SAFETY ASSURANCE OF PECANS BY IRRADIATION WITHOUT A DETRIMENTAL EFFECT ON QUALITY

A Dissertation

by

ISIN KARAGOZ

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Approved by:

Co-Chairs of Committee,	Elena Castell-Perez
	Rosana G. Moreira
Committee Members,	Leonardo Lombardini
	Luis Cisneros-Zevallos
Head of Department,	Stephen Searcy

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ABSTRACT

Pecan nuts might become contaminated with foodborne pathogens, such as *Salmonella* and *E. coli.,* through birds and other potential sources of contamination that can lead to serious illness or even death, as well as financial losses. For example, the outbreak of *Salmonella* in pecan products in Texas caused major product recalls in 2010. Irradiation with electron beams could be an effective method of preventing potential outbreaks without changing the pecans' taste, color and flavor and without causing any risk of recontamination before the product reaches the consumer. However, when irradiation is applied alone, the shelf life of the product is decelerated because of the detrimental effect of lipid oxidation. Therefore, to extend the shelf of the pecans while assuring their safety, irradiation of pecans under modified atmosphere packing (MAP) conditions could be a viable option.

This research showed that when treated with electron beams, surrogates of *Escherichia coli* (a cocktail of BAA-1427, BAA-1428, and BAA-1430), and *Salmonella* (*S.* Typhimurium LT2) were more resistant to ionizing radiation (higher D_{10} values) when packed under vacuum (VP) than under air or other MAP conditions.

This research also showed that lipid oxidation in pecans (due to exposure to ionizing radiation) shows a lag phase, probably due to the antioxidants present in pecan nuts. The lag phase represents a delay period before the pecan nuts start to get rancid (increase in PV formation), and it is best described by a modified Gompertz model. Kinetic evaluation of the lipid oxidation reaction suggests that the dose level has a more

drastic effect in PV formation than the type of package used during the irradiation treatment, e.g., vacuum packed versus nitrogen-packed.

Moreover, accelerated shelf life studies (4 weeks at 48.9^{o}C) showed that vacuum-packed (VP) pecans can be stored at -25°C up to three years, while irradiated (at 3.0 kGy) VP pecans can be stored only for eight months, without the detrimental effects of lipid oxidation. Therefore, irradiation of pecans under air at 3.0 kGy reduces the shelf life of the nuts in terms of rancidity, but vacuum-packaging can be used to extend their shelf-life. Irradiation in oxygen packaging increases rancidity and the oxidation reaction rate accelerates with increasing dose. Irradiation under nitrogen packaging requires lower doses to achieve the almost same number of log reductions in microbial population. The use of nitrogen packaging also inhibits the oxidative reaction leading to rancidity in pecans. Although there are some drawbacks to the application of nitrogen packaging in an irradiation plant (special machinery and packaging films (permeability specifications for N_2 gas)), the savings induced by avoiding recalls may make this technology worthy of consideration.

DEDICATION

To

my marvelous sister Merve Karagöz

and

my brilliant husband Dr. Mustafa Uğur Karakaplan.

You are my love, joy and inspiration

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CHAPTER I

INTRODUCTION

Advances in food technology innovations geared towards fulfilling the constantly increase human need have also led to an exponential increase in foodborne diseases. Therefore, specific safety precautions regarding raw nut tree products have been implemented; without treatment, there is a high risk that raw nuts will carry pathogens. Pasteurization is a process of killing the most resistant microorganisms associated with a specific food. Pathogen outbreaks can be controlled by applying pasteurization techniques, either thermal or cold. Thermal pasteurization is the application of a required temperature for a defined length of time, followed by immediate cooling. Cold pasteurization, also known as radiation, is the elimination of pathogens by irradiating the food at a particular dose, and is an alternative to the heating process. Other sanitation treatments such as chemical treatments and thermal pasteurization are not viable options to use for raw nutmeats.

For instance, chemical applications may change the water content of the nuts, the level of which is important for microbial inactivation and for retaining the dryness of the nuts. Chemical applications may also causes a residue to remain on the treated product (Kwakwa and Prakash, 2006). Similarly, thermal applications require high temperatures to achieve microbial safety (Jeong et al., 2009) or sometimes not even sufficient to kill large populations (Beuchat and Mann, 2010); such temperatures cause a decrease in the shelf life of the nut (Ozdemir, 2001) and alter the nut's aroma, taste, and color. On the

contrary, irradiation treatments applied after packaging prevents the risk of microbial contamination between the time of treatment and the time the product reaches the consumer. Depending upon the volume of the product, irradiation can also be competitive with other treatments in terms of cost effectiveness (Kwakwa and Prakash, 2006). Therefore, because radiation does not change the taste, reduce water content, leave residue or require any additional safety precautions post-packaging, it is one of the best solutions for raw nut kernel pasteurization.

Nuts such as pecans are rich in nutritious value, mainly because they have a high-unsaturated fatty acid oil content. However, their high oil content poses a challenge not only to thermal treatments, but also to irradiation, especially when food is irradiated to eliminate pathogens such as *Salmonella*. The greatest disadvantages of this high oil content, for both irradiation and the thermal process, are (1) the change in nutrition value after treatment, and (2) the increased the rancidity, a bitter taste formation. These disadvantages are due to a breakage of the double bonds of the unsaturated fatty acid, which is caused in both treatment processes. Though irradiation ensures a safe product, deterioration of the sensory quality makes irradiated pecans inedible. These disadvantages to irradiation are manageable by using lower doses of radiation, by applying a freezer or coating process treatment, or by modifying the packaging atmosphere (MAP) during treatment.

In order to have a 5-log reduction in pathogens, food must be irradiated to the required dose. One way to lower this dose is by using a radiosensitization technique. This technique increases the sensitivity of the target pathogens (Gomes et al., 2011) by

using low doses of ionizing irradiation along with MAP techniques. Modifying the atmosphere in the package with different gases, such as nitrogen and oxygen, may increase the effectiveness of the radiosensitization process. The oxygen has both synergetic and antagonistic effects on the irradiation of pecans. The synergetic effect decreases the number of viable microorganisms through radiosensitization; however, the antagonistic effect of the presence of oxygen within the pack accounts for the increase in rancidity of the pecans.

Therefore, removing the oxygen from the package is another potential solution for reducing the rancidity of irradiated pecans. While ionizing radiation kills the microorganisms, vacuum packaging could create a lack of oxygen that would help reduce rancidity. However, the required dose of radiation for nuts encased in vacuum packaging is higher than the necessary dose for other MAP types. In other words, when the irradiation dose increases, there is an accompanying degradation in the sensory quality of the pecans due to an increase in rancidity. Such dilemmas are the focus of researchers currently developing treatments to use with irradiation, in an effort to extend the shelf life of pecans while avoiding such detrimental effects to the quality.

Besides radiosensitization, MAP, and vacuum packaging techniques, freezing and coating are other alternative methods that may reduce rancidity and increase the shelf life of pecans. Pecans can be frozen after vacuum or air packaging, prior to any irradiation treatment. Although the irradiation of frozen pecans requires a higher dose of radiation, as compared to unfrozen pecans, freezing decelerates sensory degradation. In addition, to lower the required dose of radiation, pecans can be coated with encapsulated

antimicrobial chemicals before MAP, vacuum or air packaging. Thus, freezing or coating processes could allow pecans to tolerate higher doses of irradiation when vacuum packed, as well as help reduce the detrimental effects of the process to oxygen-packed pecans. Therefore, these treatments (i.e., radiosensitization, MAP, vacuum packaging, freezing, and coating), in combination with irradiation, may greatly extend the shelf life of pecans without impairing their quality.

The main objective of this research is to ensure the safety of pecans through irradiation without adversely affecting their quality. The specific objectives of this study are the following:

(1) Quantify the detrimental effects of oxygen on the quality of irradiated pecans;

(2) Determine the required irradiation dose to assure the safety of pecans using a *Escherichia coli cocktail (BAA-1427, BAA-1428, and BAA-1430), and Salmonella Typhimurium LT2*, under different packaging treatments;

(3) Determine the kinetic model to find the required irradiation dose to assure the quality of pecans under different treatments;

(4) Understand how the methods used in objectives (2) and (3) reduce the rancidity of pecans after irradiation;

(5) Calculate the shelf life of pecans after irradiation by using the modified kinetic model equation.

CHAPTER II

LITERATURE REVIEW

2.1 The Importance and Composition of Pecans

Recently, nuts have become more important to the human diet, especially since there has been an increase in health problems due to an unbalanced nutrition intake threatening human health. Nuts are one of the healthiest ways to obtain the desired energy from fat that all human beings need. Moreover, not only is the United States the world's largest pecan producer, with 117,162 tons of pecans (in-shell) produced in 2010 (USDA, 2010), but also Texas is in the top five pecan-producing states according to the total pecan production recorded in the years 1978 to 2009. Texas was also number one in pecan production in 2010, producing 31,751 tons of pecan (in-shell) in that year. As these facts indicate, pecans play an important role both nutritionally and economically.

Pecans may have vastly different compositions due to the pecan variety, weather conditions, harvesting time and condition, storage and handling. The general composition of pecans listed by the United States Department of Agriculture (USDA, 2011) is water (3.52%), protein (9.17%), total lipids (71.97%), ash (1.49%), minerals (0.89%) and vitamins (0.138%). For the Kanza variety, the composition is three to four percent water and 62% lipids. Pecans are mostly composed of mono-unsaturated fatty

acids. The fatty acid composition of olive oil, one of the most preferred of oils in terms of health concerns, is very similar to a pecan's fatty acid composition, as shown in Table 2.1.

Pecan oil is highly rich in oleic acid (monounsaturated fatty acid, or MUFA) that appears to be neutral relative to low-density lipoprotein (LDL), but raises the high-density lipoprotein (HDL) modestly, which is also known as "good cholesterol" (FAO, 1994). One common fact is that unsaturated fatty acids are useful for decreasing LDL as compared to saturated-fatty acids (Toro-Vazquez et al., 1999). However, Lombardini (2008) were showed that 86.9% of the people that they surveyed indicated that eating pecans make the level of LDL increase. In addition, the fact that pecans have MUFAs is also an important attribute for pecan nuts in terms of nutritional value as compared to other nuts, such as Brazilian nut and pine (Table 2.1) that have a very low content of MUFAs. Pecan oil also has higher linoleic acid concentrations than linoleic acid, (Moore et al., 1990), which is important because the intake of linoleic acid provides between four and 10 percent of energy FAO (1994), and thus is desirable.

Table 2.1 Oleic, Linoleic, and Linolenic fatty acid values of selected nuts in the literature

Nut/Oil Type	Oleic Acid (18:1)*	Linoleic Acid (18:2)	Linolenic Acid (18:3)	Citation
Pecan	40-66%	23-50%	0.65-1.94%	(Ryan et al., 2006; Miraliakbari and Shahidi, 2008; Sathe et al., 2008)
Almond	62-77%	12-33%	0.05-0.8%	(Kodad and Socias i Company, 2008; Sathe et al., 2008)
Olive oil	37-72%	8-45%	NA	(Torres et al., 2005; Sathe et al., 2008)
Pistachio	56-64%	23-31%	0.44%	
Brazilian Nut	29%	42-47%	0.2-0.8%	(Ryan et al., 2006; Miraliakbari and Shahidi, 2008)
Pine	38-39%	50%	0.65%	

*Number of double bonds in the unsaturated fatty acids, i.e., Oleic Acid has 18 pairs of Carbon atoms connected by one double bond. Unsaturated fatty acid can be saturated by adding H atoms to break double bonds.

Unsaturated fatty acids increase the pecan's susceptibility to rancidity that is also affected by increasing temperature. Therefore, temperature control during storage is essential for oil quality, as well as for the overall quality of the pecan. Shelled pecans can be stored for up to ten weeks at 21-25 °C, up to nine months at 4-7.2 °C (Baldwin and Wood, 2006) and up to 24 months at -17.77 °C (Wagner, 2007). Water content is also another important aspect affecting the pecan quality and shelf life, because higher water content triggers microbial activity. If water content stays between three and four percent of percentage kernel (wet basis) mold will not grow, and fats, proteins and carbohydrates will be more stable (Wagner, 2007).

2.2 Pathogen Contamination of Pecans

Although pecans are rich in nutritional value in terms of their high fatty acid content, like any other product consumed raw or after processing, there is a high risk of contamination from pathogens which can occur any time during any of the processing steps, especially during harvesting or packaging (Slauch et al., 1995).

Pathogen contamination does not seem to occur as often as mold, insect or rodent damage because of the pecan's low water activity (~0.6 a_w). However, recently outbreaks in nuts have been increasing.

In February of 2010, after a pecan recall (FDA, 2009), American pecan producers were urged to apply strict sanitation practices in order to increase the safety of their product. The contamination risk from *Salmonella*,, and other microorganisms exists in pecans at each step in the pre- and post-harvest continuum. In the pre-harvest period,

pecans may be contaminated by foodborne pathogens when they fall to the ground for several days before being harvested. Pecans are also contaminated through the absorption of water from the soil that is contaminated by wild- or domestic animal feces, (Beuchat and Mann, 2010), water contaminated by inadequately composted manure, or run-off water from land inhabited by livestock. In the post-harvest period, sources of contaminated water include: water used for mechanical harvesting (removal of leaves, sticks, stones, soil and other foreign matter); transportation or handling; cleaning by immersing pecans into hot or cold water, spraying, or steaming before cracking; and shelling. These cleaning applications may not contain enough chlorine to eliminate foodborne pathogens (Beuchat and Mann, 2010).

2.3 Irradiation Treatment

Irradiation, also known as cold pasteurization, is the most effective sanitation practice for pecans. Irradiation can be applied by using gamma rays, electron beams, or X-rays. The difference between these irradiation treatments is the source from which the energy comes. Food safety can be achieved through irradiation by damaging the DNA of pathogens contaminate the food. The direct effect of irradiation is that an electron hits the DNA helix (one or both helixes) and causes damage to the DNA, which results in the death of the cell. The indirect effect of irradiation is that electrons hit the water molecules and produce the following primary water radicals: H, OH, and $e^-_{(aq)}$ (Gomes et al., 2008). These radicals, especially OH, attract the chromatin fiber inside the DNA helix and increase the susceptibility of the DNA helix (Friedland et al., 1999).

The FDA has approved the irradiation of nuts for disinfestation of arthropod pests up to 1-kGy, under the phytosanitary regulations recorded in 21 CFR 179.26 (2005). However, irradiation for the pathogenic microorganism disinfestation of nuts has not yet been approved. On the other hand similar foods, such as ready-to-eat foods and seeds, can receive irradiation up to 7-kGy under the ([FDA-HHS], 2001) petition; thus, another petition will be needed to act as an amendment to include nuts (Sommers and Fan, 2006).

However, the applied dose of irradiation may impart detrimental effects on the quality of food (Turgis et al., 2008). Hence, a combination of radio sensitizers and irradiation may have a more positive overall outcome on inactivating the pathogens. In baby spinach, Gomes et al. (2011) show that a 5-log reduction of *Salmonella spp.* is achieved by using 100% O_2 MAP with a low dose (0.7-kGy) irradiation treatment. Their work shows that 100% O_2 is converted to ozone by irradiation, which radiosensitizes the pathogens while improving the shelf life of the product (Gomes et al., 2011).

Radiation treatment at doses of 0.15-0.7 kGy under specific conditions may be feasible for the control of many foodborne parasites (Farkas, 1998). Al-Bachir (2004) found that a dose of 1.5 kGy did not have any significant effect on the sensory quality or nutritional value of walnuts, and this dose was sufficient for insect disinfestation without causing detrimental effects on the sensory characteristics of walnuts. In addition, the peroxide values (PV) (mmol /kg oil) of non-irradiated and irradiated (0.5, 1, 1.5, 2 kGy) walnuts were compared. It was found that there were no significant differences between the peroxide values of non-irradiated and irradiated samples right after irradiation.

However, after 12 months of storage (at 15-18° C and 50-70% relative humidity), the PV of the samples irradiated with 0.5, 1 and 1.5 kGy doses were significantly lower, but the PV of the samples irradiated with 2.0 kGy significantly increased (0.6 mmol /kg oil) as compared to those of non-irradiated walnuts (Al-Bachir, 2004).

Irradiation doses recommended to eliminate microorganisms in the contaminated food should also be sufficient to inactivate almost all the non-spore forming pathogens. The necessary value is the radiation dose (in kGy) required to achieve a 90% reduction in viable microorganisms. The value depends upon each microorganism's type and temperature, and the composition and condition (vacuum-packed, frozen, etc.) of the product during irradiation. In order to achieve pasteurization, a 5-log CFU/g (5-D performance standard) reduction of *Salmonella* is required (USFDA, 2011). Raw almonds need at least 5 kGy to obtain a 4-log CFU/g reduction (D-value of 1.25) for *Salmonella Enteritidis* PT30, and they were found to be unacceptable to consumer panelists because of their bitter taste (Prakash et al., 2010). Mexis and Kontominas (2009a) used the 7-kGy dose on peanuts and pistachio nuts, which increased the oxidation rate and the level of saturated fatty acids while caused the decreasing in the value of unsaturated fatty acids.

It is clear that irradiation has a negative effect on the quality of nuts because of oxidative rancidity, which is the degradation of oils with the help of oxygen in the package atmosphere. Lipid oxidation occurs via three pathways: non-enzymatic non-radical photo oxidation (photo oxidation), non-enzymatic chain auto oxidation mediated by free radicals (auto oxidation), and enzymatic oxidation. Briefly, photo oxidation only

forms singlet oxygen which leads to hydroperoxide formation, which then breaks down into free radicals that trigger the initiation of auto oxidation (Laguerre et al., 2007). Auto oxidation is the key mechanism in lipid oxidations, and the process has three parts: initiation, propagation, and termination.

Initiation occurs spontaneously with triplet oxygen. The reaction can initiate via external sources such as heat; ionizing radiation; photonic impact in the UV spectrum; and chemical agents such as metal ions, free radicals and metalloproteins.

In *propagation*, the radicals that form in the initiation stage react with triplet oxygen and generate different radical species such as peroxyradicals, which form hydroperoxide (a primary oxidation compound), as well as other radicals. These "other radicals" then react with triplet oxygen and go through the same cycle. Thus, this stage is a self-sustained radical chain.

In *termination,* oxidation continues until secondary non-radical oxidation compounds form such as aldehydes, alcohols, volatile ketones and volatile compounds, non-volatile compounds and non-volatile aldehydes, oxidized triacylglycerols and their polymers. Once the polymers form, the reaction terminates. Antioxidants also help to terminate the reaction (Laguerre et al., 2007).

Thus, lipid oxidation in pecans may occur without irradiation if an appropriate storage environment is not provided, such as a controlled temperature and relative humidity. However, in the process of irradiating pecans, ionizing radiation is the initiator of the oxidation process that triggers the oxidation responsible for rancidity.

One means of reducing rancidity is to use antioxidants**,** compounds that work against oxidation, either in the initiation phase (called preventive antioxidants) or in the propagation phase (called chain-breaking antioxidants). Antioxidants can also combine different mechanisms that thwart oxidation (Laguerre et al., 2007).

Oils that are naturally rich in antioxidants such as tocopherols and carotenoids are preferred because they improve oil stability (Rudolph et al., 1992; Miraliakbari and Shahidi, 2008). Miraliakbari and Shahidi (2008) found that pecan nuts had the highest oil content (71.5 % w/w) with a peroxide value of 0.023 to 0.03 meq/kg of oil, and pecan oil had the highest total phenolic content (TPC), 783 mg/kg α-tocopherol equivalent, as determined by the chloroform/methanol extraction method, among almonds, brazil nuts, hazelnuts, pine nuts, pistachios and walnuts (Miraliakbari and Shahidi, 2008). Toro-Vazquez et al. (1999) also found the total tocopherol to be 255 mg/kg oil equivalents by HPLC (hexane/acenonitrile/2-isopropanol (98:1:1, vol//vol/vol) extraction), but the data did not show any significant relations between the fatty acid composition and α-, β-, or γ-tocopherol. Besides tocopherols, additional natural antioxidants like tyrosol, hydroxytyrosol and caffeic acid might cause high oxidative stability, as observed in pecan oils (and kernels) (Toro-Vazquez et al., 1999).

Antioxidant capacity (Rudolph et al., 1992) and tocopherol content (Toro-Vazquez et al., 1999) were significantly influenced by cultivars, as well as by the geographic and climatic conditions under which the plant was grown. Villarreal-Lozoya et al. (2007) showed that Kanza cultivar had the highest antioxidant capacity (817 μmol Trolox equivalents TE/g Orac assay and 135 mg TE/g free radical assay) and highest

total extractable phenolic content (106 mgCAE/g defatted kernel) among the other cultivars, including Desirable, Nacono, Kiowa, Pawnee, and Shawnee (Villarreal-Lozoya et al., 2007).

Therefore, irradiation is a method of ensuring the sanitary quality of food, and is a substitute for fumigants or other sanitary processes used in Asian countries and the USA. The use of higher doses of irradiation alone, or the use of lower doses of irradiation in combination (Morehouse Kim and Komolprasert, 2004) with other process such as MAP, freezing, and coating, are beginning to grow in prominence in the food preparation industry.

2.4 Modified Atmosphere Packaging (MAP)

Another way to counteract the onset of rancidity in pecans is the use of Modified Atmosphere Packaging (MAP) principles. MAP is a modification of the gaseous atmosphere within a food product's package. The concentration of this gaseous atmosphere has a huge effect on the spoilage rate of the food product during storage (Robertson, 2005), which determines the shelf-life of the food.

When the food product is packed raw, it is still alive and respiring. Therefore, the atmosphere within the package is more important than inside that of packed processed food. Irradiation, in combination with MAP, has been found to be very beneficial for the packaging of fresh fruits and vegetables (Patil., 2004; Gomes et al., 2011)

Concentrations that can be used in MAP can be high in CO_2 and low in O_2 levels (up to 10% O_2); can have high oxygen levels (up to 70% O_2); can be 100% N_2; or can

contain a varying mixture of CO_2, N_2, and/or O_2 (Jay, 2005). However, low oxygen concentrations have a minimal effect on some pathogens such *as Listeria, Yersinia*, and *Salmonella,* due to the toxin formations that occur in low oxygen levels for fresh fruits and vegetables (Patil, 2004; Gomes et al., 2011).

On the other hand, a high oxygen concentration in MAP creates toxic ozone gas when ionizing irradiation passes through the oxygen (Miller, 2005). These toxic ozone molecules are highly effective in reducing food spoilage. Therefore, using a high oxygen concentration along with an irradiation treatment increases the effectiveness of inhibiting pathogens such as. *Salmonella* (Gomes et al., 2008). The presence of oxygen is more effective in low Linear Energy Transfer (LET) than high LET (Turner, 2007).

Although oxygen-flushed MAPs have a synergistic effect with ionizing irradiation on inhibiting microorganisms due to ozone formation, oxygen existence does increase the rancidity of pecans because of auto-oxidation. Prior to irradiation, oxygen has already initiated the auto-oxidation, and irradiation speeds up the auto oxidation reaction (Laguerre et al., 2007). Therefore, the inclusion of nitrogen in MAP, vacuum packaging, coating, and freezing prior to irradiation are all alternatives for reducing rancidity.

Since nitrogen is an inert gas and vacuum packaging does not include any gas, neither will be involved in any reaction. Consequently, there is no synergistic effect on killing pathogens. In contrast, neither nitrogen nor vacuum packaging will accelerate the rancidity process due to both enjoying a lack of oxygen. Thus, both vacuum packaging

and different gas concentrations used in modified atmosphere packaging are new approaches to increasing the shelf life of pecans.

2.5 Coating Process

Coating is another alternative solution for a having safe, nutritious, stable, and high-quality food product by extending the shelf-life of the product (Wambura et al., 2010). Edible coating is a method of dipping the food product into different types of chemical solutions to create a coat on the top of the product. This edible coat provides a semi-permeable barrier to gases (Baldwin et al., 1995). Nuts are coated mainly to prevent rancidity and to maintain their texture. Several studies (Swenson et al., 1953; Senter and Forbus, 1979; Baldwin et al., 1995; Mate and Krochta, 1997; Baldwin and Wood, 2006; Wambura et al., 2010; Sayanjali et al., 2011) show that edible coatings applied to nuts have a positive effect on increasing their oxidative stability and shelf life.

Nuts have been coated with protein-, lipid- and polysaccharide-based coatings. Pecans, on the other hand, have been coated with lipid- and polysaccharide-based coatings. Polysaccharide-based coating (pectin-, cellulose-, starch-, and chitosan-coatings) possesses good gas carrier (O_2 and CO_2) properties, rather than the prevention of water loss (due to their hydrophobic structures). Lipid-based coatings such as beeswax, mineral and vegetable oils; coating with antioxidants (AOX, e.g., citric acid and phenolic compounds); butylated hydroxyanisole (BHA); and butylated hydroxytoluene (BHT)) are all good moisture barriers. Coating with AOX helps to protect against oxidative rancidity. Certain phenolic compounds also work as AOX, such

as BHA, BHT, TBHQI (tertiaty butylated hydroxyl-quinone), tocopherols, or acids (e.g., propyl gallate) (Baldwin et al., 1995).

Baldwin and Wood (2006) coated pecans (a desirable variety) with a combination of 2% CMC (carboxymethyl cellulose, a polysaccharide-based coating, to restrict oxygen contact), 0.2% lecithin (an emulsifier and surfactant), 3% propylene glycol (PG, for texture), and 0.5% α-tocopherol (an antioxidant) in DI water (distilled water). Baldwin and Wood showed that coated (the formulation of which is given above) pecans' hexanal levels 4-fold less than uncoated controls after nine months of storage in plastic zip lock films in ambient temperatures (20-25° C). Coated pecans' hexanal levels were (~30 μl/ml), while uncoated controls were (~130 μl/l). Kernels with low hexanal levels indicated less oxidation of fat, which means the pecans were less rancid (Baldwin and Wood, 2006).

Furthermore, Senter and Forbus (1979) coated pecans (Schley, Halbert, Seedling) with AMG (acetylated monoglycerides, a lipid-based coating) and evaluated them for color and PV for 24 weeks of accelerated storage at 30° C and 50% relative humidity in the dark. None of the varieties showed any significant difference ($P < 0.05$) along with the treatments after twenty-four weeks. However, there were significant differences ($P < 0.01$) between the varieties: Schley (~0.3 meq /kg oil), Halbert (~ 0.1 meq /kg oil), and Seedling (~0.8 meq /kg oil), Low PV value indicated less oxidation in fats (Senter and Forbus, 1979).

2.6 Freezing

Freezing before irradiation is a common method used in poultry and meat products. Ahn et al. (2000) showed that the radiation chemistry of refrigerated and frozen meats can be different; they suggest that temperature had a significant effect on the formation of radiolytic products because reactive intermediates of water radiolysis were trapped in deep-frozen materials, which did not let them react with each other or with substrates (Ahn et al., 2000).

On the other hand, Luchsinger et al. (1996) reported that freezing slowed down the negative effects of oxidation which were accelerated by irradiation; however, irradiation did not completely eliminate such effects (Luchsinger et al., 1996). Thus, irradiation of frozen pecans could help reduce the onset of rancidity. There are no studies showing the effects of freezing and irradiation on pecans or other nuts in the literature.

CHAPTER III

KANZA PECANS [CARYA ILLINOINENSIS (WANGENH.) K.KOCH] PROPERTIES AND E-BEAM IRRADIATION

3.1 Summary

In 2003, the FDA began putting a health claim label on nut products indicating that daily consumption of nuts as a part of a diet low in saturated fat may reduce the risk of heart disease ((21 C.F.R. §§ 101.14(e)(3) and 101.13(h))). Nuts, such as pecans, are mostly composed of oil (60-70%); therefore, they are more susceptible to lipid oxidation, which causes rancidity. The lipid oxidation accelerates when pecans are exposed to ionizing radiation, heat, or oxygen. The objectives of this study were to show those properties of pecans (of the Kanza variety) that should necessarily be the focus of future experiments testing the effects of heat, irradiation, and oxygen on the increase of lipid oxidation in pecans.

In this study, the following properties of Kanza pecans were evaluated: percentage kernels (58%), number of kernels per pound (302 kernels per pound, jumbo sized), lipid content (62.64 ± 2.6%, wet basis), moisture content (3-4 %), and color [light (gold) kernel color]. In addition to these properties of pecans, the effect of heat and irradiation on these nuts was evaluated by testing the peroxide values (PV) of commercial pecan oil, and pecan oils of the Kanza variety that were extracted in two different ways: the Hexane- and Soxhlet-methods. Then the pecan oils were irradiated at low doses, and the PVs were tested both before and after irradiation. Out of the three

pecan oil samples, the oil extracted using the Hexane-method, which has less heat treatment steps compared to the Soxhlet-method and the standard processing of commercial pecan oils, gave the lowest PVs (1.34±0.33 meq peroxide/kg sample) after irradiation. There were no significant ($P<0.05$) differences found in the pecan oils before and after irradiating them at a 1 kGy (low) dose within the same oil (oil obtained from the Hexane extraction method). Therefore, we can conclude that the heat treatment affected the rancidity of the pecan oils, and the low irradiation dose did not affect the rancidity of the pecan oils (when compared to before and immediately after irradiation). For this reason, the pecan oils that were extracted with the Hexane method were irradiated at high dose of 3 kGy and flushed with nitrogen and oxygen gases. High dose irradiation treatments and the presence of oxygen significantly affected the rancidity of the pecan oil.

3.2 Introduction

Pecans are important because of their rich lipid content, but this same rich lipid content makes pecans more prone to oxidation formation. In addition to the lipid content of pecans, several factors such as heat, irradiation, and the availability of oxygen accelerate the oxidation formation in pecans, causing a rancid taste that shortens the shelf life of the product. To begin addressing this issue, preliminary tests were conducted with pecan oil samples to observe the effects of the irradiation, heat, and oxygen factors. All experiments were conducted with one cultivar in order to achieve consistency in the results and to avoid any supplementary factors such as different nut sizes, fat and

moisture content, water activity, density, and porosity values that may increase the rancidity of pecans For example, differences in nut size may cause variations in the dose of radiation absorbed by the pecan kernels. For instance, if a small and a large kernel are irradiated at the same time, the small kernel will absorb more energy than the larger kernel because the dose is the measured absorbed energy from an ionizing radiation deposited per kilogram of matter (Alpen, 1998).

Additionally, the lipid content will also change the oxidation results. The higher the lipid content the pecan has, the faster it becomes rancid. The lipid content of pecans differs from one variety to another. Toro-Vazquez et al. (1999) showed that the protein (6.98-10.16%), water (2.41-4.6%), and lipid (70.31-79.48%) content of different pecans vary among the following varieties: Queretaro, Guanajuato, and San Louis Potosi. Also, Serter (1976) found a 72-75% pecan oil composition among the Mahan, Stuart, Cheyenne, and Shoshoni cultivars.

In this study, Kanza pecans [*Carya Illinoinensis* (Wangenh.) K. Koch] were selected as the pecan variety for testing due to previous research conducted by Villarreal et al. (2006) on the effects of e-beam treatments on the antioxidant (AOX) properties of different pecan cultivars. Villarreal et al. (2006) determined that Kanza cultivars gave the best results in terms of AOX and oxygen stability during an accelerated shelf life study at 40 °C and 55-60% relative humidity (RH) for 134 days. In addition, moisture content, water activity (a_w), and density values are also important in making dose calculations. Therefore, working with a specific cultivar of pecan kernel helped to avoid

any additional factors that might affect the rancidity of the pecans while we were testing the effects of heat, irradiation, and oxygen.

The effect of heat was compared in the commercial pecan oil and the Kanza pecan oils that were extracted using the Hexane- and Soxhlet-methods. McGlamery and Hood (1951) showed that pecans that were treated at 80° C for 15 minutes gave lower PVs than untreated pecans, which supports the notion of an inhibition of oxidative enzymes at a temperature of 80° C. Also, Buransompob et al. (2003) reported that short time heat treated (STHT) walnut kernels did not show any significant differences compared to untreated pecans. In this study, unlike McGlamery and Hood (1951) and Buransompob et al. (2003), the Hexane extraction method gave the lowest PV results because it used a low heat treatment (40° C) compared to that of the Soxhlet-method.

One reason why the Soxhlet-method (70° C) did not have low PVs could be that the amount of heat applied to pecans during the extraction process was not enough to inhibit the enzymes. Another reason could be that the results of this study may indicate that the oil oxidation that was observed was not an enzymatic reaction, as Villarreal-Lozoya et al. (2009) suggested in their research. When the dose amount increases, the probability that the electrons will hit and break the bonds in the fatty acid chains also increases. Since the oxidation reaction is a continuous process, an increase in broken fatty acid chains may, therefore, cause the pecans to get rancid at a faster rate.

The objectives of this study were (1) to determine the properties of the Kanza pecan variety used throughout this study in order to have consistent results; (2) to determine the quality attributes of Kanza pecans such as color, water activity, moisture

content, and peroxide value, which is information that will be needed for future studies; and (3) to determine the effects of heat, irradiation and oxygen on the rancidity of extracted pecan oils.

3.3 Materials and Methods

3.3.1 Sample Collection and Storage

3.3.1.1 Kanza Pecans

Kanza [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars were obtained from a fall of 2009 crop grown in the Kansas State University pecan experiment field. After they arrived on campus, the pecans were weighed both before and after removing the shell in order to calculate *the kernel percentage* (Thompson and Grauke, 2003) as

$$\text{ernel} \quad \frac{\text{ecan kernel (without shells)}}{\text{ecan with shells}} x100 \qquad [3.1]$$

Shelled pecan halves were placed in plastic Ziploc® bags and stored at -25°C until needed for further use.

3.3.1.2 Commercial Pecan Oil Sample

The industrial standard for pecans' PV is 5 meq/kg (Queenswood, 2006). However, commercially processed oil samples have higher PVs due to the refining process; for example, the international olive oil council set the upper PV limit for extra virgin olive oil at 20 meq/kg (Okogeri and Tasioula-Margari, 2002), which is very close

to the fatty acid composition of pecan oil (Table 2.1). Therefore, the commercial pecan oil's PVs are higher than those of the pecan oils extracted by the Hexane- and the Soxhlet-methods. For this reason, the commercial pecan oil sample was irradiated at a 1 kGy dose to see how the low irradiation dose would affect high PV pecan oils (such as the commercial pecan oil). Next, the commercial pecan oil PVs calculated both before and after irradiation were compared to the Kanza pecan oils' PVs extracted by the Hexane- and Soxhlet-methods. The commercial pecan oil sample that was purchased from the gourmet food mall website (GourmetFoodmall-website, 2009) was shipped in a 500 gram clear glass bottle.

