BIOACTIVE LIPID MICRO-CONSTITUENTS IN 20 DIFFERENT COMMERCIALLY-VIABLE PECAN [*Carya illinoinensis* (Wangenh.) K. Koch] CULTIVARS

by

ELIZABETH C. CARR

(Under the Direction of Ronald B. Pegg)

## ABSTRACT

Pecans are known for their healthful lipid profile, which includes the lipid micronutrients: tocopherols and phytosterols. Because the pecan has thousands of cultivars and is alternate bearing, these nutrient contents can vary. Despite this, these micronutrients are largely underreported in the USDA National Nutrient Database for Standard Reference (Release 27). Therefore, 20 different commercially-viable pecan cultivars were assayed to determine tocopherol and phytosterol contents along with effects of crop year and cultivar on these bioactives. Chromatographic methods were employed to determine the levels of tocopherols and phytosterols. All of the tocopherol and most of the phytosterol values determined were lower than that of the Database, due to the sample variability included in this study. Overall it was discovered that cultivar and crop year can significantly affect the nutrient level of pecans, and thus should be incorporated into the USDA Database.

INDEX WORDS: Pecans, Tocopherols, Phytosterols, Chromatography, Cultivar Variation

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## DEDICATION

I would like to dedicate this thesis to my parents, Kim and Loften Carr for their generous financial backing and encouragement and to my fiancé, Greg Miller, for his unconditional emotional support, constant reassurance, and unyielding love. I would have never made it through this degree without your unwavering support.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

# CHAPTER 1

# INTRODUCTION

*Carya illinoinensis* (Wangenh.) K. Koch (more commonly known as the pecan) is a species of hickory nut that is indigenous to the Southern United States.[1] It grows prominently in states located along the Mississippi river and its tributaries in addition to Southwestern states such as Texas, New Mexico, and Oklahoma.[2] The United States has produced over 50% of the worldwide pecan supply for six years (2008-2013), showing the importance of pecans in the US market.[3] The demand for pecans in international markets is also growing according to the 2014 USDA Noncitrus Fruits and Nuts Summary.[4] Pecans are a particularly important crop in Georgia, as it has been the number one producer within the US from 2007 to present.[5] Not only is the pecan a major US crop, but it also has many nutritional benefits.

Tree nuts, including pecans, have recently come into the food spotlight because of the many health benefits they can provide. In 2003 the FDA released a nut health claim stating, "Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease."[6] The favorable lipid profile of nuts, pecans in this case, (*i.e.*, unsaturated fatty acids, tocopherols and tocotrienols (vitamin E), and phytosterols) is a significant factor in the healthful profile of nuts. Multiple studies have shown that pecan consumption specifically can help lower total cholesterol, LDL cholesterol, and improve plasma lipid and lipoprotein blood profile.[7–9] Not only has the effect of pecan consumption on health been studied, but the specific effects of certain lipid bioactives like tocopherols and phytosterols have also been investigated.

Diets containing vitamin E, an antioxidant, have been linked to a reduced risk for cardiovascular disease as well as reduced incidence of deaths associated with coronary heart disease (CHD).[10,11] Moreover, occurrence of Alzheimer's[12,13] disease and some cancers has been shown to decrease with increased vitamin E consumption.[14] Phytosterols, another class of lipid bioactives, have been found to have anti-bacterial, anti-inflammatory, and anti-tumor properties and have been linked to a reduction in both overall and LDL cholesterol.[15,16] Consumption of phytosterol-rich foods has also been associated with increased levels of other cancer-fighting nutrients and bioactives such as fiber, vitamin D, retinol and flavonoids.[17] Further studies have gone on to quantify these minor bioactives in tree nuts.

Many studies have identified and quantified both tocopherols and phytosterols in various tree nuts and peanuts, but pecans are one of the least documented nuts. Previous research has reported total tocopherol contents ranging from 18.8 to 33.6 mg/100-g oil,[12,13,18,19] and phytosterol contents ranging from 129 to 184 mg/100-g nuts[13,20,21] in pecans. With the exception of Chun *et al.*[18] factors such as crop year, cultivar, and growing region/conditions were not taken into account in these studies. The aforementioned factors should be considered because they have been reported to have substantial effects on the nutrient profile of the nut.[13,22] Cultivar is particularly relevant to pecans due to the unique nature of pecan reproduction, resulting in over 1,000 named and documented cultivars worldwide.[23] Despite the numerous cultivars, research on the different cultivars is few and far between. In fact, the data for pecans found in the USDA National Nutrient Database for Standard Reference, Release 27,[24] has very limited sample numbers ($n$=9 for tocopherols and $n$=3 for phytosterols) of unknown cultivar, growing region/conditions, crop year, and handling conditions, indicating the need for research including

these distinctive factors. Therefore a study was designed to investigate the effect of crop year and cultivar on nutrient contents of the pecan.

The purpose of this investigation was to characterize and provide credible data on the content of tocopherol homologs and phytosterols in Georgia pecans, as existing literature and the USDA National Nutrient Database for Standard Reference, Release 27 [24] contain very limited data on these bioactives. To illustrate, the USDA Database provides only nine observations for three of the tocopherol homologs and only three observations for the phytosterols without any background relating to the samples analyzed. A better knowledge of the contents of these lipophilic bioactives with health-promoting properties can assist the US pecan industry in differentiating the pecan from other tree nuts and be used in targeted marketing campaigns. A further objective of this study is to provide the USDA up-to-date levels of these bioactive lipids for incorporation into their Database.

Specific objectives of the study undertaken were as follows:

1. To assess the effect of cultivar and crop year from this alternate-bearing tree nut on the levels of the tocopherols and phytosterols;
2. To isolate, chromatograph, and quantify the tocopherol homologs (vitamin E) from extracted pecan lipids of 20 different commercially-viable cultivars, and to validate the findings using a nut-based NIST standard reference material with known contents of tocopherols; and
3. To isolate, chromatograph, and quantify the phytosterols from extracted pecan lipids of 20 different commercially-viable cultivars, and to validate the findings using both internal and external commercial sterol standards.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Pecan Background

### *2.1.1 History/Growing Practices*

Pecan [*Carya illinoinensis* (Wangenh.) K. Koch] is a species of hickory nut that is indigenous to the United States.[1] Indigenous (native) pecans are distributed along the Mississippi river and its tributaries, with the oldest native cultivars found in Texas, Oklahoma, and Louisiana.[2] Several other states including Florida, Georgia, New Mexico, and North and South Carolina, successfully grow many non-native pecan cultivars.[2] Native pecans were often eaten by Native Americans and Early European explorers, which is subsequently from where the name comes.[2] The name stems from the Algonquian name "pekan" meaning "a general term for a hard nut," which was adopted by Early French settlers and translated to "pecane".[25] The pecan belongs to the Juglandaceae family and the genus *Carya*, which date back 50 and 34 million years, respectively. The Juglandaceae family also includes the walnut, butternut, hickory nut, and heart nut.[26]

Pecan trees are monoecious, heterodichogamous, and deciduous and have specific environmental requirements for proper growth, as well as variable growing patterns.[1] The trees prefer deep, fertile, and well-drained soil with a sandy loam texture and clay subsoil.[27] Because trees can grow up to 60 m tall and 3.5m wide, a long taproot is needed to keep the tree from blowing over.[1,28] Soil should not be colder than 65° F and in pH range of 6.5 to 7, and land

should be flat, or gently sloped, in order to allow airflow, while avoiding pooling water.[27] Pecan trees are alternate bearing, meaning that they have "on" and "off" years with heavy crops expected in "on" years and minimal crops expected in "off" years. This is because the high production of one year reduces the amount of carbohydrate accumulation needed to fuel growth and fruiting for the following year's crop.[28] Techniques such as fertilization and control of moisture, disease, and light exposure can help to minimize these alternate-bearing issues.[28] Adequate storage conditions for pecans are also key in maintaining kernel quality, with the most important factors being good packaging, refrigeration, and sufficient drying.[28]

*2.1.2 Composition*

The proximate composition of the pecan based on the USDA National Nutrient Database for Standard Reference, Release 27 is shown in Table 2.1 below.[24] Lipids are the most prominent component of the pecan at 71.9 g/100-g nuts in raw pecans. The next most abundant component is carbohydrate at 13.86 g/100-g nuts, with 9.6 g/100-g nuts coming from dietary fiber. The third largest component is protein at 9.17 g/100-g nuts. The final two, most minor, components of the pecan are water and ash at 3.52 and 1.49 g/100-g nuts, respectively.

**Table 2.1**: Proximate Composition (g/100g) of Pecans

| **Proximate Composition** | **Raw**[a] |
|---|---|
| Water | 3.52 |
| Total Lipid | 71.97 |
| Protein | 9.17 |
| Ash | 1.49 |
| Carbohydrate (by difference) | 13.86 |
| Fiber, total dietary | 9.6 |

[a]Data from the USDA National Nutrient Database for Standard Reference, Release 27[24]

Lipid content of pecans is of particular interest, being that lipids are the main component of the nut. Though the USDA reports that the pecan is ~72% fat, it can vary from 60.3 to 76.6%.[20,29,30] It has been shown that this value is highly dependent on cultivar and varying horticultural factors such as irrigation level, crop year, and alternate-bearing capacity.[29,31] Despite lipids being such a large component of the pecan, almost all of the lipid (96%) is triacylglycerols, with the remaining 4% being made up of various minor lipid constituents (*i.e.*, sterols, tocopherols, sphingolipids).[20] Many epidemiological studies suggest that these minor lipid components significantly affect human health.[7]

*2.1.3 Health Benefits of Nut (Pecan) Consumption*

Tree nuts have been studied extensively in order to determine the health benefits relating to consumption. Many epidemiological studies, summarized by Kris-Etherton *et al.*[7] provide strong evidence that nuts have beneficial effects on CHD risk. More specifically, an 18-51% reduction in CHD risk was reported when nuts were consumed more than 5 times per week. These health benefits have led to a FDA qualified health claim stating, "Scientific evidence suggests but does not prove that eating 1.5 ounces per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease," in addition to being incorporated into the 2010 US Dietary Guidelines.[6,32]

Another health benefit associated with nuts and nut consumption is cholesterol reduction. Numerous clinical studies, again summarized by Kris-Etherton *et al.*[7], have determined that nut consumption has a favorable affect on lipid and lipoprotein blood profile. Morgan *et al.* [8] evaluated pecan-rich diets and found that intake of pecans was associated with a reduction in all types of cholesterol (LDL, HDL, and total). Another study by Rajaram *et al.*[33] found that a diet

rich in pecans helped to increase HDL cholesterol, while lowering LDL and total serum cholesterol along with decreasing plasma triacylglycerol and lipoprotein levels.

Pecans, among other nuts, have also been found to have positive effects on weight management and associated diseases. It would be expected that high nut consumption would result in weight gain, as nuts are high in fat, but this has been disproven. Sabaté and Ang [34] reported several studies disputing nuts and weight gain as well as evidence from the Seguimiento Universidad de Navarra (SUN) study to support this claim. The SUN study, covering ~8,800 adult men and women, found that eating tree nuts ≥ 2 times per week reduced risk of weight gain by 30% and reduced odds of becoming obese by 43%.[35] Sabaté and Ang [34] also reported on the effects of nut consumption on Type II diabetes. The results found are mixed, with some finding protective effects against diabetes and some finding little or no association. In addition most of these studies are mainly applicable to women. These health benefits, in addition to others, can be attributed to specific bioactive constituents, which will be discussed later in the review.

*2.1.4 Economic Impact*

Pecans have a noteworthy economic impact both nationally and internationally. They are the third most consumed tree nuts in the US, with fairly steady consumption since the 1960s. Currently, ~3.54 pounds of pecans are consumed per person each year, indicating that they are a key US crop.[36] Pecans were valued at 460 million dollars in 2013, a 3% decrease from 2012, but preliminary data indicates a value of 508 million dollars in 2014, a 10% increase.[4,5] Worldwide production of pecans from 2008 to 2013 has been dominated by the US (59%) followed closely by Mexico (35%) with South Africa (4%), Australia (1%), and others (1%) responsible for the

remaining production. In 2013, 81,502 metric tons of pecans were produced globally, which was a 30% decrease from 2012, and overall decrease of 11% based on the six-year average.[3]

Within the US in 2013, Georgia, New Mexico, and Texas were responsible for over 70% of the 131,165 tons of in-shell US pecans produced. Georgia was the number one producer of in-shell pecans in 2013, producing approximately 44,500 tons (~33%).[5] In 2013 the Georgia Farm Gate Report, the pecan ranked tenth in the Georgia Agricultural Commodity Rankings, contributing 2.32% of Georgia's total farm gate value. Moreover, pecans were valued at 315 million dollars, which was a 27% increase from 2012.[37]

## 2.2 Factors Influencing Composition

### *2.2.1 Cultivars*

Pecans are a genetically diverse species, in that, there are over 1,000 named and documented cultivars worldwide.[28] Cultivars are either native (named pre-1920 and within the native range), seedling (man made with a single known parent), or pedigreed (man made with both parents known).[23] There are approximately 134 native cultivars, 105 seedling cultivars, 51 pedigreed cultivars, and the 522 or more remaining are of unknown origin.[23] Often cultivars are bred for certain desirable characteristics such as increased productivity, resistance to insects and disease, and more standardized nuts and nut products.[28] These "improved" cultivars are further characterized by region, western (Texas), southern (Georgia), or northern (Illinois), which incorporates factors such as tolerable growth conditions and length of growing season.[28] Thus, the types of pecans grown around the US are highly dependent on the region in which they are grown. Approximately 12 cultivars ('Caddo', 'Cape Fear', 'Creek', 'Desirable', 'Elliott', 'Forkert', 'Kanza', 'Kiowa', 'Oconee', 'Pawnee', 'Stuart', and 'Sumner") are recommended for

use in Georgia. There are also ~seven cultivars ('Amling', 'Byrd', 'Excel', 'Lakota', 'Mandan', 'McMillian', and 'Zinner') recommended for trial in Georgia.[38]

Many studies have shown that there is a difference in compositional characteristics between different cultivars of various nuts, including pecans. Rudolph *et al.* [29] showed that oil content can vary up to 16% between cultivars. Fatty acid profile variability has also been related to pecan cultivar. [39,40] Several studies reported that cultivar has a significant effect on tocopherol content in pecans.[18,22,41] Many studies have reported the phytosterol content in pecans, but few have examined the influence of cultivars on sterols. Though there have not been specific studies on how cultivar influences pecan sterols, cultivar has proven to effect sterol contents in other nuts. A study by Shin *et al.* [42] reported that cultivar had highly significant ($p< 0.001$) effects on total and individual phytosterol content of peanuts. Another study by Yada *et al.* [43] found that the content of β-sitosterol and stigmasterol is influenced by almond variety. Cultivar is an important factor in pecan composition, but there are also other noteworthy horticultural factors such as growth conditions and crop year.

*2.2.2 Growth conditions and crop year*

As was stated earlier, pecans have specific requirements for growth, and thus any variability in these conditions can have measureable effects on nutrient content. These factors can vary highly from year-to-year, indicating that crop year can be a substantial factor in nutrient composition.[44] Pre- and post-harvest conditions as well as horticultural practices such as conventional or organic practices can be critical points in the development of pecan composition.[40,45] Often, alternate bearing can effect composition as well. For example, pecans from high-yield trees tend to have a lower oil content and pecans from low-yield trees have a

higher oil content.[26] Oil content can also vary considerably between cultivars, and even vary between cultivars from the same orchard, but different crop years.[31] A study by Rudolph *et al.* [29] reported variations of 8.4% across cultivars of different crop years, which the authors attributed to both year of production and environmental effects, proving horticultural conditions do affect pecan composition.[26] Studies have also shown cultivar to have a significant effect on tocopherol content, with little or no variation due to crop year. [18,29] There are few and incomplete studies that report the effect of crop year, cultivar, growth conditions, or farming practices on the phytosterol content of the pecan. One exception is a recent study by Bouali *et al.* [46] which showed that variety and ripening stage have a considerable effect on phytosterol content of the pecan.

*2.2.3 Other factors*

Cultivar and crop year effects have proven to be central compositional factors in pecans, but several other horticultural factors have been reported to effect nutrient composition of both pecans and other nuts. Factors such as orchard management, pruning, irrigation, fertilization, *etc*. have been reported to affect the variability of pecan oil content.[31] Irrigation has been specifically linked with pecan size and kernel percentage. Another important consideration is soil type: a fine sandy loam soil with clay sub soil will provide optimum growth conditions. [28] Tree row spacing and orientation can also effect pecan composition. Several different orchard-planting schemes are possible, but spacing allowing 24 to 27 trees per acre is recommended. Local climates, which are highly variable also have substantial effects on nutrient composition. For example, the Georgia heat and humidity can often lead to pecan scab, a fungal disease that can destroy pecan

crops. [27] Lastly, specific pruning and irrigation practices can help to minimize alternate bearing, which in turn helps to produce a pecan with more consistent composition.[26]

Often times sample manipulation can also affect the composition of the pecan. An important influence on pecan composition is storage conditions.[28,45] Several studies[41,47,48] have reported that tocopherol content in nuts declined as storage time lengthened, showing changing composition is related to storage. Rudolph *et al.* [41] went on to show that increased tocopherol concentration is related to increased oxidative stability, indicating that tocopherols are a key player in the oxidative stability of pecan oil. Furthermore, research has shown that the type of assay (*i.e.*, extraction method) used can have considerable influence on the compound amounts reported.[18] Types of internal and external standards used have also been seen to influence reported values.

