LIPID CLASS CHANGES ASSOCIATED WITH OXIDATION AND DEVELOPMENT OF RANCIDITY IN PECANS

(*Carya illinoensis*)

BY

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1993

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1997

OKLAHOMA STATE UNIVERSITY

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Thesis Approved:

Thesis Advisor

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ACKNOWLEDGMENTS

I owe a very special thank to Dr. Neils O. Maness, my thesis advisor for his constant guidance, support and encouragement throughout my graduate program. I am specially grateful for his patience, sense of fairness and helpfulness that I will always remember. I am thankful to the other members of my graduate committee, Dr. Michael W. Smith and Dr. Gerald H. Brusewitz for serving on my committee. I would also like to thank Donna for her assistance in this study.

I would like to express my sincere gratitude to all those persons affiliated with the Department of Horticulture and Landscape Architecture for making this department such a comfortable and rewarding place to study. I am also very grateful to the OCAST for funding which made this project possible.

I want to express my sincere appreciations to my very special friends, especially Satish for keeping me focused.

Words cannot adequately express my heartfelt gratitude to my mother Chudamani, and also my brother Shashi and sister Suvarna for their support and encouragement. I would like to give my special appreciation to my wife, Usha, whose faith and love provided me with strength and confidence throughout this whole process.

Finally, I would like to dedicate this work to the memory of my father, Viswanath.

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CHAPTER I

INTRODUCTION

The new food pyramid encourages consumption of fruits, vegetables, and nuts for a nutritionally balanced diet (USDA,1992). From 1987 to 1992, 6 percent of all new food products introduced contained nuts. In the United States the production and consumption of nuts such as walnuts, pecans, almonds, macadamia nuts, hazel nuts, and pistachios was much greater in 1994 than in former years (USDA, 1995). The total tree nut production has increased from 445 million pounds (shelled basis) in 1975-76 to 1.1 billion pounds (shelled basis) in 1994-95, an increase of 144 %. Per capita consumption of tree nuts has increased from 1.94 pounds per year in 1975-76 to 2.27 pounds per year in 1994-95, an increase of 17% and pecans take the second place in per capita consumption (0.48 pounds) behind almonds (0.55 pounds). This is evidence of the growing importance of nuts in the everyday diet. As of 1990 the tree nut industry in particular (pecans, hazel nuts, walnuts, macadamia nuts, pistachios, and almonds) has grown by 8.3% with an average worth of $1.3 billion. The pecan market share is about 18%. The greatest actual and potential use of tree nuts will continue to be in baking, confectionery and salting trades which now use the bulk of them.

Pecans are a good source of arginine rich proteins, a fair source of vitamins, contain very low quantities of saturated fats and are cholesterol-free, low in sodium and high in unsaturated fatty acids (National Pecan Shellers Association (NPSA), 1988). From a nutritional standpoint, polyunsaturates have been desirable for their role in

lowering the risk of coronary diseases (Fraser et al., 1992; Sabate, 1993). Human diets containing oils high in monounsaturates were as effective in lowering serum cholesterol levels as were low fat diets (Grundy, 1986). As a primary component of olive oil, monounsaturated fat forms the corner stone of the "Mediteranean diet".

The 1996 pecan crop was 227 million pounds (in-shell basis), 18% below the 1995 crop (USDA, 1997). Oklahoma is eighth in total pecan production (4 million pounds which is 375% below the 1995 crop), behind Georgia (90 million pounds), Texas (45 million pounds), New Mexico (24 million pounds), Lousiana and Arizona (18 million pounds), Alabama (15 million pounds) and South Carolina (5 million pounds). Mainly native pecans are grown in Oklahoma. The pecan production is very irregular leading to surpluses and low prices during 'good' production years and shortages and high prices during 'poor' production years.

From the time of maturation, pecans begin to undergo a progressive series of largely irreversible chemical changes many of which are detrimental to the quality of the marketed product (Kays, 1987). These changes and the rate at which they occur are a function of both the unique composition of the pecan and the pre- and post harvest handling, storage and marketing conditions to which the nuts are exposed.

The major constituent of pecans is oil, accounting for 55 to 70 percent of the kernel weight (Worley, 1994). The major lipid classes found in pecans are triglycerides (three fatty acids attached to a glycerol backbone), diglycerides (two fatty acids attached to a glycerol backbone), monoglycerides (one fatty acid attached to a glycerol backbone), free fatty acids and phospholipids (one or two fatty acids plus a phosphorylated form of choline, serine, ethanolamine or inositol attached to a glycerol backbone)(Santerre, 1994).

Triglycerides compose 95% of the lipids present in pecans (Senter and Horvat, 1976). In all the lipid classes the main fatty acids are oleic, linoleic, palmitic, stearic, and linolenic. Pecan oil fatty acid composition is very high in mono-unsaturated fat (65 percent, oleic-one double bond). The closest competitors are new high oleic peanut lines (Sunoleic 95R, 80 percent-oleic) and olive oil (65-70 percent-oleic). Di and tri unsaturated fatty acids (linoleic-two double bonds and linolenic-three double bonds, respectively) make up an additional 25 to 27 percent (Maness et al., 1995). Pecan oil is very low in saturated fatty acids (8-10%, palmitic and stearic) with its closest competitors being canola and safflower oils.

While the highly unsaturated nature of component fatty acids in pecan oil offers substantial nutritional benefits, the polyunsaturated constituents also present a series of postharvest problems in that they increase pecan's susceptibility to oxidative rancidity development. Flavor deterioration, darkened color and less crispy texture are all recognized as common consequences of pecan aging and will have a negative impact on consumer acceptability of stored pecans and processed products containing those pecans.

The term "rancidity" is often used in a general sense to designate the development of any disagreeable odor and flavor in fats and oils or fatty phases of foods. Rancid off-flavors are concerned with the changes that result from hydrolytic reactions or from reactions with atmospheric oxygen. Two main types of "rancidity" can be distinguished in fats: 1) Enzymatic oxidation: Vegetable fats, as they occur in the tissues, are invariably accompanied by enzymes capable of hydrolyzing them. These lipases vary somewhat in properties with the source from which they are derived, but all are capable of decomposing neutral fats into free fatty acids and glycerol, and all are inactivated by heat.

The free fatty acids and glycerides thus formed are capable of being oxidised by enzymes called lipoxygenases. This type of rancidity is often found in products that are not heated to a high temperature during processing. 2) Non-Enzymatic oxidation: The most important, and from a scientific point of view the most interesting form of non-enzymatic rancidity is that produced by the action of oxygen of the air on the fat, referred to as oxidative rancidity. Both enzymatic and non-enzymatic mechanisms require oxygen.

Unsaturated fatty acids oxidize spontaneously when exposed to the air, oxygen attacking a double bond with the formation of highly reactive peroxides. This stage of rancidity is often referred to as the induction or initiation stage. This initiation can occur by the action of external energy sources such as heat, light or high energy radiation or by chemical initiation involving metal ions. These reactive peroxides produced during oxidation are much more potent as oxidizing agents than atmospheric oxygen itself, and are capable of bringing about or accentuating other undesirable changes. It is accepted that the first product of oxidation is an intermediate, which is itself odorless, but which breaks down to smaller molecules which do produce the off-odor and off-flavor. The precise manner in which the intermediates decompose is still largely a matter for conjecture. Some of the theories proposed indicate that these intermediates are decomposed by polymerization, isomerization, splitting, or intramolecular rearrangement of the molecule to produce compounds of lower molecular weight. This stage is referred to as the autooxidation stage. For the autooxidation to set in, the peroxides must first accumulate in sufficient quantities to accommodate continued reaction. It is the subsequent reaction of the peroxides with polyunsaturated fatty acids that determines the

accumulation of low molecular weight products, such as hexanal, and eventually the off-flavor generally recognized as "rancid"

Despite considerable progress in the understanding of rancidity in foods during the last 20 years, our knowledge is still far from complete. According to Kays (1982), the three major steps in the development of rancidity in the pecan appear to be 1) hydrolysis of component glycerides into free fatty acids 2) the oxidation of double bonds of unsaturated fatty acids to form peroxides, referred to as induction period and 3) autooxidation stage. In most pathways of lipid deterioration, the initial step is the cleavage of the fatty acid from the glycerol back bone followed by the attack of an unsaturated fatty acid by oxygen to form peroxides, which sustains a continued period of oxidative cleavage of double bonds within polyunsaturated fatty acids. Some of the strategies to retard lipid oxidation are storage of pecans at low temperature (Woodroof and Heaton, 1962), limitation of the amount of oxygen (Dull and Kays, 1988) and use of antioxidants (Rudolph, 1971). All these treatments retard lipid oxidation by lengthening the induction period thereby greatly reducing the rate of autooxidation. Certain combinations of these treatments are currently popular for handling pecans. Recently, oxygen scavenging films have been used to improve the oxidative stability of sunflower oil (Maloba et al., 1996), however it has not been tried on pecans.

Aldehydes, ketones, and hydrocarbons have been implicated as the stimuli of flavors and odors associated with oxidative rancidity (St Angelo and Ory, 1973) and their presence may be quantified as an objective measure of degree of pecan rancidity (Erickson, 1993). The rates of formation and intensities of unpleasantness produced

depend on the total number of double bonds in the lipid molecule and the condition of storage.

Increasing the shelf-life of pecans may be accomplished by lengthening the induction phase (reducing peroxide formation) or reducing the number of poly-unsaturated fatty acids available for reaction. Previous attempts to extend pecan shelf-life involved lengthening the induction period. Only two plausible systems have been suggested for reducing the number of poly-unsaturated fatty acids available for oxidation: genetic manipulation to reduce the number of poly-unsaturated fatty acids synthesised during kernel maturation, and reducing the total fat content, via partial oil extraction of the kernel. While sufficient genetic diversity appears to exist to make selection for reduced poly-unsaturation in the component fatty acids of the pecan oils possible, such a program would be lengthy and owing to the longevity of most commercial orchards (20-30 years), it would take a long time to implement. Supercritical partial oil extraction technique offers an alternate method for reducing the total lipid content of kernels without damaging the kernels, thereby reducing the number of poly-unsaturated fatty acids available for oxidation.

