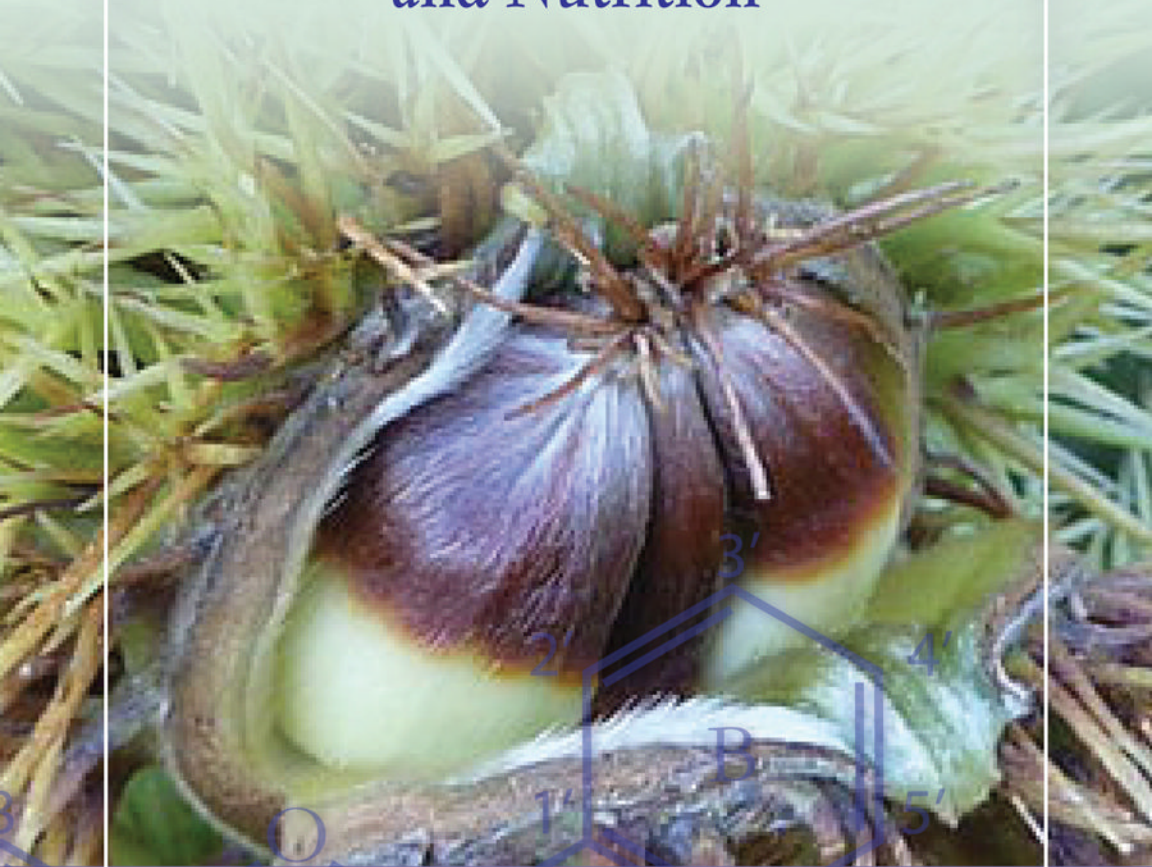


Agriculture Issues and Policies

NUTS

Properties, Consumption
and Nutrition



Isabella M. Davis
Editor

NOVA

AGRICULTURE ISSUES AND POLICIES

NUTS: PROPERTIES, CONSUMPTION AND NUTRITION

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NUTS: PROPERTIES, CONSUMPTION AND NUTRITION

ISABELLA M. DAVIS
EDITOR



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PREFACE

Several epidemiological studies have revealed that people who consume nuts regularly are less likely to suffer from coronary heart disease. Clinical trials have found that consumption of various nuts such as almonds and walnuts can lower serum LDL cholesterol concentrations. Although nuts contain various substances thought to possess cardioprotective effects, scientists believe that their Omega 3 fatty acid profile is at least in part responsible for the hypolipidemic response observed in clinical trials. This book presents current research in the study of nut properties, consumption and nutrition.

Chapter 1 - Epidemiologic studies have been remarkably consistent in showing that frequent nut consumption is negatively associated with incidences of some chronic diseases such as cardiovascular diseases, certain types of cancers, and diabetes. Besides favorable fatty acid and other macro-, micro-nutrient profiles, nuts, including almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, walnuts, and peanuts, are rich in bioactive components such as phenolics, tocopherol, and phytosterols, which are considered to be responsible for different biological effects. Specifically, nuts contain many different antioxidants. Besides vitamin A, vitamin C and β -carotene, nuts are also known to possess antioxidants such as flavonoids, isoflavones, luteolin, tocotrienols, and ellagic acid as well as plant sterols. In this chapter, bioactive compounds including phytochemical composition, biological activities, and associated health benefits in edible nuts and peanuts are extensively and critically reviewed based on a compilation of updated research.

Chapter 2 - The shea tree, *Vitellaria paradoxa*, is a tree widely distributed and usually protected in the Northern Nigeria. Both the fruit pulps and the nuts

are economically important to the rural poor. There are distinct ecological variations in the fruit and nut physicochemical attributes of Shea in Nigeria. Our studies indicated significant variation in all metric traits of fruits and nuts, except fruit length, fruit shape index and testa weight, across agro-ecological zones. All metric traits except fruit shape index also showed remarkable diversity across accessions (individual locations), with fruit length, nut length, fruit weight and nut weight ranging from 4.3-5.9 cm, 3.1-5.4 cm, 26.8-63.4 g and 8.7-22.0 g, respectively. Fruit pulp nutritional composition is significantly influenced by agroecological zone in respect of carbohydrate, protein, fibre, energy, Na, K, Mg and Fe. Fruits from the wetter southern guinea savanna zone have less fibre but higher amount of carbohydrate, energy and Na while those from the drier sudan savanna zone are richer in protein, K, Mg and Fe. The specific locations of fruit collection (accessions) have significant influence on all nutritional traits. The range in energy related proximate traits is 29.3-45.3% carbohydrate, 2.6-7.0% protein and 0.7-1.7% fat. The element Fe has significant positive statistical linkage with Zn, Mg, K and Na. All proximate traits of the shea kernel except ash content vary remarkably across ecological zones. With the exception of moisture and fibre all other proximate traits of the kernel cake are statistically similar across agroecological zones. However, all proximate traits of the shea kernel and kernel cake vary ($P < 0.05$) across sites with shea kernels from Kachia and Jalingo recording highest values for fat. Correlations between kernel and fruit pulp proximate qualities revealed a low number of significant relationships. Fatty acid profile has shown significant influence of agroecology over stearic and oleic acids content while all the four fatty acids (stearic, oleic, linoleic and palmitic acids) are significantly influenced by individual locations. The range in the stearic and oleic acids content is 45.1-49.7% and 37.2-43.4%, respectively. Generally, the fruit pulp and seed of shea have excellent nutritional properties capable of meeting the dietary needs of the rural population. Besides, both physical (metric) and nutritional traits of fruits and nuts of the shea tree have shown considerable variation across the major distribution zones in Nigeria suggesting a possibility of selection for the genetic upgrading of the species in the country.

Chapter 3 - *Anacardium occidentale* (cashew), a member of the Anacardiaceae family, is a tropical tree indigenous to Brazil. It is extensively cultivated in India and east Africa for its kernel (the cashew nut). Cashew nut shell liquid (CNSL) is a substance contained between the kernel's inner and outer shells (pericarp) in a honeycomb matrix. It is an important agricultural product of cashew nut cultivation and a unique natural source of unsaturated

long-chain phenols. Typically, solvent-extracted CNSL contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%), and traces of 2-methyl cardol. These compounds exhibit antibacterial, antifungal, and antitumor activities and also have molluscicidal, insecticidal, and fungicidal applications. They are known to be uncoupling factors of oxidative phosphorylation in the mitochondria and they show antioxidant activity and inhibitory activity against enzymes (e.g. α -glucosidase, β -lactamase, lipoxygenase, xanthine oxidase, and tyrosinase). The classes of compounds present in CNSL are also present in other plant extracts. They have identical chemical structures and their biological activities have been very extensively examined. This review focuses on recent data on the biological activities of those bioactive compounds found in both CNSL and other plants with identical chemical structures.

Chapter 4 - The present study investigated the effect of packaging material O_2 permeability, light, temperature and storage time on quality of raw ground walnuts and almonds. Samples were packaged in a) PET//LDPE, 70 μm in thickness and b) PET-SiOx//LDPE pouches, 62 μm in thickness under nitrogen. Samples were stored either under fluorescent light or in the dark at 4 or 20 $^{\circ}C$ for a period of 12 months. Quality parameters monitored were peroxide value (PV), hexanal, and the sensory attributes: odor and taste of product.

PV ranged between 0.3 meq O_2 /kg oil for fresh ground walnuts and 30.0 meq O_2 /kg oil for samples packaged in PET//LDPE pouches under N_2 , exposed to light at 20 $^{\circ}C$ after 12 months of storage. Respective values for ground almonds were 0.3 and 20.0 meq O_2 /kg oil. Hexanal ranged under 28.5 μg /kg (method detection limit) for fresh ground walnuts and 34.0 mg/kg for samples packaged in PET//LDPE exposed to light at 20 $^{\circ}C$ after 12 months of storage. Respective values for ground almonds were < 28.5 μg /kg and 9.0 mg/kg. Values for odor ranged between 8.6 (scale 9-1) for fresh walnut kernels and 1.4 for walnut kernels packaged in PET//LDPE exposed to light after 12 months of storage at 20 $^{\circ}C$. Respective values for taste were 7.8 and 1.3. Odor values for ground almonds ranged between 8.9 for fresh products and 4 for products packaged in PET//LDPE exposed to light after 12 months of storage. Respective values for taste were 8.9 and 2.2. Taste proved to be a more sensitive attribute than odor. Based mainly on sensory analysis, ground walnuts retained acceptable quality for ca. 6 months in PET//LDPE- N_2 and at least 12 months in PET-SiOx//LDPE- N_2 pouches at 20 $^{\circ}C$, with samples stored in the dark retaining higher quality than those exposed to light. Respective shelf lives at 4 $^{\circ}C$ were 6-7 and at least 12 months. Shelf life of ground almonds were ca. 6-7 months packaged in PET//LDPE and 8 months packaged

in PET-SiOx/LDPE pouches under N₂ irrespective of lighting conditions at 20 °C while at 4 °C shelf life was extended by an additional month as compared to storage at 20 °C. PET-SiOx/LDPE proved to be an effective oxygen barrier for the protection of ground walnut and almonds sensory quality.

Chapter 5 - *Vitellaria paradoxa* Gaertn or the shea tree produces kernels which have a fat content of about 35-60% usually referred to as shea butter. This butter is used traditionally in foods and medicines while on an industrial scale it used in the cosmetics and chocolate industries. The processing of fruits to obtain butter involves collection of the fruits, depulping to give nuts, cooking of the nuts, dehiscing to give the kernels, drying of kernels and oil extraction. The cooking and drying of sheanuts are critical steps in the traditional processing of shea kernels which largely determine butter quality. This work presents results on the physical properties of shea fruits and nuts which affect these critical steps and consequently butter quality. Shea fruits from 7 localities (Gashiga, Rabingha, Hina, Tchabal, Deone, Fouban and Banguoa) which cut across four ecological zones of Cameroon were harvested and their physical properties determined. The major diameters of the fruits and nuts ranged from 43.8 ± 6.3 to 69.62 ± 10.57 mm and 32.80 ± 2.91 to 44.29 ± 5.09 mm respectively. The sizes of the shea fruits and nuts analysed were highly dependent on the altitude of the sampling site. The sphericities of the fruits and nuts lay between 0.7 and 1 indicating that they essentially spherical in shape. Larger fruits were found at altitudes greater than 1200 m while smaller fruits and nuts grew generally at altitudes ranging from 200-600 m. More than 77 % of the nuts from all the sampling sites had major diameters ranging from 40-45 mm. significant differences were equally observed in the physical properties of the fruits and nuts obtained from different trees within and between sampling sites. An empirical relation was established and validated for inter-converting between the major diameter of the fruits and nuts. This relation can be used to estimate major diameters of the fruits from the nuts given that most often only the nut is available due to the highly perishable nature of the fruit pulp. Sheanut kernels are large (34-45 mm in diameter) and therefore have to be dried as thin slices in order to fasten drying times. Results on some physical properties of the kernels are also reported.

Chapter 6 - The application of anaerobically digested biosolids as a nutrient source for the pecan *Carya illinoensis* (Wangh.) K. Koch, cultivar Western, during three years was evaluated. The bearing shoot grew 16% more and nut production per tree was 11.3% higher in the biosolid treatment, on a three-year average. The accumulation of As, Cd, Cr, Hg, Ni and Pb in soil due to biosolids was very low and according to the U.S. standard, the maximum

allowable concentration would be reached in 34 years. Quantities of Cd, Cr, Ni and Pb in the kernel were below detection limits. As and Hg were found in very small quantities, and were below the limits allowed for nuts in the United Kingdom. During the preharvest, in soil fertilized with biosolids and in nuts which had contact with biosolids, the presence of *Escherichia coli* and *Salmonella* sp. were not detected.

Chapter 7 - Areca nut (AN, *Areca catechu* L.) is a popular but carcinogenic chewing material used by approximately 200–600 million people worldwide. In the past few decades, AN has been discovered to possess genotoxic, cytostatic, and cytotoxic effects on cells. Some ingredients of AN, such as AN extract (ANE), arecoline, hydroxychavicol, and oligomeric procyanidins were demonstrated to stimulate apoptotic and/or growth arresting phenotypes in treated cells. However, our recent studies showed that ANE predominantly induces the autophagic responses, albeit the simultaneous initiation of apoptotic pathway. This finding may renew the knowledge about the cytotoxic effects of AN on oral cells in physiological conditions.

Chapter 1

BIOACTIVE COMPONENTS IN EDIBLE NUTS AND HEALTH BENEFITS

Jun Yang¹, Jiaren Liu² and Dana L. Felice³

¹Frito-Lay North America RandD, 7701 Legacy Drive, Plano, TX, 75024

²Department of Anesthesia, Harvard Medical School,
300 Longwood Ave, Boston, MA, 02115

³Department of Physiology and Biophysics, University of Illinois at
Chicago, 835 S. Wolcott Ave., Chicago, IL 60612

ABSTRACT

Epidemiologic studies have been remarkably consistent in showing that frequent nut consumption is negatively associated with incidences of some chronic diseases such as cardiovascular diseases, certain types of cancers, and diabetes. Besides favorable fatty acid and other macro-, micro-nutrient profiles, nuts, including almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, walnuts, and peanuts, are rich in bioactive components such as phenolics, tocopherol, and phytosterols, which are considered to be responsible for different biological effects. Specifically, nuts contain many different antioxidants. Besides vitamin A, vitamin C and β -carotene, nuts are also

1 Corresponding author: Email: junyang97@gmail.com.

2 Email: jiarenliu@yahoo.com

3 Email: DLF34@uic.edu.

known to possess antioxidants such as flavonoids, isoflavones, luteolin, tocotrienols, and ellagic acid as well as plant sterols. In this chapter, bioactive compounds including phytochemical composition, biological activities, and associated health benefits in edible nuts and peanuts are extensively and critically reviewed based on a compilation of updated research.

