THE EFFECT OF SUPERCRITICAL FLUID EXTRACTION ON THE SHELF LIFE AND STRUCTURE OF PECANS

By

BHAGYALAKSHMI CHINTA

Bachelor of Science
Sri Sathya Sai Institute of Higher Learning
Anantapur, India
1983

Master of Science
Sri Venkateswara University
Tirupati, India
1988

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Oklahoma State University
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Thesis Approved:

Sue Knight

Thesis Adviser

Barbara J. Stoecker

Niaz Nasrin

P. Larry Claypool

Timothy J. Pettibone

Dean of the Graduate College

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INTRODUCTION

Background

Pecans (*Carya illnoinensis*) have been a part of the human diet for centuries. Pecans were an important food source for American Indians and are still a leading horticulture crop. The strong position of pecan production in the United States is explained by the American origin of the tree, early development of improved varieties, and the large area of pecan trees in commercial production.

The production of pecans has increased steadily since 1925 (Woodroof and Heaton, 1961). Today's consumer purchases nuts for special uses in cooking and in snacks (Taylor, 2001). The purchase of pecans is influenced by the supply of nuts, market price, and consumer preference. Pecans constitute an important food industry. Although pecan consumption has increased with improved storage conditions and packaging (Heaton et al., 1977), the industry still encounters problems in quality maintenance during retail marketing because of oxidation of oils (Kays, 1987) and development of rancid flavors. Further, consumers often avoid high fat foods such as nuts (Hollingsworth, 1997) as dietary recommendations call for a reduction of total calories from fat.

New markets, and new marketing strategies, are needed to sustain continued pecan market expansion and growth of the industry. Principle deterrents are related to: 1) inconsistent supply caused by "alternate bearing", that is orchard overproduction in one

season followed by one or two seasons of underproduction leading to an erratic year-to-year pricing structure; 2) problems in quality maintenance, particularly at the retail level, with kernel darkening and off-flavor development; and 3) the high fat content, which is seen as negative by a weight conscious public. Orchard overbearing can be minimized with mechanical fruit thinning of trees during an overproduction year (Smith et al., 1993).

Pecan quality and changes in quality after harvest are dictated by the chemical composition of the kernels. Pecan kernels are especially susceptible to oxidative rancidity because of their high oil content; the kernels are about 70% oil. In fact, most of the oil is made up of unsaturated fatty acids leading to flavor instability because the higher the number of double bonds, the greater the potential for reaction with oxygen and the formation of rancidity (Kays, 1987). Rancidity is the major cause of flavor deterioration in pecans and is of major interest and concern to the industry. Researchers have attempted to improve the post harvest quality of pecans and to make recommendations as to processing, handling, and storage in order to maintain optimum quality and retard rancidity. Partial oil reduction is an alternative that would not only reduce fat calories but would also reduce the number of potential sites for oxidation, thereby offering both reduction in calories and extended shelf life.

Traditionally, nut oils are obtained by pressing (which destroys the kernel) or by use of solvents such as hexane (Waters and Knight, 1985). Hexane is explosive, a potential environmental contaminant, and difficult to remove from intact kernels without causing damage. These problems can be solved by the use of a supercritical fluid extraction process. When a gas, such as carbon dioxide, is subjected to certain pressure and temperature conditions, it reaches a supercritical point where it exhibits many characteristics superior to that of a liquid solvent (Agriculture Energy Center, 1982).

A supercritical carbon dioxide extraction process has been developed at Oklahoma State University (Zhang, 1994). In the traditional solvent process the solvent must be a liquid, because liquids have a higher solvent power as compared to gas. However, supercritical fluids have the solvent power of liquids but with better mass transfer characteristics (e.g. lower viscosity and higher diffusion coefficient) than typical liquids or gases (Goodrum and Kilgo, 1987). Carbon dioxide (CO_2) offers unique advantages; it is abundant, non-reactive, non-toxic and environmentally harmless. A nondestructive oil extraction process utilizing CO_2 (Zhang et al., 1995) could alleviate two of the market expansion deterrents, rancid flavors and high fat calories, and open totally new marketing opportunities for the pecan industry.

In order to test the consumer acceptance of the supercritical extracted pecans, sensory evaluations must be conducted. Erickson and coworkers (1994) consider sensory evaluation the best method to evaluate the quality of pecans. Further it is important to study the microstructure as it helps to understand the internal structure of the nut when subjected to adverse conditions such as supercritical critical extraction procedures. Young and Schadel (1990b) studied the effects of oven roasting on peanut microstructure, the cell and its implications to the end product, peanut butter, using Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM), and light microscopy; but little has been done on pecans. Electron microscopy studies with pecans could aid in understanding the various changes in the cellular microstructure during extraction.

Statement of the Problem

Rancidity of pecans is a big problem. The biggest challenge to the investigator is to reduce the rancidity in pecans, and thus increase the marketability. It is necessary to study the key contributors to rancidity in the pecans to help in reducing their rancidity.

The specific problem in this study was to determine the sensory and structural characteristics of unextracted and extracted pecans. The dissertation format is based on the Journal of Food Science. Each experiment is written in the format of a journal paper.

Purpose and Objectives

The purpose of this study was to determine the quality of both extracted and non-extracted pecans. The specific objectives of the study were:

a) To determine the sensory parameters in order to evaluate pecans.

b) To determine the effect of retail storage conditions on the sensory quality of pecans during long-term storage.

c) To determine the effect of different packaging conditions on the sensory quality of pecans.

d) To determine the cellular and the surface structure of pecans using transmission electron microscopy and scanning electron microscopy.

Statement of Hypothesis

The following hypotheses were postulated for this research: There will be no significant differences in the sensory and structural qualities of extracted and non extracted pecans. Also there will be no difference in the pecans (both extracted and non extracted) that have been stored at retail conditions and different packaging conditions i.e., different oxygen levels.

Assumptions

The following assumptions were made in this study.

a) All the panelists will evaluate the pecans on the day they are received.

b) Panelists will not evaluate the pecans close to meal times.

c) All the panelists will perceive the characteristics similarly.

d) All the panelists will seriously and honestly make and record their perceptions.

Limitations

At the onset of the study the investigator was cognizant of the following limitations:

a) The amount of sample available for evaluation would be very small dictating a small number of panelists.

b) There could be attrition of panel members due to the long term of the study.

c) The pecan tissues are very dense and extra effort must be made in fixing proteins and fat while preparing for SEM and TEM.

Definitions

Chalky: Chalky was used as one of the descriptors, to describe the internal texture of tested pecans.

Linoleic acid: A polyunsaturated fatty acid, $C_{17}H_{31}COOH$, containing 18 carbons and two double bonds (18:2). Linoleic acid is an omega - 6 fatty acid and an essential fatty acid.

Linolenic acid: A polyunsaturated fatty acid, $C_{17}H_{29}COOH$, containing 18 carbons and three double bonds (18:3). Linolenic acid is an omega - 3 fatty acid. Linseed oil is a good source of linolenic acid.

Monounsaturated fatty acid: A fatty acid chain which contains only one double bond between the carbon molecules in the chain.

Oleic acid: The most common monounsaturated fatty acid $C_{17}H_{33}COOH$, containing 18 carbons and one double bond (18:1). Olive and nut oils are good sources.

Sensory evaluation: Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, taste, touch and hearing.

Scanning Electron Microscopy (SEM): The image looks three dimensional, which is caused by a small scanning electron beam deflected by a thin gold-paladium surface coating and causing secondary e^- to be emitted from the sample. The secondary e^- are collected and processed and the image is viewed on a cathode ray tube (CRT).

Supercritical fluid extraction: This is a process which uses liquefied gases as solvents for extracting certain components of food such as caffeine, fats, flavor extracts etc. Supercritical fluid is a gas existing above its critical temperature and critical pressure. Under a given set of conditions, a supercritical fluid may possess the density of a liquid while maintaining the diffusibility of a gas.

Tannins: Polyphenolic structures having molecular weight of > 500. Tannins contribute to the astringency of foods and also to enzymic browning reactions, although their mechanism is not well understood.

Transmission Electron Microscopy (TEM): This is a two dimensional image, which is caused by a stationary electron beam when transmitted through a specimen. The image is created of transmitted e^- (electrons) and is viewed on a florescent screen.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Pecans are the most popular of all the nuts and one of the most important horticulture crops in the state of Oklahoma. Confectioners are major nut users, in ice cream, snacks, desserts, candy and chocolate manufacturing (Taylor, 2001) Pecans add crunchiness to an otherwise flat 'mouthfeel' (Florkowski and Hubbard, 1992). The volume of pecans consumed is influenced by the market supply, market price and the quality of the nuts available (Dorn, 1996)

Composition of Pecans

The major constituent of pecans is oil accounting for 55 to 70% of total kernel weight (Kays, 1987). Pecan oil contains about 65% percent monounsaturated and 26% polyunsaturated fatty acids and is, therefore, an excellent source of unsaturated fatty acids (Maness et al., 1995). This is comparable to the unsaturation levels of walnut and sunflower oil (Greve et al., 1992). Nutritionists prefer unsaturated oil over saturated oil because of benefits to cardiovascular health (Toro-Vazquez et al., 1999). It is thought that the unsaturated fatty acids (mostly mono) lower the LDL cholesterol levels without changing the HDL levels (Wagner, 1980; Morgan and Clayshulte, 2000; Rajaram et al., 2001). The high level of monounsaturated fatty acids (oleic acid) in pecan oil can make it

similar to olive oil, a major component of the Mediterranean diet (Wise, 1994; Toro-Vazquez and Perez-Bricena et al., 1998). Unfortunately the high lipid content and degree of unsaturation of the pecans makes storage difficult. Pecans develop odors and off-flavors which detract from flavor quality (Senter et al., 1984). Pecans readily absorb lipophilic gases from the surrounding atmosphere. However the most troublesome odor and flavor defects are due to oxidative rancidity which contributes to off-flavor by forming oxidative by-products (Kay, 1987). Variations in lipid distribution and composition can result from variety, cultural conditions, maturity and past productivity of the pecan. High oleic (single double bond) and linoleic (two double bonds) acid concentrations have been found in a large number of cultivars (Woodroof and Heaton, 1961; Senter and Horvat, 1976). Cultivar differences lead to oleic acid ranging from 51 to 77% of the fatty acids present, while linoleic acid was found to range from 14 to 37% (Demir and Cetin, 1999).

Carbohydrates make up a small percentage of pecan kernels. Total carbohydrates are present in the range of 13 to 15% of the edible portion of the nut. Cell wall carbohydrates - cellulose (1.76 %) and hemicellulose (4.09%) represent a dominant group but also present are several free sugars: sucrose (1.18 %), inverted sugars (2.88 %), araban (1.95%), and methylpentosans (0.22%). Starch was absent, however small amounts of amyloid (0.59%) and tannins (0.33%) were present. Protein content varied among cultivars ranging from 7.2 to 16.9%. Lysine was the most limiting amino acid (Kays, 1991).

Volatile compounds represent a critical portion of the overall flavor of the pecan. Many of the volatile compounds emanating from the raw pecan kernel have been identified. These include a series of alcohols and aldehydes and one lactone (α -

caprolactone) (Rudolph, 1971). Pyrazines make up the backbone of the characteristics of roasted pecan aroma (Wang and Odell, 1972). They are thought to be derived via several possible mechanisms (the reactions between sugars and amino acids).

Pigmentation of pecan kernels is localized in the thin layer of surface cells making up the testa. The flavonoids and carotenoids are thought to be predominant. Carotenoids are thought to repress oxidative rancidity of pecan oils (Pyraidi and Mason, 1968). Discoloration of pecan kernels with age is caused in part by the transformation of largely, colorless leucocyanidin and leucodelphinidin to their pigmentation oxidative derivatives phlobaphenes and anthocyanidins (Senter, 1976).

Determinants of Quality

The critical quality components of pecans depend upon who is evaluating the product and its intended use. The quality parameters measured by Forbus and Senter (1976) were kernel moisture, color, aroma, peroxide values and volatile carbonyls; whereas, Heaton and co-workers (1975) looked into the fatty acid composition, sensory characteristics, and storage stability.

Color

The color of the testa is a primary attribute with light colored kernels being preferred. Use of color as a measure of quality is based largely on a characteristic darkening with time as the surface is transformed from a light golden color to a dark red-brown (Grauke et al., 1998). If the nuts are harvested as soon as they have developed

their color, the assurance of having consistently high color quality is greater. The color of testa, (skin), of pecan kernels has been used since virtually the beginning of the commercial shelling industry because as the kernels age the surface color darkens. In the United States color is used as a measure of the general quality of the kernel.

The discoloration of the testa of stored pecan kernels is caused by the oxidation of endogenous leucocyanidin and leucodelphinidin to their respective phlobaphenes and anthocyanidins (Senter et al., 1978). A final storage level of approximately 2% 0_2 gives excellent results for extending the optimum color life of kernels (Kays, 1979). Partial replacement of oxygen with inert gases appeares to be a superior replacement.
Color is measured best by a Hunter Color Difference Meter according to Forbus et al., (1983), who maintain that it is more reliable and consistent than sensory analysis. They suggested that the hue angle is the most reliable measure of color change in pecans.

Flavor

Fresh pecans have a distinctive, pleasing aroma and taste and are often consumed without roasting. Collectively, aroma and taste comprise flavor, and flavor represents the single most important quality attribute utilized by consumers. Flavor is generally not used as a measure of quality at the wholesale or retail levels, except when there is a distinctive off-flavor. The major volatile compounds given off by raw pecans include four low molecular weight alcohols (1-heptanol, 1-hexanol, 1-octanol, 1-pentanol), five low molecular weight aldehydes (amylfuran, hexanal, heptanal, nonanal, octanal) and one lactone (α - caprolactone) (Kays, 1991).

The oil content of the kernels varies widely and is closely associated with flavor (Woodroof and Heaton, 1961). The high oil content nuts are preferred over those that are low in lipids, although the relationship between fatty acid composition and flavor has not been explored. The greater the temperature and/or the higher the linoleic acid concentration the faster the nuts become rancid (Rudolph et al., 1992).

Kernel Fill and Size

Kernel fill is also an important component of quality. Nuts that are poorly filled indicate the degree of unfavorable growing conditions and do not possess the visual attractiveness, flavor quality, or textural properties of high quality nuts (Woodroof and Heaton, 1987). Size is an arbitrary measure of quality; size of the pecan influences the marketability as there is a greater demand for large nuts (Woodroof and Heaton, 1987).

Condition of the Pecan

The general physical condition of the kernels modulates assessment of their overall quality. The size of the kernels and their textural properties are important. As the moisture content rises the pecans become moist and spongy, thereby affecting the texture. Traditionally pecans were allowed to develop and cure naturally on the trees, then fall to the ground. Nuts that matured earliest for a given harvest were often exposed to adverse weather conditions, which resulted in impairment of quality (Heaton et al., 1975). However the early harvest pecans that were dried and refrigerated immediately changed at much slower rate during storage, with a better color and flavor stability.

Postharvest Factors

The factors that influence the post harvest quality are temperature, oxygen, moisture and packaging materials.

Temperature

Temperature is perhaps the single most critical environmental factor affecting the quality of nuts. Increased temperature affects the rate of reaction and increases the rate of unwanted reactions. Storage of pecans at low temperatures greatly minimizes undesirable color and flavor changes. For instance temperatures above 4.4°C result in relatively rapid discoloration, while temperatures at or below -20°C will maintain good quality for several years (Hao et al., 1989). Staleness and rancidity are detected after as little as one week at 37.8°C (Heaton et al., 1977). Fluctuations in temperature are especially undesirable when the product temperature is below the dew point temperatures of the surrounding air. In retail stores, pecans are stored and marketed under non-refrigerated conditions. This practice greatly accelerates the rate of unwanted reactions and quality is lost. Low oxygen package environments are used in an attempt to minimize the detrimental effect of high retail temperatures on quality.

Oxygen

Oxygen is a major contributor to discoloration of pecan kernels and to the development of rancidity. The detrimental effects of oxygen are concentration and temperature dependent. The higher the oxygen concentration, the more rapid the rate of discoloration. Low oxygen (controlled or modified atmosphere) environments are used commercially for several horticultural crops. While there is a distinct beneficial effect of low oxygen on pecans, it may not be practical. Wholesale storage almost universally utilizes temperature control below 0°C. With low temperature storage, oxygen concentration is much less critical since the reaction rate of oxygen within the tissue is greatly inhibited. Pecan kernels are comprised of living cells, requiring some oxygen for normal metabolic activity. Storage of kernels at very low oxygen concentrations is, therefore, undesirable and has a very pronounced detrimental effect on quality (Dull and Kays, 1985). At very low oxygen partial pressures, anaerobic conditions occur, changing the direction of the flow of carbon in the respiratory pathways toward the production of ethanol and aldehydes which give the kernels a distinct off-flavor.