## 2.3 Tocopherols (Vitamin E) Overview

### *2.3.1 Tocopherols (Vitamin E)*

Vitamin E is a fat-soluble vitamin that is an essential part of the beneficial lipid profile of the pecan. Eight tocol isomers exhibit vitamin E activity, which includes tocopherols and tocotrienols. These isomers are classified as α- β-, γ-, and δ- based on structure.[49] As can be seen in Figure 2.3, tocopherols have saturated side chains and tocotrienols have unsaturated ones. One isomer in particular, α- tocopherol, is the most abundant in nature.[50] In vegetable and nut oils tocopherols comprise the major portion of vitamin E with tocotrienols having only a minor contribution, if any.[49] Pecans, in particular, only contain tocopherols (no tocotrienols), with γ-tocopherol being the most prevalent isomer.[13]

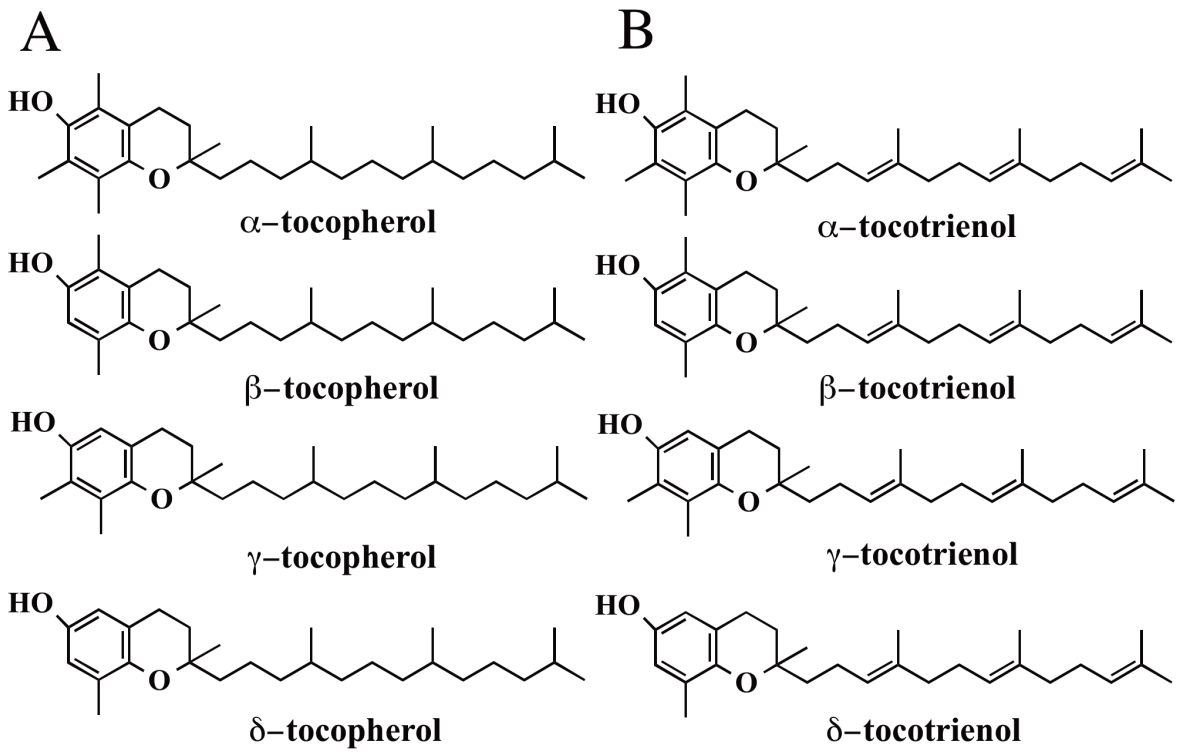


**Figure 2.3**: Chemical Structure of Tocopherols (**A**) and Tocotrienols (**B**).[50]

Vitamin E is a primary or chain-breaking antioxidant that helps to prevent propagation reactions, and thus protects the body from free radicals.[50] Many free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated naturally in the body or by varying environmental stressors.[51] The development of many chronic diseases has been linked to ROS, indicating that vitamin E is an important antioxidant in the body.[52] The various isomers of vitamin E all show different levels of antioxidant activity. Tocopherol activity generally follows the pattern of α- > β- > γ- > δ-, but it can have some dependence on concentration in a given food. It should also be noted that tocopherols have been found to have pro-oxidant activity if concentrations are too high.[49]

Although α-tocopherol has the highest antioxidant activity, γ-tocopherol also plays an important role. γ-Tocopherol has been found to be a better free-radical scavenger than α-tocopherol, helping to protect DNA and vital body proteins from peroxy-nitrite-dependent damage.[53,54] It has also been found that γ-tocopherol shows anti-inflammatory activity *in vivo*, which could contribute to human disease prevention.[55] In a study conducted by Hudthagosol *et al.* [56] patients were fed meals with 90 g of pecans, and it was found that plasma antioxidant activity increased, while oxidized-LDL cholesterol levels decreased. Therefore, it can be inferred that γ-tocopherol contributed to these beneficial antioxidant effects.

### *2.3.2 Health Benefits of Vitamin E*

Vitamin E functions as an antioxidant in the body; and as such, it imparts some health benefits upon inclusion in the diet. Vitamin E has been associated with a decreased risk for CHD related deaths.[10,11] Tocopherols consumption has also been linked to decreases in other chronic diseases. Several clinical studies summarized by Tucker and Townsend [14] have shown an association between decreased risk for Alzheimer's disease and increased α-tocopherol consumption. It has also been observed that vitamin E has a correlation with a reduced risk for several types of cancer. An aforementioned study by Hudthagosol *et al.* also emphasized the potential links between of γ-tocopherol, reduced oxidized LDL cholesterol, and increased blood antioxidant activity.[56] Other diseases such as multiple sclerosis and various kidney diseases have shown potential for treatment with either γ-tocopherol, mixed tocopherols, or tocotrienols.[57]

*2.3.3 Vitamin E Analysis*

Vitamin E can be analyzed by a number of different types of methods including biological assays, physiochemical assays, and chromatographic assays.[58] For the purpose of this review only chromatographic methods will be discussed, because they are most relevant to the current project. Tocopherols were initially analyzed *via* gas chromatography (GC) in the 1960s, which allowed for a quick assay with good reproducibility, relatively high values, and the capability to assay with other fat-soluble vitamins. Unfortunately the assay suffered from a need for excessive sample preparation, which could damage vitamin E, in addition to having poor resolution between tocopherol homologs in packed columns.[58,59] When a capillary column method was created by Marks [60] many of these problems with GC were eliminated, and it became a more usable method. In more modern times high-performance liquid chromatography (HPLC) has replaced GC; GC is often still used, especially in combination with mass spectroscopy for identification purposes. Several studies have shown the use of GC for identifying tocopherol homologs in human blood, [61] oxidation products of vitamin E,[62] and comparing specific identification methods.[63] Though GC has proved useful, HPLC is now the most commonly employed and preferred method.

HPLC is a highly efficient and effective method for analyzing tocopherols in foods. In 1973 the first analysis of vitamin E in foods was performed by Van Niekerk *et al.,*[64] highlighting advantages to using HPLC for analysis of vitamin E. These include ease and efficiency of analysis, capability to use direct injection of oil, high sensitivity provided by fluorescence, good isomer resolution, as well as good reproducibility and recovery.[58] Exhaustive research has been done to optimize sample preparation, column type, mobile phase, and detection methods for HPLC tocopherol analysis as well as to develop a food product-specific methodology. In the case

of peanuts and tree nuts, methodologies are often based on the method developed by Lee *et al.*[65] The method includes oil extraction *via* Sohxlet, oil saponification to remove interfering lipids, and normal-phase HPLC analysis using a LiChrosorb Si60 column. The mobile phase is composed of hexane and isopropanol, and detection is achieved by fluorescence with excitation and emission wavelengths of 290 and 330, respectively.

*2.3.4 Tocopherols in Pecans*

Many different studies have reported total and individual tocopherol levels in pecans. Reported total tocopherol values range from 18.8 to 33.6 mg/100-g oil.[12,13,18,19] As was aforementioned, pecans are made up of primarily γ-tocopherol (>90%), and so in many studies, this is the main tocopherol of interest.[18] Most studies either report the total tocopherol content or content specific homologs (γ in particular), but only a few studies report the content of all four tocopherol homologs. Reported pecan total tocopherol values are similar to those of other tree and groundnuts. Specifically, the values were found to be similar to walnuts, almonds, and pistachios, but higher than dry-roasted peanuts, cashews and macadamia nuts.[65] According to the FDA the daily recommended value for vitamin E is ~20 mg. The USDA National Nutrient Database for Standard Reference, Release 27,[24] states that pecans contain ~26.7 mg/100-g nuts, which, when converted, represents ~38% of the daily value. This means that pecans, among other tree nuts, can be considered to be an "excellent source of" vitamin E by the FDA.[66]

The USDA National Nutrient Database for Standard Reference, Release 27,[24] reports a total tocopherol content of ~26.7 mg/100-g tocopherols, as was previously stated. The sample size representing tocopherol content is acceptable ($n$=9), but could still be improved as samples such as almonds have exceptionally large sample sizes ($n$=90). Furthermore in the Database,

pecan samples are of unknown cultivar, growing region/conditions, crop year, and handling conditions, which have been shown to have considerable effects on nutrient composition. Overall a more comprehensive look of pecans that includes many different cultivars, crop years, *etc*. should be included to supply a fully complete Database.

## 2.4 Phytosterol Overview

### *2.4.1 Phytosterols*

Phytosterols and phytostanols, also known as plant sterols and stanols, are triterpene compounds that are found in the cell membranes of plants that have similar structure and function to cholesterol in animals.[67] Phytosterols and phytostanols are differentiated by their degree of unsaturation; phytostanols are fully saturated, whereas phytosterols are not. The sterol/stanol make-up in plant tissue is typically ~98% sterols to ~2% stanols.[68] The term phytosterols technically refers to three different classifications based on the number of methyl groups found at the C4 position in the sterol. These classifications include 4-desmethyl- (no groups), 4-methyl- (one group), and 4,4′-dimethyl- (two groups) sterols.[67] The group that is usually referred to when talking about sterols in food is the 4-desmethyl sterols, whereas the other two are typically precursors to the 4-desmethyl sterols, but are often still found in small quantities in plant tissues.[21,67]

The main purpose of phytosterols is as a functional membrane component, regulating fluidity and allowing phospholipid and protein membrane interaction.[67] Furthermore, they act as substrates for secondary metabolites and assist in cellular, developmental and membrane-associated metabolic processes.[69] In plants, phytosterols exist in four different conjugates: free sterols, esterified sterols, steryl glucosides, and acylated steryl glucosides, which are shown in

Figure 2.4.[67] The distribution of the steryl conjugates mentioned is highly dependent on the type of food being analyzed. Three sterols (β-sitosterol, campesterol, and sitgmasterol) are commonly the dominant sterol components found in plants.[21] Furthermore, these sterols can be present in any of the four conjugates mentioned; therefore, analysis techniques incorporating all four conjugates is necessary for a truly complete sterol profile of the analyzed food.

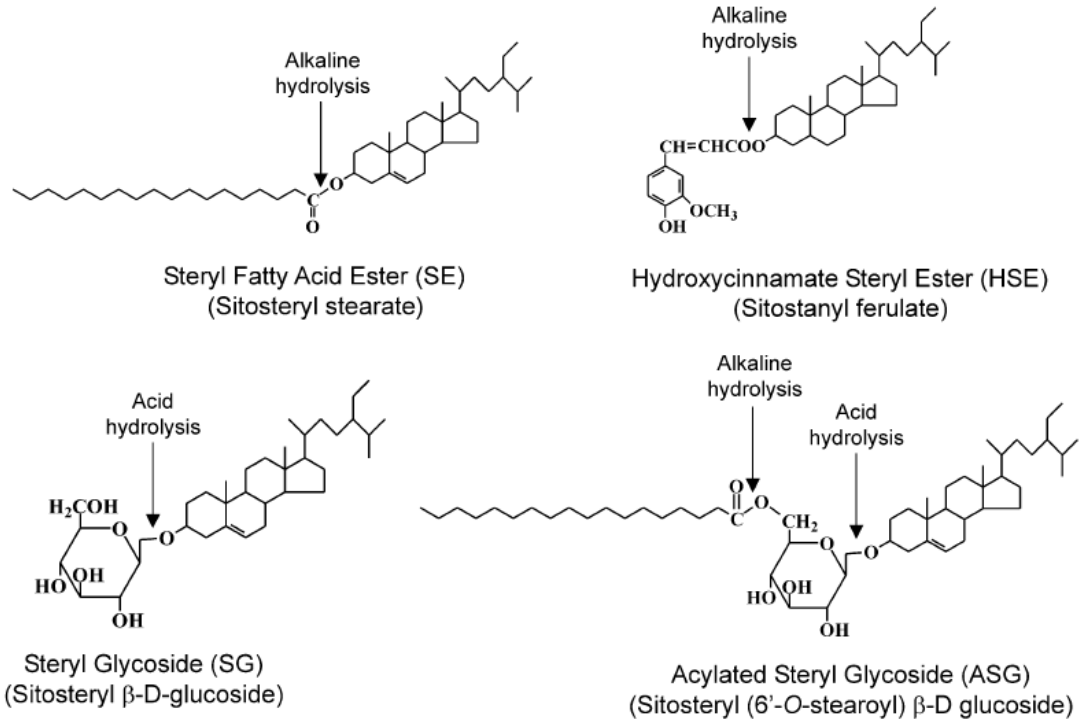


**Figure 2.4**: Chemical Structure of Phytosterol Conjugates; arrows indicate sites of acid and alkaline hydrolysis; reproduced with permission from Moreau, *et al*. [67]

*2.4.2 Health Benefits of Phytosterols*

Phytosterols are a class of lipid bioactives that have been correlated to many different beneficial health effects. The most established and well-known health benefit of phytosterols is a link between sterol consumption and reduction of LDL and total serum cholesterol, which can help to reduce risk for CHD.[15,16,69] One particular feeding study by Ostlund *et al.* [16] reported that

consumption of 300 mg sterols resulted in a 28% decrease in cholesterol absorption. Phytosterols reduce cholesterol by blocking the absorption of cholesterol in the body, potentially by out-competing cholesterol at absorption sites.[67,70] Because phytosterols are poorly absorbed in the body, they are re-excreted in bile, and thus keep serum sterol levels low.[67] Phytosterols have also been found to have anti-inflammatory, anticancer, and anti-tumor properties.[71] Awad and Fink [72] reported that phytosterols have the potential to combat cancer *via* inhibiting cell division, modifying hormones related to tumor growth, and helping stimulate tumor cell death. Furthermore, other nutrients and bioactives including fiber, vitamin D, retinol and flavonoids have been associated with consumption of phytosterol-rich foods.[17]

*2.4.3 Phytosterol Analysis*

Several types of phytosterol methods have been developed including methods for phytosterol extraction, hydrolysis and quantification of total sterols, and identification of phytosterols.[67] In general phytosterols are extracted from a food *via* the same methods that are used for lipid extraction.[67,69] In order to quantify phytosterols various hydrolysis steps must be employed. The developed AOCS method for cholesterol analysis includes direct saponification and extraction of unsaponifiables as recommended by Klatt *et al.*[73] This is effective for analyzing free and esterified sterols, but not the glucosides, making it an incomplete method. Phillips *et al.* [68] showed that total sterol content can be underestimated up to 37%, without the inclusion of steryl glucosides. This proves that all sterol conjugates must be included in analyses for a true determination of total phytosterols. Several methods [21,74,75] suggest the addition of an acid hydrolysis step in order to hydrolyze the acetal bond between the sterol and carbohydrate moiety,

thus including the steryl glucosides in the determination of total phytosterols. The different sites of hydrolysis can be seen in Figure 2.4.

Some problems with certain acid-labile glucosides have been determined in sterol analysis. One study found that $\Delta^5$-avenasterol and fucosterol are unstable in acidic conditions.[76,77] Since then, methods have been developed to quantify these acid-labile sterols. Phillips *et al.* [68] suggested using solid phase extraction (SPE) to separate glucosides and free sterols, and assaying them separately. Derivatization is then done to adjust boiling point of the compounds being analyzed so that they will volatilize when injected into the GC. The glucosides are derivatized directly, whereas the free sterols are saponified and then derivatized; the combined value of free sterols and glucosides gives an accurate quantification of $\Delta^5$-avenasterol. Both the total sterols and glucosides can be quantified by the use of TMS-ether derivatization and GC, as is shown in several different methods,[68,74,75,77] and gives a sufficient overall picture of the phytosterol profile of foods.

### *2.4.4 Phytosterols in Pecans*

The phytosterol contents of pecans have been reported in several different studies. Total phytosterol content ranged from 113 to 276 mg/100-g oil.[13,20,21,78] The wide range is a result of different extraction and quantification methods as well as whether or not steryl glucosides are included. As with most plants, the main sterol components in pecans have been determined to be β-sitosterol, campesterol, and stigmasterol, with $\Delta^5$-avenasterol also contributing a significant portion.[20,78] Upon comparison with other seeds and tree nuts, pecans fall in the middle range of phytosterol content, with relative similarity to almond, cashew, macadamia, and black walnut.[21] Values can also be affected by the aforementioned horticultural

factors, as is seen with cultivar in peanuts [42] and almonds.[43] One study monitored sterol changes over the stages of pecan ripening, noting that phytosterol contents decreased with increasing ripening.[46] Unfortunately, additional studies related to the effects of horticultural factors on phytosterols in pecans are limited, as is noted by Eitenmiller and Pegg.[26]

The USDA National Nutrient Database for Standard Reference, Release 27, reports a total phytosterol content of ~179.5 mg/100-g oil.[24] This value does reflect the inclusion of steryl glucosides, but references an extremely small sample size (*n*=3). The Database also only mentions minor sterol contributions in a footnote, though they are an integral part of the phytosterol profile of pecans. No indication of cultivars, crop years, or any other factors that could affect composition are referenced for the assayed sample; therefore, indicating additions and updates are needed to show an accurate phytosterol value in pecans. The Database should not be expected to detail the contents and composition of all potential cultivars, but a mean value including multiple different commonly consumed cultivars and various crop years would be a better overall indicator of true phytosterol composition.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Sample Preparation

### *3.1.1 Chemicals and Glassware*

Glass wool and ACS hexanes were purchased from Fischer Scientific Co., LLC (Suwanee, GA, USA).