The pecan industry has three major marketing problems: 1) inconsistent year to year production 2) quality maintenance, particularly at the retail level, caused by fatty acid oxidation, and 3) high calorie content (9 cal/g), seen as negative by weight conscious people.

Pecan trees can produce large crops one year and go virtually nutless the next. This characteristic can be minimized with technologies based on mechanical fruit thinning during an otherwise overproduction year (Smith et al.,1993).

Studies by Simms (1994) have indicated that the pecan shelf-life can be extended for one or more years by storing the product frozen inside relatively oxygen impermeable containers and in the absence of light. However, retailers indicate that frozen storage facilities are too costly for a single product like pecan. Since pecans sold in retail markets are often held in packages at room temperature, it is important to consider any methods to improve shelf-life under these conditions.

Supercritical carbon dioxide partial oil extraction seems to be promising in alleviating quality maintenance and calorie content problems, thus increasing the shelf-life (Divino et al., 1996) and producing a lower fat product. Traditionally, organic solvents have been used as extractants for oils as a means for defatting nutmeats. With increased consumer concern for the quality and safety of foods, more stringent government regulations on solvents and allowable residues, and increasing energy costs, alternate extraction methodologies that are cost effective and comply with both consumer preference and regulatory controls must be used. Supercritical fluid extraction (SFE), using carbon dioxide as extraction solvent may be a viable alternative for the traditional methods. The solvent properties of supercritical fluids have been recognized for over 100 years, but commercial application has been slow in developing, possibly due to the sophisticated and expensive high pressure equipment required. Supercritical fluid extraction is rapidly increasing in importance as it's advantages are becoming known. Advantages of carbon dioxide as a supercritical fluid are: it is relatively inexpensive, non-toxic, non-reactive, non-flammable, environmentally safe, wide range of solvent properties at different pressures and temperatures and it is easily recovered and recycled for use in subsequent extraction. Above the critical temperature (31°C) and pressure

(1070 psi) carbon dioxide has the solvent power of liquid and the diffusivity of gas. Nut oils are readily soluble in this form of carbon dioxide and can be separated from the nut in a flowing carbon dioxide stream. When pressure and temperature drops to atmospheric conditions, carbon dioxide returns to a gaseous form and is removed from the extraction matrix (the nut meat) and the extracted material (the oil), leaving no residues.

In the past, supercritical fluid extraction technique has been used to extract oils from a variety of seed matrices. Examples include roasted peanuts (Chiou et al., 1996, Santerre et al., 1994), soybean, cotton seed, corn germ (Friedrich and Pryde, 1984, List et al., 1989, List et al., 1993, Montanari et al., 1996), canola (Dunford and Temelli, 1995), evening primrose (Favati et al., 1991) and pecans (Maness et al., 1995, Zhang et al.,1995, Alexander, 1996).

Little is known about the mechanism by which pecan shelf-life is extended by partial oil extraction. A widely supported hypothesis is that the higher the lipid content of a product, the more susceptible it is to oxidative rancidity (Divino et al., 1996; Adnan et al., 1981). Thus, low-fat products are thought to develop lipid oxidation off-flavors at a slower rate than the non-extracted counterpart. Possibly, it is because the number of sites available for oxidation are reduced by partial oil extraction or maybe because partial oil extraction selectively extracts certain lipid components that are more susceptible to oxidation. The most significant discovery in the application of supercritical carbon dioxide extraction is the tenfold reduction in residual peroxidase activity of the maize germ flour (Christianson et al., 1982). This heat resistant oxidative enzyme normally is difficult to eliminate from food products even by roasting. The conditions used for supercritical carbon dioxide extraction (50°C and 8000 psi) apparently denature

peroxidase enzyme, which could be an explanation for the longer shelf-life enjoyed by partially defatted pecans.

Phospholipids are said to be less soluble in carbon dioxide (Friedrich and List, 1982). Increasing the phospholipid content by partially defatting pecans may retard the consumption of natural antioxidants and elongate the induction period, by elevating its radical-scavenging activity (Koga and Terao, 1995), thus extending the shelf-life of partially defatted pecans.

To fully evaluate whether selective lipid class extraction occurs during SFE, separation and quantitation of lipid classes is imperative. Traditional techniques for separation of lipid classes included preparative thin layer chromatography (TLC), solvent partitioning, and preparative high-performance liquid chromatography (HPLC). Recently, solid phase extraction (SPE) on either silica gel or bonded phase columns (Kaluzny et al., 1985; Kim and Salem, 1990; Prieto et al., 1992) has been explored for separation of lipid classes.

An investigation of the changes in fatty acids of different lipid classes upon aging should enhance our current understanding of rancidity. Ultimately, correlations between decrease in quantity of particular lipid class and the development of rancidity will be obtained. These correlations should be useful for implicating specific lipid class for development of rancidity, and perhaps lead to alternate methods of shelf-life extension for other high oil products.

Objectives

The research presented has three objectives:

1) to correlate the development of rancidity to changes in fatty acid composition of lipid classes during storage of non-extracted and extracted pecans;
2) to determine if partial oil extraction extends shelf-life of pecans;
3) to determine the mechanism of oxidative rancidity retardation in extracted pecans.

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CHAPTER II

LIPID CLASS CHANGES ASSOCIATED WITH OXIDATION AND DEVELOPMENT OF RANCIDITY IN PECANS

(*Carya illinoinensis*)

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Abstract

The non-extracted and supercritical carbon dioxide extracted (22 and 27%) native pecan kernels packed in standard air mixture (21% O_2, 79% N_2), stored for up to 37 weeks at 25°C and 55% RH, were subjected to color analysis, hexanal analysis, sensory analysis, weight change determinations, and determination of lipid class changes, that occur as the pecans age. Pecan nutmeat lightened by partial oil extraction. Pecan testa darkened, became more red (higher a*) and less yellow (lower b*) with storage time. Most color changes occurred in the first 18 weeks. Hexanal concentration of extracted pecans was negligible throughout the storage, while non-extracted pecans reached excessive levels by 22 weeks. Hexanal analysis was in agreement with the sensory analysis. The hexanal threshold levels for objectionable rancidity development appears to

be between 7 and 11 mg/kg pecans. Except in 27% extracted pecans, the weight change was negligible during storage. Free fatty acids increased with storage and were significantly higher in non-extracted pecans than the 27% extracted pecans at 10, 18, 22, 32, and 37 weeks. There is a potential to extend shelf-life of pecans by supercritical carbon dioxide partial oil extraction process. In addition to decreasing the total amount of oil available for oxidation, the free fatty acid lipid component of the oil, that's best correlated with the development of rancidity, was also reduced by supercritical carbon dioxide extraction.

Key words: pecan, shelf-life, hexanal, sensory, rancidity, supercritical carbon dioxide extraction, fatty acid methyl ester, lipid class.

Introduction

Pecans are a semiperishable product that must be refrigerated if quality is to be preserved over extended periods. In practice, however, storage of pecans during retail distribution is often at ambient temperatures. Consequently, quality deterioration in terms of pecan's susceptibility to rancidity and associated off flavor development is enhanced. The off-flavors associated with rancidity are caused, to a large extent, by the products of oxidative cleavage of polyunsaturated fatty acids (Erickson, 1993). Pecans range from 55 to 70 percent oil (Worley, 1994), with a high degree of unsaturation for component fatty acids (90% unsaturated fat, with 65% monounsaturated and 25% polyunsaturated, Maness et al., 1995). The major lipid classes found in pecans are triglycerides (three fatty acids attached to a glycerol backbone), diglycerides (two fatty acids attached to a glycerol

backbone), monoglycerides (one fatty acid attached to a glycerol backbone), free fatty acids and phospholipids (one or two fatty acids plus a phosphorylated form of choline, serine, ehanolamine or inositol attached to a glycerol backbone)(Santerre, 1994).

According to Kays (1982), the three major steps in the development of rancidity in the pecan appear to be 1) hydrolysis of component glycerides into free fatty acids, 2) the oxidation of double bonds of unsaturated fatty acids to form peroxides, referred to as induction period, and 3) an autooxidation process of the free fatty acid pool, referred to as the autooxidation stage. For autooxidation to occur, peroxides must accumulate in sufficient quantities to sustain continued oxidative cleavage. It is during the autooxidation stage that oxidation products such as hexanal accumulate in a sufficient quantity to allow sensory perception of "rancid" flavors (Erickson et al., 1994).

Some of the strategies that have been investigated to improve the storage stability of pecans include low temperature storage (Woodroof and Heaton, 1967), monoglyceride coating containing antioxidants (Shea, 1965; Luce, 1967; Senter and Forbus, 1979), opaque packaging materials (Heaton and Shewfelt, 1976), steam heat (Forbus and Senter, 1976), dielectric heat (Senter et al., 1984), and limitation of the amount of oxygen (Dull and Kays, 1988). Frozen storage is probably the most widely used method for extending pecan shelf-life for periods of one or more years. Since pecans sold in retail markets are often held in packages at room temperature, it is important to consider any methods to improve shelf-life under these conditions.

Supercritical carbon dioxide partial oil extraction seems to be promising in increasing shelf-life of high fat foods (Divino et al., 1996) and in decreasing fat content of foods. Traditionally, organic solvents have been used as extractants for oil extraction

from foods. With enhanced consumer concern for the quality and safety of foods, more stringent government regulations on solvents and allowable residues, and increasing energy costs, alternate extraction methodologies that are cost effective and comply with both consumer preference and regulatory controls must be used. Supercritical fluid extraction (SFE), using carbon dioxide as extraction solvent may be a viable alternative for the traditional methods.