Moisture

The higher the moisture content the greater the respiration. Respiration rate is commonly used as an indication of the general overall rate of metabolism. Hence, when the respiratory rate is high, there is a much more rapid loss of quality. When the moisture content is reduced to below the level that is generally considered safe for storage, there is

a pronounced decline in the respiratory rate (Beaudry et al., 1985). The moisture content is reduced to approximately 3.5 – 4.0% to avoid mold growth (Heaton and Woodroof, 1970; Heaton et al.,1977; Woodroof, 1979).

Packaging

Packaging is a critical component in the handling, storage, transportation, and marketing of nuts in that the package should not only contain the nuts but also provide protection against damage and oxidation. Most packaging materials allow some movement of oxygen and water molecules through the surface. Excessively low permeability rates cause unfavorable quality changes in unprocessed nuts held at room temperature (Dull and Kays, 1988).

Supercritical Fluid Extraction (SFE)

Vegetable oil is extracted in a large amount for commercial use. The process is carried out in a closed system using hot organic solvent. The oil/solvent mixture is separated from the meal and the solvent is fractionated from both for reuse. An organic solvent, such as hexane, has been the preferred solvent for extracting oil from agricultural products.

The above process is favored over mechanical expellers because it leaves less oil in the meal. Solvents have become very expensive and their possible escape from the system is a consistent air pollution and explosive hazard (Agriculture Energy Center, 1982). With the use of carcinogenic and flammable solvents coming under increased

scrutiny by the government regulatory agencies, alternative oil extraction techniques are being sought for laboratory as well as industrial applications. Hexane was used to partially extract pecan oil to extend the shelf life of pecan kernels (Waters and Knight, 1985). One alternative oil extraction technique being tested for laboratory as well as industrial applications is supercritical fluid extraction (SFE) with carbon dioxide as extracting solvent (Maness et al., 1995).

Supercritical fluid technology has gained a lot of interest and the use of liquefied gases as solvents for extracting food and other products has been the focus of recent research. Carbon dioxide stands out as one of the better gases for solvent extraction. This is relatively inexpensive and most available (Zhang et al, 1995)

Supercritical fluid (SCF) is often referred to as a dense gas. It is a gas existing above its critical temperature and critical pressure (Friedrich, 1984). The critical temperature for a substance is that temperature above which it is impossible to liquefy the gas no matter what pressure is applied (Agriculture Energy Center, 1982). Under a given set of conditions, SCF may possess the density of a liquid while maintaining the diffusivity of a gas. Supercritical CO_2 (SC-CO_2) is ideal because it is non toxic, non explosive, inexpensive, readily available, and easily removed from the extracted products (Friedrich, 1984).

A comparison done between the soybeans extracted with hexane and supercritical carbon dioxide did not show any difference in flavor (Friedrich et al., 1982). However hexane is highly flammable and explosive; as it is a petroleum fraction, it contains traces of higher boiling fractions that may be left in the oil and meal. These contaminants pose a potential health hazard (Christianson et al., 1982).

The shelf-life of SC-CO_2 extracted wheat flour was excellent as predicted by the proximate low fat content after extraction. Flavor evaluation data for initial (0-time) overall flavor score of the SC-CO_2-extracted flour was comparable to flavor scores of other vegetable protein products (Karlbrener et al.,1971) and significantly higher than scores obtained for hexane-extracted flour. No significant differences were noted in the SC-CO_2 wheat germ flour after elevated and ambient temperature storage periods. The SC-CO_2 extracted flour had lower intensity values for grassy/beany and bitter descriptions when compared with hexane-extracted flour. There was also a reduction of peroxidase activity of the supercritical-extracted germ flour (Christianson et al., 1982).

Zhang and coworkers (1995) studied the feasibility of extracting oil from pecans using a nondestructive supercritical fluid extraction method. Up to 10% of the pecan's mass (mostly oil) was extracted with static gaseous CO_2 applied for 160 min at 40° C to 100°C and 3.5 to 10 MPa. Although Zhang and coworkers did not extract all of the oil, there was an reduction. Therefore SFE extraction processes offer possible alternatives for extending pecan shelf life.

Microscopy

Increasingly, the microscope is being used to study the influence of ingredients and processing conditions on food structure, especially in the development of new food products. It shows the distribution and physical state of specific food constituents, particularly starches and fats (Flint, 1994). Published studies on the ultrastructural aspects of oil seed structure and their products are relatively few, being devoted only to

selected species (Smith, 1979). Optical and electron microscopy studies, together with biochemical techniques, have provided much information regarding the structural development of oil-bearing seeds during their development from embryos to full grown kernels. A logical extension of this type of work would appear to be the application of this knowledge and the available microscopy techniques to determine the effect of processing on the structure of oil bearing seeds. The preparation of oil bearing seeds for optical microscopy appears to be amenable to the standard methods of fixation and embedding microscopy. However, Mollenhauer and Totten (1971) stated "seed tissues are unusually difficult to process for electron microscopy" a situation which appears to be made doubly difficult in the case of oil-bearing seeds because of the oil present. The processing of oil-bearing seeds involves dehulling, followed by the application of mechanical pressures and solvents to extract oil from the broken cells of the seed. Examination of seed material after the first processing stage (heat conditioning in the presence of moisture) in the rape seed (*Brassica campestris*) showed that the oil bodies had coalesced into larger bodies and that the protein bodies had become 'lumped' to form very electron-dense bodies (Stanley et al., 1976).

Studies done by Young and Schadel (1990b,1993) show that oven roasted peanuts had pitting and pock-marks on the epidermis of the cotyledons. Also there was loss of cellular organization of the cytoplasmic network surrounding the lipid bodies and protein bodies, and heat destruction of some middle lamellae of cell-to-cell junctions.

The difficulties experienced in microscopy studies of oil seeds are exacerbated by the structure of the pecan kernel. The pecan cotelydons are divided and possess an irregular surface; and the nut is four celled at the base. Most of the endocarp consists of stone cells with brown walls. The outermost of these have very thick walls with slit-like

pits, but towards the interior the walls are thinner, with relatively large pits. Inside the endocarp there is a zone of flattened parenchyma cells with brown walls. Some of the outermost stone cells have diamond shaped crystals in their cavities. In the testa some of the cells of the outer epidermis in transverse section are square shaped and others tangentially elongated, with a radial diameter up to 18 μ and a tangential diameter of 11-32 μ. Some of these cells contain brown pigment, which may also be found in the guard cells of the stomata. The rest of the testa consists of vascular tissue and parenchyma, some cells of which contain pigment. The endosperm is normally represented by a single layer of cells, up to 16 μ in radial diameter, with small aleurone grains and oil bodies. The embryo is made of parenchyma cells of the cotyledon and has intercellular spaces. It contains aleurone grains up to 5 μ and oil drops, and may also contain some starch granules (Vanghan, 1970).

Storage Stability of Pecans

Extension of the storage period of edible products with retention of quality is a major need (Wagner, 1980). Successful storage will ensure the availability of good quality nuts throughout the year (Fourie and Basson, 1989). Stein (1980) stated that good storage practices can maintain the initial quality but cannot improve quality.

During the processing and storage of foods, several chemical changes occur that involve the internal food components and the external environmental factors. These changes cause food deterioration and reduce shelf life . The most important chemical change is associated with lipid oxidation that alters the flavor and the appearance of the food (Nawar, 1985). The presence of polyunsaturated fatty acids in foods is a prime

reason for the development of rancidity during storage as long as oxygen is available (Braddock et al., 1995). The rate of lipid oxidation is influenced by several factors: the environmental temperature, the presence of oxygen, and the loss of antioxidants.

To assure overall retention of sensory qualities, the moisture of the pecan should be reduced to 4.5 % before refrigerated storage (Heaton and Beuchat, 1980) with humidity control. Proper humidity and temperature both are the most important factors to consider for optimum pecan storability. Keeping pecans in frozen storage is most effective for maintenance of eating quality (Wagner, 1980). Williams and co-workers (1973) reported that 50% of the pecans sold in the retail stores in this country did not meet the USDA standard for overall quality. In commercial practices, the storage of pecans during retail and wholesale distribution is often at ambient temperature which will accelerate autooxidation and result in undesirable changes in color and flavor (Erickson et al., 1994).

In a eight month study done by Erickson (1993) on pecans stored at room temperature it was observed that during the early stages of storage pentanal was the predominate gas in the pecan headspace, whereas in the later stages hexanal predominated. Quantities of triglycerides recovered from pecans did not change significantly during storage, whereas a decrease in phospholipid content and an increase in free fatty acid content were seen. While losses of polyunsaturated fatty acids (PUFA) in triglycerides and free fatty acids only occurred during the last month of storage, PUFA losses were recorded in the phospholipid fraction by five months. Based on the strong negative correlation ($r = -0.98$) between hexanal and its precursor fatty acid (18:2) in phospholipids, the membrane lipids would appear to be a primary site of attack during the early stages of oxidation. The free fatty acids in pecans increased during storage (Forbus

and Senter, 1976; Erickson, 1993). Free fatty acids are thought to contribute to oxidative instability although they are not a major site for oxidation.

During oxidation of pecan oils, the tocopherol concentration of the oils decreased and the oils discolored, changing from yellow to reddish and eventually becoming colorless. Changes in color were followed by a rapid increase in rancidity products and a corresponding decrease in linoleic acid concentration (Rudolph et al., 1992).

The type of packaging materials used during storage is also important. Dull and Kays (1988) reported that pecans packaged in polyvinylidenechloride coated cellophane packaging films with low oxygen transmission rates were of acceptable quality after 6 months storage at 24°C and 60 % relative humidity (RH). In addition to being oxygen impermeable, the barrier film should also be impermeable to UV light.

Sensory Evaluation

The sensory attributes of food are major factors that influence food consumption. These attributes are all included in the senses of sight, smell, taste, touch and sound. The quality of food is evaluated by a combination of factors. These qualities are appearance (size, shape, color), texture (kinesthetics), and flavor (smell, taste) (Kramer, 1972). Increased emphasis should be placed on developing indices for sensory evaluation, that are based on objective measurements of physical, chemical and textural properties of kernels that relate more directly to maintenance of flavor, quality, and stability during storage and marketing (Forbus et al., 1983). The indices used for sensory analysis should be non technical and should, use everyday language so that it is clear and understood by all the panel members (Stone and Sidel, 1998). Sensory analysis is considered the most

accurate for evaluating pecans, but it is very time consuming and requires an adequate uniform sample. A large panel size may be required, especially in the case of an untrained consumer panel. Erickson and coworkers (1994) conducted descriptive analysis to evaluate raw and roasted pecans. They tested for crunchiness, internal lightness, rancid aroma and flavor using an 11 member trained panel. Evaluations were recorded by placing a vertical mark for each sample along a 150 mm line for each attribute. The panelists evaluated rancid aroma by three short sniffs from a slightly opened sample cup (Dull and Kays, 1988). Crunchiness was evaluated by biting the sample perpendicular to ridges of the pecan with the incisor teeth and indicating the amount of force to shatter the pecan. Panelists evaluated internal lightness of pecans under 'cool-white' fluorescent lights.

Dull and Kays (1988) conducted a series of sensory analyses of pecans using untrained panels. Pecans were stored for seven months at - 40°C. The panelists rated each sample using a nine-point hedonic scale from "like extremely (9) to "dislike extremely". They reported that as the pecans aged they became rancid (as reported by the researchers), and the mean ratings went from nine to one.

In a later study with pecans, Erickson and co-workers (1994) used a semi-trained panel to predict the relationships for sensory responses of crunchiness and rancid aroma and flavor. In their study raw and roasted pecans were stored under two different relative humidities (55 and 65%) for up to eight months. Rancid sensory scores in raw and roasted pecans during storage most closely paralleled peroxide value and thiobarbituric acid reactive substances. However the panelists were able to detect the initial flavor change before these chemical tests did.

Santerre and coworkers (1994) trained six male and four female panel members between the ages of 18 and 30 yrs to evaluate roasted peanuts and peanut butter which were extracted using supercritical fluid extraction. The panelists indicated a reduction in roasted peanut aroma intensity, fracturability, moistness of mass, and roasted peanut flavor intensity of peanuts with increasing extraction time. The adhesive property of peanut butter decreased, due to reduction in lipids.

Sensory studies of pecans have tended to center on post harvest flavor changes and how these changes seem to relate to storage, time, temperature, moisture and packaging conditions. There is a need for an objective test for flavor quality, but the tongue still seems to be the best analytical tool.

CHAPTER III

METHODOLOGY

Introduction

This chapter includes the procedures used to evaluate different structural and sensory characteristics of extracted and non extracted pecans. Included is Experiment I where the panelists studied and compared pecans extracted at 40°C and 80°C for different extraction periods. Experiment II evaluates pecans extracted at 40°C for 20 min and stored for a period of 37 weeks. Experiment III evaluates pecans stored at different atmospheric conditions for 12 months. Experiment IV was a study to observe the structural changes in SFE pecans using TEM and SEM. Experiments I, II and III were conducted sequentially.

Experiment I

Effect of Supercritical Fluid Extraction on Pecan Flavor

A large, uniform supply of shelled, intact pecan halves that had been cleaned and sized was obtained from a commercial source. Kernels with a light color, distinctive and pleasing aroma and taste, that were also free from diseases and insects and without

breakage, were selected for oil extraction tests. The nutmeats had about 4% moisture content and 66% oil content.

A Diomex Model SFE-703 (Sunnyvale, Calif.) supercritical fluid extraction instrument was utilized for the continuous CO_2 flow pecan oil extraction experiment. This system was an automated eight-cell off-line extraction instrument operational in either automatic or manual mode. The 24 ml stainless steel extraction cells installed in the temperature controlled oven chamber were rated at 68.9 MPa (10,000 psi). The flow rate of CO_2 through the extraction vessel was regulated by the flow rate of the restrictor.

Intact pecan halves (5-6 g) were weighed and loaded into 1-8 extraction cells, using glass wool plugs to retain the pecan in both ends of the cell. The filled cells were weighed, placed in the main oven chamber, and connected to the manifold and restrictors. Dionex's high flow rate (1200 ml/min) restrictors were heated to 150°C to prevent the lipids from precipitating out in the small diameter tubing during decompression. Glass wool was inserted into the inner tube and the inside of each collection vials to ensure complete trapping of pecan oil. It was found best to leave a space of approximately 2 cm between the glass wool and the upper end of the inner tube to allow penetration of the restrictor needle into the vial. Each vial was weighed and inserted into the chilled collection zone. The pecan kernels remained in the extraction vessels until the extraction cycle was completed and the pressure returned to the normal conditions. Pecans were extracted at 40°C for 20 and 150 min and 80°C for 10, 60 and 120 min. Following the extraction the samples were placed in low oxygen permeable bags, labeled and frozen (-18°C) for the sensory evaluations.

Sensory Evaluation

A research methods class of 19 graduate students performed the sensory evaluations. Their training sessions consisted of identifying and rating basic flavor solutions in different concentrations so that they were able to identify and differentiate the basic flavors (sweet, sour, bitter and salt). They also performed the thresholds of the basic flavors and measured the flavor interaction on time scale. Before the actual testing began, the panelists were given samples of non extracted pecans and, as a group along with the researchers, arrived at the flavor descriptors that would be on the evaluation score sheets (Appendix 1). This exercise was of particular importance since the students were from different parts of the country and some of them were not familiar with pecans. Therefore, the students tasted, trained, discussed and described until all agreed on descriptors with a common meaning. The students were instructed on the importance of avoiding bias due to interaction with other panelists or distracting conditions. The evaluation sessions were held during the first part of the class at one week intervals for 3 weeks. For the first session, members were given a control (not extracted), and pecans extracted for 150 min at 40°C and 120 min at 80°C. The second week pecans extracted for 20 min at 40°C and 10 min at 80°C and a control were given. At the third session four samples, all extracted at 80°C for 10, 60, 120 min, and a control were given. The panelists evaluated the pecans on the following criteria: appearance, texture, pecan flavor (sweet flavor, nutty flavor, oily flavor), off flavors (tannin, sour, rancid and toasted) and acceptability. The pecan samples were drawn from larger samples that had been chopped

into small pieces so that evaluators would not be biased by the characteristics of a single kernel.

The results, discussion and the conclusions from this study are discussed in chapter IV of the dissertation.

Experiment II

Storage Study I

The samples were extracted similarly to the pecans extracted in Experiment I. The pecans in this study were extracted at 40°C for 20 and 480 min. The pecans were packaged in 25 g bags and labeled before storage. Extracted and control pecans were then stored at 25°C and 55% relative humidity for up to 37 weeks. Three sample bags from control and the extracted pecan sample (20 and 480 min) were randomly drawn. This was replicated three times. The pecans were initially taken out every two weeks for the first eight weeks and then they were taken out after every month for the remaining weeks. Once the pecans were taken out they were put in a dehydrator for 1 hour at 90° F to let any moisture in the pecans dry out. They were then chopped into small pieces and put in six color coded containers and sealed in a vacuum bag and given to the panelists. Therefore the panelists received each treatment in triplicate. The pecans were evaluated on the basis of the evaluation sheet prepared in Experiment I (Appendix I).