### *3.1.2 Collection of Samples*

Twenty different pecan cultivars were collected from various test orchards in Tifton, GA, over three months. Collection was completed for two different crop years, 2012 and 2013. Samples were shipped to UGA Department of Food Science and Technology raw and in-shell. Upon arrival samples were vacuum-sealed (Henkelman 600, Henkelman BV, The Netherlands) in labeled pouches (Sealed Air Corporation, Elmwood Park, NJ, USA) and stored at -80 °C until further analysis. Prior to analysis pecans were removed from the freezer, shelled, and nutmeat was collected. Approximately 20 g were collected and then refrozen overnight at -80 °C to facilitate grinding. The nut samples were removed from the freezer and immediately ground into a fine powder using a coffee mill (Grind Central Coffee Grinder, Cuisinart, East Windsor, NJ, USA). Immediate grinding was performed in order to keep oil from leeching out, and to ensure uniform particle size.

*3.1.3 Lipid Extraction*

Lipids were extracted using a Soxhlet apparatus. Approximately 20 g of the pecan 'powder' were placed into a cellulose extraction thimble (single thickness, 43 mm I.D. and 123 mm external length, Whatman International Ltd., Maidstone, England) and the mass was recorded. Glass wool was inserted into the top of the thimble in order to keep the sample in place during extraction. Extraction was performed using ~250 mL hexanes per sample over ~18 h. After extraction, the oil was evaporated to dryness using a Büchi Rotavapor R-210 (Büchi Corporation, New Castle, DE, USA) and hexanes were collected and reused for further samples. All hexanes were either fresh or used exclusively on pecans. The lipid extracts were transferred to amber vials, flushed with $N_2$, capped, and stored at -80 °C until further analysis.

**3.2 Tocopherols**

*3.2.1 Chemicals and Glassware*

HPLC-grade hexanes and isopropanol were purchased from Fischer Scientific Co., LLC (Suwanee, GA, USA). Butylated hydroxytoluene (BHT) was procured from MP Biomedicals, LLC (Solon, OH, USA).

*3.2.2 Tocopherol and Tocotrienol Analysis*

Sample Preparation: The lipid extracts were allowed to warm to room temperature prior to analysis. One gram of oil was dissolved in a BHT-spiked mobile phase (0.85% IPA in hexanes with 0.01% BHT) and brought to volume in a 5-mL volumetric flask. The BHT was used in order to prevent vitamin E degradation during sample preparation. The samples were filtered into HPLC vials through a 13-mm metal filter attached to a glass syringe using 0.45-μm nylon filter

paper (Millipore, Billerica, MA, USA). The vials were filled and immediately capped in order to avoid $O_2$ exposure. Additionally all procedures were carried out under yellow light in order to prevent oxidation.

HPLC Quantitation*:* Method: The oil samples were injected into an HPLC system comprised of a Shimadzu CBM-20A Prominence communications bus module, DG-14A degasser, LC-10AT controller/pump, RF-10AXL fluorescence detector, and EZ Start chromatography software (Shimadzu Corp., Columbia, MD, USA). An isocratic mobile phase composed of 0.85% (v/v) isopropanol in hexanes at a flow rate of 0.8 mL/min was employed. Prior to use in the system, the mobile phase was vacuum filtered *via* a 0.45-µm nylon filter (MSI, Westboro, MA, USA). A normal phase LiChrosorb Si 60 column (4 mm x 250 mm, 5-µm particle size; Hibar Fertigsäule RT, Merck, Darmstadt, Germany) in combination with a LiChroCART 4-4 guard column packed with LiChrospher Si 60 (5 µm) was used for analysis. Wavelengths for excitation and emission in tocopherol and tocotrienol analysis were 290 and 330 nm, respectively. Twenty microliters of sample were injected *via* a Rheodyne loop per run with a sampling frequency of 2 Hz. Samples were quantified using tocopherol standard curves constructed with pure α-, β-, γ-, and δ-tocopherol standards and the areas of the peak tocopherol responses. A 20-µL mixed tocopherol standard was injected each day for standard curve verification.

### 3.3 Phytosterols

#### *3.3.1 Chemicals and Glassware*

Ethanol (95%), 5-mL Reacti-vials, magnetic stir bars, ACS-grade hexanes, ether, and methanol, were purchased from Fischer Scientific Co., LLC (Suwanee, GA, USA). ACS-grade

hydrochloric acid, potassium hydroxide, pyridine, and toluene were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). ACS-grade pyrogallol (99%) was obtained from the Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Plant sterol standards including β-sitosterol, stigmasterol, campesterol, and steryl glucosides (all 98% purity), and 5α-cholestane (the internal standard; purity >98%) were purchased from Matreya, LLC (Pleasant Gap, PA, USA). The silanizing reagent, 5% dimethyldichlorosilane (DMDCS) in toluene, was procured from Supleco Chemical Co. (Bellefonte, PA, USA) and the derivatization reagent, N,O-*bis* (trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS), was acquired from Thermo Scientific (Rockford, IL, USA).

*3.3.2 Phytosterol Analysis*

Sample Preparation: All glassware was silanized with 5% DMDCS in toluene and then rinsed three times with toluene followed by a final rinse of anhydrous methanol. Glassware was air dried for 24 h prior to experimentation.

An acid hydrolysis step in addition to saponification was used in order to ensure full sterol extraction. Both the acid hydrolysis step and the saponification step were done according to the method of Shin *et al.*[42] Briefly for the acid hydrolysis, 1 mL of internal standard (0.5 mg/mL of 5α-cholestane in hexanes) was pipetted into a glass culture tube and evaporated to dryness with an N-EVAP (Organomation Associates, Inc., Berlin, MA, USA). Approximately 300 to 500 mg of oil were weighed into each tube and then ethanol (1 mL) was added to dissolve both the oil and internal standard. The tubes were shaken and sonicated for about two min to ensure full dissolution. Next 5 mL of 6M HCL were added, the tubes were flushed with $N_2$ to protect against sample degradation, and then capped. The tubes were placed in a OLS200 Grant

combined orbital/linear shaking water bath (Grant Instruments Cambridge, UK) at 80 °C for 1 h. Upon removal, the tubes were cooled for approximately 15 min before continuing analysis. Ethanol (5 mL) was added to each tube and the contents were then vortexed for about 30 s. In order to separate the organic solvent layer, 7 mL of a 1:1 (v/v) mixture diethyl ether:hexanes were added to the tubes, in addition to 12 mL of deionized water, as a washing step. The organic layer was then extracted and transferred to a new tube. This process was repeated a total of three times, and extracts were pooled and evaporated to dryness at 40 °C using the N-EVAP.

For the saponification step, the dried hydrolysis extract was re-suspended in 8 mL of 3% (w/v) pyrogallol in ethanol. Saturated KOH (0.5 mL) was added, tubes were flushed with $N_2$, tightly capped and heated in a shaking water bath at 80 °C for 30 min. When saponification was complete, tubes were allowed to cool. Once cool, 12 mL of deionized water were added followed by 7 mL of 1:1 mixture diethyl ether:hexanes. The organic layer was extracted in triplicate, then the three extracts were pooled and evaporated to dryness at 40 °C using the N-EVAP.

Sterols are not volatile compounds and therefore must be derivatized to trimethylsilyl (TMS) ethers in order to carryout GC-FID analysis. The dried, saponified sterol residues were re-suspended in ~3 mL hexanes, transferred to 5-mL Reacti-vials, and evaporated to dryness with the N-EVAP. Once dried, 250 µL of anhydrous pyridine, followed by 250-µL BSTFA + 1% TMCS, and a Reacti-vial magnetic stirrer were added to the Reacti-vials. The vials were then immediately capped and placed into a Reacti-Block B-1 aluminum block, preheated to 70 °C *via* a Reacti-Therm III Heating/Stirring Module (Thermo Fischer Scientific, Rockford, IL, USA). Vials were maintained at 70 °C with gentle stirring for ~1 h. After derivatization, the samples were cooled in a cool Reacti-Block B-1 aluminum block and evaporated to dryness with the N-EVAP. Once the samples were dried, the residues were re-suspended in 1.0-mL hexanes. To

fully purify the sterol extracts, the residues were filtered with 0.45-µm syringe filters (MSI, Westboro, MA, USA) into GC vials, and capped.

One particular sterol, $\Delta^5$-avenasterol, is acid labile and is degraded during acid hydrolysis.[68] The steryl glycosides associated with this sterol must therefore be analyzed separately. The glycosides were isolated with solid phase extraction (SPE), derivatized, and quantified *via* the method of Shin *et al.*[42] Briefly, a Sep-Pak classic silica cartridge (500 mg, 6 cc, 80-µm particle size; Waters Corp., Milford, MA, USA) was conditioned with two cartridge volumes of $CHCl_3$. Approximately 100 mg of the lipid extract were dissolved in $CHCl_3$ and quantitatively transferred to the cartridge, and then the column was washed with an additional 2 mL of $CHCl_3$ in order to elute the free and esterified sterols. The steryl glucosides were then eluted using ~ 4 mL of $CH_3OH$ and were then dried with the N-EVAP at 50 °C. The fraction was then derivatized using 250 µL of anhydrous pyridine and 250-µL BSTFA + 1% TMCS reagent, and incubated at 75 °C in a Reacti-Block B-1 aluminum block for 2 h. The TMS ethers were analyzed by GC-FID as described below. A separate portion of the pecan lipid extract was subjected to alkaline hydrolysis alone, derivatized, and then assayed for free and esterified sterols. The total content of $\Delta^5$-avenasterol was calculated by summing the quantity of steryl glucosides determined by the SPE assay with those found *via* saponification. As there is no commercial standard for $\Delta^5$-avenasterol or its derivatives, $\Delta^5$-avenasterol was quantified by constructing a calibration curve using a steryl glucoside standard mixture containing sitosteryl, campesteryl, and stigmasteryl glucosides (catalog no. 1117, purity 98%) from Matreya LLC.

GC Quantitation: GC-FID analysis was carried out using an Agilent Technologies 6890N GC system with a HP-5 capillary column (30 m × 0.32 mm *i.d.*, 0.25-µm film thickness). Ultra high-purity helium was used as the carrier gas and was under constant flow mode at a flow rate

of 1.9 mL/min. The fuel gases used for the FID were ultra high-purity hydrogen and scientific-grade air with flow rates of 30 and 300 mL/min, respectively. For each run, 1 μL of sample was injected with an injection temperature of 300 °C and a 50:1 split ratio. A temperature ramp of 3 °C/min was employed with a starting temperature of 260 °C, a final temperature of 300 °C, and a 5-min hold at the final temperature (300°C). The total time of the analysis was roughly 18 min. Quantification was achieved using relative response factors (RRFs) generated using the response of 5α-cholestane in five replicate analyses. The RRFs were calculated as follows:

$$RRF = \frac{PA_{sterol}}{W_{sterol}} \times \frac{W_{IS}}{PA_{IS}}$$

where, $PA_{sterol}$ is the peak area of the sterol, $W_{sterol}$ is the mass (mg) of the sterol, $PA_{IS}$ is the peak are of internal standard, and $W_{IS}$ is the mass of the internal standard. Because 5α-cholestane RRFs were generated, it was then used as the internal standard as per the methods reported by Robbins *et al.*[13] The equation below was used.

Sterols (mg/100-g oil)

$$= \frac{PA_{sterol}}{PA_{IS}} \times \frac{1}{RRF} \times \frac{W_{IS}}{W_{sample}} \times 100$$

where, $PA_{sterol}$ is the peak area of the sterol, $PA_{IS}$ is the peak are of internal standard, $W_{IS}$ is the mass of the internal standard, $W_{sample}$ is the mass of the tree nut oil. Some sterols such as $\Delta^5$-avenasterol, and other minor sterol constituents were quantified using the response of β-sitosterol being that no commercial standards were available.

### 3.4 Statistical Analysis

Both phytosterol and tocopherol contents were reported as mean ± standard deviation (SD) (mg/100-g oil). Each different cultivar was analyzed in triplicate for each assay employed.

Cultivar and crop year effects were investigated using a two-way or general linear model ANOVA with randomized blocking by year. Significant differences between means *(p*<0.05) were determined using Tukey's HSD multiple comparisons test. Analysis incorporating both crop year and cultivar for all samples as well as analysis comparing cultivars against themselves from each crop year were also performed. All analysis was completed using Statistical Analysis System software, Version 9.0 (SAS Institute, Cary, NC).

### 3.5 Method Validation

Both tocopherol and phytosterol methods were validated using SRM 2387 (peanut butter), as no tree nut standard reference material (SRM) is available from NIST. Each assay was replicated five times and data was compared against certified values. The bias, relative standard deviation (%$RSD_r$), and percent accepted values were calculated *via* the following equations:

bias = accepted value – analytical value

%$RSD_r$ = standard deviation/mean x 100

% accepted value = (analytical value x 100) /accepted value

For tocopherols, specifically, validation was done using α-, β-, γ-, and δ-tocopherol standards to determine calibration curve linearity. Standard solutions of five different concentrations ranging from 0.01 to 0.09 mg/mL were prepared for each tocopherol isomer and the assay was performed in triplicate. Standard addition done with both the sample and the peanut butter SRM was used to determine reproducibility. This was completed by the use of known quantities of each tocopherol isomer at three different concentrations. Recovery was then calculated *via* the following equation:

$$R(\%) = \frac{C_s - C_p}{C_a} \text{ x } 100$$

where, *R* (%) is the percent recovery of added standard, $C_s$ is the tocopherol content in the spiked sample, $C_p$ is the tocopherol concentration in the non-spiked sample, and $C_a$ is the amount of tocopherol standard added. Limits of detection (LODs) and limits of quantification (LOQs) were also calculated for each tocopherol.

For phytosterol methodology, specifically, validation was done using 5α-cholestane (IS), campesterol, stigmasterol, and β-sitosterol standards to determine standard curve linearity. Standard solutions at five different concentrations were prepared for each sterol, and triplicate analysis was completed. The standard solutions were ranged from 0.2 to 1.0 for 5α-cholestane, 0.025 to 0.125 for campesterol, 0.12 to 0.60 for stigmasterol, and 0.2 to 1.0 µg/1-µL injection for β-sitosterol. Phytosterol recoveries *R* (%) were determined *via* spiking of the peanut butter SRM, and were calculated in the same way as the tocopherol recoveries. The LODs and LOQs for each sterol were calculated on the basis of a minimal accepted value of a signal-to-noise ratio of three and ten, respectively.

# CHAPTER 4

# RESULTS

## 4.1 Method Validation

High-purity tocopherol standards (Cat. No. 613424, Calbiochem) were prepared using the method of Lee *et al.* [65] All of the tocopherol homologs had very high standard curve linearity ($r^2$ >0.9999). The tocopherol standard recoveries ranged from 100.4 to 107.4% and SRM recoveries ranged from 97.2 to 106.6%. Repeatability precision (%$RSD_r$) ranged from 2.1 to 6.2%, bias ranged from 0.2 to 2.0, and percent accepted values ranged from 97 to 106%. The LOD values for α-, β-, γ-, and δ-tocopherol were 0.035, 0.039, 0.035, and 0.023 μg/mL, respectively and the LOQ values were 0.12, 0.13, 0.12, and 0.079 μg/mL, respectively. The FID responses of the IS (5α-cholestane), campesterol, stigmasterol, and β-sitosterol for the concentrations measured also had very high linearity ($r^2$ >0.9999). The samples spiked with campesterol, stigmasterol, or β-sitosterol had mean percent recoveries of 98.4 ± 2.2, 97.5 ± 4.3, and 98.7 ± 4.7, respectively. These relatively high recoveries help to demonstrate that no significant loss of phytosterols occurs when the pecan oils are subjected to acid hydrolysis. Repeatability precision (%$RSD_r$) from intraday analyses of the samples was found to be between 1.5 and 1.9%. The LOD values for campesterol, stigmasterol, and β-sitosterol, were 0.19, 0.21, and 0.23 μg/mL, respectively and the LOQ values were 0.59, 0.51, and 0.52 μg/mL, respectively.

**4.2 Tocopherol Analysis**

The twenty different cultivars studied were all analyzed in order to determine α-, β-, γ-, and δ-tocopherol and tocotrienol contents in 2012 (Table 4.1.1) and 2013 (Table 4.1.2). Standard curves developed from commercial standards were used for tocopherol quantification. A curve was established for each homolog and the linearity of all curves was found to be ~0.99. No tocotrienols were detected in any of the tested samples, and this can be seen in Figure 4.1. Additionally cultivars were compared to themselves, within crop year to determine which cultivars varied more year-to-year (Table 4.1.3). Comparisons of all components from year-to-year were also determined (Table 4.1.4). The results show the influence of cultivar and crop year on the tocopherol content of pecans.