3.3.2 Pecan Properties

3.3.2.1 Number of Half Kernels per Pound

The number of half kernels per pound was calculated after the shells and center walls of the pecans were removed. Samples were measured in triplicate. The size classification of pecans can be specified according to the number of halves per pound (Table 3.1). The size classification is important for future irradiation and microbiology experiments in order to facilitate consistent dose distribution calculations. The size of the pecan kernels will be consistent; therefore, the amount of the dose absorbed by the pecan kernels will also be consistent for all kernels treated with the ionizing radiation.

Table 3.1 Pecan halves size classification

Size classifications for halves	Number of halves per pound
Mammoth	250 or less
Junior mammoth	251-300
Jumbo	301-350
Extra large	351-450
Large	451-500
Medium	551-650
Small (topper)	651-700

Source: (USDA, 1997)

3.3.2.2 Moisture Content

The moisture content of the pecans was measured using two methods. In the first method, the pecan kernels were minced with a food processor, and 5 grams of the minced pecan kernels (Erickson et al., 1994; Buransompob et al., 2003; Villarreal-Lozoya et al., 2009) were dried at 60-65º C (>13.3 kPa) to a constant weight (for about 10-12 h) in a vacuum oven (Squared Lab Line Instruments, Melrose Park, IL, USA), following the AOAC method 930.04 (AOAC, 1995). The second method also followed the AOAC method 930.04 (AOAC, 1995) with the exception that two whole pecan halves were dried instead of minced. The reason whole pecan halves were used was to determine if there would be any moisture content changes due to deformation of the pecan kernel. Samples were tested in triplicate, with two replications per test.

3.3.2.3 Water Activity (a_w)

The water activity of the pecans was determined using a Rotronic Hydrometer (Rotronic Instrument Corp., Huntington, NY, USA) at room temperature. The minced (2 g) pecan samples were placed in an airtight chamber, and the corresponding water activity and temperature were recorded through a display panel that was connected to that airtight chamber. The instrument was calibrated using Magnesium Chloride (a_w= 0.327±0.001, at 22° C). Samples were tested in triplicate, with two replications per test.

3.3.2.4 Lipid Extraction Methods

Soxhlet-Henkel Method:

Pecans were ground with a small chopper and the minced pecans (60 grams) were dried in an oven (Lab-Line Instruments Model 3618-5, IL). About 4 grams of minced pecans were weighed in a cellulose thimble (model 2800256, Whatman, England) throughout the oil extraction and closed with cotton. The weight of the desiccated empty metal cups (previously dried in a conventional oven at 105° C for 15 minutes and cooled in the desiccator) where oil was to be collected was recorded. The pecan kernels were defatted between 2 and 2.5 hours in the Soxhlet unit (Tecator Soxtec System HT 1043 Extraction Unit Pertorp, Inc., Silver Spring, MD, USA), by using petroleum ether (50 ml) as a solvent. After extraction, the residual traces of petroleum either were removed and the sample weight was recorded to calculate the lipid content of the pecans as

$$\text{ipid}(\quad) \quad \frac{f \quad 0}{p} \text{x } 100 \qquad [3.2]$$

where,

W_f= final weight of metal cup (g)

W_0= initial weight of metal cup (g)

W_p= minced pecans (approximately 4 g)

Hexane Method:

This method was used to obtain pecan oil for further experiments, such as the measurement of peroxide values and oil irradiation tests. Pecans were minced (using a food processor) and weighed 24 grams into 500 ml beaker and filled with hexane (1:20 w/v) chemical (Villarreal-Lozoya et al., 2007). The beaker was sealed with parafilm and kept under the hood overnight. Samples were filtrated with a Buchner funnel and slow-filtration rate filter paper (Fisher filter paper number 4). After defatting the cake (remainings) two more times, the hexane was evaporated by using a rotary evaporator (Heidolph Laborota 4001, Germany). The oil was flushed with 100% N_2 for 15 minutes, weighed and recorded.

Mechanical Extraction Method:

Ten grams of pecans were weighed, recorded, and placed into three Ziploc® packs, which had already been weighed and recorded. The pecans were pressed in a hydraulic press (Baileigh Industrial Model HSP-10H, Baden-Württemberg,

Deutschland). After pressing, the pecans were removed and the oil and Ziploc® bags were weighed (final weight). The oil percentage was calculated as

$$W_{oil} = W_{Ziploc+oil} - W_{ziploc} \quad [3.3]$$

Since the weight of pecans was 10 grams,

$$\%Oil = 10\ x\ W_{oil} \quad [3.4]$$

3.3.2.5 Peroxide Value (PV)

The oil was obtained using the Hexane extraction method, which was described in section 3.3.2.4. were used to determine the PV according to the A.O.C.S. official method Cd 8b-90 (Villarreal-Lozoya et al., 2009). This iodometric method calculates the hydroperoxides that are primary oxidation products, generally referred to as peroxides (Gray, 1978), as

$$\text{Peroxide value (me peroxide kg oil sample)} \quad \frac{(\quad)\ \text{x}\quad \text{x}\ 1000}{\text{eight of sample (g)}} \quad [3.5]$$

where,

B = volume of titrant, ml of blank

S = volume of titrant, ml of sample

N = normality of sodium thiosulfate solution (0.01 N)

Test was performed in duplicate.

3.3.2.6 Density (Bulk and True)

Two different methods were reported by different authors to determine the bulk density of foods. In the first method, the pecan kernels' bulk density was measured as the mass occupying a 200-ml volume beaker volume at room temperature (Nelson, 1981; Aydin, 2003; Moreno et al., 2007). In the second method, the bulk density was measured by a li uid displacement techni ue using u oyancy force (Archimedes' principle) (Lozano et al., 1980; Thompson and Grauke, 2003) with toluene (Moreira et al., 2009). Toluene (EMD Millipore chemicals, Billerica, MA) was used instead of water as a displacement liquid for three reasons: (1) the low surface tension; (2) the low dissolution power (Aydin, 2003) because pecans dissolve in water and leave a light yellow color and residuals; and (3) the low density (0.87 g/ cm^3) of the toluene, so that the pecans will not float on the surface and the liquid displacement volume will cover the whole pecan volume (1 g/cm^3) shown in figure on page 45. Both the liquid displacement and mass occupation of bulk density methods were calculated as

$$\rho_b = \frac{m_{pecans}}{V_b} \qquad [3.6]$$

where V_b is the bulk volume of the pecans. For the mass occupation technique, V_b was 200 ml and for the liquid displacement technique V_b was the volume of displaced liquid without air space (cm^3) and m_{pecans} was the mass of the pecans (g). The method was

applied to both unfrozen (at room temperature, 21°C) and frozen (taken out from a -25°C freezer and used immediately) whole pecan halves, with five replications.

True density, also called particular density, is based on the particular volume, which includes the internal pores (Nelson, 1981; Lozano et al., 1983). The true density of both the frozen and unfrozen whole pecan halves (~ 10 g) was measured using a helium gas multi-pycnometer (Quantachrome & Trade, NY, USA) (Moreira et al., 2009). The helium pycnometer was used for accurately measuring the volume of the material, including the air spaces, using the principle of oyle's aw. The volume of the pecans was calculated by using two pressure readings (P_1 and P_2) taken from the replacement of the helium gas in a known reference volume (Vr) and a known cell (Vc),

$$V_t = V_c - V_r \cdot \left(\frac{P_1}{P_2} - 1\right) \quad [3.7]$$

where Vc and Vr were 151.906 and 94.265 (cm^3), respectively. Therefore, the true density was calculated as

$$\rho_t = \frac{m_{pecans}}{V_t} \quad [3.8]$$

where ρ_t is the true density, m_{pecans} is the mass of pecans and V_t was calculated from Eq. [3.7]. The true density values were determined with five replications each for both

unfrozen (at room temperature) and frozen (taken out from -25° C freezer and used immediately) pecans.

Finally, the Porosity, ϕ, of both the unfrozen and frozen pecans was calculated using the following equation:

$$\phi = 1 - \frac{\rho_{bulk}}{\rho_{true}} \quad [3.9]$$

Knowing the porosity of the pecans is necessary for understanding the internalization of microorganisms during microbiological experiments and the D_{10} value calculations (Chapter 4).

3.3.2.7 Color

In this study, the different parts of the pecan kernel (the top and bottom parts) and minced pecan kernels were evaluated in terms of consistency of color. One individual pecan half was used in each reading to measure the top and bottom parts of the pecan kernels' color, and ten readings were recorded for each side. A total of 16 pecans were minced (~ 24 grams) and used for the minced pecan color measurements. The mean values were used to determine the color coordinates L* (lightness - darkness), a* (redness/greenness), and b* (yellowness/blueness). A Labscan XE (16437) colorimeter (Hunter Lab, Inc., Reston, VA, USA) with the CIELAB system with a measuring aperture diameter of 36 mm and illuminant/viewing geometry of D65/10o

was used. The colorimeter was calibrated using the standard white and black plates (Aydin, 2003).

3.3.3 Pecan Oil Irradiation

Two different sets of pecan oil experiments were conducted to evaluate the effects of (1) the oil extraction methods on the oxidative rancidity of pecans after irradiation and (2) the presence of oxygen on rancidity after irradiation.

In the first set of experiments, the Kanza pecan oil extracted using the Hexane-method and the Soxhlet-method, and the commercial pecan oil (purified) were irradiated, and the PVs of each extraction methods were tested before and after the irradiation treatment. The mechanical oil extraction method was not used in this set of experiments because the oil that was extracted from mechanical extraction method was not clear enough (the residuals blur vision) and required continued processing with the hexane method to obtain cleaner oil. In the second set of experiments, the effects of the oxygen and a high dose of irradiation (3 kGy) on the pecan oil's rancidity were evaluated. In conclusion, the Kanza pecan oil (extracted with the hexane method) was irradiated under two different atmospheric conditions, N_2 (100%) and O_2 (100%), at a 3-kGy dose. Non-irradiated samples for both the N_2 and O_2 flushed packages served as controls. The objectives of the second set of experiments were to (1) evaluate the effects of a high dose of irradiation (3-kGy) and the presence of oxygen on oil rancidity and (2) to determine the impact of the combination treatment of a high dose of irradiation and oxygen packaging on accelerating the rancidity of pecans.

3.3.3.1 Experiments to Determine the Effects of Oil Extraction Methods on Rancidity After Irradiation

Pecan Oil Sample Preparation:

One gram each of three types of pecan oils (one extracted using the hexane method, one the soxhlet method, and one the commercial pecan oil) were weighed into 12 conically shaped small tubes (VWR polyethylene 1.7 ml centrifuge tubes) and sealed with parafilm (Figure 3.1a). The samples were examined with two replications.

Irradiation of Pecan Oils:

Irradiation of the samples was carried out using a 1.35 MeV electron beam Van De Graaff accelerator (low energy) located at Texas A&M University. The pecan oils in the conical tubes were irradiated from the front (0.75 kGy) and the back (0.75 kGy) for a total dose of 1.0 kGy. The dose calculations were made by using the conical tubes' geometry and the density of the pecan oil. The source energy input spectrum and source size were entered into the Monte Carlo N-Particle radiation transport code (MCNP5) to obtain the dose distributions in the conical tube of pecan oil irradiated with a 1.35-MeV e-beam accelerator. The e-beam source was assumed to be a parallel plane (Figure 3.1b) large enough to cover the target (Figure 3.1c) and the electrons were emitted in a plane and distributed evenly within the scan (Kim et al., 2007). Samples were irradiated at room temperature.

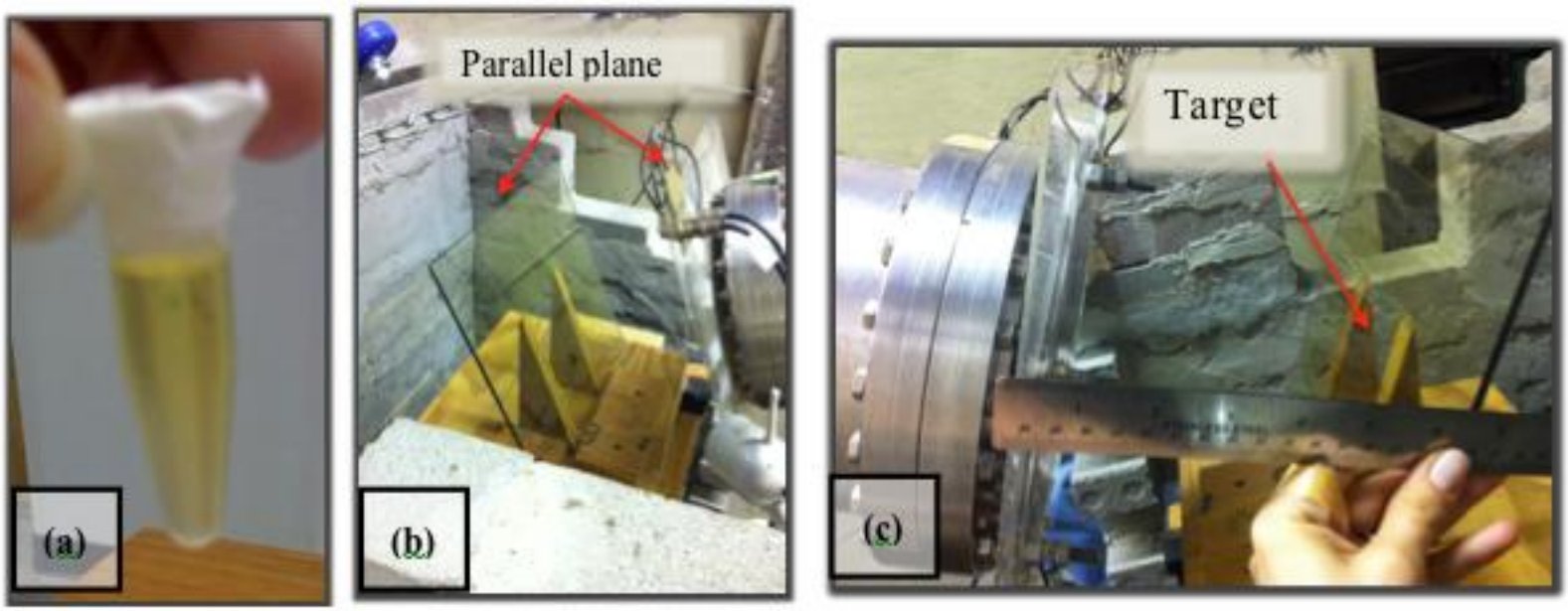

Figure 3.1 Pecan oil irradiation. (a) Pecan oil placed in a 1.7 ml conical tube. (b,c) Pecan irradiation set up in the Van De Graaff accelerator. The e-beam source was assumed to be a parallel plane (b) and large enough to cover the target (c).

3.3.3.2 Experiments to Evaluate the Effects of Oxygen and High Doses of Irradiation on Pecan Oil Rancidity

Pecan Oil Sample Preparation:

The pecan oil was extracted using the Hexane extraction method that was described in Section 3.3.2.4. Then, five grams of pecan oil was weighed into Mylar bags (Zip eal™, 8.64 x 10.16 cm, 48Ga ET E 0.00035 Foil D E, orbent ystems, Impak Co., Los Angeles, CA, USA), for a total of 18 Mylar bags. Mylar bags have an excellent oxygen barrier with a transmission rate of 0.0006 cm^3/ 100 in^2 /24 hrs. Next, either 100% oxygen or 100% nitrogen gas was flushed into the Mylar bags (six bags for each gas) until the bags were completely filled. After filling, the bags were immediately sealed and carried to the Van De Graaff accelerator (1.35 MeV) located at Texas A&M University.

Irradiation of Pecan Oils:

For a total of 12 pecan oil samples, six packs were flushed with N_2 gas and six were flushed with O_2 gas. Then, three bags of pecan oil samples for each gas were irradiated with a 3.0 kGy dose. Two groups of three non-irradiated bags, each containing only N_2 or only O_2, served as controls. Dose calculation was performed using the Mylar bag geometry (assumed to be a cylinder) and the density of the pecan oil. The source energy input spectrum and source size were entered into the Monte Carlo N-Particle radiation transport code (MCNP5) to obtain the dose distributions of the pecan oil in the Mylar bags that were irradiated with the 1.35-MeV e-beam accelerator. The e-beam

source was assumed to be a parallel plane large enough to cover the target (Figure 3.1b) and the electrons were emitted in a plane and distributed evenly within the scan (Kim et al., 2007). One Mylar bag was irradiated each time. For the dose of 3.0 kGy irradiated pecan oil, the 1.5 kGy front part of the Mylar bag was irradiated; then the bag was flipped to the back and irradiated again with a dose of 1.5 kGy. After irradiation, the pecan oil samples were stored for 3 months at 21°C until the PV tests were performed to calculate the rancidity of the pecan oils.

3.4 Results and Discussion

3.4.1 Pecan Properties

3.4.1.1 Pecan Kernel Percentage and Dimensions

Kernel Percentage:

A 58% pecan kernel percentage was obtained using Equation [3.1], which is very close to what the literature cited, a 54% kernel percentage (Thompson et al., 1997) in which the pecan kernel percentage varied from 40-60 % (Villarreal-Lozoya et al., 2009). Kanza pecan nut size classifications were obtained from the United States Grade Standards for Shelled Pecans (USDA, 1997) by calculating the number of pecan halves per pound, which was 302. As a result, the Kanza pecans were classified in the jumbo category (Table 3.1). According to the US grade standard, the minimum diameter of each pecan should be 7.9 mm.

Dimensions:

The dimensions of the pecans halves (both unfrozen and frozen) were not significantly ($P < 0.05$) different (Table 3.2). The average length, width, and height dimensions of the Kanza pecans were 24.33±1.66 mm, 17.29±0.68 mm, 7.26±0.37 mm, respectively (Figure 3.2).

3.4.1.2 Moisture Content

The moisture content of the non-irradiated milled pecans was 3.01 ± 0.1% (wet basis), and 3±0.7 % (w.b.) for the whole pecans. Therefore, the moisture content of the pecans could be found using either the minced or the whole pecan kernels. Increasing the surface area (by mincing the pecans) did not affect the pecan kernels' moisture content.

Table 3.2 Dimensions of frozen and unfrozen pecan halves

	a, Length (mm)	b, Width (mm)	c, Height (mm)
Frozen	24.55 a	17.17 a	7.35 a
	±1.86	±0.76	±0.43
Unfrozen	24.33 a	17.29 a	7.26 a
	±1.66	±0.68	±0.37

Values are means of ten replications. a, b Means within the columns not followed by a common superscript letter are significantly different ($P < 0.05$).

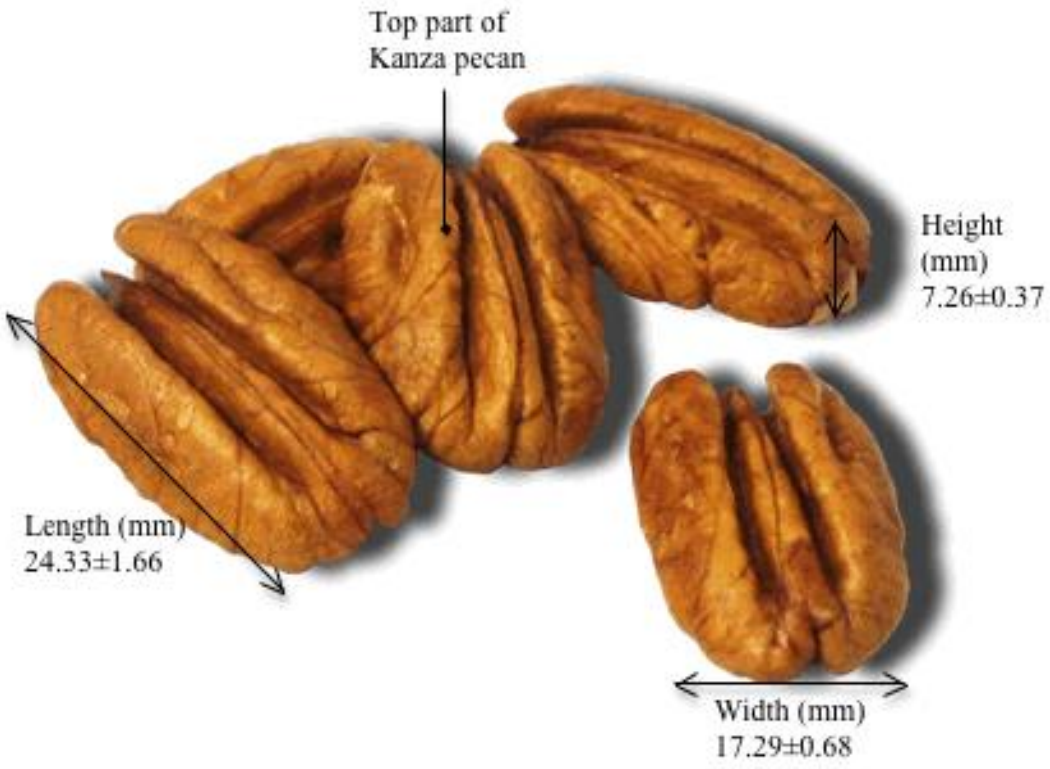

Figure 3.2 Kanza pecan dimensions (unfrozen kernel).

3.4.1.3 Water Activity (a_w)

The water activity of a food is an indication of the unbounded water (or free water) available in the food that microorganisms can use to grow. A food product that has <0.6 water activity is considered a low moisture product (Fennama, 2000). Low moisture and low a_w foods have less of a probability of microbial growth, unwanted fermentation, and many undesirable biochemical changes (Venkatachalam and Sathe, 2006). Minimum limit for yeast and mold growth is a_w =0.61 (Beuchat, 1981). For instance, Venkatachalam and Sathe (2006) showed that no mold growth was observed in nuts (almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, and walnuts) that had an $a_w \leq 0.53$ when the nuts were stored at 25° C for six months. Bacterial growth will not be observed in low water activities (a_w <0.75, Halophilic bacteria) (Beuchat, 1981). However, pathogenic bacteria can be hosted by low water activity foods such as nuts for long periods of time, and such foods are reported as sources of pathogens (Beuchat and Mann, 2010).

The water activity of non-irradiated pecans was found to be 0.57 ± 0.01, which may make the Kanza variety of pecans a poor environment for bacteria, yeast, and mold growth, but the low water activity may make the pecans a possible host for pathogenic microorganisms.

3.4.1.4 Comparisons of Pecan Oil Extraction Methods in Terms of Yield and Effect on Rancidity

The yield of oils obtained from the three different extraction methods that were used in this study are shown in Figure 3.3. The oil content of the Kanza pecans as determined by the Soxhlet-, Mechanical-, and Hexane-methods were 62.64 ± 2.6, 43.84 ±3.3, and 59.89 ± 3.7, respectively (Table A.1 O, Appendix A).

Even though the oil yield of the Hexane-method sample was not significantly ($P<0.05$) different from the Soxhlet-method, the highest yield of oil out of the three methods was obtained from the Soxhlet-method (Figure 3.4). Therefore, the Soxhlet-method was used to determine the oil content of the pecans. However, the Soxhlet method of oil extraction was not used to evaluate further experiments such as the PV determination because the PV of the oil that was extracted by the Soxhlet-extraction method increased significantly ($P <0.05$) after the irradiation treatment (1 kGy) . In other words, the heating process that occurs during the Soxhlet extraction method had a significant effect on the quality of irradiated oil.

The mechanical extraction method does not involve a heating process, but the oil extracted from the mechanical method was very cloudy and needed additional purification steps such as dissolving the oil in the Hexane solvent, filtrating the oil-Hexane mixture to get a clearer oil, and evaporating the Hexane solution to obtain the oil. Therefore, the mechanical method was not used in any experiments. Unlike the mechanical method, neither the Hexane nor the Soxhlet methods required any additional purification steps to obtain clear pecan oil.

In conclusion, the pecan oil extracted by the Hexane method was used for the remainder of the experiments that required pecan oil samples because the Hexane extraction method had no significant ($P<0.05$) impact on the rancidity of the pecan oil after the irradiation treatment (Figure 3.3).

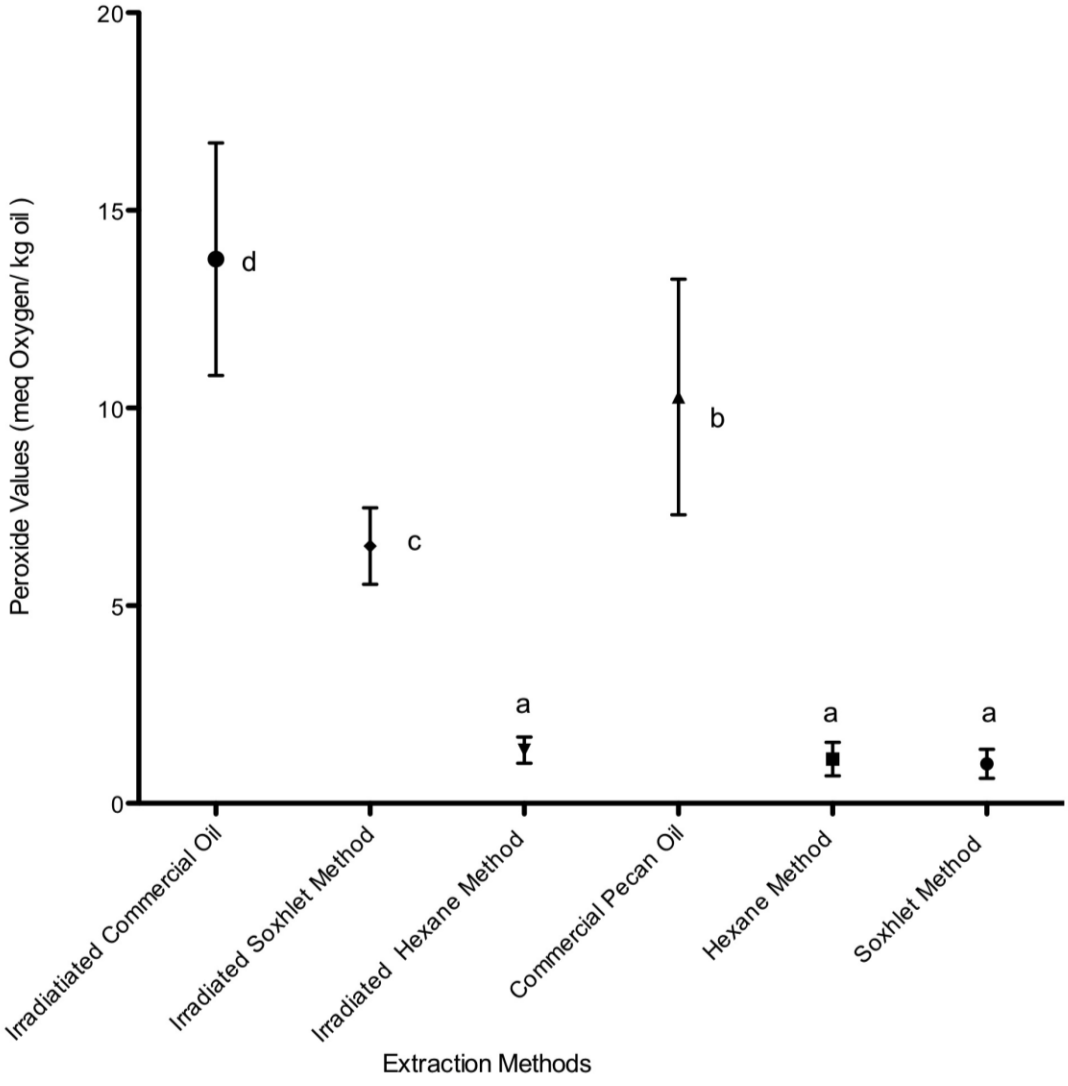

Figure 3.3 Effect of oil extraction method on peroxide values (in meq oxygen/kg of oil) of irradiated pecan oil.

3.4.1.5 Density and Porosity of Frozen and Unfrozen Pecans

The bulk densities of both the frozen and unfrozen pecans that were calculated by the mass occupation technique were found to be 0.45±0.007 g/cm^3, which is very similar to the results shown on the (Aqua-calc) website, 0.46 g/cm^3. The bulk densities calculated by the liquid displacement method were two times higher than those found using the mass occupation method. In the mass occupation method, when the pecan kernels were placed into a 200-ml beaker, there were too many spaces in the beaker volume that could not be covered by the pecan kernels. However, these spaces counted as part of the pecan volume in the mass occupation method of calculation. Yet, in the li uid displacement method, the pecan kernels' volume was completely covered by the displacement liquid (toluene), and the real kernel volume was measured. Therefore, the bulk densities of both the unfrozen and frozen pecans were calculated using the volume displacement method (Moreira et al., 2009). Then the porosity values were calculated using Equation [3.9]. The porosity and the bulk and true densities of the unfrozen and frozen pecan values are shown in Table 3.3. The frozen and unfrozen samples' bulk densities were not significantly ($P<0.05$) different; however, the true densities of both the unfrozen and frozen pecans were significantly different ($P <0.05$) (Table 3.3). The frozen pecan

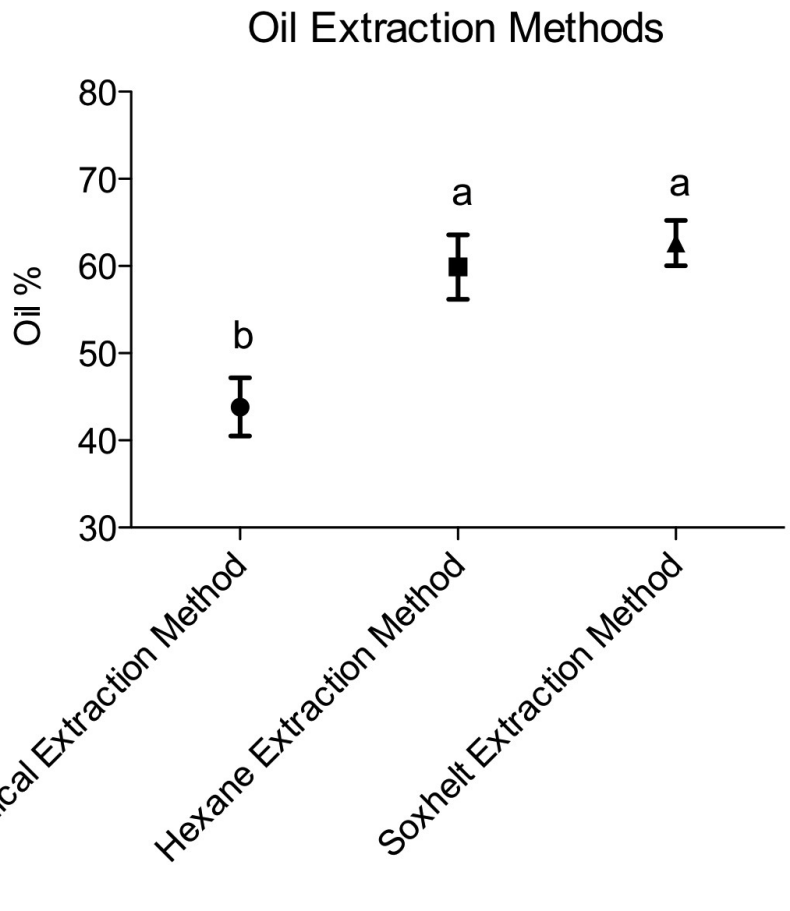

Figure 3.4 Means, standard deviation, and statistical values of pecan oil extraction methods: Soxhlet-, Hexane-, and Mechanical-methods. Bars show the standard deviation of each method. The a,b means which are not followed by a common letter are significantly different ($P < 0.05$).

Table 3.3 Bulk density, true density and porosity of frozen and unfrozen pecans

	Unfrozen	Frozen
True density (g/cm^3)	1.02 ± 0.02^{a}	1.21 ± 0.07^{a}
Bulk density (g/cm^3)	0.91 ± 0.01^{a}	0.94 ± 0.025^{b}
Porosity	0.11 ± 0.01	0.22 ± 0.04

* whole pecan halves were used for both density calculations. The ^{a,b} means which are not followed by a common letter within a row are significantly different ($P < 0.05$).

samples had a slightly higher true density (+0.19 g/cm^3) compared to the unfrozen samples, which can be observed as specific gravity (the ratio of the densities) by the naked eye (Figure 3.5) when the frozen kernels were placed in deionized water (density of water= 0.998 g/cm^3 at 20° C). Moreover, the porosity of the frozen kernels was twice as much as that of the unfrozen pecan kernels.

3.4.1.6 Color

The top (Figure 3.2) and bottom parts of the pecan halves, and also the minced pecan color measurements were compared (Table 3.4). The top part of the pecan was found to contain more yellow (*b) and red (*a) colors than the bottom part of the pecan, and significant ($P < 0.05$) differences were observed between the bottom and top parts of the pecan in all *L (brightness), *a (redness/greenness), and *b (yellowness/blueness) values. The bottom parts of the pecan halves had some white parts (the connection point of the two pecan halves), which made the *L value significantly ($P < 0.05$) higher than that obtained from the top parts of the pecans. The *L and *b values were almost 12-17 points, and the *a value was 3 points lower than the Villarreal-Lozoya et al. (2009) findings for the Kanza pecan cultivar. In the Heaton et al. (1975) color value findings for the Schley, Stuart, and Wichita cultivars, the *L value was 6-10 points lower and the *a and *b values were very similar to what was found in this study. These differences may be due to the color measurements being taken from different parts of the pecan, such as the bottom or top parts, or because of different cultivars or harvesting time differences.

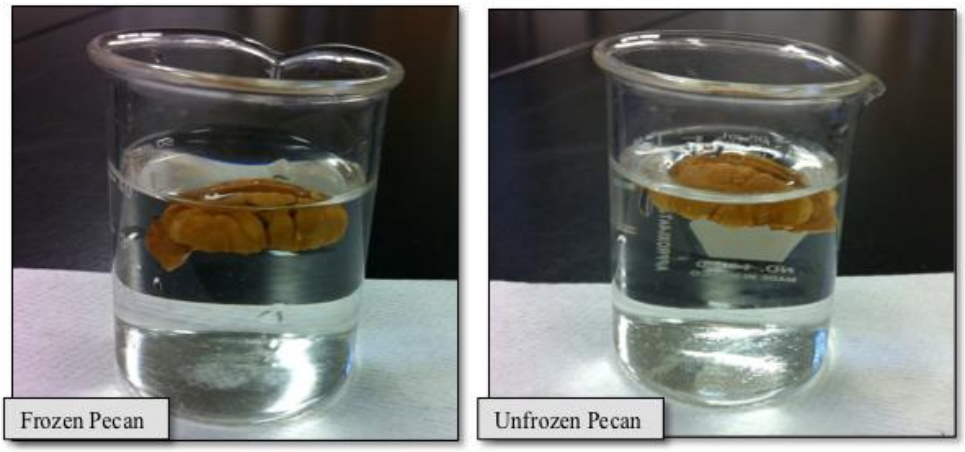

Figure 3.5 Frozen and unfrozen pecan halves in-deionized water.

Heaton et al. (1975) reported that early harvest pecans demonstrated an improved color and flavor as compared to late harvest pecans within the same cultivar.