The amount of sample available was so small that it was impossible to have a very large panel group, since each of the panel members received 5 g of the sample. Four

panel members from the permanent faculty of the College of Human Environmental Sciences were selected, and trained in flavor identification and determination of flavor threshold levels (Experimental Foods Manual, 1998).

The results, discussion and conclusion from Experiment II, a storage study of SFE pecans kernels, are given in Chapter V of the dissertation.

Experiment III

Storage Study II

The samples were extracted similarly to the extraction in Experiment II i.e., at 40°C but for 2 hours. They were stored at 2%, 10% and 21% oxygen for a year at 25°C and 55% relative humidity. The control and the extracted pecans were then placed in small plastic bags. 5-g samples were prepared so that the control and the extracted pecan was rated three times. Every three months the samples were taken out of the storage and were evaluated. The evaluation was similar to Experiment II. The same panelists continued in this study which started only after the conclusion of Storage study I (Experiment II).

The results, discussion and conclusions from this experiment concerning the effects of oxygen level on sensory quality during storage are given in Chapter VI of the dissertation.

Experiment IV

Microscopy Study

Samples for this study were drawn from the same large frozen batch of pecans used in Experiments I, II and III. The treatments were control pecans, pecans extracted at 40°C for 8 hours, pecans extracted at 80°C for 1 hour, and pecans extracted in a commercial extraction plant. These treatment pecans were selected for TEM and SEM observations.

Transmission Electron Microscopy (TEM)

Preparation of pecan samples: A pecan kernel was cut into pieces of 1 approximately $1mm^3$ (Young and Schadel, 1990). The samples were fixed in a mixture of 8% glutaraldehyde and phosphate buffer in a 1:1 ratio for 4 hours in a vacuum system. The samples were washed in phosphate buffer (hereafter referred to as buffer) three times for 20 minutes. Then they were post fixed for two hours in 1% osmium tetraoxide and buffer in a 1:1 ratio. After post fixation, the pieces were washed in buffer 3 times for 20 minutes. Then they were dehydrated at room temperature in a graded series (7 times) of aqueous ethanol (50, 70, 90, 95, 100, 100, 100%) solutions.

Preparation of pecan sections: The dehydrated pecan pieces were put in propylene oxide and the embedding resin. The bottle was then closed with a lid and was placed in the

fume hood for a week. The samples were removed and placed in molds containing the embedding resin spurr's and allowed to dry at 70°F for 24 hours. Ultrasection sections were cut using a Reichert ultramicrotome (Appendix II) and were stained with 4% uranyl acetate, followed by 0.4% lead citrate. The sections were examined with a JOEL 100S TEM (Appendix III).

Scanning Electron Microscopy (SEM)

Whole pecans were placed in 8% glutaraldehyde and buffer in a 1:1 ratio for 2 hours and were washed in buffer 3 times for 20 minutes. Then the pecans were post-fixed in osmium tetraoxide and buffer in a 1:1 ratio for 2 hours. They were washed in buffer 3 times for 20 minutes, and were dehydrated in a graded series of aqueous ethanol (50, 70, 90, 95, 100, 100, 100%) solutions. The dehydrated sample was critical point dried in a Tousimis PVT-3B unit using liquid CO_2. Subsequently the dried samples were mounted on aluminum specimen stubs with double-sided tape and silver conducting paint. Prepared stubs were coated with 30 nm gold-palladium alloy at room temperature in a Hummer V sputter coater (Appendix IV). The samples were viewed with a JOEL 800S (AppendixV) Scanning Electron Microscope.

The results, discussion and conclusion are reported in chapter VII of the dissertation.

Statistical Analysis

The data obtained in Experiment I, II, and III from the sensory evaluation were analyzed using the Statistical Analysis System (SAS, Institute, Inc., 1985). Analysis of variance using the GLM procedure and Least Squares differences to compare the means was performed to analyze the data of Experiment I and II but for Experiment III Proc Mixed analysis was used to analyze the data. The significance level was established at $P \leq 0.05$.

Experimental Design

As only limited amounts of sample were available, a randomized block design was set up. The panelists were the blocks and the extraction time and extraction temperature was the treatments in Experiment I. In Experiment II and III the storage time and the temperature were the treatments.

CHAPTER IV

EFFECT OF SUPERCRITICAL FLUID EXTRACTION (SFE), TEMPERATURE AND TIME ON SENSORY QUALITITES OF PECAN KERNELS

B. CHINTA, S. KNIGHT, B. STOECKER

Department of Nutritional Sciences
Oklahoma State University, Stillwater, OK 74078

N. O. MANESS

Department of Horticulture
Oklahoma State University, Stillwater, OK 74078

P. L. CLAYPOOL

Department of Statistics
Oklahoma State University, Stillwater, OK 74078

Abstract

Oil, the major constituent of pecan kernels, accounts for up to 70% of the kernel weight. Thus pecans are high in calories, and the high degree of polyunsaturation makes them susceptible to oxidative deterioration. Oil reduction in pecans was achieved by supercritical fluid extraction process conducted at 69 MPa, and temperatures of 40 °C and 80 °C with carbon dioxide a natural nontoxic gas as extractant. The objective of this study was to compare the sensory characteristics of control pecans (non-extracted) and pecans extracted at two different temperatures (40 °C and 80 °C)for various time periods to affect different levels of oil reduction. A panel of graduate students ranging from 24 – 45 years, was semi-trained to evaluate the pecans for interior color, oily flavor, toasted

flavor, nutty flavor, and acceptability. Differences were determined, but few were consistent throughout the study except for the trend for the interior color becoming white and having less oily flavor for extracted pecans versus control pecans. There was a decrease in nutty flavor in the extracted pecans but neither treatment significantly reduced acceptability compared to controls.

Introduction

Pecans are fruit seeds enclosed in a leathery, woody covering and are edible tree nuts. The optimum quality of nuts is reached soon after they attain full maturity (Heaton et al., 1975). Pecan production is an important agricultural industry. Although pecan consumption has increased with improved storage conditions and packaging (Heaton et al., 1977), the pecan industry still encounters problems in quality maintenance during retail marketing because of fatty acid oxidation (Kays, 1987) and associated development of rancid flavors.

Changes in quality after harvest are dictated by the chemical composition of kernels. Pecan kernels are especially susceptible to oxidative rancidity because of their high polyunsaturated oil content. The kernels contain approximately 70% oil, an oil that is high in unsaturated fatty acids. Over time oxidative cleavage of polyunsaturated fatty acids causes flavor instability (Kays, 1987). In addition the high fat content of the pecan is considered detrimental by a weight-conscious public (Florkowski and Hubbard, 1992). Partial oil reduction would not only reduce the fat calories but would also reduce the number of potential sites for oxidation, thereby offering both reduction in calories and extended shelf life. Traditionally, nut oils are obtained by pressing (which destroys the

kernels) or by use of solvents such as hexane. Hexane, a potential environmental contaminant, is explosive and can be difficult to completely remove from intact kernels without negatively affecting quality. These problems can be solved by the use of a supercritical fluid extraction (SFE) process. When a gas such as carbon dioxide is subjected to certain pressure and temperature conditions, it reaches a supercritical point where it exists a semi-liquid state. A supercritical carbon dioxide process has been developed for partial oil extraction of pecans at Oklahoma State University (Zhang, 1994). In the traditional solvent extraction process, the solvent should be a liquid because of its high solvent power compared with gas. However, supercritical fluids have the solvent power of liquids and have better mass transfer characteristics (e.g. lower viscosity and higher diffusion coefficient) than typical liquids. Using carbon dioxide (CO_2) as the supercritical fluid offers unique advantages: it is abundant, non-reactive, non-toxic and environmentally harmless.

Pecans must taste good if they are to be purchased and then repurchased by customers. Although flavor and texture of pecans are important, there are relatively few sensory studies available. Resurreccion and Heaton (1987) studied both sensory and objective measurements on early and traditionally harvested pecans. Ocon and co-workers (1995) studied texture of pecans using sensory and instrumental methods. Good quality in pecan kernels was characterized by Senter and Forbus (1979) as light color, crisp texture and freedom from staleness and rancidity. Waters and Knight (1985) reported sensory qualities of reduced oil pecans with hexane as extractant, but no reports were found describing the sensory qualities of reduced oil pecans using CO_2 as extractant.

A possible disadvantage of oil removal from pecans is a concomitant removal of some characteristic pecan flavors resident in the oil (Maness et al., 1995). Therefore the objective of this study was to determine the sensory qualities of pecans extracted for different times and temperatures and compare them to non-extracted pecans. These data will be used to optimize extraction parameters.

Materials and Methods

Supercritical Fluid Extraction (SFE)

The apparatus used for SFE was a Dionex 703 extractor (Sunnyvale, Calif.) with four fifty ml extraction vessels. Clean, empty vessels were installed prior to extraction, and a blank extraction was conducted to purge the SFE system of any oil remaining from prior extractions. Pecan samples were loosely packed into the extraction vessels between glass wool plugs which had been inserted into the inlet and outlet ports to retain the samples inside the cell. Extractions were carried out simultaneously in four extraction vessels at 69 MPa (final pressure) and 40°C for 20 and 150 minutes and 80°C for 10, 60 and 120 minutes. The gaseous flow rate and total flow were determined from on-board flow meters for each vessel. Flow rates ranged from 510 to 680 ml/min at 69 MPa. Upon completion of extraction, the extracted oil was quantitatively transferred into vials (Maness et al., 1995). The pecan kernels remained in the extraction vessels until the extraction cycle was completed and the pressure was returned to normal conditions.

These samples were then placed into individual bags and frozen at –18°C until sensory evaluations were conducted.

Sensory Evaluation

A panel of 19 graduate students performed the sensory evaluations. They were trained on solutions of four basic flavors (salt, bitter, sugar and sour) at the threshold levels and in different concentrations, to establish their ability to identify and differentiate the basic flavors. As a part of training, panelists were given samples of control pecans and, as a group along with the researchers, arrived at the flavor descriptors used on the evaluation score sheets. During evaluations, panelisits were separated by wooden panels to avoid bias due to interaction, and sessions were held at one-week intervals for three weeks. The pecans were chopped into small pieces, approximately 1 cm^2 so that evaluations would be based on a mixture of kernel pieces. For the first evaluation session, members were given control and pecans extracted for150 min at 40°C and 120 min at 80°C. The second week, pecans extracted for 20 min at 40°C and for 10 min at 80°C and a control were given. At the third session samples extracted at 80°C for 10, 60, and 120 min and a control were given. The panelists evaluated the pecans on the following criteria: appearance (outer and inner color of the kernel); texture (chalkiness and crispiness of the kernel); pecan flavors (sweet flavor, nutty flavor, oily flavor); off flavors (tannin, sour, rancid and toasted); and acceptability. A five-point scale was used to measure the characteristics. The skin color was based on a subjective scale, which was measured by allocating 1 for light tan and 5 for dark brown. The inner color of the pecan

kernel was evaluated with 1 being white and 5 being yellow tan. The other characteristics were evaluated on a scale where 1 was none and 5 was most. An overall acceptability score was given where 1 was least acceptable and 5 was most acceptable.

Statistical Analysis

The data obtained from the sensory evaluation were analyzed using the Statistical Analysis System (SAS Institute, Inc., 1985), with Analysis of Variance using the GLM procedure and least square means to compare the means. The significance level was established at $P \leq 0.05$.

Experimental Design

To most efficiently use the limited amount of samples available, a randomized block design was used. The panelists were the blocks and the extraction temperature and extraction time were the treatments.

Results and Discussion

The panelists rated the pecans on these characteristics: appearance, texture, pecan flavor (sweet flavor, nutty flavor, oily flavor), off flavors (tannin, sour, rancid and toasted) and acceptability. Unless noted otherwise, only results significant at $P \leq 0.05$ are discussed. Table 1 shows the results from pecans with extended extraction times (150

min at 40°C and 120 min at 80°C). Eller and King (2000) observed that higher temperatures used to extract oil resulted in greater percentage of oil recovery. The nut meat of the control pecans was a pale yellow-tan in color. The extracted pecan nut meats were whiter than the control, and the pecans extracted at 80°C (120 min) had a whiter interior color than the pecans extracted at 40°C (150 min). The extracted oil had a pale yellow color. Santerre et al. (1994) reported similar changes in color with peanuts. Sweetness decreased in the extracted pecans which was unexpected since sugar is not fat soluble. Nutty flavor was highest in the control. The panelists perceived the control pecans to have a more oily taste compared to the extracted nuts. Similar results were observed by Yackinous and Guinard (2000). The tannin flavor was stronger in the extracted pecans than in the control pecans. Rancidity and acceptability were not significantly affected by SFE.

Table 1: Sensory characteristics of the control pecans and pecans extracted at two different temperatures (40°C and 80°C) at extended extraction times (n=19) §

Characteristics	40°C (150 min)	80°C (120 min)	Control
Interior color♣	2.35^{b}	1.64^{c}	4.00^{a}
Sweet⊗	2.52^{b}	2.35^{b}	3.29^{a}
Nutty⊗	2.70^{b}	2.70^{b}	3.47^{a}
Oily perception⊗	2.35^{b}	2.00^{b}	3.64^{a}
Tannins⊗	2.64^{a}	2.35^{a}	1.94^{b}
Rancid⊗	1.94^{a}	2.05^{a}	2.05^{a}
Acceptability♦	2.88^{a}	2.88^{a}	3.52^{a}

§ Means in the row with the same letter are not significantly different

♣ white 1 - yellow-tan 5

⊗ none 1 - strong 5

♦ least 1- most 5

Table 2 shows the results at shorter extraction times (10 min at 80°C and 20 min at 40°C). The interior color of the extracted pecans was lighter when compared to the control pecans.

Control pecan had lower chalkiness scores, indicating when the oil was extracted the texture became more dry and chalky. The pecans extracted at 80°C (10 min) had a more toasted flavor than the pecans extracted at 40°C (20 min) or the control pecans because of the higher extaction temperature that was used to extract oil. The pecans at 40°C (20 min) had the least rancid flavor when compared to control and 80°C (10 min). The oily perception was not significantly different. When the pecans are extracted the oil comes to the surface (testa) of the kernel and some of it remain on the testa. This residual oil on the testa might have given the panel members a false perception. The control had the strongest level of rancid flavor but was not significantly different from the 80°C (10 min). The acceptability scores were not significantly different.

Table 2: Sensory characteristics of the control and extracted pecans at short extraction times at two different temperatures (40°C and 80°C) (n=19)[§]

Characteristics	40°C (20 min)	80°C (10 min)	Control
Interior color[♣]	2.83[b]	2.44[b]	3.66[a]
Chalky[*]	2.50[a]	2.66[a]	2.05[b]
Oily perception[*]	2.16[a]	2.22[a]	2.38[a]
Rancid[*]	1.72[b]	2.11[a]	2.38[a]
Toasted[*]	2.11[b]	2.77[a]	2.05[b]
Acceptability[♦]	2.83[a]	3.11[a]	2.67[a]

§ Means in a row with same letter are not significantly different
♣ white 1 - yellow-tan 5
* none 1 - strong 5
♦ least 1 – most 5

Table 3 shows data for pecans extracted at the same temperature (80°C) but for different times. There was no difference in skin color among the extracted pecans, but all were lighter than the control. The control pecans had a darker skin color, although the control pecans as well as the extracted pecans, were all stored at -18°C. The interior color of control pecans and the ones extracted for 60 min were not significantly different (somewhat cream color). The interior for pecans extracted at 10 min had more yellow color than the controls. Pecans extracted for 120 min had whiter interior. The control pecans had a crisper texture and a stronger toasted flavor than the extracted pecans. Palazoglu and Balaban (1998) have reported that sensory evaluation of lipids extracted from roasted pistachio showed a reduction in roasted pistachio flavor intensity following SC-CO_2. Similarly, the flavor that our panelist described as "toasted" declined in pecans extracted for longer duration but all were significantly less than control. Nutty flavor, oil perception, tannin flavor, sour and rancid flavors also were not significantly different. Although the panelists were able to detect several differences among the pecan treatments and the control, there were no significant differences in overall acceptability. None of the extraction conditions for pecans in this study were not enough to reduce the total flavor components of the pecans to an unacceptable level. Though not significant the 10 and 60 min extraction had higher acceptable scores than 120 min extraction.

