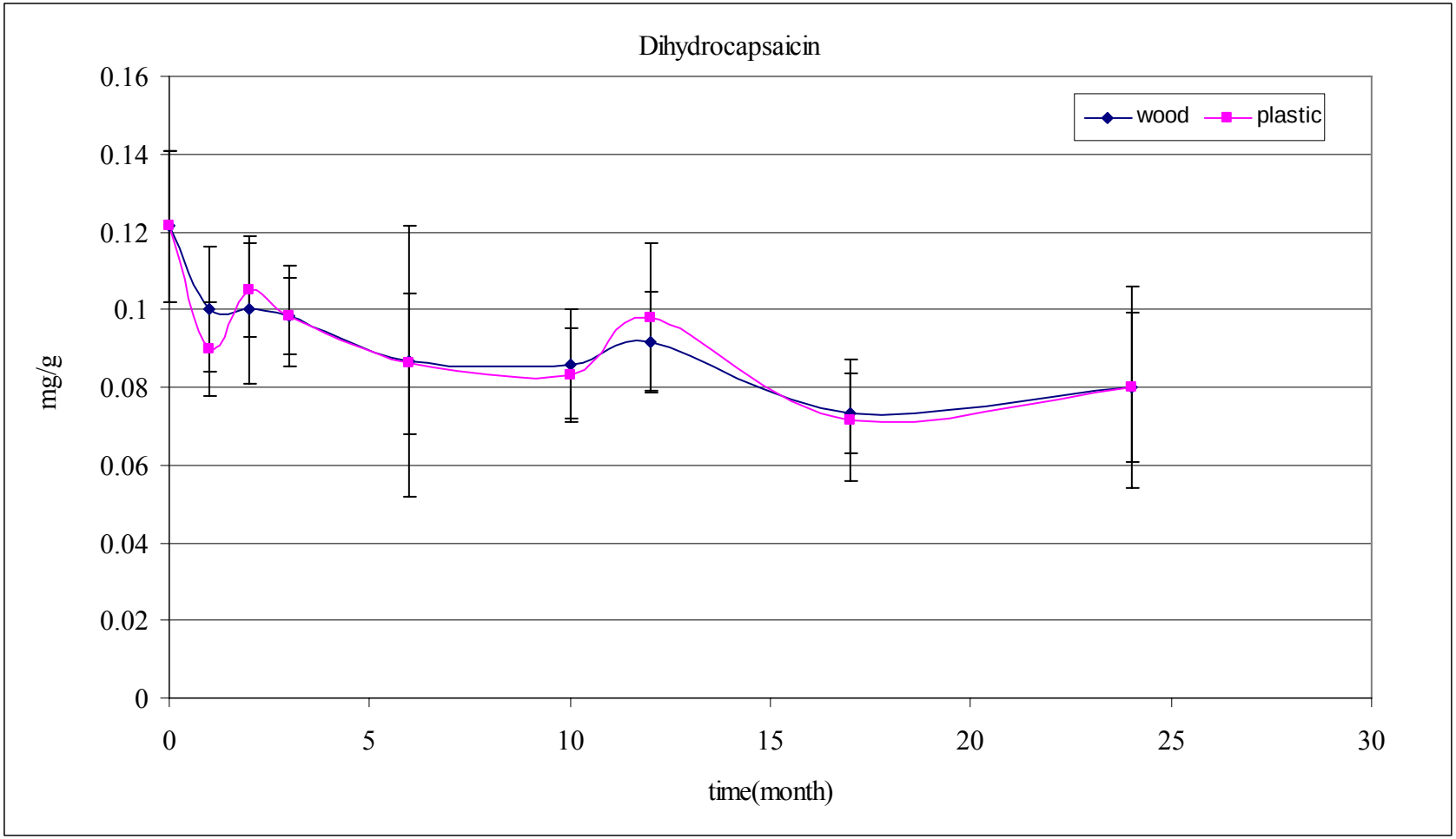


Figure 14b: The mean value of dihydrocapsaicin (fresh salted, n=7; fermented mash, n=12) on the basis of freeze dried pepper mash quantified from HPLC chromatogram.

CHAPTER 5
SUMMARY AND CONCLUSIONS

Louisiana has long been known for its famous hot sauce production state especially sauce made from Tabasco pepper. Mature fresh pepper fruit are usually fermented and aged before sauce production. Aging helps pepper to develop flavors, bouquet and unique odor. Pepper fermentation is also a kind of food preservation method to prevent undesirable changes in food and food products during storage.