INTRODUCTION

Nut consumption is inversely associated with incidences of some chronic diseases such as cardiovascular diseases, certain types of cancers, and diabetes. In July 2003 the U.S. Food and Drug Administration (FDA) approved a new qualified health claim for nuts and heart disease - “Scientific evidence suggests but does not prove that eating 1.5 ounces (42 grams) per day of most nuts as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease.” Tree nuts are cholesterol-free and full of nutrients, including fat, protein and fiber. Nuts are also a great source of vitamins such as folic acid, niacin and vitamins E and B₆, and minerals like magnesium, copper, zinc, selenium, phosphorus and potassium. Some nuts are good sources of antioxidants such as vitamin E, selenium, and certain phytochemicals. Tree nuts and peanuts are rich in a number of bioactive components with health-promoting benefits. The common bioactive components, including phytochemicals such as carotenoids, phenolics, and alkaloids, present in tree nuts and peanuts are listed in Figure 1. As consumers become increasingly aware of healthy diets, the bioactive component profile of edible nuts would help them make informed decisions on selecting and consuming these nutritious foods.

NUT BIOACTIVE COMPONENTS

Commonly, the most popular and commercially important edible nuts are almonds (*Prunus dulcis*), cashews (*Anacardium occidentale*), Brazil nuts (*Bertholetia excelssa*), hazelnuts (*Corylus avellana*), macadamias (*Macadamia integrifolia*), pecans (*Carya illinoensis*), pine nuts (*Pinus pinea*), pistachios (*Pistachia vera*), walnuts (*Juglans regia*), and peanuts (*Arachis hypogaea*). Phytochemicals, broadly classified as alkaloids, nitrogen-containing

compounds, carotenoids, organosulfur compounds, phenolics, and phytosterols, are defined as bioactive non-nutrient components in plant foods.

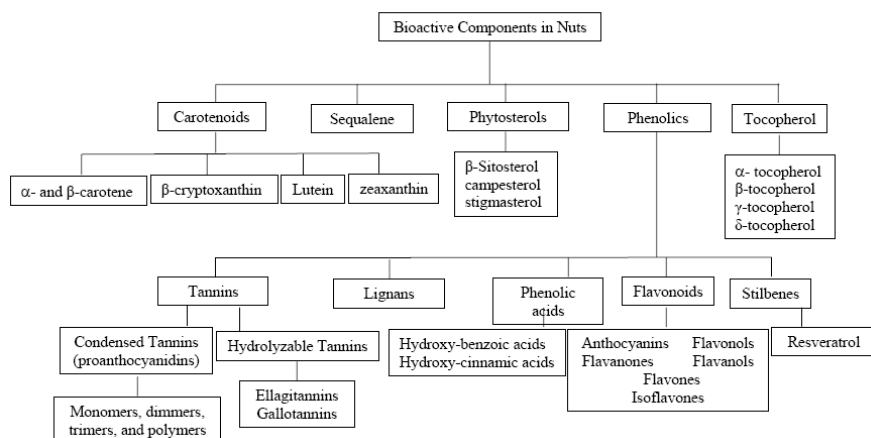


Figure 1. Bioactive components in tree nuts and peanuts.

Nuts contain bioactive constituents such as phenolics, carotenoids, phytosterols, tocopherols and squalene, which have been found to possess biological effects against cardiovascular disease, cancers, and other types of chronic diseases.

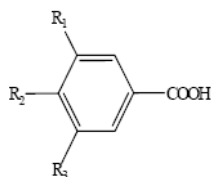
1. Phenolics

Phenolics constitute one of the largest and most ubiquitous groups of phytochemicals. They can be grouped into more than ten subtypes based on their chemical structure (Strack, 1997). Phenolics share a common chemical structure and differ in their linkages to other compounds. All phenolics possess an aromatic ring bearing one or more hydroxyl groups (Figure 2, 3, and 4). The majority of phenolics have a sugar residue, such as a monosaccharide, disaccharide, or oligosaccharide, linked to the carbon skeleton. Other residues include amines, organic acids, carboxylic acids, and lipids. The thousands of identified phenolic structures greatly vary from simple compounds such as phenolic acids with a C6 ring structure to highly polymerized molecules such as tannins.

Total phenolics have been quantified in tree nuts and peanuts. The profiles of total phenolics and flavonoids, including both soluble free and bound forms,

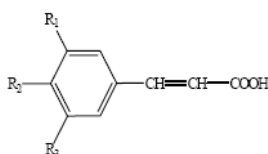
were investigated by utilizing solvent extraction, base digestion, and solid-phase extraction methods (Yang et al., 2009a).

1) Benzoic Acid



Benzoic acid Derivatives	Substitutions		
	R ₁	R ₂	R ₃
<i>p</i> -Hydroxybenzoic acid	H	OH	H
Protocatechuic acid	H	OH	OH
Vanillic acid	OCH ₃	OH	H
Syringic acid	OCH ₃	OH	OCH ₃
Gallic acid	OH	OH	OH

2) Cinnamic Acid



Cinnamic acid Derivatives	Substitutions		
	R ₁	R ₂	R ₃
<i>p</i> -Coumaric acid	H	OH	H
Caffeic acid	OH	OH	H
Ferulic acid	OCH ₃	OH	H
Sinapic acid	OCH ₃	OH	OCH ₃

3) Resveratrol

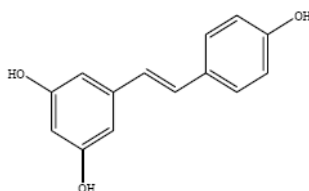


Figure 2. Chemical structures of common phenolics in tree nuts and peanuts.

Walnuts contained the richest total phenolic and flavonoid contents (1580.5 ± 58.0 mg/100 g, 744.8 ± 93.3 mg/100 g in dry nuts, respectively). The amount of total phenolics in 10 different types of nuts was analyzed (Kornsteiner et al., 2006). The average content of total phenolics ranged from 32 mg in pine nuts to 1625 mg gallic acid equivalents/100 g in fresh walnuts (Table 1).

Phenolic acids in almond, pine nut, and black walnut were extracted by methanol-HCl and analyzed as their methyl esters/trimethylsilyl derivatives by GLC-MS (Senter et al., 1983).

Table 1. Total phenolic and flavonoid contents of 9 tree nuts and peanuts (Kornsteiner et al., 2006; Yang et al., 2009)

Edible Nut Seeds	Phenolics (mg/100g dry weight)			Total Phenolics (mg/100g fresh weight)	Flavonoids (mg/100g dry weight)		
	Free Form	Bound Form	Total	Range	Free Form	Bound Form	Total
Almonds	83.0 ± 1.3	129.9 ± 13	212.9 ± 12.3	130 - 456	39.8 ± 2.0	53.7 ± 11.9	93.5 ± 10.8
Brazil Nuts	46.2 ± 5.7	123.1 ± 18.4	169.2 ± 14.6	100 - 133	29.2 ± 7.2	78.6 ± 9.2	107.8 ± 6.0
Cashews	86.7 ± 8.1	229.7 ± 15.1	316.4 ± 7.0	131 - 142	42.1 ± 3.8	21.6 ± 5.2	63.7 ± 2.1
Hazelnuts	22.5 ± 1.1	292.2 ± 48.4	314.8 ± 47.3	101 - 433	13.9 ± 2.3	99.8 ± 28.5	113.7 ± 30.2
Macadamia Nuts	36.2 ± 2.6	461.7 ± 51.2	497.8 ± 52.6	45 - 46	9.4 ± 0.7	128.5 ± 9.3	137.9 ± 9.9
Peanuts	352.8 ± 22.2	293.1 ± 25.0	645.9 ± 47.0	326 - 552	145.5 ± 10.0	44.2 ± 5.2	189.8 ± 13.1
Pecans	1227.3 ± 8.4	236.6 ± 28.1	1463.9 ± 32.3	1022 - 1444	639.3 ± 17.0	65.4 ± 12.7	704.7 ± 29.5
Pine Nuts	39.1 ± 0.6	113.8 ± 14.3	152.9 ± 14.1	30 - 34	13.0 ± 1.5	32.0 ± 6.8	45.0 ± 5.4
Pistachios	339.6 ± 15.1	232.2 ± 13.3	571.8 ± 12.5	492 - 1442	87.4 ± 14.0	55.9 ± 13.6	143.3 ± 18.7
Walnuts	1325.1 ± 37.4	255.4 ± 25.0	1580.5 ± 58.0	1020 - 2052	535.4 ± 71.5	209.4 ± 22.1	744.8 ± 93.3

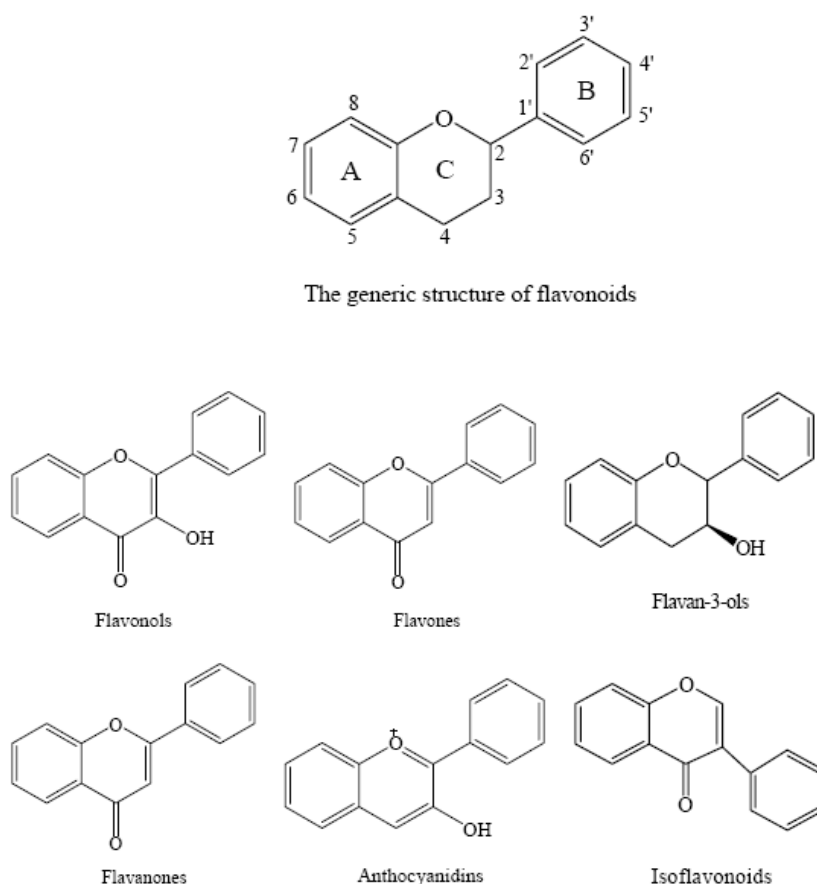


Figure 3. Chemical structure of main classes of dietary flavonoids.

The isolated and identified phenolic acids included *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, vanillic, protocatechuic, syringic, gallic, caffeic and ferulic acids. It was observed that caffeic acid was the predominant acid in pine nuts; protocatechuic acid was the major one in almonds. Amarowicz et al. (2005) examined phenolic composition and antioxidant activity in defatted almond seeds by using 80% aqueous acetone. The crude extract was used in a Sephadex LH-20 column. The column was eluted by ethanol to form fraction I. Fraction II was obtained using water-acetone (1:1, v/v) as the mobile phases. The results showed that vanillic, caffeic, *p*-coumaric, and ferulic acids (after basic hydrolysis), quercetin, kaempferol and isorhamnetin (after acidic

hydrolysis), delphinidin and cyanidin (after n-butanol-HCl hydrolysis) and procyanidin B2 and B3 were observed in almond crude extract.

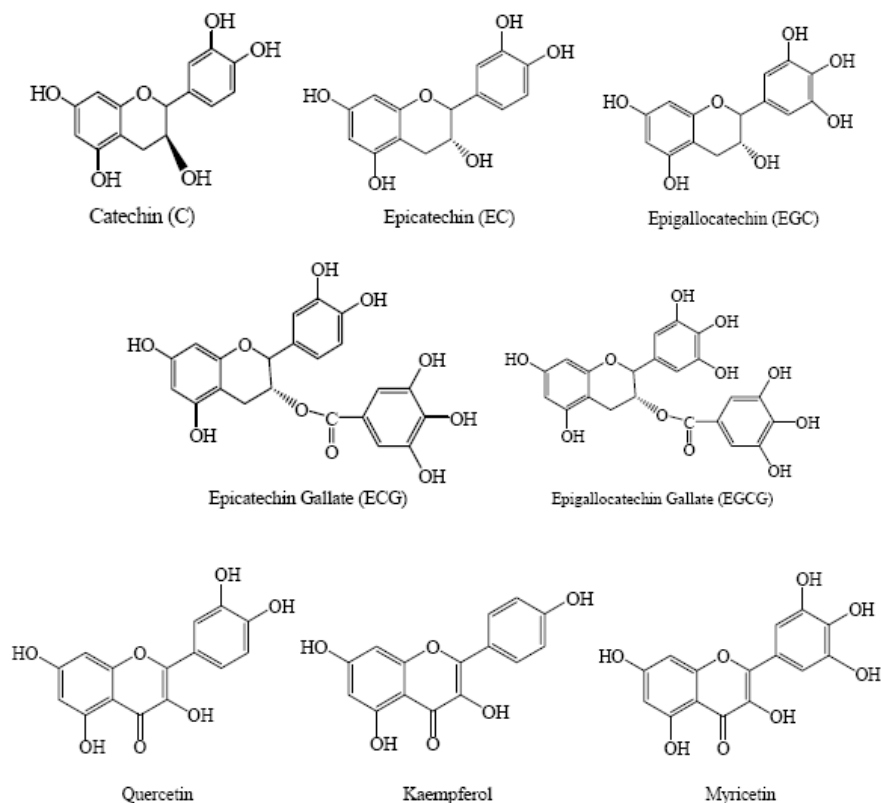


Figure 4. Chemical structures of major flavonoids present in tree nuts and peanuts.

The content of tannins in fraction II was 10 times higher than that in the crude extract. The total antioxidant activity of tannin fraction was 3.93 mmol Trolox/g, whereas the crude extract and fraction I showed values of only 0.24 and 0.09 mmol Trolox/mg, respectively. In addition, Alasalvar et al. (2006) have used 80% ethanol (v/v) and 80% acetone (v/v) to extract phytochemicals in hazelnut kernel and hazelnut green leafy cover. The results exhibited significant differences ($p < 0.05$) in total phenolics, condensed tannins, and total antioxidant activity. Among four extracts, hazelnut green leafy cover extracted by 80% acetone exhibited the highest level of total phenolics (201 mg of catechin equivalents/g of extract), condensed tannins (542 mg of

catechin equivalents/g of extract), and total antioxidant activity (1.29 mmol of TE/g of extract). Total phenolic content correlated well with total antioxidant activity ($R^2 = 0.97$).

Total phenolics, flavonoids, and phenolic acids in California almond (*Prunus dulcis*) skins and kernels among the main almond varieties (Butte, Carmel, Fritz, Mission, Monterey, Nonpareil, Padre, and Price) were determined by HPLC with electrochemical detection and UV detection (Milbury et al., 2006). The predominant flavonoids and phenolic acids were verified through HPLC and tandem MS. Total phenolics ranged from 127 to 241 mg gallic acid equivalents/100 g of fresh nut. Among 18 flavonoids, the principal ones were isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside (in combination), catechin, kaempferol-3-*O*-rutinoside, epicatechin, quercetin-3-*O*-galactoside, and isorhamnetin-3-*O*-galactoside with 16.81, 1.93, 1.17, 0.85, 0.83, and 0.50 mg/100 g of fresh almonds, respectively.