Moreover, the bottom and top parts of the pecans, which represent the actual colors of the pecan, were significantly ($P < 0.05$) different from the *L and *b color values of the minced pecans. Therefore, the minced pecan color measurements were not used for further shelf life studies. According to USDA (1997) pecan kernel color standards, PEC- MC-1, consisting of plastic models of pecan kernels, illustrate the color intensities implied by the terms "golden," "light brown," "medium brown," and "dark brown. Therefore, the Kanza pecan kernel color can be specified as golden.

Table 3.4 Color parameters at the bottom and top parts of the pecan halves and of the minced pecans

	*L	*a	*b
+Bottom	a 33.93 ±1.74	a 9.97 ±0.7	a 23.53 ±1.8
+Top	b 29.76 ±1.44	b 10.51 ± 0.7	b 25.09 ±1.5
Minced	c 60.52 ±0.16	c 4.72 ± 0.1	a 22.48 ±0.23

The a,b,c means within the columns which are not followed by a common superscript letter are significantly different ($P < 0.05$).

3.4.2 Pecan Irradiation

3.4.2.1 Comparison of Commercial Pecan Oil and Extracted Pecan Oil on Rancidity After Irradiation

The pecan oils that were extracted by the Soxhlet extraction method were significantly ($P<0.05$) more susceptible to rancidity after the irradiation treatment because of the heating process used in this method, while the oils extracted by the Hexane method were not ($P<0.05$) (Section 3.4.1.4.). In addition to the extracted Kanza pecan oils, the commercial pecan oil samples were also evaluated to see the effect of heating on the rancidity of pecans after an irradiation treatment. As shown in Figure 3.3, the commercial pecan oil samples always had the highest peroxide values before and after the irradiation treatment, as compared to the extracted Kanza pecan oils, regardless of the method used. This can be explained by the refining steps that the commercial pecan oil underwent. Therefore, it can be concluded that the heating process element of the pecan extraction methods significantly affected the rancidity of the pecans after the irradiation treatment. Consequently, the hexane method is a more appropriate extraction method to use for further experiments in order to examine the oxidation and irradiation effects on pecans without experiencing the detrimental effects caused by a particular extraction method.

3.4.2.2 Comparisons of the Effects of High Irradiation Dose and the Presence of Oxygen on Extracted Kanza Pecan Oils

Irradiation at 3.0 kGy has a significant ($P< 0.05$) impact on the rancidity of pecan oils (Table 3.5). The PV of irradiated pecan oils (under oxygen and nitrogen modified atmosphere conditions) were significantly higher than for the non-irradiated controls. As expected, the presence of oxygen alone (non-irradiated pecans under oxygen-packaging) increased the rancidity (PV value) of the pecan oil significantly ($P < 0.05$) when compared to the PV values of the samples irradiated under nitrogen. When comparing the type of MAP conditions (nitrogen vs. oxygen packing), samples packed under nitrogen had lower ($P < 0.05$) PV values. Furthermore, the combined effect of exposure to ionizing radiation and oxygen increases the PV values three-fold (Table 3.5). Therefore, irradiation of pecan oil with a high dose (3.0 kGy) without packaging (under air) will cause a serious detriment to the quality of pecans due to lipid oxidation and packaging may help reduce the overall effect on the quality of the nuts.

Table 3.5 Effect of packaging atmosphere on the peroxide value (PV) of extracted Kanza pecan oils irradiated at 3.0 kGy and stored at 21° C for three months

	Non-irradiated	Irradiated with 3- kGy dose
Nitrogen-packed	4.55 ± 0.02^{a}	5.45 ± 0.11^{b}
Oxygen-Packed Pecan oils	11.53 ± 0.13^{c}	15.97 ± 0.35^{d}

Values are means of three replications, and ± standard deviation. The ^{a,b,c} means which are not followed by a common letter within a row are significantly different ($P < 0.05$).

3.5 Conclusion

In this study, some physicochemical properties of Kanza variety pecans were characterized and the effect of heat, irradiation treatment, and presence of oxygen on their rancidity was evaluated.

Moisture content and water activity (a_w) of raw, non-irradiated Kanza variety pecans were measured for use in the analysis of radiation D_{10} values. The porosity of frozen pecan kernels was twice as high as that of unfrozen kernels, be due to the slow freezing process.

Determining the method to use when extracting oil from the pecans was a challenge for this study. Although the Soxhlet extraction method yielded the highest oil yield from the Kanza pecans, a preliminary study showed that the oil extracted from irradiated pecan using the Hexane method had significantly lower peroxide values (PVs) compared to the oils obtained from the other extraction methods. Hence, the Hexane method is the recommended method when considering the monitoring the irradiated oils.

Furthermore, the preliminary data showed that irradiation of pecan oils at a higher dose (3.0 kGy), the presence of oxygen, and the combination of irradiation and oxygen increased ($P<0.05$) the PVs of the pecan oil. Hence, irradiation in an atmosphere not containing any oxygen could be a solution for retarding or reducing the onset of rancidity. However, further studies are needed to determine the maximum dose that will not exceed the limits of acceptable quality for pecans in industrial use and to evaluate the usefulness of different packaging types for minimizing the effects of irradiation on pecans.

CHAPTER IV

D_{10} VALUES FOR *SALMONELLA TYPHIMURIUM* LT2 AND AN *E.COLI* COCKTAIL IN PECAN NUTS (KANZA CULTIVAR)

4.1 Summary

In the preliminary portion of this study, dose mapping and recovery of water were investigated. The dose mapping was conducted using Monte Carlo simulation and an ion chamber. Four different tests were carried out to assure that the absorbed dose in the pecan half was the same as the calculated dose using the Monte Carlo simulation. The first test was performed with alanine dosimeters, and the other three were performed with radiochromic films (RF), which were placed in different locations within the pecan kernel. The pecan kernels' entrance dose was 0.48 ± 0.06 kGy and the exit dose was 0.8 ± 0.04 kGy, when irradiated only on one side and at 0.5 kGy (target dose). The accumulated absorbed dose in the middle of the kernel was 0.94 ± 0.09 kGy; the doses at the entrance and exit points were 0.57 ± 0.09 each, when the pecans were irradiated on each side at 0.5 kGy. The other preliminary study (conducted to determine the change in the water activity of pecans after inoculations) found that the water activity of pecans remained constant after the air-dried pecans were inoculated.

The pecans [Carya *illinoinensis* (Wangenh) K. Koch cv. Kanza] were inoculated with either *Salmonella* Typhimurium *LT2* or an *Escherichia coli* cocktail of BAA-1427, BAA-1428, and BAA-1430. Four pecan halves were placed inside Ziploc® bags; two sets of 15 bags were flushed for 90 seconds with 100% nitrogen gas (NP) and 100%

oxygen gas (OP), and sealed immediately. Another set of 15 bags were vacuumed and then sealed (VP). Each set of bags was irradiated (three bags per dose) at room temperature at 0.2, 0.4, 0.6, and 0.8-kGy using a 1.35 MeV Van De Graaff electron beam accelerator (low energy). For each treatment (MAP condition), non-irradiated samples served as controls. Immediately after irradiation, appropriate dilutions of the inoculums were plated in duplicate and incubated for 18 hours at 37° C before enumerating colonies.

The D_{10} values were obtained from the slope of the log CFU/g versus the dose plot. The D_{10} values for the *E. coli* cocktail were 0.36, 0.40, and 0.46-kGy for the OP, NP, and VP samples, respectively. The D_{10} values for *Salmonella* Typhimurium LT2 were 0.34, 0.38, and 0.44-kGy for the OP, NP, and VP samples, respectively. As expected, the D_{10} values for the samples irradiated under vacuum were significantly ($P<0.05$) higher for both microorganisms. These results confirm that irradiation of pecans under vacuum will increase the microorganism's resistance to irradiation and irradiation using nitrogen-packaging (NP) could be a feasible option.

4.2 Introduction

Increases in outbreaks of foodborne illnesses caused by the consumption of raw nuts have resulted in greater interest in ways to reduce pathogen contamination in nuts. A recall of pecans in February of 2010, after the Food and Drug Administration (FDA) determined a possible contamination with *Salmonella*, is an example of the importance of ensuring the safety of fresh produce, including nuts (FDA, 2010).

One approach to assuring safety in nuts is to use ionizing radiation; however, the high oil content of nuts such as pecans makes the use of irradiation treatments very challenging because such treatments accelerate the degradation of fat which results in a loss of quality. Therefore, a solution for decreasing the degradation of fat caused by irradiation could be obtained if the pecans were packed in a modified atmosphere package before they were irradiated.

It is also known that in nuts, the higher the irradiation dose, the faster the onset of rancidity (Uthman et al., 1998; Al-Bachir, 2004; Mexis et al., 2009; Mexis and Kontominas, 2009a, b, c; Villarreal-Lozoya et al., 2009; Prakash et al., 2010). However, when the nuts are irradiated at low doses, the effectiveness of the irradiation to neutralize microorganisms decreases. Thus, theneed to determine the minimum dose required to achieve a 5-log reduction in undesirable microbial populations.

Exposure to ionizing radiation kill the microorganisms either directly by breaking a single or double DNA strain, or indirectly by interacting with water molecules and creating OH- ions that interact with microorganisms. Thus causing irreversible DNA damage. In both the direct and indirect killing effects of irradiation, vital microorganisms either die or suffer irreversible DNA damage (Moreira et al., 2010). The degree of effectiveness of the indirect effects of irradiation with regards to inhibiting microorganisms depends upon the a_w level of the medium (in this case, pecans). Because of the nature of the inoculation process, the water activity of air-dried inoculated pecans was measured and compared with the water activity levels of non-

inoculated pecan kernels to determine whether the inoculation process caused changes in this property.

Hence, the objectives of this study were to (1) verify that the pecans received the actual (applied) dose; (2) verify that the pecan kernels' water activity levels were unchanged after inoculation;(3) determine the dose (kGy) required to produce a 1-log reduction (D_{10} values) in the populations of the *E. coli* cocktail and *Salmonella* Typhimurium LT2 in inoculated pecans as a function of MAP conditions; and (4) determine the best irradiation/MAP combination when treating pecans using a low energy electron beam accelerator (1.35 MeV).

4.3 Materials and Methods

4.3.1 Preliminary Studies

4.3.1.1 Dose Mapping

Monte Carlo Simulation:

Packages with pecans, which had 4 pecan halves, 6 grams, in different atmospheric condition (MAP) were irradiated (Figure 4.1) with a 1.35-MeV Van de Graaff accelerator (High Voltage Engineering Corp., Cambridge, MA) located at the Department of Biological & Agricultural Engineering in the Hobgood building at the Texas A&M University west campus. The irradiation dose distributions in pecan kernels at a low energy treatment (1.35 MeV electrons, Van de Graaff accelerator) were determined by a Monte Carlo N-Particle radiation transport code (MCNP5) (Kim et al., 2010).

First, a multi-slice Computer Tomography (CT) scan determined the pecan kernel's 3-D geometry data. Second, to obtain dose distributions, the geometry and density of the pecan kernel, the type of source energy input spectrum, and the source size were all entered into the MCNP5. The simulator was run on a parallel computer platform (Dell TM PowerEdge TM 6650, 4 CPU) located at the Department of Biological and Agricultural Engineering at Texas A&M University. The e- beam source was assumed to be a parallel plane large enough to cover the target, and the electrons were emitted in a plane and distributed evenly within the scan (Kim et al., 2007).

Ion chamber Calculations:

A Farmer ionization chamber (Marcus Chamber, type 23343) was used for the calibration procedure. The Farmer ion chamber was placed at a 22.5° angle, 6 cm away from the parallel plate transmission ion chamber, which was directly attached to the exit e-beam window. Once the e-beam began to generate, the parallel plate ion chamber measured the charge C resulting from the electrons passing through it(Yang, 2009); the dose that was exposed to the farmer ion chamber was measured with the exposure meter in Rontgen (R) units. Then, the dose count was calculated by a linear regression between the counts obtained by the parallel plate and the dose measured by the farmer ion chamber. To find the hot spot, the farmer ion chamber was placed at different points along the Cartesian coordinate's generated using dose counts.

After the hot spot was determined, the radiochromic film (FWT-60 Series, Far West Technologies, Goleta, CA) was placed into the hotspot and irradiated with the

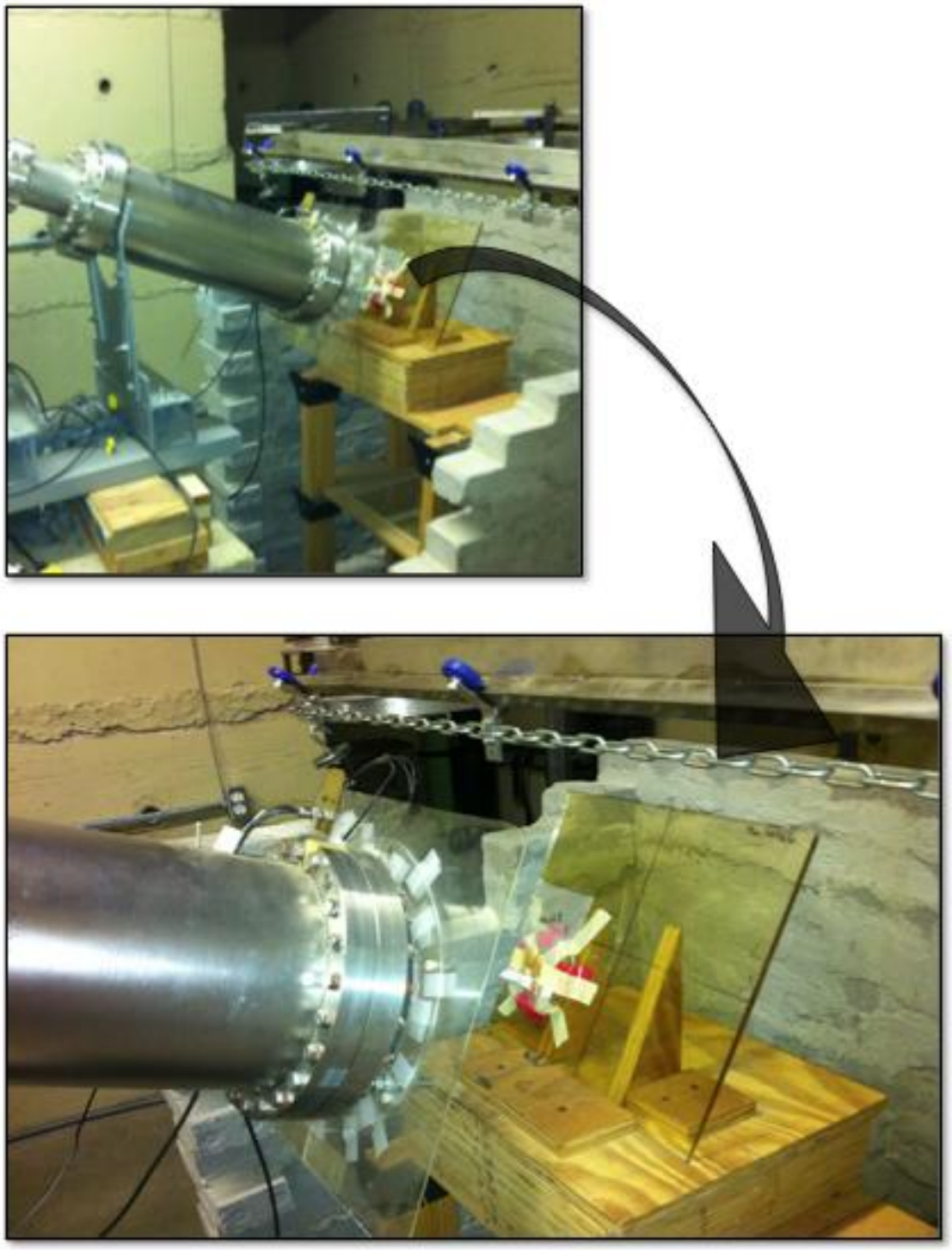

Figure 4.1 Pecans placed on the glass plate ~15 cm away from the electron gun in a 1.35 MeV Van De Graaff accelerator.

needed dose by using dose count numbers that were obtained earlier. After the irradiation, the optical density of the RF was read by the digital radiochromic reader (Model FWT-92D, Far West Technologies, Goleta, CA). The optical density of the RF and the dose linear relationship were calculated and used for further absorbed-dose calculations.

4.3.1.2 Absorbed Dose Calculations with Alanine Dosimeter

The absorbed dose was measured with L-alpha alanine dosimeter pellets (Gamma-Service Produktbestrahlung GmbH, Germany). The pecan halves were carved and the alanine dosimeters, which have a radius of 2.9 mm and a diameter of 4.8 mm, were placed into the carved holes (the holes were small enough so that the alanine dosimeters did not fall through) at seven different locations within each pecan half (Figure 4.2). Pellet numbers 3 and 4 were filled with the carved pecan pieces and then vacuum packed (FoodSaver® V2220 Vacuum Sealer) (Figure 4.2.a). For further detail, see Figure 4.2.b.

The packages with pecans were irradiated with a total of a 1.0 kGy dose (0.5 kGy on each side, front and back) as determined from the ion chamber calculations (the dose count calculations) with a 1.35-MeV Van de Graaff accelerator. Then, the alanine dosimeters were carried to the Electron Beam Food Research Facility to read the absorbed dose from the alanine pellets using E-scan electron paramagnetic resonance spectroscopy (Bruker BioSpin Corp., Billerica, Mass.).

Figure 4.2 Placement of the alanine dosimeters in the pecan halves. (a) first package alanine pellets were placed into carved pecans on the right and left side of front pecan (#2,3) and in the middle of back part of pecan (#4, 5) (b) after pellets (# 2, 3, 4, 5) were placed in the pecan halves pellet #3, and 4 filled with pecan pieces and vacuum packed. (c) location of pellet # 1, 7, 8, last pecan half was without pellet (d)Vacuum packed demonstration of second package, pellet #1 and 7 filled with pecan pieces.

4.3.1.3 Absorbed Dose Calculations with Radiochromic Film

Pecan halves were irradiated with RFs placed in the front, back (Figure 4.3), and middle (sandwich model) of each pecan half.

Radiochromic film placed in Front and Back:

The RFs were placed on the pecan halves, as shown in Figure 4.3a. Four pecan halves that had RFs placed (2 RFs inside each aluminum pouch) on top of them were vacuum packed with a FoodSaver® V2220 Vacuum Sealer. A total of two packages were made, for a total of 16 RFs. The packages were then placed such that the RF facing the exit e-beam window (Figure 4.3d, the left package) irradiated only one side at a 0.5 kGy dose with a 1.35-MeV Van de Graaff accelerator.

These front readings were calculated as an entrance dose. The same procedure was applied to pecans with RFs placed on the back of each pecan half, and all were irradiated on only one side at a 0.5 kGy dose with the 1.35-MeV Van de Graaff accelerator. This time the RFs did not directly face the exit e-beam window, because the pecan half was between the exit e-beam window and the RF Figure 4.3d, the package on the right). Therefore, we concluded that the RFs placed on the back of the pecan halves irradiated on only one side would show the exit dose. In addition, one package was made with 4 pecan halves, which had 2 RFs placed on the front and 2 RFs placed on the back part of the pecan half. These pecans were placed diagonally (Figure 4.4b and c: b is front part of pack, c is back part of the pack) and packed together. This last package was also irradiated only from the front side (Figure 4.4b). This package was prepared to monitor the entrance and exit doses together.

Figure 4.3 Absorbed dose measurement with RFs were placed on the front and back parts of the pecans, and irradiated on only one side at 0.5 kGy. (a) Demonstration of how RF was placed on the front part of pecan half; (b)RFs were placed on the front part of pecans and also RFs placed on the back part of pecans and placed diagonally and irradiated from this side (c) demonstration of the back part of the same package in (b); (d) Three packages that were irradiated only from front side right RFs placed on the back, middle RFs placed diagonally, left RFs placed on the front of the pecan.

Sandwich Model (Accumulated Dose):

This calibration was made to calculate the accumulated dose in the middle part of each pecan. For this reason, the pecan halves were cut in half with a very sharp razor, without breaking off any part; the RFs then were placed exactly in the middle of each pecan piece (Figure 4.4.a). Note that the RFs were shown in Figure 4.4.a only as a demonstration of where the RFs would be when they were placed inside each pecan half. The RFs should always remain inside the aluminum pouches and never be exposed to light because the light changes the optical density results (Figure 4.4.b).

After this step in the process, the two sides of each pecan half were put back together, and closed with the help of paper tape (Figure 4.5.a). The tape could not be too thick, but rather just thin enough to hold the pieces together. Therefore, the RFs (two films in each aluminum pouch) remained in between the pecan halves like a sandwich (Figure 4.5.b). Then each set of four-sandwiched pecan halves were placed into packs and vacuum sealed (Figure 4.6). A total of two packages were prepared, for a total of 16 RFs. The packages were irradiated at 0.5 kGy doses on each side, for a total of 1.0 kGy dose.

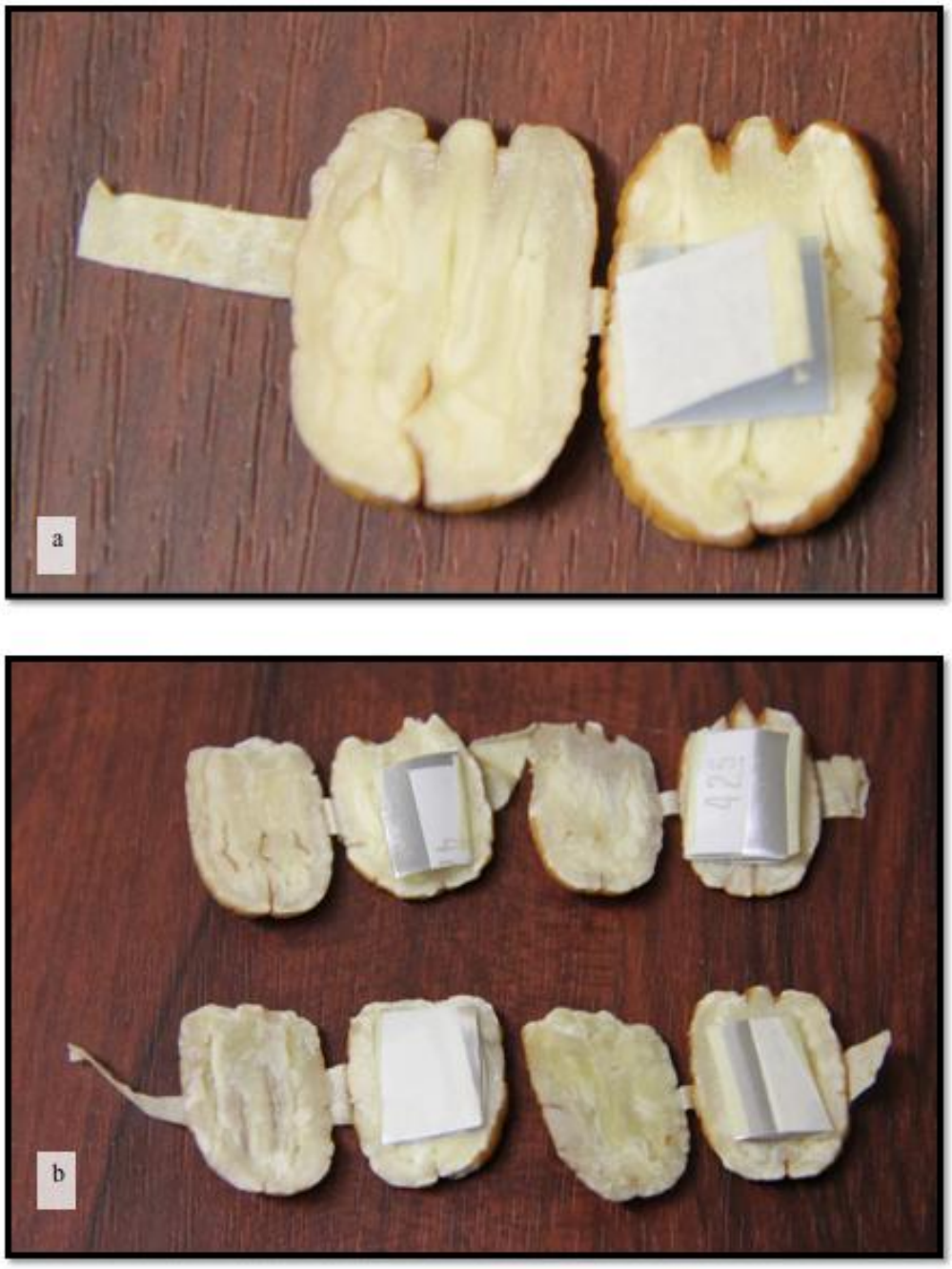

Figure 4.4 Radiochromic film (RF) placed inside each pecan half previously cut in half. a.) Each RF was centered in the middle of each pecan half. b.) Each RF inside the aluminum pouch was placed in the middle of each pecan; the RF was centered in the middle of each pecan half.

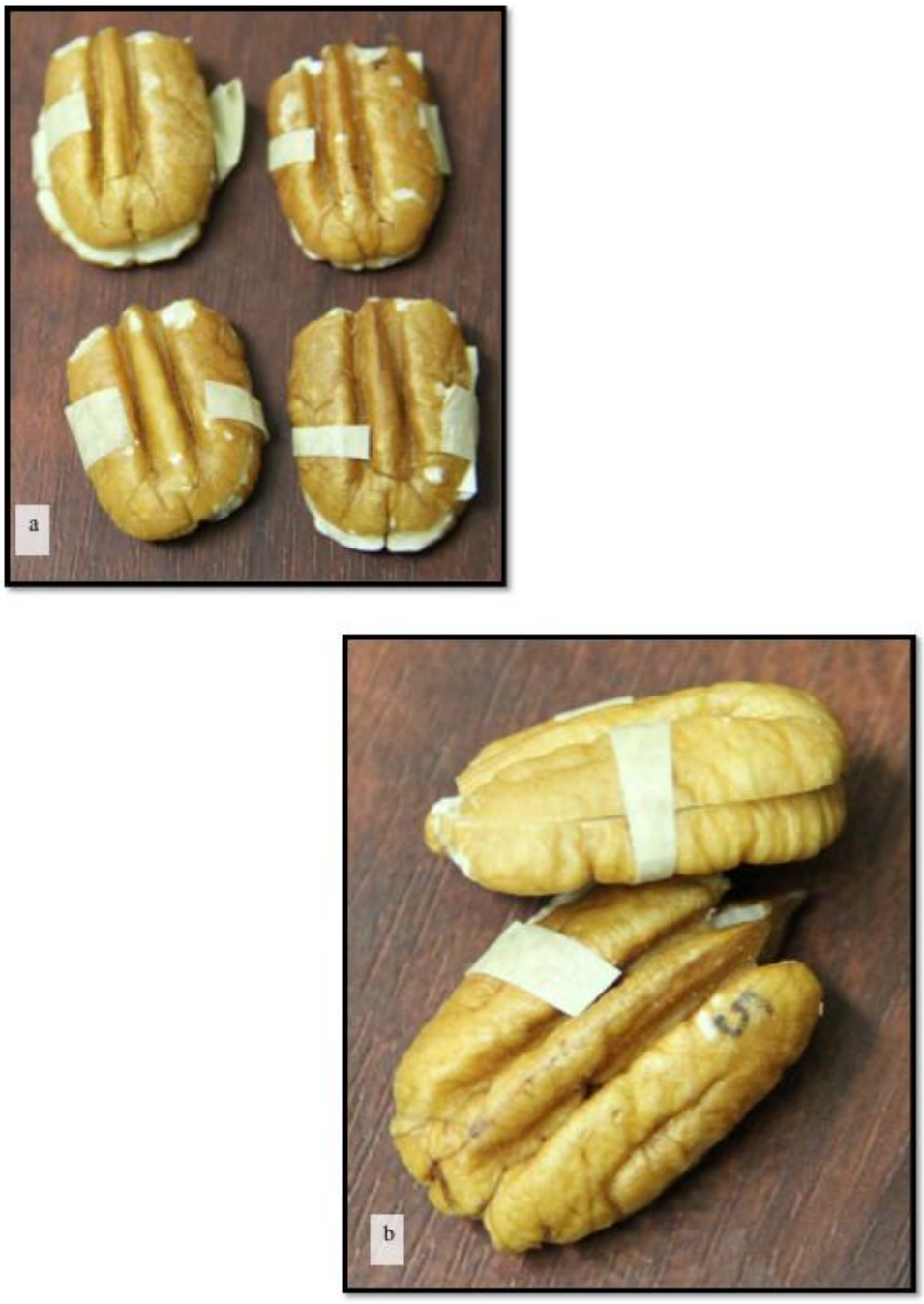

Figure 4.5 Absorbed dose measurement with each RF placed in the middle of a pecan half, the "sandwich" model (a) front view of after pecans halves were sandwiched and taped tightly on the sides; (b) side view of sandwiched pecans no spaces left within the pieces.

Figure 4.6 Vacuum packed "sandwich" model pecans. Each RF was placed in the middle of a pecan half and taped with thin tape. Then the four pecan halves were packed together under vacuum packaging.

Radiochromic films placed on the dorsal grooves (valleys) of the pecan halves:

The pecan halves were sprayed with a multi-purpose rubber coating spray (Plasti dip from Performix) to avoid the pecan oil coming in contact with the RFs (Figure 4.7a). The RFs were placed within the dorsal grooves, as shown in Figure 4.7b, to measure the dose values in the valleys and on top of the dorsal grooves to measure the peak dose value. Both RFs were placed in pecans, as seen in Figures 4.7d and e. Each pecan half was irradiated alone and without packaging. The pecan halves were stuck onto the sticky side of a piece of tape, which was then placed in front of the e-beam exit window. See Figures 4.7f and g. The pecan halves were irradiated with a 0.5 kGy dose, and only on one side. The absorbed doses were calculated by reading the optical densities of the RFs that were placed on the peaks and dorsal grooves of each pecan.

4.3.1.4 Recovery of Water Activity (a_w) of Pecans After Inoculation

This study used distilled water instead of the actual inoculum to simulate the inoculation treatments, according to Biological Laboratory Safety level 2 (BL2) requirements (Gomes, 2010). In order to simulate a real case of inoculation, 40-µl of distilled water was dropped into the pecan kernels and left to air dry for two hours. After the pecans were dried at room temperature inside a hood, the pecan kernels' water activity levels were tested, as described in Section 3.3.2.3.

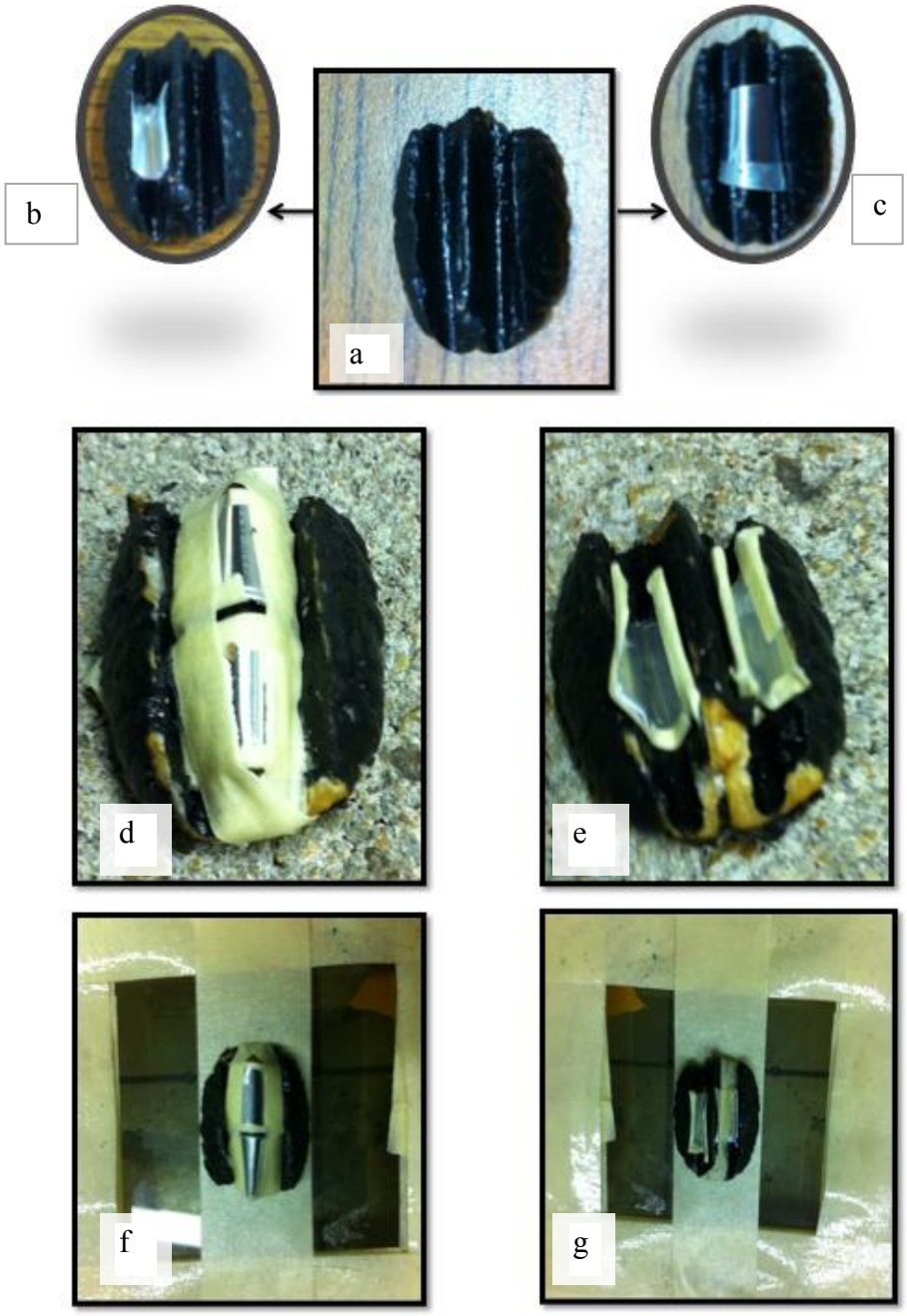

Figure 4.7 The absorbed dose measurements taken from the pecan dorsal grooves. (a) Each pecan half was sprayed with a rubber coating spray to prevent potential oil contamination. Each RF was placed within the dorsal grooves (b,e), and on top (c,d). Then each half was stuck onto a piece of paper tape (d,g) and placed in front of the exit beam window for irradiation.

4.3.1.5 Inoculation Method Determination

Before irradiation, the pecan kernels were first inoculated with surrogates and then packed in different atmospheres (MAP). With regards to the inoculation of the pecans, three different methods were tested in an effort to obtain the highest initial concentration of microorganisms after inoculation. The reason for this effort was that higher initial values of microorganisms help underscore the effects of dose on reducing the number of microorganisms. Consequently, the best inoculation method was determined by comparing three different methods: the drop method, the dip method, and the shaking method. The method that yielded the highest number of initial microorganisms was used as the inoculation method for further D_{10} value determinations. The inoculum (*Salmonella* LT2 and *E. coli* cocktail) used in these methods was prepared exactly as the preparation described in Section 4.3.2.3 (Inoculum Preparation).