In our study, we investigated the effect of fermentation barrels (wood and plastic) on the pepper fermentation quality. Many studies have been done on the effect of fermentation material on wine or beer quality. However, physiocochemical changes such as, dry weight, pH, titrable acidity, soluble carbohydrate, pectin and capsaicinoids during pepper fermentation process were unknown. Wooden containers have been a traditional fermentation tank in many fermented products such as beer, wine, sauerkraut, pickled and also pepper. Due to their relative permeability, the primary disadvantages of wooden containers are leakage and contamination. Also, wooden barrels are relatively expensive. In contrast, plastic barrels have longer usage time, lower cost, lower maintenance, less leakage, and are easier to sanitize. Upon this emphasis, this research study was attempted to substitute the traditional wooden barrel with polyethylene plastic barrel to overcome the cost and sanitation problems. The results of this study indicate that physiocochemical changes in pepper mash in 24 months fermentation is not significantly influences by barrel type.

Dry weight significantly decreased when the fermentation started for pepper mash fermented in both wood and plastic barrels. At the end, pepper mash fermented in wood barrel have a higher dry weight due to the natural porous of the oak wood. However, the average dry weight was not significantly different. Fresh ground peppers have an average pH of 5; after 24 months fermentation, final pH decreased to 4. Fresh pepper started out with 0.58% titrable acidity (expressed as lactic acid) increased to 1.5% after 24 month. Wood and plastic fermented pepper mash yielded the same results throughout the fermentation. A very low concentration of sucrose was present in the freshly ground red pepper pod. However, at the end of fermentation, sucrose, fructose, and glucose were apparently still available in pepper mash despite lactic acid fermentation. Total uronide content have an unexpected increased at 3 months to 10 months, however, the concentration remain stable from 10s month to 24 months. Chelator-soluble pectin was extensively degraded after 1 month fermentation. This indicated that the mature fresh red pepper sample was already extensively but not fully degraded to galacturonic acid residues. At this stage, pectin polymers were tightly bound so they were not released from fresh tissue into water, unless the cell wall structure was disrupted allowing some dissolution. However, pepper mash did not turn watery at the end of fermentation process. Other pectins such as oxalate-soluble pectin, acid-soluble pectin and insoluble pectin was not investigated in the research study. Fresh salted pepper mash has 0.32mg/g of capsaicin and 0.12 mg/g of dihydrocapsaicin. Although, capsaicinoids content decreased slightly when the fermentation started, capsaicin and dihydrocapsaicin remain stable throughout 24 months of fermentation process.

As a conclusion, all the physiocochemical properties of pepper mash fermented in wood and plastic barrels was not significantly different. Therefore, plastic barrels can be a substitution for wood barrels in pepper fermentation process and produces high qualities pepper mash for sauce production.

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APPENDIX
DATA OF PHYSICOCHEMICAL ANALYSIS

Appendix 1: Freshly ground pepper pod (*C. frustescen*)

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
Fresh1	3.83	4.98	0.58	0.29	0.12	0.07846	15.33251	19.65836	173.64
Fresh 2	3.71	4.97	0.57	0.3	0.11	0.069542	15.99528	20.51132	124.65
Fresh 3	3.79	4.99	0.58	0.32	0.12	0.056	16.17111	20.60884	86.14
Mean	**3.7767**	**4.9800**	**0.5755**	**0.303333**	**0.116667**	**0.068**	**15.83297**	**20.2595**	**128.1436**
SD	**0.0611**	**0.0100**	**0.0046**	**0.015275**	**0.005774**	**0.011309**	**0.442237**	**0.522889**	**43.8538**

Appendix 2: Freshly ground pepper pod (*C. frustescen*) with 8% of salt

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
Fresh+salt1	4.47	4.73	0.53	0.25	0.09	0.00	6.67	8.42	132.09
Fresh+salt2	4.63	4.67	0.57	0.28	0.11	0.00	6.06	8.34	122.07
Fresh+salt3	4.59	4.71	0.62	0.36	0.14	0.00	8.08	9.73	120.52
Fresh+salt4	4.58	4.80	0.54	0.36	0.14	0.00	8.51	11.17	124.17
Fresh+salt5	4.42	4.73	0.43	0.32	0.12	0.00	7.41	10.32	115.22
Fresh+salt6	4.65	4.68	0.53	0.37	0.14	0.00	5.72	7.77	83.25
Fresh+salt7	4.73	4.59	0.56	0.28	0.11	0.00	10.50	12.83	167.93
Mean	**4.5807**	**4.7014**	**0.5405**	**0.3171**	**0.1214**	**0.0000**	**7.5660**	**9.7972**	**123.6102**
SD	**0.1057**	**0.0649**	**0.0570**	**0.0479**	**0.0195**		**1.6459**	**1.8027**	**24.9940**