The α-tocopherol content ranged from 0.83 mg/100-g oil in 'Curtis' to 1.91 mg/100-g oil in 'Stuart' in 2012, and 0.73 mg/100-g oil in 'Schley' to 1.91 mg/100-g oil in 'Stuart' in 2013. If both crop years are included α-tocopherol content ranged from 0.81 mg/100-g oil in 'Schley' to 1.91 mg/100-g oil in 'Stuart.' The mean using all cultivars and crop years was determined to be 1.18 ± 0.32 mg/100-g oil. The cultivar with the highest amount of α-tocopherol was found to be 'Stuart' in both 2012 and 2013; in fact, it was significantly ($p$<0.05) different from all other cultivars. 'Caddo' and 'Wichita' were the cultivars that were significantly different ($p$<0.05) from themselves between crop years.

The β-tocopherol content in 2012 ranged from 0.01 mg/100-g oil in 'Zinner' to 0.77 mg/100-g oil in 'Elliot' and was not detected in 'Excel.' In 2013, β-tocopherol content ranged from 0.01 mg/100-g oil in 'Caddo' to 0.54 mg/100-g oil in 'Elliot' with no detection in 'Wichita' and 'Excel.' If both crop years are included, data ranged from 0.02 mg/100-g oil in 'Wichita' to 0.65 mg/100-g oil in 'Elliot' and no detection in 'Excel' The all-inclusive mean was determined

to be 0.22 ± 0.20 mg/100-g oil. 'Elliot' had the highest amount of β-tocopherol for both crop years tested and was significantly ($p$<0.05) different than all other cultivars except 'Caddo,' 'Kiowa,' and 'Schley.' Cultivars that were significantly different ($p$<0.05) from themselves between crop years included 'Kiowa' and 'Zinner.'

In 2012, the γ-tocopherol content was found to range from 17.35 mg/100-g oil in 'Excel' to 35.61 mg/100-g oil in 'Lakota', and in 2013 it ranged from 20.94 mg/100-g oil in 'Caddo' to 33.57 mg/100-g oil in 'Western Schley.' The overall range for both crop years was from 19.31 to 34.11 mg/100-g oil in 'Excel' and 'Western Schley,' respectively. The mean including all crop years and cultivars was 25.97 ± 3.88 mg/100-g oil. One noteworthy observation is that 'Western Schley' was either the highest or second highest in both tested crop years. Two cultivars, 'Western Schley' and 'Excel' had relevant significant ($p$<0.05) differences from the other cultivars. 'Western Schley' was different from all cultivars except 'Byrd,' and 'Lakota,' while 'Excel' was different from all cultivars except 'Amling,' 'Caddo,' 'Stuart,' and 'Wichita.' Cultivars that were significantly different ($p$<0.05) from themselves between crop years included 'Byrd,' 'Caddo,' 'Elliot,' 'Forkhert,' 'Kiowa,' and 'Lakota.'

The 2012 δ-tocopherol ranged from 0.01 to 0.13 mg/100-g oil in 'Stuart' and 'Elliot,' respectively. Additionally there were several cultivars ('Amling', 'Byrd' 'Curtis,' 'Excel,' 'Forkhert,' 'Kiowa,' 'McMillian,' 'Western Schley,' and 'Wichita') where δ-tocopherol was not detected. In 2013, δ-tocopherol ranged from 0.04 mg/100-g oil in 'Lakota' and 0.11 mg/100-g oil in 'Caddo,' with 'Pawnee,' 'Stuart,' and the previously stated cultivars having no detection. The range with all crop years and cultivars included was 0.01 mg/100-g oil in 'Stuart' to 0.10 mg/100-g oil in 'Elliot,' and the cultivars mentioned in the 2012 summary all showed no detection. The overall mean was determined to be 0.031 ± 0.035 mg/100-g oil. 'Elliot' was

found to be significantly ($p$<0.05) different from all cultivars except for ‘Caddo;’ ‘Caddo’ in turn was only similar to ‘Cape Fear,’ ‘Desirable,’ and ‘Schley.’ Cultivars that were significantly different ($p$<0.05) from themselves between crop years included ‘Elliot’ and ‘Pawnee.’

Lastly, the total tocopherol content in 2012 was lowest in ‘Excel’ (18.58 mg/100-g oil) and greatest in ‘Lakota’ (36.74 mg/100-g oil). In 2013 the total tocopherol content was lowest in ‘Caddo’ (22.23 mg/100-g oil) and highest in ‘Western Schley’ (35.13 mg/100-g oil). With both crop years included, ‘Excel’ (20.54 mg/100-g oil) was lowest in total tocopherols and ‘Western Schley’ (35.69 mg/100-g oil) was highest. The mean of all cultivars and crop years was determined to be 27.40 ± 3.96 mg/100-g oil. Cultivars that were significantly different ($p$<0.05) from themselves between crop years included ‘Byrd,’ ‘Caddo,’ ‘Elliot,’ ‘Forkhert,’ and ‘Lakota.’ Additionally, ‘Western Schley’ was had the highest or second highest total tocopherol content in both crop years. It was also found to be significantly ($p$<0.05) different than all other cultivars except ‘Lakota.’ This is reflective of γ-tocopherol, which makes sense because it comprises ~95% of the tocopherols found in the pecan. One other observation of note is that tocopherol content was significantly ($p$<0.05) different between crop years for all homologs, with the 2012 crop year having higher content overall.

### 4.3 Phytosterol Analysis

Approximately nine different phytosterols (namely campesterol, stigmasterol, clerosterol, β-sitosterol, $\Delta^5$-avenasterol + β-sitostanol, $\Delta^{5,24(25)}$-stigmastadienol, cycloartenol, 24-methylene-cycloartanol, and citrostadienol) were detected in the 20 different examined cultivars in 2012 (Table 4.2.1 and 4.2.2) and 2013 (Table 4.2.3 and 4.2.4). The five most prominent sterols, in descending order, were β-sitosterol, cycloartenol, $\Delta^5$-avenasterol + β-sitostanol, citrostadienol,

and campesterol. These five sterols were detected in all 20 cultivars, whereas the other four (namely clerosterol, stigmasterol, $\Delta^{5,24(25)}$-stigmastadienol, and 24-methylene-cycloartanol) were only found in selected cultivars. A representative chromatogram is depicted in Figure 4.2. Variations between the same cultivar in the different crop years were also examined in both major (Table 4.2.5) and minor phytosterols (Table 4.2.6). Lastly, differences between crop years of each component were also reported (Table 4.1.4).

In crop year 2012 the most prominent sterol, β-sitosterol, ranged from 78.80 in 'Western Schley' to 138.6 mg/100-g oil in 'Lakota.' In crop year 2013, it ranged from 65.08 in 'Curtis' to 121.2 mg/100-g oil in Wichita. When both crop years are included β-sitosterol ranged from 81.84 to 120.7 mg/100-g oil in 'Cape Fear' and 'Lakota,' respectively. The overall average including both crop years was calculated to be 100.7 ± 10.26 mg/100-g oil. No cultivar was found to be significantly different ($p$<0.05) from four or less other cultivars, but content was found to be significantly ($p$<0.05) different between crop years. Cultivars that were significantly different ($p$<0.05) from themselves across the two crop years include 'Cape Fear,' 'Curtis,' 'Desirable,' 'Forkhert,' 'Kiowa,' 'Lakota,' 'Pawnee,' 'Sumner,' 'Western Schley,' and 'Wichita.'

Cycloartenol, the second most prominent sterol, ranged from 14.23 in 'Desirable' to 34.77 mg/100-g oil in 'McMillian' in crop year 2012. In 2013, cycloartenol ranged from 10.46 in 'Curtis' to 33.45 mg/100-g oil in 'Schley.' If both 2012 and 2013 crop years are included, cycloartenol content ranged from 14.69 to 33.67 mg/100-g oil. The mean with all cultivars and crop years included was determined to be 22.38 ± 4.64 mg/100-g oil. 'Schley' was found to be significantly ($p$<0.05) different from all other cultivars except 'McMillian' and 'Pawnee.' Moreover, it was determined that the cycloaretnol content varied significantly ($p$<0.05) year-to-

year. Cultivars that were not significantly different ($p>0.05$) from themselves between the two crop years include 'Caddo,' 'Desirable,' 'Elliot,' 'Forkhert,' 'Oconee,' and 'Schley.' All others were different between the 2012 and 2013 crop year.

$\Delta^5$-Avenasterol + β-sitostanol content, the third most prominent sterol, was lowest in 'Desirable' at 11.34 mg/100-g oil and greatest in 'Byrd' at 28.19 mg/100-g oil in 2012, and in 2013 it was lowest in 'Desirable' at 12.08 mg/100-g oil and highest in 'Byrd' at 33.21 mg/100-g oil. In all cultivars and crop years, $\Delta^5$-avenasterol + β-sitostanol ranged from 11.34 in 'Desirable' to 33.21 mg/100-g oil in 'Byrd.' The mean including all variables was found to be 22.07 ± 4.21 mg/100-g oil. Overall in both 2012 and 2013 'Byrd' had the greatest $\Delta^5$-avenasterol + β-sitostanol content, and 'Desirable' had the lowest. No cultivar was found to be significantly different ($p<0.05$) from four or less other cultivars nor was crop year found to be significantly different ($p<0.05$). Additionally, no cultivars were determined to be significantly different ($p<0.05$) from themselves between crop years in this particular sterol.

In 2012, the citrostadienol content ranged from 6.83 in 'Zinner' to 14.44 mg/100-g oil in 'Oconee.' In 2013, it ranged from 5.55 in 'Desirable' to 12.98 mg/100-g oil in 'Byrd.' If both crop years are included the data ranges from 6.47 in 'Desirable' to 11.82 mg/100-g oil in 'Byrd.' The overall mean citrostadienol content was determined to be 9.39 ± 1.56 mg/100-g oil. No cultivar was found to be significantly different ($p<0.05$) from four or less other cultivars, but content was found to vary significantly ($p<0.05$) between the two crop years. Cultivars that were not significantly different ($p>0.05$) from themselves between the two crop years include 'Amling,' 'Elliot,' 'Excel,' 'Kiowa,' 'McMillian,' 'Pawnee,' 'Schley,' 'Stuart,' and 'Zinner.' All others were different between the 2012 and 2013 crop year.

Campesterol content in 2012 ranged from 1.65 to 6.08 mg/100-g oil in 'Western Schley' and 'Curtis,' respectively. Both 'Excel' and 'Zinner' showed no detection of campesterol in 2012. The range in 2013 was from 1.71 to 5.28 mg/100-g oil in 'Curtis' and 'Wichita,' respectively. Campesterol was undetected in one cultivar, 'Forkhert,' in 2013. The range when including both 2012 and 2013 was found to be from 1.74 to 5.46 mg/100-g oil in 'Zinner' and 'Kiowa,' respectively. The mean including all factors was 4.80 ± 1.20 mg/100-g oil. One interesting observation is that 'Curtis' is highest in one year and lowest in the other, which indicates high variability. No cultivar was found to be significantly different ($p$<0.05) from four or less other cultivars on top of campesterol content not being significantly ($p$<0.05) different the two crop years. Cultivars that were significantly different ($p$<0.05) from themselves between the two crop years include 'Curtis,' 'Excel,' 'Forkhert,' and 'Zinner.'

Stigmasterol and clerosterol were detected in only select cultivars. Stigmasterol was determined in 'Byrd,' 'Curtis,' 'Lakota,' 'Stuart,' and 'Zinner,' in 2012 and in 'Amling,' 'Cape Fear,' 'Elliot,' 'Oconee,' 'Wichita,' and 'Western Schley,' for 2013. Values in 2012 ranged from 0.99 in 'Curtis' to 5.05 mg/100-g oil in 'Byrd.' Values in 2013 ranged from 1.50 in 'Amling' to 8.77 mg/100-g oil in 'Cape Fear.' There were no cultivars that were significantly ($p$<0.05) different than four or less cultivars. Clerosterol was detected in 'Western Schley' and 'Forkhert,' in 2012, and in 'Byrd,' 'Cape Fear,' 'Curtis,' 'McMillian,' and 'Sumner,' in 2013. Values in 2012 ranged from 2.63 to 14.75 mg/100-g oil in 'Forkhert' and 'Western Schley,' respectively, and in 2013 values ranged from 1.11 to 27.67 mg/100-g oil in 'Cape Fear' and 'Curtis,' respectively. Clerosterol content in 'Curtis' was determined to be significantly ($p$<0.05) different from all other cultivars except 'Western Schley.' These two sterols were undetected in all unmentioned cultivars. Overall averages were not reported due to the high amount of non-

detection. It was also noted that stigmasterol and clerosterol contents were not significantly different ($p<0.05$) between crop years 2012 and 2013. Cultivars that were significantly different ($p<0.05$) from themselves between the two crop years include 'Byrd,' 'Cape Fear,' 'and 'Western Schley,' for stigmasterol and 'Curtis,' 'McMillian,' and 'Western Schley,' for clerosterol.

Two phytosterols, $\Delta^{5,24(25)}$-stigmastadienol and 24-methylene-cycloartanol, were only found in two or less cultivars per year. In 2012, $\Delta^{5,24(25)}$-stigmastadienol was only detected in the two cultivars 'Schley' (1.43 mg/100-g oil) and 'McMillian' (3.22 mg/100-g oil), and in 2013 it is was only detected in Amling (1.11 mg/100-g oil). 'McMilian' was significantly ($p<0.05$) different from all other cultivars except 'Amling' and 'Schley.' $\Delta^{5,24(25)}$-Stigmastadienol content was determined to be not significantly different ($p>0.05$) for crop years 2012 and 2013. 24-Methylene-cycloartanol was detected in only one cultivar, 'Pawnee' (2.50 mg/100-g oil) in 2012, and was not detected in any cultivars in 2013. As Pawnee was the only cultivar with 24-methylene-cycloartanol detection, it was found to be significantly ($p<0.05$) different than all other cultivars. It was also noted that crop years 2012 and 2013 were significantly ($p<0.05$) different in terms of 24-methylene-cycloartanol content. The overall averages for these sterols were not reported because of non-detection skewing the data. Cultivars that were significantly different ($p<0.05$) from themselves between the two crop years include 'Amling,' 'McMillian,' 'and 'Schley,' for $\Delta^{5,24(25)}$-stigmastadienol and 'Pawnee' for 24-methylene-cycloartanol.

The total phytosterol content for each year was also determined. In 2012 the total phytosterol content was lowest in 'Zinner' (137.8 mg/100-g oil) and greatest in 'Lakota' (211.3 mg/100-g oil). In 2013 the total phytosterol content was lowest in 'Cape Fear' (120.7 mg/100-g oil) and highest in 'Byrd' (183.1 mg/100-g oil). With both crop years included, 'Cape Fear'

(138.0 mg/100-g oil) was lowest in total phytosterols and ‘Lakota’ (184.2 mg/100-g oil) was highest. The mean of all cultivars and crop years was determined to be 161.0 ± 14.35 mg/100-g oil. Additionally, no total phytosterol content was found to be significantly ($p$<0.05) different than from four or less other cultivars. Though no one cultivar stands out from the rest, the two crop years, 2012 and 2013, were found to be significantly ($p$<0.05) different from one another in terms of total phytosterols. Cultivars that were significantly different ($p$<0.05) from themselves between the two crop years include ‘Amling,’ ‘Cape Fear,’ ‘Curtis,’ ‘Desirable,’ ‘Forkhert,’ ‘Kiowa,’ ‘Lakota,’ ‘Oconee,’ ‘Pawnee,’ and ‘Wichita.’

**Table 4.1.1**: Tocopherol Content of 20 Different 2012 Pecan Cultivars (mg/100-g oil, *n*=3)[a]

| Sample | α-Tocopherol | β-Tocopherol | γ-Tocopherol | δ-Tocopherol | Total |
|---|---|---|---|---|---|
| Amling | 0.92 ± 0.01 | 0.25 ± 0.003 | 21.05 ± 0.27 | 0.002 ± 0.001 | 22.23 ± 0.28 |
| Byrd | 1.02 ± 0.03 | 0.07 ± 0.003 | 26.08 ± 0.54 | nd | 27.17 ± 0.57 |
| Caddo | 1.53 ± 0.04 | 0.57 ± 0.01 | 26.11 ± 0.54 | 0.08 ± 0.01 | 28.28 ± 0.60 |
| Cape Fear | 1.14 ± 0.04 | 0.32 ± 0.03 | 29.50 ± 1.38 | 0.07 ± 0.01 | 31.03 ± 1.45 |
| Curtis | 0.83 ± 0.04 | 0.14 ± 0.02 | 26.02 ± 1.42 | nd | 27.00 ± 1.47 |
| Desirable | 1.53 ± 0.04 | 0.32 ± 0.01 | 26.65 ± 0.65 | 0.08 ± 0.005 | 28.58 ± 0.71 |
| Elliot | 1.38 ± 0.02 | 0.77 ± 0.01 | 28.69 ± 0.31 | 0.13 ± 0.01 | 30.97 ± 0.33 |
| Excel | 1.23 ± 0.01 | nd | 17.35 ± 0.20 | nd | 18.58 ± 0.21 |
| Forkert | 1.41 ± 0.05 | 0.23 ± 0.02 | 27.44 ± 1.29 | nd | 29.08 ± 1.37 |
| Kiowa | 1.56 ± 0.02 | 0.71 ± 0.01 | 27.95 ± 0.50 | nd | 30.22 ± 0.53 |
| Lakota | 0.87 ± 0.01 | 0.19 ± 0.01 | 35.61 ± 0.44 | 0.07 ± 0.004 | 36.74 ± 0.46 |
| McMillan | 1.34 ± 0.01 | 0.08 ± 0.002 | 24.20 ± 0.03 | nd | 25.62 ± 0.03 |
| Oconee | 1.51 ± 0.03 | 0.27 ± 0.01 | 29.88 ± 0.59 | 0.03 ± 0.004 | 31.68 ± 0.62 |
| Pawnee | 0.87 ± 0.04 | 0.22 ± 0.02 | 24.11 ± 1.07 | 0.04 ± 0.01 | 25.24 ± 1.14 |
| Schley | 0.89 ± 0.04 | 0.54 ± 0.03 | 27.47 ± 1.18 | 0.06 ± 0.005 | 28.95 ± 1.25 |
| Stuart | 1.91 ± 0.03 | 0.22 ± 0.01 | 24.42 ± 0.36 | 0.01 ± 0.01 | 26.56 ± 0.40 |
| Sumner | 1.01 ± 0.01 | 0.03 ± 0.004 | 23.78 ± 0.13 | 0.05 ± 0.02 | 24.86 ± 0.14 |
| W. Schley | 1.49 ± 0.00 | 0.07 ± 0.01 | 34.66 ± 0.07 | 0.004 ± 0.005 | 36.22 ± 0.07 |
| Wichita | 1.40 ± 0.03 | 0.04 ± 0.01 | 22.24 ± 0.47 | nd | 23.68 ± 0.49 |
| Zinner | 1.11 ± 0.02 | 0.01 ± 0.01 | 27.54 ± 0.58 | 0.06 ± 0.01 | 28.71 ± 0.62 |

[a]*n*= number of independent original samples; data represent the mean ± standard deviation.