The drop method consisted of a 40-µl of inoculum being dropped into the top parts of the pecan cracks (Figure 4.8). A total of 160-µl of inoculum per package was used (Singh et al., 2002). The dip method inoculation consisted of a 40-µl inoculum being scraped onto each pecan half *(Singh et al., 2002).* The shaking method inoculation consisted of four pecan halves (6 g) being placed into polyethylene bags (18 oz. Whirl Pak bags) and 480µl (120-µl per pecan half) of inoculum being added to each bag. Then each bag was shaken by hand for about two minutes (about 150 times) (Beuchat and Mann, 2010).

Each method used three replications of four pecan halves (one package) and were conducted under a hood and within a sterile environment. After inoculation, the inoculum was allowed to dry at room temperature for two hours. Once the pecan halves dried, the inoculated samples were subsequently transferred into sterile polyethylene bags (18 oz. Whirl Pak bags) and pummeled with 20 ml of DIFCO™ buffered peptone water (pH 7.0). Samples of 100 µl serial dilution in 0.1% peptone water were plated on MacConkey Agar (MCAR; Sigma, St. Louis, MO) and incubated at 37°C until visible colonies were countable with the use of a magnifier counter (the detection limit was 10 CFU/g of pecan halves). The maximum number of microorganisms was obtained in the sample inoculated by the drop method (10^8 CFU/ml of microbial cocktail).

4.3.2 Preparation of Pecan Kernels and Inoculum

4.3.2.1 Pecan Preparation

Shelled Kanza pecan [*Carya illinoinensis* (Wangenh.) K. Koch] kernels purchased from the Kansas State University pecan experiment orchards were used. Kanza pecans that were previously unshelled and stored at -25º C were removed from the freezer; and allowed to reach room temperature before they were used for experiments.

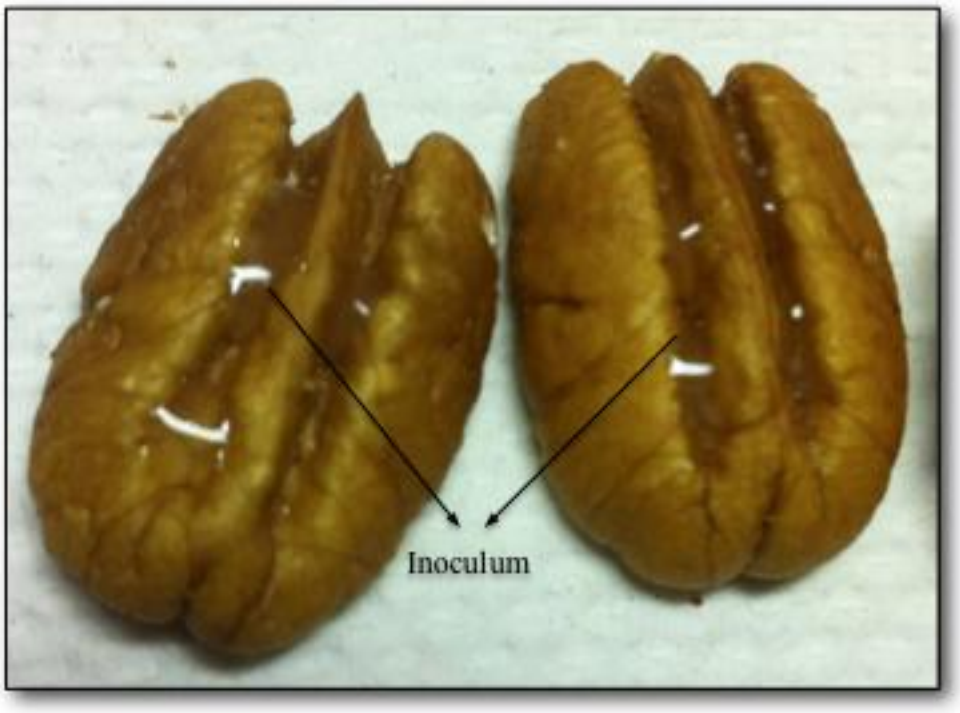

Figure 4.8 Pecan halves inoculated within the cracks by 40 µl of 10^8 CFU/ml of the microbial cocktail.

4.3.2.2 Microorganisms

Rifampicin resistant (Rif+, Sigma-Aldrich; St. Louis, MO) microorganisms (80 μg/mL) *Salmonella Typhimurium LT2, E. coli BAA-1427, BAA-1428, and, BAA-1430* were obtained from Dr. A. Castillo's Food Microbiology Laboratory (Animal Science Department, Texas A&M University). The surrogates carry exactly the same characteristics as the actual pathogenic microorganisms, except with regards to pathogenicity (Rodriguez et al., 2006). Frozen stocks were maintained at -80° C. Prior to use, an inoculum was removed from the frozen culture with a loop, streaked onto 10 ml of Tryptic oy r oth (T ; DIFCO™, Detroit, MI) and incubated at 37° C for 24 hours. Single colony isolates were obtained from TSB plates through two successive transfers to Tryptic oy Agar slant (T A; DIFCO™, Detroit, MI). The colonies were stored on a TSA slant at 25° C as working cultures and used during the following month.

4.3.2.3 Preparation of Inoculum

Bacteria strains (*Salmonella* Typhimurium LT2*; E. coli* BAA-1427, BAA-1428, and, BAA-1430) for inoculation were prepared by transferring a loop inoculum from the working cultures at 25° C to 9 ml TSB test tubes (a set of four tubes per strain) and incubated at 37° C for 18 hours. After incubation, each set of four test tubes from each strain was transferred into four different sterile centrifuge tubes. Each centrifuge tube was centrifuged (3000 x *g* for 15 min) (Centrifuge B4i, Jouan, Winchester, VA) and washed three times with an e ual volume of sterile DIFCO™ buffered peptone water (BPW). After the final wash, the remaining pellet was resuspended in 0.1% peptone water (, DIFCO™).

An estimated initial concentration of approximately 10^8 CFU/ml at an absorbance of 0.5% (Milton Roy Spectronic 20D turbidity meter, optical density (OD) 600 nm, Milton Roy Co, CA) was confirmed by making serial dilutions of the inoculum suspension in 9 ml test tubes of 0.1% peptone water, plated on MacConkey Agar supplemented with 80 μg/ml of rifampicin (MCAR; Sigma, St. Louis, MO) and incubated at 37° C until visible colonies were counted.

4.3.3 Inoculation of Pecans

Each pecan half was inoculated by the drop method, as described earlier. Forty μl of the 10^8 CFU/ml bacteria strains (*Salmonella* Typhimurium LT2*; E. coli* BAA-1427, BAA-1428, and, BAA-1430), as described in section 4.3.2.3, was dropped in between the cracks of the pecan halves (Figure 4.1). After inoculation, the inoculum was allowed to dry at room temperature for two hours. Four pecan halves (~ 6 grams of pecans) were placed into small Ziploc® (5 x 8 cm) bags. Each pecan half was placed in the same direction that the inoculated surface was facing, which was upwards. After placing four pecan halves into a small plastic Ziploc® bag, the bag was flushed with either nitrogen (Nitrogen-packed, NP) or oxygen (Oxygen-packed, OP) gases without turning the pecan halves onto their opposite sides. Then each small Ziploc® bag was placed into a larger FoodSaver® plastic bag and immediately sealed with a FoodSaver® V2220 Vacuum Sealer. For the Vacuum-Packed (VP) and Air-Packed (AP) samples, after placing four inoculated pecan halves into a small Ziploc® bag, the bag was placed into a larger bag and either sealed with (VP) or without vacuum (AP), using a FoodSaver® V2220

vacuum sealer double bag storage used for both microbial safety and easy sealing ability of the bags. Next, the bags were approximately 0.07 mm in thickness; they did not affect the simulation calculations regarding the dose distribution of the pecans. Therefore, sets of 20 bags were packed per each of the following treatments: NP, OP, VP, and AP. The samples were stored at 10°C until the following day, when they were irradiated.

4.3.4 Irradiation of Pecans

The packed pecans were irradiated with a 1.35-MeV Van de Graaff accelerator (High Voltage Engineering Corp., Cambridge, MA). Four independent samples packed in each treatment (NP, OP, VP, and AP) were irradiated only on one side and at each dose level (0.2, 0.4, 0.6 and 0.8 kGy). Pecan packages under vacuum (VP) were placed (Figure 4.2) on a glass plate that was previously calibrated to find the location of maximum electrons emitted on the plate (approximately 15 cm away from the electron gun). Non-irradiated samples served as controls. .

4.3.5 Microbiological Analysis

After irradiation, the samples were kept at 10°C and subsequently transferred to polyethylene bags (18 oz. hirl ak bags) and pummeled with 20 ml of DIFCO™ buffered peptone water (pH 7.0). Samples of 100 µl from a serial dilution in 0.1% of peptone water were plated on an MCAR and incubated at 37° C until visible colonies were counted with the use of a magnifier counter (the detection limit was 10 CFU/g of pecan halves). The radiation D_{10} value was determined by finding the slope of survivors

versus the dose plot (Kim et al., 2010) which was determined after counting the number of colony-forming units per gram of sample (CFU/g) for each treatment (NP, OP,VP) of each microorganism. Then, a logarithm of CFU/g versus dose (0.0, 0.2, 0.4, 0.6, 0.8 kGy) was plotted, and over the inverse of the slope of this graph was given as the D_{10} value.

4.3.6. D_{10} Value Theory

The D_{10} value is the radiation dose required to neutralize 90% of the viable microorganism (Rodriguez et al., 2006). The D_{10} value determination was proposed to be describe by a "target theory" (Lea, 1955) model and a single hit inactivation model (Alpen, 1998), which assumes that the microorganism will not survive when one or more types of ionizing radiation energy hit the target of the cell (can be DNA) (Gomes et al., 2008). This classic first-order relationship of logarithmic survival on dose was written as:

$$S = \frac{N}{N_0} = e^{-D/D_0} \qquad [4.1]$$

Natural logarithm of S

$$lnS = \frac{D}{D_0} \qquad [4.2]$$

where,

N= the number of remaining cells after dose D,

N= the initial number of undamaged cells,

D= the dose,

D_0= the lethal dose, a required dose to reduce the survival fractions to 1/e=37% of the initial value.

Therefore,

$$S = \frac{N}{N_0} = 10^{-D/D_0} \quad [4.3]$$

Decimal logarithm of S

$$logS = -\frac{D}{D_{10}} \quad [4.4]$$

D_{10} is the radiation dose required to eliminate 90% of the initial number of undamaged cells (one logarithmic cycle reduction) (Dion et al., 1994).

When [4.3] and [4.4] and are computed, we get:

$$D_0 = -\frac{D_{10}}{2.303} \quad [4.5]$$

4.3.7 Statistical Analysis

The D_{10} value was determined for the two types of microorganisms irradiated under different packaging-treatments (VP, OP, and NP). For each MAP condition (VP, OP, NP), three samples were used per dose for a total of 15 samples per treatment. This experiment was replicated three more times, on different days. The results were analyzed by an analysis of variance (ANOVA) using a JMP Mac-Version 9 (SAS Institute Inc., Cary, NC, 1999-2010); mean ($P < 0.05$) comparisons between each treatment were based on Tukey's Honestly ignificant Differences (H D).

4.4 Results

4.4.1 Absorbed Dose by Whole Pecan Half and Pecan Dorsal Grooves and Recovery of Water Activity

All the target doses were calculated using the counts per dose of 482, 426 counts/kGy numbers obtained from the ion chamber. These were the doses that the pecans were assumed would to receive. In order to find how much of each dose the pecan kernels really absorbed, the absorbed doses were measured using alanine dosimeters (Table 4.1). The pellet locations can be seen in Figure 4.2, organized by pellet number. The target dose for this experiment was 1.0 kGy. However, the dose received by the alanine pellets was actually higher ~1.19 kGy, probably due to calibration errors. When pellets were irradiated with 0.5 kGy, the pellet reading was 0.62 kGy (see, for example, pellet # 6 on Table 4.1). This means that the calculated target dose is underestimated, or that the pecans receive a larger dose because of the pellet size.

In addition, the doses obtained in the pecan kernels were also accumulated doses. According to pellet #6 dose calculations, the expected dose values would be greater than 1.19 kGy, because of the double-sided irradiation setup (0.62 x 2 kGy). However, some pellets received higher doses, such as pellets # 4, 5, 2, 8, and 3. The location of these pellets may have affected the scattering of electrons. For example, pellets # 7 and #1 could have had a scattering effect. The reason for the pellets at different locations in pecans having different absorbed doses is that when the electrons hit the surface they may have a scattering effect, which can also be caused by the neighbors. Hence, pellets # 8, 3,2 may affect the amount of dose absorbed by the pecans by scattering electrons from their neighbors.

From the results of these experiments, the absorbed dose would differ from one part of the pecan to another. To further test this result, the RFs were placed in different parts of the pecan half.

The absorbed doses at the different parts of the pecan sample are shown in Table 4.2. When the pecans were irradiated only on one side, the entrance (0.48 ± 0.06 kGy) and exit dose (0.08 ± 0.06 kGy) could be calculated. When the RF was placed in the middle of the pecan half and irradiated on both sides (0.5 kGy each), the accumulated dose was 0.94 ± 0.09 kGy. When the RF was placed on both sides of the pecan sample and irradiated on both sides, the accumulated absorbed dose at both the entrance and exit points could be determined.

Table 4.1 Absorbed dose measured by alanine dosimeters plugged into pecan halves (Figure 4.2) and irradiated at a 0.5-kGy dose on both sides (1.0 kGy target dose). Pellet number 6 was irradiated alone, one time, at a 0.5 kGy dose.

Pellet number	Absorbed Dose (kGy)	Pellet location in pecan	Irradiated dose 0.5-kGy on both sides
4	1.32	BACK center	1.0 kGy
5	1.33	BACK center	1.0 kGy
1	1.19	BACK center	1.0 kGy
2	1.44	FRONT right	1.0 kGy
8	1.31	FRONT right	1.0 kGy
3	1.45	FRONT left	1.0 kGy
7	1.2	CENTER	1.0 kGy
6	0.62	Alanine pellet alone	0.5 kGy

The double-sided and one-sided irradiation treatments are illustrated in Figure 4.9 and Figure 4.10, respectively. The dose absorbed by the dorsal grooves (valleys) of the pecans (0.72 kGy) was 40% higher than the target dose (0.5 kGy) (Figure 4.9). Figure 4.10 shows the accumulated doses (calculated using Monte Carlo simulation) in pecans irradiated on both sides (front and back) at 0.5 kGy each. The Figure 4.11 shows the entrance and exit doses. The entrance dose is the amount of dose that starts to touch the pecan surface and it then penetrates into the pecans. The pecan half depth was about 0.7 cm. In this simulation, after the pecan half was irradiated on both sides, the middle of the nut had the highest dose absorption, basically due to the accumulated dose. The dose calculated for the one-sided irradiation setup at a target dose of 1.0 kGy is shown in Figure 4.12. The highest accumulated dose was at a depth of 0.23 cm and not at the center of the pecans, mainly because after the electrons continue to travel into the pecan, they lose some of their energy, and the exit dose is lower.

In brief, when pecan halves are irradiated with a single beam (or one side) using a low energy accelerator, the dose distribution within the sample is not uniform. Therefore, the best method to irradiate the pecan halves is using a double-sided irradiation setup.

Table 4.2 RF absorbed-dose calculations on pecans irradiated at a 0.5 kGy dose on both sides (total of 1.0 kGy), where the RFs were placed on the front (Figure 4.2.a) and in the middle of the pecan half. RF absorbed-dose calculations after the pecans were irradiated at a 0.5 kGy dose only on one side, where the RFs were placed on the front (see Figure 4.3.b) and the back (see Figure 4.3.c) of each pecan half.

RF location	Irradiation direction	Number of sides	Total applied dose (kGy)	Absorbed dose (kGy)
FRONT	FRONT	1 side	0.5 kGy	0.48±0.06
BACK	FRONT	1 side	0.5 kGy	0.08±0.04
FRONT	FRONT & BACK	2 side	1.0 kGy	0.57±0.09
MIDDLE	FRONT & BACK	2 side	1.0 kGy	0.94±0.09

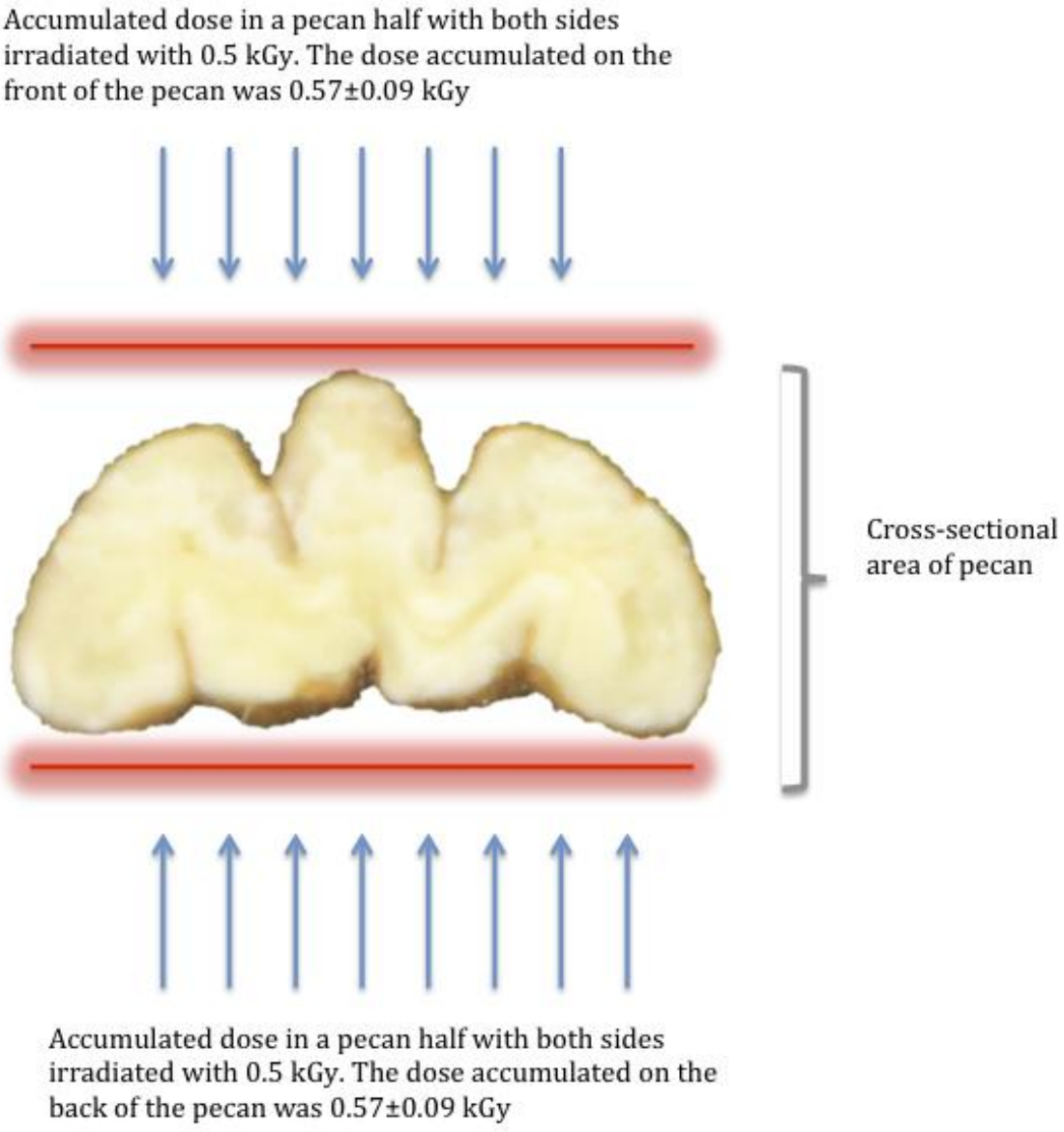

Figure 4.9 Illustration of dose absorbed by the pecan halves after irradiation on both sides at 0.5 kGy. Also shown are the accumulated entrance and exit parts of each pecan.

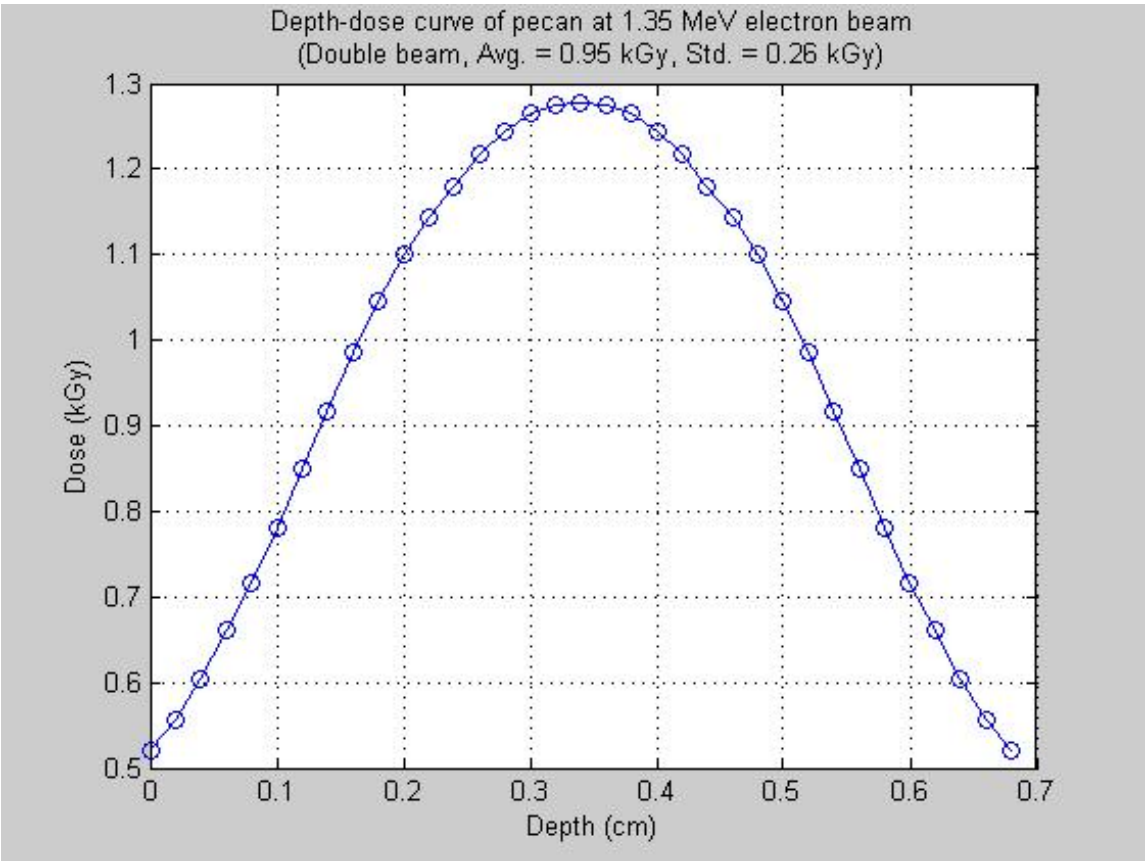

Figure 4.10 Depth-dose curve calculated dose using Monte Carlo simulation for the double-sided (double beam set up) irradiation (0.5 kGy in each side) of a pecan half.

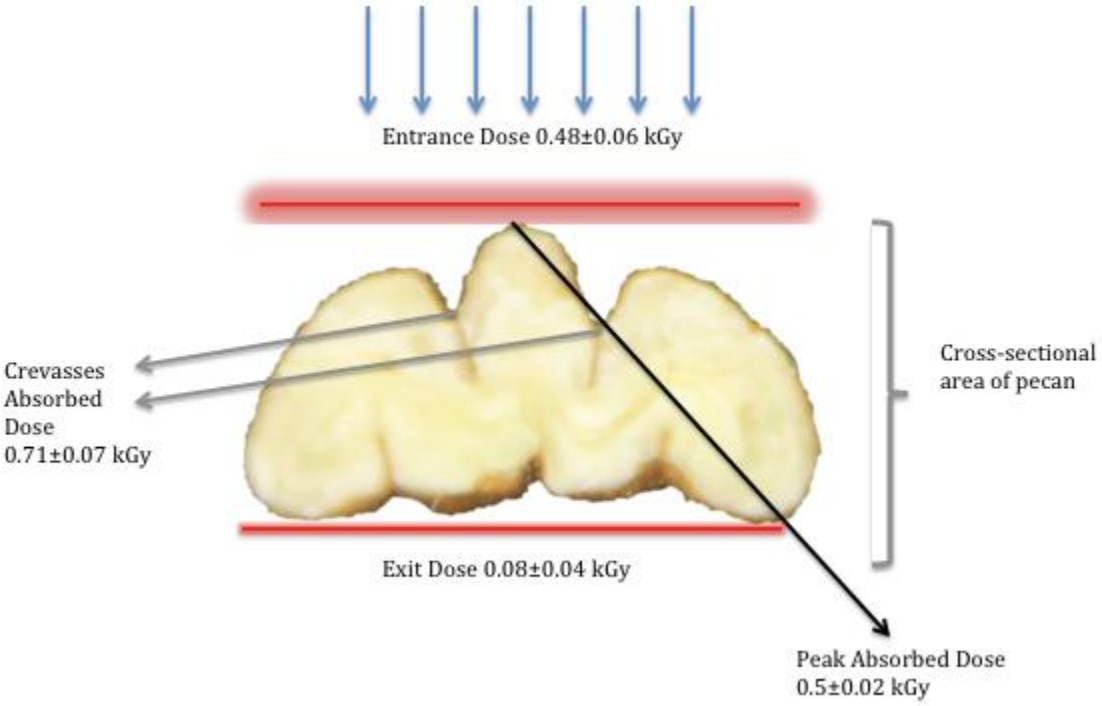

Figure 4.11 Illustration of dose absorbed in pecan halves after irradiating on only one side at 0.5 kGy. The entrance and exit doses can be found using this method.

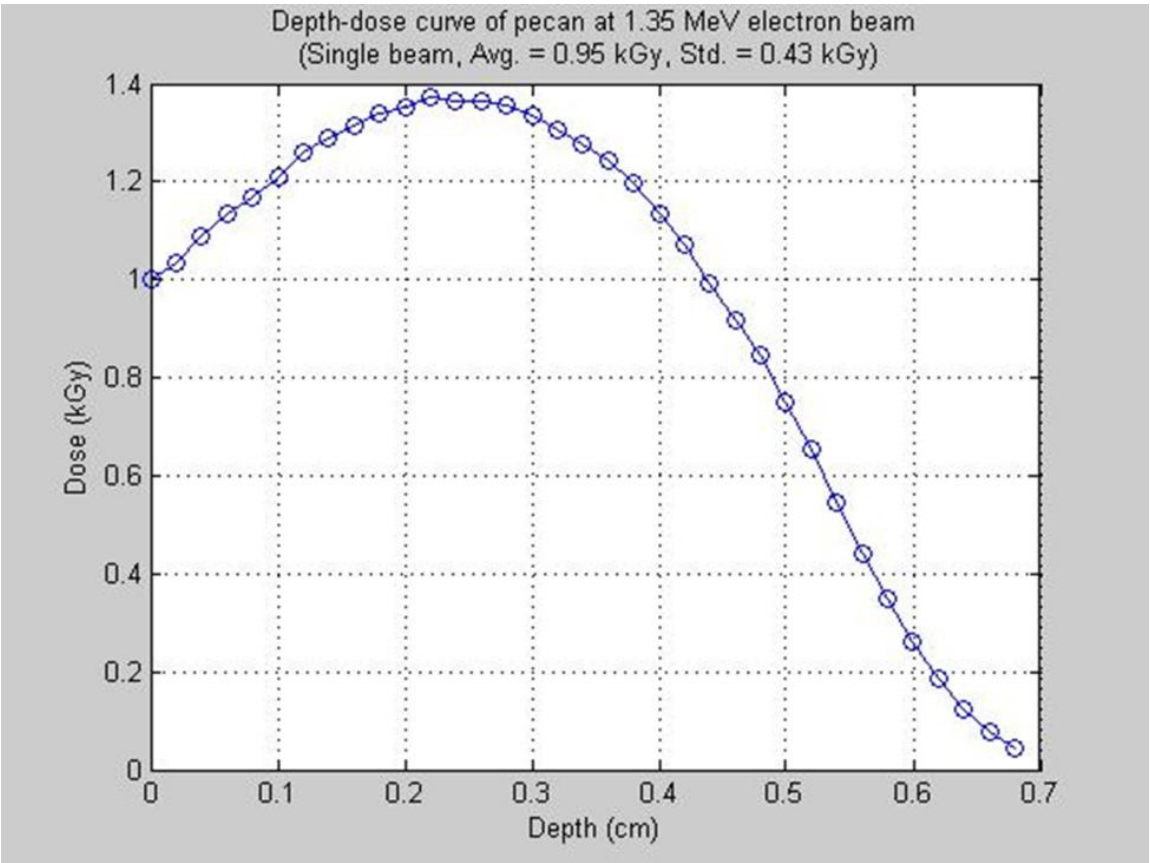

Figure 4.12 Depth-dose curve calculated dose using Monte Carlo simulation for the one-sided (single beam set up) irradiation (1.0 kGy) of a pecan half.

No significant ($P<0.05$) differences were found in the water activity levels after inoculation. The a_w level of the simulated inoculation was approximately 0.57 ± 0.03 while the value of non-irradiated pecans was 0.57 ± 0.01 (see Chapter III).

4.4.2. D_{10} Values of an *E. coli* Cocktail and *Salmonella* Typhimurium LT2 in Pecans Under Different MAP Conditions

There were no significant ($P < 0.05$) differences in D_{10} values within the different sets of data, which were conducted on different days for both *Salmonella* Typhimurium LT2 and the cocktail. In other words, the methodology produced consistent results. The D_{10} values for the *E. coli* cocktail in VP, OP, and NP pecans were 0.46, 0.36, and 0.4 kGy doses, respectively (see Table 4.3). Similarly, the D_{10} value for *Salmonella* Typhimurium *LT2* for the VP, OP, and NP samples to be 0.44, 0.34, and 0.38 kGy, respectively (Table 4.3).

For a specific MAP treatment, the D_{10} values for *Salmonella* Typhimurium LT2 and the *E. coli* cocktail were similar ($P > 0.05$). However, irradiating under vacuum (VP) significantly ($P < 0.05$) increased the D_{10} value of both microorganisms compared to irradiation under oxygen (OP), nitrogen (NP), and air (AP, which served as the control). Irradiating under vacuum (VP) and 100% oxygen (O) gave the most significance ($P<0.05$) difference on D_{10} values, confirming the impact of the absence/presence of oxygen on the radiation sensitivity of microorganisms, regardless of the type of microorganism.

Table 4.3 D_{10} values for the cocktail and *Salmonella* Typhimurium LT2 on pecans irradiated under different MAP conditions using a 1.35 MeV electron beam accelerator

	D_{10} value (kGy)	
MAP condition	*Salmonella* Typhimurium LT2	(cocktail)
Vacuum-Packed	$_{w}0.44 \pm 0.06^{a}$	$_{w}0.46 \pm 0.05^{a}$
Nitrogen-Packed	$_{x}0.38 \pm 0.05^{ab}$	$_{x}0.4 \pm 0.04^{ab}$
Oxygen-Packed	$_{y}0.34 \pm 0.04^{b}$	$_{y}0.36 \pm 0.05^{b}$
Control (Air-Packed)	$_{z}0.36 \pm 0.01^{ab}$	$_{z}0.38 \pm 0.005^{ab}$

a,b, c within each column, items not followed by a common superscript letter are significantly different ($p < 0.05$).
w,x,y z within a row, items, which are not preceded by a common superscript letter are significantly different ($P<0.05$). Each set value is the mean of nine samples ± S.D.

The D_{10} values found in this study were lower than in studies conducted with other nuts. For instance, in almonds, D_{10} values for *Salmonella* (*S*. Anatum, *S*. Hartford, *S*.PT30) ranged between 1.06 and 1.25 kGy when irradiated using electron beam irradiation (Prakash et al., 2010), and 0.92 to 0.72 kGy for *Salmonella* Enteritidis PT 30 and *Salmonella* Senftenberg when using electron beams (Cuervo 2011, unpublished thesis). One possible explanation is that the growth medium could be too rich for the organisms used in this study (Pillai, 2012). On the other hand, it is common knowledge that microorganisms existing in low moisture content foods are more resistant to e-beam radiation than those existing in higher moisture content foods (Sommers and Fan, 2006).

The reason for this is that in survival cell theory, microorganisms survive according to either indirect or direct damage. For low energy applications, indirect damage is more effective than direct damage, which is the radiolysis of water. It was found that not only the tonicity of the cell affected the sensitivity of the microorganism, but also variations in the osmatic pressure affected the size and the water content of the cell (Michaels and Hunt, 1977). When the water content increases, the yield of indirect effect damage also increases (Michaels and Hunt, 1977).

This theory supports the notion that in a less water-saturated environment, the D_{10} values of the microorganisms that try to survive under stress (a lack of water) are more resistant to irradiation. However, Thayer et al. (2003) found no significant difference in the D_{10} values of microorganisms in alfalfa seeds (~0.90 kGy) when the moisture content was reduced from 23.92% (a_w = 0.96) to 10.48% (a_w = 0.50). Therefore, adaptations to the environment could show differences within the same

strains of the same microorganisms as well as microorganisms of different strains (pathogens vs. non-pathogens), and inoculation time and temperature, as well as product structure and other factors such as pH and protein content may all affect the sensitivity of organisms to irradiation (Prakash et al., 2010).

For instance, the study performed by Rodriguez et al. (2006) found a significant difference between the D_{10} value of *Escherichia* coli O157:H7 933(0.13kGy), *Listeria* monocytogenes ATCC 51414(0.18kGy), and *Salmonella* Poona (0.13kGy) and those for the surrogates' D_{10} value: E. coli K-12 MG1655 (0.45kGy), *Listeria* innocua Seeliger *1983*; NRRL B-33003 (0.66kGy) and NRRl B-33014 (0.72kGy), *Enterobacter* aerogenes B199A (1.92kGy), and *Salmonella* LT2 (0.12kGy) in a model system (10% w/w gelatin system).

However, this study showed that the absorbed dose in pecan kernels can be different, depending on which part of the kernel is inoculated. Since the inoculations were made within the dorsal grooves of the pecan kernel (which absorbed 40% more radiation irradiation than the target dose) this explains why the D_{10} values of the pecan kernels were lower than those found in the literature.