The total phenolics of defatted peanut skin was documented to be 140 - 150 mg/g dry skin (Nepote et al., 2002), and to be 90 - 125 mg/g dry skin, including phenolic acids, flavonoids and resveratrol (Yu et al., 2005). The composition of ethanolic extracts of peanut skin obtained from direct peeling, peeling after blanching, and peeling after roasting was determined by HPLC and LC-MS (Yu et al., 2006). It was concluded that total phenolics in peanut skins after the different processing methods were 130, 124, and 14.4 mg/g dry skin, respectively. Total catechins, procyanidin dimers, trimers and tetramers in directly peeled peanut skin were 16.1, 111.3, 221.3 and 296.1 mg/100 g, respectively, vs. 8.8, 143.5, 157.5 and 203.9 mg/100 g, respectively, in roasted dry skin.

Flavonoids include over thousands of known compounds, and this number is constantly growing due to the great structural diversity arising from various hydroxylation, glycosylation, methoxylation, and acylation. The generic structure of flavonoids consists of two aromatic rings (A and B rings) linked by 3 carbons that are usually in an oxygenated heterocycle ring called the C ring (Figure 3). Based on differences in the heterocycle C ring, flavonoids are categorized as flavonols (quercetin, kaempferol, and myricetin), flavones (luteolin and apigenin), flavanols (catechins, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate), flavanones (naringenin), anthocyanidins, and isoflavonoids (genistein, daidzein, dihydrodaidzein, and equol) (Figure 4). For naturally occurring flavonoids, they are mostly conjugated in glycosylated or esterified forms but can occur as aglycones, especially as a result of the effects of food processing.

Table 2. Flavonoid Content in Tree Nuts and Peanuts (mg/100 g of fresh weight) (Harnly et al., 2006)

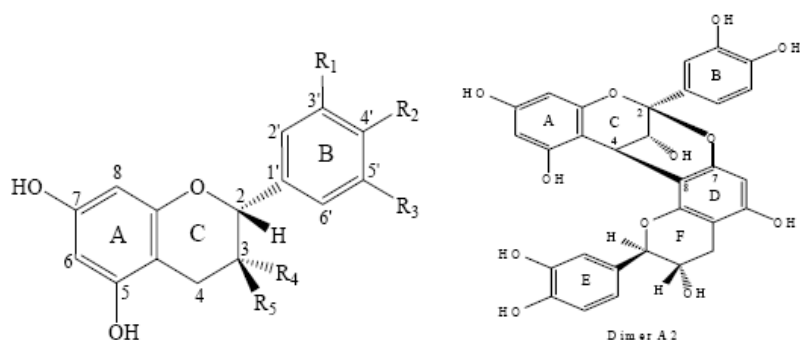
Tree nuts and Peanuts	NNDDB No	Source	Flavan-3-ols					Anthocyanins		Flavanones	Flavonols		
			C	EC	ECG	EGC	EGCG	GCG	Cya	Del	Nari	Kaem	Quer
Almonds	12061	Harnly et al	0.1 ± 0.1	0.3 ± 0.2		2.6 ± 0.6		0.46 ± 0.31	2.46 ± 1.63				
		USDA	1.9 ± 0.9	0.7 ± 0.3						0.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.3	
Brazil Nuts	12078	Harnly et al											
		USDA											
Cashews	12086	Harnly et al		0.9 ± 0.5	0.2 ± 0.3								
		USDA											
Hazelnuts or filberts	12120	Harnly et al	1.2 ± 1.1	0.2 ± 0.2		2.8 ± 2.7	1.1 ± 1.0	0.4 ± 0.4	6.7 ± 3.1				
		USDA											
Macadamia	12131	Harnly et al											
		USDA											
Pecans	12143	Harnly et al	7.2 ± 1.4	0.8 ± 0.2		5.6 ± 3.9	2.3 ± 1.2	0.8 ± 0.4	10.7 ± 4.0	7.3 ± 2.5			
		USDA											
Pine Nuts	14149	Harnly et al											
		USDA											
Pistachios	12151	Harnly et al	3.6 ± 2.7	0.8 ± 1.2		2.1 ± 2.2		0.5 ± 1.0	7.2 ± 3.9				
		USDA											
Walnuts (English)	12155	Harnly et al											
		USDA											
Peanuts	16089	Harnly et al											
		USDA											

Abbreviations: C, catechin; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GCG, gallicocatechin gallate; Cya, cyanidin; Del, delphinidin; Nari, naringenin; Kaem, kaempferol; and Quer, quercetin.

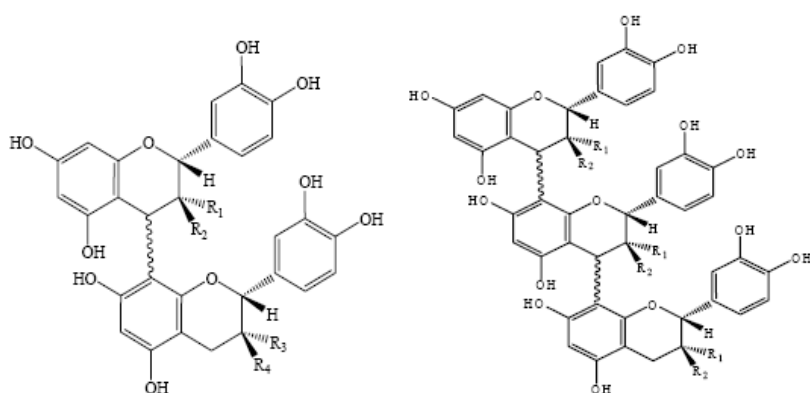
The Nutrient Data Laboratory at USDA established a flavonoid database in 2003, and a proanthocyanidin database in 2004, which include edible nuts.

The flavonoid content in 9 tree nuts and peanuts was documented both in the flavonoid database established in 2003 by the Nutrient Data Laboratory at USDA and in a flavonoid profile compiled and published by the USDA (Harnly et al., 2006) (Table 2). Ranked by descending order, the nuts with the highest total flavonoid levels were pecans, almonds, pistachios, and hazelnuts. The 20 flavonoids listed in the database include 8 flavan-3-ols (catechin, catechin gallate, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, gallic catechin, and gallic catechin gallate), 6 anthocyanins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin), 2 flavanones (hesperetin and naringenin), 2 flavones (apigenin and luteolin), and 2 flavonols (myricetin and quercetin). Among eight flavan-3-ols, neither catechin gallate nor gallic catechin was found in 9 tree nuts and peanuts. Interestingly, no eight flavan-3-ols were detected in macadamia, pine nuts, walnuts (English), or peanuts. Of six anthocyanins, cyanidin was found in almonds, hazelnuts, pecans, and pistachios. Delphinidin was only detected in pecans. No flavones were found in 9 tree nuts and peanuts. In terms of flavanones, only naringenin was reported to be present in almonds. Kaempferol and myricetin, both flavonols, were identified only in almonds. From the USDA database, flavonoids have been identified in most nuts by their aglycone profiles. The total flavonoid contents found in pecan, almond, pistachios, and hazelnuts are 34, 15, 12, and 12 mg/100 g, respectively. There are no flavonoids detected in Brazil or macadamia nuts. Flavan-3-ols, occurring as monomers, oligomeric and polymeric forms, are abundant flavonoids in nuts. However, flavan-3-ols present in tree nuts and peanuts differed in concentration, type of interflavan linkage, structural composition, and degree of polymerization (Lou et al., 1999).

Nuts are rich in tannins (Bravo 1998). The most common structural monomeric units of proanthocyanidins in plants are (epi)afzelechin, (epi)catechin, and (epi)gallic catechin (Figure 5). Some of these units could be esterified with other molecules such as gallic acid and glucose. A-type procyanidins have an additional ether type bond between the C-2 position of the top unit and the hydroxyl group at C-5 or C-7 of the lower unit. B-type procyanidins are monomers linked through the C-4 position of the top unit and the C-6 or C-8 positions of the terminal unit (Figure 5). Proanthocyanidins are polymers of catechin and are found in almonds, cashews, hazelnuts, pecans, pistachios, peanuts, and walnuts. Lou et al (1999) investigated A-type proanthocyanidins from peanut skins.



Flavan-3-ols	Substitutions				
	R ₁	R ₂	R ₃	R ₄	R ₅
Catechin	H	OH	OH	H	OH
Epicatechin	H	OH	OH	OH	H
Afzelechin	H	OH	H	H	OH
Epiafzelechin	H	OH	H	OH	H



Dimer	Substitutions				Trimer	Substitutions	
	R ₁	R ₂	R ₃	R ₄		R ₁	R ₂
B1	OH	H	H	OH	C1	OH	H
B2	OH	H	OH	H	C2	OH	OH

Figure 5. Chemical structures of proanthocyanidins.

From water-soluble fraction of peanut skins, 6 A-type proanthocyanidins were isolated and identified: epicatechin-(2 β →O→7, 4 β →4)-catechin, epicatechin-(2 β →O→7, 4 β →6)-ent-catechin, epicatechin-(2 β →O→7, 4 β →6)-

ent-epicatechin, proanthocyanidin A-1, proanthocyanidin A-2 and epicatechin-(2 β →O→7, 4 β →8)-ent-epicatechin. Anti-hyaluronidase activity was observed by these six compounds. The flavan-3-ol composition and antioxidant capacity of roasted skins developed from industrial processing of almond, hazelnuts, and peanuts, as well as fractions containing low and high molecular weight (LMW and HMW) flavan-3-ols, were recently studied by Monagas et al. (2009). The results demonstrated that roasted hazelnut and peanut skins contained similar total phenolic levels, which are much higher than that of almond skins, but their flavan-3-ol profiles differed considerably. From a structure standpoint, flavan-3-ols in peanut and almond skins presented both A- and B-type proanthocyanidins. However in peanuts the A forms (up to DP12) were predominant, whereas in almonds the B forms (up to DP8) were more abundant. The antioxidant activity from whole extracts in roasted peanut and hazelnut skins was higher than that in almond skins.

Proanthocyanidins reported in hazelnuts, pecans, pistachios, almonds, walnuts, peanuts, and cashews are 501, 494, 237, 184, 67, 16, and 9.11 mg/100 g of nuts (**Table 3**). Venkatachalam and Sathe (2006) extracted and quantified nonpolar and polar tannins in nuts by using both absolute MeOH and acidified MeOH (1% v/v HCl). The total amount of tannin ranged from 0.01-0.88%. It showed that higher amounts of tannin were extracted by acidified methanol from almonds, cashew nut, hazelnut, pecan, pistachio, and peanut, indicating the presence of measurable amounts of polar tannins. Both solvents extracted similar amounts of total tannins among Brazil nut, macadamia, and pine nut, suggesting the tannins in these nuts to be mainly nonpolar in nature. In addition, almonds, hazelnuts, and pistachios appear to contain significant proportions of polar tannins.

It was found that almond skin contains 70-100% of the total phenolics that exist in the nut, including flavonoids and nonflavonoids (Milbury et al., 2006, Sang et al., 2002). Flavanol monomers (+)-catechin, (-)-epicatechin and dimers constituted by these units (procyanidins B1, B3, and B4) in almond skin were identified by Brieskorn and Betz (1998). By using *n*-butanol-HCl hydrolysis in almond seed, procyanidins B2, B3, delphinidin and cyanidin were observed (Amarowicz et al., 2005). Flavonols, including 3-*O*-glucosides, -galactosides, and -rutinosides of quercetin, kaempferol, isorhamnetin, and their corresponding aglycones, morin and dihydrokaempferol, and flavanones, including naringenin-7-*O*-glucoside, eriodictyol-7-*O*-glucoside, and eriodictyol-7-*O*-galactoside and their corresponding aglycones have been identified in almond skins (Sang et al., 2002; Wijeratne, et al., 2006; Milbury, et al., 2006).

Table 3. Proanthocyanidin Content in Tree Nuts and Peanuts (mg/100 g of nuts) a (Gu et al., 2004)

Nuts	Moisture %	Monomers	Dimers	Trimers	4–6 mers	7–10 mers	> 10 mers	Total Proanthocyanidins	Type ^c
Almonds	5.2	7.8 ± 0.9	9.5 ± 1.6	8.8 ± 1.7	40.0 ± 8.5	37.7 ± 8.4	80.3 ± 28.1	184.0 ± 48.2	PP, PC
Cashews	5.2	6.7 ± 2.9	2.0 ± 0.4	nd ^b	nd	nd	nd	8.7 ± 3.2	PC
Hazelnuts	5.3	9.8 ± 1.6	12.5 ± 3.8	13.6 ± 3.9	67.7 ± 20.3	74.6 ± 21.9	322.4 ± 102.5	500.7 ± 152.0	PC, PD
Peanuts, roasted	2.0	5.1 ± 1.0	4.1 ± 0.7	3.7 ± 0.5	2.8 ± 0.2	nd	nd	15.6 ± 2.3	A, PC
Pecans	3.5	17.2 ± 2.5	42.1 ± 5.4	26.0 ± 2.0	101.4 ± 10.4	84.2 ± 12.9	223.0 ± 59.1	494.1 ± 86.2	PC, PD
Pistachios	4.0	10.9 ± 4.3	13.3 ± 1.8	10.5 ± 1.2	42.2 ± 5.2	37.9 ± 4.9	122.5 ± 37.1	237.3 ± 52.0	PC, PD
Walnuts	4.1	6.9 ± 3.4	5.6 ± 0.9	7.2 ± 1.2	22.1 ± 3.3	5.4 ± 0.8	20.0 ± 9.3	67.3 ± 14.7	PC

a: Values are means ± SD (n = 4-8).

b: nd: not detected.

c: PP: propelargonidins, PC: procyanidins, PD: prodelphinidins, and A: A-type proanthocyanidins.

Phenolic composition of almond (*Prunus dulcis* (Mill.) D.A. Webb) skins have been extensively studied by using of HPLC-DAD/ESI-MS technique (Monagas et al. 2007). The proanthocyanidins present in almond skin were characterized by MALDI-TOF MS. A total of 33 compounds, classified as flavanols, flavonols, dihydroflavonols, flavanones, and other nonflavonoid compounds, have been identified. Flavanols and flavonol glycosides were found to be the most abundant phenolics in almond skins, accounting for 38% - 57%, and 14% - 35% of the total phenolics, respectively. It was also observed that there existed a series of A- and B-type procyanidins and propelargonidins. The antioxidant activity (Oxygen Radical Absorbance Capacity: ORAC) ranged from 0.398-0.500 mmol Trolox/g of almond skins.

Catechins, B-type procyanidin dimers, trimers, tetramers, and oligomers were reported to be present in peanut skin (Lazarus et al., 1999).

Resveratrol has been found in peanuts and pistachios at 84 and 115 $\mu\text{g}/100\text{ g}$ (Tokusoglu et al., 2005).