Table 3: Sensory characteristics of the control and pecans extracted at the same temperature (80°C) for different times (n=19)[§]

Characteristics	10 min	60 min	120 min	Control
Skin color†	2.81[b]	2.87[b]	2.81[b]	4.93[a]
Interior color•	3.25[a]	2.12[b]	1.43[c]	2.75[b]
Crisp texture+	2.93[b]	2.75[b]	2.75[b]	4.37[a]
Toasted*	2.50[b]	2.31[b]	1.93[b]	3.25[a]
Nutty*	3.25[a]	2.93[a]	2.81[a]	2.81[a]
Oily perception*	2.81[a]	2.81[a]	2.25[a]	2.56[a]
Tannin*	2.56[a]	2.62[a]	2.12[a]	2.56[a]
Sour*	2.00[a]	1.87[a]	1.93[a]	2.00[a]
[a]Rancid*	2.00[a]	2.06[a]	2.00[a]	2.56[a]
Acceptability♦	3.19[a]	3.19[a]	2.44[a]	3.06[a]

[§] Means in a row with same letter are not significantly different

† Light tan 1 - very dark brown 5

• White 1 - yellow-tan 5

+ Soggy 1 – crisp 5

* None 1 – strong 5

♦ least 1- most 5

Conclusion

This study was done to investigate how extraction conditions affected pecan flavors to establish extraction parameters related to acceptability. The dominant extraction effects were that the pecans extracted at lower temperature had more chalky texture, less rancid flavor and lighter interior color than the controls. With longer extraction time, pecans had a lighter interior color. Partial oil extraction affects some flavors and textural attributes of pecans. However, the flavor, color and textural differences due to extraction are not severe enough to cause significant losses in acceptability ratings. The differences might be even less important if consumers realized

that they were associated with fewer calories and possibly less rancidity. Information obtained from this study will be used for future research on the shelf life of the extracted pecans.

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CHAPTER V

THE EFFECT OF OIL REDUCTION BY SUPERCRITICAL FLUID EXTRACTION (SFE) ON THE STORAGE OF PECAN KERNELS

B. CHINTA, S. KNIGHT, B.STOECKER

Department of Nutritional Sciences
Oklahoma State University, Stillwater, OK 74078

N. O. MANESS

Department of Horticulture
Oklahoma State University, Stillwater, OK 74078

P. L. CLAYPOOL

Department of Statistics
Oklahoma State University, Stillwater, OK 74078

Abstract

The effect of oil reduction using the supercritical fluid extraction (SFE) process on the shelf life of pecans was studied in this experiment. Pecans were extracted at 40 ℃ for 20 and 480 min. All the pecans were stored at 25 ℃ in a dark place at 55% relative humidity. These pecans were removed for evaluation at 2, 4, 6, 8, 10, 14, 18, 22, 26, 32, 37 weeks respectively. The extracted pecans had lower hexanal scores when compared to the control, which tended to parallel the panelists' evaluation of the nuts. The control pecans increased in rancid flavor as the storage time increased but this flavor was not so

pronounced in the extracted pecans, indicating the extraction of oil from the pecans might increase the shelf life

Introduction

Pecan marketing efforts are hindered by two factors, both of which are related to the fat content of pecans. Pecans have a higher total fat content than most nuts and a substantial percentage of the oil is in the form of polyunsaturated fatty acids (PUFA). The high oil content and associated high caloric value, is one factor limiting marketability. The relatively high percentage of PUFA predisposes pecans to susceptibility to oxidation and development of rancid flavors which is the second factor limiting pecan marketability.

Most commercially shelled pecans are refrigerated from the time they are purchased by the sheller until distribution to food processors and retailers. This helps in maintaining the flavor and texture (Hoa et al., 1989). However once the pecans reach the retailers they are most often held at room temperature (Forbus et al., 1980). Because of the high susceptibility to oxidative deterioration and associated off flavor development, pecan kernels have a shorter shelf life compared to competing nuts which have lower oil content (Fourie and Basson, 1989). The high oil content and degree of polyunsaturation of the fatty acids in pecan kernels complicate successful storage. Extension of the storage period of edible products with retention of quality is one of the major needs of the food industry. When customers purchase and use off-flavored pecans, potential repeat sales are lost.

The high fat and calorie content of pecans has received a negative response from weight-conscious consumers. Reducing the oil content of pecans will produce a lower fat product and may extend shelf life, thereby enhancing consumer appeal. Cold pressing, solvent extraction, or supercritical fluid extraction (SFE) can reduce oil content. SFE, with carbon dioxide, can extract oil from pecans without damaging kernel structure and without using organic solvents (Zhang et al., 1995; Alexander, 1996). SFE has been used with other products. Eldridge and coworkers (1986) reported that the usual grassy, beany, and bitter flavors of hexane-defatted soybeans were only minimally detected in the beans extracted by SFE. Further the SFE extracted beans had high quality protein which was more stable even when stored in adverse conditions.

Forbus and coworkers (1980) described the sequence of flavor deterioration in pecan as follows; there is a loss of readily volatile substances increasing the bland flavor; followed by the onset of oxidation which causes darkening of color; then development of stale aroma, flavor, and also hydrolysis of fats resulting in increase of free fatty acid and development of acrid flavor. Erickson (1993) reported that during the early stages of pecan storage, pentanal predominated in the head space of the GC when pecans were being analyzed, where as at later stages hexanal was predominant. Hexanal production in foods has been used to measure degree of rancidity. Fritch and Gale (1977) found that the onset of rancid odors in stored cereals occurred at hexanal concentrations between 5 and 10 ppm. Hofland et al. (1995) found that flavor intensity of roasted sunflower kernels correlated with hexanal levels and that 6 ppm was the maximum acceptable hexanal content for good storage quality as determined by sensory analysis. Based on a strong negative correlation ($R = -0.98$) between hexanal and its precursor fatty acid (18:2)

in phospholipids, the results suggest that membrane lipids are the primary site of attack during the early stages of oxidation (Erickson 1993).

The best method for evaluating pecan kernel quality is subjective evaluation for aroma and flavor by trained panelists (Erickson et al., 1994). However, the method is time consuming, expensive, and may not be practical for quality control on a production basis (Forbus et al, 1980). To reduce the time and expense associated with sensory analysis, studies have been conducted to establish a correlation between objective and subjective measurements. Researchers have found that physiochemical measurements varied in ability to predict sensory responses of rancid flavor and aromas. In this study an effort was made to evaluate pecans by both subjective and chemical measurements. The objectives of this study were to evaluate the pecans using sensory panel and quantitative hexanal analysis as measures of rancid flavor development in pecans and to determine the effect of oil reduction by SFE on extended shelf life of pecans.

Materials and Methods

Oklahoma native pecan halves containing 65% oil by weight, that had been frozen (-20°C) since harvest, were allowed to warm to room temperature. Oil was extracted using supercritical Coleman grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) at 69 MPa and 40°C for 20 or 480 min to reduce oil content by 22 or 28% of the original oil content respectively. SFE was conducted using a Dionex 703 extractor (Dionex Corp., Sunnyvale, CA) with four 50 ml extraction vessels holding approximately 15 g (16 halves) each. After extraction, sound kernels were saved for in airtight freezer

bags and stored at –18°C until a sufficient quantity was extracted for the entire storage test. During the extraction process some of the pecans kernels were damaged so they were discarded.

Packaging and Storage

Extracted and control pecans were taken from cold storage and allowed to thaw inside a freezer bag at room temperature for at least six hour. Pecan halves were randomly selected from each replication and extraction level (control, 20, 480 min extraction time). Pecans were then randomly distributed into 108 groups (3 extraction levels x 12 storage times x 3 reps), of 20 pecans each and were weighed and packaged. The kernels were placed into bags (approximately 15.2 cm X 15.2 cm), and then evacuated from ambient pressure (98kPa) to less than 0.3kPa, back-flushed with a standard air mixture of 21% 0_2 in N_2 (Air Products and Chemicals, Chicago, IL) to 88 kPa, and sealed in a Multivac A316 vacuum packaging machine (Multivac, Inc., Kansas City MO). The bags used were made of 13 μ Saran-coated Mylar (polyester) laminated to 63.5 μ polyethylene (The Packaging Group, Woodbridge, Ontario). The water vapor and oxygen transmission rates, obtained from supplier data sheets, for this packaging material were 60mg $H_2O/100cm^2 \cdot$/24 hr and $90ml O_2/100cm^2 \cdot$/24hr.

Packaged pecans were stored in an environmental chamber with circulated air at 25°C and 55% RH (relative humidity) with no lighting. Three replicates from each SFE level were removed from storage at 2, 4, 6, 8, 10, 14, 18, 22, 26, 32, and 37 weeks and analyzed for hexanal content and sensory properties..

Hexanal Analysis

Ten kernels from each replicate utilized were for hexanal analysis. Kernels were ground to a particle size less than 1 mm, and six samples of 50 mg each were placed into two-dram vials. A known amount of 4-heptanone, dissolved in canola oil, was added as internal standard (Erickson, 1993) just prior to sealing of vials with open top caps and Teflon-lined silicon septa, then incubated at 90°C for 150 min in a dry block heater. Head space gas (1 ml) was taken immediately and analyzed using a gas chromatograph equipped with a split injector (split ratio 1:50) and an FID detector. Injector temperature was 275°C and detector temperature was 300°C. Separations were carried out on a DB 23 fused silica capillary column (30 m x 0.25 mm i.d., 10.25 μm film thickness, J and W Scientific, Inc., Rancho Cardova, CA). Column oven temperature was maintained at 50°C for 2 min, then raised at 10°C/min for 4 min. Oven temperature was then returned to 50°C to await the next injection.

Sensory Analysis

The samples were frozen for 1 to 2 days at –18°C so that the panelists received the samples at the same time. The pecans were thawed in the bags for approximately four hours at room temperature and then were taken out of the bag. Once the pecans were taken out, they were put in a dehydrator for one hour at 90°F to remove any moisture in the pecans. They were then chopped into small pieces and put in color-coded containers, sealed in vacuum bags and given to the panelists.

The amount of sample available was small so it was impossible to have a very large panel group. Four panel members from the faculty of the Human Environmental Sciences College were selected and trained with the basic four flavors sweet, salt, sour and bitter. They were also given different concentration levels of these flavors to see if they could detect at the threshold levels and rank concentration levels (Experimental Foods Manual, 1998). The panelists were trained on the rancid aspect by giving some fresh and stale (not so fresh pecans) pecans and being asked to differentiate. The evaluation sheet they used was based on the following criteria: appearance (outer and inner color of the kernel), texture (chalky and crispness of the kernels), pecan flavor (sweet flavor, nutty flavor, oily flavor), off flavors (tannin, sour, rancid and toasted) and acceptability. A five-point scale was used to measure the characteristics. The skin color was evaluated where 1 was light tan to 5 being very dark brown. The inner color was evaluated with 1 white and 5 yellow tan. The other characteristics were evaluated on a scale where 1 was none and 5 was most. Based on all the above characteristics a overall acceptability score was given where 1 was least.

Statistical Analysis

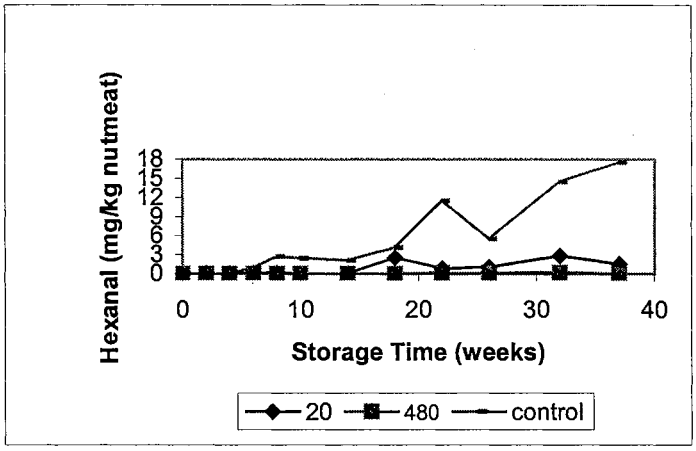
Analysis of variance (ANOVA) procedures were performed to determine effects of oil reduction and storage time on pecan color and hexanal content, using the Statistical Analysis System (SAS Institute, Cary, NC). Within each replication the data were analyzed as split plot unit experiment in a randomized block design where the panelists were the blocks, extraction time was the main unit treatment factor and storage time was the subunit treatment factor. A significance level of $P \leq 0.05$ was used.

Results and Discussion

Hexanal Analysis

Hexanal production increased with storage time is in agreement with Forbus et al., (1980). Hexanal levels ranged form 0 to 16.99 ppm for full-oil (control) pecans; 0 to 4.44 ppm for20 min extraction time pecans (22% reduced oil), and 0 to 0.56 ppm for 28% reduced-oil pecans (480 min extraction time) during 37 weeks of storage (Figure 1)

Figure 1: A graph showing the hexanal concentration during storage



The main effects of extraction level and storage duration, as well as their interaction on hexanal production, were statistically significant ($P \leq 0.01$). No significant differences in hexanal concentration were detected in any treatments until the 22nd week of storage, when full-oil pecans showed significantly higher levels of hexanal than 20 min extraction and 480 min extraction pecans. Also, at 18 weeks, hexanal levels in the full-

oil pecans rose above 6 ppm, determined by Hofland et al., (1995). At no times during storage were hexanal levels for extracted pecans above 6 ppm or statistically different from each other. For all storage times, except when hexanal levels for all oil reduction levels were negligible (up to 6 weeks of storage), mean hexanal levels were always higher for full-oil pecans. Reducing the oil content of pecans does have a positive effect on curtailing hexanal production and lengthening shelf life. There was no significant difference in the 20 min and 480 min extraction time, but both were lower than control.

Sensory Analysis

Samples for the 37th week were not evaluated because the full oil pecans were too rancid for ingestion. Only those characteristics that were statistically different in the study are discussed in the results.

Table 1 shows that the skin color darkened after the 10th week. The darkening of the skin color has been associated with quality deterioration (Woodroof, 1967; Senter et al., 1978). Processors and consumers associate light colored kernels with desirable quality so this has a great impact on the economic aspect of trade. There was no particular trend with the chalkiness, sour flavor or nutty flavor. Toasty flavor seemed to increase in the 32nd week of the storage.

Table 1: Treatment means for the characteristics* that were significantly different during the storage period

Storage Time (Weeks)	Skin Color♣	Chalky Texture⊗	Nutty Flavor⊗	Sour Flavor⊗	Toasty Flavor⊗
2	2.76^{bc}	2.24^{cd}	3.14^{abc}	1.80^{bc}	2.11^{c}
4	3.05^{abc}	1.96^{d}	2.36^{d}	1.86^{bc}	2.00^{cd}
6	2.53^{c}	3.33^{a}	3.17^{abc}	2.76^{a}	2.87^{ab}
8	3.29^{ab}	2.48^{bc}	2.97^{abc}	1.70^{c}	2.10^{c}
10	2.92^{bc}	2.45^{bcd}	2.63^{cd}	1.81^{bc}	2.07^{c}
14	3.37^{a}	2.66^{bc}	3.41^{a}	1.28^{cd}	2.31^{bc}
18	3.40^{a}	2.67^{bc}	2.82^{bcd}	1.29^{cd}	1.47^{de}
22	3.55^{a}	2.83^{ab}	2.93^{abc}	1.93^{bc}	2.57^{abc}
26	3.17^{ab}	2.90^{ab}	2.81^{bcd}	1.12^{d}	1.27^{e}
32	3.61^{a}	2.40^{bcd}	3.36^{ab}	1.73^{b}	3.10^{a}

* Means with the same letter in a column are not significantly different

♣ light tan 1 – dark 5

⊗ none 1 – strong 5

In Table 2, skin color was lighter for control and darker for the 20 and 420 min extracted pecan, indicating that the extracted pecan had a darker skin. The extracted pecans had a more chalky texture than the control pecan, because partial oil extraction left the kernel with a woody texture. The control pecan had a higher nutty flavor when compared with the pecans extracted for 480 min. The oil flavor was higher in the control pecan. The 20 min extraction had oil recovery of approximately 22% of the original oil content and 480 min extraction had approximately 28% of the original oil content.

Table 2: Treatment means of characteristics* that were significantly different for extraction time

Treatment Means	Skin Color♣	Chalky Texture⊗	Nutty Flavor⊗	Oily Flavor⊗
Control	2.80^{b}	2.20^{b}	3.20^{a}	3.25^{a}
20 min	3.30^{a}	2.72^{a}	2.91^{ab}	2.23^{b}
480 min	3.40^{a}	2.84^{a}	2.76^{b}	2.08^{b}

* Means with the same letter in a column are not significantly different

♣ light tan 1 - dark 5

⊗ none 1 - strong 5

In Table 3, the control pecan had higher scores indicating that the color was light yellow when compared to the whiter appearance of the pecan extracted for 20 min or 480 min presumably because of the higher oil content. Beginning in the 14^{th} week of storage the color tended to increase for the control pecan. It was not possible to identify a pattern for the extracted pecans.

Table 3: Treatment means of interior color*§π for different storage and extraction times

Storage Time (weeks)	Control	20 min	480 min
2	$2.70^{cd\clubsuit}$	$2.37^{a\clubsuit}$	$1.55^{bc\heartsuit}$
4	$3.68^{ab\clubsuit}$	$2.25^{a\heartsuit}$	$1.95^{ab\heartsuit}$
6	$2.80^{d\clubsuit}$	$2.31^{ab\clubsuit}$	$1.01^{c\heartsuit}$
8	$2.64^{cd\clubsuit}$	$1.47^{bcd\clubsuit}$	$1.17^{c\heartsuit}$
10	$2.42^{d\clubsuit}$	$1.47^{bcd\heartsuit}$	$1.84^{abc\heartsuit}$
14	$3.26^{abc\clubsuit}$	$1.33^{d\heartsuit}$	$2.02^{ab\heartsuit}$
18	$2.97^{bcd\clubsuit}$	$2.02^{abc\heartsuit}$	$2.39^{a\clubsuit}$
22	$3.46^{ab\clubsuit}$	$1.28^{d\heartsuit}$	$1.06^{c\heartsuit}$
26	$3.91^{a\clubsuit}$	$1.39^{bcd\heartsuit}$	$1.87^{abc\heartsuit}$
32	$2.91^{abc\clubsuit}$	$1.06^{d\heartsuit}$	$1.53^{bc\heartsuit}$

* Means with the same letter in a column are not significantly different

§ Means with same symbol in a row are not significantly different

π White 1- yellow tan 5

As shown in Table 4 control pecans were crisper than the extracted pecan. Length of storage had no consistent effect on crispiness.