This study also showed that the dorsal grooves received greater doses than the peaks or the flat surfaces of the pecan because of the scattering effects of electron beam irradiation; therefore, microorganisms, which internalize into the pecan dorsal grooves will be more difficult to destroy than those at the surface. In addition, the D_{10} value for a particular microorganism may vary when it is present in different food systems, even when the composition is similar. In other words, it is the structure of the food system and

not the type of food, which makes a difference in the radiation resistance of a microorganism. For instance, Rodriguez et al. (2006) found a D_{10} value of 0.45 for *E. coli* K-12 MG1655 in gelatin systems, and 0.18 kGy in fresh-cut cantaloupe. Conversely, Chimbombi et al. (2011) found a D_{10} value of 0.177 kGy in fresh-cut cantaloupe inoculated with *Salmonella* LT2 compared to 0.12 kGy in a gelatin system (Rodriguez et al., 2006).

4.5 Conclusions

The water activity of pecan kernels remains constant after inoculation with the drop method technique. Regarding irradiation, pecan kernels may absorb different doses in different parts of the nut due to the scattering effects of irradiation and the positioning of the sample in front of the electron beam. Therefore, not only the water activity of the pecan kernels, but also the surface of the product is important when considering applying irradiation to pecans using electron beam accelerators. In addition, irradiation of pecans in vacuum packaging (VP) yielded the highest D_{10} value for both the *Salmonella* and *E.* coli surrogates (hindered killing effect of irradiation), while irradiation in the O_2-packed (OP) pecans yielded the lowest values (enhanced killing effect). However, the D_{10} values obtained from irradiation in N_2-packages (NP) were not different ($P > 0.05$) from the values obtained from irradiation in O_2-packages (OP). Thus, irradiation of pecans in nitrogen-packages is a feasible alternative to reduce the dose required to cause a significant reduction in the microbial population, since irradiation in 100% oxygen has a detrimental effect in quality (rancidity).

CHAPTER V

MODELING LIPID OXIDATION REACTION (PEROXIDE VALUE) IN PECAN NUTS (KANZA CULTIVAR) AS A FUNCTION OF RADIATION DOSE AND MAP CONDITIONS

5.1 Summary

This study provides a mathematical description of lipid oxidation (PV values) in irradiated pecan nuts and kinetic reaction rates for each MAP condition and dose level (0 to 0.9 kGy). Irradiation applied to food assures its safety by killing pathogens; however, many of the mechanisms of the chemical changes that occur during and after irradiation still need to be better understood. The aim of this study was to understand the mechanisms and kinetics of lipid oxidation in pecans after exposure to ionizing radiation when packaged using different methods. As a result, irradiation applications in high lipid-content foods such as nuts can be optimized.

In this study, preliminary data were collected for coated and frozen pecans packed under vacuum (VP). Regardless of the MAP condition, frozen pecans under VP reached high peroxide values (PVs) right after irradiation at 0.8 kGy. Coated pecans had a 5-fold increase in PVs which had an undesirable effect on their quality (taste and appearance). Therefore, neither frozen nor coated pecans were used in the kinetic study.

To understand the lipid oxidation reactions under different MAPs, the following experiments were carried out. Pecan halves were packed in four different MAP conditions - Vacuum (VP), Air (AP), Nitrogen (NP), and Oxygen (OP) - and irradiated

at doses of 0.3, 0.5, 0.7, and 0.9 kGy using a 1.35 MeV accelerator. Non-irradiated pecans packed under each MAP condition served as controls. After irradiation, the packages were stored at 48.9° C and 13% relative humidity (RH) for a month. Right after irradiation (Week 0), peroxide value (PV) analyses were performed every week for a month. The characteristic quality index is a parameter that helps to determine the loss of quality in that particular food and the peroxide value was the one used in this study was.

PV data were fitted into the Gompertz model, a Modified Gompertz Model, the Logistic model, and the reaction order (n^{th} order) equation to determine the order of the lipid oxidation reaction as a function of applied dose and MAP condition. The best-performing curve-fit model was obtained by the Modified Gompertz equation.

Packaging type (MAP condition) had a significant ($P<0.05$) impact on the onset of rancidity in irradiated pecans. Nitrogen and Vacuum-packed pecans were the best options in terms of quality (rancidity measured as peroxide value) when irradiating at doses up to 0.9 kGy.

5.2 Introduction

Food quality is the degree of acceptability of the food characteristics (attributes) that are being led by consumers' demand (Caswell, 1996; Taoukis et al., 1997). When the change in these characteristics; for example, chemical: not rancid taste in nut; physical: crispiness in chips; or microbial: no molds on strawberries, can be controlled, the quality loss will be delimited during the processing, storage and distribution.

The quality loss over time can be determined by measuring the change in the characteristic quality index (A) of that particular food. The characteristic quality index is the dominant parameter in which any change in this parameter will change the quality of that particular food product, and it allow the determination of the shelf life of the product. In this study, it was the peroxide value (rancidity) formation in the pecans during storage. This change can be written as a function of time (dA/dt) (Fu and Labuza, 1997). For example, let us say the quality index is A and the reaction for the change in the amount of A with time is

$$A + B \rightarrow C + D \qquad [5.1]$$

then the reaction rate can be shown by the following kinetic equation:

$$r \text{ (rate)} = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = \frac{d[C]}{dt} = \frac{d[D]}{dt} = k[A][B] \qquad [5.2]$$

$$f(A) = \frac{d[A]}{dt} = k[A]^n \quad \text{(rate law)} \qquad [5.3]$$

where $f(A)$ is the quality function, also called reaction rate; ($d[A]/dt$) is the change in the characteristic quality index (A) with time (this equation is also called rate law); k is a rate constant ($1/time$) and n is a power factor called reaction order. The shape of the deterioration curves (amount of A, [A], versus time plot) also can be determined by the

order of the reaction, *n*, if the environmental factors are held constant (Fu and Labuza, 1997). Therefore, deriving the kinetic equation will give the basic kinetic information of that particular food, which will help to predict the changes in food quality during processing and storage (Van Boekel, 1996).

Kinetic models have been applied to the microbial (Zwietering et al., 1994) (McMeekin et al., 2002; van Boekel, 2002), physical, and chemical (van Boekel, 2001; Giannakourou and Taoukis, 2003; Peleg et al., 2009) changes in food. The chemical kinetic models are widely used to predict the changes on high oil content foods. More specifically, the main deteriorative chemical reaction that controls the shelf life for the high oil content foods is lipid oxidation (Simon et al., 2000; Jin et al., 2001; Tan et al., 2001; Pu and Sathivel, 2011; Shim and Lee, 2011).

The strategies that are designed to inhibit the progression of the deteriorative impact of the oxidative reactions (Kama- Eldin and Yanishlieva, 2005) can be achievable by understanding the nature of these reactions [5.4-8] (Özilgen and Özilgen, 1990; Laguerre et al., 2007; Van Boekel, 2009a).

$$^3O_2 \rightarrow {}^1O_2 \text{ Singlet oxygen formation (initiation)} \quad [5.4]$$

$$RH + {}^3O_2 \rightarrow ROOH \text{ (initiation)} \quad [5.5]$$

where, RH is fatty acid chain double bond and ROOH hydroperoxide

$$R_1H \xrightarrow{\text{initiator}} R_1 + H^{\cdot} \text{ free radical formation (initiation)for autoxidation} \quad [5.6]$$

Free radicals are highly unstable, and stabilize by abstracting an H atom from other chemicals(Laguerre et al., 2007)

$$ROOH \rightarrow ROO\cdot + H\cdot \text{ (initiation)} \quad [5.7]$$

In the initiation phase of autoxidation reaction; hydroxyl (·OH) and hydroperoxyl (HOO) radicals, as well as lipid alkoxyl (RO·) and peroxyl (ROO·) radicals were formed and initiate the lipid oxidation reactions.

$$R\cdot + {}^3O_2 \rightarrow RO_2\cdot \text{ (propagation)} \quad [5.8]$$

R· radical reacts with triplet oxygen and forms peroxyradicals ROO·

$$RO_2\cdot + RH \rightarrow RO_2H + R. \text{ (propagation)} \quad [5.9]$$

ROO· then will capture another H atom from RH (unsaturated fatty acid bond) and forms hydroperoxide (ROOH) and other R· radicals. Formation of ROOH peaks in propagation stage.

$$R. + RO_2. \rightarrow RO_2R \text{ (termination)} \quad [5.10]$$

In termination stage peroxide and secondary non-radical oxidation compounds forms. ROOR is the peroxides.

$$RO_2 + RO_2 \cdot \rightarrow RO_2R + O_2 \text{ (termination)} \quad [5.11]$$

Most frequently, Peroxide values (PV) (which determine the primary oxidation of products) and Thiobarbituric Acid Reactive Substances Assay (TBARS) (which determine the secondary oxidation of products) are used to determine rancidity, depending upon the food. For nuts, a PV method can be used to determine the kinetics of the oxidation. In this chapter, PV tests were used to determine the kinetic models for coated, frozen and irradiated at different doses (0.0-0.8 kGy), and raw pecans, which were packed in different MAP conditions and irradiated at different doses (0.0-0.9 kGy).

Experimental data were fitted into several kinetic models, including the Gompertz model, the Modified Gompertz model, and the reaction order equation commonly used to describe the kinetics of lipid oxidation in the literature. These models are explained in section 5.3.2.3 and the best-fitted model for the experimental data found in this study, the Modified Gompertz model, is discussed in the Results and Discussion section.

The objectives of this study were to (1) evaluate the effectiveness of coating and freezing treatments on reducing the rancidity of irradiated pecans, (2) quantify the change in quality (rancidity, as measured by PV test) in irradiated pecans (Kanza cultivar) (coated, frozen, MAP); and, (3) determine the best MAP/irradiation

combination that would delay the rancidity in irradiated pecans as a function of applied dose.

5.3 Materials and Methods

5.3.1 Preliminary Studies with Coating and Freezing of Pecans

5.3.1.1 Coating

Kanza pecans, previously unshelled and stored at -25º C, were taken out of the freezer and left for 20 minutes (until they reached room temperature) before they were used in the coating experiments. The coating experiments were all held at room temperature (21-25° C). Based on preliminary data obtained from our laboratory (Martinon, 2011; Mantilla, 2012 ; Sipahi, 2012, unpublished theses), the following solutions were prepared: 1.5% pectin, 1.5% calcium chloride and 1.5 % chitosan-antimicrobial (trans-cinnamaldehyde in beta-cyclodextrin) to manufacture an edible coating with an incorporated antimicrobial compound using a layer-by-layer procedure.

1.5% Pectin Solution (w/v):

1.5% pectin (USP, Citrus, Spectrum NJ, USA)solution was prepared by weighting 1.5 gram pectin and slowly dissolving it on a stir hot plate with previously heated at 45° C sterile distilled water until it completely dissolved.

1.5% Calcium chloride Solution (w/v):

1.5 grams of calcium chloride (USP, Mallinckrodt Baker, Phillipsburg, NJ, USA) were weighted and dissolved with sterile distilled water at room temperature until it completely dissolved.

Antimicrobial agent:

A mixture of 2.11g trans-cinnamaldehyde (99+%, Sigma-Aldrich, St. Louis, MO), 18.16g beta-cyclodextrin (hydrate, Alfa Aesar Johnson Matthey, Lancashire, UK) and one liter of distilled water were mixed in a laboratory stirrer for 24 hours. After filtrating the suspension with a 0.45 µm nylon filter (VWR vacuum filtration systems, VWR international, West Chester, PA, USA), then the filtrate frozen at –18°C and freeze-dried at -50°C under 5 mtorr (9.67 x 10-5 psi) vacuum for 48 hours in a Labconco Freeze Dry-5 unit (Labconco, Kansas City, MO, USA). The powder of trans-cinnamaldehyde in beta-cyclodextrin compound (about 17.5g) was kept in a desiccator placed inside a freezer (-20°C) until further use (Martinon 2011).

Chitosan Solution:

0.5 % Tween 20 (molecular biology grade, VWR International, West Chester, PA, USA) (w/v), 2% glycerin (USP, Mallinckrodt Baker, Phillipsburg, NJ, USA) (w/v), and 1% acetic acid (Glacial, Mallinckrodt Baker Inc., Paris, KY, USA) (w/v) was weighted and dissolved slowly with distilled water. Upon that, 2% chitosan (medium molecular weight, Sigma-Aldrich, St. Louis, MO, USA) (w/v) was added, while heating on a stirring got plate at 45° C until completely dissolves. The anti-microbial agent (2 % beta-cyclodextrin, w/v) was added later to the solution while it was continuing to stir.

Coating Procedure:

The coating procedure was adopted from Martinon (2011). The layer-by-layer procedure was performed in four steps to ensure the proper coating of the pecans

Twenty-four pecan halves were dipped into each coating solution for two minutes; the coating was then allowed to drip off for two minutes before submerging the samples into the next solution. The order in which the coating solutions were applied to the pecans is as follows: first, the calcium chloride solution; then, the chitosan-antimicrobial solution; then, the pectin solution; and finally, a second dip into the calcium chloride solution (Figure 5.1).

The coated pecans were air dried at room temperature (21-22° C) over night in a dark place to avoid any acceleration effects that might occur from exposure to light. The pecans were then vacuum packed and sealed with a Foodsaver® V2220 Vacuum Sealer prior to irradiation.

5.3.1.2 Packing Coated and Frozen Pecans

Both the coated and the raw pecans (which were later used to prepare the frozen pecan samples) were packed as follows: four pecan halves (6g total) were placed inside a 8x5 cm Foodsaver® sealer bag. The bags are made of four layers of Polyethylene (PE) film with a fifth layer of nylon (for strength and rigidity). Then, twenty packs each of the coated and the raw pecans were packed and sealed under vacuum conditions (referred to here as the Vacuum Packed (VP) coated and Vacuum Packed frozen samples). Then, only twenty raw pecan packs were packed and sealed under atmospheric pressure (referred to here as the Air Pack (AP) frozen sample) using a Foodsaver® V2220

Figure 5.1 Coating the pecans. Twenty-four pecan halves were dipped into each coating solution; first, the calcium chloride solution; then, the chitosan-antimicrobial solution; then, the pectin solution; and finally, a second dip into the calcium chloride solution, for two minutes and allowed to drip off for two minutes before submerging the samples into the next solution.

Vacuum Sealer. The raw pecans, which were packed under vacuum and atmospheric pressures, were kept at -25° C in the freezer until they were prepared for irradiation.

5.3.1.3 Freezing of Pecans

Pecans that were kept in a freezer (-25 °C) for at least five days before irradiation were immediately taken out to the irradiation facility. Immediately prior to irradiation, the packages containing frozen pecans were dipped into liquid nitrogen (obtained from the Texas A&M University Biology department) for ten minutes. This process was necessary to keep the pecans in the frozen stage during irradiation, because the pecan halves thaw in five to eight minutes if they are left to stand at room temperature (21-25° C). Preliminary tests were conducted to calculate these times by immersing a thermocouple (Purple E type, Omega Stamford, Connecticut) inside the pecan packages. The changes in temperature with time were recorded by a computer program (Lab Windows/CVI software for windows), and then change in temperature in time was plotted in Matlab (Mathworks, Inc., Natick, Mass, version 2007 b for windows). In order to keep the pecans in the frozen stage during the irradiation treatment (for about maximum of fifteen minutes), dipping the packages into the liquid nitrogen for about fifteen to twenty minutes was the optimum time for these pecans. Therefore, the pecan packs that were dipped into liquid nitrogen for at least fifteen minutes remained at the frozen stage throughout the irradiation treatment (Figure A5 in Appendix).

5.3.1.4 Irradiation of Coated and Frozen Pecans

The irradiation of pecan samples was carried out using a Van De Graaff accelerator (1.35 MeV) located at Texas A&M University, where samples were irradiated at 0.2, 0.4, 0.6 and 0.8 kGy at room temperature.

5.3.1.5 Peroxide Value of Coated, Frozen and MAP Packed Pecans

After irradiation, the pecans were brought to the Physical Properties Laboratory, after which PV measurements (AOCS (Cd 8b-90, 1997)were immediately taken with duplicate samples. Non-irradiated (0.0 kGy) samples for both the coated and the frozen pecans in each package type (AP and VP) served as controls.

5.3.2 Irradiation of Pecans in Modified Atmosphere Packages (MAP)

5.3.2.1 Preparation of Modified Atmosphere Packages (MAP)

This study was conducted using uncoated, unfrozen, raw pecan halves that were packed in four different atmospheric conditions: NP, OP, AP and VP. First, four pecan halves were placed into a polyethylene film bag (8x5 cm Foodsaver® sealer bags); then, the packages were each flushed with one of four gases, either (1) Nitrogen (100% N_2 research grade, NP) or (2) Oxygen (100% O_2 research grade, OP) gas, or (3) packed at atmospheric pressure (AP), or (4) vacuumed (VP) with the Foodsaver® V2220 Vacuum Sealer. Each of these packages were sealed immediately with the Foodsaver® V2220

Vacuum Sealer. One hundred packages were made for testing in each atmospheric condition.

5.3.2.2 Irradiation of Modified Atmosphere Packed Pecans and Experimental Design

For each atmospheric condition, one hundred packages were divided into five groups of twenty, for a total of four hundred packages. The first group of twenty were irradiated at 0.9 kGy, the second group of twenty were irradiated at 0.7 kGy, the third group of twenty were irradiated at 0.5 kGy, the forth group of twenty were irradiated at 0.3 kGy, and the last group of twenty served as a control (0.0 kGy). Then, all of the packages were kept at an accelerated temperature for four weeks at 48.9 °C with 13% RH. Each week, four pecan packages of the twenty packages allocated to each dose level were tested for PV to determine the rancidity, color, and texture. Testing began at 0 week and continued up to the fourth week (Figure 5.2). PV test results were used to determine the kinetic equations. In addition, the color and texture results are presented in the shelf life studies discussed in Chapter 6.

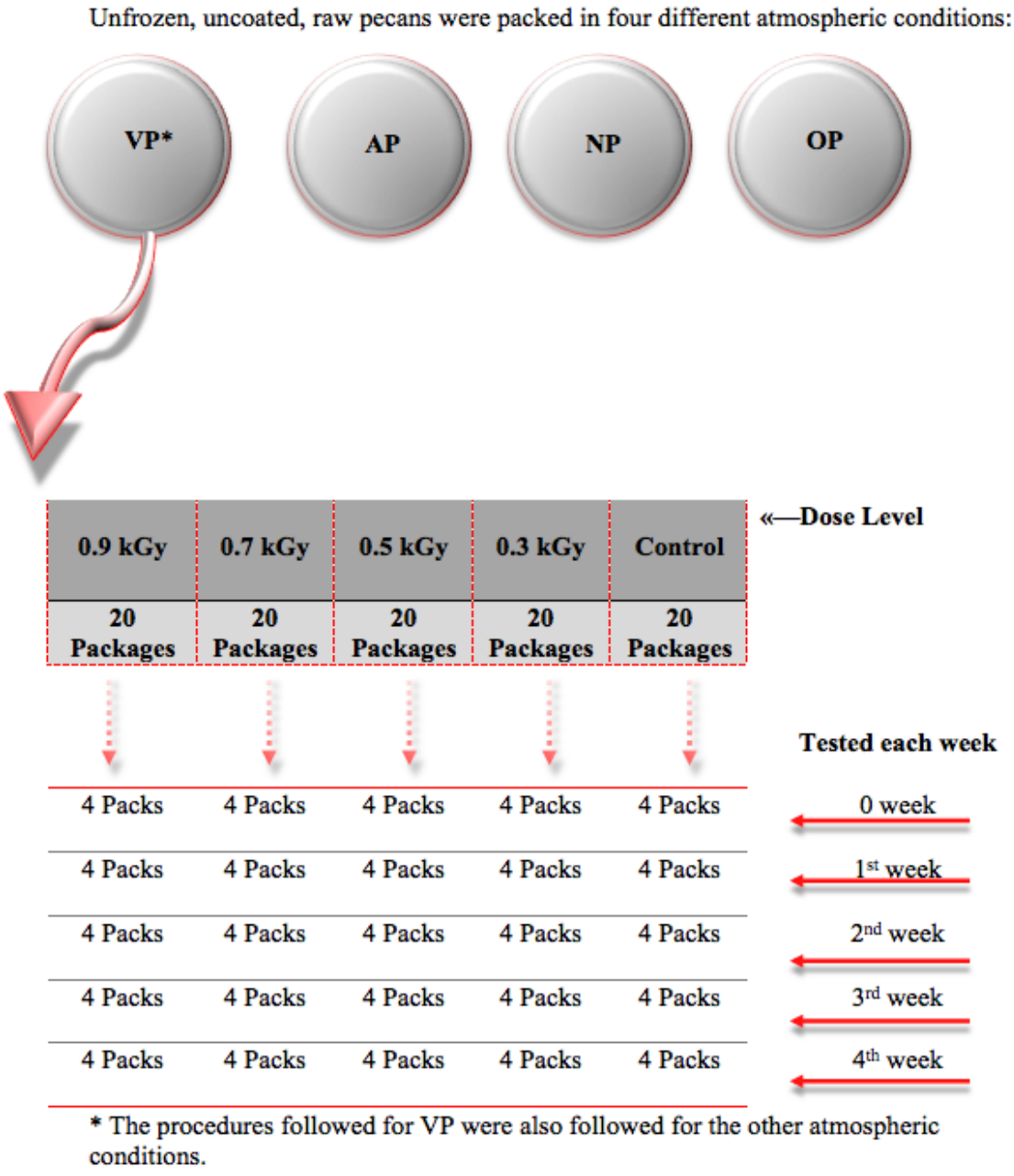

Figure 5.2 Experimental design. Experiment was a completely randomized full factorial design. Randomly selected pecans were irradiated at four different doses (kGy); nonirradiated samples served as a control.

5.3.2.3 Kinetics of Quality Changes for MAP Packed Pecans

At the end of the fourth week, PV results were used to quantify the oxidation process on the pecans that were packed in VP, AP, OP, and NP conditions. Thus, PV data were first fitted into the n^{th} order equations to calculate the order of the reaction and then fitted into the following different models: Gompertz, Modified Gompertz, Logistic, and the multi-response model.

Calculating zero, first, second and nth order reactions: To calculate the order of the equations, the differential equation [5.3] (which was in the form of a power law expression) was integrated with respect to time, where c_0 is the initial concentration ($t=0$), c is the final concentration at the time we tested ($t=t$), k is the reaction rate constant, and dC/dt is the change in the amount of c concentration with respect to time. Therefore,

- Zero order equation, when n=0:

$$\frac{dC}{c^n} = -k \cdot dt \quad \text{if n=0} \qquad [5.12]$$

$$\int_{c_0}^{c} \frac{dC}{1} = \int_{0}^{t} -k \cdot dt \qquad [5.13]$$

$$c - c_0 = -k(t - 0) \qquad [5.14]$$

$$\text{Then,} \quad c = c_0 - kt \qquad [5.15]$$

- First order equation, when *n*=1:

$$\frac{dC}{c^n} = -k \cdot dt \quad \text{if n=1} \tag{5.16}$$

$$\int_{c_0}^{c} \frac{dC}{c} = \int_{0}^{t} -k \cdot dt \tag{5.17}$$

$$\ln\left(\frac{c}{c_0}\right) = -k(t-0) \tag{5.18}$$

$$\text{Then,} \quad c = c_0 \exp(-kt) \tag{5.19}$$

- Second order equation, when *n*=2:

$$\frac{1}{c} = \frac{1}{c_0} + kt \tag{5.20}$$

- Third order equation, when *n*=3:

$$\frac{1}{c^2} = \frac{1}{c_0^2} + 2kt \tag{5.21}$$

- Then the general formula for the reaction rate becomes:

n^{th} order $\quad c^{1-n} = c_0^{1-n} + (n-1)kt \quad$ for every n ≥0 except for n ≠1 [5.22]

Gompertz Model:

The Gompertz equation [5.23] was developed by Gompertz in 1825, and designed to predict human mortality as a function of age (Van Boekel, 2009a).

$$y = a \cdot \exp[-\exp(b - ct)] \quad [5.23]$$

where *a, b, c* are the sigmoidal curve's mathematical parameters. The parameters would represent a chemical reaction meaning when modified for oxidation reaction.

Modified Gompertz (Model for predicting the oxidation kinetics):

Equation [5.23] was modified by Zwietering et al. (1990) to model the bacterial growth curve, which is a sigmoidal curve and shows the phases, lag, and exponential and asymptotic phases. In bacterial growth curves, specific growth rates starts from a zero value and accelerate until they reaches the maximum (μ_{max}) value in a certain period of time, resulting in a lag time (λ, t_{lag}).

When a growth rate reaches the maximum value, it starts to decelerate, and finally reaches zero where an Asymptote (*A*) is achieved (Zwietering et al., 1990). Likewise, the PV data collected in the experimental design (Figure 5.2) against the time plots also follows the sigmoidal shape.

Therefore, a similar approach can be applied to the PV (characteristic quality index characteristic quality index) curves. Zwietering et al. (1990) Modified Gompertz model was modified (Equation [5.34]) to describe preliminary oxidation process. Hence, μmax is defined as the maximum specific reaction rate, λ is the time period before the PV reaction accelerates, and *A* is an asymptotic value (y_{max}) where PV reaches its maximum value for that reaction. Then, the sigmoidal PV against the time curve has a lag phase which starts from t=0 and lasts until t=*tlag*, then continues with the exponential phase where the reaction rates accelerate. This phase is followed by the asymptotic phase, where the reaction rate converges to zero (Figure 5.3).

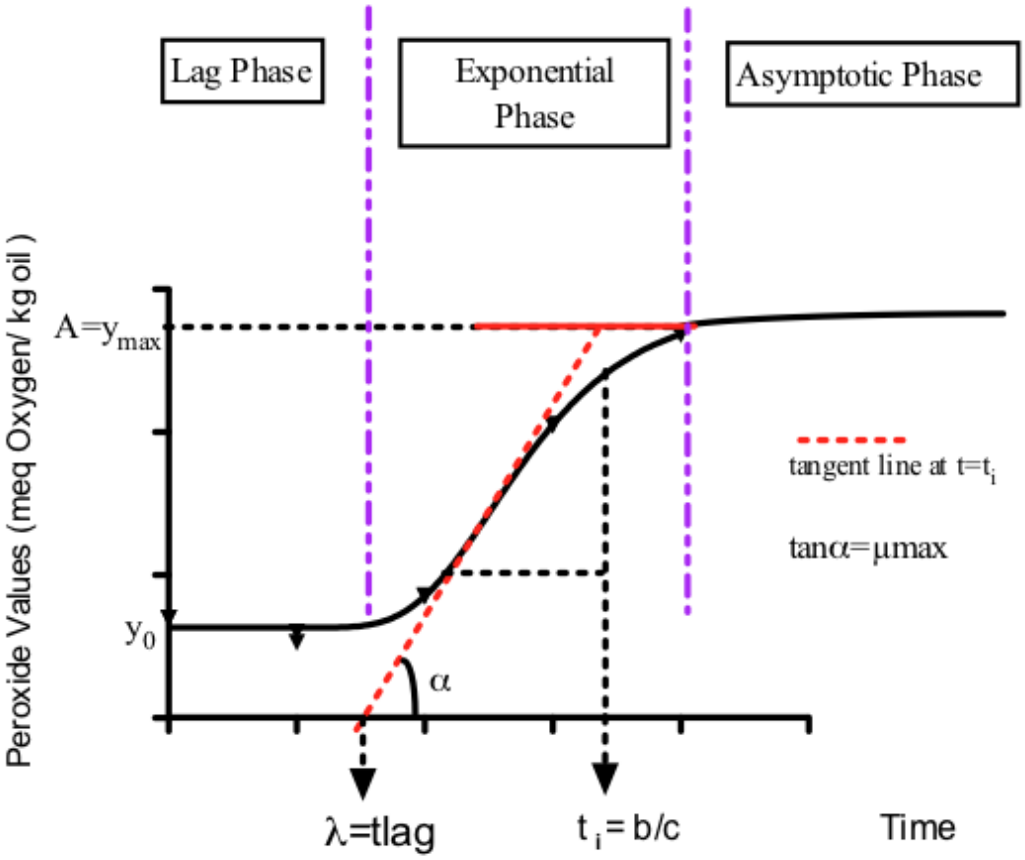

Figure 5.3 Peroxide value (PV) of irradiated pecans versus time after storage at 48.9 °C for four weeks.

Modified Gompertz Model Calculations:

The lag time is the t-axis intercept of the tangent through the inflection point of the curve (Zwietering et al., 1990). Therefore, must determine the inflection point of the curve, then the slope of the tangent line through the inflection point of the curve, and finally the lag time of the curve.

$$\frac{dy}{dt} = \frac{a \cdot c \cdot e^{b-ct}}{e^{e^{b-ct}}} \qquad [5.24]$$

$$\frac{d^2y}{dx^2} = \frac{a \cdot c^2 \cdot e^{b-ct}(e^{b-ct} - 1)}{e^{e^{b-ct}}} \qquad [5.25]$$

The second derivative of the function [5.23] is zero at the inflection point, where $t=t_i$.

$$\frac{d^2y}{dx^2} = 0 \quad e^{b-ct} = 0 \qquad \text{then } t_i = b/c \qquad [5.26]$$

The first derivative of the function [5.23] at the inflection point gives the slope of the tangent line to the curve, which is μ_{max} (specific reaction rate):

$$\tan \alpha = \mu_{max} = \left.\frac{dy}{dt}\right|_{t_i} = \frac{ac}{e} \qquad [5.27]$$

Then, the tangent line at the inflection point is

$$y = \mu_m \cdot (t - t_i) + \frac{a}{e} \qquad [5.28]$$

Finally, the lag time (Eq. [5.30]) is equal to t-axis intercept of the tangent line through the inflection point (Zwietering et al., 1990):

$$0 = \mu_m \cdot (\lambda - t_i) + \frac{a}{e} \quad \text{where } t_i = b/c \qquad [5.29]$$

$$\lambda = \frac{(b-1)}{c} \qquad [5.30]$$

If the parameters in the Gompertz equation, *a*, *b* and *c* are

$$c = \frac{\mu_{max} \cdot e}{a} \qquad [5.31]$$

$$b = \frac{\mu_{max} \cdot e}{a}\lambda + 1 \qquad [5.32]$$

When time converges to infinity, y approaches the asymptotic value A (y_{max}).

$$\text{when } t \to \infty \text{ then } y \to \text{A and } a = y_{max} - y_0 \qquad [5.33]$$

The initial PV value is also added into the equation because when t=0 there already has been some peroxide formation. Adding the initial value into the equation will only shift the curve on the y-axis.

Then, the final modified Gompertz equation becomes:

$$y = y_0 + (y_{max} - y_0) \cdot exp\left\{-exp\left[\frac{\mu_{max} e}{y_{max} - y_0}(\lambda - t) + 1\right]\right\} \quad [5.34]$$

5.3.3 Statistical Analysis to Determine Goodness of Fit

Non-linear regression was used to fit the experimental data into the equations and the goodness of fit values was calculated automatically, depending upon the model used by the program to fit the experimental data. To see the interaction effect of the package type x dose x time, the variance (ANOVA) test was also performed for the full factorial experimental design, which was used to investigate the interaction effects among the treatments, using JMP Mac-Version 9 (SAS Institute Inc., Cary, NC, 2010) and a mean (<0.05) comparison based on Tukey's Honestly ignificant Differences (HSD).

5.4 Results and Discussion

5.4.1. Coated and Frozen Pecans

PV data for each of the coated (VP only) and frozen pecans (AP and VP) were plotted against time. The values for vacuum-packed (VP) frozen pecans were significantly (P<0.05) different from the Air-packed (AP) frozen pecans and the vacuum-packed (VP) coated pecans before irradiation. The initial PV results (before

irradiation) for VP frozen pecans were almost 5- fold higher than the other samples (Table 5.1). However, after irradiation (Week 0) the PV values of VP and AP frozen samples were not significantly ($P>0.05$) different regardless of the dose, though they were significantly different ($P<0.05$) from the vacuum-packed (VP) coated pecans. In addition, regardless of the package type and initial PV value, both frozen pecan samples reached very high rancidity values (3.09-3.5 meq O_2/kg oil at 0.9 kGy, Table 5.1) when exposed to the highest dose (0.9 kGy). One reason for this result is that both VP and AP samples may follow similar oxidation patterns after irradiation.

The PV values of the VP coated pecans were significantly different ($P<0.05$) from those for the VP frozen pecans, regardless of the dose. The PV values for the VP-coated pecans were only significantly ($P<0.05$) different from those for the AP-frozen pecans at a higher dose (0.9 kGy, Table 5.1). The slope of the plots of PV versus time for VP-coated and AP-frozen pecans were not significantly ($P>0.05$) different from each other, but they were different from the VP-frozen pecans. In addition, the coated pecans had a white residue left from the coating procedure, which did not look very tempting (Figure 5.4). Furthermore, the coated pecans had very noticeable cinnamon taste and odor. Therefore, neither the freezing nor the coating treatments were selected as a means to extend the shelf life of pecan nuts in addition to modified atmospheric packaging. For this reason, no kinetic models were evaluated for these samples.

Figure 5.4 Coated Pecans with visible whitish residual. The coated pecans were air dried at room temperature (21-22° C) overnight in a dark place to avoid any acceleration effects that might occur from exposure to light

Table 5.1. PV of frozen and coated pecans in AP and VP packages irradiated at room temperature using a 1.35 MeV electron beam accelerator

Dose (kGy)	AP FROZEN PV	VP FROZEN PV	VP COATED PV
0.8	w3.09 ±0.13[d]	w3.50 ±0.14[c]	x1.9 ±0.14[d]
0.6	w2.09 ±0.42[c]	w2.40 ±0.00[b]	x1.4 ±0.00[c]
0.4	w0.79 ±0.00[b]	x2.30 ±0.14[b]	y1.2 ±0.00[c]
0.2	w0.89 ±0.70[b]	x2.19 ±0.00[b]	w0.8 ±0.00[b]
0.0*	w0.30 ±0.14[a]	x1.78 ±0.25[a]	w0.4 ±0.00[a]

*Non-irradiated samples served as controls.
VP= Vacuum pack, AP=Air pack, N_2=Nitrogen pack, O_2=Oxygen packed pecan samples.
All samples were irradiated from 0.2 through 0.8 kGy with a 0.2 kGy increment.
[a, b, c] within each column, items not followed by a common superscript letter are significantly different ($p < 0.05$).
w, x, y within a row, items, which are not preceded by a common superscript letter are significantly different ($P<0.05$). Each set value is the mean of nine samples ± S.D.

5.4.2. MAP Experiments

To evaluate the effect of irradiation on pecans' rancidity under different MAP conditions, the peroxide values (raw data) were plotted as a function of time (weeks) for each dose level at each atmospheric condition. Surprisingly, the PV did not accelerate linearly with time and there was a fluctuation within the first two weeks of the experiment, as shown in Figure 5.5 to Figure 5.9. The nonlinear relationship between PV and time suggests that the oxidation reaction does not follow the first order (Equation [5.19]). Thus, the order of the reaction (PV formation in irradiated pecans) must be determined.