2. Carotenoids

Carotenoids are a class of natural fat-soluble pigments found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They belong to the category of tetraterpenoids, and are generally classified into carotenes and xanthophylls. Structurally they are in the form of a 40-carbon polyene chain which could be considered the backbone of the molecule. This chain may be terminated by cyclic end-groups (rings) and may be complemented with oxygen-containing functional groups. The unoxygenated carotenoids such as α -carotene, β -carotene, and lycopene are known as carotenes, which typically contain only carbon and hydrogen. Carotenoids containing oxygen are named xanthophylls such as lutein and zeaxanthin. Carotenoids are composed of eight isoprenoid units, where the arrangement of isoprenoid units is reversed at the center of the molecule. The centrally located and extended conjugated double-bond system in carotenoids is responsible for their properties and functions. Over 600 naturally-occurring carotenoids have been characterized, and new carotenoids continue to be detected and identified. Those most frequently found in human blood and tissues are α - and β -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin. The chemical structure of carotenoids commonly present in nuts is shown in Figure 6.

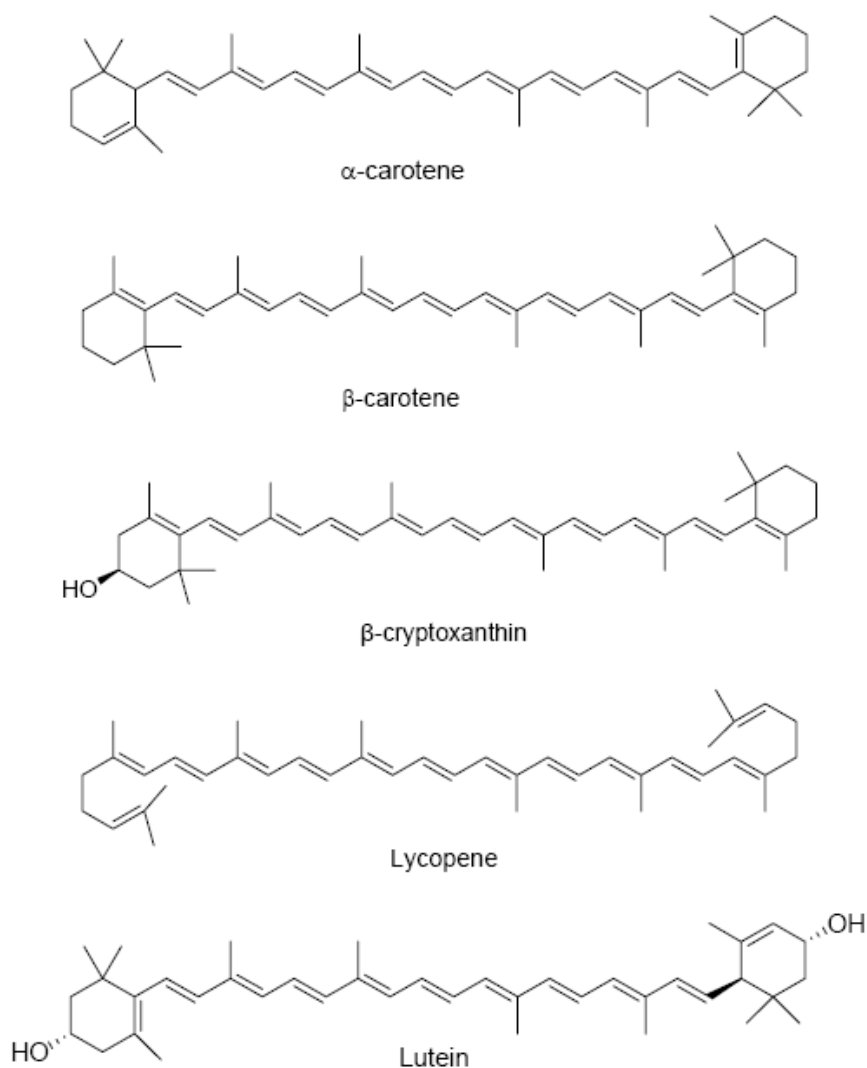


Figure 6. Chemical structures of carotenoids in tree nuts and peanuts.

The literature regarding the content of carotenes in nuts is scarce. No carotenes or retinol were identified in walnut kernels (Lavedrine et al., 1997). Carotenoids, including α - and β -carotene, zeaxanthin, lutein, cryptoxanthin and lycopene, in nine tree nuts and peanuts from the unsaponifiable matter were screened by Kornsteiner et al. (2006). Analyses of the unsaponifiable

matter were undertaken with 2 g of oil. The results demonstrated that there were no carotenoids found in the tested nuts except pistachios. β -carotene and lutein are detected in pistachios at 0.21 and 2.32 mg/100 g dry weight, respectively.

3. Tocopherols

Tocopherols are a class of chemical compounds, many of which have vitamin E activity (Figure 7). They are powerful antioxidants and have been shown to reduce the risk of CHD through inhibition of LDL cholesterol oxidation. Karle et al. (2004) have reviewed the α -tocopherol content (in mg) of nine types of nuts contained in a database to be used in the estimation of vitamin E intake. Almonds and hazelnuts had a value of more than 20% of the Recommended Dietary Allowances (RDA, 15 mg of α -tocopherol/day); Brazil nuts and peanuts possessed a value between 10.0 and 19.9%; cashews, macadamia nuts, pecans, pistachios, and walnuts contained a value of less than 10%. These data indicate that although nuts vary highly in their content of α -tocopherol, they can contribute to meeting the RDA for vitamin E.

Maguire et al. (2004) and Ryan et al. (2006) have reported that the contents of total tocopherols are dramatically different among various types of nuts, ranging from 60.8 in cashews to 452.0 $\mu\text{g/g}$ oil in almonds (**Table 4**). The decreasing order of total tocopherol level was almonds > hazelnuts > walnuts > pistachios > pine nuts > Brazil nuts > pecans > peanuts > macadamias > cashews. α -Tocopherol was detected in contents ranging from 3.6 ± 1.4 in the cashews to 439.5 ± 4.8 $\mu\text{g/g}$ oil in almonds. γ -Tocopherol concentration ranged from 12.5 ± 2.1 in almond to 300.5 ± 31.0 $\mu\text{g/g}$ oil in walnuts. The content of α -tocopherol in macadamias was reported to be 122.3 ± 24.5 $\mu\text{g/g}$ oil, which is much higher than previously reported (< 1.1 $\mu\text{g/g}$ lipids) (Kaijser et al., 2000). However, Kornsteiner et al. (2006) reported that no tocopherols were detected in macadamias.

The content of tocopherols (α -, β -, γ -, and δ -) in nine tree nuts and peanuts was fully assessed in the unsaponifiable matter (Kornsteiner et al., 2006). It was found that α -tocopherol content was the highest in hazelnuts with 31.4 mg/100 g of oil among all nuts tested. Almonds were also rich in α -tocopherol (24.2 mg/100 g extracted oil). Small amounts of α -tocopherol (less than 7 mg/100 g of oil) were measured in Brazil nuts, peanuts, and pine nuts.

Tocopherol Homologues	Substitutions		
	R ₁	R ₂	R ₃
α- tocopherol	CH ₃	CH ₃	CH ₃
β- tocopherol	CH ₃	H	CH ₃
γ- tocopherol	H	CH ₃	CH ₃
δ- tocopherol	H	H	CH ₃

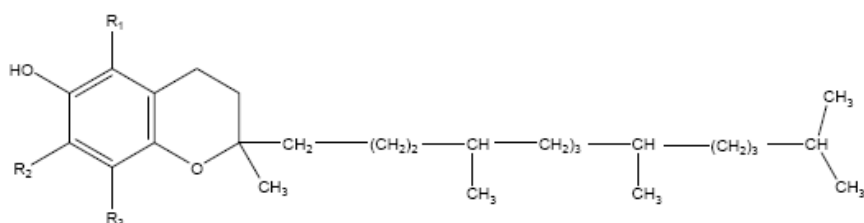


Figure 7. Chemical structure of tocopherols in tree nuts and peanuts.

Both β- and γ-tocopherols were prevalent in Brazil nuts, cashews, peanuts, pecans, pine nuts, pistachios and walnuts, with 5.1 in cashews and 29.3 mg/100 g extracted oil in pistachios. Traces of δ-tocopherol were detected in cashews, hazelnuts, peanuts, pecans, pines, pistachios, and walnuts. The α-tocopherol equivalents (TEs) greatly varied from non-detectable in macadamias to 33.1 mg/100 g extracted oil in hazelnuts. The average TEs in descending order were hazelnuts > almonds > peanuts > pistachios > pines > walnuts > Brazil nuts > pecans > cashews > macadamias (Table 4).

4. Phytosterols

Phytosterols, structurally similar to cholesterol, possess a cyclopentanoperhydrophenanthrene ring but differ in the side chain at C24 and/or the position and configuration of unsaturated double bonds and the optical rotation at chiral carbons from cholesterol (Goat 1991). Phytosterols have been categorized on the basis of the number of methyl groups at the C4 position. The primary plant sterols in the tree nuts and peanuts are sitosterol, stigmasterol, and campesterol (Figure 8). Phytosterols have been shown to lower blood cholesterol, to reduce the risk of certain types of cancer, as well as to enhance immune function (Moreau et al., 2002; Ling and Jones, 1995; Awad and Fink, 2000; Bouic 2001; Ostlund 2004).

Table 4. Tocopherol content of oil extracted from different nuts (Maguire et al., 2004; Ryan et al., 2006; Kornsteiner et al., 2006)

Oil Sample	Tocopherol ($\mu\text{g/g oil}$) ^a		Tocopherol (mg/100 g extracted oil)			
	α -Tocopherol	γ -Tocopherol	α -Tocopherol	β - & γ -Tocopherol	δ -Tocopherol	α -TE ^d
Almonds	439.5 $\pm$ 4.8	12.5 $\pm$ 2.1	nd to 34.9	0.5 – 10.4	nd	2.6 – 35.2
Brazil Nuts	82.9 $\pm$ 9.5	116.2 $\pm$ 5.1	nd to 2.2	8.2 – 17.9	nd	2.1 – 6.7
Cashews	3.6 $\pm$ 1.4	57.2 $\pm$ 6.2	nd	4.8 – 5.3	0.3 – 0.4	1.2 – 1.3
Hazelnuts	310.1 $\pm$ 31.1	61.2 $\pm$ 29.8	15.7 – 42.1	4.3 – 9.4	nd to 0.3	16.8 – 44.4
Macadamias	122.3 $\pm$ 24.5	Tr ^b	nd ^c	nd	nd	nd
Peanuts	87.9 $\pm$ 6.7	60.3 $\pm$ 6.7	1.7 – 10.4	5.4 – 10.0	1.4 – 2.4	3.1 -12.9
Pecans	12.2 $\pm$ 3.2	168.5 $\pm$ 15.9	nd	2.1 – 23.8	nd to 0.7	0.5 – 6.0
Pine nuts	124.3 $\pm$ 9.4	105.2 $\pm$ 7.2	2.2 – 6.0	6.4 – 9.8	nd to 0.7	3.8 – 8.5
Pistachios	15.6 $\pm$ 1.2	275.4 $\pm$ 19.8	nd	10.0 – 43.4	nd to 2.3	2.5 – 10.8
Walnuts	20.6 $\pm$ 8.2	300.5 $\pm$ 31.0	nd	12.4 – 32.8	2.3 – 5.4	3.1 – 8.2

^a: Results are the mean $\pm$ standard error of the mean for at least three independent experiments.

^b: Tr: trace amounts.

^c: nd: not detectable.

^d: Vitamin E activity in different nuts is represented as α -Tocopherol equivalent (α -TE).

α -TEs = α -Tocopherol (mg) $\times$ 1.0 + β - and γ -Tocopherol (mg) $\times$ 0.25 + δ -Tocopherol (mg) $\times$ 0.01.

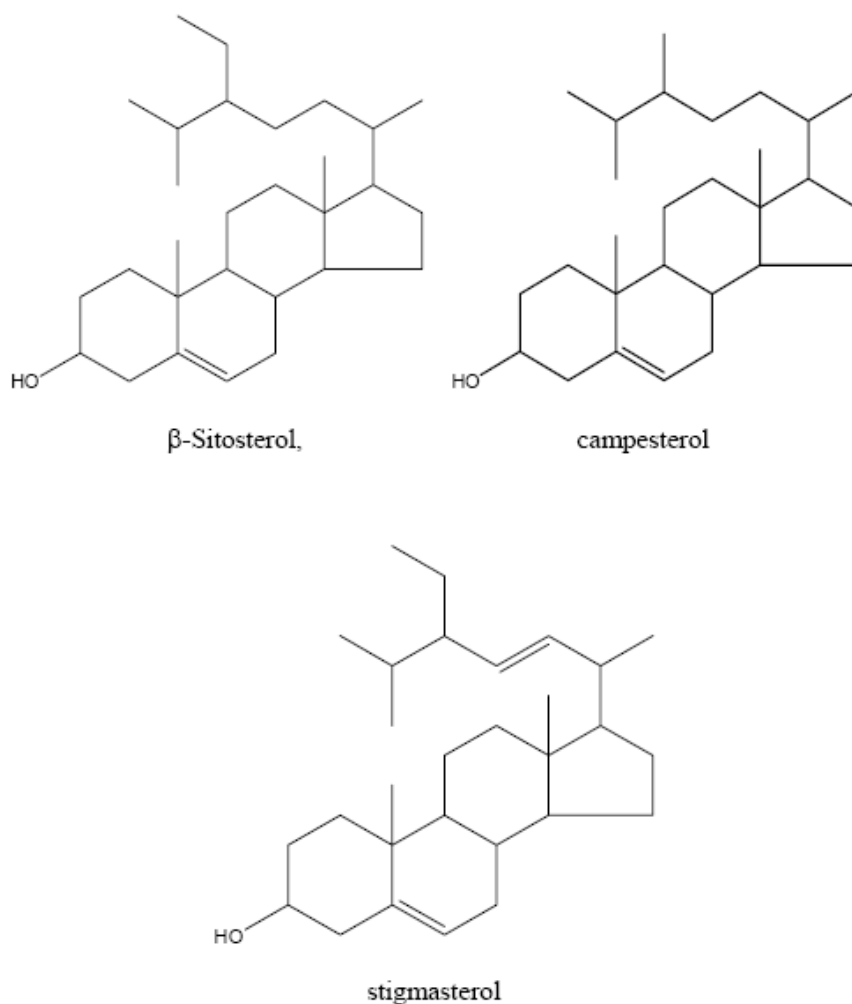


Figure 8. Chemical structure of β -Sitosterol, campesterol, and stigmasterol.

The FDA has approved the following claim for phytosterols: "Foods containing at least 0.4 gram per serving of plant sterols, eaten twice a day with meals for a daily total intake of at least 0.8 gram, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease."

Nuts are good sources of phytosterols. A summary of phytosterol content in tree nuts and peanuts has been compiled previously (Weihsrauch and Gardner, 1978; Breinholder et al., 2002; Maguire et al., 2004; Thompson et al., 2006; and Phillips et al., 2005). The amount of total phytosterols present in

nine tree nuts and peanuts is summarized in Table 5. According to the data reported by Maguire et al (2004) and Ryan et al (2006), the decreasing order of total phytosterol content was pistachios > pine nuts > almonds > cashews > pecans > Brazil nuts > peanuts > macadamias > walnuts > hazelnuts. β -Sitosterol was the most prevalent phytosterol, ranging in concentration from 1325.4 to 4685.9 $\mu\text{g/g}$ oil among freshly ground Brazil, pecan, pine, pistachio and cashew nuts. The pistachios contained the richest phytosterol content with 5586 $\mu\text{g/g}$ oil; while the lowest content was found in hazelnuts with 1096 $\mu\text{g/g}$ oil. There is approximately a 5-fold difference in phytosterol content between the highest and the lowest ranked nuts. β -Sitosterol was the principal plant sterol in nuts, ranging from 991.2 ± 73.2 in hazelnuts to 4685.9 ± 154.1 $\mu\text{g/g}$ oil in pistachios, which was approximately a 4.8-fold difference.