For fat reduction or elimination from foods, SFE operations have been used for total extraction, deodorization, and fractionation. Zhang et al. (1995) investigated the important parameters in using supercritical carbon dioxide that affect extraction of oil from intact pecan halves. The important finding of this study was both pressure and temperature, in the range of 17.7 to 68.9 MPa and 40°C to 80°C respectively, had an effect on oil recoveries. Oil recoveries ranged from 41% for a pressure and temperature combination of 41.34 MPa and 40°C, to 77% for 68.90 MPa and 80°C combination for 160 min of extraction. In a study conducted by Friedrich and Pryde (1984), supercritical carbon dioxide extracted oils from flaked soybean, corn germ, and flaked cottonseed were found to have a lighter color with a lower phosphorous concentration that resulted in lower refining losses compared to hexane-extracted-oil. It was also found that extractability was affected by particle size. Oil yields were quite low with cracked soybeans, but good oil yields (nearly theoretical yields) were obtained from ground or thinly flaked (<0.010 in) seeds. Supercritical carbon dioxide has also been used to degum the hexane extracted crude soybean oil (List et al., 1993). Other examples involving supercritical carbon dioxide are oil extraction from canola (Dunford and Temelli, 1995),

evening primrose (Favati et al., 1991), roasted peanuts (Chiou et al., 1996), and sunflower (Calvo et al., 1994).

Little is known about the mechanism by which pecan shelf-life is extended by partial oil extraction. A widely supported hypothesis is that the higher the lipid content of a product, the more susceptible it is to oxidative rancidity (Divino et al., 1996; Adnan et al., 1981). Thus, low-fat products are thought to develop lipid oxidation off-flavors at a slower rate than the non-extracted counterpart. An investigation of the changes in fatty acids of different lipid classes upon aging should enhance our current understanding of rancidity, and may further define the mechanism by which partial oil extraction extends shelf-life.

The purpose of this work was to determine whether partial oil extraction extends shelf-life, and then determine the mechanism of oxidative rancidity retardation in extracted pecans. The development of rancidity was correlated with changes in fatty acid composition of lipid classes during storage of non-extracted and extracted pecans. These correlations should be useful for implicating specific lipid class for development of rancidity, and perhaps lead to alternate methods of shelf-life extension for other high oil products.

MATERIALS AND METHODS

Pecans and partial oil extraction:

Oklahoma native pecan halves that had been frozen (-4°C) were allowed to come to room temperature. The oil was partially extracted using a Dionex 703 supercritical fluid extraction unit (Dionex Corp., Sunneyvale, CA) with coleman grade CO_2 (Air Products and Chemicals, Inc., Allentown, PA) as extraction solvent. Four 50 ml extraction vessels were loaded with 15 gm of pecan halves (~16 halves) and the extractions were carried out at 690 Atm and 40°C for 20 min and for 8 h. Oil from the pecans was collected in chilled hexane. After extraction, broken halves were separated out leaving only sound kernels and were frozen in airtight freezer bags until a sufficient quantity was extracted for the entire storage study. The oil from 20 min partial extraction was pooled, and the oil from 8 h partial extraction was pooled, blanketed with nitrogen and frozen, for use in further analysis.

Packaging and storage:

The bags used for storage were made of packaging material consisting of 13μ saran coated mylar (polyster) laminated to 63.5μ polyethylene (The Packaging Group, Woodbridge, Ontario). The water vapor and oxygen transmission rates for this packaging material were 0.06 g/100 cm^2/24h and 0.09 ml/100 cm/24h, respectively.

Three replicated samples of 20 pecans each from each extraction level for 12 sampling times were placed into bags (about 15.2 cm×15.2 cm), evacuated from ambient pressure (98 kPa) to less than 0.3 kPa, back-flushed with a standard air mixture of 21%

(v/v) O_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 packaged pecans were stored in a controlled environment chamber at 25°C and 55% RH in dark. Three replicated samples from each SFE treatment and a non extracted control were removed at 0, 2, 4, 6, 8, 10, 14, 18, 22, 26, 32 and 37 weeks after storage for quality analysis.

Color analysis:

Five pecan halves were randomly selected from each replication of each extraction treatment and color evaluation was performed using a Minolta CR300, Chromameter (Minolta Corp., Ramsey, NJ). As pecans were smaller than the aperture of the chromameter (8 mm), a black disk with a 3 mm hole was secured over the aperture to block out side light effects. CIELAB color parameters L*, a* and b* were measured at five points on each pecan half. Two measurements of seed coat (testa) color were taken on the dorsal side and two on the ventral side of the kernel and one measurement of nutmeat color at the point of detachment (POD) where the two cotyledons separated. Color measurements were averaged by site, ten of dorsal testa color, ten of ventral testa color, and five of POD nutmeat color per replication.

Hexanal analysis:

Ten pecan halves from each bag (replicate) were ground in a Warring blender to a particle size less than 1mm. Six 0.5 g aliquots from each sample were weighed into 3.7 ml glass vials fitted with Teflon-lined silicone septa caps. A known amount of 4-heptanone was added to each vial as an internal standard prior to sealing with the caps. The samples were then incubated at 90°C for 15 minutes in a dry-block heater. A 1.0 ml

sample of headspace gas was removed from the vial immediately following the incubation period and injected onto a Tracor 540 gas chromatograph (Tracor Instruments, Austin, TX) 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 × 0.25 μm film thickness; J and W Scientific Inc., Rancho Cardova, CA) with helium carrier gas at a linear flow rate of 20 cm/sec. 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. Peak areas are obtained using a Spectra-Physics 4270 integrator (Spectra-Physics Inc., San Jose, CA). Hexanal was identified according to coelution with an authentic standard and quantified relative to 4-heptanone as internal standard.

After hexanal analysis the remaining ground sample was blanketed with nitrogen and frozen, to be used for quantitative oil extraction.

Taste panel evaluation:

The ten remaining kernels from each replication in storage were used for sensory evaluation. Kernels were chopped into about 3 mm^2 pieces and supplied to a trained taste panel. Samples were evaluated for rancid flavor on a numbered scale, with 0 being no rancid flavor and 5 being very rancid flavor. A score of 3 or above indicated objectionable levels of rancidity.

Sample preparation for quantitative SFE:

The remainder of ground pecan samples from hexanal analysis were utilized for quantitative oil extraction. Prior to opening, all samples were allowed to reach room temperature to prevent moisture condensation onto nutmeats.

Quantitative supercritical CO_2 extraction:

A Dionex 703 (Dionex Corp., Sunneyvale, CA) supercritical fluid extraction instrument was utilized for quantitative extraction of pecan oil essentially as described by Maness et al. (1995). Eight 2.5 ml extraction vessels (7.9 mm × 50 mm) rated to 69 MPa (Keystone Scientific, Inc., Bellefonte, PA) were used during each extraction. Coleman grade CO_2 with a 14 MPa He headspace and dip tube was obtained from Air Products (Air Products and Chemicals, Inc., Allentown, PA). Prior to extraction clean empty vessels were installed and a blank extraction was conducted to purge the SFE system components of oil remaining from the prior extraction. For each storage replicate, four ground samples were weighed (500 mg), wrapped in a Kimwipe and inserted into the extraction vessels with glass wool plug inserted at the outlet end to prevent clogging of the restrictors by pecan particles. Extractions were carried out simultaneously in eight extraction vessels (2 samples replicated 4 times) at 69 MPa (final pressure) and 75°C using 250 ml/min restrictors for 64 min. At the beginning of each run, a two stage ramp in pressure from 0 to 25 MPa and from 25 to 50 MPa, with durations of 2 min each was necessary to prevent restrictor clogging by the extract. Restrictors were maintained at 150°C. The oil from each extraction vessel was channeled into vials containing 15 ml of chilled hexane (2°C). Expanded CO_2 flow rate and total flow were determined from on-board flow meters for each vessel.

Upon completion of extraction, the extracts were quantitatively transferred into tared two-dram vials using hexane and dried *in vacuo* with a Speed Vac sample concentrator equipped with an ultralow sample condenser and an organic vapor trap

(Savant Inc., Farmingdale, NY). Oil yields were determined gravimetrically. The oil was then blanketed with nitrogen and frozen, to be used for lipid class separation.

Lipid class separation:

The oil samples from quantitative extractions and partial extractions, that was frozen were used for lipid class separations. Prior to opening, the frozen oil samples were allowed to reach room temperature to prevent moisture condensation on to the oil. Bond Elut Aminopropyl bonded phase columns with stainless steel frits (500 mg; Varian, Harbor City, CA) were used for the separation and isolation of lipid classes. This solid phase separation technique involves creating selectivity in the isolation of compounds by serially altering the mobile phase. There is evidence that the lipid classes can be isolated essentially to homogeneity with greater than 95% recovery (Kaluzny et al., 1985). The following table gives the solvents used in the Bond Elut isolation of pecan oil lipid classes.

Name	Solvents	Lipids eluted
A	Chloroform-2-propanol 2:1	All neutral lipids
B	2% Acetic acid in diethyl ether	Fatty acids
C	Methanol	Phospholipid
D	Hexane	Cholestrol esters
E	1% Diethyl ether, 10% methylene chloride in hexane	Triglycerides
F	5% Ethyl acetate in hexane	Cholestrol
G	15% Ethyl acetate in hexane	Diglycerides
H	Chloroform-methanol 2:1	Monoglycerides

Procedure outlined by Kaluzny et.al (1985) was followed with a few modifications. First, 0.5 mg of oil was dissolved in a minimal volume of chloroform (~ 0.5 ml). Aminopropyl columns were preconditioned twice under vacuum with 2 ml portions of hexane. The vacuum was released immediately after the second hexane wash to prevent the columns from becoming completely dry. Oil in chloroform was applied to the column and the chloroform allowed to elute at normal atmospheric pressure. This leaves the entire lipid on the column. The column was first eluted with 4 ml of solvent A. This eluate 1 (neutral lipids) was saved and new collection tube was placed in the rack and was then eluted with 4 ml of solvent B, eluate 2 (free fatty acids) was saved. A new collection tube was placed in the rack and the column was then eluted with 4 ml of solvent C, eluate 3 (phospholipid) was saved.