**Table 4.1.2:** Tocopherol Content of 20 Different 2013 Pecan Cultivars (mg/100-g oil, *n*=3)[a]

| Sample | α-Tocopherol | β-Tocopherol | γ-Tocopherol | δ-Tocopherol | Total |
|---|---|---|---|---|---|
| Amling | 0.73 ± 0.02 | 0.19 ± 0.01 | 22.86 ± 0.30 | nd | 23.77 ± 0.33 |
| Byrd | 0.82 ± 0.08 | 0.09 ± 0.02 | 32.71 ± 3.30 | nd | 33.62 ± 3.40 |
| Caddo | 0.94 ± 0.04 | 0.25 ± 0.02 | 20.94 ± 0.71 | 0.11 ± 0.01 | 22.23 ± 0.78 |
| Cape Fear | 0.82 ± 0.54 | 0.15 ± 0.02 | 26.43 ± 1.66 | 0.07 ± 0.02 | 27.47 ± 2.04 |
| Curtis | 1.17 ± 0.05 | 0.24 ± 0.01 | 27.50 ± 1.17 | 0.001 ± 0.002 | 28.90 ± 1.24 |
| Desirable | 1.37 ± 0.01 | 0.07 ± 0.01 | 24.68 ± 0.22 | 0.05 ± 0.01 | 26.17 ± 0.25 |
| Elliot | 1.03 ± 0.16 | 0.54 ± 0.12 | 24.28 ± 4.35 | 0.08 ± 0.03 | 25.93 ± 4.66 |
| Excel | 1.24 ± 0.15 | nd | 21.26 ± 2.76 | nd | 22.50 ± 2.91 |
| Forkert | 1.35 ± 0.02 | 0.24 ± 0.02 | 22.20 ± 0.43 | nd | 23.80 ± 0.47 |
| Kiowa | 1.36 ± 0.27 | 0.23 ± 0.02 | 25.80 ± 0.57 | nd | 27.39 ± 0.83 |
| Lakota | 0.98 ± 0.06 | 0.13 ± 0.01 | 24.83 ± 1.12 | 0.04 ± 0.03 | 25.98 ± 1.22 |
| McMillan | 1.07 ± 0.07 | 0.01 ± 0.00 | 24.66 ± 1.53 | nd | 25.74 ± 1.60 |
| Oconee | 1.39 ± 0.08 | 0.14 ± 0.01 | 27.16 ± 1.00 | 0.04 ± 0.01 | 28.74 ± 1.08 |
| Pawnee | 0.88 ± 0.03 | 0.19 ± 0.01 | 25.46 ± 0.67 | nd | 26.52 ± 0.70 |
| Schley | 0.73 ± 0.46 | 0.53 ± 0.02 | 23.61 ± 0.69 | 0.08 ± 0.01 | 24.95 ± 1.02 |
| Stuart | 1.91 ± 0.05 | 0.17 ± 0.02 | 21.74 ± 0.59 | nd | 23.83 ± 0.66 |
| Sumner | 0.86 ± 0.00 | 0.22 ± 0.02 | 26.83 ± 0.14 | 0.05 ± 0.01 | 27.97 ± 0.15 |
| W. Schley | 1.52 ± 0.01 | 0.06 ± 0.01 | 33.57 ± 0.12 | 0.004 ± 0.005 | 35.16 ± 0.12 |
| Wichita | 0.91 ± 0.08 | 0.004 ± 0.01 | 24.22 ± 1.98 | nd | 25.13 ± 2.07 |
| Zinner | 1.08 ± 0.04 | 0.45 ± 0.77 | 27.45 ± 0.91 | 0.04 ± 0.01 | 29.03 ± 0.21 |

[a]*n*= number of independent original samples; data represent the mean ± standard deviation.

**Table 4.1.3**: Tocopherol Content of 20 Pecan Cultivars in Two Crop Years (mg/100-g oil)

| Cultivar | α-Tocopherol[b] | β-Tocopherol | γ-Tocopherol | δ-Tocopherol | Total |
|---|---|---|---|---|---|
| Amling | | | | | |
| 2012 (*n*=3) | 0.92 ± 0.01a | 0.25 ± 0.003a | 21.05 ± 0.27a | nd | 22.23 ± 0.28a |
| 2013 (*n*=3) | 0.73 ± 0.02a | 0.19 ± 0.01a | 22.86 ± 0.30a | nd | 23.77 ± 0.33a |
| Byrd | | | | | |
| 2012 (*n*=3) | 1.02 ± 0.03a | 0.07 ± 0.003a | 26.08 ± 0.54a | nd | 27.17 ± 0.57a |
| 2013 (*n*=3) | 0.82 ± 0.08a | 0.09 ± 0.02a | 32.71 ± 3.30b | nd | 33.62 ± 3.40b |
| Caddo | | | | | |
| 2012 (*n*=3) | 1.53 ± 0.04a | 0.57 ± 0.01a | 26.11 ± 0.54a | 0.08 ± 0.01a | 28.28 ± 0.60a |
| 2013 (*n*=3) | 0.94 ± 0.04b | 0.25 ± 0.02a | 20.94 ± 0.71b | 0.11 ± 0.01a | 22.23 ± 0.78b |
| Cape Fear | | | | | |
| 2012 (*n*=3) | 1.14 ± 0.04a | 0.32 ± 0.03a | 29.50 ± 1.38a | 0.07 ± 0.01a | 31.03 ± 1.45a |
| 2013 (*n*=3) | 0.82 ± 0.54a | 0.15 ± 0.02a | 26.43 ± 1.66a | 0.07 ± 0.02a | 27.47 ± 2.04a |
| Curtis | | | | | |
| 2012 (*n*=3) | 0.83 ± 0.04a | 0.14 ± 0.02a | 26.02 ± 1.42a | nd | 27.00 ± 1.47a |
| 2013 (*n*=3) | 1.17 ± 0.05a | 0.24 ± 0.01a | 27.50 ± 1.17a | nd | 28.90 ± 1.24a |
| Desirable | | | | | |
| 2012 (*n*=3) | 1.53 ± 0.04a | 0.32 ± 0.01a | 26.65 ± 0.65a | 0.08 ± 0.005a | 28.58 ± 0.71a |
| 2013 (*n*=3) | 1.37 ± 0.01a | 0.07 ± 0.01a | 24.68 ± 0.22a | 0.05 ± 0.01a | 26.17 ± 0.25a |
| Elliot | | | | | |
| 2012 (*n*=3) | 1.38 ± 0.02a | 0.77 ± 0.01a | 28.69 ± 0.3a | 0.13 ± 0.01a | 30.97 ± 0.33a |
| 2013 (*n*=3) | 1.03 ± 0.16a | 0.54 ± 0.12a | 24.28 ± 4.35b | 0.08 ± 0.03a | 25.93 ± 4.66b |
| Excel | | | | | |
| 2012 (*n*=3) | 1.23 ± 0.01a | nd | 17.35 ± 0.20a | nd | 18.58 ± 0.21a |
| 2013 (*n*=3) | 1.24 ± 0.15a | nd | 21.26 ± 2.76a | nd | 22.50 ± 2.91a |
| Forkhert | | | | | |
| 2012 (*n*=3) | 1.41 ± 0.05a | 0.23 ± 0.02a | 27.44 ± 1.29a | nd | 29.08 ± 1.37a |
| 2013 (*n*=3) | 1.35 ± 0.02a | 0.24 ± 0.02a | 22.20 ± 0.43b | nd | 23.80 ± 0.47b |
| Kiowa | | | | | |
| 2012 (*n*=3) | 1.56 ± 0.02a | 0.71 ± 0.01a | 27.95 ± 0.50a | nd | 30.22 ± 0.53a |
| 2013 (*n*=3) | 1.36 ± 0.27a | 0.23 ± 0.02b | 25.80 ± 0.57b | nd | 27.39 ± 0.83a |
| Lakota | | | | | |
| 2012 (*n*=3) | 0.87 ± 0.01a | 0.19 ± 0.01a | 35.61 ± 0.44a | 0.07 ± 0.004a | 36.74 ± 0.46a |
| 2013 (*n*=3) | 0.98 ± 0.06a | 0.13 ± 0.01a | 24.83 ± 1.12b | 0.04 ± 0.03a | 25.98 ± 1.22b |
| McMillian | | | | | |
| 2012 (*n*=3) | 1.34 ± 0.01a | 0.08 ± 0.002a | 24.20 ± 0.03a | nd | 25.62 ± 0.03a |
| 2013 (*n*=3) | 1.07 ± 0.07a | 0.01 ± 0.00a | 24.66 ± 1.53a | nd | 25.74 ± 1.60a |
| Oconee | | | | | |
| 2012 (*n*=3) | 1.51 ± 0.03a | 0.27 ± 0.01a | 29.88 ± 0.59a | 0.03 ± 0.004a | 31.68 ± 0.62a |
| 2013 (*n*=3) | 1.39 ± 0.08a | 0.14 ± 0.01a | 27.16 ± 1.00a | 0.04 ± 0.01a | 28.74 ± 1.08a |
| Pawnee | | | | | |
| 2012 (*n*=3) | 0.87 ± 0.04a | 0.22 ± 0.02a | 24.11 ± 1.07a | 0.04 ± 0.01a | 25.24 ± 1.14a |
| 2013 (*n*=3) | 0.88 ± 0.03a | 0.19 ± 0.01a | 25.46 ± 0.67a | ndb | 26.52 ± 0.70a |
| Schley | | | | | |
| 2012 (*n*=3) | 0.89 ± 0.04a | 0.54 ± 0.03a | 27.47 ± 1.18a | 0.06 ± 0.005a | 28.95 ± 1.25a |
| 2013 (*n*=3) | 0.73 ± 0.46a | 0.53 ± 0.02a | 23.61 ± 0.69a | 0.08 ± 0.01a | 24.95 ± 1.02a |
| Stuart | | | | | |
| 2012 (*n*=3) | 1.91 ± 0.03a | 0.22 ± 0.01a | 24.42 ± 0.36a | 0.01 ± 0.01a | 26.56 ± 0.40a |
| 2013 (*n*=3) | 1.91 ± 0.05a | 0.17 ± 0.02a | 21.74 ± 0.59a | nd | 23.83 ± 0.66a |

| | | | | | |
|---|---|---|---|---|---|
| Sumner | | | | | |
| 2012 (*n*=3) | 1.01 ± 0.01a | 0.03 ± 0.004a | 23.78 ± 0.13a | 0.05 ± 0.02a | 24.86 ± 0.14a |
| 2013 (*n*=3) | 0.86 ± 0.003a | 0.22 ± 0.02a | 26.83 ± 0.14a | 0.05 ± 0.01a | 27.97 ± 0.15a |
| W. Schley | | | | | |
| 2012 (*n*=3) | 1.49 ± 0.004a | 0.07 ± 0.01a | 34.66 ± 0.07a | nd | 36.22 ± 0.07a |
| 2013 (*n*=3) | 1.52 ± 0.01a | 0.06 ± 0.01a | 33.57 ± 0.12a | nd | 35.16 ± 0.12a |
| Wichita | | | | | |
| 2012 (*n*=3) | 1.40 ± 0.03a | 0.04 ± 0.01a | 22.24 ± 0.47a | nd | 23.68 ± 0.49a |
| 2013 (*n*=3) | 0.91 ± 0.08b | nda | 24.22 ± 1.98a | nd | 25.13 ± 2.07a |
| Zinner | | | | | |
| 2012 (*n*=3) | 1.11 ± 0.02a | 0.01 ± 0.01a | 27.54 ± 0.58a | 0.06 ± 0.01a | 28.71 ± 0.62a |
| 2013 (*n*=3) | 1.08 ± 0.04a | 0.45 ± 0.77b | 27.45 ± 0.91a | 0.04 ± 0.01a | 29.03 ± 0.21a |

[a]*n*= number of samples;data represents mean ± SD of each cultivar using year as a blocking variable.
[b]Letters that are different for production years within a cultivar indicate that the means are significantly different by Tukey's multiple-range test ($p<0.05$).

**Table 4.1.4**: Overall Means of Each Bioactive Constituent per Crop Year (mg/100-g oil, $n$=60)[a]

| Bioactive Compound | 2012[b] | 2013 |
|---|---|---|
| α-tocopherol | 1.25a | 1.11b |
| β-tocopherol | 0.25a | 0.20b |
| γ-tocopherol | 26.54a | 25.41b |
| δ-tocopherol | 0.033a | 0.028b |
| **Total Tocopherols** | **28.07a** | **26.74b** |
| campesterol | 4.24a | 3.92a |
| stigmasterol | 1.00a | 0.61a |
| clerosterol | 1.87a | 0.87a |
| β-sitosterol | 104.6a | 96.71b |
| $\Delta^5$-avenasterol +β-sitostanol[c] | 22.37a | 21.77a |
| $\Delta^{5,24(25)}$-stigmastadienol | 0.23a | 0.055b |
| cycloartenol | 25.55a | 19.21b |
| 24-methylene-cycloartanol | 0.13a | ndb |
| citrostadienol | 9.84a | 8.94b |
| **Total Phytosterols** | **167.8a** | **154.1b** |

[a]$n$= number of samples;data represents mean ± SD of each cultivar using year as a blocking variable.
[b]Letters that are different in the same row indicate that the means are significantly different by Tukey's multiple-range test ($p$<0.05).
[c]Peaks are listed together as they often co-elute.

**Table 4.2.1**: Phytosterol Content of 20 Different 2012 Pecan Cultivars (mg/100-g oil, *n*=3)[a]

| Sample | Campesterol | Stigmasterol | Clerosterol | β-sitosterol | $\Delta^5$-avenasterol +β-sitostanol[b] | Total[c] |
|---|---|---|---|---|---|---|
| Amling | 5.08 ± 0.08 | nd | nd | 106.0 ± 1.32 | 27.02 ± 0.79 | 177.0 ± 3.30 |
| Byrd | 2.49 ± 3.52 | 5.05 ± 7.14 | nd | 100.4 ± 2.39 | 28.19 ± 1.06 | 173.3 ± 1.30 |
| Caddo | 4.89 ± 0.23 | nd | nd | 104.1 ± 4.48 | 20.13 ± 1.14 | 161.0 ± 4.03 |
| Cape Fear | 5.10 ± 0.21 | nd | nd | 96.84 ± 3.75 | 16.28 ± 1.07 | 155.2 ± 8.20 |
| Curtis | 6.08 ± 0.12 | 0.99 ± 1.40 | nd | 112.3 ± 0.88 | 24.53 ± 0.46 | 182.6 ± 1.18 |
| Desirable | 5.27 ± 0.11 | nd | nd | 112.3 ± 3.12 | 11.34 ± 0.51 | 150.6 ± 4.93 |
| Elliot | 5.34 ± 0.01 | nd | nd | 101.5 ± 1.33 | 12.34 ± 0.04 | 153.8 ± 2.09 |
| Excel | 0.00 ± 0.00 | nd | nd | 92.80 ± 1.27 | 24.99 ± 0.54 | 157.3 ± 1.83 |
| Forkert | 4.24 ± 0.01 | nd | 2.63 ± 3.73 | 99.60 ± 0.06 | 25.02 ± 0.02 | 166.7 ± 5.61 |
| Kiowa | 5.84 ± 0.28 | nd | nd | 125.2 ± 5.60 | 27.21 ± 0.82 | 191.0 ± 7.46 |
| Lakota | 5.42 ± 0.15 | 1.20 ± 1.69 | nd | 138.6 ± 3.97 | 20.98 ± 0.74 | 211.3 ± 4.12 |
| McMillan | 4.37 ± 0.03 | nd | nd | 102.3 ± 0.01 | 20.70 ± 0.30 | 175.6 ± 0.23 |
| Oconee | 4.97 ± 0.25 | nd | nd | 112.3 ± 6.25 | 15.22 ± 0.80 | 166.2 ± 8.24 |
| Pawnee | 5.24 ± 0.13 | nd | nd | 122.4 ± 4.50 | 22.21 ± 0.58 | 192.7 ± 6.51 |
| Schley | 5.53 ± 0.25 | nd | nd | 107.3 ± 7.06 | 24.14 ± 1.49 | 183.6 ± 15.04 |
| Stuart | 4.14 ± 0.02 | 2.06 ± 2.91 | nd | 90.96 ± 4.49 | 23.11 ± 0.28 | 153.7 ± 1.29 |
| Sumner | 5.36 ± 0.14 | nd | nd | 118.5 ± 1.95 | 23.65 ± 0.27 | 173.9 ± 2.01 |
| W. Schley | 1.65 ± 2.33 | nd | 14.75 ± 0.84 | 78.80 ± 2.65 | 26.65 ± 0.43 | 147.1 ± 2.79 |
| Wichita | 3.75 ± 0.18 | nd | nd | 87.01 ± 4.43 | 20.48 ± 0.93 | 146.6 ± 7.32 |
| Zinner | 0.00 ± 0.00 | 2.84 ± 4.02 | nd | 82.86 ± 3.90 | 21.27 ± 0.98 | 137.8 ± 1.63 |

[a]*n*= number of samples; data represent the mean ± standard deviation.
[b]Peaks are listed together as they often co-elute.
[c]Total includes both major and minor sterol contents.