Appendix 3 : One month fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA1M	3.38	4.02	1.73	0.20	0.10	2.15	13.76	7.44	144.74
PB1M	3.80	3.94	1.32	0.24	0.12	2.11	14.04	5.90	136.93
PC1M	3.81	3.94	2.52	0.22	0.10	1.90	12.19	6.59	148.83
PD1M	3.77	4.01	1.25	0.20	0.10	0.00	6.81	1.30	137.39
PE1M	4.01	4.03	1.17	0.18	0.08	0.00	6.40	1.23	62.54
PF1M	3.19	3.07	1.98	0.14	0.06	2.13	11.38	2.42	114.39
PG1M	3.96	3.59	1.19	0.18	0.08	1.88	11.00	5.07	50.08
PH1M	4.32	4.41	0.93	0.18	0.08	1.58	8.41	1.79	103.41
PI1M	3.96	4.00	1.22	0.18	0.08	1.88	10.99	5.06	151.61
PJ1M	4.11	3.60	1.24	0.20	0.10	1.31	10.90	8.74	110.20
PK1M	4.09	4.28	0.97	0.20	0.08	8.89	8.91	3.75	120.94
PL1M	3.51	3.73	1.12	0.18	0.10	2.03	4.60	3.77	71.66
Mean	**3.8233**	**3.8850**	**1.3872**	**0.1917**	**0.0900**	**2.1552**	**9.9494**	**4.4204**	**112.7259**
SD	**0.3265**	**0.3519**	**0.4645**	**0.0248**	**0.0160**	**2.2555**	**2.9528**	**2.4725**	**34.8087**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 4 : One month fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA1M	3.86	3.19	1.80	0.18	0.08	1.65	12.83	8.07	15.31
WB1M	3.83	3.47	1.58	0.20	0.10	1.67	12.93	8.13	66.65
WC1M	3.91	3.17	2.10	0.24	0.12	13.86	9.97	1.32	44.41
WD1M	3.95	3.68	1.37	0.18	0.10	1.74	9.81	5.79	96.13
WE1M	3.65	3.84	1.69	0.18	0.08	3.01	9.86	3.39	70.77
WF1M	4.14	3.74	1.42	0.22	0.10	2.65	8.69	2.99	31.49
WG1M	3.93	3.87	1.32	0.24	0.12	11.32	10.06	2.77	124.06
WH1M	3.77	3.94	1.40	0.20	0.10	2.49	14.53	2.68	61.91
WI1M	4.15	3.39	1.71	0.20	0.10	1.43	9.49	9.89	92.64
WJ1M	4.44	4.60	1.04	0.20	0.10	2.95	12.00	12.23	114.58
WK1M	3.66	3.22	1.59	0.20	0.10	7.02	4.97	2.50	129.58
WL1M	3.56	4.13	1.23	0.20	0.10	3.48	8.46	4.65	156.90
Mean	**3.9038**	**3.6867**	**1.5201**	**0.2033**	**0.1000**	**4.5267**	**10.4684**	**5.4329**	**77.0479**
SD	**0.2480**	**0.4287**	**0.4157**	**0.0206**	**0.0121**	**3.3923**	**2.9847**	**2.9563**	**41.3184**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 5 : Two months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA2M	3.21	3.95	1.77	0.16	0.08	10.35	8.76	15.60	231.45
PB2M	3.40	4.05	1.22	0.26	0.12	11.08	7.33	2.30	153.41
PC2M	3.95	4.12	1.50	0.24	0.12	0.00	3.75	0.63	58.62
PD2M	3.60	4.05	1.23	0.26	0.12	8.39	7.21	3.09	181.94
PE2M	4.16	4.16	1.23	0.22	0.10	9.08	10.29	4.77	121.07
PF2M	4.05	4.08	1.20	0.18	0.08	7.21	11.90	4.27	168.94
PG2M	3.77	4.08	1.27	0.18	0.08	10.31	12.70	6.83	107.13
PH2M	3.80	4.19	1.02	0.20	0.10	8.32	13.71	4.39	131.20
PI2M	4.13	4.28	1.05	0.28	0.14	0.94	13.73	8.59	138.45
PJ2M	4.13	4.65	0.59	0.28	0.12	1.74	6.71	8.37	143.41
PK2M	4.17	4.70	0.83	0.22	0.10	1.18	15.08	13.16	149.03
PL2M	4.17	4.62	0.80	0.22	0.10	1.42	11.67	8.14	40.46
Mean	**3.8742**	**4.2591**	**1.1425**	**0.2250**	**0.1050**	**5.8351**	**10.2356**	**6.6776**	**135.4276**
SD	**0.3283**	**0.2696**	**0.3189**	**0.0401**	**0.0193**	**4.3585**	**3.4760**	**4.4076**	**51.3729**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 6: Two months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA2M	3.92	4.06	1.40	0.20	0.10	6.59	10.72	9.63	145.36