Phytosterols in commonly consumed nuts in the United States were determined by utilizing new analytical methodology that measures total free, esterified, and glycosidic sterols (Phillips et al., 2005). Total lipids were extracted from 1-2 g of each sample by acid hydrolysis and then alkaline saponification. An aliquot of the total lipid extract was analyzed for total free, esterified, and glycosidic sterols after acid hydrolysis. Free sterols were analyzed as trimethylsilyl derivatives by capillary GC-FID and GC-MS. Δ^5 -Avenasterol was quantified after alkaline saponification. It was found that, among all nuts and seeds tested, Brazil nuts contained the lowest total phytosterol content with 95 mg/100g, and pistachio had the highest in phytosterols with 270-289 mg/100 g. The data also showed that the predominant phytosterols in nuts were β -sitosterol, Δ^5 -avenasterol, and campesterol. Campestanol ranged from 1.0 to 12.7 mg/100 g. The phytosterol content in almonds, Brazil nuts, cashews, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, and walnuts are 187, 95, 138, 120, 198, 150, 198, 280, and 113 mg/100 g of wet weight of nuts, respectively, which exhibited different amounts of sterol from the data reported by Maguire et al. (2004) and Ryan et al. (2006). In this study, phytosterol levels were higher than reported in other food composition databases, probably due to the inclusion of steryl glycosides, which represent a significant portion of total sterols in nuts.

Table 5. Phytosterol content of oil extracted from ten edible nuts (Maguire et al., 2004; Ryan et al., 2006; Phillips et al., 2005)

Nuts	Phytosterol ($\mu\text{g/g oil}$) ^a			Phytosterol (mg / 100 g of nuts)							
	β -Sitosterol	Campesterol	Stigmasterol	β -Sitosterol	campesterol	Stigmasterol	Δ^5 -avenasterol	sitostanol	campestanol	others ^c	total
Almonds	2071.7 $\pm$ 25.9	55.0 $\pm$ 10.8	51.7 $\pm$ 3.6	143.4	4.9	5.0	19.7	3.2	3.3	19.6	199
Brazil Nuts	1325.4 $\pm$ 68.1	26.9 $\pm$ 4.4	577.5 $\pm$ 34.3	65.5	2.0	6.2	13.6	4.1	2.0	3.4	95
Cashews	1768.0 $\pm$ 210.6	105.3 $\pm$ 16.0	116.7 $\pm$ 12.6	112.6	8.9	< 1.2	13.7	< 1.2	2.0	13.3	150
Hazelnuts	991.2 $\pm$ 73.2	66.7 $\pm$ 6.7	38.1 $\pm$ 4.0	102.2	6.6	< 2.5	2.6	4.0	3.0	2.5	121
Macadamias	1506.7 $\pm$ 140.5	73.3 $\pm$ 8.9	38.3 $\pm$ 2.7	143.7	9.6	nd ^b	13.3	nd	2.9	17.0	187
Peanuts	1363.3 $\pm$ 103.9	198.3 $\pm$ 21.4	163.3 $\pm$ 23.8	76.8	13.2	12.1	17.8	< 1.2	1.6	15.0	137
Pecans	1572.4 $\pm$ 41.0	52.2 $\pm$ 7.1	340.4 $\pm$ 29.5	116.5	5.9	2.6	14.6	< 1.7	2.8	14.1	157
Pine nuts	1841.7 $\pm$ 125.2	214.9 $\pm$ 13.7	680.5 $\pm$ 45.7	132.0	19.8	< 1.7	40.3	5.9	3.8	34.2	236
Pistachios	4685.9 $\pm$ 154.1	236.8 $\pm$ 24.8	663.3 $\pm$ 61.0	209.8	10.1	2.3	26.2	1.2	5.0	24.6	279
Walnuts	1129.5 $\pm$ 124.6	51.0 $\pm$ 2.9	55.5 $\pm$ 11.0	114.4	4.7	< 1.7	29.5	< 2.5	2.6	25.8	177

5. Squalenes

Squalene is a hydrocarbon steroid precursor with a linear configuration and 30 carbons in length (Figure 9). It can be found in both plant and animal cells. Goodwin (1996) has reported that squalene is converted to phytosterols in plant cells. Squalene is a powerful antioxidant which can inhibit lipid oxidation, effectively quench singlet oxygen, suppress sodium arsenite-induced sister chromatid exchanges in Chinese ovary-K1 cells, and protect against H_2O_2 -induced SCE in Chinese hamster V79 cells (Fan et al., 1996; Kohno et al., 1995; O'Sullivan et al., 2002; Yang 2009b).

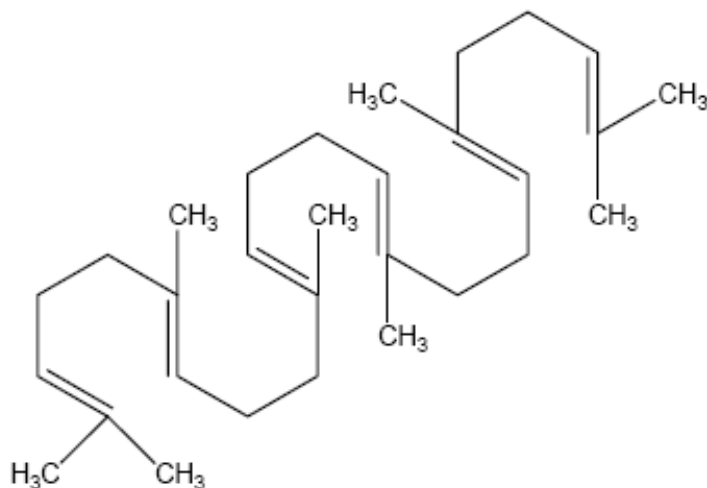


Figure 9. Chemical structure of squalenes.

Brazil nuts contained the highest squalene content with 1377.8 ± 8.4 $\mu\text{g/g}$ oil, followed by hazelnuts (186.4 ± 11.6), macadamias (185.0 ± 27.2), pecans (151.7 ± 10.8), peanuts (98.3 ± 13.4), almonds (95.0 ± 8.5), pistachios (91.4 ± 18.9), cashews (89.4 ± 9.7), pine nuts (39.5 ± 7.7), and walnuts (9.4 ± 1.8). The lowest squalene level occurred in the walnut, with around a 147-fold difference in squalene between the highest and the lowest ranked nuts (Ryan et al., 2006).

6. Others

Phytoestrogens, isoflavonoids, and lignans are also found in edible nuts (Figure 10). By using gas chromatography - mass spectrometry methods, Thompson et al. (2006) have quantified nine phytoestrogens in 6 tree nuts and peanuts available in Ontario, Canada, including four isoflavones (formononetin, genistein, daidzein, and glycitein), four lignans (secoisolariciresinol, matairesinol, pinoresinol, and lariciresinol), and coumestan (coumestrol). Nuts with decreasing contents of total phytoestrogens are pistachios, walnuts, almonds, cashews, hazelnuts, peanuts, and pecans (Table 6). Decreasing levels of lignans are found in pistachios, almonds, cashews, walnuts, hazelnuts, peanuts, and pecans. Coumestrol, one of the richest sources of phytoestrogens, was identified in six tree nuts and peanuts. Pistachios contained the highest total isoflavones, total lignans, and total phytoestrogens with 176.9, 198.9, and 382.5 $\mu\text{g}/100$ grams of wet weight, respectively.

BIOLOGICAL ACTIVITIES AND MECHANISMS

Edible nuts have been reported to contain the essential nutrients, including fatty acids such as polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) as well as linoleic acid, vitamins and minerals, and fiber (Allen, 2008). More importantly, nuts are a good source of phytonutrients, including phytosterols, carotenoids, flavonoids, and proanthocyanidins, which are responsible for antioxidant, anti-inflammatory, insulin resistance and anticancer properties. In this section, the effects of nuts on biological activities and their possible mechanisms are reviewed.

7. Antioxidant

Antioxidation from Nut Components

The antioxidant activities of nuts mainly come from such components as phenolics, lutein, carotenoids, and tocopherols. As shown in Table 7, total antioxidant capacity of tree nuts is determined by the methods of ORAC, ferric reducing antioxidant power (FRAP), total reactive antioxidant potentials (TRAP), trolox-equivalent antioxidant capacity (TEAC), and total oxyradical scavenging capacity (TOSC).

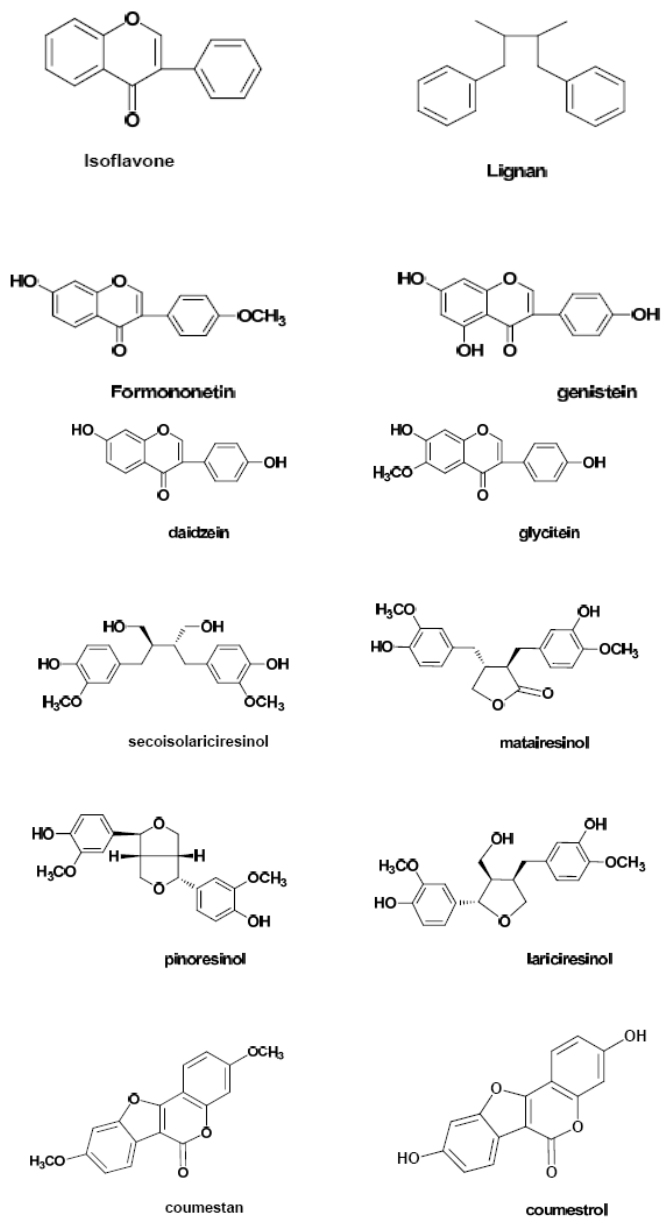


Figure 10: Chemical structure of phytoestrogens, isoflavones, and lignans in tree nuts and peanuts.

**Table 6. Isoflavone, Lignan, and Phytoestrogen Content of in Nuts ($\mu\text{g}/100$ grams of wet weight)
(Thompson et al., 2006)**

Tree nuts and Peanu ts	Formo noneti n	Daidzei n	Geniste in	Glycite in	Total Iso flavon es	Matairesi nol	Lariciresi nol	Pinoresi nol	Secoiso lariciresi nol	Total Ligna ns	Cou me strol	Total Phyto estrog ns
Almo nds	0.8	2.1	14.4	0.6	18.0	0.3	32.2	9.0	70.3	111.8	1.5	131.1
Cashe ws	10.0	1.4	10.3	0.4	22.1	0.3	60.5	1.1	37.5	99.4	0.4	121.9
Hazel nuts	1.2	3.6	24.8	0.5	30.2	1.2	14.3	1.1	60.5	77.1	0.3	107.5
Pecan s	0.7	1.6	0.9	0.3	3.5	0.6	8.4	1.2	14.8	25.0	0.3	28.8
Pistac hios	0.2	73.1	103.3	0.4	176.9	0.1	123.0	31.2	44.6	198.9	6.7	382.5
Walnu ts	0.9	35.2	16.4	0.8	53.3	0.2	7.2	0.2	78.0	85.7	0.6	139.5
Peanu ts	0.3	1.7	4.9	0.4	7.3	0.1	0.9	0.8	25.3	27.1	0.1	34.5

Table 7. Total Antioxidant Activity of Tree Nuts and Peanuts
(Wu et al., 2004; Pellegrini et al., 2006; Yang et al. 2009)

Edible Nut	ORAC (L+H) ^a ($\mu\text{mol TE}^{\text{b}}/\text{g}$)	FRAP ($\mu\text{mol Fe}^{2+}/\text{g}$)	TRAP ($\mu\text{mol TE}/\text{g}$)	TEAC ($\mu\text{mol TE}/\text{g}$)	TOSC ($\mu\text{mol VE}^{\text{d}}/\text{g}$)
Almonds	44.54	41.34	6.33	13.36	25.4 $\pm$ 2.0
Brazil nuts	14.19	- ^c	-	-	16.0 $\pm$ 1.2
Cashews	19.97	-	-	-	29.5 $\pm$ 2.7
Hazelnuts	96.45	42.31	6.90	12.02	7.1 $\pm$ 0.9
Macadamias	16.95	-	-	-	13.4 $\pm$ 0.4
Peanuts	31.66	15.46	3.30	4.76	81.3 $\pm$ 3.2
Pecans	179.40	-	-	-	427.0 $\pm$ 21.6
Pine Nuts	7.19	13.42	1.54	5.25	14.6 $\pm$ 1.1
Pistachios	79.83	192.67	25.92	61.46	75.9 $\pm$ 1.2
Walnuts	135.41	453.94	31.85	137.01	458.1 $\pm$ 14.0

^a:Lipophilic (L) and hydrophilic (H) ORAC assay values combined.

^b: Trolox Equivalents.

^c: not determined.

^d: Vitamin C Equivalents.

Walnut ranks number one in total antioxidant capacity amongst tree nuts. A study of 28 hypercholesterolemic adults who consumed 3 isoenergetic diets, with or without pistachios for 4 weeks each, has shown that participants had greater plasma lutein and γ -tocopherol than that of the lower-fat control diet (Kay et al. 2010). Participants also exhibited lower serum oxidized-LDL concentrations in pistachios diet than that in the control diet. On the pistachio nut diet, percent energy from saturated fat was found to be statistically significantly decreased, and percent energy from polyunsaturated fat and fiber intake also statistically significantly increased when compared to the control diet, which suggests that inclusion of pistachios in the diet contributes to the decrease in the serum oxidized-LDL concentration (Sheridan et al. 2007). In addition, on the pistachio diet, the levels of serum TC/HDL-C and LDL-C/HDL-C were statistically significantly reduced and the HDL-C level significantly increased in comparison with the control diet groups.