Table 4: Treatment means of crispiness[*§π] for different storage and extraction times

Storage Time (weeks)	Control	20 min	480 min
2	$4.11^{a\clubsuit}$	$3.36^{a\heartsuit}$	$1.90^{c\spadesuit}$
4	$3.86^{ab\clubsuit}$	$2.69^{abc\heartsuit}$	$2.05^{bc\heartsuit}$
6	$4.51^{a\clubsuit}$	$2.87^{abc\heartsuit}$	$3.05^{ab\heartsuit}$
8	$3.60^{ab\clubsuit}$	$2.61^{abc\heartsuit}$	$1.58^{c\spadesuit}$
10	$3.38^{ab\clubsuit}$	$2.16^{c\heartsuit}$	$2.24^{bc\heartsuit}$
14	$3.92^{ab\clubsuit}$	$2.38^{bc\heartsuit}$	$2.15^{bc\heartsuit}$
18	$3.04^{b\clubsuit}$	$3.16^{ab\clubsuit}$	$3.03^{ab\clubsuit}$
22	$3.48^{ab\clubsuit}$	$2.34^{bc\heartsuit}$	$1.94^{bc\heartsuit}$
26	$3.33^{ab\clubsuit}$	$3.57^{a\clubsuit}$	$3.60^{a\clubsuit}$
32	$4.17^{a\clubsuit}$	$2.74^{abc\heartsuit}$	$2.93^{ab\heartsuit}$

* Means with the same letter in a column are not significantly different
§ Means with the same symbol in a row are not significantly different
π None 1 more 5

As shown in Table 5, rancid flavor was highest at weeks 22 and 32 for the control pecan whereas the mean scores did not increase significantly for the extracted pecans. Apparently reducing the oil content lowered the rancid scores. The decrease in rancidity at week 26 noted by panelists was consistent with chemical hexanal analysis (Figure 1) which showed a dip in concentration to 6 μg hexanal /g pecan at week 26 compared to 12 μg hexanal/g pecan at week 20 and 14 μg hexanal/g pecan at week 32. The high lipid content in pecan kernels complicates successful storage. The storage of pecans during retail distribution is often at ambient temperatures, resulting in rancidity which is associated with off-flavor development. The off-flavors associated with rancidity are

caused to a large extent by products of oxidative cleavage of polyunsaturated fatty acids (Erickson, 1993). Previous research indicates that the deterioration in fats and lipids is associated with a high free fatty acid content (Robertson et al., 1985). With decreased lipids there will be lower free fatty acid available for oxidation. It is also possible that the extraction process may have altered the lipids in pecans making them less susceptible to attack by oxygen. This will have major implications on retail sales.

Table 5: Treatment means of rancid flavor*§π for different storage and extraction times

Storage Time (weeks)	Control	20 min	480 min
2	$1.49^{d\clubsuit}$	$1.45^{c\clubsuit}$	$1.57^{ab\clubsuit}$
4	$2.15^{bcd\clubsuit}$	$2.00^{abc\clubsuit}$	$1.77^{ab\clubsuit}$
6	$2.96^{ab\clubsuit}$	$3.06^{a\clubsuit}$	$2.57^{a\clubsuit}$
8	$1.93^{bcd\clubsuit}$	$2.09^{abc\clubsuit}$	$1.69^{ab\clubsuit}$
10	$2.04^{bcd\clubsuit}$	$1.65^{bc\clubsuit}$	$1.47^{ab\clubsuit}$
14	$2.38^{bc\clubsuit}$	$1.46^{c\heartsuit}$	$1.36^{b\heartsuit}$
18	$1.82^{cd\clubsuit}$	$1.54^{bc\clubsuit}$	$1.47^{ab\clubsuit}$
22	$3.75^{a\clubsuit}$	$1.31^{c\heartsuit}$	$1.40^{b\heartsuit}$
26	$1.92^{bcd\clubsuit}$	$2.47^{ab\clubsuit}$	$1.72^{ab\clubsuit}$
32	$3.42^{a\clubsuit}$	$1.97^{abc\heartsuit}$	$1.39^{b\heartsuit}$

* Means with the same letter in a column are not significantly different
§ Means with same symbol in a row are not significantly different
π Least 1 – most 5

In Table 6, the acceptability scores decreased up to the 22nd week and increased for the 26th week and then again decreased for 32nd week for the control pecans. The scores remained the same or increased for the extracted pecan. The partial extraction of oil helped in retaining the quality of the pecans and increasing the shelf life of pecans. Similar results were observed by Kanamangala and coworkers (1999).

Table 6: Treatment means of acceptability*§π for different storage and extraction times

Storage Time (weeks)	Control	20 min	480 min
2	$3.76^{a\clubsuit}$	$3.31^{a\clubsuit}$	$2.44^{bc\heartsuit}$
4	$3.04^{ab\clubsuit}$	$2.97^{ab\clubsuit}$	$1.98^{c\heartsuit}$
6	$2.79^{abc\clubsuit}$	$2.06^{c\clubsuit}$	$2.01^{c\clubsuit}$
8	$3.11^{ab\clubsuit}$	$2.36^{bc\clubsuit}$	$2.37^{bc\clubsuit}$
10	$2.74^{bc\clubsuit}$	$2.06^{c\clubsuit}$	$2.63^{bc\clubsuit}$
14	$3.85^{a\clubsuit}$	$2.93^{ab\heartsuit}$	$3.03^{ab\heartsuit}$
18	$2.55^{bc\clubsuit}$	$2.58^{abc\clubsuit}$	$3.04^{ab\clubsuit}$
22	$2.09^{c\heartsuit}$	$3.25^{a\heartsuit}$	$3.81^{a\clubsuit}$
26	$3.46^{ab\clubsuit}$	$2.43^{abc\clubsuit}$	$2.71^{bc\clubsuit}$
32	$2.46^{bc\clubsuit}$	$2.59^{abc\clubsuit}$	$3.05^{ab\clubsuit}$

* Means with the same letter in a column are not significantly different
§ Means with same symbol in a row are not significantly different
π Least 1 – most 5

Conclusion

The supercritical CO_2 partial pecan oil extraction process seems to be promising for increasing shelf life and decreasing the fat content of high fat foods. The control pecans seems to undergo changes resulting increasing in rancid flavor. The extracted pecans did not have a significant change in rancid flavor. Supercritical fluid extraction method has been successfully used in a laboratory for a number of food products, mainly milk, meat, peanuts, barley, etc., to reduce fat content. These processes, if commercially viable, can have a tremendous impact on the industry where people are leaning towards low fat products.

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CHAPTER VI

EFFECT OF OXYGEN CONCENTRATION ON THE STORAGE OF PECAN

B. CHINTA, S. KNIGHT, B. STOECKER

Department of Nutritional Sciences
Oklahoma State University, Stillwater, OK 74078

N. O. MANESS

Department of Horticulture
Oklahoma State University, Stillwater, OK 74078

P. L. CLAYPOOL

Department of Statistics
Oklahoma State University, Stillwater, OK 74078

Abstract

The effect of oxygen content on supercritical fluid extracted pecans was studied. The pecans were extracted at 40 ℃ for 20 minutes reducing the content of oil by approximately 22% of the original amount. The pecans were packaged in bags which were made of 13 μ Saran coated mylar (polyester) and stored at 2%, 10% and 21% oxygen content with a mixture of nitrogen with 55% relative humidity in a dark place. Sample packages were selected every three months for evaluation (0, 3, 6, 12 months). The non extracted pecans developed rancid flavors much sooner than the extracted pecans. Storage time was the single most influential factor in causing rancidity and

lower acceptability. Under the conditions of this study oxygen levels did not seem to play a significant role in causing rancidity or lowering acceptability.

Introduction

Rancidity is defined as development of off-flavors that makes food unacceptable for the consumer and is generally a major problem in nuts during storage (Labuza, 1971). This off-flavor is due to lipid oxidation (Juan et al., 1996). It begins as a slow process; however, the human palate is very discriminating; and, when even as few as one in a thousand double bonds have reacted with oxygen, rancidity is already detectable (Berger and Hamilton, 1996). The detrimental effect of oxygen is both concentration and temperature dependent. Maintaining a relatively low oxygen concentration therefore, is much more critical at the retail level where the nuts are subjected to non-refrigerated storage and marketing conditions (Kays, 1987).

Pecan kernels, being comprised of live cells, require some oxygen for normal metabolic activity. Storage of kernels at very low oxygen concentrations is undesirable and has a very pronounced detrimental effect on quality (Kays, 1991). At very low oxygen partial pressures (less than 2%) anaerobic conditions occur, changing the direction of the flow of carbon in the respiratory pathways toward the production of ethanol and aldehydes, which give the kernels a distinct off-flavor. Packaging materials must be selected that allow some oxygen movement through the material into the interior to prevent anaerobic conditions from occurring. Without a continual low level of diffusion of oxygen into the package, the nuts, which utilize oxygen in their normal

respiratory process, pull the internal concentration downward eventually reaching an anaerobic concentration.

Upon leaving the wholesale storage environment, product temperature increases substantially, thereby making low oxygen environments of distinct value. The easiest and most economical method for maintaining the kernels under a low oxygen environment at this stage is through the use of retail packages in which there has been a partial replacement of oxygen with nitrogen. The use of nitrogen appears to be superior to carbon dioxide in replacing oxygen. The optimum oxygen concentration has not yet been established, but is thought to be in the 2% range at 21°C (Kays 1991). The primary problem is not the precise concentration, but is the fact that the conditions within the package are not constant. The more pecans within the package and/or the higher the temperature, the faster the oxygen is utilized. Most packages, however, are not completely impermeable to oxygen. As a consequence, a small amount of oxygen from outside the package moves across the surface into the package. The rate that external oxygen moves in is a function of the type of packaging material, the surface area of the package, the ambient temperature and the differential in the oxygen concentration between the exterior and interior of the package. The objective of this study was to study the effect of oxygen levels on the quality of pecans during storage (on both extracted and non extracted kernels).

Materials and Methods

Pecans

Oklahoma native pecan halves containing 65% oil by weight, that had been frozen (-20°C) since harvest, were allowed to warm to room temperature. Oil was extracted using supercritical Coleman grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) at 69 MPa and 40°C for 20 minutes to reduce oil content by approximately 22%. Supercritical fluid extraction (SFE) was conducted using a Dionex 703 extractor (Dionex Corp., Sunnyvale, CA) with four 50 ml extraction vessels holding approximately 15 g (16 halves) each. After extraction, only sound half kernels were selected for storage tests. Pecans were placed in airtight freezer bags and stored at –20°C until a sufficient quantity was extracted for the entire storage test.

Packaging and Storage

Pecans were taken from cold storage and allowed to thaw at room temperature for at least six hours. Ten pecan halves were randomly selected for initial value analyses. Pecans were then randomly distributed into 60 groups {2 extraction times (0 and 20 min extraction) x 5 storage times (0, 3, 6, 9, 12 months) x 3 levels of oxygen (2, 10, 21%) and x 3 reps of 10 halves each (A, B, C)}, weighed and then packaged at the same time as corresponding replicate packages from each of the SFE treatments. Packaged pecans were then stored in a environment chamber in the dark with circulated air at 25°C and

55% relative humidity. The kernels were placed into bags (approximately 15.2 cm X 15.2 cm) then evacuated from ambient pressure (98 kPa) to less than 0.3 kPa, back-flushed with a standard air mixture of three levels of 0_2 in N_2 (Air Products and Chemicals, Chicago, IL) to 88 kPa, and sealed in a Multivac-A316 vacuum packaging machine (Multivac, Inc., Kansas City MO). The bags used were made of packaging material consisting of 13 μ Saran-coated Mylar (polyester) laminated to 63.5 μ polyethylene (The Packaging Group, Woodbridge, Ontario). The water vapor and oxygen transmission rates, obtained from supplier data sheets, for this packaging material were 60 mg/100 cm^2·24 hr, and 90 mg/100 cm^2·24 hr respectively.

Eighteen samples were removed from storage at 0, 3, 6, 9 and 12 months analyzed for sensory properties. After the samples were taken out of storage, the samples were frozen for approximately two days. This was done so that all the panel members could be available for testing at the same time.

Sensory Analysis

The pecans were allowed to thaw in the bags for approximately 4 hrs at room temperature and then were taken out of the bag. Once the pecans were taken out, they were put in a dehydrator for one hour at 90°F to remove any moisture in the pecans. They were then chopped into small pieces and put in color coded containers and sealed in a vacuum bag to maintain dryness and given to the panelists. The panelists evaluated the pecans on the following criteria: appearance (outer and inner color of the kernel), texture (chalkiness and crispness of the kernels), pecan flavor (sweet flavor, nutty flavor, oily

flavor), off flavors (tannin, sour, rancid and toasted) and acceptability. A five-point scale was used to measure the characteristics. The skin color was evaluated where 1 was light tan to 5 very dark brown. The inner color was evaluated with 1 white and 5 yellow tan. The other characteristics were evaluated on a scale where 1 was none and 5 was most. Based on all the above characteristics an overall acceptability score was given where 1 was least acceptable and 5 was most acceptable.

The sample available was small so it was impossible to have a very large panel group. Ten pecan halves had to be chopped and divided among the panelists. Therefore, four panel members, from the faculty of Human Environmental College were selected and trained with the basic four flavors sweet, salt, sour and bitter. They were also given different concentrations of these flavors to see if they could detect basic flavors at threshold levels and detect concentration increases (Experimental Foods Manual, 1998). The panelists were also trained in detecting pecan flavor and were given different pecans ranging from very fresh to stale (not so fresh) to help familiarize them with a range of flavor changes in pecans.

Statistical Analysis

Proc GLM procedures were performed to determine effects of oil reduction and storage time on the sensory properties using the Statistical Analysis System (SAS Institute, Cary, NC). The data were analyzed as a split plot unit experiment in a randomized block design where the panelists were the blocks, extraction (yes or no) and oxygen (2, 10, 21%) levels were the main unit treatment factors and storage time (0, 3, 6,

9, 12 months) was the subunit treatment factor. A significance level of $P \leq 0.05$ was used. A test of effect slices was used to compare treatments.

Results and Discussion

The data were analyzed and only statistically significant ($P < 0.05$) data are discussed here. There was a significant interaction between time and treatment for some of the characters. Table 1 through 6 show the comparisons for each character interaction at different time and treatment.

For up to six months the interaction between extraction and time of storage was significant. However, for storage times greater than six months, there were no significant differences in mean skin color between extracted and control pecans (Table 1). The scores of extracted pecans were higher than the control indicating that the extracted pecans had a darker skin.

Table 1: Comparison of control (C) and extracted (E) pecans at each storage time for skin color$^{\Theta\delta}$

Storage Time for Control (C) and Extracted (E) Pecans$^{\spadesuit}$	Control	Extracted Pecans
0	$2.19\pm0.75^{*b}$	$3.95\pm1.20^{a\clubsuit}$
3	$3.18\pm1.10^{a*}$	$3.80\pm0.78^{a\clubsuit}$
6	$2.55\pm1.01^{*a}$	$3.53\pm0.93^{a\clubsuit}$
9	$3.25\pm0.99^{\clubsuit a}$	$4.04\pm1.08^{a\clubsuit}$
12	$3.33\pm1.71^{\clubsuit a}$	$3.41\pm1.27^{a\clubsuit}$

$\spadesuit$ The numbers correspond to the month when the sample was taken out of the storage
Θ Means with the same letter are not significantly different
δ Means with same symbol in row are not significantly different

In Table 2, the interior color for control and the extracted pecans were significantly different through out the storage period except for the third month. The control pecans had a more yellowish color and the extracted pecans had a cream color. The pecan oil has a dark yellow color and a reduction in the oil makes the kernel less yellow in color.