Eluate 1 was dried under nitrogen and reconstituted in ~ 0.2 ml of hexane, then loaded onto a new preconditioned column. Then solvents D (4 ml), E (12 ml), F (4 ml), G (4 ml) and H (4 ml) are serially passed through the column and the eluates 4, 5, 6, 7 and 8 (cholestryl esters, triglycerides, cholesterol, diglycerides and monoglycerides respectively) were saved. It was necessary to increase the elution volume of solvent E (from 6 ml to 12 ml) to accommodate increased amounts of triglycerides in pecan oil, relative to other classes and also the elution volume of solvent F was decreased (from 12 ml to 4 ml) because the cholesterol in pecan oil is almost zero.

Eluates (2 to 8) were quantitatively transferred into tarred 2 dram vials and dried *in vacuo* with a speed vac sample concentrator equipped with an ultralow sample condenser and an organic vapor trap (Savant Inc., Farmingdale, NY). The weights of the

eluates (lipid classes) were determined. Lipid classes were methanolised and then subjected to fatty acid analysis.

Samples of oil from duplicated storage replications were run three times. Separations were conducted with standard lipids (lipid standard (178-6), that contained 33.3% each of monoolein, diolein, and triolein; L-α-phosphatidylinositol (P-0639); L-α-phosphatidylcholine (P-3556); oleic acid (O-1008); linoleic acid (L-1376); Sigma Chemical Co., St. Louis, MO) to substantiate results from the original method, and to accommodate optimization of the method for pecan oil separation.

The lipid class separation procedure was used for two experiments in this study. The objective of the first experiment was to determine if any selectivity for lipid classes occurred as a consequence of partial oil extraction. For this, the oils from non-extracted, 22% extracted, 27% extracted pecans (0 time) and the pooled oils from 20 min partial extraction (obtained during partial oil extraction of the 22% extracted pecans), 8 h partial extraction (obtained during partial oil extraction of the 27% extracted pecans) were subjected to lipid class separation procedure. Since, the 20 min partial extract from a number of runs were pooled together and 8 h partial extract from different runs were pooled together, we couldn't make direct comparisons between the partial extracts and the oil that remained in the pecans. Instead we compared them to the oil from non-extracted pecans separately.

The objective of the second experiment was to determine the mechanism of shelf-life extension of extracted pecans. For this, the quantitatively extracted oils from non-extracted, 22% extracted, 27% extracted pecans of storage weeks 0, 10, 18, 22, 26, ,32,

and 37 were subjected to lipid class separation procedure and the lipid classes of these three extraction levels were compared.

Fatty acid methyl ester preparation and analysis:

Heptadecanoic acid (HDA) as internal standard was added to the vials containing the lipid classes. The amount of HDA was adjusted to the weight of the eluates (600nm HDA/mg eluate). Two-hundred µl of methanolic HCL (3% HCL in methanol, prepared by adding 0.5 ml acetic anhydride to 10 ml methanol) and 50 µl methyl acetate (as a water scavenger) was added and vials were sealed with teflon lined caps and incubated for 2 h at 90°C in a dry heating block. It was necessary to mix the vial contents by vortexing during the first 15 min of the incubation period to assure equilibrium of the eluates into a single phase for methanolysis. Following incubation vials were cooled and 10 drops of tertiary butanol was added to co-evaporate the HCL and the samples are dried under nitrogen gas. Fatty acid methyl esters were brought up into hexane, with the volume of hexane based on the weight of the eluate (600 µl hexane/mg eluate). One µl aliquot was utilized for gas chromatography.

Gas chromatography was conducted using a Tracor model 540 gas chromatograph (Tracor Instruments, Austin, TX), equipped with a split injection port (split ratio of 50:1) and flame ionization detector. Separations were performed using a DB 23 fused silica capillary column (30m×0.25 µm film thickness; J and W Scientific Inc., Rancho Cardova, CA) with helium carrier gas at a linear flow rate of 20 cm/sec. The injector temperature was 275°C and the detector temperature was 300°C. The initial column temperature was 50°C for 2 min. Fatty acid methyl esters were then separated using a linear temperature program from 50°C to 180°C at 10°C/min, a hold at 180°C for 5 min, and a second linear

temperature program from 180°C to 240°C at 5°C/min and a hold at 240°C for a final 5 min period. Individual FAME peaks are identified according to co-elution with an authentic standard (FAME preparation 2; Sigma Chemicals Co., St. Louis, MO). Peak areas are obtained using a Spectra-Physics 4270 integrator (Spectra-Physics Inc., San Jose, CA) and quantified relative to HDA as internal standard.

Alternative method for determination of free fatty acid content:

AOCS Official Method Ca 5a-40 was used in free fatty acids determination, with a few modifications. According to this procedure, the weight of the oil sample for a free fatty acid range of 0.2-1.0% is 28.2 ± 0.2 g. Because we had a small quantity of oil, the sample weight was scaled down to 0.282 g. Proportionally the amounts of hot neutralized alcohol (scaled down from 50 ml to 500 µl) and phenolphthalein indicator solution were adjusted (scaled down from 2 ml to 20 µl). Percent free fatty acids as oleic was calculated by (ml of alkali × normality of alkali × 28.2) / weight of sample.

Statistical design and analysis:

Analysis of variance (ANOVA) and mean separation procedures were performed to determine effects of oil reduction and storage time on pecan color, hexanal concentration, taste panel evaluation, and weight using the Statistical Analysis System (SAS Institute, Cary, NC). Data were analyzed as a 3 (oil content) × 12 (storage times) factorial experiment with significance's at $P \leq 0.05$.

A split-plot experimental design with the pecan extraction levels designated as main plot and storage time as the sub plot was used. Independent variables were extraction levels and storage duration, the dependent variables were quantity of each lipid class and the fatty acid profile of the lipid classes. Data was analyzed using trend analysis.

Where appropriate the protected LSD was used to identify significant ($p<0.05$) differences among treatment means.

Results

Partial and quantitative oil extraction:

The pecans that were extracted for 20 min and 8 h lost 22% and 27% oil respectively (Table 1). Percent oil reduction was arrived at using the formula (total gm oil of reduced oil pecan/total gm oil of full oil pecan) × 100. Oil content of non-extracted, 20 min extracted and 8 h extracted were 64%, 50% and 47% (w/w) respectively. There were no differences in oil yields in these 3 samples across storage, which indicates that there was no loss in oil during storage. Weight/pecan and percent weight reduction as a consequence of extraction is presented in Table 1.

Weight loss:

Over the course of the experiment, pecan weight gain was slight, most of which occurred within the first 4 to 6 weeks (Appendix A). The 27% extracted pecans had significantly higher weight gain (1.26%) than 0% (0.14%) and 22% (0.11%) extracted pecans.

Color analysis:

Oil reduction significantly affected all color parameters, except dorsal L* (Table 2). Ventral and POD L* values increased with oil reduction, indicating lightened pecan testa and nutmeats. The a* values showed that oil reduction caused testa color to be significantly more red (higher a*). The POD became more white (less yellow). Extracted pecans had higher b* values than non-extracted pecans. The storage duration also affected testa color. Pecan testa darkened (decreasing L*) at a relatively rapid rate during the first

4 weeks of storage, then continued to darken at a slower, almost linear rate. Pecan testa a* values increased and b* values decreased during storage (Table 2).

Hexanal analysis:

Hexanal production was affected by storage time and oil extraction (Fig 1). Hexanal levels ranged from 0.0 to 17.0 mg/kg nutmeat for non-extracted (0% extracted) pecans, 0.0 to 4.4 mg/kg nutmeat for 22% extracted pecans, and 0.0 to 0.6 mg/kg nutmeat for 27% extracted pecans during 37 weeks of storage. Hexanal values for non-extracted pecans were statistically greater than extracted pecans at weeks 22, 32 and 37. Although 22% extracted samples consistently exhibited higher hexanal values than 27% extracted samples, there was no statistical difference between the two extraction levels over the duration of the experiment.

Taste panel evaluation:

Sensory panel results indicated only marginally detectable (score of 1) to mild (score of 2) rancid flavor in pecans of all oil levels up to the 22nd week of storage (Fig. 2). Objectionable levels of rancidity (score of 3 or above) were noted for non-extracted pecans in weeks 22, 32 and 37, but were never noted for extracted pecans. The 26 week samples were not judged objectionably rancid by the sensory panel which was in agreement with the decreased hexanal production. There was a significant decline in hexanal production for 26 week samples (7 mg/kg nutmeat), compared to 22 week (11 mg/kg nutmeat) and 32 week (14 mg/kg nutmeat) samples.

Fatty acid analysis of whole (unfractionated) oil:

Fatty acid analysis of unfractionated oils were conducted and were found to be similar across extraction level and storage duration (Appendix B). The main fatty acids

were oleic, linoleic, palmitic, stearic, and linolenic and the grand means of these fatty acids in mg/g oil were 659, 246, 61, 22, and 12, respectively. The changes in the fatty acid profiles of non-extracted, 22% extracted, and 27% extracted pecans across storage were not significant ($P < 0.05$).

Selectivity experiment:

The oils from 20 min partial extraction, 8 h partial extraction, and quantitatively extracted oils from non-extracted (0 week), 22% extracted (0 week), and 27% extracted (0 week) pecans were subjected to lipid class separation to determine if any selectivity for lipid classes occurred as a consequence of partial oil extraction. The lipid classes and their fatty acid profiles of the oils from non-extracted, 22% extracted, 27% extracted pecans (0 time) and the oils from 20 min partial extraction, 8 h partial extraction are compared in Table 3. Triglycerides were the major component, followed by monoglycerides, diglycerides, and free fatty acids. Phospholipids were a very minor component.

Oil obtained from 20 min partial extraction and 8 h partial extraction had a significantly higher free fatty acid content compared to the oil from full oil pecans. This corresponded to the reduced amounts of free fatty acids in the remaining oil that was obtained from 22% extracted and 27% extracted pecans (Table 3). Also the oil obtained from the 20 min partial extraction had more free fatty acids than the oil from 8 h partial extraction. The palmitic and stearic acids in the oil from 20 min and 8 h partial extraction were significantly higher than that of the oil from non-extracted pecans.