In one study, extracts from almond and its co-products including whole seed, brown skin, and green shell cover showed potent antioxidant activities (Wijeratne et al. 2006). These extracts inhibited human LDL oxidation, DNA scission, and metal ion chelation activities. Further HPLC analysis of extracts revealed the presence of quercetin, isorhamnetin, quercitrin, kaempferol 3-O-rutinoside, isorhamnetin 3-O-glucoside, and morin. In another study, extracts of defatted raw hazelnut kernel and hazelnut byproducts (skin, hard shell, green leafy cover, and tree leaf) were evaluated for total antioxidant activity (TAA), and free-radical scavenging activity (hydrogen peroxide, superoxide radical and 2,2-diphenyl-1-picrylhydrazyl radical) (Shahidi et al. 2007). Five phenolic acids (gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and sinapic acid) in both free and esterified forms were tentatively identified and quantified in the extracts. Hazelnut extracts exhibited antioxidant activities based on a β -carotene-linoleate model system, inhibition of oxidation of human LDL cholesterol, and inhibition of strand breaking of supercoiled deoxyribonucleic acid.

The acute bioavailability of polyphenols from both walnuts and almonds was measured in a recent human study (Torabian et al., 2009). After an overnight fast, walnuts, almonds or a control meal in the form of smoothies were consumed by 13 subjects. There was a significant increase in plasma polyphenol concentration following the nut meals, indicated by increased total antioxidant capacity and reduced plasma lipid peroxidation.

Increasing Antioxidant Enzymes

Besides acting as classical antioxidants characterized by their ability to directly scavenge or neutralize reactive oxidative species, nuts also contribute to building endogenous antioxidant defenses (Ros 2009). As a constituent of selenoproteins, selenium has structural and enzymatic roles, in the latter context being best-known as an antioxidant and catalyst for the production of active thyroid hormone. Brazil nut supplementation (5 g/day) increased blood levels of Se and glutathione peroxidase (GSH-Px) activity in hemodialysis patients to improve their antioxidant status (Stockler-Pinto et al., 2009).

In another *in vitro* study, powder of pistachio seed and skin scavenged the superoxide anion with IC_{50} of 3.25 ± 0.19 and 0.25 ± 0.02 mg, respectively (Tomaino et al. 2010). A non blinded, cross-over, placebo-controlled trial randomly assigned 22 volunteers (60% overweight and 40% obese) to a walnut meal (WM) or control meal (CM) during two different 5 week periods. The volunteers given the WM had significantly increased serum catalase (CAT) activity, total glutathione and oxidized glutathione (GSSG) in comparison with CM (Canales et al. 2007).

Extension of Lag Time in Oxidation of LDL

Anderson et al. (2001) investigated components analyzed by LC-ELSD/MS and antioxidant activity determined by TEAC in English walnuts (*Juglans regia*). Walnut extract significantly inhibited 87% LDL oxidation induced by 2, 2'-Azobis'(2-amidino propane) hydrochloride (AAPH). The extract also inhibited 84% LDL oxidation-mediated by a copper. Plasma thiobarbituric acid reacting substance (TBARS) formation was significantly inhibited by walnut extract in a dose-dependent manner, and the extract exhibited a TEAC value greater than that of α -tocopherol. These data indicated that polyphenol-rich extract from walnuts inhibits *in vitro* plasma and LDL oxidation and showed the effects of α -tocopherol on LDL during oxidative stress.

Polyphenolics (0.12-2.0 μ mol/L gallic acid equivalents) from almond skin reduced tryptophan (Trp) oxidation by 6.7-75.7%, increased the polarity of the Cu^{2+} -induced generation of conjugated dienes in human LDL by 21.0-81.5% at 90 min, and decreased the ratio of LDL- to total LDL by 38.2-83.8% at 5 h in a dose-dependent manner. In addition, almond skin polyphenolics lower the oxidative modification of apo B-100 and stabilize LDL conformation in a dose-dependent manner (Chen et al., 2007). Regular

peanut consumption lowers serum triacylglycerol (Alper and Mattes 2003). Jenkins et al. (2002) showed an almond diet was associated with significant reduction of conjugated dienes (a marker of lipid oxidation) in LDL cholesterol by comparison with a control diet. Thus, some bioactive compounds in nuts likely counteract the pro-oxidant effect of PUFA on LDL and protect PUFA *in vivo* against oxidative modification (Ros et al., 2004; Kris-Etherton et al., 2008).

8. Anti-Inflammatory

Nuts contain diverse macro- and micronutrients as well as other bioactive components such as magnesium, fiber, α -linolenic acid, L-arginine, and phytochemicals. These components in nuts may protect against inflammation (Ma et al. 2008; Jiang et al. 1998). Nut consumption has also been shown to decrease the plasma concentration of C-reactive protein (CRP), interleukin-6 (IL-6) and some endothelial markers in recent clinical trials (Salas-Salvado et al., 2008a). A cross-sectional analysis of data from 6,080 participants aged 45-84 years was examined for associations between nut consumption and CRP, IL-6, and fibrinogen in the Multi-Ethnic Study of Atherosclerosis (Jiang and Jacobs 2006). The results showed that frequent nut intake was linked to lower levels of inflammatory markers. The CRP level was decreased from 1.98 mg/L in subjects who rarely or never ate nuts to 1.72 mg/L in subjects who consumed nuts 5 times/wk ($P<0.01$), IL-6 decreased from 1.25 to 1.15 pg/mL ($P<0.01$) and adjusted fibrinogen also decreased from 343 to 331 mg/dL ($P<0.01$). Another prospective and cross-sectional study of 987 diabetic women who had no history of cardiovascular disease investigated the relationship between a Mediterranean dietary pattern and higher plasma adiponectin concentrations (Mantzoros et al. 2006). Adiponectin is an adipose tissue - secreted cytokine which improves insulin sensitivity and regulates lipid metabolism. The results demonstrated that nuts, as components of the Mediterranean diet, had the strongest association with adiponectin concentrations. A third cross-sectional study performed in 339 men and 433 women aged between 55 and 80 years at high cardiovascular risk evaluated associations between components of the Mediterranean diet and circulating markers of inflammation (Salas-Salvado et al. 2008b). The results indicated that subjects with the highest consumption of nuts had the lowest concentrations of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), IL-6 and CRP ($P<0.01$).

9. Insulin Resistance and Type II Diabetes

Nut consumption, including peanuts, protects against not only CHD but also against diabetes, the CHD associated with diabetes, and other metabolic syndrome diseases (Jenkins et al., 2008). Acute feeding studies have demonstrated the ability of nuts, when eaten with carbohydrate (bread), to depress postprandial glycemia due to nuts possessing a beneficial nutritional profile: high in phytochemicals, MUFA, PUFA, and vegetable protein as well as low in carbohydrates (Kendall et al., 2010a). Epidemiological studies indicated that regular consumption of nuts is unlikely to contribute to obesity or increased risk of diabetes. It may help to regulate body weight by suppressing appetite and fat absorption. Nut consumption counteracts dyslipidemia and has the capacity to improve circulatory function through the actions of multiple constituents (arginine, polyphenols) on endothelial mechanisms (Coates and Howe 2007).

10. Anticancer Activity

Regular intake of nuts may have beneficial effects against oxidative stress-mediated diseases such as cancer. Although the results are not conclusive, a protective effect on colon and rectum cancer was reported (Rajamanickam and Agarwal 2008). Likewise, some studies show a possible protective effect on prostate cancer, but there is insufficient data on other tumor locations (Gonzalez and Salas-Salvado 2006). Walnut extracts were assayed for their antiproliferative effectiveness using human renal cancer cell lines A-498 and 769-P and the colon cancer cell line Caco-2. All extracts exhibited concentration-dependent growth inhibition toward human kidney and colon cancer cells (Carvalho et al., 2010). Su et al. (2010) reported that dietary intake of fiber and nuts during adolescence could influence subsequent risk of breast disease from 29,480 women who completed a high school diet questionnaire. It may suggest a viable means for breast cancer prevention. Several natural products present in nuts, including phenolics, flavonoids, carotenoids, alkaloids, nitrogen-containing as well as organosulfur compounds, confer protective effects against a wide range of cancers (Rajamanickam and Agarwal 2008). β -Escin, a principle component of horse chestnut, inhibited colonic aberrant crypt foci formation in a rat model. β -Escin also induced cell cycle arrest at the G1/S phase together with an induction of p21^{Cip1} and an associated reduction in the phosphorylation of

retinoblastoma protein in HT-29 cells (Patlolla et al., 2006). Ellagic acid, a polyphenolic compound found in nuts, has been shown to possess growth-inhibiting and apoptosis-promoting activities in cancer cell lines *in vitro*. Its molecular targets include NF- κ B, cyclin D1, p21^{cip1/waf1} and p53, all involved in regulation of cell cycle and apoptosis (Fjaeraa and Nanberg 2009). Yang et al. (2009a) reported that nine types of tree nuts and peanuts showed antiproliferative activity in HepG₂ and Caco-2 cells.

11. Others

Protection of DNA from oxidative injury has also been demonstrated *in vitro* for extracts of almonds and hazelnuts (Wijeratne et al., 2006; Shahidi et al., 2007). Almond consumption (84 g/d) significantly reduced oxidative DNA damage among smokers following a 4 wk study (Jia et al., 2006; Li et al., 2007). Flavonoids from almond skins possess antioxidant capacity *in vitro*; they are bioavailable and act in synergy with vitamins C and E to protect LDL against oxidation in hamsters (Chen et al., 2005). Regular peanut consumption not only reduced serum TAG, but also increased serum magnesium concentration in a human study (Alper and Mattes 2003). In systemic inflammation, a high magnesium intake was associated with lower concentrations of markers including CRP, IL-6, sVCAM-1, sICAM-1 and E-selectin (Chacko et al., 2010; Song et al., 2007).

HEALTH BENEFITS

12. Cardiovascular Disease

As comprehensively summarized by Ros (2009), the development of CVD involves damage to endothelial walls of blood vessels, accumulation of LDL cholesterol on the arterial wall, and oxidation of the lipids followed by an inflammatory response. It is not surprising then, that a healthy diet can help prevent CVD by altering serum lipid profiles, and reducing oxidative stress and inflammation. While dietary saturated fat has long been associated with blood cholesterol and coronary heart disease (Keys 1980), unsaturated fats have been more recently recognized as having beneficial effects on markers of cardiovascular health (Ros and Mataix, 2006). Nuts are unique in that although they are a relatively high-fat food, they are abundant in poly- and

monounsaturated fatty acids (PUFAs and MUFAs, respectively). The correlation between dietary fat and serum lipid levels has been so well-studied that one can mathematically predict a decrease in LDL cholesterol based on the extent to which dietary fat intake has been reduced. The decrease seen in response to consuming nuts and peanuts is more dramatic than predicted by such calculations, suggesting nuts contain components other than the fatty acids which can modulate serum lipid levels (Griel and Kris-Etherton, 2006), such as phytosterols. Other components found in nuts that may also contribute to aspects of cardiovascular health are fiber, potassium, calcium, magnesium, tocopherols, phenolics, resveratrol and L-arginine (Kris-Etherton et al., 2008).

Four major epidemiology studies (The Adventist Health Study, 1992; Nurses' Health Study, 1998; Physician's Health Study, 2002; and Iowa Women's Health Study, 2006) revealed a dose-response relationship between increasing nut consumption and decreasing risk of CHD (Fraser et al., 1992; Hu et al., 1998; Ellsworth et al., 2001; Albert et al., 2002). Pooling the results of these studies resulted in an overall lower risk of CHD in the group of individuals consuming the highest intake of nuts compared to the lowest (RR=0.65, CI: 0.47-0.89). Specifically for fatal CHD, the RR was 0.61 (CI: 0.35-1.05) and for nonfatal myocardial infarction the RR was 0.68 (CI: 0.47-1.00) (Kris-Etherton et al., 2008). Peanuts appeared to more potently decrease CHD risk (Hu et al., 1998). For example, consuming peanuts more than twice per week reduced relative risk of CHD to 0.66 (CI: 0.46-0.94) while the same frequency of consuming tree nuts reduced RR to only 0.79 (CI: 0.50-1.25).

A highly controlled, randomized crossover feeding trial found differential effects of walnuts versus fish on serum lipid levels (Rajaram et al., 2009). The study followed 25 normal to mildly hyperlipidemic subjects after 4 weeks on each of 3 different diets: the walnut diet (42.5 g walnuts/10.1 MJ), the fish diet (113 g salmon twice/week), or the control diet (devoid of fish and nuts). All 3 diets contained 30% fat, with less than 10% saturated fat. Controlling for saturated fat content is a strength of the study, since differences in saturated fat content between control and experimental diets may confound results of serum lipid studies. Another strength of the study is the controlled meal preparation, enabling the isolation of effects of specific dietary components. The "doses" of fish and walnuts were chosen based on current dietary recommendations for the prevention of CHD (Rajaram et al., 2009). Interestingly, the walnut diet resulted in lower total serum cholesterol (4.87 ± 0.18 mmol/L) and LDL cholesterol (2.77 ± 0.15 mmol/L) compared to the fish diet (5.33 ± 0.18 and 3.2 ± 0.15 mmol/L) and to the control diet (5.14 ± 0.18 and 3.06 ± 0.15 mmol/L; $P < 0.001$), while reduced serum triglycerides were seen in the fish

diet (1.0 ± 0.11 mmol/L; control diet: 1.12 ± 0.11 ; walnut diet: 1.11 ± 0.11 mmol/L) as well as improved HDL levels (1.23 ± 0.05 ; control diet: 1.19 ± 0.05 ; walnut diet: 1.18 ± 0.05 mmol/L). These findings highlight the value of whole foods and dietary patterns for optimizing health benefits, rather than recommending one particular nutrient. Rajaram's (2009) results are consistent with a recent pooled analysis of studies on the effects of nut consumption on serum lipid levels (Sabate et al., 2010). Among 25 studies included in the analysis, consumption of on average 67 g nuts/day correlated with a decrease in total cholesterol, LDL, LDL:HDL ratio, and total cholesterol:HDL ratio (change of 5.1%, 7.4%, 8.3% and 5.6%, respectively), regardless of the type of nut, and of the age or gender of the subjects. Interestingly, nut consumption more dramatically reduced cholesterol levels in subjects with more elevated starting levels of LDL, which may have important clinical implications. Subjects with lower BMI benefitted from a greater reduction in cholesterol in response to nut consumption as well. On the contrary, subjects with obesity or metabolic syndrome did not see changes in blood lipid levels in response to high intake of walnuts or cashews (Mukuddem-Petersen et al., 2007). Nuts altered serum lipids more dramatically in comparison to a typical Western diet than compared to a Mediterranean or a low-fat diet, and when consumed in place of saturated fat compared to olive oil or carbohydrates. Importantly, a dose-response became evident when the control diet was devoid of nuts.

Similar trends were seen in a meta-analysis of walnut feeding studies measuring effects on serum lipid levels and other risk factors for CVD (Banel and Hu, 2009). Compared to other tree nuts which contain abundant MUFAs, walnuts contain mostly PUFAs, namely the omega-3 FA α -linolenic acid and the omega-6 linoleic acid. The meta-analysis covered 13 studies including 365 subjects total. Subjects had normal cholesterol levels in 4 studies, modest hypercholesterolemia in 6 studies, and either diabetes, obesity, or metabolic syndrome in each of the 3 remaining studies. The meta-analysis revealed a decrease in total cholesterol of 10.29 mg/dL and 9.23 mg/mL in LDL cholesterol after a walnut diet compared to controlled diets (weighted mean difference; $P < 0.001$ for both values). HDL cholesterol levels did not change significantly, and although the change in triglycerides was not significantly different, there was a larger decrease after the walnut diet versus baseline than after the control diet (Banel and Hu, 2009).