WB2M	3.70	3.99	1.82	0.18	0.08	3.34	14.30	3.86	179.61
WC2M	3.94	3.99	1.43	0.22	0.10	3.48	11.34	1.58	79.28
WD2M	3.75	3.98	1.50	0.20	0.10	8.50	20.29	2.74	107.19
WE2M	3.65	3.92	1.60	0.18	0.08	10.45	10.96	3.74	192.25
WF2M	3.95	4.08	1.39	0.24	0.12	6.42	11.20	6.51	187.56
WG2M	3.95	4.00	1.35	0.22	0.10	11.95	10.68	4.80	82.79
WH2M	3.77	4.07	1.37	0.20	0.10	8.47	20.21	2.73	158.00
WI2M	4.03	4.17	1.32	0.24	0.10	5.37	11.35	9.86	171.02
WJ2M	4.22	4.25	1.22	0.22	0.10	5.13	10.83	9.42	166.32
WK2M	3.99	4.26	1.49	0.20	0.10	5.77	14.36	7.50	65.08
WL2M	3.90	4.32	1.35	0.26	0.12	11.43	14.36	7.01	181.47
Mean	**3.8950**	**4.0908**	**1.4368**	**0.2133**	**0.1000**	**7.2422**	**13.3822**	**5.7823**	**142.9946**
SD	**0.1591**	**0.1294**	**0.1555**	**0.0246**	**0.0121**	**2.9171**	**3.5269**	**2.9335**	**46.5433**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 7: Three months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA3M	3.52	3.87	1.55	0.30	0.12	0.00	8.26	7.33	330.03
PB3M	3.00	3.91	1.49	0.24	0.10	0.00	12.35	6.91	225.36
PC3M	3.90	4.61	0.94	0.26	0.10	1.04	17.14	18.63	216.95
PD3M	3.76	4.36	1.00	0.26	0.10	0.68	11.88	12.65	134.87
PE3M	3.92	4.23	0.96	0.26	0.10	0.00	15.43	14.44	576.70
PF3M	3.96	3.98	1.05	0.26	0.10	0.00	10.17	2.63	153.07
PG3M	3.73	4.49	0.73	0.24	0.08	0.04	9.31	16.39	206.25
PH3M	3.86	4.60	0.77	0.26	0.10	0.00	6.53	2.22	245.85
PI3M	3.74	4.47	0.87	0.28	0.10	0.00	9.21	13.77	251.03
PJ3M	3.69	4.07	1.14	0.26	0.10	0.20	16.59	18.76	238.88
PK3M	3.66	4.63	0.59	0.24	0.08	0.78	16.27	19.37	228.18
PL3M	3.85	4.10	0.96	0.26	0.10	0.23	18.99	11.85	190.93
Mean	**3.7146**	**4.2767**	**1.0044**	**0.2600**	**0.0983**	**0.2473**	**12.6773**	**12.0795**	**249.8404**
SD	**0.2573**	**0.2851**	**0.2819**	**0.0171**	**0.0103**	**0.3708**	**4.0795**	**6.0592**	**114.2218**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 8: Three months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA3M	2.96	3.92	1.61	0.28	0.12	0.00	9.75	7.03	233.04
WB3M	3.63	3.95	1.96	0.22	0.08	2.96	16.05	1.87	163.01
WC3M	3.63	3.98	1.28	0.22	0.08	0.10	15.44	12.53	294.02
WD3M	3.61	3.92	1.57	0.26	0.10	0.41	16.15	15.60	297.34
WE3M	3.62	3.90	1.65	0.28	0.10	0.00	8.08	7.48	262.24
WF3M	3.46	3.95	1.77	0.26	0.10	0.00	16.50	3.07	224.58
WG3M	3.79	4.03	1.50	0.30	0.12	0.00	9.17	10.74	164.62
WH3M	3.25	4.02	1.73	0.24	0.10	1.20	18.66	7.99	281.53
WI3M	3.78	4.54	1.15	0.26	0.10	0.36	15.67	20.13	296.97
WJ3M	3.46	4.37	0.95	0.22	0.08	0.00	20.87	16.66	165.51
WK3M	3.83	4.28	1.10	0.28	0.10	0.07	16.50	16.83	228.83
WL3M	3.72	4.30	1.67	0.30	0.10	0.00	17.29	6.92	211.54
Mean	**3.5592**	**4.0967**	**1.4953**	**0.2600**	**0.0983**	**0.4248**	**15.0110**	**10.5706**	**235.2693**
SD	**0.2508**	**0.2162**	**0.3063**	**0.0295**	**0.0134**	**0.8711**	**3.9336**	**5.8049**	**52.0306**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 9: Six months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA6M	3.38	3.96	1.44	0.20	0.08	2.08	15.72	7.64	472.10