Table 2: Comparison of control (C) and extracted (E) pecans at each storage time for interior color[Θδ]

Storage Time for Control (C) and Extracted (E) Pecans[♠]	Control	Extracted Pecans
0	2.71±1.33[*b]	1.97±0.81[a♣]
3	2.92±1.21[*ab]	2.39±1.20[*a]
6	3.30±0.96[*a]	2.44±1.33[a♣]
9	3.50±1.14[*a]	1.33±0.87[b♣]
12	3.55±1.19[*a]	2.53±1.18[a♣]

♠ The numbers correspond to the month when the sample was taken out of the storage
Θ Means with the same letter are not significantly different
δ Means with same symbol in row are not significantly different

The crispiness of the pecan was not significant at different times of the storage period except for the six month and the twelve month periods (Table 3). The reason for this can be attributed to a) the sample was small so the panel was not able to accurately analyze the pecans, b) the pecan absorbed some moisture from the surrounding atmosphere. The control pecans had a higher scores than the extracted pecans. Anzaldua-Morales and coworkers (1998) observed that fracturability measured by the Instron Analyzer was 21% lower for the extracted pecan when compared to the control. Fracturability is the closet objective test for comparing crispiness.

Table 3: Comparison of control (C) and extracted (E) pecans at each storage time for crispiness[Θδ]

Storage Time for Control (C) and Extracted (E) Pecans[♠]	Control	Extracted Pecans
0	3.56±0.98[*b]	3.50±1.14[*a]
3	4.59±0.54[*a]	4.33±0.64[*a]
6	4.05±0.95[*a]	3.12±1.33[♣a]
9	4.29±0.81[*a]	4.04±1.04[*a]
12	4.55±0.61[*a]	2.82±1.07[♣b]

♠ The numbers correspond to the month when the sample was taken out of the storage
Θ Means with the same letter are not significantly different
δ Means with same symbol in row are not significantly different

A reduction in the oil content gave the extracted pecan a fibrous structure, which was attributed as chalkiness. The control was significantly different from the extracted pecan except after six and twelve months of storage (Table 4). The extracted pecans had a higher chalky structure than the controls.

Table 4: Comparison of control (C) and extracted (E) pecans at each storage time for chalkiness[Θδ]

Storage Time for Control (C) and Extracted (E) Pecans[♠]	Control	Extracted Pecans
0	1.58±0.75[*ab]	2.64±1.26[♣ab]
3	2.03±1.52[*a]	2.82±1.63[♣a]
6	1.77±1.29[*ab]	2.44±1.39[*ab]
9	1.17±0.38[*b]	2.83±1.34[♣a]
12	1.94±1.26[*ab]	2.00±1.00[*b]

♠ The numbers correspond to the month when the sample was taken out of the storage
Θ Means with the same letter are not significantly different
δ Means with same symbol in row are not significantly different

The oily perception was significantly different between control an extracted pecan for the first six months and then the differences reduced (Table 5). The control pecans had higher oily perception scores than the extracted pecans. The reason for this may be that the oil flavor was masked by the onset of rancid flavor. Kanamangala and coworkers (1999) observed that the lipid oxidation was noticeable from 18th week on wards for the control and 22nd week onward for the extracted pecan.

Table 5: Comparison of control (C) and extracted (E) pecans at each storage time for oily perception$^{\Theta\delta}$

Storage Time for Control (C) and Extracted (E) Pecans$^{\spadesuit}$	Control	Extracted Pecans
0	3.39±1.26^{*a}	2.00±0.78$^{\clubsuit b}$
3	0.35±1.42^{*ab}	2.20±0.77$^{\clubsuit b}$
6	3.86±0.84^{*a}	3.03±1.19$^{\clubsuit a}$
9	2.25±1.62^{*b}	1.83±1.05^{*b}
12	2.78±1.86^{*ab}	2.88±1.87^{*ab}

♠ The numbers correspond to the month when the sample was taken out of the storage
Θ Means with the same letter are not significantly different
δ Means with same symbol in row are not significantly different

There was a significant difference in toasted flavor between the control and the extracted pecans, although there was no set pattern (Table 6). The reason may be that the panel did not ingest as soon as the sample was given to them. As the sample is exposed to the regular room temperatures for longer period of time it may pick the moisture and other aromatic flavors from the surroundings thus losing its original flavor.

Table 6: Comparison of control (C) and extracted (E) pecans at each storage time for toasted flavor[Θδ]

Storage Time for Control (C) and Extracted (E) Pecans[♠]	Control	Extracted Pecans
0	1.54±0.51[*c]	1.56±0.59[*b]
3	1.33±0.63[*c]	2.05±1.62[♣b]
6	2.05±1.61[*b]	1.68±1.04[*b]
9	2.62±1.61[*b]	2.00±1.06[♣b]
12	4.55±0.85[*a]	3.52±1.07[*a]

♠ The numbers correspond to the month when the sample was taken out of the storage
Θ Means with the same letter are not significantly different
δ Means with same symbol in row are not significantly different

From the data analyzed it was observed that levels of oxygen did not significantly affect the storage properties of the pecan. The data shown in Table 7 gives an overall indication of rancid flavor and acceptability scores for three oxygen levels.

Table 7: The effect of oxygen levels on rancidity and acceptability (Mean ± Sem)*

Oxygen Level (%)	Rancidity		Acceptability	
	Control	Extracted	Control	Extracted
2	2.27 ± 1.31[a]	1.73 ± 1.05[a]	2.89 ± 1.50[a]	2.81 ± 1.11[a]
10	2.20 ± 1.42[a]	1.90 ± 1.19[a]	2.71 ± 1.37[a]	2.57 ± 1.20[a]
21	2.26 ± 1.41[a]	1.76 ± 1.07[a]	2.76 ± 1.07[a]	2.58 ± 1.13[a]

* Means with same letter in a row for rancidity/acceptability are not significantly different

The rancid scores of control were higher when compared to the extracted pecans, indicating that the reduced oil in the pecan kernel slows down rancidity process. Similar results were observed by Whitelock and coworkers (1996). The acceptability scores were

similar for both control and extracted pecans. The rancidity and acceptability scores were not significantly different for the control and extracted pecans.

Conclusion

Time had a greater effect on the development of rancidity than did oxygen levels, and the extracted pecans developed rancidity at a much lower level. There were significant differences between the control and extracted pecans in skin color and interior color. Chalkiness and crispiness characteristics showed that there was significant difference between control and extracted pecans. The oily perception of the control and extracted pecan were significantly different for up to six months. The results would have been more precise if larger samples and a larger sensory group were possible. The overall acceptability decreased with time. Oxygen levels had no significant impact on pecan characteristics studied (control and extracted).

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CHAPTER VII

TRANSMISSION AND SCANNING ELECTRON MICROSCOPY OF PECANS BEFORE AND AFTER SUPERCRITICAL EXTRACTION PROCESS

B. CHINTA, S. KNIGHT

Department of Nutritional Sciences
Oklahoma State University, Stillwater, OK 74078

G. BAKER

Electron Microscopy Laboratory
Oklahoma State University College of Osteopathic Medicine
Tulsa, OK, 74107

Abstract

Pecans (Carya illinoensis) are nuts very rich in oil ranging from 55 to 70%, with a high degree of unsaturated fatty acids. Oil content of the pecans has been reduced by approximately 22 - 28% using a supercritical fluid extraction (SFE). The objective of this study was to determine the effect of SFE on the structure of the pecans using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Four samples i.e., control pecans, pecans extracted at 40 ℃ for eight hours, pecans extracted at 80 ℃ for one hour and pecans extracted in a commercial pilot plant at 40 ℃ for two hours were used. The SEM micrographs showed blisters on the extracted pecans probably caused by CO_2 exit during the depressurization extraction phase. The

TEM micrographs showed that the SFE process destroyed the cell structure allowing the fat bodies to escape outside the cell structure.

Introduction

Recently there has been a great emphasis on the microstructure of food ingredients. The microstructure of oil-bearing seeds is in many respects similar to many other seeds. Some species of oil bearing seeds contain oil in quantities large enough to be extracted economically. They are a very important commercial crop. The use of scanning electron microscopy in recent studies of food structure has been an invaluable technique in providing excellent information about the dynamic structure of plant food products, requiring minimal sample (Swanson et al 1985).

Increasingly, the microscope is being used to study the influence of ingredients and processing conditions on food structure, especially in the development of new food products. By showing the distribution and physical state of specific food constituents, particularly starches and fats, the microscope can give a visual explanation as to why foods of similar chemical constitution have markedly different textures. It is important to preserve what is often a fragile structure, especially when it is the precise nature of that structure which is of interest. Histological methods have evolved to suit intact tissues which can withstand fixation, wax embedding and lengthy staining procedures. Any one of these stages may alter a food product and so the information that is subsequently obtained can be totally misleading. It is an important principle in food microscopy that the less done to the specimen the better (Flint, 1994)

The elongated pecan nut is approximately 35 mm long with a thin shell (endocarp) and has a smooth surface. The cotyledons are divided and possess an irregular surface, and the nut is four celled (endocarp, testa, endosperm and embryo). The pigment that gives color is present in the testa. The endosperm, represented by a single layer of cells, is made of small aleurone grains and oil bodies. The parenchyma cells of the cotyledon have intercellular spaces and contain aleurone grains and oil bodies and a minute amount of starch (Vanghan, 1970). The objective of this study is to determine the structural differences of the pecan kernels before and after the SFE process using TEM and SEM procedures.

Materials and Methods

Pecans were obtained from Young Pecan company (Florence, SC). There were four treatments used for both TEM and SEM. One treatment was extracted at 40°C for 8 hours, a second was extracted at 80°C for one hour using a Dinex 703. Treatments were subjected to the supercritical extraction procedure at the Oklahoma State University laboratory. A third sample was extracted at a pilot plant (Flavex, Rhelengen, Germany) at 40°C for 2 hours. There was also a control treatment (non extracted pecan).

Preparation for TEM

Samples from the different treatments were thawed to room temperature. The samples were taken from the middle of the kernel and cut into 1 mm^3. The tissues were

immediately fixed in a buffered 8% glutaraldehyde . The pH was adjusted to be approximately 7. The vials were placed under vacuum for 2 hours at 23°C. The tissue was washed three times in buffer for approximately 20 minutes each time. The samples were post fixed in 1% osmium tetraoxide mixed with 1:1 phosphate buffer. The samples were washed for 20 minutes in water before dehydration. The samples were dehydrated in 50, 70, 90, 95, 100, 100, 100% of ethanol for 20 minutes in each solution. The samples were washed in propylene oxide three times each for 20 minutes each time, and were embedded in 1:1 propylene oxide and Poly/Bed (in capped vials under the hood) overnight. The vials were uncapped and placed in a vacuum desicator for seven hours. The samples were labeled and embedded in 100% embedding medium (Spurrs), and placed in a vacuum oven at 60-70°C for 24 hours to harden.

Glass knives were made using a LKB 2208 Multiplate. Using the knives in a ultramicrotomy (Porter-Blum MT-2) (Appendix II), thin sections were cut (60 -100 μm). The sections were between gray and silver color. Approximately five sections were picked on to the dull side of the grid. The grids were then stained with uranyl acetate and later with a lead stain. The sections were then examined with a JEOL JEM-100CX transmission electron microscope (Appendix III).

Preparation for SEM

Four samples were fixed in 1.6% glutaraldehyde and in 0.1 M cacodylate buffer (room temperature) for two hours. The samples were rinsed three times in buffer for approximately 20 minutes each time; they were then placed in 1% O_SO_4 (osmium

tetroxide) in 0.1M cacodylate buffer (room temperature). The samples were again rinsed three times in buffer for approximately 20 minutes. The pecan samples were then dehydrated in five concentrations of ethanol solution i.e., 50, 70, 90, 95 and 100%. The dehydrated samples were critical point dried in a Denton DCP-1 apparatus (Appendix IV) using liquid CO_2. Dried samples were mounted on aluminum specimen stubs with a double sided tape and silver conducting paint. The stubs with the specimens were then coated with 30 nm gold-palladium alloy. The samples were then examined with a JOEL 800S scanning electron microscope (Appendix V).

Results and Discussion

SEM: Pecan Kernel

The outer surface of the control pecan kernel was rough with wax deposit (Figure 1) that has been left behind on the kernel after shelling. The kernel of the pecan had characteristic pits and cracks made by the wax deposits. Similar findings were observed by Engquist and Swanson (1992) when working with Adzular bean.

Blisters were seen on the surface of the pecans extracted at 40°C and 80°C. During the SFE process, the epidermal cell of the outer and inner surface became swollen as a result of heating of cellular contents and poc marks appeared as a result of the escape of internal steam and oil released from the lipid bodies. Similar results were observed in roasted peanuts Young and Schadel, (1993). The pecans extracted at 80°C had smaller blisters when compared to the pecans extracted at 40°C. The reason for smaller blisters

was the longer the heating time and exposure to high pressure the greater the disruption of the exterior structure networks (Figures 3 and 4). The pecans that were extracted in a commercial pilot plant at 40°C for two hours had evenly distributed small blisters (Figure 2). Apparently the larger equipment and volume used in the pilot plant provides a less harsh process then that of laboratory extraction. Also in the commercial process 4200 psi was used versus 10000 psi in the lab. Both time and pressure reduction ware much greater commercially than with the lab equipment.

TEM: Pecan Kernel

The TEM micrographs showed cell-to-cell junctions which were characterized by a distinct lamella existing between the parenchymal cells of the control pecans (Figure 5). A similar system was observed by Young and Schadel (1990) while studying peanuts. The parenchymal cells of the mid region of the raw pecan cotyledon contained a cytoplasmic network that surrounded the subcellular organelles which included starch and protein bodies and the spaces (the gray and white spaces) once covered by the oil bodies (the lipids were removed during the alcohol dehydration during the specimen preparation).

The supercritical extraction process put a lot of pressure on the pecan kernel so a lot of disruption in the cell structure was seen. The TEM of pecan kernels that were subjected to 40°C for eight hours showed a total loss of cellular organization. The cell wall was broken allowing the oil bodies and the other cellular organelles to disorient (Figure 7). The TEM of the pecan kernels extracted at 80°C for one hour showed broken

cell walls in two sides. The contents of the cell were more or less intact (Figure 8). Perhaps the longer the kernels were exposed to high pressure the greater was the destruction. Therefore the kernels extracted at 40°C for eight hours had greater disoriented cell structure than the kernels that were extracted at 80°C for two hours. However the TEM of kernels extracted in a commercial pilot plant (40°C for two hours) showed less disorientation when compared to the kernel extracted at the university laboratory (Figure 6). This indicates that the process in the pilot plant was more uniform or in some way less destructive of the pecan cells.

Conclusion

The process of supercritical fluid extraction does alter the microstructure of the pecan kernels. The SEM micrographs showed blisters on the extracted kernels and the TEM micrographs showed that the structure has been disrupted because of the supercritical extraction process. However the pilot plant extraction was less destructive than the laboratory extractions.

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Figure 1: SEM of the control