**Table 4.2.2**: Minor Phytosterol Content of 20 Different 2012 Pecan Cultivars (mg/100-g oil, *n*=3)[a]

| Sample | $\Delta^{5,24(25)}$-Stigmastadienol | Cycloartenol | 24-Methylene-cycloartanol | Citrostadienol | Minor Total[b] |
|---|---|---|---|---|---|
| Amling | nd | 29.11 ± 0.80 | nd | 9.74 ± 0.32 | 38.85 ± 1.12 |
| Byrd | nd | 26.58 ± 1.30 | nd | 10.65 ± 0.16 | 37.23 ± 1.14 |
| Caddo | nd | 21.00 ± 2.17 | nd | 10.89 ± 0.36 | 31.89 ± 1.81 |
| Cape Fear | nd | 26.88 ± 2.83 | nd | 10.11 ± 0.35 | 36.99 ± 3.18 |
| Curtis | nd | 27.46 ± 0.45 | nd | 11.20 ± 0.91 | 38.66 ± 1.37 |
| Desirable | nd | 14.23 ± 0.79 | nd | 7.39 ± 0.41 | 21.62 ± 1.20 |
| Elliot | nd | 27.20 ± 0.67 | nd | 7.49 ± 0.11 | 34.69 ± 0.78 |
| Excel | nd | 31.02 ± 0.05 | nd | 8.43 ± 0.07 | 39.45 ± 0.03 |
| Forkert | nd | 25.99 ± 1.68 | nd | 9.21 ± 0.23 | 35.20 ± 1.91 |
| Kiowa | nd | 22.93 ± 0.25 | nd | 9.82 ± 0.51 | 32.76 ± 0.76 |
| Lakota | nd | 31.23 ± 0.71 | nd | 13.81 ± 0.25 | 45.04 ± 0.95 |
| McMillan | 3.22 ± 0.54 | 34.77 ± 1.15 | nd | 10.25 ± 0.13 | 48.24 ± 0.49 |
| Oconee | nd | 19.23 ± 0.36 | nd | 14.44 ± 0.58 | 33.67 ± 0.93 |
| Pawnee | nd | 29.33 ± 1.13 | 2.50 ± 0.15 | 11.00 ± 0.02 | 42.83 ± 1.30 |
| Schley | 1.43 ± 2.02 | 33.88 ± 3.72 | nd | 11.22 ± 0.50 | 46.53 ± 6.25 |
| Stuart | nd | 23.60 ± 4.83 | nd | 9.85 ± 1.69 | 33.45 ± 3.13 |
| Sumner | nd | 16.48 ± 0.19 | nd | 9.89 ± 0.01 | 26.37 ± 0.20 |
| W. Schley | nd | 18.38 ± 0.44 | nd | 6.91 ± 0.75 | 25.29 ± 1.19 |
| Wichita | nd | 27.78 ± 1.06 | nd | 7.60 ± 0.72 | 35.38 ± 1.78 |
| Zinner | nd | 23.95 ± 0.43 | nd | 6.83 ± 0.34 | 30.78 ± 0.77 |

[a]*n* = number of samples; data represent the mean ± standard deviation.
[b]Total includes only sterols listed in this table.

**Table 4.2.3**: Phytosterol Content of 20 Different 2013 Pecan Cultivars (mg/100-g oil, *n*=3)[a]

| Sample | Campesterol | Stigmasterol | Clerosterol | β-sitosterol | $\Delta^5$-avenasterol +β-sitostanol[b] | Total[c] |
|---|---|---|---|---|---|---|
| Amling | 2.54 ± 3.60 | 1.50 ± 2.13 | nd | 94.77 ± 9.32 | 26.91 ± 2.99 | 158.2 ± 8.26 |
| Byrd | 5.22 ± 0.39 | nd | 1.81 ± 2.57 | 111.2 ± 6.15 | 33.21 ± 2.00 | 183.1 ± 6.72 |
| Caddo | 5.08 ± 0.18 | nd | nd | 99.74 ± 4.67 | 15.90 ± 0.55 | 149.4 ± 6.08 |
| Cape Fear | 3.36 ± 0.38 | 8.77 ± 8.21 | 1.11 ± 1.57 | 66.84 ± 10.17 | 16.25 ± 1.49 | 120.7 ± 2.81 |
| Curtis | 1.71 ± 2.42 | nd | 27.67 ± 0.17 | 65.08 ± 2.77 | 19.83 ± 0.05 | 132.8 ± 1.53 |
| Desirable | 4.25 ± 0.45 | nd | nd | 92.32 ± 8.50 | 12.08 ± 0.52 | 130.0 ± 11.73 |
| Elliot | 2.59 ± 3.66 | 1.51 ± 2.14 | nd | 98.58 ± 2.95 | 19.21 ± 0.05 | 153.4 ± 3.95 |
| Excel | 4.50 ± 0.39 | nd | nd | 96.50 ± 5.42 | 22.27 ± 1.68 | 146.0 ± 7.49 |
| Forkert | nd | nd | nd | 83.45 ± 0.19 | 18.27 ± 3.70 | 132.1 ± 5.51 |
| Kiowa | 5.09 ± 0.19 | nd | nd | 107.8 ± 1.53 | 18.57 ± 0.82 | 157.9 ± 2.74 |
| Lakota | 5.19 ± 0.18 | nd | nd | 102.7 ± 2.41 | 20.76 ± 0.56 | 157.2 ± 3.44 |
| McMillan | 4.60 ± 0.30 | nd | 4.06 ± 5.75 | 104.8 ± 3.94 | 29.83 ± 0.87 | 179.7 ± 2.61 |
| Oconee | 5.10 ± 0.53 | 1.81 ± 2.55 | nd | 105.4 ± 9.85 | 24.91 ± 0.93 | 162.3 ± 9.95 |
| Pawnee | 4.41 ± 0.52 | nd | nd | 100.8 ± 10.73 | 25.32 ± 1.76 | 165.8 ± 12.80 |
| Schley | 5.07 ± 0.06 | nd | nd | 98.47 ± 0.55 | 20.61 ± 0.34 | 167.3 ± 0.38 |
| Stuart | 3.96 ± 0.11 | nd | nd | 94.73 ± 0.52 | 23.97 ± 5.93 | 146.2 ± 6.44 |
| Sumner | 5.03 ± 0.07 | nd | 2.87 ± 4.06 | 101.2 ± 1.31 | 23.63 ± 0.56 | 163.8 ± 3.62 |
| W. Schley | 1.90 ± 2.69 | 4.80 ± 0.11 | nd | 95.42 ± 8.67 | 21.88 ± 1.04 | 144.0 ± 8.98 |
| Wichita | 5.28 ± 0.02 | 1.53 ± 2.16 | nd | 121.2 ± 1.93 | 24.92 ± 0.34 | 182.4 ± 0.46 |
| Zinner | 3.48 ± 0.10 | nd | nd | 93.27 ± 0.59 | 28.98 ± 0.08 | 149.1 ± 1.41 |

[a]*n* = number of samples; data represent the mean ± standard deviation.
[b]Peaks are listed together as they often co-elute.
[c]Total includes both major and minor sterol contents.

**Table 4.2.4**: Minor Phytosterol Content of 20 Different 2013 Pecan Cultivars (mg/100-g oil, *n*=3)[a]

| Sample | $\Delta^{5,24(25)}$-Stigmastadienol | Cycloartenol | 24-Methylene-cycloartanol | Citrostadienol | Minor Total[b] |
|---|---|---|---|---|---|
| Amling | 1.11 ± 1.57 | 20.50 ± 1.52 | nd | 10.88 ± 1.72 | 32.49 ± 1.67 |
| Byrd | nd | 18.69 ± 0.44 | nd | 12.98 ± 0.31 | 31.67 ± 0.75 |
| Caddo | nd | 21.37 ± 0.45 | nd | 7.30 ± 0.22 | 28.67 ± 0.67 |
| Cape Fear | nd | 17.66 ± 0.11 | nd | 6.74 ± 0.66 | 24.40 ± 0.55 |
| Curtis | nd | 10.46 ± 0.65 | nd | 8.05 ± 1.35 | 18.51 ± 2.00 |
| Desirable | nd | 15.78 ± 1.71 | nd | 5.55 ± 0.55 | 21.33 ± 2.26 |
| Elliot | nd | 24.61 ± 1.12 | nd | 6.90 ± 0.08 | 31.51 ± 1.04 |
| Excel | nd | 13.06 ± 0.33 | nd | 9.70 ± 0.33 | 22.75 ± 0.01 |
| Forkert | nd | 23.53 ± 0.45 | nd | 6.88 ± 1.55 | 32.21 ± 0.54 |
| Kiowa | nd | 16.91 ± 0.19 | nd | 9.50 ± 0.39 | 26.41 ± 0.20 |
| Lakota | nd | 19.19 ± 0.02 | nd | 9.36 ± 0.27 | 28.55 ± 0.29 |
| McMillan | nd | 24.38 ± 1.73 | nd | 11.99 ± 0.25 | 36.36 ± 1.98 |
| Oconee | nd | 16.17 ± 0.61 | nd | 8.89 ± 0.58 | 25.06 ± 1.19 |
| Pawnee | nd | 24.86 ± 2.83 | nd | 10.43 ± 2.63 | 35.29 ± 0.20 |
| Schley | nd | 33.45 ± 0.61 | nd | 9.64 ± 0.05 | 43.09 ± 0.66 |
| Stuart | nd | 15.27 ± 0.13 | nd | 8.25 ± 0.25 | 23.52 ± 0.13 |
| Sumner | nd | 23.29 ± 1.71 | nd | 7.83 ± 0.21 | 31.12 ± 1.50 |
| W. Schley | nd | 11.01 ± 0.92 | nd | 9.00 ± 0.92 | 20.00 ± 1.84 |
| Wichita | nd | 18.19 ± 0.60 | nd | 11.24 ± 0.02 | 29.42 ± 0.58 |
| Zinner | nd | 15.77 ± 0.62 | nd | 7.62 ± 0.03 | 23.39 ± 0.65 |

[a]*n* = number of samples; data represent the mean ± standard deviation.
[b]Total includes only sterols listed in this table.

**Table 4.2.5**: Phytosterol Content of 20 Pecan Cultivars in Two Crop Years (mg/100-g oil)

| Cultivar[a] | Campesterol[b] | Stigmasterol | Clerosterol | β-sitosterol | $\Delta^5$-avenasterol +β-sitostanol[c] | Total |
|---|---|---|---|---|---|---|
| Amling | | | | | | |
| 2012 (*n*=3) | 5.08 ± 0.08a | nda | nda | 106.0 ± 1.32a | 27.02 ± 0.79a | 177.0 ± 3.30a |
| 2013 (*n*=3) | 2.54 ± 3.60a | 1.50 ± 2.13a | nda | 94.77 ± 9.32a | 26.91 ± 2.99a | 158.2 ± 8.26b |
| Byrd | | | | | | |
| 2012 (*n*=3) | 2.49 ± 3.52a | 5.05 ± 7.14a | nda | 100.4 ± 2.39a | 28.19 ± 1.06a | 173.3 ± 1.30a |
| 2013 (*n*=3) | 5.22 ± 0.39a | ndb | 1.81 ± 2.57a | 111.2 ± 6.15a | 33.21 ± 2.00a | 183.1 ± 6.72a |
| Caddo | | | | | | |
| 2012 (*n*=3) | 4.89 ± 0.23a | nd | nd | 104.1 ± 4.48a | 20.13 ± 1.14a | 161.0 ± 4.03a |
| 2013 (*n*=3) | 5.08 ± 0.18a | nd | nd | 99.74 ± 4.67a | 15.90 ± 0.55a | 149.4 ± 6.08a |
| Cape Fear | | | | | | |
| 2012 (*n*=3) | 5.10 ± 0.21a | nda | nda | 96.84 ± 3.75a | 16.28 ± 1.07a | 155.2 ± 8.20a |
| 2013 (*n*=3) | 3.36 ± 0.38a | 8.77 ± 8.21b | 1.11 ± 1.57a | 66.84 ± 10.17b | 16.25 ± 1.49a | 120.7 ± 2.81b |
| Curtis | | | | | | |
| 2012 (*n*=3) | 6.08 ± 0.12a | 0.99 ± 1.40a | nda | 112.3 ± 0.88a | 24.53 ± 0.46a | 182.6 ± 1.18a |
| 2013 (*n*=3) | 1.71 ± 2.42b | nda | 27.67 ± 0.17b | 65.08 ± 2.77b | 19.83 ± 0.05a | 132.8 ± 1.53b |
| Desirable | | | | | | |
| 2012 (*n*=3) | 5.27 ± 0.11a | nd | nd | 112.3 ± 3.12a | 11.34 ± 0.51a | 150.6 ± 4.93a |
| 2013 (*n*=3) | 4.25 ± 0.45a | nd | nd | 92.32 ± 8.50b | 12.08 ± 0.52a | 130.0 ± 11.73b |
| Elliot | | | | | | |
| 2012 (*n*=3) | 5.34 ± 0.01a | nda | nd | 101.5 ± 1.33a | 12.34 ± 0.04a | 153.8 ± 2.09a |
| 2013 (*n*=3) | 2.59 ± 3.66a | 1.51 ± 2.14a | nd | 98.58 ± 2.95a | 19.21 ± 0.05a | 153.4 ± 3.95a |
| Excel | | | | | | |
| 2012 (*n*=3) | nda | nd | nd | 92.80 ± 1.27a | 24.99 ± 0.54a | 157.3 ± 1.83a |
| 2013 (*n*=3) | 4.50 ± 0.39b | nd | nd | 96.50 ± 5.42a | 22.27 ± 1.68a | 146.0 ± 7.49a |
| Forkhert | | | | | | |
| 2012 (*n*=3) | 4.24 ± 0.01a | nd | 2.63 ± 3.73a | 99.60 ± 0.06a | 25.02 ± 0.02a | 166.7 ± 5.61a |
| 2013 (*n*=3) | ndb | nd | ndb | 83.45 ± 0.19b | 18.27 ± 3.70a | 132.1 ± 5.51b |
| Kiowa | | | | | | |
| 2012 (*n*=3) | 5.84 ± 0.28a | nd | nd | 125.2 ± 5.60a | 27.21 ± 0.82a | 191.0 ± 7.46a |
| 2013 (*n*=3) | 5.09 ± 0.19a | nd | nd | 107.8 ± 1.53b | 18.57 ± 0.82a | 157.9 ± 2.74b |
| Lakota | | | | | | |
| 2012 (*n*=3) | 5.42 ± 0.15a | 1.20 ± 1.69a | nd | 138.6 ± 3.97a | 20.98 ± 0.74a | 211.3 ± 4.12a |
| 2013 (*n*=3) | 5.19 ± 0.18a | nda | nd | 102.7 ± 2.41b | 20.76 ± 0.56a | 157.2 ± 3.44b |

| | | | | | | |
|---|---|---|---|---|---|---|
| McMillian | | | | | | |
| 2012 (*n*=3) | 4.37 ± 0.03a | nd | nda | 102.3 ± 0.01a | 20.70 ± 0.30a | 175.6 ± 0.23a |
| 2013 (*n*=3) | 4.60 ± 0.30a | nd | 4.06 ± 5.75b | 104.8 ± 3.94a | 29.83 ± 0.87a | 179.7 ± 2.61a |
| Oconee | | | | | | |
| 2012 (*n*=3) | 4.97 ± 0.25a | nda | nd | 112.3 ± 6.25a | 15.22 ± 0.80a | 166.2 ± 8.24a |
| 2013 (*n*=3) | 5.10 ± 0.53a | 1.81 ± 2.55a | nd | 105.4 ± 9.85a | 24.91 ± 0.93a | 162.3 ± 9.95b |
| Pawnee | | | | | | |
| 2012 (*n*=3) | 5.24 ± 0.13a | nd | nd | 122.4 ± 4.50a | 22.21 ± 0.58a | 192.7 ± 6.51a |
| 2013 (*n*=3) | 4.41 ± 0.52a | nd | nd | 100.8 ± 10.73b | 25.32 ± 1.76a | 165.8 ± 12.80b |
| Schley | | | | | | |
| 2012 (*n*=3) | 5.53 ± 0.25a | nd | nd | 107.3 ± 7.06a | 24.14 ± 1.49a | 183.5 ± 15.04a |
| 2013 (*n*=3) | 5.07 ± 0.06a | nd | nd | 98.47 ± 0.55a | 20.61 ± 0.34a | 167.3 ± 0.38a |
| Stuart | | | | | | |
| 2012 (*n*=3) | 4.14 ± 0.02a | 2.06 ± 2.91a | nd | 90.96 ± 4.49a | 23.11 ± 0.28a | 153.7 ± 1.29a |
| 2013 (*n*=3) | 3.96 ± 0.11a | nda | nd | 94.73 ± 0.52a | 23.97 ± 5.93a | 146.2 ± 6.44a |
| Sumner | | | | | | |
| 2012 (*n*=3) | 5.36 ± 0.14a | nd | nda | 118.5 ± 1.95a | 23.65 ± 0.27a | 173.9 ± 2.01a |
| 2013 (*n*=3) | 5.03 ± 0.07a | nd | 2.87 ± 4.06a | 101.2 ± 1.31b | 23.63 ± 0.56a | 163.8 ± 3.62a |
| W. Schley | | | | | | |
| 2012 (*n*=3) | 1.65 ± 2.33a | nda | 14.75 ± 0.84a | 78.80 ± 2.65a | 26.65 ± 0.43a | 147.1 ± 2.79a |
| 2013 (*n*=3) | 1.90 ± 2.69a | 4.80 ± 0.11b | ndb | 95.42 ± 8.67b | 21.88 ± 1.04a | 144.0 ± 8.98a |
| Wichita | | | | | | |
| 2012 (*n*=3) | 3.75 ± 0.18a | nda | nd | 87.01 ± 4.43a | 20.48 ± 0.93a | 146.6 ± 7.32a |
| 2013 (*n*=3) | 5.28 ± 0.02a | 1.53 ± 2.16a | nd | 121.2 ± 1.93b | 24.92 ± 0.34a | 182.4 ± 0.46b |
| Zinner | | | | | | |
| 2012 (*n*=3) | nda | 2.84 ± 4.02a | nd | 82.86 ± 3.90a | 21.27 ± 0.98a | 137.8 ± 1.63a |
| 2013 (*n*=3) | 3.48 ± 0.10b | nda | nd | 93.27 ± 0.59a | 28.98 ± 0.08a | 149.1 ± 1.41a |

[a]*n*= number of samples; data represents mean ± SD of each cultivar using year as a blocking variable.
[b]Letters that are different for production years within a cultivar indicate that the means are significantly different by Tukey's multiple-range test ($p<0.05$).
[c]Peaks are listed together as they often co-elute.