PB6M	3.38	3.94	1.35	0.34	0.14	2.20	12.99	13.57	349.31
PC6M	3.29	4.00	1.31	0.20	0.08	3.52	18.76	7.24	392.02
PD6M	3.30	4.11	1.22	0.20	0.08	1.91	15.55	15.14	417.83
PE6M	3.26	4.12	1.05	0.20	0.08	2.30	17.84	15.79	370.27
PF6M	3.38	4.15	1.26	0.20	0.08	3.02	16.07	13.86	366.73
PG6M	3.31	4.06	1.17	0.16	0.06	1.86	18.99	19.96	92.34
PH6M	3.58	4.36	1.15	0.40	0.18	2.26	18.29	15.58	273.62
PI6M	3.33	3.83	1.35	0.18	0.08	8.89	14.97	6.51	407.46
PJ6M	3.26	3.91	1.53	0.16	0.06	2.47	20.05	17.09	181.80
PK6M	3.37	3.99	1.07	0.16	0.06	1.94	15.27	12.06	298.44
PL6M	3.36	3.93	0.97	0.18	0.08	0.30	19.53	20.27	339.59
Mean	**3.3519**	**4.0554**	**1.2244**	**0.2108**	**0.0862**	**2.6613**	**16.8510**	**13.7962**	**333.2116**
SD	**0.0827**	**0.1631**	**0.1685**	**0.0738**	**0.0350**	**2.0109**	**2.1703**	**4.4476**	**102.1808**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 10: Six months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA6M	3.58	4.03	1.30	0.26	0.10	1.44	14.10	14.29	434.30
WB6M	3.93	4.42	1.22	0.28	0.12	2.19	15.48	13.74	394.86
WC6M	3.36	3.98	1.35	0.18	0.08	2.20	12.68	14.76	280.37
WD6M	4.06	4.07	1.23	0.24	0.10	1.23	11.99	10.96	227.54
WE6M	3.78	4.12	1.14	0.16	0.06	2.28	16.09	14.28	391.78
WF6M	3.58	3.99	1.24	0.22	0.08	1.96	17.50	15.08	441.73
WG6M	3.38	4.18	1.22	0.24	0.10	2.78	17.66	12.88	293.80
WH6M	3.56	4.04	1.12	0.18	0.08	1.75	13.72	14.33	289.24
WI6M	3.81	4.05	1.08	0.18	0.08	1.64	13.08	14.06	134.88
WJ6M	3.11	3.85	1.84	0.18	0.08	19.69	13.59	8.50	444.68
WK6M	3.35	4.36	1.35	0.24	0.10	2.33	18.57	21.06	402.64
WL6M	3.69	4.23	1.17	0.14	0.06	1.52	14.32	14.93	326.77
Mean	**3.3260**	**3.8064**	**1.2709**	**0.2083**	**0.0867**	**3.4184**	**14.8978**	**14.0727**	**338.5496**
SD	**0.2717**	**0.1633**	**0.1980**	**0.0439**	**0.0178**	**5.1444**	**2.1419**	**2.9143**	**96.7445**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 11: Ten months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA10M	3.48	3.96	1.25	0.24	0.08	0.54	15.04	10.49	230.56
PB10M	3.60	4.13	1.34	0.20	0.08	0.85	12.90	3.46	139.27
PC10M	3.15	4.00	1.25	0.24	0.10	0.45	16.77	6.53	348.11
PD10M	3.46	4.04	1.63	0.20	0.10	1.73	18.17	8.70	193.26
PE10M	3.57	3.95	1.86	0.20	0.08	1.29	14.22	2.41	239.62
PF10M	3.51	4.26	1.13	0.20	0.10	1.34	9.60	1.75	23.06
PG10M	4.08	4.07	1.50	0.20	0.08	1.25	16.80	3.27	60.90
PH10M	3.55	3.92	1.58	0.18	0.10	1.11	11.05	2.58	228.22
PI10M	2.74	3.96	1.17	0.20	0.08	0.00	5.94	3.39	242.75
PJ10M	3.66	4.18	1.41	0.22	0.06	0.26	17.37	2.86	228.46
PK10M	3.65	4.15	1.32	0.20	0.06	0.28	17.23	6.23	176.40
PL10M	3.28	4.02	1.31	0.24	0.08	1.39	18.51	7.03	167.63
Mean	**3.4746**	**4.0533**	**1.3947**	**0.2100**	**0.0833**	**0.8740**	**14.4687**	**4.8920**	**189.8530**
SD	**0.3230**	**0.1063**	**0.2131**	**0.0200**	**0.0144**	**0.5538**	**3.9067**	**2.8188**	**86.6731**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 12: Ten months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA10M	3.72	4.04	1.91	0.24	0.10	0.00	12.70	12.87	190.55