In lipid oxidation, the reaction order has been generally found to be zero (Gallagher et al., 2011) or of the first (Fu and Labuza, 1997) order. For example, Gomez-Alonso et al. (2004) found a zero order reaction rate for the primary oxidation products, and a first order reaction rate for the secondary oxidation products of olive oil, which has a very similar lipid composition and tocopherol content to pecan oil (Villarreal-Lozoya et al., 2007). A nonlinear relationship between PVs and time was also found by Villarreal and others (2009).

5.4.2.1. Reaction Order

The order of the reaction can be determined only by experimental data. The order of the reaction does not give *direct* information on the mechanisms of the reaction, but instead describes the experimental data (Van Boekel, 1996). Orders are representative of the mechanistic step, which limits the rate of the whole reaction; however, determination

of the each radical during limit oxidation reaction (Equation 5.4-11) is practically impossible due to the rapid kinetics (Bondet et al., 1997). In addition, it should be noted that for a complex reaction (such as lipid oxidation reaction), the order of the reaction may not always equals to their stoichiometric coefficients (Bondet et al., 1997).

Therefore, in this study the change in the PV value was observed (time dependent concentration), and each individual MAP condition for each dose was evaluated separately. In order to find the reaction order, the steps below were followed:

1. The reaction order equations; zero [5.15], first [5.19], second [5.20] and third [5.21] were calculated by integrating the rate law equation [5.3] with respect to time,
2. PVs data from each MAP condition (AP, VP, N_2, O_2) were irradiated at different doses were plotted as a function of time into each reaction order equations (from step 1).
3. Rate constant, *k* (unit vary depend on *n*), was determined from the slope of each linearized plot,
4. R^2 was used to determine the goodness of fit. The highest R^2 of each plot were selected as the reaction order for that individual MAP condition and dose level.
5. Van Boekel (2009b) indicates that R^2 alone is not enough to determine the goodness of fit, and the reporting residuals are better indicators to describe the goodness of fit and residual plots should randomly be distributed, and should not follow a pattern. Therefore, the residuals of each reaction order were obtained by

using the Graph Prism 5 for Mac software (GraphPad Software, La Jolla California USA) and were plotted using GraphPad software to compare each reaction order. This process was applied to each MAP of each dose in order to determine each reaction order.

6. The results are shown in Table 5.2.
7. Then PV values were plotted as a function of time into each reaction order equation, best fit was selected as the order of the reaction. R^2 values lower than 0.75 were statistically unacceptable to represent the goodness of the fit.
8. Non-irradiated samples served as controls.
9. VP= Vacuum pack, AP=Air pack,N_2=Nitrogen pack, O_2=Oxygen packed pecan samples.

The lipid oxidation reactions were of the zero, first, second, and third (only O_2 packages (OP) irradiated at 0.9 kGy order. Vacuum-packed (VP) samples initially followed a zero order reaction regardless of the dose, which suggests that a change in PV value will not affect the reaction rate. In other words, the rate of the reaction is a constant (from rate law equation [5.3]; when n=0 → r (rate)=k, please be noted that k is the reaction rate constant).

Table 5.2 Reaction orderto describe the PV formation reaction in pecans irradiated up to 0.9 kGy in MAP conditions at room temperature using an electron beam accelerator.

R^2	Pack and Dose	Reaction Order
0.91	VP control	1 or 0
0.72	VP 0.3 kGy	**0**
0.79	VP 0.5 kGy	0
0.78	VP 0.7 kGy	0 or 1
0.84	VP 0.9 kGy	0
0.81	AP control	1
0.64	AP 0.3 kGy	**0**
0.73	AP 0.5 kGy	**2**
0.28	AP 0.7 kGy	**0**
0.52	AP 0.9 kGy	**0**
0.75	N2 control	1
0.91	N2 0.3 kGy	1
0.82	N2 0.5 kGy	1
0.61	N2 0.7 kGy	**2**
0.67	N2 0.9 kGy	**2**
0.85	O2 control	1
0.85	O2 0.3 kGy	1
0.96	O2 0.5 kGy	2
0.96	O2 0.7 kGy	2
0.91	O2 0.9 kGy	3

Numbers in bold represent $R^2 < 0.75$

For the pecans irradiated in air-packages (AP), only the data for the control samples fitted well (R^2>0.075), and the PV formation for all the control samples, regardless of MAP condition, followed a first order reaction. From equation [5.3], for the first order reactions, the rate is directly proportional to the concentration of the PV ($r=k[A]$, r is rate, k is rate constant, [A] is concentration of PV), and for the second order reactions, the rate of reaction is directly proportional to the square of the concentration of the PV ($r=k[A]^2$). Therefore, the higher the reaction order is, the faster the reaction.

For the nitrogen-packaged (NP) samples, only the control, and the samples irradiated with 0.3 and 0.5 kGy intermediate dose levels followed the first order reaction rate, and the N_2-packed pecans (at 0.7 and 0.9 kGy) and the Air-packed (all doses) samples did not fit any equation because R^2 values were less than 0.75.When the irradiation dose increased, the PV reaction did not follow a linear trend in the NP-packed samples. One reason for this trend is that the AOX in pecans induce a lag phase and the lack of oxygen in the packages enhanced the lag phase formation.

Finally, for the O_2-packed (OP) samples, the order of the oxidation reaction increased up to the third order (Table 5.2) because the dose increased with the presence of high oxygen concentration in the package. In other words, both the presence of oxygen in the package and irradiation dose accelerate the reaction, but once the AOX's in pecan are oxidized, the PVs in the NP-packed samples will not increase rapidly; however, the oxidation reaction in the OP-packed samples increases at a faster rate than before because there is nothing left to hold them to produce the oxidation reaction.

In contrast, for the vacuum-packed (VP) samples, the rate of reaction remained constant as the reaction progressed, regardless of the irradiated dose. As expected, the lack of oxygen in the environment helps inhibit the PV formation as time passes and the increase in dose does not have a significant effect on PVs. One possible reason for that is that the electrons could not find any matter to interact with; therefore, the PVs may not be affected by the change in dose.

In summary, the kinetics of PV formation in irradiated pecans varies depending upon the MAP condition used for irradiation. Irradiation may create different radicals in AP, NP, and OP samples, where they cannot create anything in VP. Therefore, this may be the reason why VP samples reaction orders stay as n=0 even though the dose increases. This result confirms the benefit of applying irradiation under specific atmospheric conditions to delay the onset of rancidity in the nuts.

5.4.2.2 Modified Gompertz Model

As stated before, when the peroxide values (raw data) were plotted over time (Figure 5.5 to Figure 5.9), the values did not increase linearly with time. For example, in Figure 5.8 AP and NP (0.7 kGy) samples were dropped down on the second week and then raised up, or Figure 5.6 and Figure 5.9 all the packages were increased rapidly after second week. This means that there is a time delay until the PV values increase almost 2-fold very rapidly. This time delay is called a lag phase, which may occur due the presence of antioxidants in Kanza pecans. It has been reported by several authors (Ragnarsson and Labuza, 1977; Bondet et al., 1997; Buransompob et al., 2003;

Buranasompob et al., 2007; Laguerre et al., 2007)that the antioxidants (such as alpha-tocopherol) act as a free radical scavenger by donating hydrogen atoms to lipid radicals. In other words, ROO hold H atoms from antioxidants (Equation [5.35]), instead of breaking double bonds of fatty acids (Equation [5.9]). Thus, eliminating the ROO radicals to break double bonds results in the delay of oxidative rancidity, which may produce the lag phase in the reaction (Figure 5.6):

$$AH + ROO \rightarrow A + ROOH \quad \text{AH =antioxidant} \qquad [5.35]$$

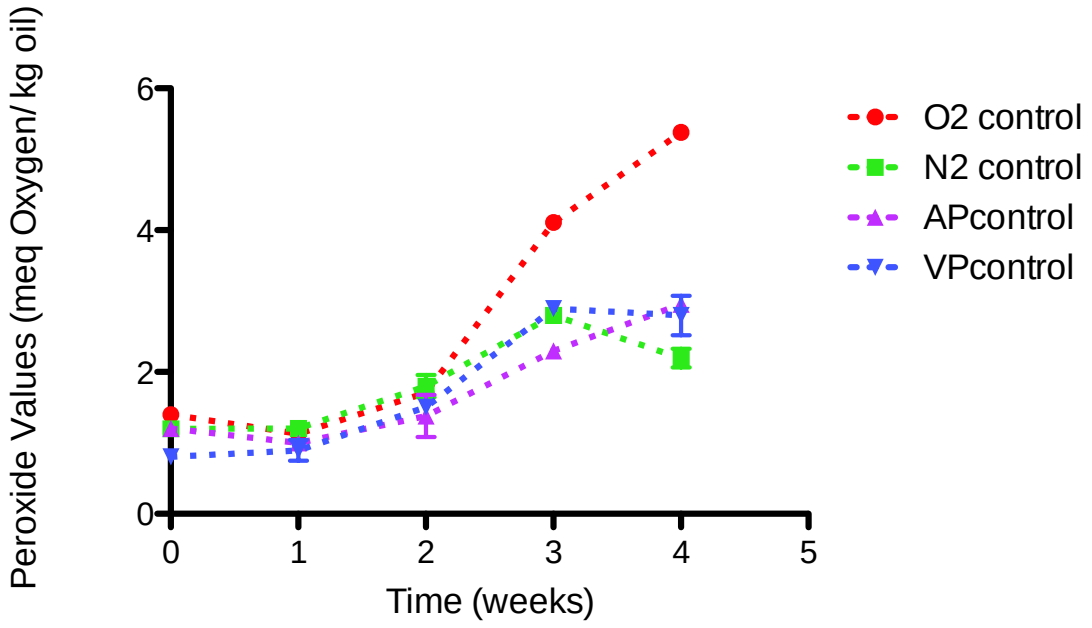

Figure 5.5 Peroxide value against time for non-irradiated Air Pack (AP), Vacuum Pack (VP), Nitrogen Pack (NP), and Oxygen Pack (OP) packages.

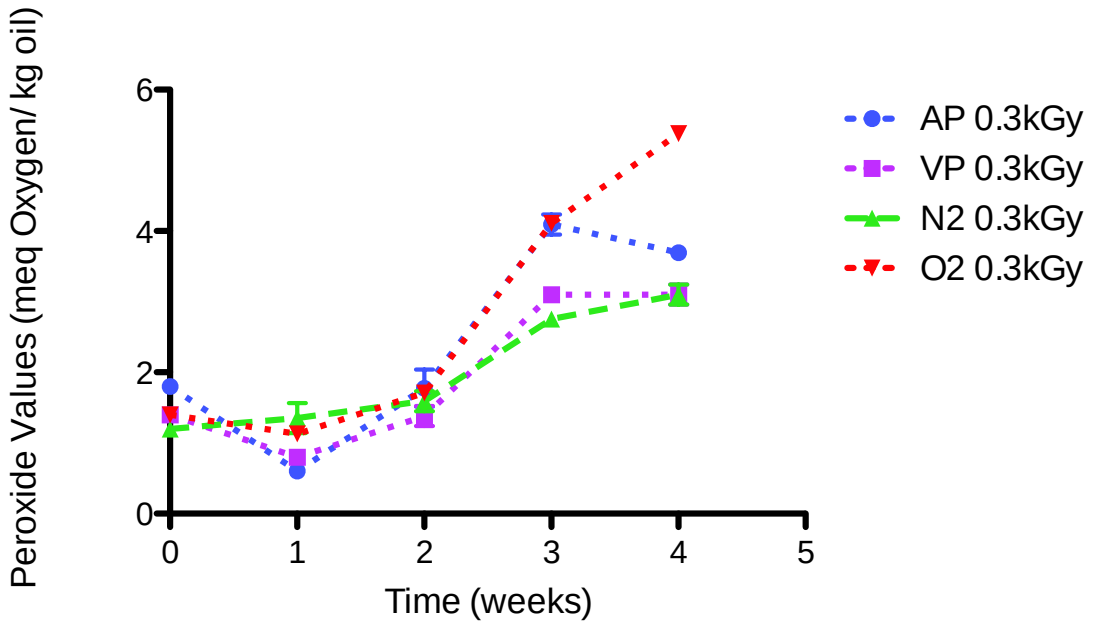

Figure 5.6 Peroxide value against time for Air Pack (AP), Vacuum Pack (VP), Nitrogen Pack (NP), and Oxygen Pack (OP) packages irradiated at 0.3-kGy using a 1.35 MeV Van de Graaff accelerator at room temperature.

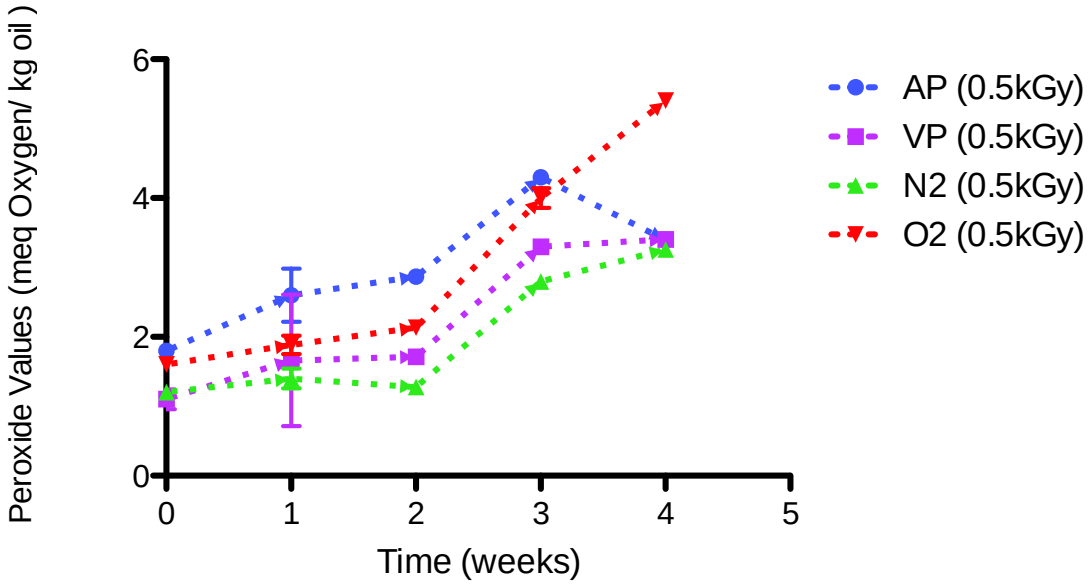

Figure 5.7 Peroxide value against time for Air Pack (AP), Vacuum Pack (VP), Nitrogen Pack (NP), and Oxygen Pack (OP) packages irradiated at 0.5-kGy using a 1.35 MeV Van de Graaff accelerator at room temperature.

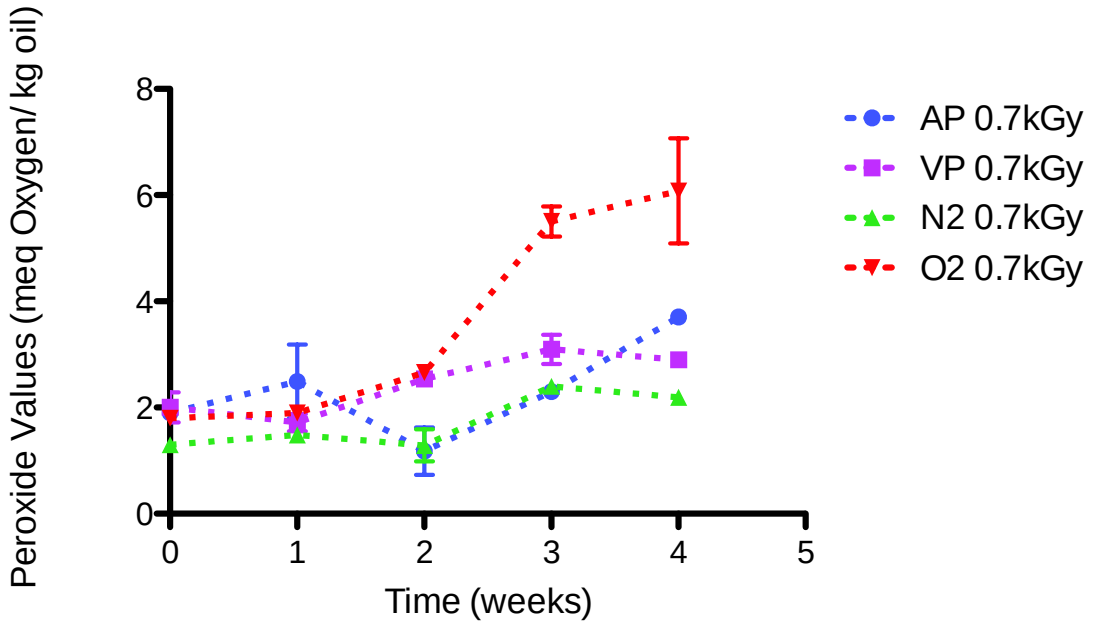

Figure 5.8 Peroxide value against time for Air Pack (AP), Vacuum Pack (VP), Nitrogen Pack (N_2), and Oxygen Pack (OP) packages irradiated at 0.7-kGy using a 1.35 MeV Van de Graaff accelerator at room temperature.

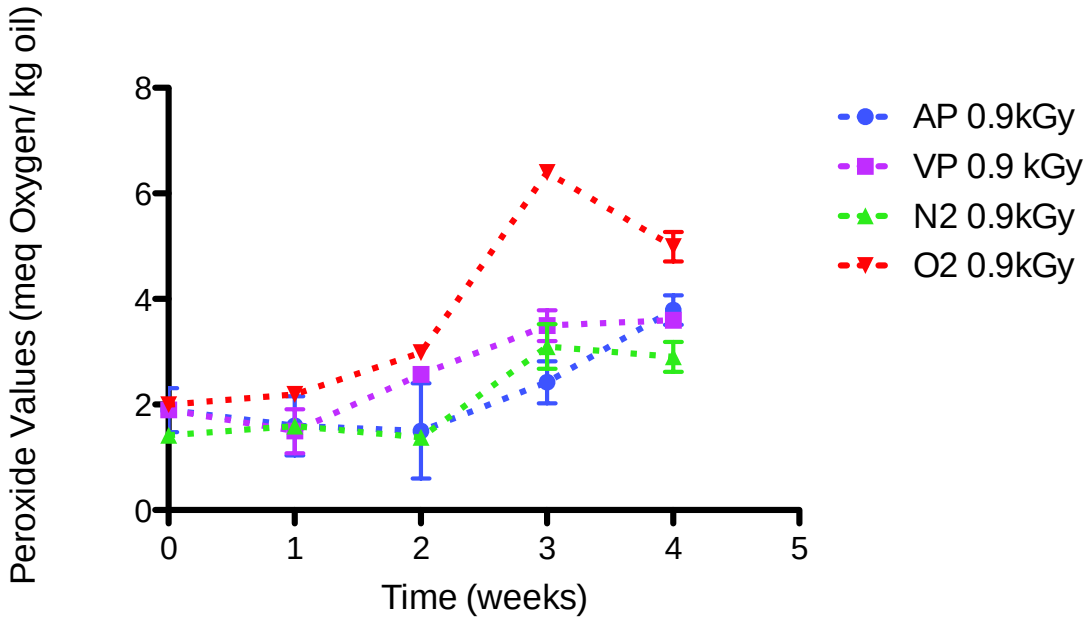

Figure 5.9 Peroxide value against time for Air Pack (AP), Vacuum Pack (VP), Nitrogen Pack (NP), and Oxygen Pack (OP) packages irradiated at 0.9-kGy using a 1.35 MeV Van de Graaff accelerator at room Temperature.

5.4.2.3. Lag Phase

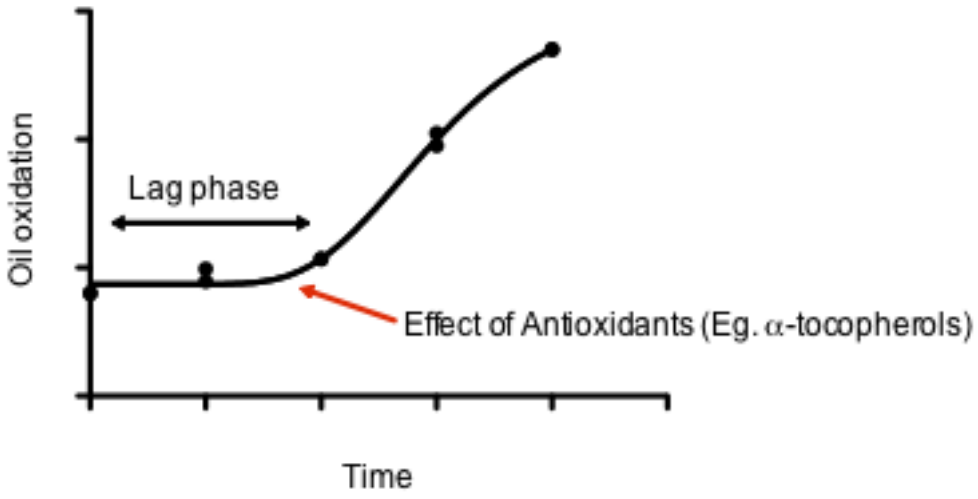

Figure 5.10 Lag phase in oil oxidation reaction (PV formation) due to the action of antioxidants present in pecans

In this study, the peroxide values generally (depending on MAP type and irradiation dose) dropped down in the first two weeks of storage at 49.8°C, and then rapidly started to increase (more than 2- fold) by the third (21st day) week. Based on the lag phase hypothesis (Figure 5.10), tocopherol content should start to drop down by week three. Villarreal-Lozoya et al. (2009) reported that the antioxidant (AOX) capacity of Kanza pecans (irradiated at 1.5 and 3.0 kGy, and non-irradiated control samples), which were obtained by the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay, increased surprisingly until 7 days of storage (28% and 20% for the samples irradiated at 1.5 kGy and 3.0, respectively) and the controls had no significant changes. The DPPH values then dropped to their lowest values on the 21th day and no significant changes

were found at the end of storage at 40°C and 55% RH). A possible reason for this result was provided by these authors as a higher rate of lipid oxidation within the outer cell layers of the kernel once the tocopherol has been depleted in that area.

The results obtained in this study support the idea that chain-breaking antioxidants (e.g., tocopherols) in pecans prevent the substrate from being substantially oxidized, resulting in a lag phase during the first or second week. The PV values significantly ($P < 0.05$) increased after the lag phase ended at $t > t_{lag}$. Laguerre et al. (2007) also verified that once the antioxidant disappears, the peroxidation rate rises sharply until it reaches the same rate as that experienced during uninhibited oxidation. Thus, the non-linear regression method was used to better explain the effect of dose on the rate of the oxidation reaction, and to determine the best MAP condition to slow down the reaction.

5.4.2.4. Modified Gompertz Model Results

The model's mathematical calculations were described in section 5.3.2.3. The results at each dose treatment for each MAP are shown in Figure 5.11to Figure 5.15. The maximum reaction rate (μ_{max}), the maximum (A) and minimum (y_{min}) PV values, and the lag time ($\lambda=t_{lag}$) of the reaction were all determined and are shown in Table 5.3.

Table 5.3 Parameters describing the kinetics of peroxide formation (PV) using the Modified Gompertz Model (Equation [5.34]).

R^2	Absolute Sum of Squares	Sy.x	Pack and Dose	y_{min}	A	μ_{max}	t_{lag}
0.9971	0.08	0.12	O2 control	1.27	5.77	2.68	1.90
0.8923	0.41	0.26	N2 control	1.20	2.50	6.57	1.91
0.9756	0.14	0.15	AP control	1.10	3.32	1.00	1.78
0.9859	0.12	0.14	VP control	0.85	2.85	9.48	1.93
0.9013	1.69	0.53	AP 0.3 kGy	1.20	3.89	26.13	1.98
0.956	0.41	0.26	VP 0.3 kGy	1.10	3.10	4.91	1.95
0.9813	0.11	0.14	N2 0.3 kGy	1.27	3.16	1.38	1.79
0.9971	0.08	0.12	O2 0.3-kGy	1.27	5.77	2.68	1.90
0.7166	2.68	0.67	AP 0.5 kGy	2.16	3.40	12.49	2.09
0.8726	1.23	0.45	VP 0.5 kGy	1.38	3.40	2.60	1.88
0.9956	0.06	0.10	N2 0.5 kGy	1.29	3.37	2.80	2.41
0.9945	0.12	0.14	O2 0.5 kGy	1.74	6.15	2.08	1.91
0.6754	2.45	0.64	AP 0.7 kGy	1.86	3.98	1.88	2.77
0.8902	0.32	0.23	VP 0.7 kGy	1.86	3.00	5.00	1.86
0.8977	0.24	0.20	N2 0.7 kGy	1.37	2.23	11.17	2.08
0.968	1.11	0.43	O2 0.7 kGy	1.85	6.15	3.68	1.79
0.8004	1.71	0.53	AP 0.9 kGy	1.66	4.00	2.14	2.65
0.9393	0.44	0.27	VP 0.9 kGy	1.70	3.60	1.83	1.52
0.9349	0.39	0.26	N2 0.9 kGy	1.46	3.00	30.41	2.17
0.928	2.11	0.59	O2 0.9 kGy	2.09	5.69	20.63	1.96

y_{min}=time (weeks), A= maximum meq O_2/kg oil, $\boldsymbol{\mu_{max}}$ = meq O_2/oil) weeks^{-1}

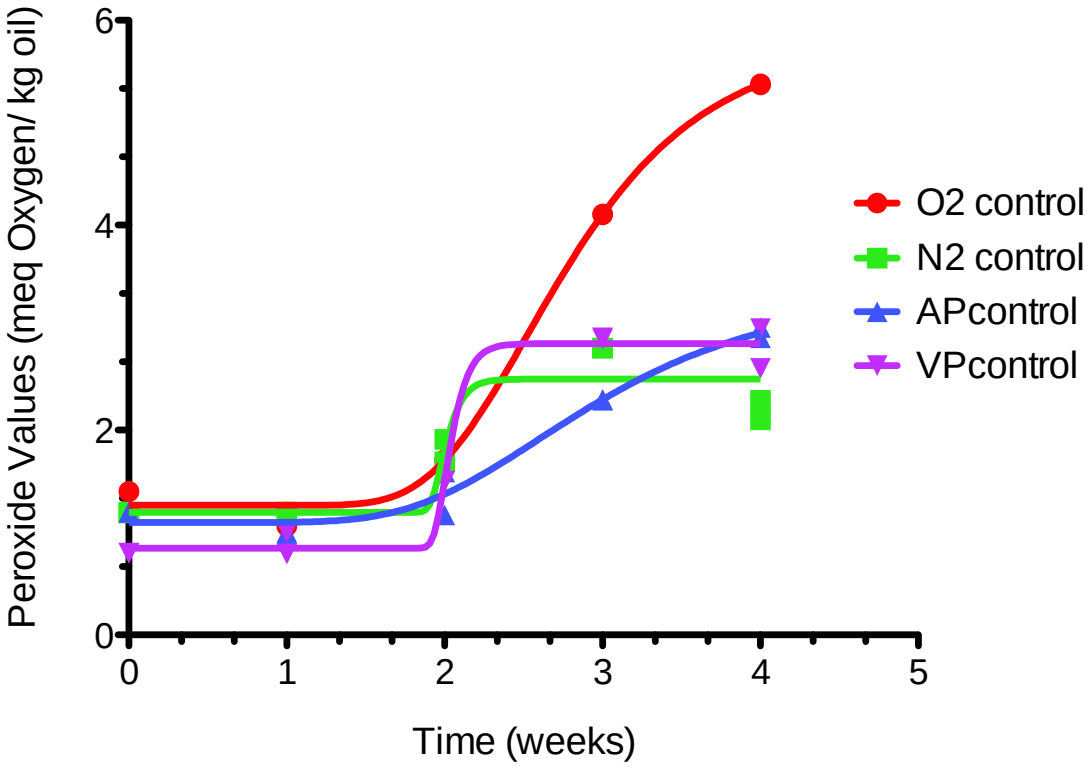

Figure 5.11 Modified Gompertz Model ([5.34]) of peroxide value formation in non-irradiated pecans under air (AP, control), vacuum (VP), O_2 (OP) and N_2 (NP) stored at 48.9°C and 13% RH for 4 weeks

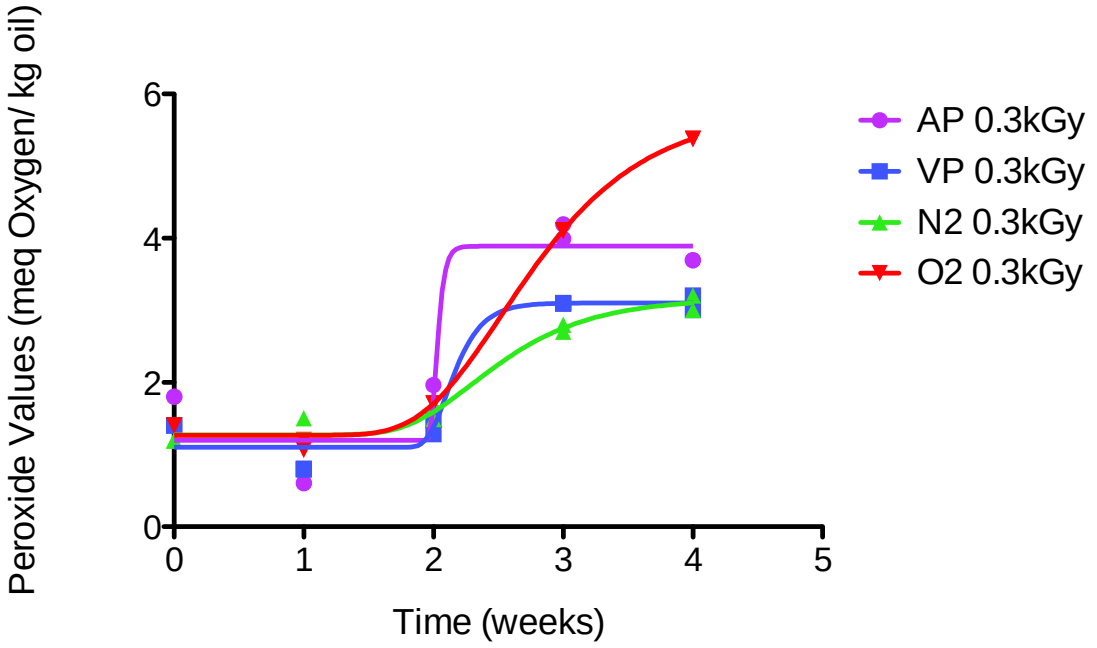

Figure 5.12 Modified Gompertz Model ([5.34]) of peroxide value formation in pecans irradiated with a dose of 0.3 kGy under air (AP, control), vacuum (VP), O_2 (OP) and N_2 (NP) stored at 48.9 °C and 13% RH for 4 weeks.

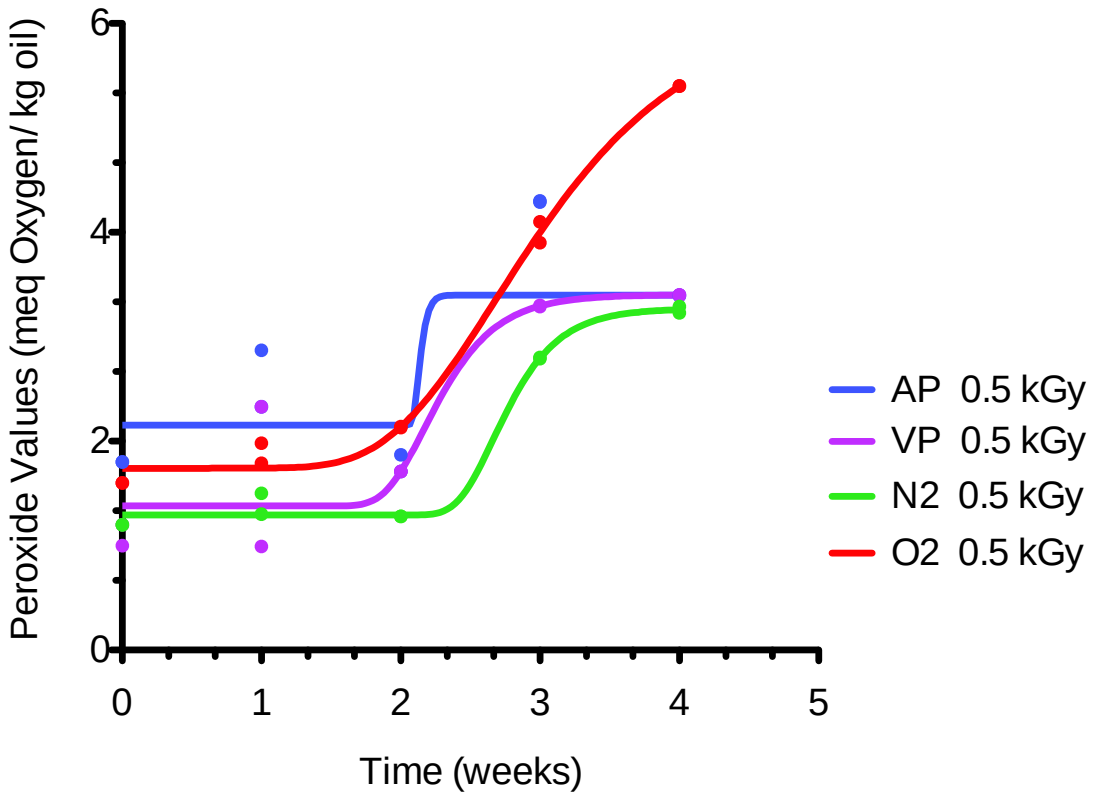

Figure 5.13 Modified Gompertz Model ([5.34]) of peroxide value formation in pecans irradiated with a dose of 0.5 kGy under air (AP, control), vacuum (VP), O_2 (OP) and N_2 (NP) stored at 48.9 °C and 13% RH for 4 weeks.