**Table 4.2.6**: Minor Phytosterol Content of 20 Pecan Cultivars in Two Crop Years (mg/100-g oil)

| Cultivar[a] | $\Delta^{5,24(25)}$-stigmastadienol[b] | Cycloartenol | 24-methylene-cycloartanol | Citrostadienol | Minor Total |
|---|---|---|---|---|---|
| Amling | | | | | |
| 2012 (*n*=3) | nda | 29.11 ± 0.80a | nd | 9.74 ± 0.32a | 38.85 ± 1.12a |
| 2013 (*n*=3) | 1.11 ± 1.57b | 20.50 ± 1.52b | nd | 10.88 ± 1.72a | 32.49 ± 1.67b |
| Byrd | | | | | |
| 2012 (*n*=3) | nd | 26.58 ± 1.30a | nd | 10.65 ± 0.16a | 37.23 ± 1.14a |
| 2013 (*n*=3) | nd | 18.69 ± 0.44b | nd | 12.98 ± 0.31b | 31.67 ± 0.75b |
| Caddo | | | | | |
| 2012 (*n*=3) | nd | 21.00 ± 2.17a | nd | 10.89 ± 0.36a | 31.89 ± 1.81a |
| 2013 (*n*=3) | nd | 21.37 ± 0.45a | nd | 7.30 ± 0.22b | 28.67 ± 0.67a |
| Cape Fear | | | | | |
| 2012 (*n*=3) | nd | 26.88 ± 2.83a | nd | 10.11 ± 0.35a | 36.99 ± 3.18a |
| 2013 (*n*=3) | nd | 17.66 ± 0.11b | nd | 6.74 ± 0.66b | 24.40 ± 0.55b |
| Curtis | | | | | |
| 2012 (*n*=3) | nd | 27.46 ± 0.45a | nd | 11.20 ± 0.91a | 38.66 ± 1.37a |
| 2013 (*n*=3) | nd | 10.46 ± 0.65b | nd | 8.05 ± 1.35b | 18.51 ± 2.00b |
| Desirable | | | | | |
| 2012 (*n*=3) | nd | 14.23 ± 0.79a | nd | 7.39 ± 0.41a | 21.62 ± 1.20a |
| 2013 (*n*=3) | nd | 15.78 ± 1.71a | nd | 5.55 ± 0.55b | 21.33 ± 2.26a |
| Elliot | | | | | |
| 2012 (*n*=3) | nd | 27.20 ± 0.67a | nd | 7.49 ± 0.11a | 34.69 ± 0.78a |
| 2013 (*n*=3) | nd | 24.61 ± 1.12a | nd | 6.90 ± 0.08a | 31.51 ± 1.04a |
| Excel | | | | | |
| 2012 (*n*=3) | nd | 31.02 ± 0.05a | nd | 8.43 ± 0.07a | 39.45 ± 0.03a |
| 2013 (*n*=3) | nd | 13.06 ± 0.33b | nd | 9.70 ± 0.33a | 22.75 ± 0.01b |
| Forkhert | | | | | |
| 2012 (*n*=3) | nd | 25.99 ± 1.68a | nd | 9.21 ± 0.23a | 35.20 ± 1.91a |
| 2013 (*n*=3) | nd | 23.53 ± 0.45a | nd | 6.88 ± 1.55b | 32.21 ± 0.54a |
| Kiowa | | | | | |
| 2012 (*n*=3) | nd | 22.93 ± 0.25a | nd | 9.82 ± 0.51a | 32.76 ± 0.76a |
| 2013 (*n*=3) | nd | 16.91 ± 0.19b | nd | 9.50 ± 0.39a | 26.41 ± 0.20b |
| Lakota | | | | | |
| 2012 (*n*=3) | nd | 31.23 ± 0.71a | nd | 13.81 ± 0.25a | 45.04 ± 0.95a |
| 2013 (*n*=3) | nd | 19.19 ± 0.02b | nd | 9.36 ± 0.27b | 28.55 ± 0.29b |

| | | | | | |
|---|---|---|---|---|---|
| McMillian | | | | | |
| 2012 (*n*=3) | 3.22 ± 0.54a | 34.77 ± 1.15a | nd | 10.25 ± 0.13a | 48.24 ± 0.49a |
| 2013 (*n*=3) | ndb | 24.38 ± 1.73b | nd | 11.99 ± 0.25a | 36.36 ± 1.98b |
| Oconee | | | | | |
| 2012 (*n*=3) | nd | 19.23 ± 0.36a | nd | 14.44 ± 0.58a | 33.67 ± 0.93a |
| 2013 (*n*=3) | nd | 16.17 ± 0.61a | nd | 8.89 ± 0.58b | 25.06 ± 1.19b |
| Pawnee | | | | | |
| 2012 (*n*=3) | nd | 29.33 ± 1.13a | 2.50 ± 0.15a | 11.00 ± 0.02a | 42.83 ± 1.30a |
| 2013 (*n*=3) | nd | 24.86 ± 2.83b | ndb | 10.43 ± 2.63a | 35.29 ± 0.20b |
| Schley | | | | | |
| 2012 (*n*=3) | 1.43 ± 2.02a | 33.88 ± 3.72a | nd | 11.22 ± 0.50a | 46.53 ± 6.25a |
| 2013 (*n*=3) | ndb | 33.45 ± 0.61a | nd | 9.64 ± 0.05a | 43.09 ± 0.66a |
| Stuart | | | | | |
| 2012 (*n*=3) | nd | 23.60 ± 4.83a | nd | 9.85 ± 1.69a | 33.45 ± 3.13a |
| 2013 (*n*=3) | nd | 15.27 ± 0.13b | nd | 8.25 ± 0.25a | 23.52 ± 0.13b |
| Sumner | | | | | |
| 2012 (*n*=3) | nd | 16.48 ± 0.19a | nd | 9.89 ± 0.01a | 26.37 ± 0.20a |
| 2013 (*n*=3) | nd | 23.29 ± 1.71b | nd | 7.83 ± 0.21b | 31.12 ± 1.50b |
| W. Schley | | | | | |
| 2012 (*n*=3) | nd | 18.38 ± 0.44a | nd | 6.91 ± 0.75a | 25.29 ± 1.19a |
| 2013 (*n*=3) | nd | 11.01 ± 0.92b | nd | 9.00 ± 0.92b | 20.00 ± 1.84b |
| Wichita | | | | | |
| 2012 (*n*=3) | nd | 27.78 ± 1.06a | nd | 7.60 ± 0.72a | 35.38 ± 1.78a |
| 2013 (*n*=3) | nd | 18.19 ± 0.60b | nd | 11.24 ± 0.02b | 29.42 ± 0.58b |
| Zinner | | | | | |
| 2012 (*n*=3) | nd | 23.95 ± 0.43a | nd | 6.83 ± 0.34a | 30.78 ± 0.77a |
| 2013 (*n*=3) | nd | 15.77 ± 0.62b | nd | 7.62 ± 0.03a | 23.39 ± 0.65b |

[a]n= number of samples;data represents mean ± SD of each cultivar using year as a blocking variable.
[b]Letters that are different for production years within a cultivar indicate that the means are significantly different by Tukey's multiple-range test ($p<0.05$).

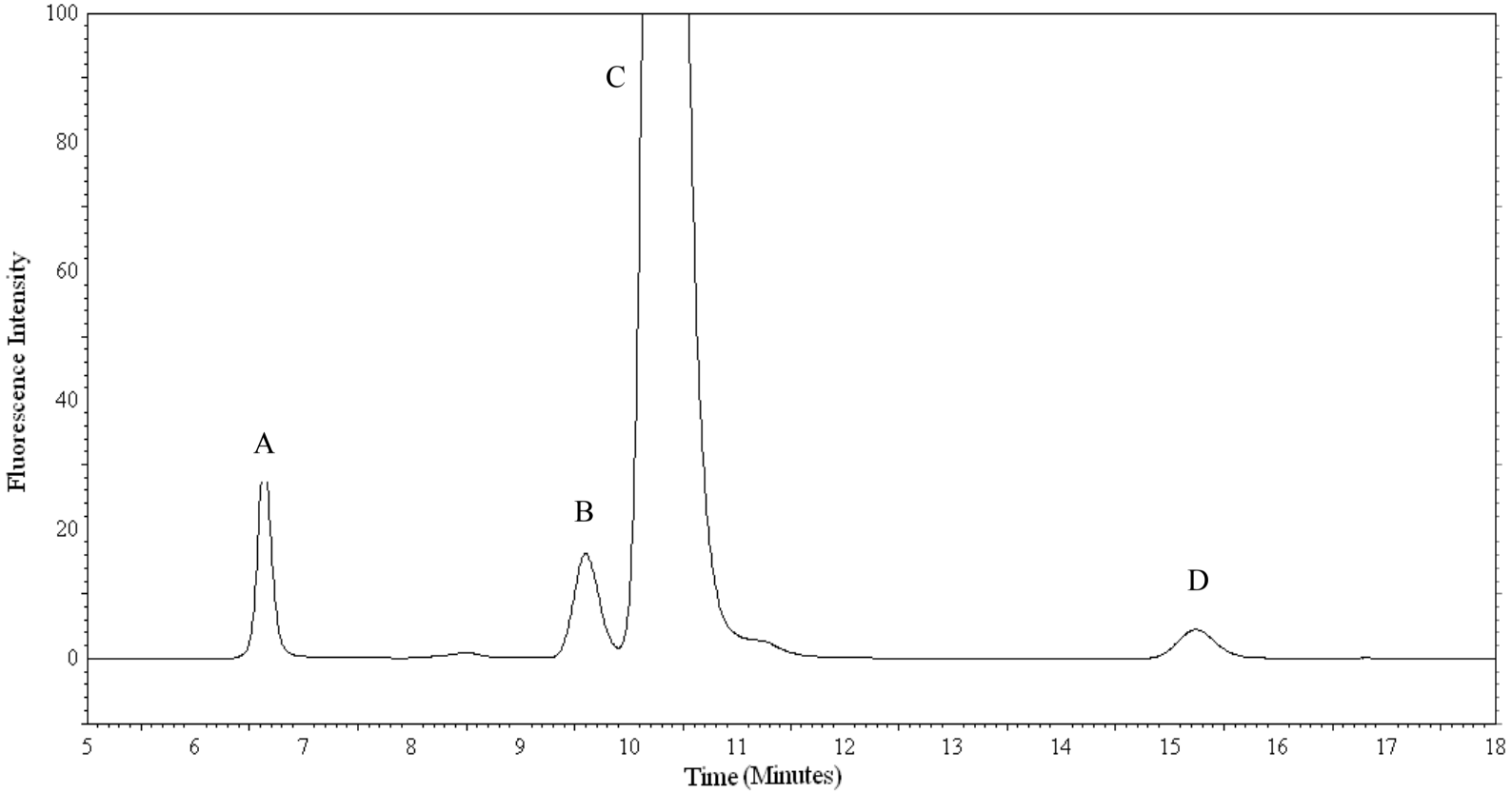


**Figure 4.1:** Representative Chromatogram of Tocopherols in 'Elliot' Pecan. Peaks: A, α-tocopherol; B, β-tocopherol; C, γ-tocopherol; D, δ=tocopherol.

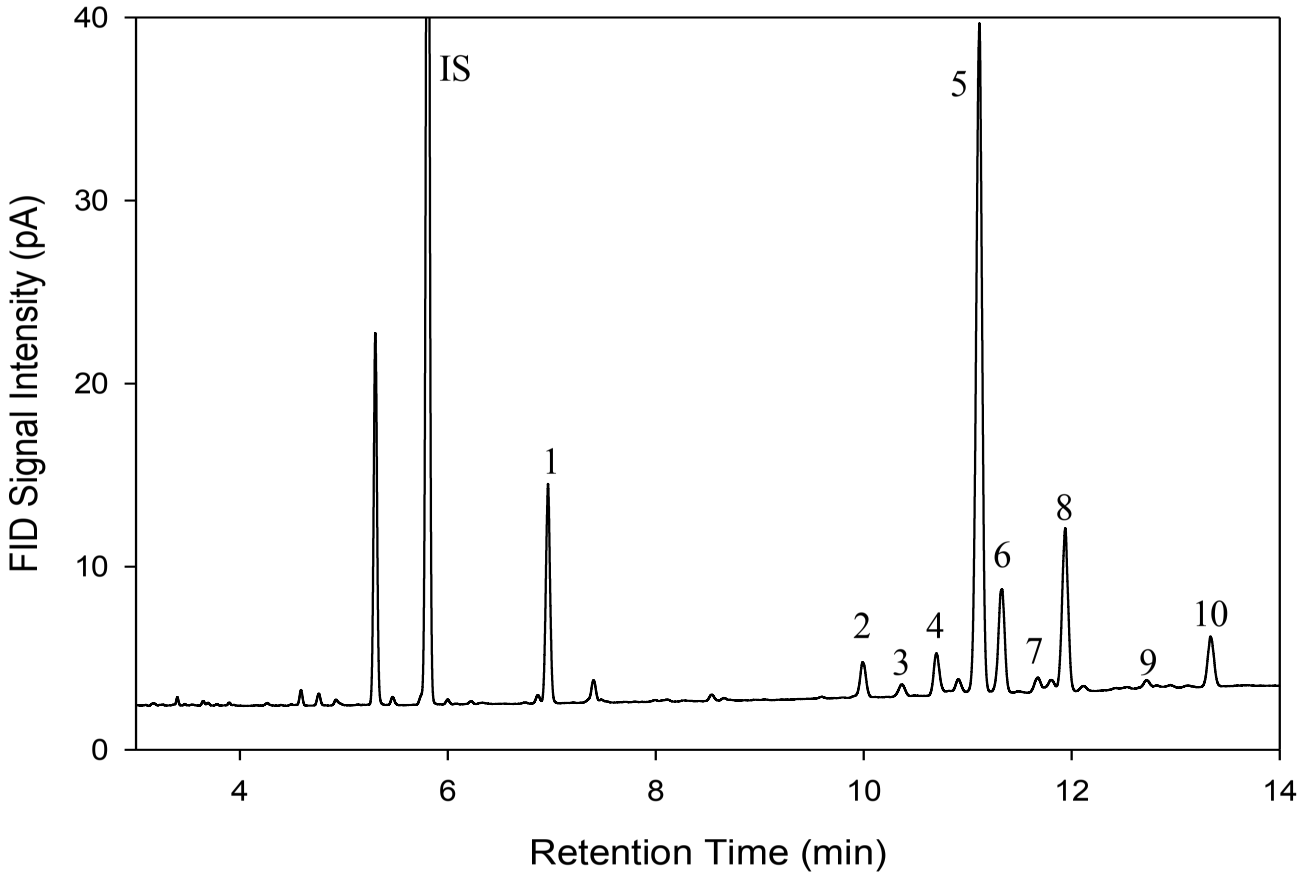


**Figure 4.2:** Representative Chromatogram of TMS Ether Derivatives of Phytosterols in 'Sumner' Pecan [internal standard (IS), 5α-cholestane]. Peaks: 1, fatty acid; 2, campesterol; 3, stigmasterol; 4, clerosterol; 5, β-sitosterol; 6, $\Delta^5$-avenasterol + β-sitostanol (possible coelution); 7, $\Delta^{5,24(25)-}$stigmastadienol; 8, cycloartenol; 9, 24-methylenecycloartenol (below LOD); 10, citrostadienol.