In the large, prospective Physicians' Health Study, which followed 21,078 male physicians for more than 20 years (1982-2008), no association was found between nut consumption and either total or ischemic stroke (Djousse et al., 2010). Interestingly, the correlation with hemorrhagic stroke had a J-shaped

relationship, where those consuming the lowest intake of nuts (<1 time/week) had an intermediate hazard ratio of risk (1.13, 95% CI 0.78-1.62), an intermediate intake of nuts (2-4 times/week) had the lowest risk (0.49, 95% CI 0.27-0.89), and the highest intake of nuts (≥ 7 times/week) correlated with the highest risk (1.84, 95% CI 0.95-3.57). The explanations for this trend may be physiological, technical (i.e. study design or analysis), or some combination thereof. While PUFAs, which prevent platelet aggregation, may be beneficial in preventing some aspects of CVD such as atherosclerosis, the same property may contribute to the risk of other conditions such as hemorrhagic stroke. Similarly, individuals with low BMI and low LDL have a reduced risk of ischemic stroke, but an increased risk of hemorrhagic stroke (Knekt et al., 1991). The authors note that while both the entire sample size and the number of strokes which occurred were large and the follow-up time was long, the number of the different types of strokes among the different groups of nut consumption were small, and therefore recommend the statistical results be interpreted “with caution.” Their results, however, remind us that no single food can be a cure-all. Rather, it is the overall diet which will contribute most to our health, and dietary recommendations may best be made for each individual, based on specific health needs and risks.

Dietary antioxidants that protect against LDL oxidation include tocopherols and phenolic compounds, both found in nuts. MUFAs, abundant in nuts, are refractory to oxidation (Reaven and Witztum, 1996). The PUFAs in nuts, which can be oxidized into a product called malondialdehyde (MDA), are protected by the other antioxidant components in the nuts (Kris-Etherton et al., 2008; Reaven and Witztum, 1996). Pistachios, for example, have been found to improve oxidative status in two different studies. In one study, 44 healthy volunteers were divided into two groups: their regular diet, or a pistachio diet (Kocyigit et al., 2006). The pistachio diet included pistachios to account for ~20% of daily energy intake (65-75g/day), which replaced some calories from other high-fat foods such as meat, oils, margarine and butter. At the end of the 3-week intervention, those in the pistachio group had consumed less SFA, and more MUFA, PUFA and fiber than the control diet group. Antioxidant potential (AOP) of the serum, a measure of the dynamic balance between oxidants and antioxidants, increased significantly in the pistachio group compared to baseline, increasing from 1.83 ± 0.85 to 2.68 ± 1.57 $\mu\text{mol/L}$ ($P < 0.05$). The control diet group saw an insignificant increase in AOP from 1.79 ± 0.65 to 1.84 ± 0.81 $\mu\text{mol/L}$. Similarly, the pistachio diet resulted in a significant decrease in serum MDA (from 2.11 ± 0.72 nmol/L to 1.68 ± 0.44 nmol/L ($P < 0.05$), while the decrease after the control diet was insignificant

(from 2.19 ± 0.62 to 2.12 ± 0.56 nmol/L). Of note, the pistachio diet significantly improved total cholesterol and HDL levels, while having no effect on body weight (Kocyigit et al., 2006).

More recently, healthy adults with mild hypercholesterolemia participated in a randomized, crossover controlled-feeding study, following 3 diets for 4 weeks each (Kay et al., 2010). The control diet contained no pistachios, the 1 PD diet derived 10% of energy from pistachios (32 to 63 g/d), and the 2 PD diet derived 20% (63 to 126 g/d). The differing amounts of pistachios altered the amount of unsaturated fats in the diets. Otherwise, all 3 diets were matched for total energy, saturated fat (8%), cholesterol, antioxidant vitamins A, C and E, tocopherols, lutein, selenium and folate, and total fat accounted for 25 to 35% of energy. Serum levels of LDL-cholesterol and oxidized-LDL were significantly decreased after the pistachio diets compared to the control diet, while levels of the antioxidants lutein, γ -tocopherol and β -carotene were increased. The diets did not affect serum lipid hydroperoxides or glutathione. The authors speculate the lack of effect on serum lipid hydroperoxides or glutathione may be due to dynamic rates of protein oxidation (such as lipoproteins) versus lipids, activity of other serum antioxidants, sensitivity of the specific assays used, and/or a blunted response in healthy individuals. In summary, the authors conclude pistachios contribute to a heart-healthy diet by decreasing LDL oxidation, through lowered LDL levels and increased serum antioxidant levels (Kay et al., 2010).

While there are some inconsistencies between studies of almond trials and markers of oxidation Ros (2009) concludes MUFA-rich nuts may help to decrease oxidation while nuts higher in PUFAs, namely walnuts, show only a slight improvement or none at all. It is important to note that more than 50% of the antioxidants reside in the skins of nuts (Blomhoff et al., 2006). Many studies do not specify whether skins were included, and this may partly account for differing results across studies of oxidative stress markers. In addition, not controlling for saturated fat and antioxidants between experimental and control diets also may contribute to inconsistent findings (Kay et al., 2010).

Many components of nuts, such as the omega-3 PUFA α -linolenic acid (ALA; found in walnuts), the phenolic compound ellagic acid, L-arginine, and antioxidant vitamins such as tocopherols, fiber and magnesium, may modulate inflammation (Jiang et al., 2006; Ros 2009). As summarized by Ros (2009), several studies failed to show significant changes in CRP levels after consumption of almonds, walnuts, cashews, or mixed nuts. However, several studies have shown walnuts to decrease levels of the inflammatory cytokines

ICAM-1 and VCAM-1 (Ros et al., 2004) and E-selectin (Cortes et al., 2006) in the serum, and mixed nuts to decrease IL-6, ICAM-1 and VCAM-1 (Estruch et al., 2006). Therefore, evidence exists that nuts may differentially affect biomarkers of inflammation, but clearly further research in this area is needed.

Omega-3 PUFAs, L-arginine, tocopherol, phenolic antioxidants, folic acid, and magnesium have been associated with healthy endothelial function. Walnuts are rich in all of these components, and have been shown to improve endothelial function in intervention studies (Ros 2009).

13. Diabetes

Nearly one-third of American adults has metabolic syndrome, a collection of conditions including abdominal obesity, atherogenesis dyslipidemia (blood fat disorders), elevated blood pressure, insulin resistance or glucose intolerance, prothrombotic state and proinflammatory state (American Heart Association, 2010). Those with metabolic syndrome have about a 5-fold increase in risk of diabetes, and in turn, diabetics have a 2- to 5-fold increase in risk of CVD, the risk higher for women than men (Kendall et al., 2010b). The maintenance of healthy body weight and dietary modification are important in maintaining glycemic control for diabetics as well as in preventing the onset of diabetes, which currently has no cure.

The type of fat consumed has more influence on, and therefore may be a better predictor of, type 2 diabetes risk than total fat intake itself (Jiang et al., 2002). Nuts are about 70-80% fat, but the majority of the fat found in nuts is mono- and polyunsaturated. There is some evidence that increased intake of unsaturated fats can improve insulin sensitivity and lower diabetes risk, while higher intakes of saturated and trans fats may have the opposite effects. In addition to fats, nuts are rich in fiber, magnesium, vitamins, minerals and antioxidants, and have a lower glycemic index, all factors which may be protective regarding development of diabetes. Fiber, magnesium, and the relatively low glycemic index of nuts are associated with improved insulin sensitivity and reduced risk of type 2 diabetes. Jiang et al. (2002) utilized the Nurses' Health Study, ultimately using data from 83,818 female participants, age 30 to 55 at baseline and followed for 16 years (1980 to 1996) to determine whether a correlation exists between nut and peanut consumption and incidence of type 2 diabetes. Food frequency questionnaires listed four categories for nut consumption: never/almost never, less than one time per week, 1 to 4 times per week, and 5 or more times per week. Comparing the

most frequent nut consumers (5 or more times per week) with the least frequent (never/almost never), the age-adjusted relative risk of diabetes incidence (RR) was 0.55 (95% CI, 0.45-0.66). Controlling for BMI, the reduced risk was still significant: RR 0.74 when BMI was used as a categorical variable (95% CI 0.61-0.89, *P* for trend < 0.001) and RR 0.72 when used as a continuous variable (95% CI, 0.59-0.87, *P* for trend < 0.001). Controlling for other dietary factors (glycemic load, multivitamin use, and intakes of polyunsaturated fats, saturated fat, trans-fat, cereal fiber, magnesium, whole grains, vegetables, fruits, and fish) changed the RR only slightly, to 0.71 (95% CI, 0.57-0.87), while adjusting for family history, family history of diabetes, physical activity, smoking, alcohol consumption, and total energy intake did not change the relative risk of diabetes incidence. Those women who consumed peanut butter the most frequently (5 or more times per week) also had a reduced risk of type 2 diabetes compared to those who ate it least frequently (never/almost never; RR 0.79, 95% CI, 0.68-0.91). After adjusting for age, family history of diabetes, physical activity, smoking, alcohol use and baseline weight, the amount of weight gained over the 16 year follow-up was statistically the same across all four categories of nut consumption. The authors conclude that in this cohort, increased nut consumption did not affect body weight but did reduce risk of developing type 2 diabetes (Jiang et al., 2002).

Commenting on the findings of Jiang et al. (2002) and the Nurses' Health Study, Parker et al. (2003) looked into data from the Iowa Women's Health Study, a prospective cohort of 35,988 post-menopausal women and a 12-year follow-up using similar categories of nut (one serving 28.5g) or peanut butter (1 serving 1 tablespoon) consumption (<1 time per month, <1 time per week; 1 to 4 times per week, or 5 or more times per week). However, this study failed to replicate the trends seen in Jiang et al. Compared to those consuming the least amount of nuts, those who ate nuts < 1 time per week or those who consumed 1 to 4 times per week had a RR of 0.85, while the highest consuming group had a RR of 0.98 (*P* for trend 0.88), a trend which was made less dramatic or even positive upon adjusting for various confounding factors. The authors speculate the differences between this and the Nurses' Health Study may be due to analysis of multiple dietary factors versus only one in the Iowa study, different measures of diabetes diagnosis, and the age of the cohort. As such, the authors hesitate to recommend nuts as a means of decreasing diabetes risk until further research has been done. In response, Jiang et al. speculate over-adjustment for dietary components actually found in nuts may account for the insignificant results in the Iowa study, while such associations

did not change the results of the Nurses' study. Jiang et al. re-analyzed the data stratifying by menopausal status but still found an inverse correlation (premenopausal: RR 0.67, 95% CI 0.46-0.97; postmenopausal: RR 0.73, 95% CI 0.57-0.95). However, when using 1986 as baseline to shorten follow-up, the association was reduced (RR 0.89, 95% CI 0.69-1.14). Jiang et al. maintain that nuts can be recommended as "part of a healthy dietary pattern."

In individuals predisposed to insulin resistance or diabetes, excess caloric intake leads to elevated levels of intracellular glucose and FFA (free fatty acid) oxidation, which both generate ROS (reactive oxygen species; Franzini et al., 2008). The resulting oxidative stress does not necessarily cause insulin resistance but can worsen the condition as well as cause death of pancreatic B-cells, which secrete insulin. This chain of events may lead to diabetes and CVD (cardiovascular disease). In a review exploring the relationship between diet-induced oxidative stress, excess calories, insulin resistance and diabetes, Franzini et al (2008) suggest antioxidants may help to interrupt this "vicious circle." While it is possible to improve oxidative balance through consumption of antioxidants, studies using supplements of specific compounds have been less conclusive than studies measuring antioxidant intake from whole foods. Franzini et al. describe many reasons why this may be so. For example, ROS have very short half-lives (on the order of fractions of seconds), and therefore total antioxidant capacity of the blood may be a better marker of oxidative stress. In addition, many components of the diet contribute to circulating antioxidant load in the blood, such as polyphenols as well as vitamins, and so singling out one antioxidant may not be sufficient to provide the whole picture. Two studies illustrate the point: In an 8-week intervention trial, a diet including fish high in omega-3 FAs (fatty acids) more effectively reduced plasma MDA and increased plasma antioxidant status than did an isocaloric diet including fish oil supplements in place of fish (Parra et al., 2007). In another 8-week trial, post-menopausal women who consumed soy nuts experienced a more effective decrease in inflammatory markers (E-selectin, interleukin-18, Tumor Necrosis Factor- α (TNF- α), and C-Reactive Protein (CRP)) than those who were given soy protein (Azadbakht et al., 2007). One of the many detriments of smoking is generation of ROS. Smokers given almond powder as a supplement to their diet had significantly increased plasma antioxidant capacity and significantly decreased oxidative stress markers (Li et al., 2007). These studies highlight the benefits of consuming whole foods, rather than supplements, to improve antioxidant balance, which may protect against various chronic diseases such as diabetes, CVD, and cancer (Franzini et al., 2008).

As summarized by Kendall et al. (2010b), insulin sensitivity can be increased, hyperinsulinemia can be prevented, and glycemic control improved in type 2 diabetics by consuming foods that do not dramatically increase blood glucose or insulin levels after consumption. Nuts are a good example of such a food, since they are a low in carbohydrates. In addition, when eaten with carbohydrates, nuts can reduce postprandial glycemia, insulinemia, and oxidative stress (Josse et al., 2007; Jenkins et al., 2006), thus highlighting that it is the whole food, and even combinations of foods, providing benefits beyond those conferred by individual nutrients or isolated compounds. As Jenkins et al. note, such observations point toward making “food-based recommendations in addition to macronutrient based recommendations” for prevention of chronic disease. Four of eight trials studying glycemic control, reviewed by Kendall et al., show improvements of at least one marker due to consumption of nuts, but none saw significant effects on HbA1c, which measures long-term glycemic control. Two studies show improved fasting insulin (12-week feeding trial of 50 patients with metabolic syndrome given control diet or nut diet, which included 15 g walnuts, 7.5 g almonds, and 7.5 g hazelnuts per day: Casas-Agustench et al., 2009; year-long study of 50 overweight diabetic patients following low-fat diet plus 30 g walnuts per day: Tapsell et al., 2009) and one showed improved fasting glucose (772 healthy adults at high risk of CVD, assigned to either low fat diet, Mediterranean diet with 1L/week olive oil, or Mediterranean diet with 30g/d walnuts: Estruch et al., 2006). Interestingly, the fasting glucose due to nut consumption was improved over the control, but not over the olive oil group, suggesting similar beneficial activities in the two foods. Inconsistencies between studies may be attributed to varying treatment durations or low baseline levels of markers (Kendall et al., 2010a). Long-term studies of effects of nuts on glycemic control in subjects with diabetes or metabolic syndrome do not consistently support the hypothesis that nuts may improve relevant markers. However, the authors note that since diabetics have increased risk of CVD, and nuts have been shown to significantly reduce risk of CVD, addition of nuts to the diet may be of benefit to the overall health of diabetics (Kendall et al., 2010a).

14. Cancer

Nuts contain many components which have been correlated with protection from cancer, such as fiber, vegetable protein, MUFAs, vitamin E, phenolic compounds, selenium, folic acid, and phytoestrogens (Gonzales et al.,

2006). However, few studies have been designed to specifically answer the question of whether consumption of nuts can confer protection against cancer. Often in such studies linking diet with disease outcomes, nuts are classified with seeds and legumes, making it difficult to distinguish effects of the food groups independently of one another (Gonzalez and Salas-Salvado 2006).