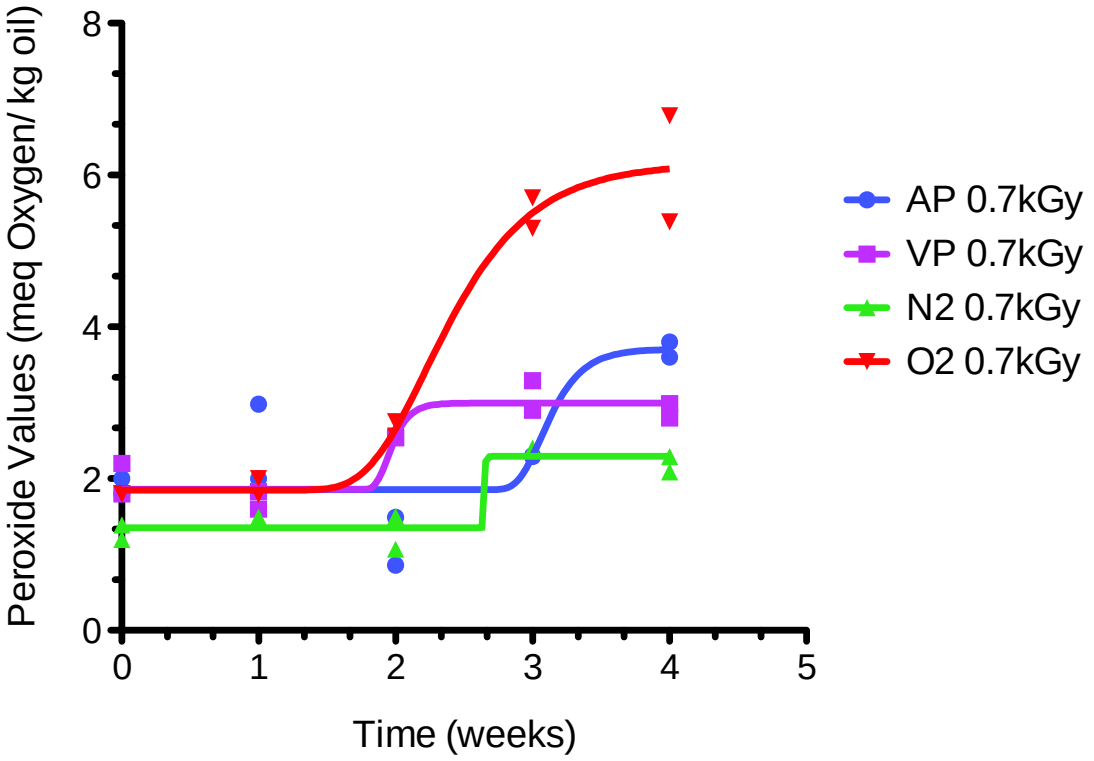

Figure 5.14 Modified Gompertz Model ([5.34]) of peroxide value formation in pecans irradiated with a dose of 0.7 kGy under air (AP, control), vacuum (VP), O_2 (OP) and N_2 (NP) stored at 48.9 ℃ and 13% RH for 4 weeks.

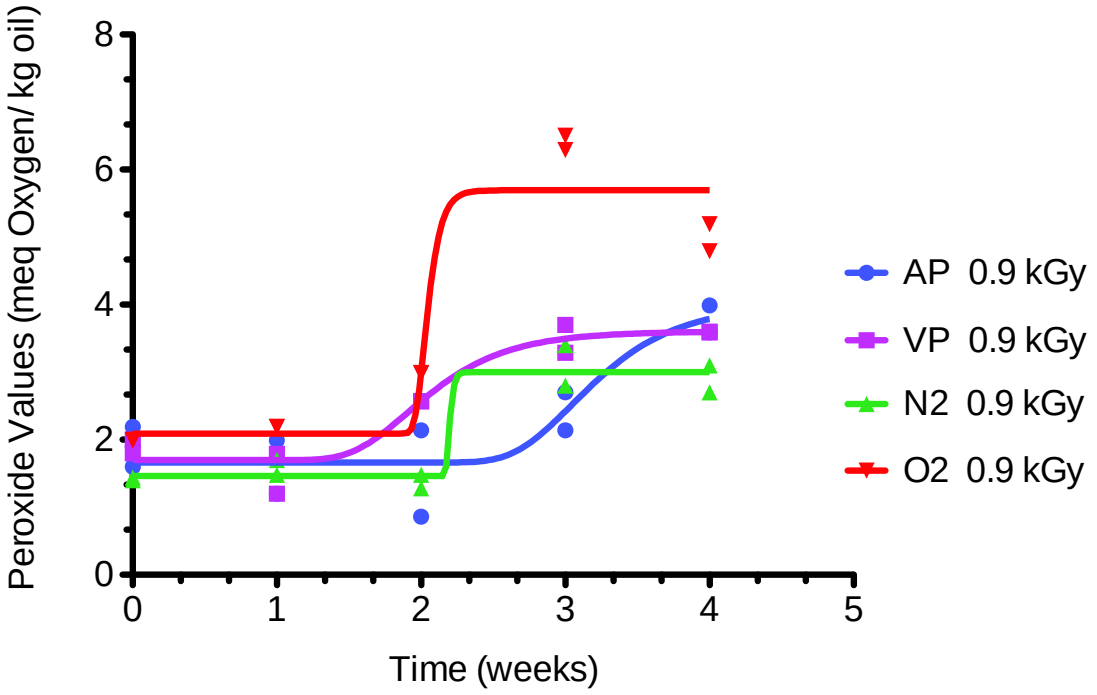

Figure 5.15 Modified Gompertz Model ([5.34]) of peroxide value formation in pecans irradiated with a dose of 0.9 kGy under air (AP, control), vacuum (VP), O_2 (OP) and N_2 (NP) stored at 48.9 °C and 13% RH for 4 weeks.

The Modified Gompertz Model (equation [5.34] plays an important role in explaining how and why the lag time occurs in irradiated pecan rancidity (PV) curves. The order of the reaction will help to determine the reaction rate the reaction rate constant, *k* (1/slope*)*. Similarly, the μ_{max} calculated by the tangent line passing through the inflection point (b/c, [5.26]) of the curve also gives the maximum reaction rate [5.27] of the reaction (PV formation). However, the maximum reaction rate only shows the maximum value at which the reaction was reached.

For example, the μ_{max} value of the control N_2-packed (NP) sample was higher (1.5 to 10 times) than in the other MAP conditions for the control group (Table 5.3). The N_2-packed (NP) control sample had the highest t_{lag} time than the other MAP conditions in the control group. Thus, the N_2-packed pecans were significantly ($P<0.05$) less rancid than the other pecans in the control group until $t=t_{lag}$. In other words, the oxidation reaction might have been delayed (perhaps due to the AOX) until $t>t_{lag}$. Then, after all the AOXs were used in the reaction, the reaction might have jumped to higher values (as compared to the previous value of the PV in the lag phase) within a short period of time.

Therefore, the maximum reaction rate of this particular N_2 -packed control samples might have higher values (μ_{max}) than the others. However, simply comparing the t_{lag} values of the curve is not enough to make a decision with regards to determining the best MAP condition to use for that particular dose, based on a Modified Gompertz Curve. Therefore, three other parameters were checked: *A*, μ_{max}, and the time when the reaction reaches its *A* value.

- If the quality parameter versus the time curve is linear, the reaction rate (the slope=μ_{max}) is the same at every point on the curve and one can determine the best MAP condition by looking at the reaction rate (or the maximum reaction rate if they are the same). The bigger the slope, the higher the reaction rate; the higher the reaction rate, the faster the product will become rancid. Therefore, the MAP condition that has the lowest reaction rate is the best one.
- However, if there is a lag phase, neither the maximum reaction rate (μ_{max}) nor the lag time of the curve should be used as the sole parameter when determining the best MAP condition.
- Therefore, if the oxidation reaction has a lag phase due to the oxidation inhibitors (in this study the inhibitors are assumed to be the AOXs because of the Kanza pecans' high tocopherol content) which prevent the substrate from being substantially oxidized until the AOXs are used, then the reaction rate alone is not a sufficient criterium for describing the mechanisms of the oxidation reaction. Hence, besides the maximum reaction rate of the curve, the lag phase (t_{lag}=λ), the Asymptotic value (A value=y_{max}), and the time when the curve reached its A value should also be taken into consideration. New two cases illustrated in here to show this idea:

Case 1:

Figure 5.16 shows the case of a high reaction rate in the curve with a longer lag phase. Data regarding the N_2 -packed pecans irradiated at 0.7 kGy shows that a longer lag phase results in a lower *A* value at the end of four weeks of storage at 48.9°C. In this case, the maximum reaction rate (μ_{max}) only indicates that the rancidity reaction (PV formation) of N2 -packed pecans (irradiated at 0.7 kGy) accelerated 2.5 times more than that of the O_2 -packed pecans (also irradiated at 0.7 kGy); however, the maximum PV values were significantly different, with PV (N_2) =2.23 and PV(O_2) = 6.15 meq O_2/kg oil. At the end of the 4 weeks of storage, N_2 -packed pecans had the lowest PVs ($A_{nitrogen}<A_{oxygen}$). Therefore, it can be concluded that the longer lag phase time may help inhibit the onset of rancidity.

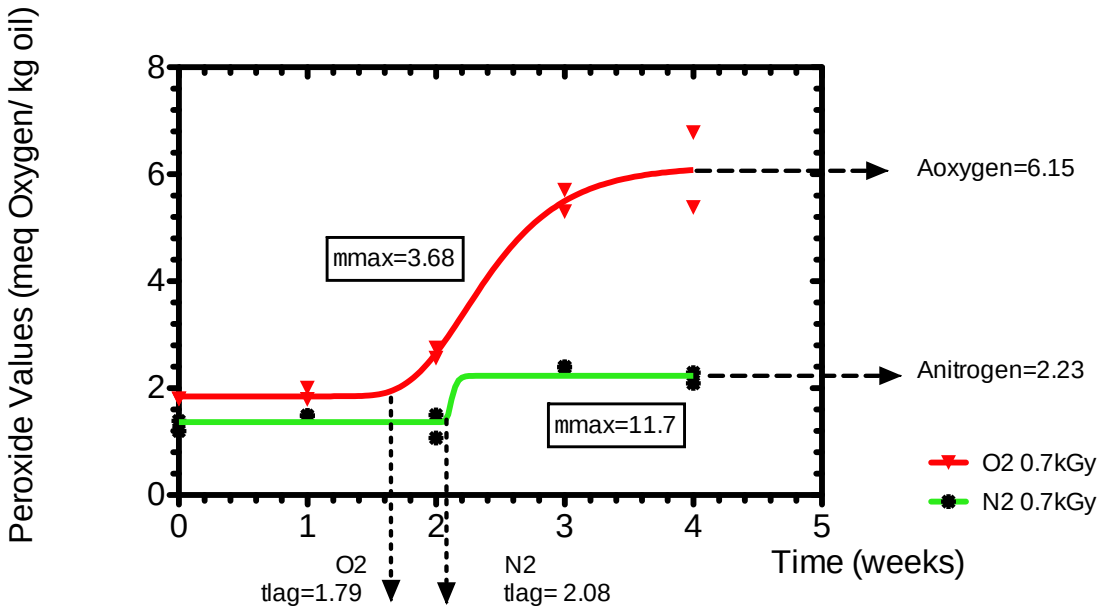

Figure 5.16 Comparison of μ_{max} and t_{lag} for Case 1: High reaction rate, longer lag phase, and low *A* value. Values obtained using the Modified Gompertz model (Equation [5.34]).

Case 2:

The AP -packaged pecans (irradiated at 0.9 kGy) had a longer lag time (t_{lag}= 2.65 weeks) and higher μ_{max} (2.14 rate change in PV in time) than the VP-packed pecans (also irradiated at 0.9 kGy), as can be seen in Figure 5.17 . However, the *A* values of both AP and VP-packed pecans (irradiated at 0.9 kGy) reached at the end of 4 weeks of storage were slightly similar (A_{VP}=3.4, A_{AP}=4 weeks). Therefore, Case 2 shows that a longer lag time may not always result in low *A* values. In other words, in order to determine the best MAP condition to irradiate to irradiate pecans, one should check lag time, asymptotic value (*A*), and maximum reaction rate (μ_{max}). One possible reason why the *A* value was high even though the lag time was longer, is that by the end of the lag period, the AP-packed sample did have more oxygen gas to help continue to induce preliminary oxidation (Hydroxyl peroxide formation, tested by PV test) in the product.

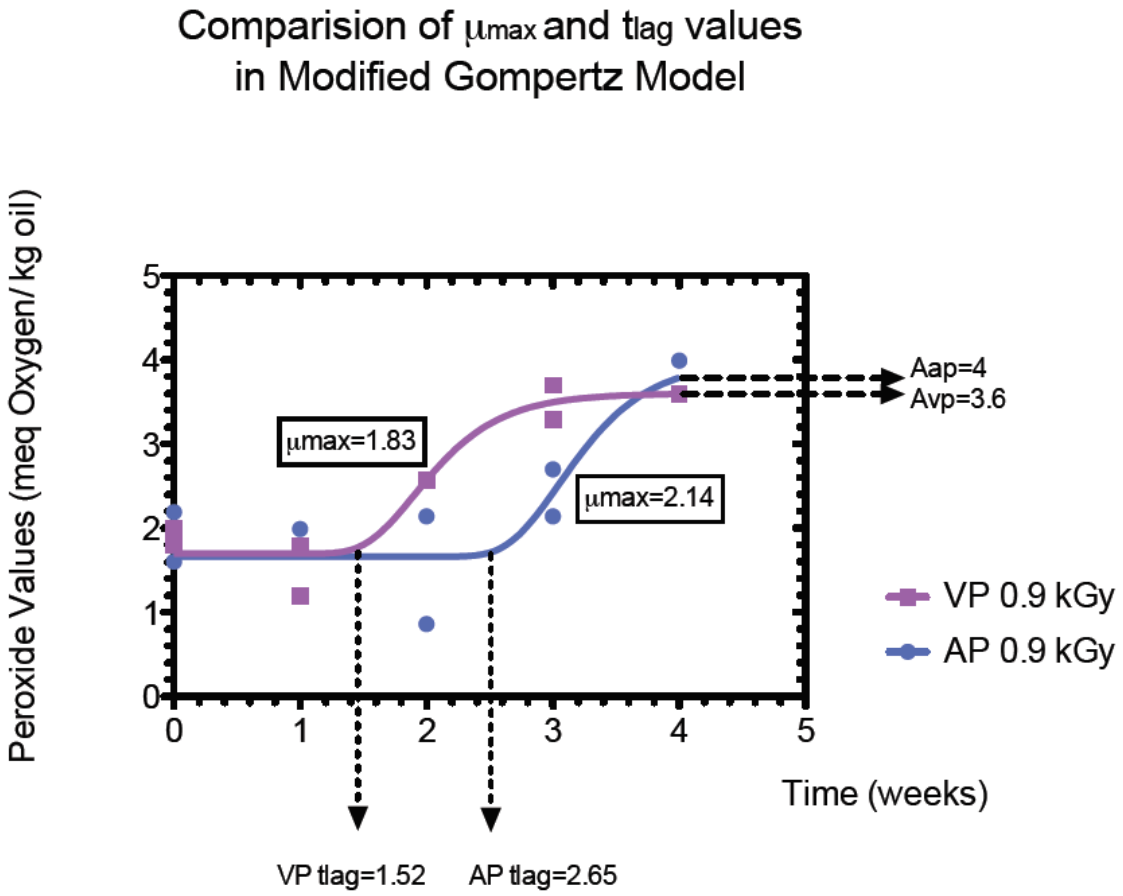

Figure 5.17 Comparison of μ_{max} and t_{lag} for Case 2: High reaction rate, longer lag phase, and high *A* value. Values obtained using the Modified Gompertz model (Equation [5.34])

In summary, the best MAP condition is N_2, then, VP, AP and finally, O_2 . This order does not change with an increase in applied dose. However, for the control sample, the N_2 and VP-samples at low doses give similar results, but as the dose increases, the N_2 -packed samples were better in quality than the VP-samples.

- Control Best -N_2<VP<AP<O_2-Bad
- 0.3 kGy Best-N_2=VP<AP<O_2-Bad
- 0.5 kGy Best -N_2<VP=AP<O_2-Bad
- 0.7 kGy Best -N_2<VP<AP<O_2-Bad
- 0.9 kGy Best -N_2<VP<AP<O_2-Bad

5.4.2.5. Other Models: Linear, Logistic, and Multi Response Models

In addition to the linear models, the logistic models described by (Özilgen and Özilgen, 1990) were also fitted into this equation. However, this model did not fit well the experimental data collected in this study. When the lag phase increased, the applicability of the logistic model deviated because of the time delay in the reaction (Ozilgen, 2012). Moreover, multi-response modeling was considered to explain the oxidation reaction, which required at least two different experiments strongly related to one another (Boekel, 2012); for instance, the PV (primary oxidation products) and TBARS (secondary oxidation products) tests.

5.4.2.6 Overall Interaction (ANOVA)

ANOVA tests were first performed with all the experimental data in order to see if there is a three-way interaction (dose x week x time interaction). For $P<0.05$, there was no three-way interaction. However, there were two-way interactions between the pack × dose and the pack × week, but there was not a week x dose interaction. This means:

1. The effect of the MAP condition should be evaluated after setting the dose. For example, when the dose =0.5 kGy, the best MAP condition is N_2 after four weeks of accelerated storage at 48.9° C
2. The PV data follows a similar pathway over time, regardless of the dose. This conclusion also explains how the Modified Gompertz model fits for most of the experimental data.

Then, the ANOVA test was performed again, individually, by plotting the PV changes over time for the four MAP condition at each dose level.

The results showed that the PV values of pecans irradiated in oxygen packages were significantly ($P<0.05$) different from the pecans irradiated in the other packages, regardless of the dose. The PV values of pecans in N_2-packages were slightly different from the other packages at lower doses and significantly ($P<0.05$) different from the other packages when the dose was equal to or more than 0.5 kGy. Therefore, the use of

Nitrogen packaging is a promising MAP approach when the pecans are irradiated at doses > 1.0 kGy.

5.5 Conclusions

Predicting changes in the quality of a particular food as a function of time depends upon processing type (irradiation doses), environmental conditions (package type and storage temperature), and time. For this reason, mathematical models were used in an effort to understand the kinetics of the oxidation reaction in pecan nuts packed in different atmospheric conditions and irradiated at different doses.

According to preliminary studies, initial PV values of VP-frozen pecan samples were 5 times higher than in AP-frozen pecans. After the irradiation treatment, AP-frozen pecan PV values increased rapidly with an increase in dose and both AP- and VP-frozen pecans PVs reached very high values (~3-3.5 meq O_2/kg oil) right after irradiation at 0.8 kGy. On the other hand, PV values of VP-coated samples also increased when the irradiation dose increased, but the PV values of VP-coated pecans were 1/3 lower than the PV values of AP and VP-frozen pecans when irradiated at 0.8 kGy. Therefore, AP- and VP-frozen pecan samples were not suitable for shelf life studies as well as kinetic calculations because of the high PV levels reached after irradiation at 0.8 kGy. Although the coating technique (layer-by-layer) and the coating material can be an alternative for coating studies for pecans, because of the cinnamon taste and white residuals, VP-coated pecans were not further evaluated in this study.

The reaction order calculations showed that peroxide formation in Vacuum-packed (VP) samples (at all dose levels) is a zero order reaction, which means that the rate of the reaction is constant regardless of dose. This supports the application of irradiation of pecans and other nuts under vacuum packaging when high doses are required for safety purposes (pathogen decontamination).The Modified Gompertz model was used to explain the oxidation mechanism (PV formation) in irradiated pecans under MAP. In this study, the presence of the lag phase was confirmed. Oxidation inhibitors such as AOXs may help to create a lag phase, which, in turn, slows down the reaction. However, packaging atmospheres (such as the presence or absence of oxygen) play an important role in the rate of the lipid oxidation reaction. The PVs of pecans irradiated in O_2- packages were significantly ($P<0.05$) different regardless of the level of irradiation (dose). Moreover, the Nitrogen-packages helped to slightly inhibit the rancidity in pecans at low doses (0.3 and 0.5 kGy) and in the non-irradiated controls. Therefore, both Nitrogen and Vacuum Packaging are feasible MAP conditions to significantly ($P<0.05$) inhibit the rancidity of high oil content products irradiated up to 0.9 kGy using electron beams.

CHAPTER VI

ACCELERATED SHELF LIFE TIME (ASLT) STUDIES IN PECAN NUTS (KANZA VARIETY)

6.1 Summary

Many chemical changes occur both before and after the harvesting process. Deteriorative chemical changes are a key factor in defining the shelf life of the product. The end of a food product's shelf life is determined by when it loses its desired quality. Therefore, the speed of these chemical reactions, called the reaction rate, delimits the shelf life of the product. The faster the reaction rate, the shorter the shelf life.

In Chapter IV, the highest D_{10} values were obtained when irradiating pecans under vacuum (VP). Hence, the VP pecans were selected for the shelf life experiments presented in this Chapter. In Chapter V, it was shown that dose has a more significant effect on PV formation than the packaging type (MAP condition), and both VP and N_2-packed pecans had the lowest lipid oxidation levels. Since, the VP packages can be comparable with N_2 packages (NP), the VP pecans were irradiated at dose of 3.0 kGy to determine their shelf life under accelerated conditions of 37.8°C and 48.9°C at13% RH for four weeks.

The samples were taken out each week and the PV values, which determine the primary oxidation of the product, measured. After the PV values were obtained, the reaction orders were calculated by the method described in Chapter V; then, the parameters of the quality changes over time for both temperatures were computed using

the Arrhenius equation. The temperature quotients (Q_{10}) and the activation energy (Ea) for the peroxidate formation reaction in irradiated pecans were 1.50 and 28.53 kJ/mole.K for the non-irradiated VP samples, and 1.4 and 25.24 kJ/mole. K for the 3.0- kGy irradiated samples.

6.2 Introduction

The food products that we consume, whether processed or unprocessed, continue to experience certain chemical reactions. Depending upon the results of these reactions, the food quality may change over time. When the quality of the food is not deemed to be acceptable by consumers, or the food product is not microbiologically safe enough to consume, the product has reached the end of its shelf life. Thus, determining the shelf life is important to minimizing any loss of the product, as well as for eliminating the possibility of any illnesses that might be caused by a microbiologically unsafe product. A determination of the product's shelf life can be accomplished by monitoring the physical and microbiological quality of the food product, both of which are affected by environmental factors. Therefore, a food product's shelf life can be defined as a change in the food product's physical and microbial uality as affected by environmental factors, and as a function of time (Fu and Labuza, 1993).

To determine the shelf life of a food product, a quality parameter must be selected, which can be physical or microbial quality parameter. This parameter varies from one food to another. To determine the shelf life of the pecan nut kernels, both microbial and physical quality parameters were used. Lipid oxidation (rancidity) was

used as the physical quality parameter, where the amount of dose necessary to achieve a 90% reduction in the microorganisms, the D_{10} value (calculated in Chapter IV), was used as the microbial quality parameter. These parameters were chosen because lipid oxidation is one of the most common food deterioration reactions in high lipid-content foods, and microbial quality is important for food safety. Both reduce the shelf life of the product. Therefore, the pecan samples were tested in terms of dose (the required dose from microbial quality parameter, e.g., 3.0 kGy) and MAP condition (best condition from physical quality parameter, rancidity, e.g., vacuum-packing) to perform the shelf life study by using the Accelerated Shelf Life Test (ASLT) technique.

6.3 Materials and Methods

Cultivar Kanza [*Carya illinoinensis* (Wangenh.) K. Koch] is one of the newest pecan cultivars, released in May of 1996 by the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) (Thompson et al., 1997). The Kanza tree has an extremely high level of productivity, nut quality (also, a very satisfying taste), cold tolerance, disease resistance, and scab resistance, as well as a moderate resistance to fungal leaf scorch, leaf phylloxera (Phylloxera notabalis Pergande), stem phylloxera (P. devastatriz Pergande), and other pecan insects (Thompson et al., 1997), when compared to other cultivars such as Osage, Caddo Mohawk, Pawnee, and Creek. The Kanza nut kernels were selected as a representative of good pecan kernel quality. Therefore, the effect of low quality kernels was minimized for the tests designed to predict shelf life. In

the shelf life tests, as with the other experiments, only unbroken, whole pecan halves were used.

This study was conducted using uncoated, unfrozen, raw pecan halves that were packed under vacuum (VP). The reason for this was that in the previous study (Chapter V), the highest D_{10} values were obtained when pecans were irradiated under vacuum (VP) conditions (0.48 kGy for *E.* coli*)*, and the required dose for a 5-log reduction would be $5D_{10}$ (~ 2.48 for *E.* coli). However, that study showed that the pecan dorsal grooves absorbed 40% more of the dose than the entrance level dose. As a result, an irradiation dose of 3.0 kGy instead of 2.48 kGy was selected for the shelf life study.

Four pecan halves were placed into a polyethylene film bag (8x5 cm Foodsaver® sealer bags were used); the packages were then vacuum packed (VP) with the Industrial Vacuum Sealer. Eighty packages were made to test each dose and temperature condition.

6.3.1 Irradiation of Vacuum Packed Pecans and Accelerated Shelf Life Test (ASLT)

The eighty VP pecan packages were divided into four groups of twenty. The first two groups of twenty were irradiated at 3.0 kGy, and the second two groups of twenty served as the control (0.0 kGy). Then a twenty-package group from each dose (3.0 kGy and 0 kGy) (for a total of forty packages) was kept at an accelerated temperature of 37.8° C and 13% relative humidity (RH) for four weeks. The other twenty packages from each dose (3 kGy and 0 kGy) (for a total of sixty packages) were kept at an accelerated temperature at 48.9°C and 13% RH for four weeks. Each week, four pecan packages from the twenty packages allocated to each dose level were tested for PV to determine

their rancidity. Testing began at week 0 and continued up through the fourth week. In addition, the pecans were kept in the freezer (-25°C) and tested every 6 months for PV test results were used to determine the kinetic equations, reaction order, and reaction rate, as calculated in Chapter V. These values were used in an Arrhenius equation (Equation [6.1]) in order to calculate the Temperature Quotient (Q_{10}) value.6.3.2 Arrhenius Equation and Q_{10} value

The Arrhenius equation was used to monitor the effect of temperature on the rate of deterioration (Robertson, 2005). The integrated form of the Arrhenius equation is shown in Equation [6.1]:

$$k = Aexp\left(\frac{E_a}{Rt_a}\right) \qquad [6.1]$$

where, *A* is the frequency factor (pre-exponential factor), *k* is the reaction rate constant for the deteriorative reaction (lipid oxidation), *Ea* is the activation energy (kJ/mole), *R* is the universal gas constant (8.314 J/mole K), and *ta* is the absolute temperature (K). In order to determine the rate constant (*k*), the reaction order (*n*) must first be found. The reaction rate constant is determined from the slope of the particular reaction in which the reaction order was determined. The calculations of the *n* and *k* values are explained in detail in Chapter V, in Section 5.3.2.3.

Two inherent assumptions were made for the Arrhenius equation, which are that Ea and A will not change with the temperature. However, Robertson (2005) indicated that when the temperature span exceeds 40°C, the *Ea* may vary. Therefore, the Ea for

both 37.8℃ and 48.9℃ for each dose were also calculated. The Temperature Quotient, or Q_{10} value, is the value which describes the ratio of the reaction rate constants when the temperature differs by 10℃:

$$Q_{10} = \left(\frac{k_{T+10}}{k_T}\right) = \frac{Qs_{T+10}}{Qs_T} \quad [6.2]$$

where, k_T is the reaction rate at temperature T, and k_{T+10} is the reaction rate at a temperature increased by 10° C, Qs_T shelf life at temperature T(°C) and Qs_{t+10} is the shelf life at temperature $T+10$ (℃). This ratio is also equivalent to a shelf life time at the temperature T over a shelf life time at a temperature 10° C higher,

$$Q_{10}^{\Delta/10} = \frac{Qs_{T1}}{Qs_{T2}} \quad [6.3]$$

where, Qs_{T1} shelf life at temperature T_1 (℃) and Qs_{T2} shelf life at temperature T_2 (°C),and Δ e uals to T_2-T_1.

If the Q_{10} value is represented by the Arrhenius Equation, the following equation is:

$$lnQ_{10} = \frac{10Ea}{RT^2} \quad [6.4]$$

The temperature effect on the quality parameter PV was evaluated by analyzing the control samples that were used in the ASLT; the irradiation effects on the quality parameter was quantified by analyzing the 3,0-kGy irradiated pecan samples that were used in the ASLT. Both the irradiated and non-irradiated pecans were vacuum packed and raw uncoated, unfrozen pecan kernels were used. Therefore, the shelf life was determined by using the Q_{10} value.

6.3.2 Color

A Labscan XE (16437) colorimeter (HunterLab Inc., Reston, Va., U.S.A.) with the CIELAB system with measuring aperture diameter of 36 mm and illuminant/viewing geometry of D65/10o were used. The colorimeter was calibrated using the standard white and black plates. Ten pecan kernels were used for each treatment (dose). Three readings were made on each pecan half.. The mean values were used to determine the color coordinates L* (light- ness – darkness), a* (redness – greenness), and b* (yellowness – blueness).

6.3.3 Texture

Texture Profile Analysis hardness and fracturability of pecans at room temperature (21°C) were measured using a Texture Analyzer (TA.XT2i, Texture Technologies Corp., Scardale, N.Y., U.S.A.) equipped with Rounded end Probes (TA-18: 1 2" dia ball)(Surjadinata et al., 2001). 16 measurements will be performed for each treatment (irradiated and control).

6.3.4 Sensory Test

Thirty students, staff, and faculty at Texas A&M University were evaluated samples by visual inspection of color, odor, texture, flavor and overall quality for 2 and 4 weeks of storage (49°C temperature and 13% RH) of control and 1 kGy irradiated VP pecans. The panelists were scored the samples using a 9-hedonic scale (Carr et al., 1999), where a score of 1 represents attributes most disliked and a score of 9 represented attributes most liked. Scores higher or equal to 5 were considered acceptable. Each treatment was labeled with a random 3-digit number (Gomes et al., 2008). One pecan kernel was used for each treatment and panelists were rinse their mouth with water and eat a cracker (or a unsalted toasted bread) with ricotta cheese in between samples (Gou et al., 2000).

6.4 Results and Discussion

The pecan industry standard is limited at < 5 meq/kg by the Queenswood Company (Queenswood, 2006). This limit number is also same as the PV limit number for almonds that were specified by almond board of California (ABC, 2010). The shelf life of irradiated and non-irradiated vacuum-packed (VP) pecans was first determined with an ASLT at 37.8°C. At the end of the four weeks, the PV levels increased, up to 3.72 meq O_2/kg sample for the non-irradiated controls while the irradiated VP samples PV values' were reached to 4.72 meq O_2/kg oil sample (Table 6.1), which is very close to the limit value of 5 meq O_2/kg oil sample. The ASLT at 48.9° C for four weeks,

yielded samples with a maximum PV of 4.48 meq O_2/kg sample for the nonirradiated controls while the VP samples reached values 5.64> 5 after four weeks (Table 6.1).

Therefore, the VP pecan samples that were irradiated at 3.0 kGy were exceeded the industry PV limits on the third week of the ASLT where the control sample did not reach to the limit value (5 meq O_2/kg oil sample) at the end of ASLT.

6.4.1. Reaction Order

The reaction orders for the PV formation in non-irradiated and irradiated VP pecans were calculated by integrating the rate law equation [5.3] with respect to time and then plotted into each equation, as described in Section 5.4.1. Then the best-fitted straight line among the plots was accepted as the reaction order of the equation, and the slope of that plot was accepted as rate constant k (units vary depending on reaction order, n). The reaction orders, rate constants, and R^2 values are shown in Table 5.3.

The PV formation in non-irradiated vacuum-packed pecans stored at 37.8° C and 48.9° C was best described as a zero order reaction while the reaction followed a second order for the irradiated samples. These findings are compatible with the ones described in Chapter V for vacuum-packed pecans used as a control (0 or 1st order). Therefore, the reaction order did not change with an 11° C increase in temperature; however, the reaction order increased with irradiation dose where reaction order was 1 for VP pecans irradiated with 0.9 kGy (n=1), and n = 2 for those irradiated with 3.0 kGy.

Table 6.1 Peroxide values for irradiated (3.0 kGy) and non-irradiated (0.0 kGy) vacuum-packed (VP) pecans stored at ASLT temperatures of 37.8° C and 48.9° C for four weeks. Values are means of two replications

	PV (meq O_2/kg oil) at 37.8° C			PV (meq O_2/kg oil) at 48.9° C	
Time	*0 kGy	3 kGy	Time	*0 kGy	3 kGy
0 week	$_{w}$ 1.64 [a]	$_{x}$ 2.18 [a]	0 week	$_{w}$ 1.64 [d]	$_{x}$ 2.18 [a]
	[1] (0.08)	0.1		[1] (0.08)	0.1
1st week	$_{w}$2.77 [b]	$_{x}$ 2.49 [ab]	1st week	$_{wx}$ 2.5 [c]	$_{x}$ 2.5 [a]
	0.86	0.9		0.87	0.57
2nd week	$_{w}$ 2.86 [b]	$_{wx}$ 2.94 [ab]	2nd week	$_{x}$ 2.48[c]	$_{y}$ 3.11 [b]
	0.47	0.82		0.49	0.33
3rd week	$_{w}$ 2.8 [bc]	$_{w}$ 2.85[bc]	3rd week	$_{x}$ 3.25 [b]	$_{y}$ 5.71[c]
	0.77	0.5		0.04	0.5
4th week	$_{w}$ 3.72 [c]	$_{wy}$ 4.72 [c]	4th week	$_{y}$ 4.48 [a]	$_{x}$ 5.64 [c]
	0.91	0.6		0.64	0.69

[1] Standard Deviation

[a,c]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).

[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

*non-irradiated controls

The increase in reaction order is an indicator of how fast the reaction will be. Van Boekel (2009b) indicates that an increase in temperature will increase the reaction rate up to a maximum point, and the reaction rate may not increase with an increase in temperature if the reaction has already reached that high of a temperature point.

Therefore, this may be the reason why the reaction order did not change when the temperature increased. On the other hand, it has been shown that the order of the reaction increases with the irradiation dose. This result demonstrates the sensitivity of lipids to being oxidized by ionizing radiation at the levels used in this study.

6.4.2. Activation Energy Determination

The activation energy defines the minimum required energy to activate the reaction, i.e. PV formation. In irradiated samples, *Ea* (25.24 kJ/mole K) was lower compared to that for non-irradiated samples (37.9 kJ/mole K), which means the reaction happens faster (Table 6.2). Thus, the energy required to initiate the reaction (PV

Table 6.2 Kinetic parameters for PV formation in vacuum-packed irradiated (3 kGy) and non-irradiated pecans stored at 48.9°C and 37.8°C for four weeks.

	0.0 kGy (non-irradiated)	3.0 kGy irradiated
$k_{48.9° C}$ Eq. [6.1]	0.637 M/s	-0.07888 1/Ms
$n_{48.9° C}$	0 Eq. [5.15]	2 Eq. [5.20]
$R^2_{48.9° C}$	0.91	0.94
$k_{37.8° C}$	0.365 M/s	-0.0544 1/Ms
$n_{37.8° C}$	0	2
$R^2_{37.8° C}$	0.77	0.88
Q_{10} Eq.[6.2]	1.65	1.4
Ea Eq.[6.4]	37.9 kJ/mole K	25.24 kJ/mole K

M =molarity or amount of PV

formation) can be obtained from the electrons that were sent to pecans during the irradiation treatment. This result supports the previous finding that the reaction rate order increases with an increase in dose. Activation energies for the lipid oxidation reaction are between 41-105 kJ/mole (Ozdemir, 2001; Robertson, 2005) and were also calculated in this study using Equation 1.5. Hence, irradiation at a high dose (3.0 kGy in this study) has a significant impact on PV formation in pecans at constant temperature, even when the pecans are packed under vacuum.