WB10M	3.26	4.10	1.38	0.16	0.08	0.54	24.87	7.90	245.55
WC10M	3.73	3.95	1.96	0.24	0.10	0.33	9.14	8.54	204.54
WD10M	4.22	4.06	1.40	0.24	0.10	0.00	15.62	12.16	266.12
WE10M	3.17	3.93	1.64	0.26	0.10	2.62	13.04	9.93	239.82
WF10M	3.66	4.08	1.56	0.24	0.10	0.08	16.47	12.62	231.00
WG10M	3.70	4.04	1.69	0.20	0.08	0.52	18.69	6.88	228.66
WH10M	3.26	3.96	1.44	0.18	0.08	0.68	16.13	14.88	141.04
WI10M	3.87	4.00	1.64	0.20	0.08	0.74	15.29	9.83	20.20
WJ10M	2.93	3.85	1.55	0.16	0.06	0.32	12.44	6.26	186.56
WK10M	3.96	4.28	1.25	0.20	0.08	0.00	15.08	20.97	251.53
WL10M	3.50	3.99	1.64	0.20	0.08	0.96	18.62	4.92	312.15
Mean	**3.6023**	**4.0177**	**1.5875**	**0.2077**	**0.0862**	**0.5578**	**16.1609**	**10.3706**	**212.2761**
SD	**0.3614**	**0.1047**	**0.2003**	**0.0332**	**0.0124**	**0.7004**	**4.2288**	**4.4747**	**74.1145**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 13: Twelve months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA12M	2.76	3.95	1.40	0.26	0.10	15.49	20.09	1.99	154.39
PB12M	3.22	4.10	1.34	0.28	0.10	11.35	21.10	4.24	152.85
PC12M	3.23	4.11	1.72	0.24	0.08	0.96	15.93	0.49	145.86
PD12M	3.46	3.95	1.54	0.26	0.10	2.70	15.48	4.16	166.14
PE12M	3.66	4.12	1.07	0.24	0.08	7.50	12.58	3.76	278.73
PF12M	3.42	4.21	1.04	0.22	0.08	7.25	15.11	4.47	301.46
PG12M	3.48	4.09	1.03	0.26	0.10	12.95	12.13	1.82	194.44
PH12M	3.52	4.07	1.69	0.24	0.10	8.55	13.58	0.85	99.58
PI12M	3.97	4.49	1.04	0.28	0.10	2.43	10.72	2.62	121.29
PJ12M	3.86	4.33	1.22	0.22	0.10	6.57	12.16	4.93	112.54
PK12M	3.67	4.48	1.07	0.26	0.10	0.65	12.31	2.33	204.62
PL12M	3.70	4.13	1.50	0.16	0.06	0.00	8.19	1.78	182.14
Mean	**3.4942**	**4.1692**	**1.3039**	**0.2433**	**0.0917**	**6.3673**	**14.1144**	**2.7854**	**176.1706**
SD	**0.3239**	**0.1788**	**0.2635**	**0.0328**	**0.0134**	**5.1316**	**3.7059**	**1.4843**	**62.0559**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 14: Twelve months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA12M	3.58	4.03	1.71	0.22	0.08	2.23	16.81	14.23	200.52
WB12M	3.15	3.98	1.74	0.26	0.10	7.38	19.21	7.08	151.92
WC12M	3.31	4.03	1.85	0.24	0.10	7.84	20.11	3.02	199.74
WD12M	3.58	4.08	2.19	0.30	0.12	8.18	11.53	0.42	148.83
WE12M	3.33	3.97	2.11	0.22	0.08	6.97	18.14	6.69	192.97
WF12M	3.51	3.99	1.95	0.24	0.10	18.67	15.32	2.73	264.22
WG12M	3.93	4.04	1.77	0.36	0.14	13.43	8.16	2.92	142.39
WH12M	3.74		0.00	0.20	0.08	10.83	14.37	2.56	246.77
WI12M	4.21	4.04	1.99	0.30	0.12	12.55	7.63	2.73	151.61
WJ12M	4.21	4.37	1.12	0.26	0.10	0.00	14.12	10.20	191.41
WK12M	3.74	4.40	1.17	0.22	0.08	6.87	20.95	17.39	306.96
WL12M	3.55	4.20	1.26	0.26	0.10	13.49	10.54	6.50	155.65
Mean	**3.6485**	**4.1092**	**1.5535**	**0.2554**	**0.0985**	**9.1885**	**15.0127**	**6.4245**	**195.0211**
SD	**0.3196**	**0.1474**	**0.5862**	**0.0433**	**0.0191**	**4.9407**	**4.4491**	**4.9858**	**50.3530**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 15: Seventeen months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA17M	3.19	3.82	2.01	0.18	0.08	4.05	9.79	2.11	117.76