# CHAPTER 5

# DISCUSSION

## 5.1 Tocopherols

The tocopherol values found in this study were compared to similar studies, in addition to values from the USDA National Nutrient Database for Standard Reference, Release 27.[24] The average total tocopherol content found in this study, 27.40 ± 3.96 mg/100-g oil, was similar to the value of 37.10 mg/100-g oil reported in the USDA Database. The same similarity was found for the α-, β-, γ-, and δ-tocopherol homologs with study values of 1.18 ± 0.32, 0.22 ± 0.20, 25.97 ± 3.88, and 0.03 ± 0.03 mg/100-g oil and USDA values of 1.95, 0.54, 33.96, and 0.65 mg/100-g oil, respectively. Though the values in this study are lower, this is likely due to the fact that this study includes a broad range of cultivars and a significantly higher sample size ($n$=120) than the USDA Database ($n$=3).

The data in this study also show some similarities to past tocopherol and pecan studies. The general tendency of the data in this study being lower than past reported data remains true. Robbins *et al.* [13] and Miraliakbari and Shahidi [20] found total tocopherol values of 33.56 and 49.11 mg/100-g oil, respectively, both of which are higher than those reported in this study. Chun *et al.* [18] also reported highly similar data for four different individual cultivars, three of which were the same as in this study. For example, both studies assayed the 'Stuart' cultivar, reporting total tocopherol content values of 25.60 and 25.19 mg/100-g oil, respectively. This tendency could be seen in the tocopherol homologs as well as other cultivar-to-cultivar

comparisons. These cultivar similarities help show that cultivar is a significant factor effecting nutrient composition of the pecan. In addition, an earlier study by Rudolph *et al.* [29] covered many different cultivars and found values that were again higher than those found here (*i.e.*, 30.9 *vs* 27.0 mg/100-g oil for 'Schley'). The study by Chun *et al.* [18] took into account crop year an additional factor, which is also reflected in the current study.

Crop year as an influential horticultural factor is not specifically addressed in many studies. Rudolph *et al.* [29] showed differences in proximate composition of the pecan over several crop years; they found that crop year was particularly influential on oil content. As tocopherols are a component of pecan lipids, varying oil content would result in varying tocopherol content. Shin *et al.* [22] showed crop year to be an influential factor on the α- and β-tocopherol content in peanuts. The aforementioned study by Chun *et al.* takes crop year into account in relation to tocopherol content of pecans, but found that crop year was not a significant factor in predicting tocopherol content.[18] This study drew the opposite conclusion, stating that crop year was a significant factor influencing tocopherol content. Despite the disagreement that exists with the Chun *et al.* [18] study, both the studies by Rudolph *et al.* [29] and Shin *et al.* [22] agree with our results. Because pecans have an alternate bearing nature,[26] crop year being an important factor, is, in fact, a logical conclusion.

In this study among others, horticultural factors were found to have significant effects on tocopherol content of pecans. In all tocopherol homologs, including total tocopherol content, the 2012 and 2013 crop year were found to be significantly different ($p<0.05$). In addition the 2012 values were observed to be higher in all cases. Significant differences ($p<0.05$) were less definitive in terms of cultivar. In most cases there was not one overall cultivar that was different than all others, and there was a large amount of overlap between cultivars. The most significant

differences of note are that 'Stuart' is significantly different ($p$<0.05) than all other cultivars in α-tocopherol content and that 'Western Schley' was found to be significantly different ($p$<0.05) than all other cultivars, except for 'Lakota', in total tocopherol content.

It is important to incorporate these factors into the Database so that an accurate nutritional profile of the pecan is represented. When consumers or other analytical chemists go to the USDA Database they expect accurate values that are well supported by ample data. An example of this can be seen in testing of commercial nut oils. Gong *et al.* [79] found that several commercial pecan oils were exceedingly high in α-tocopherol, which is incorrect because pecans are primarily γ-tocopherol as seen in both this study and the USDA Database. This indicates that the oils could have potentially been adulterated with α-tocopherol (say from almond oil) in order to enhance the nutritional profile, but without the accurate Database values as reference this might not have been discovered. If more subtle adulteration issues were to arise, the current nine-sample base for pecan tocopherol data may not be precise or accurate enough to indicate if a problem exists. Overall incorporation of this data (as well as other study data) into the Database will help to increase the overall accuracy and precision of the pecan data represented.

**5.2 Phytosterols**

The average total phytosterol content determined in this study, 161.0 ± 14.35 mg/100-g oil, was lower than the 215.9 mg/100-g oil value reported in the USDA National Nutrient Database for Standard Reference, Release 27.[24] Only some of the phytosterols reported in this study are listed in the USDA Database because it does not list values for many of the minor sterols that were found in this investigation. Those sterols are campesterol, stigmasterol, β-sitosterol, and $\Delta^5$-avenasterol + β-sitostanol (these two sterols are listed together as they often

co-elute), which have study values of 4.08 ± 1.20, 0.80 ± 1.14, 100.7 ± 10.26, and 22.07 ± 4.21 mg/100-g oil and USDA values of 8.34, 4.17, 162.57, and 19.87 mg/100-g oil, respectively. These values are lower than previously reported values much like total phytosterols with the exception of $\Delta^5$-avenasterol + β-sitostanol. In addition, more minor sterols were found in this study than there are listed in the Database. Similar to the tocopherol data, this discrepancy is likely due to the variation of cultivars, and the subsequently larger sample size used in this study.

Values in this study were not only compared with the USDA Database, but also other sterol pecan studies. One overarching problem with data comparison is that there is not one clear and consistent unit throughout phytosterol analysis. Oftentimes values are reported in unclear or uncommonly employed units. Other times, all necessary conversion details are not given and thus must be converted with best available knowledge; only those with clear enough background information were converted and compared to in this report. In general, the findings from this study is lower than previously reported values, much like with the USDA Database data. The total phytosterol content determined in this study, 161.0 mg/100-g oil, fell in the middle range of the data surveyed. Two studies, Robbins *et al.* [13] and Miraliakbari and Shahidi [20], reported higher values of 253 and 262 mg/100-g oil, respectively, whereas two other studies by Phillips *et al.* [21] and Kornstiener-Krenn *et al.* [78] found lower values of 157 mg/100-g oil and 113 mg/100-g oil, respectively. This wide variation could be due to method, genetic, or horticultural variations, illustrating the need for a study like this, which incorporates multiple factors effecting nutritional content.

Some of the individual sterols in pecans had more similarities to other studies than the overall total phytosterol content. The most prominent phytosterol in pecans, β-sitosterol, had a concentration of 100.7 mg/100-g oil, which was similar to a few studies, while being much lower

than all others. Data from Korstiener-Krenn *et al.* [78] and Phillips *et al.* [21] reported reasonably similar values of 105.8 and 116.5 mg/100-g oil, respectively. Values from other examined studies ranged from 167.0 to 178.0 mg/100-g oil[13,20,80] and were much higher than the contents reported in this investigation. Campesterol, stigmasterol, and clerosterol contents (Table 4.1.4) were low when compared to all other study values. The reported data was most similar to that of Phillips *et al.*,[21] but not all investigations gave values for the same sterols, leading to some inconsistency among comparisons. The lower values reported here are skewed by the wide range of contents, as related to both crop year and cultivar.

The phytosterol content of one other sterol, $\Delta^5$-avenasterol +β-sitostanol, was higher than all other previously reported values. A value of 22.07 ± 4.21 mg/100-g oil for $\Delta^5$-avenasterol +β-sitostanol was noted in this study. Values of the other studies include 17.24,[13] 14.60,[21] 11.00,[80] and 10.00 [20] mg/100-g oil; thus, this study showed significantly higher $\Delta^5$-avenasterol +β-sitostanol contents than any of these studies as well as the USDA Database. This again could be due to the aforementioned factors, but could also be affected by sterol specific methods. As $\Delta^5$-avenasterol +β-sitostanol is acid labile, it must be determined separately by assaying and quantifying both the glycoside and free sterol portions, with the combined number representing the true value.

Minor sterols make up ~20% of the sterol profile of the pecan, which makes them worthy of investigation. The minor sterols quantified here, only appear in one other study examined. The minor sterol contents reported in this study (Table 4.1.4) were similar to those reported by Robbins *et al.* [13] Overall the total minor sterol content was found to be 32.03 mg/100-g oil, which was higher than Phillips *et al.* [21] and the USDA Database, [24] but lower than Robbins *et al.*[13] This variability could be due to horticultural and method variation, much like with the other

sterols discussed. Despite the fact that these sterols are considered 'minor,' they should still be included in the USDA Database, as they do make up a substantial portion of pecan phytosterols.

In terms of horticultural factors that effect phytosterol content there are very few studies addressing this. Eitenmiller and Pegg [26] state that little to no research has been done to characterize the various genetic, environmental, and other factors that can have significant influence on pecan nutrient composition. There are several studies such as Rudolph *et al.,*[29] Wakeling *et al.*,[81] and Wells *et al.* [31] that examine the relationship between cultivar, crop year, proximate composition, fatty acid composition, and tocopherol content, but not phytosterol content. There are also a few studies that deal with specific environmental factors. For example Rudolph *et al.*[41] addresses some compositional changes resulting from oxidation during storage, and Bouali *et al.*[46] addresses nutrient changes during the ripening process. Phytosterols remain largely undiscussed in either of these papers, with almost no known work relating to pecan cultivar and crop year effects.

This study helps to fill the knowledge gap of horticultural effects on phytosterol composition of the pecan. In general the major phytosterol means were not significantly different ($p>0.05$) between crop years, but the minor phytosterol means were. Two exceptions, however, were β-sitosterol, which was significantly different from 2012 to 2013 and $\Delta^{5,24(25)}$-stigmastadienol, which was not significantly different ($p>0.05$) between the two years. Total phytosterol content showed similar results to β-sitosterol, and was significantly different ($p<0.05$) between 2012 and 2013. For those values with significantly different means, 2012 values were observed to be higher in all cases, much like with the tocopherols. There were not many definitive significant differences ($p<0.05$) between cultivars or one overall cultivar that was different than all others, as there was a large amount of overlap between cultivars. The

differences of note were that ‘Curtis’ and ‘Pawnee’ are significantly different ($p$<0.05) than all other cultivars in clerosterol and 24-methylene-cycloaretenol content, respectively, and ‘McMillian’ and ‘Schley’ are similar to only two other cultivars in $\Delta^{5,24(25)}$-stigmastadienol and cycloartenol content, respectively.

Accurate and precise phytosterol data is an important component of the USDA Database, much like with the tocopherols. Though there are not specific adulteration examples in relation to sterols, variation in sterol composition can be seen in the various assayed pecan oils in the Gong *et al.* [79] study. The basis of pecan phytosterol data only includes three samples, so it is nearly impossible to know if differences are due to natural variations or the oil has actually been tampered with. Though phytosterols are not something that are typically affected by oil adulteration, it would still be important to know if the sterol profile is at all correct. Perhaps if oil were adulterated with another type of oil such as almond, these differences would be reflected in the sterol profile. Overall, this data should be incorporated into the Database to help improve accuracy and precision of pecan data.

The critical question in relation to cultivars and crop years is, “is a pecan, a pecan, a pecan (meaning all pecans have reasonably similar nutrient profiles)?” Though it would be nice to have a definitive answer to this question, a large number of cultivars in conjunction with year-to-year natural variability limits the capability to answer this question. Because some cultivars did show significant differences ($p$<0.05) from all others in terms of certain nutrients, it cannot be concluded that all cultivars are the same. Yet, the significant overlap of many cultivars also indicates all cultivars are not exceedingly different from one another. In order to show overall influence of cultivar and crop year on nutrient values, a chart comparing the standard error with or without incorporation of these factors was generated (Figure 5.1). It is clear that standard error

is lower when cultivar and crop year factors are incorporated, giving the impression that perhaps a pecan, is not a pecan, is not a pecan.

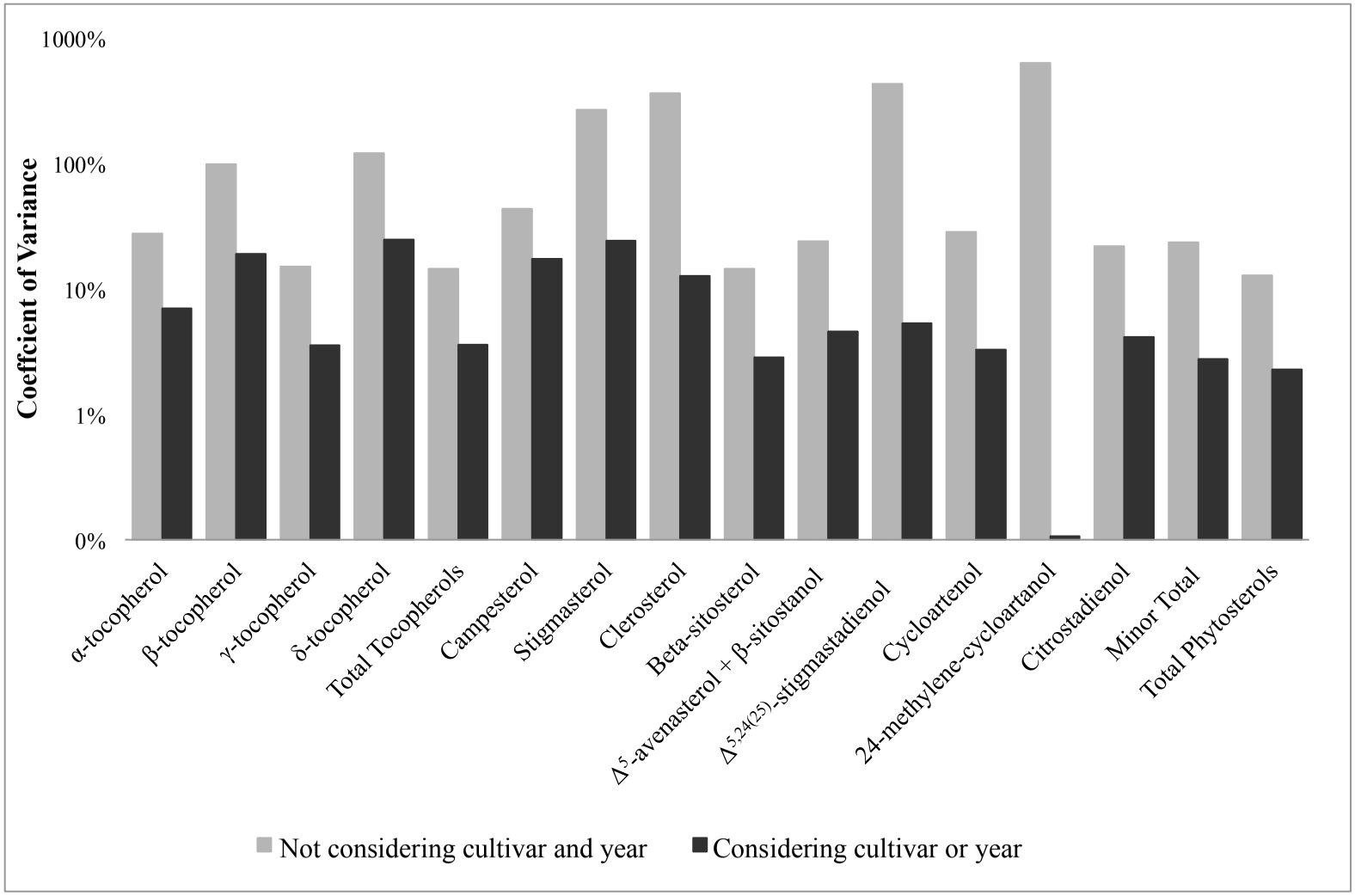


**Figure 5.1**: Comparison of the Coefficient of Variance of all Minor Lipid Bioactives with and without Horticultural Factors

# CHAPTER 6

# SUMMARY OF RELEVEANT RESULTS AND CONCLUSIONS

Overall this study provides credible data on the minor lipid nutrient composition of the pecan. As was discussed, values were similar to both the USDA Database and studies that have been conducted on pecan tocopherol and phytosterol content. Even though some numbers were lower than reported values, it is likely due to the inclusion of many different cultivars of different crop years in this data set. Some pecans were found to be high in certain tocopherol or phytosterol components (*i.e.*,'Stuart' pecans were highest in α-tocopherol), but there was no cultivar found to be nutritionally better than all others. Additionally, cultivar and crop year were determined to influence the tocopherol content of pecans, which is reflected in some earlier studies as well. It was also found that these factors could affect the content of phytosterols, with variation depending on the individual sterol.

Pecan cultivars being virtually the same in minor lipid content is good news for the pecan industry. This conclusion helps allow pecan farmers and growers to continue to produce already established pecan cultivars, rather than having to switch to new or different varieties. Because pecan trees take many years for proper cultivation, this is an especially important conclusion for pecan economics. Though all cultivars were grown in Georgia, it was seen that pecans from the western part of the US did not have tocopherol and phytosterol levels greater than those cultivars from the southeastern US. Yielding pecans of equal nutritional value to other states helps to bolster Georgia's involvement in pecan production. Marketing can further point out that any and

all pecans are healthful and not just one particular variety/type; this can help to increase the presence of pecans in the marketplace. On the whole, the conclusion that pecans of all types are truly healthful foods is also solidified by this investigation.

In conclusion, it was discovered that cultivar and crop year have significant effects on the bioactive lipid composition of the pecan. Pecan cultivars and crop years are factors that should be incorporated into the USDA Database in order to provide a more accurate and precise record of pecan minor nutrient composition. Including pecan data ranges is also suggested so that consumers can get a more accurate idea of nutrient variability in the pecan. This data would also help to increase the pecan sample size making the Database an even more valid and useful tool. Even though more research is needed to accurately answer the question, "is a pecan, a pecan, a pecan?," this investigation has helped to show that most pecan cultivars have closely related nutrient values. This study along with others can help to validate and substantiate pecan data for current and future research of all kinds.

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