Differences in phospholipid recovery (mg/g oil) or their composition was not significantly affected by partial oil extraction.

Triglyceride recovery was also not significant in either of these comparisons. There was a significant difference in the triglyceride fatty acid composition in many cases, notable among them was that the oleic acid was statistically greater in the oil from 20 min and 8 h partial extractions than the oil from non-extracted pecan and linoleic was lower in the oil from 20 min and 8 h partial extraction than the oil from non-extracted pecan.

Diglyceride recovery (mg/g oil) was significantly higher in the oil from partial extractions (20 min and 8 h) than the oil from non-extracted pecan. Levels of oleic and linoleic were significantly higher in the oil from partial extractions than the oil from non-extracted pecans.

Monoglyceride recoveries were significantly different in both the comparisons and their composition was also significantly different in a few cases.

Storage experiment:

Quantitatively extracted oils from non-extracted, 22% extracted, and 27% extracted pecans of storage weeks 0, 10, 18, 22, 26, 32, and 37 were subjected to lipid class separation to investigate the changes in the different lipid classes and their fatty acid profile with storage. To account for relative kernel oil content, we converted values from mole percent to mg/pecan. At 0 week the free fatty acid/pecan was significantly lower in 22% and 27% extracted pecans than non-extracted (0% extracted) pecans. Free fatty acid/pecan in all three extraction levels increased with storage time (Table 4). Free fatty acid linearly increased with storage in non-extracted pecans, and quadratically increased with storage time in 22% extracted pecans. In the 27% extracted pecans, free fatty acid increased untill 22 weeks of storage, then there was a decrease at 26 week, followed by a

further increase at 32 weeks of storage, giving it a cubic response. The free fatty acid pool in non-extracted pecans was statistically greater than 22% extracted pecans at 10, 32, 37 weeks of storage and was statistically greater than 27% extracted pecans at 10,18, 22, 32, and 37 weeks of storage.

Phospholipid recoveries (mg/pecan) were significantly lower in extracted pecans than the non-extracted pecans throughout the storage study (Table 4). Phospholipids in non-extracted and 27% extracted pecans decreased with storage time. A quadratically decreasing response with storage, for non-extracted pecans and a linearly decreasing response with storage, for 27% extracted pecans was observed.

Triglycerides (mg/pecan) in the extracted samples were nearly stable during storage and were consistently lower than in non-extracted pecans (Table 4). Triglycerides in non-extracted pecans decreased untill week 26 and increased at week 32, giving a cubic response.

Diglycerides (mg/pecan) in all three extraction levels increased quadratically with storage. There was no significant difference between the three extraction levels for any storage duration (Table 4).

In contrast to diglycerides, monoglycerides decreased with storage in all 3 extraction treatments (Table 4). A quadratically decreasing response, with storage was observed in all three extraction levels. There was no significant difference between the three extraction levels at any of the storage weeks.

The main fatty acids in free fatty acid fraction were stearic, palmitic, oleic, and linoleic. Free fatty acid in non-extracted pecans had a significantly higher amounts of saturated fatty acids (palmitic and stearic), than the extracted pecans throughout the

storage (Table 5). Palmitic acid in non-extracted pecans increased linearly with storage and stearic acid showed an increase during the first three weeks, after which they started to decline, and again started to increase towards the end of the storage. In 22 and 27% extracted pecans no significant trend was observed for palmitic and stearic acids. At 0 weeks, free fatty acid had lower amounts of oleic and linoleic in the extracted pecan than the non-extracted pecan. With storage, the extracted pecans consistently exhibited higher oleic and linoleic acid values, but there was no statistical difference between them, except for weeks 18 and 22 where there was a significant difference between 22% extracted and non-extracted pecans (Table 4). With storage, oleic acid increased linearly in non-extracted pecans, and increased quadratically in 27% extracted pecans, but in 22% extracted pecans oleic increased till 22 week , decreased at 26 week and again started to increase (cubic response). Linoleic acid in non-extracted, 22% extracted, and 27% extracted pecans showed a quadratically increasing response with storage.

The main fatty acids in phospholipid fraction were palmitic and stearic (Table 6). Both palmitic and stearic acids were significantly lower in extracted pecans than non-extracted pecans throughout storage.

Palmitic, stearic, oleic, linoleic, and linoleic acids were the main fatty acids in triglyceride fraction. The amounts of these fatty acids were close to the amounts in the whole oil, and all these fatty acids were significantly lower in extracted pecans than non-extracted pecans throughout storage (Table 7). In non-extracted pecans linoleic decreased quadratically with storage, whereas in 22% and 27% extracted pecans, the decrease was linear.

The composition of diglycerides changed during storage. The main fatty acids in diglycerides were oleic, linoleic, palmitic, and stearic. All the fatty acids increased with storage, in all three extraction levels (Table 8). There was no significant difference in oleic acid between the three extraction levels, during the storage. Twenty-two percent reduced oil-pecans had a significantly lower linoleic than non-extracted pecans at weeks 22 and 26, the 27% extracted pecans had a significantly lower linoleic than non-extracted pecans at week 32. Both oleic and linoleic acids, increased quadratically with storage in all three extraction levels.

The main fatty acids in monoglycerides were also oleic, linoleic, palmitic, and stearic. In contrast to diglycerides, all the fatty acids in monoglyceride decreased with storage in all 3 extraction levels (Table 9). Twenty-two and 27% extracted pecans had a significantly lower oleic concentration than non-extracted pecans at weeks 10 and 18. But there was no significant difference in linoleic acid in any of the extraction levels. Both oleic and linoleic acids decreased quadratically with storage in all three extraction levels.

Discussion

The partial oil extraction process is nondestructive and whitens pecan nutmeats (Table 2), imparting a fresh appearance to the extracted pecan halves. Flavor of the partially defatted pecans is slightly milder; probably because the oil contains most of the flavor (Woodroof, 1983). Partial oil extraction provides a new reduced-calorie product, thereby enhancing product variety for consumers and providing producers a new market for a unique product for confectionery and retail pecan distribution.

The marginal weight gain during storage of 27% extracted pecans (Appendix A) is probably due to moisture uptake. The 27% extracted pecans have less oil, and will perhaps have a higher equilibrium moisture content. This explains the greater weight gain by the 27% extracted pecans than non-extracted and 22% extracted pecans. Forbus et al. (1980) who reported that testa L* and b* values decreased linearly during storage; however in our study the changes were rapid during the first 4 weeks of storage, after which the changes were slow and linear.

Pecan shelf-life can be extended with partial oil extraction. Hexanal is a direct oxidative breakdown product of linoleic acid, and increased levels indicated increased oil oxidation. The resistance of oils to oxidation, indicated by the time required for the onset of sustained hexanal production, was increased from 6 weeks for non-extracted pecans to 18 weeks for 22% extracted pecans to 22 weeks for 27% extracted pecans (Fig. 1). Also, at 22 weeks hexanal levels in the non-extracted pecans rose above 6 mg/kg pecan, determined by Hofland et al.(1995) to be undesirably high in sensory analyses. At no time during storage were 22% and 27% extracted pecan hexanal levels above the 6 mg/kg

pecan. Sensory evaluation was conducted to substantiate actual rancid flavor development in stored samples. The taste panel evaluation was in agreement with hexanal production; pecans were judged objectionably rancid (average sensory scores over 3) at weeks 22, 32, and 37. The 26 week samples were not judged objectionably rancid by the sensory panel (Fig. 2). This was reflected by a noticeable decline in hexanal production for 26 week samples, compared to 22 and 32 week samples, probably caused by inherent variation in pecan rancidity development. For pecans, the hexanal threshold necessary for sensory detection of objectionable rancidity appeared to be between 7 mg hexanal/kg pecan (26 weeks) and 11 mg hexanal/kg pecan (22 week). This is slightly above threshold levels reported by Hofland et al. (1995).

Decrease in autooxidation as a result of partial oil extraction from peanuts has been reported, and the explanation given was "decreasing the oil content would result in less substrate available for attack and less free radical formation" (Divino, et al., 1996; Adnam et al., 1981)). We examined the changes in the lipid classes during pecan aging and correlated them to development of rancidity in an attempt to examine the mechanism of rancidity development in non-extracted pecans and rancidity retardation in extracted pecans.

The increase in free fatty acid levels obtained by the lipid class separation procedure was substantiated by the AOCS procedure (Appendix C). Increases in free fatty acids during storage of pecan kernels has been noted by Erickson (1993) and Forbus and Senter (1976) and free fatty acids are thought to contribute to oxidative instability of many products. At 0 week, the significantly lower free fatty acid levels (Table 4) in extracted pecans was apparently due to selective extraction of free fatty acids during the

partial oil extraction. Higher free fatty acid levels in the oil from 20 min and 8 h partial extractions (Table 3), compared to the oil from total pecan extraction, indicates some selectivity for free fatty acids during partial oil extraction. Free fatty acids, because of their lower molecular weight, tend to be more soluble in supercritical carbon dioxide (Friedrich and Pryde, 1984) than other lipid classes. The extracted pecans went into storage with a lower free fatty acid content than the non-extracted pecans, and consequently autooxidation was delayed by the reduction in substrate for continued peroxide formation. Free fatty acids did not increase during this storage period (37 weeks) for autooxidation to set in and to be perceived as rancid. At no point in storage did the free fatty acid levels in extracted pecans exceed 2.7 mg/pecan, while the non-extracted pecans had a maximum free fatty acid level of 3.4 mg/pecan. At 26 weeks the free fatty acid levels were significantly lower than at 22 weeks, which agrees with hexanal and taste panel results.

In contrast to the earlier findings (Erickson, 1993), free fatty acids had more saturated fatty acids than unsaturated fatty acids (Table 5). The observed differences may arise from the different lipid extraction procedures (chloroform : methanol 2:1 vs SFE) as well as different lipid class separation procedures (thin layer chromatography vs aminopropyl bonded phase columns) employed in these two studies.