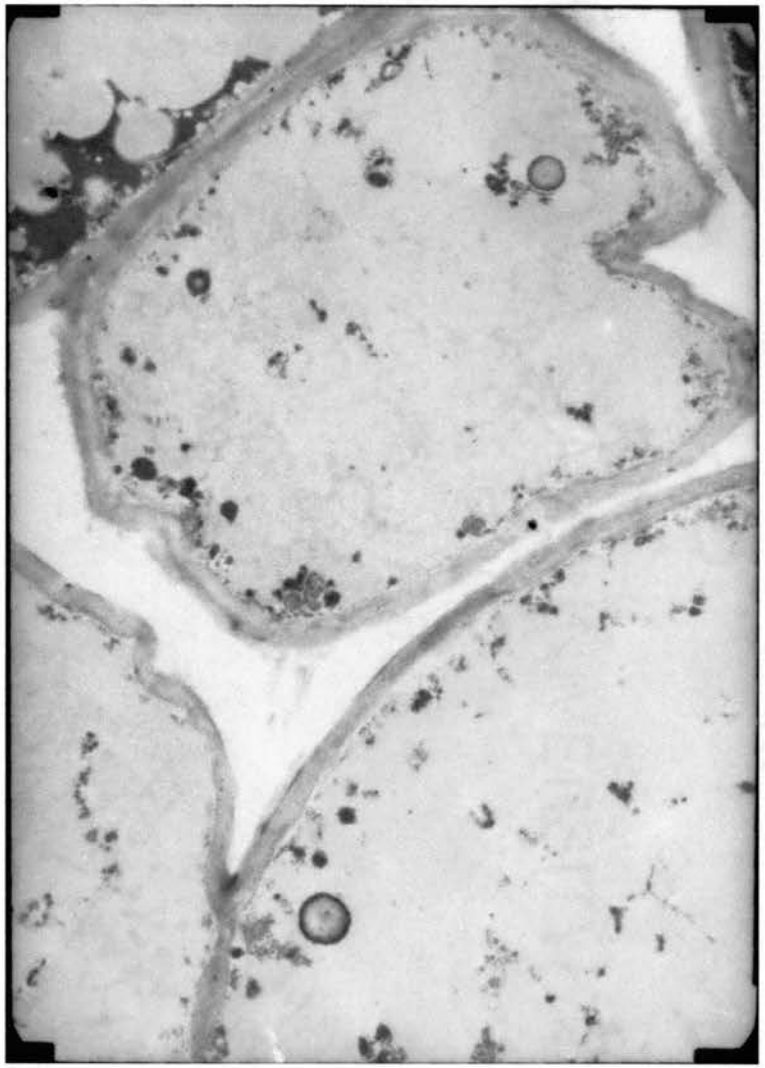

Figure 2: SEM of the commercial extracted pecan

Figure 3: SEM of the pecan extracted at 40° C

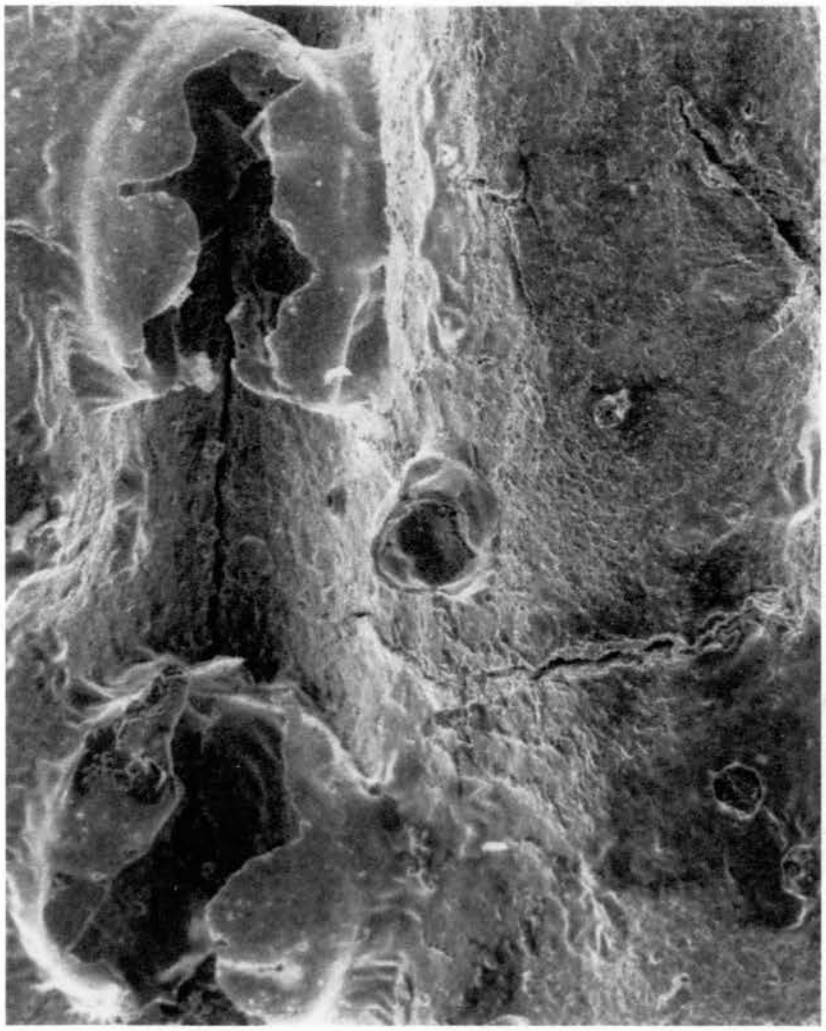

Figure 4: SEM of the pecan extracted at 80° C

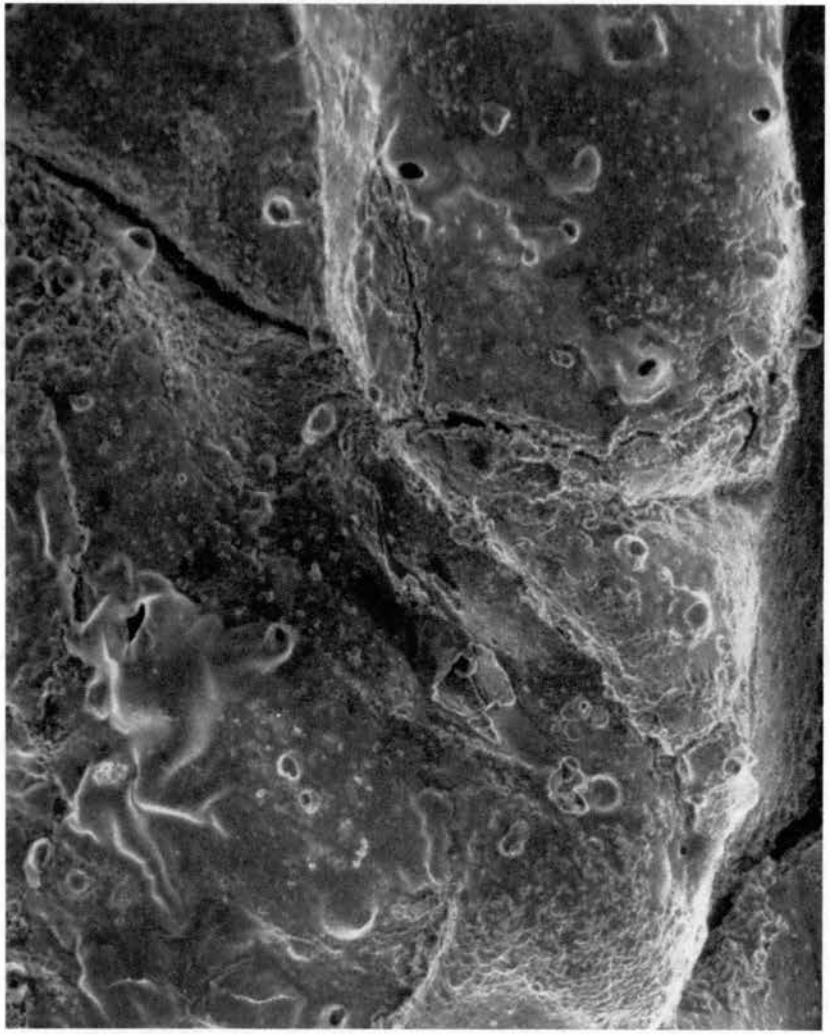

Figure 5: TEM of the control

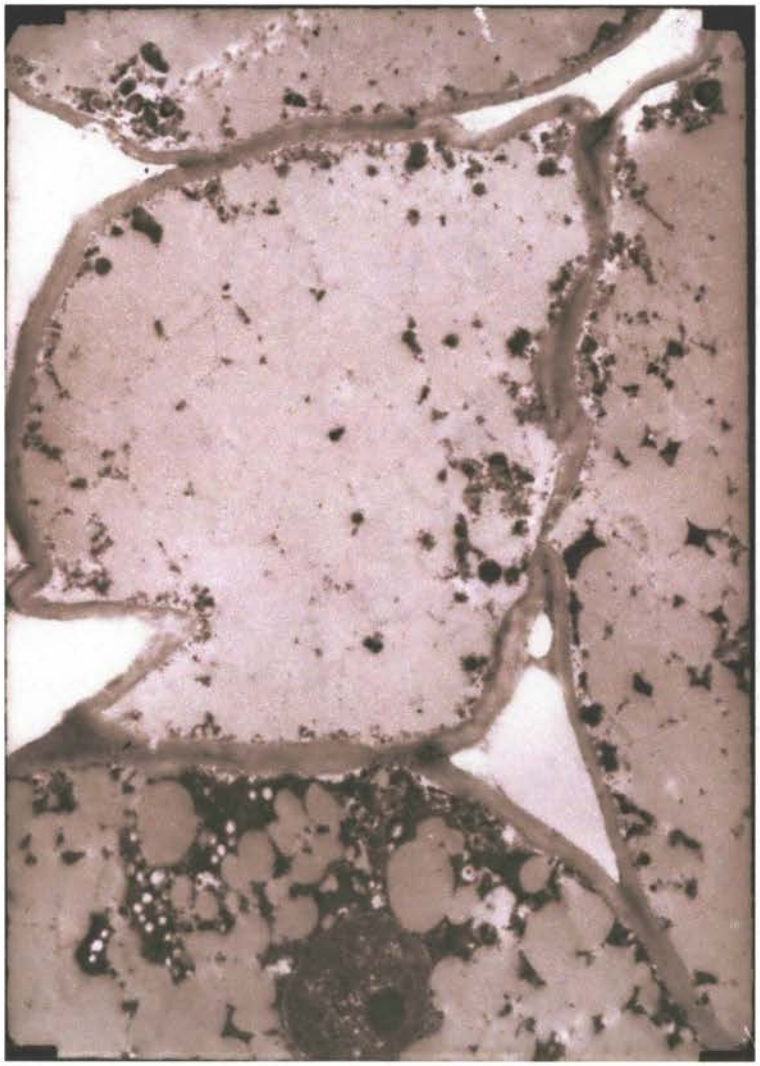

Figure 6: TEM of the commercial extracted pecans

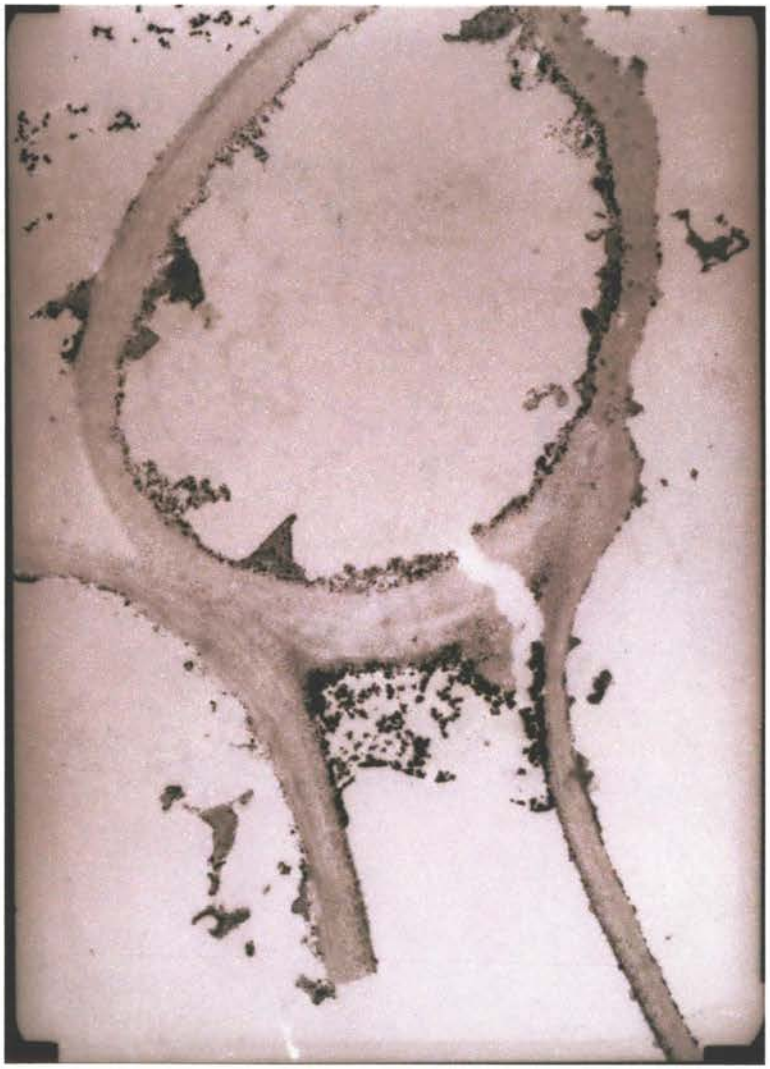

Figure 7: TEM of the pecan extracted at 40° C

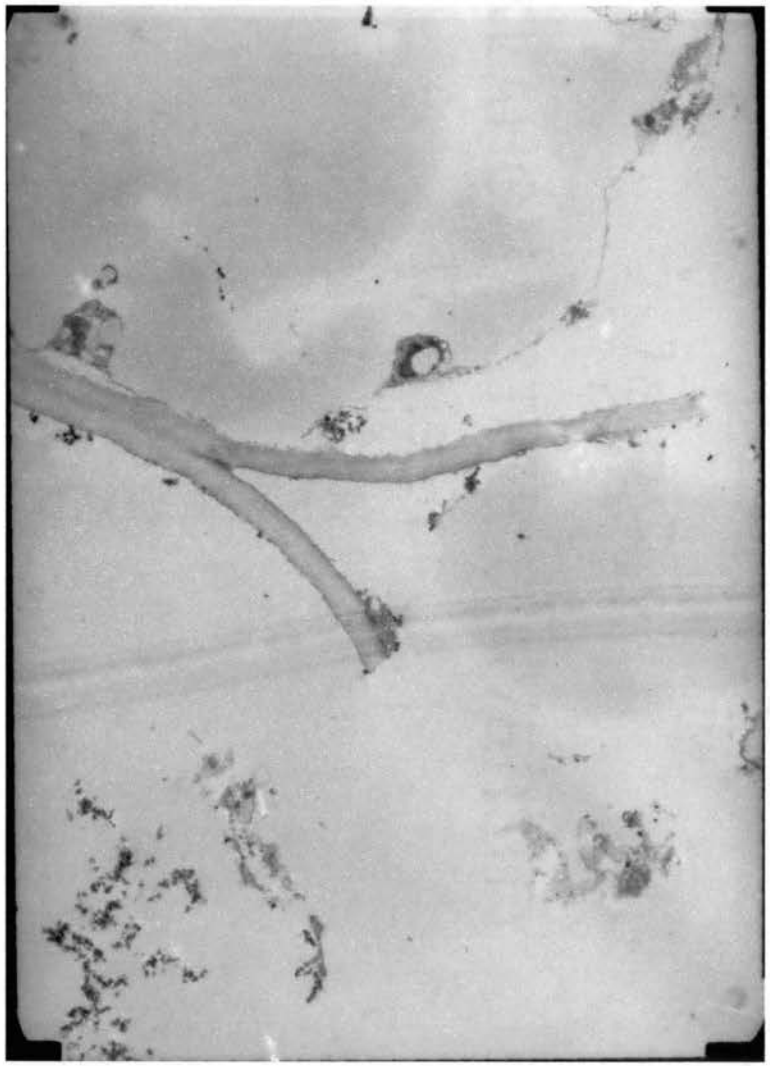

Figure 8: TEM of the pecan extracted at 80° C

CHAPTER VIII

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that supercritical fluid extraction (SFE) of pecans using CO_2 lowered the fat content and extended the unrefrigerated shelf life by several weeks. This has great economic advantages because suppliers need to have a shelf life of at least six months to assure that the product can move through the distribution system and sit on the shelf before purchase and still be an acceptable product for the consumer. Further, partial removal of the fat, reduces the caloric value of a given volume of pecans, thus increasing their appeal for the growing number of weight-conscious consumers. Oxygen levels during storage study did not have as great an effect as storage time in development of rancid flavors. However, extraction of oil had a greater reduction in rancidity. This indicates that reduction of oil content could have a greater economic impact than packaging material for room temperature storage. Studies with larger amounts of samples for testing could allow the use of larger panels. This would allow further refinement of sensory findings in the experiment.

The electron microscope studies clearly showed that the extraction process was quite disruptive to the internal tissues. Apparently the longer the time the kernel was subjected to the pressure the greater the damage. The surfaces of the nutmeats extracted in the pilot plant were more uniform in apparent surface texture with little disruption of the surface as compared to the kernels extracted in the small laboratory extractor. The

larger extraction vessels seemed to produce a more uniform, less damaged product. This is very beneficial, since commercial scale extraction would be in large volumes.

A recommendation for further study is that more work be done to see if a slower reduction of pressure in a controlled study would decrease the amount of disruption to the internal tissues of the pecan meats.

In conclusion, one of the most important findings in this research was that hexanal analysis closely followed sensory detection of rancidity. This could be very important in the development of a sensitive objective and chemical test for rancidity.

A recommendation is that further recipe development should be done to incorporate the defatted pecans into common foods. Also consumer information such as calorie charts showing the calorie reductions, and the calculations involved in changing the weights in recipes if measurements are being done by weight, (since a given volume of defatted pecans would weigh less after the extraction) are needed as well. However, recipes based on volume would require no changes. But, the extracted kernels are more fragile, as confirmed by EM studies, so procedures followed in preparing recipes might need adaptation.