PB17M	3.23	4.10	0.33	0.18	0.08	4.90	20.57	4.57	138.01
PC17M	3.00	4.14	1.36	0.16	0.06	3.92	9.03	3.24	153.83
PD17M	3.57	3.92	1.58	0.20	0.08	0.77	10.00	1.96	154.02
PE17M	3.31	3.86	1.82	0.20	0.08	0.95	16.67	2.42	188.05
PF17M	3.67	4.18	1.32	0.16	0.06	2.21	15.00	12.36	194.43
PG17M	3.63	3.99	1.86	0.16	0.06	3.94	16.32	6.00	155.50
PH17M	3.11	3.98	1.69	0.14	0.06	4.15	15.41	3.35	117.71
PI17M	3.22	4.07	1.26	0.18	0.08	2.90	5.11	3.46	214.49
PJ17M	3.89	4.09	1.36	0.20	0.08	0.71	11.74	3.60	204.92
PK17M	3.66	4.02	1.42	0.16	0.06	0.77	17.36	1.54	119.54
PL17M	3.46	4.13	1.31	0.16	0.08	0.00	18.79	2.87	203.31
Mean	**3.4100**	**4.0250**	**1.4435**	**0.1733**	**0.0717**	**2.4381**	**13.8146**	**3.9553**	**163.4631**
SD	**0.2734**	**0.1143**	**0.4300**	**0.0197**	**0.0103**	**1.7312**	**4.6229**	**2.9108**	**36.2801**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 16: Seventeen months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA17M	3.55	4.07	1.42	0.14	0.06	2.72	10.53	9.63	144.24
WB17M	3.74	4.01	1.32	0.02	0.08	2.57	6.57	2.07	181.30
WC17M	4.02	3.91	1.90	0.22	0.10	2.29	8.79	8.34	165.44
WD17M	4.47	4.08	1.67	0.18	0.08	2.15	5.50	4.70	195.18
WE17M	3.50	3.95	1.82	0.16	0.06	2.87	10.88	8.77	178.74
WF17M	3.86	3.99	1.85	0.16	0.06	4.03	16.40	5.44	136.64
WG17M	3.73	4.21	1.70	0.18	0.08	2.43	15.23	13.05	207.03
WH17M	3.62	4.06	1.59	0.16	0.06	4.31	17.51	5.81	182.31
WI17M	3.97	4.05	1.77	0.18	0.08	2.98	13.15	7.84	233.80
WJ17M	3.55	3.85	2.06	0.22	0.10	2.90	10.38	4.07	239.50
WK17M	3.81	4.27	1.06	0.14	0.06	2.79	9.29	2.03	217.33
WL17M	3.45	3.99	1.52	0.16	0.06	5.09	15.47	3.76	194.63
Mean	**3.7700**	**4.0367**	**1.6410**	**0.1600**	**0.0733**	**3.0930**	**11.6421**	**6.2923**	**189.6790**
SD	**0.2870**	**0.1174**	**0.2765**	**0.0512**	**0.0156**	**0.9008**	**3.9004**	**3.3072**	**31.9923**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 17: twenty four months fermented pepper mash in plastic barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
PA24M	3.57	3.87	1.95	0.22	0.08	6.55	17.80	16.75	144.15
PB24M	3.30	4.30	1.30	0.24	0.10	7.98	9.54	0.64	159.50
PC24M	3.37	3.99	1.52	0.28	0.10	7.50	5.71	1.60	181.58
PD24M	3.41	4.00	2.09	0.28	0.12	6.14	17.44	2.43	117.58
PE24M	2.88	4.04	1.95	0.16	0.06	9.04	20.22	2.70	145.60
PF24M	3.49	4.21	1.36	0.18	0.08	5.33	8.90	2.73	161.30
PG24M	3.38	3.97	1.65	0.16	0.06	6.26	20.91	2.71	208.99
PH24M	3.32	3.97	1.85	0.16	0.06	9.41	13.22	6.63	155.10
PI24M	3.43	4.11	0.51	0.18	0.06	5.43	9.07	2.78	121.46
PJ24M	3.32	4.02	1.62	0.22	0.08	9.54	4.36	1.84	176.40
PK24M	4.14	4.16	1.43	0.22	0.08	0.11	10.73	2.29	167.92
PL24M	3.72	4.09	1.72	0.18	0.08	4.51	17.49	2.31	243.34
Mean	**3.4429**	**4.0608**	**1.5802**	**0.2067**	**0.0800**	**6.4846**	**12.9487**	**3.7834**	**165.2441**
SD	**0.2938**	**0.1192**	**0.4188**	**0.0438**	**0.0191**	**2.6097**	**5.6797**	**4.3214**	**35.1399**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