There was a decrease in phospholipid, in the non-extracted pecans during the storage (Table 4), which agrees with Erickson's (1993) results. Considering that phospholipids represent a very small percentage of the total lipid, oxidation of phospholipids may be negligible compared to oxidation of triglycerides. Phospholipids were said to contain more of unsaturated fatty acids, but in our study phospholipids

mainly had saturated fatty acids with little or no unsaturated fatty acids (Table 6). It could be because the recovered phospholipids, since they are sparingly soluble in supercritical carbon dioxide (Dunford and Temelli, 1995), were not representative of the total phospholipid pool in pecans.

Triglyceride recovery (Table 4) corresponded closely to that reported by Senter and Horvat (1976) for total pecan lipids. Losses of linoleic in the triglyceride fraction of non-extracted pecans occurred during storage, but it did not coincide with hexanal results. The inability to correlate losses of polyunsaturated fatty acids with increases in peroxide value during the same time period has been observed by Adnan et al. (1981) in reconstituted partially defatted peanuts. It is possible that sufficient PUFA buildup is required to detect the oxidation products.

There is a potential to extend the shelf-life of pecans by supercritical carbon dioxide partial oil extraction process. It has long been the custom to associate deterioration in oils and fats with a high free fatty acid content (Robertson et al., 1984, 1985), and the acid value has been and is still very widely used in the specification of products of edible quality. Both the total amount of fat available for oxidation that could replenish the free fatty acid pool and the free fatty acid pool itself was reduced with supercritical carbon dioxide partial oil extraction. These results could be crucial in exploring alternate methods for extending shelf-life for high oil products, besides pecans.

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CHAPTER III

SUGGESTIONS FOR FUTURE STUDY

In our study the partially extracted pecans never went rancid. A longer storage study (longer than 9 months) could be conducted to determine when these partially extracted pecans will go rancid, which would further give an insight into rancidity development process.

A study is needed to further investigate the biochemical basis for the development of rancidity in pecans.

Examination of the effect of pecan cultivars on shelf-life extension of pecans by supercritical carbon dioxide partial oil extraction would be useful to determine if there are any differences among cultivars.

It would be interesting to investigate the effect of supercritical carbon dioxide partial oil extraction of pecans on the tocopherol content of pecans. If tocopherol is not selectively extracted, then the concentration of tocopherol in the pecan increases. This could be a factor contributing to the shelf-life extension of extracted pecans. If tocopherol is being selectively extracted, then the extracted oil will have a high concentration of tocopherol, and is likely to enjoy a longer shelf-life.

A study could be conducted combining supercritical carbon dioxide partial oil extraction of pecans with modified atmosphere packaging (packaging materials like polyvinylidene chloride coated cellophane, nitrocellulose coated cellophane, oxygen

scavenging films) of these partially extracted pecans to see if the shelf-life is further extended.

Table 1: Mean oil content[z], oil reduction, weight/pecan, and weight reduction of non-extracted, 20 min extracted and 8 h extracted pecans.

Extraction	Oil content (%)	Oil reduction (%)	Wt/pecan (g)	Wt. Reduction (%)
None	64 ± 2.5[y]	0	0.86 ± 0.05	0.0
20 min	50 ± 1.6	22	0.69 ± 0.04	19.8
8 h	47 ± 1.3	27	0.64 ± 0.04	25.6

[z] All results are a grand mean for all the extractions of all storage durations.

[y] Numbers following the ± sign are the standard deviation.

Table 2: Color parameters[a] by site, oil reduction, and storage time.

Color parameter	Measurement site	Oil reduction (%)	Storage time (weeks) 0	18	37
L*	Dorsal	0	66.7 ± 0.9	61.3 ± 0.4	59.9 ± 1.2
		22	65.4 ± 0.9	61.0 ± 1.3	60.5 ± 0.4
		27	67.5 ± 0.9	61.7 ± 1.5	59.3 ± 0.8
	Ventral	0	62.4 ± 1.2	58.7 ± 1.0	56.5 ± 1.1
		22	62.8 ± 1.5	59.4 ± 0.5	56.4 ± 0.8
		27	63.2 ± 1.0	59.1 ± 1.5	57.3 ± 0.2
	POD	0	68.7 ± 0.3	71.6 ± 1.3	71.7 ± 2.4
		22	77.0 ± 3.5	77.7 ± 1.1	79.7 ± 1.5
		27	81.4 ± 1.4	78.8 ± 1.6	80.2 ± 0.9
a*	Dorsal	0	6.0 ± 0.7	9.3 ± 0.2	8.9 ± 0.4
		22	7.2 ± 0.8	9.4 ± 0.9	9.5 ± 0.6
		27	8.4 ± 0.3	11.4 ± 0.4	10.7 ± 0.5
	Ventral	0	5.5 ± 0.7	8.3 ± 0.8	7.8 ± 0.2
		22	7.0 ± 0.2	9.2 ± 1.0	8.4 ± 0.8
		27	7.7 ± 0.6	10.3 ± 0.2	9.8 ± 0.4
	POD	0	0.1 ± 0.1	1.3 ± 0.3	1.1 ± 0.3
		22	-0.2 ± 0.4	0.6 ± 0.2	-0.2 ± 0.1
		27	-0.4 ± 0.2	0.6 ± 0.1	0.1 ± 0.1
b*	Dorsal	0	18.5 ± 0.4	15.1 ± 0.7	14.5 ± 1.3
		22	19.9 ± 1.3	15.5 ± 1.0	15.8 ± 0.5
		27	22.5 ± 1.0	16.6 ± 1.3	14.7 ± 1.3
	Ventral	0	16.9 ± 1.7	12.9 ± 1.2	11.4 ± 1.2
		22	18.2 ± 1.3	14.2 ± 0.5	11.7 ± 0.8
		27	19.4 ± 1.6	14.1 ± 1.4	12.8 ± 0.4
	POD	0	11.8 ± 0.7	11.3 ± 0.6	12.2 ± 0.5
		22	12.3 ± 1.4	12.0 ± 0.2	13.0 ± 0.3
		27	13.5 ± 0.5	13.0 ± 0.2	13.7 ± 0.7

[a] Means (n=3) ± SD

Table 3. Comparison of the lipid class recoveries and their fatty acid composition for oils from non-extracted, 22% extracted, 27% extracted pecans and, oils from 20 min partial extraction and 8 h partial extraction. Means are average of six runs[z].

Oil extracted[y]	Recovery (mg/g oil)	mg fatty acid/g oil				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
		Free fatty acids				
None	4.1	1.3	1.4	1.0	0.4	-
22%	3.7	1.4	1.4	0.5	0.3	-
27%	4.4	1.7	1.9	0.6	0.3	-
$LSD_{0.05}$[x]	NS[w]	NS	0.4	NS	NS	-
20 min	5.9	1.8	2.0	1.6	0.5	-
8 h	4.8	1.8	1.9	0.8	0.4	-
$LSD_{0.05}$	0.5	0.3	0.4	0.4	NS	-
		Phospholipids				
None	2.0	0.9	0.9	0.1	-	-
22%	1.8	0.9	0.8	0.1	-	-
27%	1.9	0.8	1.0	0.1	-	-
$LSD_{0.05}$	NS	NS	NS	NS	-	-
20 min	2.3	1.1	1.1	0.1	-	-
8 h	2.0	0.9	1.0	0.1	-	-
$LSD_{0.05}$	NS	NS	NS	NS	-	-
		Triglycerides				
None	947.2	58.1	20.1	661.9	248.8	13.6
22%	940.3	51.9	19.5	642.9	215.6	10.3
27%	960.0	54.7	17.3	616.4	259.5	12.7
$LSD_{0.05}$	NS	1.8	NS	NS	21.1	NS
20 min	941.9	51.4	16.7	561.7	216.8	9.4
8 h	936.3	56.4	19.0	614.2	235.5	11.2
$LSD_{0.05}$	NS	3.5	1.7	32.2	16.7	NS
		Diglycerides				
None	2.9	0.4	0.2	1.6	0.7	-
22%	3.3	0.5	0.2	1.9	0.7	-
27%	3.0	0.4	0.2	1.7	0.7	-
$LSD_{0.05}$	NS	NS	NS	NS	NS	-
20 min	4.7	0.7	0.4	2.7	0.9	-
8 h	4.0	0.5	0.2	2.3	1.0	-
$LSD_{0.05}$	1.1	NS	NS	0.5	0.2	-
		Monoglycerides				
None	9.5	1.1	0.6	4.4	3.4	-
22%	13.8	1.7	1.0	5.7	5.4	-
27%	13.5	1.8	1.1	5.9	4.7	-
$LSD_{0.05}$	2.9	NS	NS	1.3	1.0	-

20 min	17.8	2.3	1.4	8.7	5.3
8 h	13.0	1.6	0.9	5.5	5.0
$LSD_{0.05}$	2.6	0.6	NS	1.3	1.0

[z] Recoveries obtained by aminopropyl column separations as described in mate methods.

[y] Comparison between oils from non-extracted pecans versus 22% extracted, a extracted pecans, and between oils from non-extracted pecans versus oils obt during the 20 min and 8 h partial extraction.

[x] Least significant difference at 5% probability.

[w] NS = nonsignificant.

Table 4: Lipid class recoveries (mg/pecan) for oils from non-extracted, 22% extracted, and 27% extracted pecans as affected by storage duration. Means are average of six runs[z].