6.4.3. Q_{10} Value Calculation

As expected, the Q_{10} values for the non-irradiated samples (Q_{10}= 1.65) were higher than for the irradiated samples (Q_{10}= 1.4), which means the shelf-life of irradiated pecans will be shortened by two months compared to the shelf -life of non-irradiated pecans (four months) at 19° C (room temperature) (Table 6.2). However, it can be stated that vacuum packed pecans have a longer shelf life when compared with air packed pecans stored up to 3 months at 21° C (Baldwin and Wood 2006). The predicted shelf life values at specific temperatures are shown in **Error! Reference source not found.**3 and data from literature are shown in Table 6.4.

Table 6.3 Predicted shelf life (Equations [6.3]) of irradiated (3.0 kGy) and non-irradiated (0.0 kGy) VP pecans at different storage temperatures

	48.9° C	19° C	4° C	-25° C
0 kGy	4 weeks	4 months	8 months 25 days	3 years 1 month
3 kGy	3 weeks	8 weeks	3 months 5 days	8 months

The basis for shelf life prediction was peroxide value

Table 6.4 Literature values of shelf life of non-irradiated VP pecans

Shelf Life	T (°C)	Reference
3 months	21° C	Baldwin and Wood 2006
9 months	4.4° C	Baldwin and Wood 2006
24 months	-17.77° C	Wagner 2007

6.4.4. Color, Texture and Sensory Characteristics

The effect of ionizing radiation at 3.0 kGy on the color attributes (L*, a*, b* values) of the vacuum packed pecan kernels that were stored at 48.9° C for a month were shown in Table 6.5. All irradiated and non-irradiated samples, brightness(L*), redness (a*), and yellowness (b*) values were significantly changed after first week, and they were continued to changed significantly ($P>0.05$) throughout storage. The brightness (L*) and redness (a*) of the VP pecans were not affected by the dose, but the temperature. However, after four weeks of storage, the yellowness (b*) of the pecan kernels was affected by both temperature and the dose, and 3.0 kGy samples showed significantly lower b* values (more yellowish) than the control samples at the end of ASLT (Table 6.5).

Table 6.5 Effect of dose and storage (weeks) on color (L*brightness, a* redness, b* yellowness) of VP pecans stored for 4 weeks at 49°C and 13% R.H.

	L*(+white)		a*(+ red)		b*(+ yellow)	
Time	0 kGy	3 kGy	0 kGy	3 kGy	0 kGy	3 kGy
0 week	$_{w}$ 29.85 ^{a}	$_{w}$ 28.4 ^{a}	$_{x}$ 10.35 ^{c}	$_{w}$ 11.19 ^{c}	$_{w}$ 25.62 ^{a}	$_{w}$ 26.91 ^{a}
	^{1} (1.43)	(0.8)	^{1} (0.82)	(0.52)	^{1} (1.39)	(0.71)
1st week	$_{w}$ 27.31 ^{b}	$_{w}$ 25.9 ^{b}	$_{x}$ 11.94 ^{b}	$_{w}$ 12.75 ^{b}	$_{w}$ 21.67 ^{b}	$_{w}$ 20.82 ^{b}
	(1.45)	(1.29)	(0.77)	(0.68)	(0.75)	(1.26)
2nd week	$_{w}$ 25.17 ^{c}	$_{x}$ 23.63 ^{c}	$_{w}$ 12.95 ^{a}	$_{w}$ 13.39 ^{ab}	$_{w}$ 17.92 ^{c}	$_{w}$ 17.11 ^{c}
	(1.11)	(1.42)	(0.59)	(0.54)	(0.94)	(1.22)
3rd week	$_{w}$ 23.22 ^{d}	$_{w}$ 22.45^{c}	$_{w}$ 13.49 ^{a}	$_{w}$ 13.8^{a}	$_{w}$ 17.78 ^{c}	$_{w}$ 16.53^{cd}
	(0.75)	(1.95)	(0.63)	(0.71)	(1.20)	(1.99)
4th week	$_{w}$ 23.61 ^{d}	$_{w}$ 22.82 ^{c}	$_{w}$ 13.47 ^{a}	$_{w}$ 13.61 ^{a}	$_{x}$ 17.29 ^{c}	$_{w}$ 14.87 ^{d}
	(0.95)	(1.50)	(1.02)	(0.61)	(1.0)	(1.56)

[1]Standard deviation

^{a,b,c,d}Means within a column which are not followed by a common superscript letter are significantly different ($P < 0.05$).

$_{w,x,}$Means within a row(L*, a*, b* evaluated individually), which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Each treatment (dose) has ten measurements and each measurement was the mean value of three readings that was taken from the top part of the pecan half,

Irradiation had no effect on the force required to bite the pecan kernel (Table 6.6). The irradiated pecans were not significantly ($P < 0.05$) harder than then the controls throughout the storage. However, the temperature had some effect on the control samples but no effects on the irradiated samples, i.e. the textures of the irradiated pecan kernels were stayed similar at the end of shelf life.

However, the sensory test was conducted for VP and irradiated at 1.0 kGy pecans were showed that at the end of shelf life pecans texture was significantly different than the 1.0 kGy irradiated pecans; therefore the even the lower dose (1.0 kGy) of irradiation was affecting the texture characteristic that were tasted by panelists (Table 6.7).

On the other hand, 1.0 kGy VP irradiated pecans were still acceptable in terms of color, odor, texture, flavor and over all sensory characteristics (Table 6.7). Only flavor and texture; therefore, the overall sensory characteristics were significantly ($P>0.05$) lower than the non-irradiated control samples and the sensory scores were very close (1 kGy flavor at 4 week is 5.9±2.11, Table 6.7) to the limit score, which is 5.

Table 6.6 Effect of dose and storage (weeks) on texture (Force, [N]) of VP pecans stored for 4 weeks at 48.9°C and 13% R.H.

Time	0 kGy	3 kGy
0 week	$_{w}$ 34.69 ^{c}	$_{w}$ 31.85 ^{a}
	^{1} (7.33)	(8.43)
1st week	$_{w}$ 37.06 ^{bc}	$_{w}$ 32.28 ^{a}
	(7.45)	(11.12)
2nd week	$_{w}$ 41.22 ^{a}	$_{w}$ 38.69 ^{a}
	(12.63)	(13.68)
3rd week	$_{w}$ 38.17 ^{ab}	$_{w}$ 30.07^{a}
	(14.56)	(14.33)
4th week	$_{w}$ 31.71 ^{ab}	$_{w}$ 35.19 ^{a}
	(10.23)	(11.15)

[1]Standard deviation

[a,b,c,d]Means within a column which are not followed by a common superscript letter are significantly different ($P < 0.05$).

[w,x,]Means within a row, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Sixteen measurements were performed for each treatment (irradiated and control).

Table 6.7 Sensory results for vacuum packed pecans irradiated with 1.0 kGy and stored for 4 weeks at 48.9° C and 13% RH

	Color		Odor		Texture		Flavor		Over all	
Dose / Time	0 kGy*	1 kGy	0 kGy	1 kGy	0 kGy	1 kGy	0 kGy	1 kGy	0 kGy	1 kGy
2 weeks	$_{w}$ 7.5 ^{a} (1.07)^{SD}	$_{w}$ 7.39^{a} 1.31	$_{w}$ 6.39 ^{a} (1.7)	$_{w}$ 7 ^{a} (1.24)	$_{w}$ 7.57 ^{a} 0.99	$_{w}$ 7.67 ^{a} (0.94)	$_{w}$ 7.5 ^{a} (1.43)	$_{w}$ 7.42 ^{a} 1.52	$_{w}$ 7.64 ^{a} (1.16)	$_{w}$ 7.5 ^{a} (1.07)
1month	$_{w}$ 7.5 ^{a} 1.02	$_{w}$ 7.39 ^{a} 1.17	$_{w}$ 6.42 ^{a} 1.69	$_{w}$ 6.21 ^{a} 1.82	$_{w}$ 7.48 ^{a} 1.03	$_{w}$ 6.91 ^{b} 1.54	$_{w}$ 6.9 ^{a} 1.61	$_{x}$ 5.9 ^{b} 2.11	$_{w}$ 7.24 ^{a} 1.48	$_{x}$ 6.24 ^{b} 1.82

Values are means of 30 panelists

^{SD}Standard deviation

^{a,c}Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).

^{x,y}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

*non-irradiated controls

6.5 Conclusion

From the kinetic modeling studies (Chapter V), the irradiation dose has a stronger effect than the MAP condition in terms of extending the shelf life of the pecans. The choice of MAP condition (vacuum-packaging) for the shelf life experiments was based upon two factors: (1) it was the one that increased the dose requirement (both for the *E. coli* cocktail and the *Salmonella* LT2) e.), and (2) the one that helped reduce rancidity in irradiated pecans (VP, or N_2- MAP).

The shelf life results support the findings from the kinetic model study on the significant effect of dose level and the positive effect of vacuum packaging on extending the shelf life of irradiated and non-irradiated pecans. This study also showed that irradiation of pecans at a dose as high as 3.0 kGy in VP packages reduced the shelf life of pecans (based on peroxide formation) by half when the packages were kept at room temperature (19° C). Thus, as the irradiation dose increased, the Q_{10} value decreased and shelf -life was reduced. The brightness and redness of color values and texture characteristics of 3.0 kGy VP irradiated pecans were not significantly different than the control samples at the end of ASLT, which could be because of the vacuum packaging.

However, the consumer panelists noticing a "off" flavor in irradiated pecans (1.0 kGy) compared to the non-irradiated controls, which is correlated to PV results in this study. Therefore, irradiation dose changing the PV as well as taste of pecans, but VP packages were helping to reduce th detrimental effects of the dose.

As expected, storage at refrigeration temperature (~ 4° C) will help extend the shelf life of VP-packed pecan nuts; moreover, non-irradiated VP pecans will last for

almost 9 months, while irradiated (3.0 kGy) VP pecans will be of good quality for more than 3 months at 4° C. Thus, storage of irradiated pecans at refrigeration temperature could be a good practice for pecans in AP and VP (MAP conditions). When frozen (-25° C), the shelf life of VP-packed non-irradiated pecans could be more than 3 years, while the literature and some pecan selling websites (2012) indicate that pecans have a 2 year shelf-life when stored in a home freezer (-18°C) using regular packaging. The predictive equations obtained in this study are a useful tool to estimate the shelf life of pecans at different storage temperatures, based on the simplified assumption of peroxide formation.

In brief, exposure to high doses of ionizing radiation (~ 3 kGy), MAP conditions, and storage temperature have an impact on the shelf life of raw pecans. Exposure to ionizing radiation has the most deleterious effect on pecans quality (in the form of rancidity). These results indicate that irradiation treatment of pecans using electron beams can be used as an intervention strategy to decontaminate the nuts from pathogens such as *Salmonella* and *Escherichia* coli. However, irradiation treatment of pecans should be carried out in combination with MAP technologies (vacuum or N_2-packaging) and the pecans should be stored at refrigeration temperature to prevent the loss of quality due to rancidity.

CHAPTER VII

RECOMMENDATIONS FOR FUTURE STUDIES

1. Conduct a shelf life study using nitrogen flushed unfrozen pecan packages irradiated at high doses (>3.0 kGy), and after irradiation (not during) stored at different temperatures. Measure other quality parameters (texture, flavor, odor, color) in addition to the rancidity measurements.
2. To understand the mechanism of lipid oxidation in irradiated pecans, determine pecans' fatty acids, tocopherols, volatile compounds, and peroxide values and determine the kinetics of formation of these compounds.
3. Evaluate the feasibility of modeling the oxidation reactions using the Weibull model.
4. The development of a suitable antimicrobial coating should be carried out to evaluate the feasibility of reducing the required dose and enhancing the shelf life of pecans using MAP technology.

CHAPTER VIII

CONCLUSIONS

The effectiveness of irradiation using electron beams as a means to kill populations of an *E. coli* cocktail and *Salmonella* LT2 in pecan nuts was investigated. Since irradiation of pecans has a deleterious effect on their quality (rancidity), the effect of different modified atmosphere packaging (MAP) conditions was explored as a means to extend the shelf life of irradiated pecans.

First, the required dose to produce a 5-log reduction in *E. coli* and *Salmonella* surrogates under different MAP conditions was established. In addition, dose absorption in different parts of the pecan kernels was also investigated. In this study, the most notable findings were as follows:

1. Due to their complex shape, there is a difference in the dose absorbed throughout the nut kernel.
2. The dorsal grooves (valleys) of pecans receive almost 40% more dose than other parts of the pecan. This may be due to the scattering effects of electron beam irradiation; therefore, microorganisms, which internalize into the pecan grooves, could be easily destroyed than those at the surface (since they will receive up to 40% more dose).
3. The entrance dose in the pecan was different (0.48 kGy) than the exit dose (0.08 kGy) and the dose in the dorsal grooves (0.71 kGy), when irradiated on one side (single beam setup) at 0.5 kGy.

4. The best way to irradiate pecans with a low energy electron beam accelerator is using a double -sided (double beam) setup.
5. The water activity levels of pecan kernels remain constant after the kernels have been inoculated using the drop method inoculation technique.
6. The food safety concerns (contamination with *E. coli*) for high oil content nuts such as pecans can be addressed by irradiating the pecans with up to 2.2 kGy, 1.9 kGy, and 1.7 kGy when irradiating under vacuum (VP), nitrogen (NP) and oxygen (OP), respectively.
7. The food safety concerns (contamination with *Salmonella*) for high oil content nuts such as pecans can be addressed by irradiating them up to 2.3 kGy, 2.0 and up to 1.8 kGy when irradiating under vacuum (VP), nitrogen (NP) and oxygen (OP), respectively.
8. The surrogate microorganisms evaluated in this study were more resistant to irradiation when packed under vacuum (VP packages), due to two reasons: (1) no radiosensitization effect because of the lack of oxygen and, (2) the microorganisms became more resistant to irradiation under stress (because of a lack of oxygen).
9. Irradiation of pecans in O_2 -packages produced the lowest D_{10} values among all the MAP conditions, and the values for the N_2-packed pecans were not significantly different from the ones obtained for the O_2-packed pecans ($P>0.05$), suggesting that N_2-atmospheres may be a suitable alternative to irradiation under air when using doses higher than 1.0 kGy.

After determining the required dose values for pecan kernels in each MAP condition, the reduction of the detrimental effects on the quality of pecans (the lipid oxidation) caused by irradiation, was investigated. The conclusions of this study are as follows:

10. After irradiating frozen pecans packed under vacuum and air, peroxide values (PV) of vacuum- and air-packed pecans were ten and three-fold higher than for the non-irradiated frozen pecans (controls). However, when pecans were packed in the unfrozen stage, PV changes were not significant after irradiation.
11. The kinetics of PV formation at different doses (0.3, 0.5, 0.7, and 0.9 kGy) under different MAP conditions (AP, VP, N_2, O_2) was different.
12. The PV formation in samples irradiated in Vacuum packaged (VP) packages was a zero order reaction, and it did not change with the increase in dose.
13. The PV formation in samples irradiated in nitrogen-packaged (NP) pecans irradiated at 0.7 and 0.9 kGy and all pecans irradiated under air (AP) could not be determined because the data did not fit well any of the kinetic models tested.
14. The order of the PV formation in samples irradiated in oxygen-packaged depended upon the dose level --the reaction order increased when the dose increased. This finding confirms that the combination of exposure to ionizing radiation in the presence of oxygen is deleterious to the quality of pecans in terms of rancidity.
15. A low dose of irradiation is critical to delay the oxidation reaction rate; however, even if given low doses of irradiation, the oxygen-packed pecans had accelerated

oxidation reaction rates. Therefore, irradiation treatment at low doses (< 1.0 kGy) is only feasible under Nitrogen or vacuum MAP conditions.

16. A model was proposed to understand the lipid oxidation reactions in irradiated pecans as a function of dose level and MAP condition.
17. The presence of a lag phase in the peroxide formation reaction was confirmed and it was quantified by the modified Gompertz model.
18. The Nitrogen (NP) and Vacuum (VP) MAP options helped to extend the lag phase; hence the shelf life of the product.

After determining the best MAP conditions for irradiation of pecans (NP and VP), a shelf life study was conducted. Pecans under vacuum irradiated with 3.0 kGy were selected for this study because the microorganisms were more resistant to irradiation under VP conditions.

19. Irradiation dose did not significantly change the brightness and redness and the texture of the pecan kernels, but decreased the yellowness of the pecans compared to non-irradiated VP control samples.
20. Activation energy values in pecans show that irradiation at a high dose (3.0 kGy) has a significant impact on PV formation in pecans at constant temperature, even when the pecans are packed under vacuum.
21. The shelf life of irradiated pecans (3 kGy) was estimated to be eight weeks at room temperature (19° C) and eight months in a laboratory type freezer (-25° C). Storage at 4° C was estimated to extend the shelf life of the irradiated pecans by 2.5 times.

22. The shelf life of non-irradiated pecans stored under vacuum was estimated as four months and five days at room temperature (19° C), nine months (4° C) and three years in a laboratory type freezer (-25° C).
23. Irradiation dose level has the most impact on pecans' shelf life, then storage temperature, and then packaging condition.

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APPENDIX

Table A.1 Oil extraction methods peroxide value results before and right after irradiated at 3.0 kGy dose.

Extraction Methods	Non-Irradiated	Irradiated
Hexane-Method	1.12 ± 0.42^{a}	1.34 ± 0.33^{a}
Soxhlet-Method	0.99 ± 0.36^{a}	6.50 ± 0.93^{b}
Commercial Pecan oil	10.28 ± 2.97^{c}	13.77 ± 2.94^{d}

[a,b,c,d,] Means, which are not followed by a common letter are significantly different ($P < 0.05$).

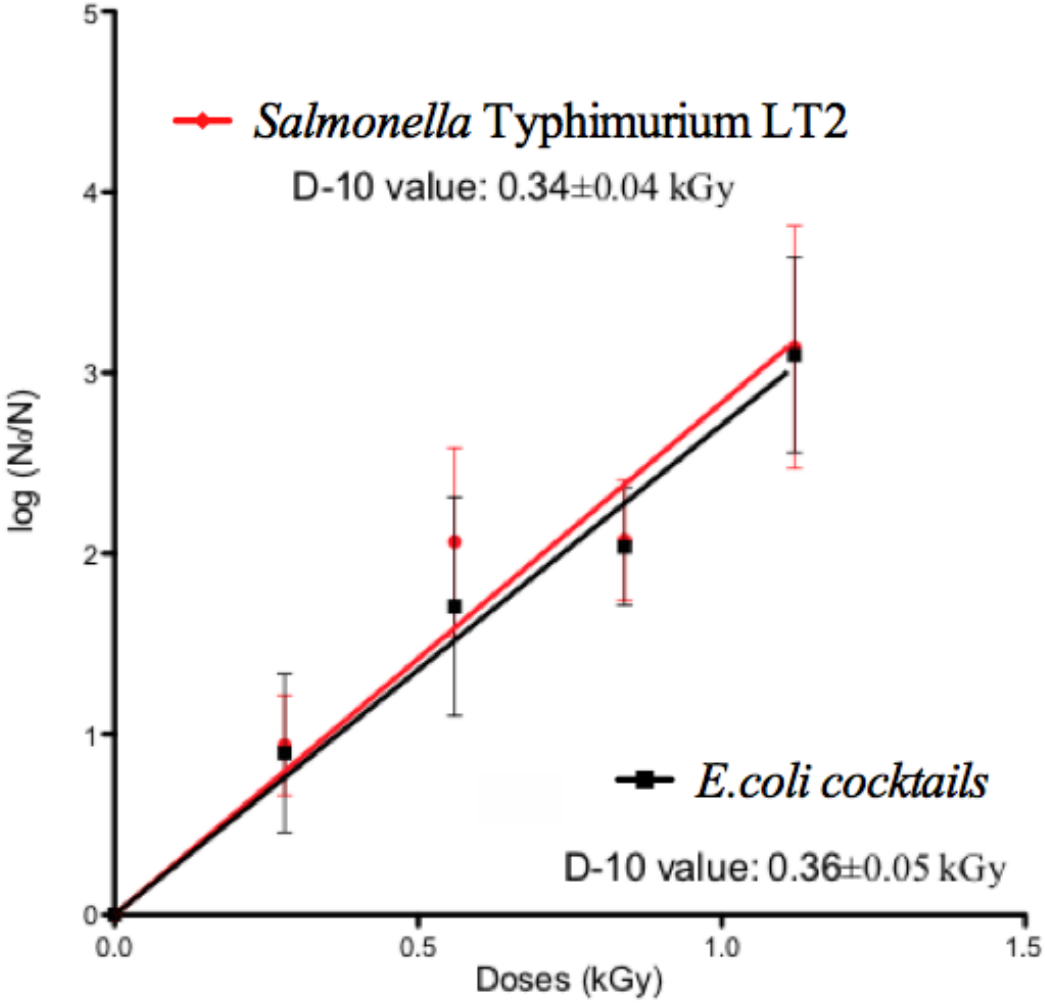

Figure A.1 D_{10} values of *Salmonella* Typhimurium LT2 and an *E. coli* cocktail in Oxygen-packed (OP) pecans irradiated at room temperature.

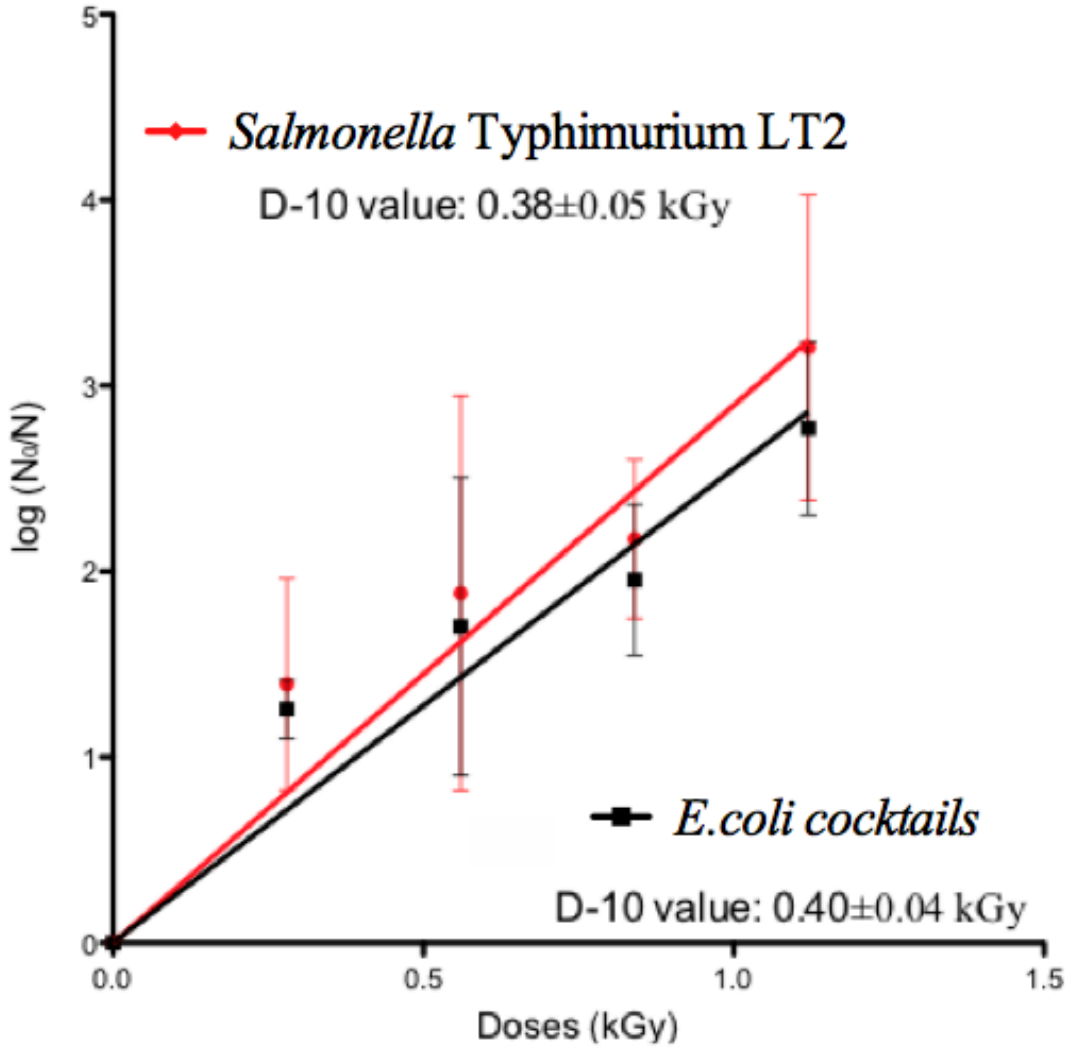

Figure A. 2 D_{10} values of *Salmonella* Typhimurium LT2 vs. *E. coli* cocktail in Nitrogen-packed (NP) pecans irradiated at room temperature.

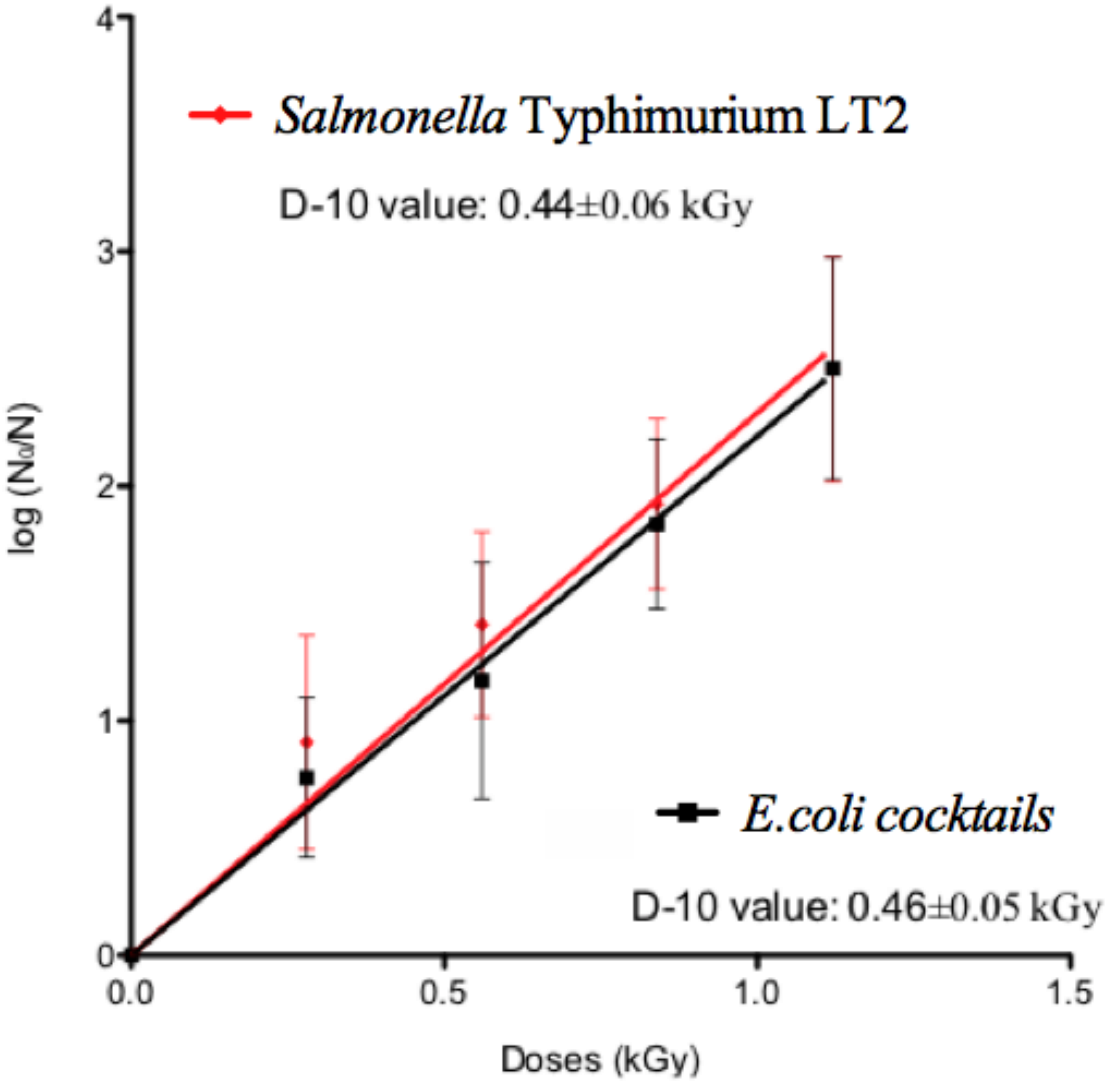

Figure A.3 D_{10} values of *Salmonella* Typhimurium LT2 and *E. coli* cocktail in Vacuum-packed (VP) pecans irradiated at room temperature.

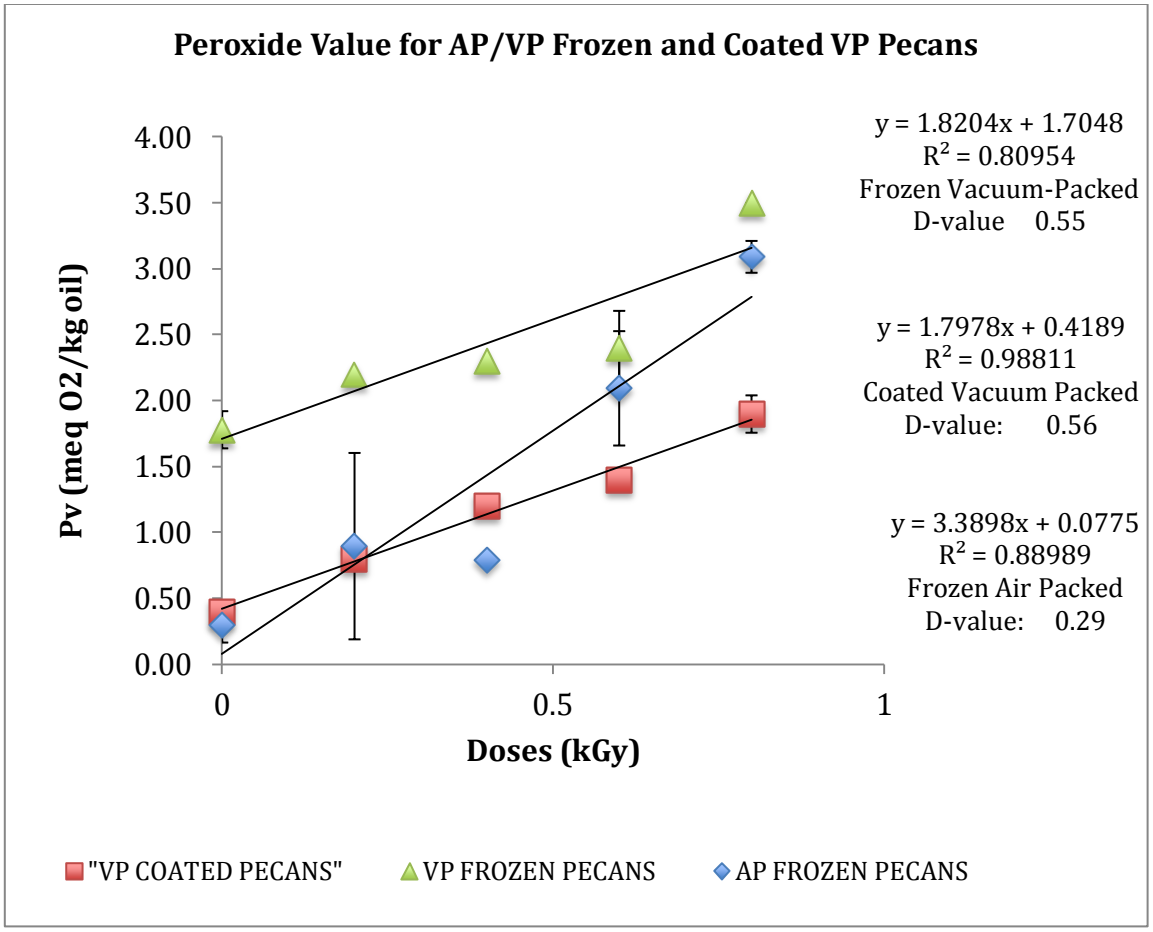

Figure A.4 PVs of frozen (AP and VP) and coated pecans irradiated at 0.2, 0.4, 0.6, and 0.8 kGy using a low energy e-beam accelerator

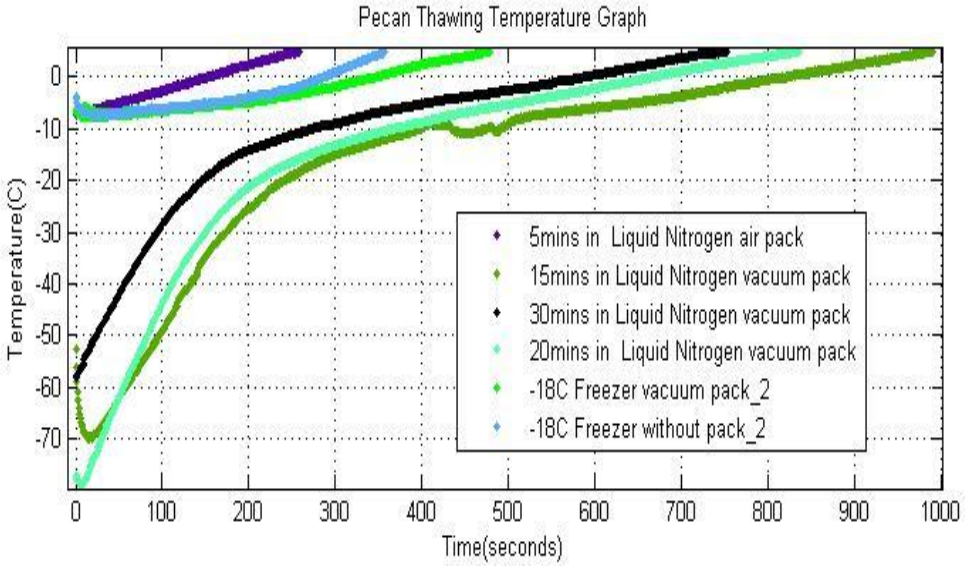

Figure A.5 Pecan Thawing Temperature. Data were analyzed by using Matlab 2007b for Windows

For walnuts freezing time -.6.7° C and for peanuts -8.3° C (Jay, 2005)