Appendix 18: twenty four months fermented pepper mash in wood barrel

	Mean DW	pH	TA	Capsaicin (mg)/g	Dihydrocapsaicin (mg)/g	Sucrose (mg/g)	Fructose (mg/g)	Glucose (mg/g)	Pectin (mg/g)
WA24M	4.08	4.09	1.86	0.20	0.08	8.62	14.24	13.40	221.30
WB24M	4.00	3.84	2.05	0.18	0.08	10.09	9.80	8.55	149.17
WC24M	4.25	4.19	1.68	0.18	0.08	0.00	11.60	3.78	170.59
WD24M	3.92	4.06	1.74	0.24	0.10	10.92	14.74	11.50	182.39
WE24M	3.58	4.37	1.36	0.28	0.12	11.89	11.11	7.39	186.04
WF24M	3.89	3.98	1.68	0.18	0.08	11.33	10.51	9.58	168.46
WG24M	3.78	4.20	1.68	0.18	0.06	11.26	10.52	7.00	185.02
WH24M	3.93	3.93	1.67	0.16	0.06	9.80	15.47	8.78	330.06
WI24M	3.34	3.93	2.05	0.18	0.08	9.25	19.50	5.96	170.00
WJ24M	3.65	4.18	1.27	0.12	0.04	11.41	5.57	1.86	165.33
WK24M	3.71	3.99	1.95	0.16	0.06	9.49	17.57	5.52	160.69
WL24M	3.82	4.06	1.60	0.22	0.08	9.47	12.78	7.59	134.52
Mean	**3.8268**	**4.0681**	**1.6173**	**0.2200**	**0.0800**	**9.4608**	**12.7847**	**7.5745**	**185.2980**
SD	**0.2533**	**0.1479**	**0.4479**	**0.0578**	**0.0264**	**3.7220**	**4.2955**	**3.2983**	**50.3479**

TA = Titratable Acidity (expressed as lactic acid); SD = Standard Deviation; DW = Dry Weight
Sucrose, fructose, and glucose expressed on dry weight basis
Pectin expressed on per gram of air-dried tissues
Capsaicin and dihydrocapsaicin expressed on per gram of freeze dried tissue

VITA

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