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APPENDIXES

APPENDIX I

FLAVOR DESCRIPTORS FOR EVALUATION

Appearance

Skin Color	1 Light tan	2	3	4	5 Dark tan
Interior Color	1 White	2	3	4	5 Yellow tan

Texture

Chalky	1 None	2	3	4	5 Most
Crispiness	1 Soggy	2	3	4	5 Crispy

Pecan Flavors

Sweet	1 Least	2	3	4	5 Strong
Nutty	1 Least	2	3	4	5 Strong
Oily	1 Least	2	3	4	5 Strong

Off Flavors

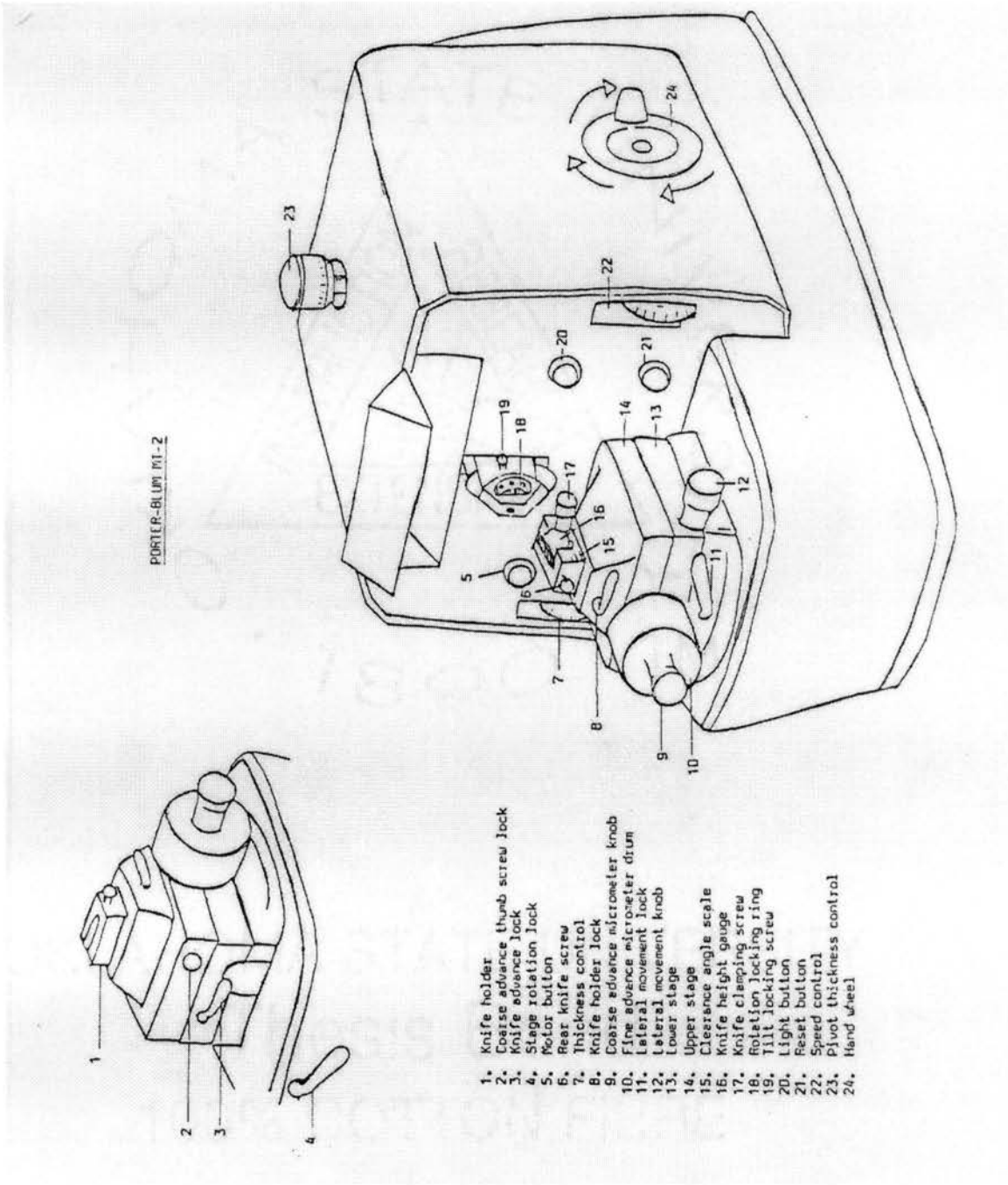
Tannin	1 Least	2	3	4	5 Strong
Sour	1 Least	2	3	4	5 Strong
Rancid	1 Least	2	3	4	5 Strong
Toasted	1 Least	2	3	4	5 Strong

Overall Acceptability

	1 Least	2	3	4	5 Most

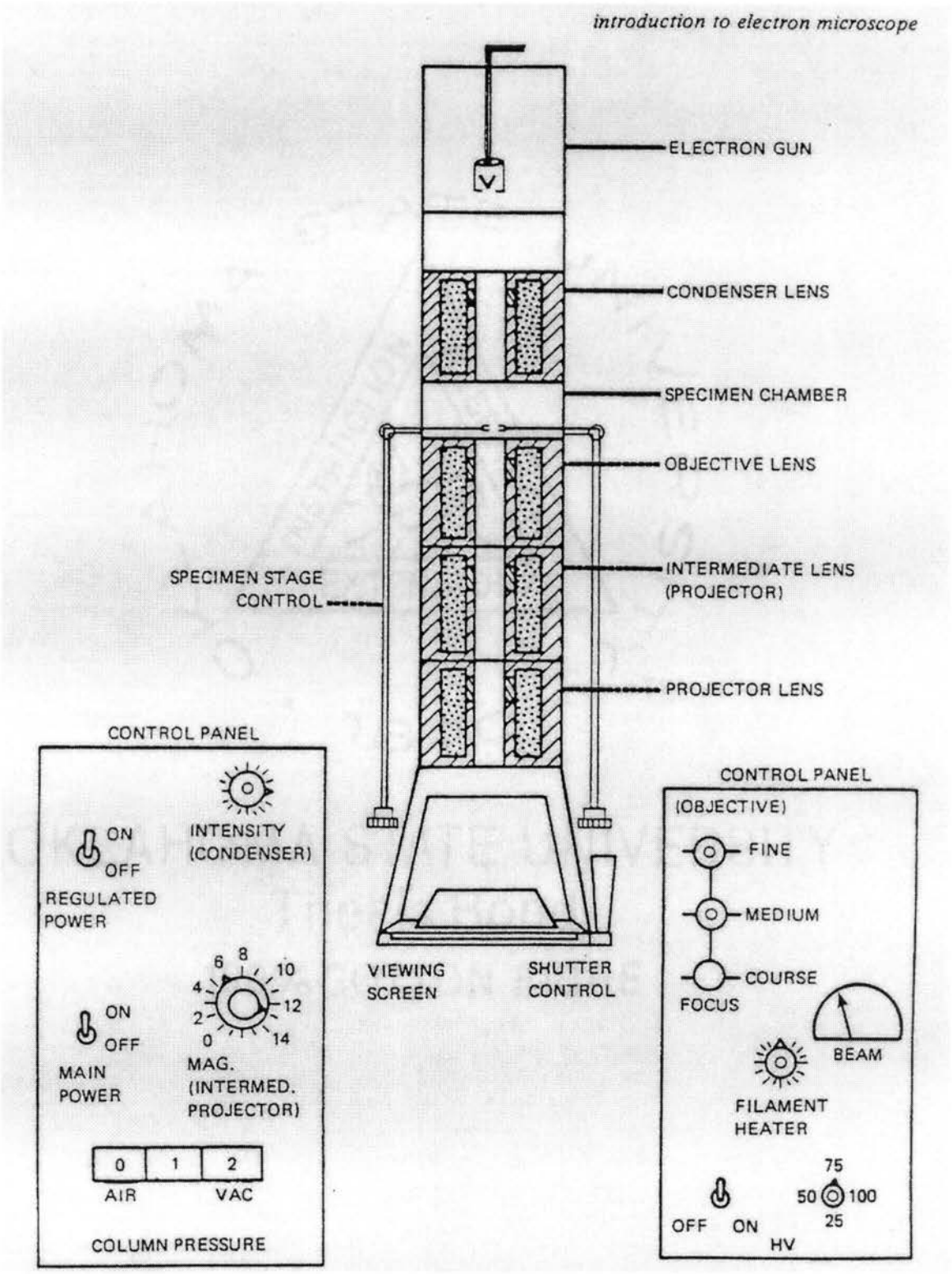
APPENDIX II

PORTER-BLUM MT-2
(ultramicrotomy)



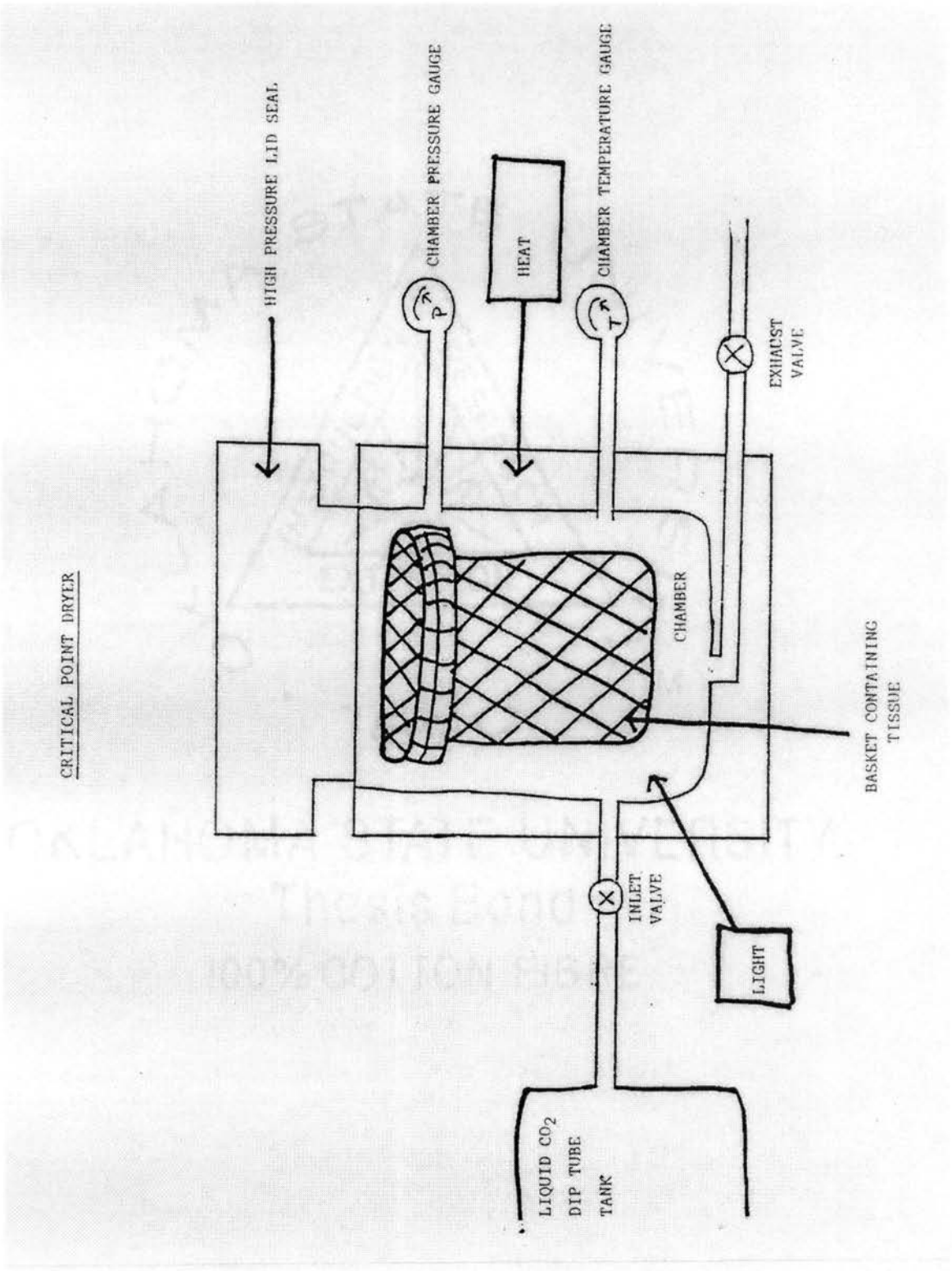
APPENDIX III

ELECTRON MICROSCOPE



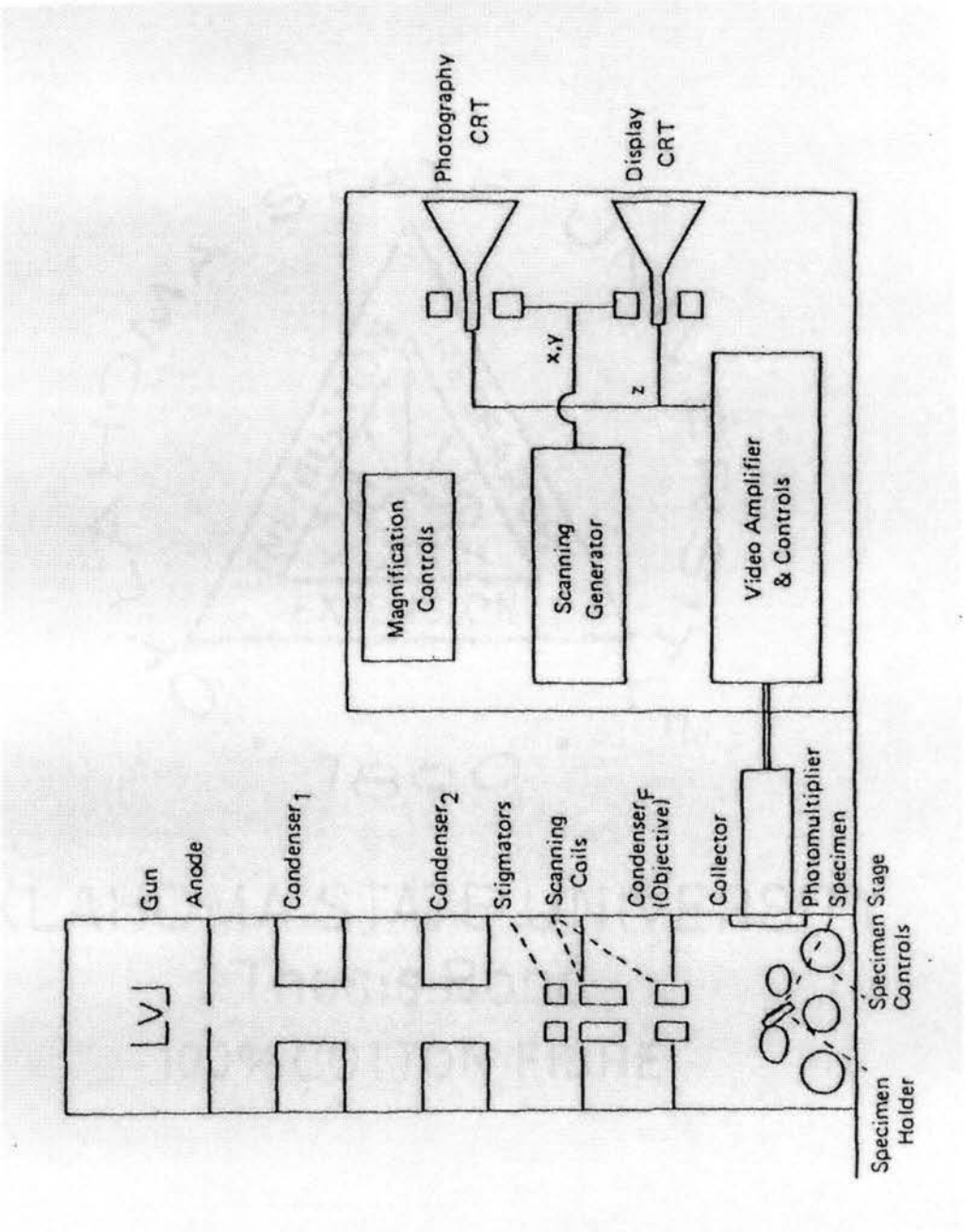
APPENDIX IV

CRITICAL POINT DRYER



APPENDIX V

SCANNING ELECTRON MICROSCOPE



APPENDIX VI

INSTITUTIONAL REVIEW BOARD APPROVAL

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 07-19-95 **IRB#:** HE-96-003

Proposal Title: SENSORY EVALUATION OF ADVANCED FOOD COMPANY INCORPORATED FLAVORED CHICKEN BREASTS

Principal Investigator(s): Sue B. Knight, Debessu Tideg, Bhaggi Chinta

Reviewed and Processed as: Exempt

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING.
APPROVAL STATUS PERIOD VALID FOR ONE CALENDAR YEAR AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Reasons for Deferral or Disapproval are as follows:

Signature: _____ Date: July 19, 1995

Chair of Institutional Review Board

VITA

Bhagyalakshmi Chinta 2

Candidate for the Degree of

Doctor of Philosophy

Thesis: THE EFFECT OF SUPERCRITICAL FLUID EXTRACTION ON THE SHELF LIFE AND STRUCTURE OF PECANS

Major Field: Food Science

Biographical:

Personal Data: Born in Andhra Pradesh, India, the daughter of Subba Reddy and Subbalakshmi on January 27, 1961. Married to Dr. E. S. Reddy and have three daughters, Sujata, Nirmala, and Taruna, and a son, Sriranga.

Education: Graduated from Kendriya Vidyalaya, I.I.T, Kharagpur, India in June 1980; received Bachelor of Science degree in Home Science in 1983 and a Master of Science in Home Science in 1988. Completed the requirements for Doctor of Philosophy with a major in Food Science at Oklahoma State University in July 2002.

Experience: Graduate research associate, Oklahoma State University, Dept. of Nutritional Sciences, Sept. 1993 to Dec. 1996. Graduate teaching assistant, Oklahoma State University, Dept. of Nutritional Sciences, Sept. 1993 to Dec. 1996.

Professional Memberships: Institute of Food Technology, American Society for Quality Control.