Storage duration (Weeks)	mg/pecan[y]		
	Non-extracted	22% extracted	27% extracted
	Free fatty acids		
0	2.12	1.33	1.30
10	2.51	2.05	2.00
18	2.94	2.42	2.04
22	2.96	2.64	2.35
26	2.50	2.10	1.92
32	3.35	2.68	2.57
37	2.98	2.51	2.12
Linear	**	**	*
Quadratic	NS	**	**
Cubic	NS	NS	*
$LSD_{0.05}$ (for same storage week)[x] = 0.44			
	Phospholipids		
0	1.00	0.63	0.57
10	0.95	0.60	0.59
18	0.84	0.57	0.52
22	0.81	0.67	0.56
26	0.81	0.65	0.52
32	0.96	0.60	0.46
37	0.93	0.63	0.47
Linear	NS	NS	*
Quadratic	**	NS	NS
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.13			
	Triglycerides		
0	518.0	333.8	288.8
10	481.4	322.0	294.3
18	507.1	346.8	289.3
22	497.8	333.3	288.1
26	496.2	331.3	293.0
32	511.5	348.7	305.3
37	502.2	341.2	281.1
Linear	NS	*	NS
Quadratic	*	NS	NS
Cubic	**	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 14.3			
	Diglycerides		
0	1.50	1.18	0.91
10	5.72	5.64	4.72
18	4.82	5.53	6.07

22	6.97	5.70	5.72
26	8.01	6.68	5.82
32	7.67	6.14	5.90
37	5.92	6.81	6.17
Linear	**	**	*
Quadratic	**	**	**
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 2.2			
	Monoglycerides		
0	4.93	4.88	4.05
10	5.70	3.64	3.69
18	4.12	2.26	2.53
22	1.61	2.68	1.36
26	1.10	2.69	1.50
32	1.54	1.45	1.41
37	1.08	0.46	0.50
Linear	*	**	NS
Quadratic	**	*	**
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 2.1			

NS, *, **, Nonsignificant or significant at $P \leq 0.05, 0.01$ respectively

[z] Recoveries obtained by aminopropyl column separations as described in materials and methods.

[y] mg of representative fatty acids/pecan obtained by fatty acid analysis using gas chromatography, as described in material and methods.

[x] Pairwise comparisons between oil levels at a particular storage week.

Table 5: Fatty acid profile of free fatty acid lipid class (mg/pecan) for oils from non-extracted, 22% extracted, and 27% extracted pecans as affected by storage duration. Means are average of six runs[z].

Storage duration (Weeks)	mg/pecan[y]		
	Non-extracted	22% extracted	27% extracted
	Palmitic acid		
0	0.66	0.51	0.50
10	0.74	0.57	0.60
18	0.87	0.65	0.49
22	0.83	0.61	0.52
26	0.75	0.57	0.52
32	0.91	0.63	0.58
37	0.82	0.60	0.51
Linear	**	NS	NS
Quadratic	NS	NS	NS
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.10			
	Stearic acid		
0	0.75	0.52	0.56
10	0.90	0.64	0.64
18	0.93	0.61	0.45
22	0.88	0.68	0.52
26	0.80	0.54	0.55
32	1.09	0.63	0.57
37	1.12	0.61	0.52
Linear	**	NS	NS
Quadratic	*	NS	NS
Cubic	**	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.11			
	Oleic acid		
0	0.51	0.19	0.17
10	0.49	0.55	0.81
18	0.70	1.07	0.71
22	0.66	1.07	0.70
26	0.57	0.62	0.77
32	0.91	0.81	0.89
37	0.67	0.82	0.73
Linear	*	NS	**
Quadratic	NS	**	*
Cubic	NS	*	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.24			
	Linoleic acid		
0	0.21	0.11	0.07
10	0.39	0.39	0.51

18	0.44	0.59	0.39
22	0.50	0.49	0.35
26	0.34	0.38	0.45
32	0.43	0.60	0.53
37	0.32	0.48	0.36
Linear	NS	**	**
Quadratic	**	**	*
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.13			

NS, *, **, Nonsignificant or significant at $P \leq 0.05, 0.01$ respectively

[z] Recoveries obtained by aminopropyl column separations as described in materials and methods.

[y] mg of representative fatty acids/pecan obtained by fatty acid analysis using gas chromatography, as described in material and methods.

[x] Pairwise comparisons between oil levels at a particular storage week.

Table 6: Fatty acid profile of phospholipid lipid class (mg/pecan) for oils from non-extracted, 22% extracted, and 27% extracted pecans as affected by storage duration. Means are average of six runs[z].

Storage duration (Weeks)	mg/pecan[y]		
	Non-extracted	22% extracted	27% extracted
	Palmitic acid		
0	0.45	0.31	0.25
10	0.44	0.30	0.27
18	0.39	0.26	0.26
22	0.39	0.32	0.29
26	0.40	0.33	0.23
32	0.40	0.27	0.18
37	0.40	0.27	0.23
Linear	*	NS	*
Quadratic	NS	*	NS
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.05			
	Stearic acid		
0	0.49	0.29	0.30
10	0.51	0.30	0.32
18	0.42	0.26	0.26
22	0.42	0.29	0.27
26	0.41	0.32	0.25
32	0.46	0.29	0.25
37	0.50	0.28	0.22
Linear	NS	NS	**
Quadratic	*	NS	NS
Cubic	*	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.07			

NS, *, **, Nonsignificant or significant at $P \leq 0.05$, 0.01 respectively

[z] Recoveries obtained by aminopropyl column separations as described in materials and methods.

[y] mg of representative fatty acids/pecan obtained by fatty acid analysis using gas chromatography, as described in material and methods.

[x] Pairwise comparisons between oil levels at a particular storage week.

Table 7: Fatty acid profile of triglyceride lipid class (mg/pecan) for oils from non-extracted, 22% extracted, and 27% extracted pecans as affected by storage duration. Means are average of six runs[z].

Storage duration (Weeks)	mg/pecan[y]		
	Non-extracted	22% extracted	27% extracted
	Palmitic acid		
0	30.03	18.43	16.44
10	27.84	18.50	16.94
18	27.30	19.83	15.90
22	28.00	19.50	17.01
26	29.93	19.10	16.76
32	30.66	19.61	16.79
37	27.87	19.80	16.56
Linear	NS	*	NS
Quadratic	*	NS	NS
Cubic	**	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 1.3			
	Stearic acid		
0	10.36	6.94	5.20
10	7.78	5.32	5.61
18	8.11	7.26	5.25
22	8.07	6.93	5.81
26	10.17	5.58	6.56
32	12.12	7.83	6.82
37	11.45	7.69	6.95
Linear	**	**	**
Quadratic	**	**	NS
Cubic	**	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.92			
	Oleic acid		
0	341.96	228.23	185.41
10	326.38	223.68	199.39
18	347.08	239.12	195.30
22	316.16	220.98	199.81
26	330.63	228.17	194.30
32	351.99	219.92	202.09
37	336.73	229.99	193.21
Linear	NS	NS	NS
Quadratic	**	NS	*
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 10.1			
	Linoleic acid		
0	128.54	76.54	78.05
10	114.47	71.01	68.94

18	118.65	76.88	69.33
22	119.42	81.75	62.95
26	119.93	75.25	71.57
32	110.52	78.50	75.82
37	110.47	79.31	61.06
Linear	NS	*	*
Quadratic	*	NS	NS
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 8.8			
	Linolenic acid		
0	7.02	3.67	3.82
10	4.98	3.39	3.19
18	5.75	3.58	3.47
22	6.39	4.07	2.54
26	5.54	3.26	3.87
32	6.50	5.15	4.48
37	5.61	4.27	3.34
Linear	NS	**	NS
Quadratic	*	*	*
Cubic	**	NS	**
$LSD_{0.05}$ (for same storage week)[x] = 0.66			

NS, *, **, Nonsignificant or significant at $P \leq 0.05$, 0.01 respectively

[z] Recoveries obtained by aminopropyl column separations as described in materials and methods.

[y] mg of representative fatty acids/pecan obtained by fatty acid analysis using gas chromatography, as described in material and methods.

[x] Pairwise comparisons between oil levels at a particular storage week.

Table 8: Fatty acid profile of diglyceride lipid class (mg/pecan) for oils from non-extracted, 22% extracted, and 27% extracted pecans as affected by storage duration. Means are average of six runs[z].

Storage duration (Weeks)	mg/pecan[y]		
	Non-extracted	22% extracted	27% extracted
	Palmitic acid		
0	0.22	0.19	0.13
10	0.53	0.57	0.44
18	0.49	0.50	0.58
22	0.67	0.32	0.58
26	0.77	0.40	0.51
32	0.72	0.61	0.37
37	0.73	0.73	0.63
Linear	**	**	**
Quadratic	NS	NS	**
Cubic	NS	**	*
$LSD_{0.05}$ (for same storage week)[x] = 0.21			
	Stearic acid		
0	0.11	0.09	0.06
10	0.25	0.23	0.23
18	0.24	0.26	0.28
22	0.32	0.13	0.30
26	0.39	0.18	0.31
32	0.47	0.35	0.29
37	0.50	0.44	0.50
Linear	**	**	**
Quadratic	NS	NS	NS
Cubic	NS	**	**
$LSD_{0.05}$ (for same storage week)[x] = 0.13			
	Oleic acid		
0	0.83	0.67	0.50
10	3.04	3.10	2.63
18	2.71	3.18	3.39
22	3.78	3.22	3.23
26	4.38	3.05	3.18
32	4.32	3.20	3.50
37	2.97	3.59	4.00
Linear	**	**	*
Quadratic	**	*	**
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 1.2			
	Linoleic acid		
0	0.33	0.25	0.22
10	1.91	1.74	1.42

18	1.39	1.59	1.82
22	2.21	1.39	1.61
26	2.48	1.01	1.82
32	2.18	1.98	1.42
37	1.93	2.05	1.65
Linear	**	**	*
Quadratic	*	*	*
Cubic	NS	NS	NS

$LSD_{0.05}$ (for same storage week)[x] = 0.74

NS, *, **, Nonsignificant or significant at $P \leq 0.05$, 0.01 respectively

[z] Recoveries obtained by aminopropyl column separations as described in materials and methods.

[y] mg of representative fatty acids/pecan obtained by fatty acid analysis using gas chromatography, as described in material and methods.

[x] Pairwise comparisons between oil levels at a particular storage week.

Table 9: Fatty acid profile of monoglyceride lipid class (mg/pecan) for oils from non-extracted, 22% extracted, and 27% extracted pecans as affected by storage duration. Means are average of six runs[z].

Storage duration (Weeks)	mg/pecan[y]		
	Non-extracted	22% extracted	27% extracted
	Palmitic acid		
0	0.59	0.59	0.54
10	0.73	0.37	0.24
18	0.52	0.29	0.22
22	0.29	0.31	0.24
26	0.30	0.29	0.16
32	0.31	0.31	0.21
37	0.34	0.22	0.15
Linear	**	NS	NS
Quadratic	NS	NS	*
Cubic	**	**	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.21			
	Stearic acid		
0	0.32	0.36	0.34
10	0.34	0.14	0.10
18	0.30	0.14	0.09
22	0.13	0.18	0.08
26	0.17	0.17	0.12
32	0.14	0.16	0.10
37	0.11	0.13	0.10
Linear	*	NS	NS
Quadratic	NS	NS	**
Cubic	**	**	**
$LSD_{0.05}$ (for same storage week)[x] = 0.14			
	Oleic acid		
0	2.27	2.04	1.77
10	2.26	0.71	0.81
18	2.11	0.78	0.65
22	0.73	0.81	0.21
26	0.42	0.85	0.18
32	0.61	0.61	0.12
37	0.85	0.09	0.17
Linear	**	*	NS
Quadratic	*	**	**
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 1.1			
	Linoleic acid		
0	1.75	1.90	1.4
10	1.17	0.96	0.55

18	1.19	0.56	0.17
22	0.46	0.47	0.13
26	0.21	0.27	0.14
32	0.38	0.37	0.32
37	0.47	0.03	0.07
Linear	**	*	NS
Quadratic	*	**	**
Cubic	NS	NS	NS
$LSD_{0.05}$ (for same storage week)[x] = 0.67			

NS, *, **, Nonsignificant or significant at $P \leq 0.05$, 0.01 respectively

[z] Recoveries obtained by aminopropyl column separations as described in materials and methods.

[y] mg of representative fatty acids/pecan obtained by fatty acid analysis using gas chromatography, as described in material and methods.

[x] Pairwise comparisons between oil levels at a particular storage week.

List of Figures

Figure 1

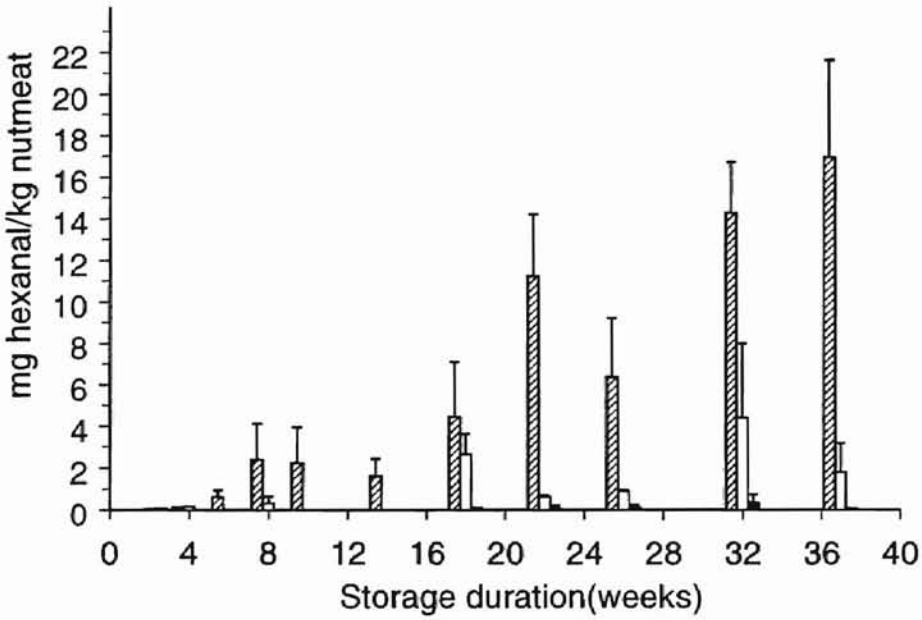
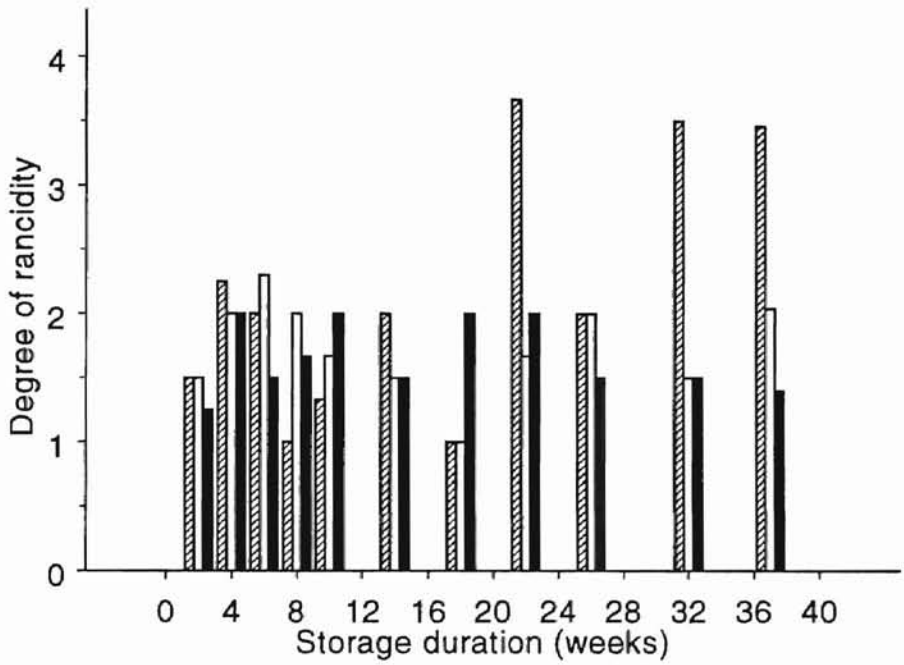

mg hexanal/kg nutmeat
0
2
4
6
8
10
12
14
16
18
20
22
0
4
8
12
16
20
24
28
32
36
40
Storage duration(weeks)

Figure 2


Degree of rancidity
0
1
2
3
4
0 4 8 12 16 20 24 28 32 36 40
Storage duration (weeks)

APPENDICES

APPENDIX A

PECAN WEIGHT CHANGE DURING STORAGE

Storage duration (Weeks)	Weight change[z] (%)		
	Non-extracted	22% extracted	27% extracted
0	0.06	0.00	0.15
2	-0.11	0.09	0.45
4	0.15	0.24	1.34
6	0.04	0.10	1.25
8	0.15	-0.27	1.69
10	0.28	0.48	1.53
14	0.30	0.39	1.84
18	0.28	0.23	1.84
22	0.06	0.29	1.17
32	0.27	-0.22	1.43
37	0.04	-0.13	1.16

[z] Weight change of non-extracted, 22% extracted, and 27% extracted pecans during storage. Weight change in percent of initial.

APPENDIX B

FATTY ACID PROFILES OF UNFRACTIONATED OILS

Oil extracted	Storage duration (weeks)	mg fatty acid/g oil[z]				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
None	0	63.0	26.5	654.1	242.0	13.5
22%	0	60.0	29.2	657.7	240.6	12.1
27%	0	62.3	24.0	650.6	249.6	13.6
None	10	61.1	20.6	651.1	255.0	12.3
22%	10	63.2	20.9	650.1	252.9	12.8
27%	10	60.2	21.9	665.2	240.4	12.5
None	18	60.5	18.3	656.6	252.6	12.0
22%	18	59.6	22.6	666.4	240.7	11.1
27%	18	61.1	19.8	666.3	240.4	12.5
None	22	61.3	20.7	650.0	254.1	13.9
22%	22	61.9	22.1	652.9	251.1	12.2
27%	22	61.4	21.8	662.2	243.4	11.4
None	26	60.8	20.7	661.8	245.3	11.5
22%	26	59.0	20.2	665.4	243.8	11.8
27%	26	58.7	19.8	660.8	248.9	12.0
None	32	59.5	22.2	656.5	250.6	11.3
22%	32	59.8	21.4	648.7	256.5	13.8
27%	32	60.0	21.8	661.9	244.7	11.9
None	37	58.6	20.8	668.8	239.7	12.1
22%	37	59.8	21.4	669.6	236.9	12.4
27%	37	59.2	20.8	669.0	239.2	11.9

[z] Fatty acid profiles of unfractionated oils from non-extracted, 22% extracted, and 27%extracted pecans across storage, obtained by gas chromatography.

APPENDIX C

PERCENT FREE FATTY ACID OBTAINED BY AOCS

OFFICIAL METHOD Ca 5a-40

Storage duration (Weeks)	Free fatty acid (%)[z]			
	Replication	Non-extracted	22% extracted	27% extracted
0	1	0.16	0.11	0.10
	2	0.14	0.10	0.11
10	1	0.21	0.13	0.14
	2	0.20	0.16	0.15
	3	0.20	0.15	0.15
	4	0.21	0.14	0.12
18	1	0.21	0.18	0.16
	2	0.18	0.20	0.15
	3	0.20	0.16	0.19
	4	0.21	0.15	0.16
22	1	0.32	0.18	0.15
	2	0.31	0.18	0.16
	3	0.41	0.20	0.16
	4	0.28	0.16	0.18
26	1	0.24	0.18	0.23
	2	0.20	0.20	0.20
	3	0.24	0.18	0.20
	4	0.25	0.21	0.21
32	1	0.33	0.23	0.20
	2	0.35	0.22	0.20
	3	0.30	0.23	0.22
	4	0.42	0.25	0.23
37	1	0.30	0.25	0.22
	2	0.37	0.23	0.20
	3	0.38	0.20	0.20
	4	0.35	0.25	0.21

[z] Percent free fatty acid of oils from non-extracted, 22% extracted, and 27% extracted pecans across storage.

VITA

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