Few recent prospective studies have looked specifically at nut consumption and cancer incidence. In a cohort study of 10 European countries, called the European Prospective Investigation into Cancer and Nutrition, consumption of nuts and seeds was associated with decreased risk of colon cancer in women (>6.2 g/d vs. nonconsumers: OR 0.69; 95% CI 0.50-0.95) while no significant difference was seen in men (Jenab et al. 2004). Similarly, a Taiwanese cohort study found a dramatically reduced colon cancer risk in women consuming peanut products more than 2 times/week (OR 0.42; 95% CI 0.21-0.84), but not at all in men. This study followed ~24,000 people for 10 years (Yeh et al., 2006). Finally, a much smaller case-control study in Greece (84 cases and 84 controls) found that consumption of nuts, seeds and legumes reduced risk of endometrial cancer in women (OR 0.64; 95% CI 0.47-0.86; Petridou et al., 2002).

A Canadian case-control study of 396 men (half cases and half controls), interviewed between 1989 and 1993, demonstrated an association between higher intake of nuts and a significantly reduced risk of prostate cancer (OR 0.43; 95% CI 0.22-0.85; $P=0.01$; Raimondi et al., 2010). Other foods associated with a decreased risk included legumes (OR 0.40; 0.22-0.74; $P=0.006$) and seafood (OR 0.54; 0.30-0.97; $P=0.05$), while dairy, particularly milk, was associated with increased risk (comparing groups with highest intake versus lowest, OR 2.19; 95% CI 1.22-3.94; $P=0.03$). The protective effects of nuts may be due to their high vitamin E content. Although there is some evidence vitamin E may protect against prostate cancer (Kristal et al., 1999; Heinonen et al., 1998) and other studies were unable to show such an effect (Peters et al., 2008; Lippman et al., 2009; Gaziano et al., 2009), the current study did correlate higher vitamin E with reduced prostate cancer risk (Raimondi et al., 2010).

Women who have been diagnosed with benign breast disease (BBD) have an increased risk of developing breast cancer (Su et al., 2010). Several studies that have looked at fiber intake during adulthood and risk of BBD have been somewhat inconsistent, with case-control and some cohort studies showing an inverse association, and prospective studies showing no association (for example the Nurses' Health Study II cohort). During childhood and adolescence, breast tissue may be more vulnerable to carcinogenic insults and

therefore, beneficial dietary components may confer more protection during those years. In addition, retrospective studies are subject to inaccurate reporting, as a diagnosis can influence recall of prior dietary intake. The current study was an analysis of the Nurses' Health Study II, with additional cases, and done in a prospective fashion. The analysis included 29,480 women who responded to a questionnaire in 1998 regarding food intake during their high school years. Between 1991 and 2001, 682 cases of benign breast disease were identified among the cohort. Ultimately, high intake of fiber was associated with a 25% reduced risk of proliferative BBD (HR 0.75; 95% CI 0.59-0.96; highest vs. lowest quintile). When adjusted for nut intake, the beneficial effect of nuts was somewhat attenuated (HR 0.80; 95% CI 0.62-1.02). Higher intake of peanuts (≥ 1 serving/week) during high school significantly reduced the risk of BBD by 34% (HR 0.66; 95% CI 0.51-0.86). Intake of total nuts had a similar effect, lowering the risk of BBD by 36% (HR 0.64; 95% CI 0.48-0.85; ≥ 2 servings/week vs. <1 serving/month). Interestingly, adjusting for fiber made little difference, suggesting that other components of nuts, in addition to the fiber, may be involved in this protective mechanism (Su et al., 2010).

A major risk factor for non-Hodgkin's lymphoma (NHL) is immunosuppression, although not every case of NHL can be correlated with this factor (Thompson et al., 2010). Some studies have demonstrated a protective effect of dietary fruits and vegetables. Since immune system cells are vulnerable to attack by reactive oxygen species (ROS), and dietary antioxidants have been found to accumulate in immune system cells, the Iowa Women's Health Study was employed to determine whether correlations between intake of particular food groups and incidence of NHL exist. Over 41,000 women aged 55 to 69 years at baseline were followed for nearly 20 years. In this cohort, no significant association was seen between consumption of nuts and overall incidence of NHL, or two specific subtypes, diffuse large B-cell lymphoma or follicular lymphoma. Inverse associations were, however, seen with fruits, vegetables, carotenoids, vitamin C, proanthocyanidins and manganese (Thompson et al., 2010).

There are two cases where nuts are correlated with adverse health outcomes. In the case of aflatoxin, nut consumption may be indirectly associated with liver cancer, as the fungus found on crops such as peanuts produces the causative agent. In the case of betel quid chewing, components of the areca nut may directly cause oral cancers.

Aflatoxin, a potent liver carcinogen, is produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Liu and Wu, 2010). These fungi are found

on peanut and corn crops throughout the world, namely in regions which do not have regulations and/or methods of controlling aflatoxin exposure. As a result, aflatoxin-induced liver cancers are prevalent in such regions as sub-Saharan Africa, Southeast Asia and China. Particularly susceptible are individuals infected with hepatitis B virus. Since the carcinogenic agent derives from the fungus and not from the nuts themselves, we will not discuss this topic in detail here. For a thorough analysis of the world-wide burden of aflatoxin-induced liver cancer, see Liu and Wu (2010).

Betel quid (BQ) chewing is a popular habit in many countries, including South Africa, India, Taiwan, Papua New Guinea, and throughout Southeast Asia (Jeng et al., 2001; Angadi and Rao, 2010). The ingredients vary from place to place, but generally contain some combination of areca nut (also known as betel nut or supari) and lime, and may contain tobacco or *Piper betle* leaf. This habit is associated with various pathologies of the oral cavity, in particular leukoplakia and oral submucous fibrosis (OSF), which unfortunately are incurable. Between 2.3 and 7.6% of cases progress to oral cancers. Chewing of BQ exposes the oral cavity to many damaging agents, such as alkaloids, tannins, some polyphenols, reactive metabolites, nitrosamines, and reactive oxygen species. Consistent exposure to such compounds depletes the cells' natural antioxidant defenses (e.g. glutathione) and DNA repair capacity, thus inducing the multi-step process of carcinogenesis: initiation (pre-cancerous lesions), promotion and progression to clinical tumors. Epidemiology studies measuring the association between BQ chewing and pathologies of the oral cavity, as well as in vitro and in vivo studies investigating mechanisms of action of particular compounds and metabolites, are extensively reviewed by Jeng et al. (2001) and Angadi and Rao (2010). Various plausible mechanisms have been described, although researchers continue to seek out a definitive etiology and a cure. In the meantime, it is safe to say prevention of the disease by avoiding BQ chewing is preferable and advisable.

The connections between aflatoxin and liver cancer and between areca nut chewing and oral cancers are well-established. However, the role of dietary consumption of nuts in the prevention of cancer is less clear. The current research indicates that nuts may confer protection in women against colon cancer, endometrial cancer, and benign breast disease (when consumed in adolescence). In men, nuts may protect against prostate cancer. Further studies are needed for several reasons: 1. to investigate the gender differences in the colon cancer studies, 2. to investigate effects of nut consumption on risk of

other types of cancers, and 3. to distinguish between protective effects conferred by nuts versus seeds and legumes (Sabate et al., 2009).

15. Weight Management

Since nuts are an energy-dense, relatively high fat food (45-75% fat by weight, mostly unsaturated), there has been some concern that nuts may contribute to weight gain (Sabate, 2003). This is particularly important for those individuals at high risk of developing, or who have, chronic diseases exacerbated by overweight and obesity, such as cardiovascular disease and diabetes. A growing body of evidence is shedding light on this concern and revealing that nuts may not lead to weight gain. In fact, including nuts in the diet may be associated with lower body weight and may aid in weight loss programs.

Several epidemiology studies have not seen significant weight gain in subjects who consumed more nuts overall. For example, nut consumption was not correlated with body weight in the Iowa Women's Health Study, or with BMI in the Physician's Health Study. Interestingly, the Adventist Health Study saw an inverse association between nut consumption and obesity, while the Nurses' Health Study saw an inverse association between nut consumption and BMI (Sabate, 2003; Mattes et al., 2008).

Similarly, clinical trials observing effects of nut consumption on various health-related markers which also documented body weight or BMI contribute to the evidence. In various trials where nuts were added to the diet, the actual weight gain by the end of the study was far less than the predicted weight gain (ranging from 2 to 28% of predicted) based upon the energy content of the nuts. In other cases, no weight change was seen (Mattes et al., 2008). In a meta-analysis of 13 published studies on effects of walnuts on blood lipids, no significant change in body weight was seen in the subjects consuming walnut diets compared to control diets. These 13 studies followed a total of 365 individuals for 4 to 24 weeks. Walnut diets ranged from 10-24% of total calories (Banel and Hu, 2009).

A few recent studies have investigated the effects of nut consumption on body weight and energy balance. For example, Hollis and Mattes (2007) sought to determine whether addition of 1440 kJ of almonds (~60 g) to subjects' usual diet each day would alter energy balance and body composition. The study followed 20 women who consumed their normal diet, with or without almonds, for 10 weeks each in a crossover design. The authors

found that addition of almonds did not significantly alter body weight, mostly due to the individuals freely compensating for the almonds by eating less of other foods (accounting for ~74% of the almonds' total energy). Almonds are high in protein and fiber and have a crunchy texture - all qualities which promote satiation. The remaining energy balance of the almonds can be accounted for by changes in energy expenditure and by the inefficient absorption of energy from almonds during digestion. There were no differences in resting metabolic rate or thermic effect of food during the almond diet compared to the control diet (Hollis and Mattes 2007). Interestingly, some feeding studies have demonstrated increased resting energy expenditure after peanut consumption (Alper and Mattes, 2002). It is unclear whether this discrepancy is due to study design, variation among subject groups, or differences in the composition of various types of nuts. However, increased satiety, dietary compensation, and inefficient energy absorption appear to hold true across varieties of nuts (Mattes et al., 2008; Sabate, 2003).

A few studies have been designed to specifically observe the relationship between nut consumption and weight loss. Individuals given diets supplemented with nuts tended to achieve greater reductions in overall weight, BMI and waist circumference as well as improvements in lipid markers of cardiovascular disease compared to the group given diets supplemented with complex carbohydrates. Importantly, diets were matched for total calories, protein, cholesterol, and saturated fat (Wien et al., 2003). In a comparison of a low-fat diet without nuts versus a moderate-fat diet with peanuts, subjects in both groups lost comparable amounts of weight and saw favorable changes in serum lipids. However, the positive changes in lipids (i.e. increased HDL and decreased triglycerides) were sustained beyond the weight-loss period, unlike in the low-fat diet group (Pelkman et al., 2004). A third study demonstrated that those who included nuts in the weight-loss diet tended to adhere to the diet longer, lose more weight, and have higher overall nutritional quality of the diet compared to those who avoided nuts (McManus et al., 2001).

In summary, nut consumption does not lead to weight gain when substituted for other foods in order to maintain consistent caloric intake. Nuts can contribute to increased satiety and yet not all of the energy content is actually absorbed. By comparison, some studies show more efficient absorption of nutritional components, such as lipids, from ground nuts versus whole nuts (Jenkins et al., 2008; Ellis et al., 2004). Nuts can be included as part of a healthy weight-loss program and may improve not only the nutritional value of such diets, but the likelihood of successful weight loss.

16. Gallbladder Disease

Gallbladder disease affects between 10 and 25% of U.S. adults (Tsai et al., 2004a; Tsai et al., 2004b; Shaffer, 2006). Risk factors for developing gallstones are both non-modifiable (female gender, aging, heredity) and modifiable (obesity, metabolic syndrome, rapid weight loss, cirrhosis, Crohn's disease, and gallbladder stasis from other causes). In the U.S. up to 80% of gallstones are cholesterol stones which can result from either biliary hypersecretion of cholesterol, or low serum LDL and high triglyceride levels. As the majority of the fat in nuts is mono- and polyunsaturated (MUFAs and PUFAs), nuts have been shown to favorably improve blood lipid levels, and MUFAs and PUFAs are known to inhibit formation of cholesterol gallstones, it is plausible that consumption of nuts may help to prevent formation of gallstones. Fiber, magnesium, phytochemicals and antioxidant vitamins such as vitamin E, all found in nuts, may also promote gallbladder health. For example, fiber reduces recirculation of bile acids in the intestine, phytosterols inhibit absorption of dietary cholesterol, and insulin sensitivity can be improved by magnesium as well as fiber (Tsai et al., 2004a; Tsai et al., 2004b; Shaffer, 2006).

Two relatively recent studies have investigated the association between nut consumption and gallstone disease in men (Tsai et al., 2004a) and in women (Tsai et al., 2004b). The Health Professionals Follow-up Study followed 51,529 US male dentists, veterinarians, optometrists, osteopathic physicians and podiatrists between ages 40 and 75 at baseline in 1986 (Tsai et al., 2004a). Diet was assessed by questionnaire at baseline and then again in 1990 and 1994. Ultimately, 43,823 men were considered eligible for the study on gallbladder disease. Nut consumption was divided into 4 categories: less than once per month, 1-3 times per month, once per week, 2-4 times per week, and 5 or more times per week. Those participants who consumed nuts most frequently tended to also be more physically active, drink more alcohol but less caffeine, consume less trans-fat and saturated fat, and more PUFAs, MUFAs and fiber. As obesity can be a risk factor for gallstone disease, it is important to note body mass index did not significantly differ between frequent and infrequent nut consumers. From baseline (1986) to 1998, 1,833 new cases of gallstone disease were diagnosed and of those, 1,033 patients required cholecystectomy. Using cumulatively updated data on nut consumption and gallbladder disease to discern effect of long-term consumption, the authors determined those who consumed nuts the most frequently (≥ 5 times/week) had a 30% lower risk compared to those who

rarely consumed nuts (RR 0.70; 95% CI: 0.60 – 0.86; $P_{\text{trend}} < 0.001$; adjusted for age and multiple confounding factors). Adjusting for intake of specific types of fat (trans, saturated, MUFA and PUFA) only slightly modified the results (RR 0.73; 95% CI: 0.58-0.92; $P_{\text{trend}} = 0.005$). This indicates components of nuts other than the fatty acids may also be contributing to the protective effect, such as fiber, magnesium, and vitamin E (Tsai et al., 2004a).

A large cohort of women from the Nurses' Health Study was observed for a correlation between gallbladder disease (using cholecystectomy as an endpoint) and nut consumption. The study included 80,718 women who were followed from 1980 to 2000, during which 7,831 cholecystectomies occurred. Similar to the men in the Health Professionals Follow-up Study described above, women who ate more nuts tended to be more physically active, drink more alcohol but less caffeine, and consume more PUFAs and fiber. Unlike the men, however, women who ate more nuts tended to weigh less and consume less carbohydrate. After adjusting for age, several confounding factors, and intakes of particular types of fat, women who consumed nuts more frequently (≥ 5 times/week) had a significantly reduced risk of undergoing cholecystectomy than those who ate nuts the least frequently (RR 0.78; 95% CI: 0.68-0.88; $P_{\text{trend}} < 0.0001$). Analyzing the data for effects of long-term consumption altered the reduction in risk only slightly, to RR 0.85 (95% CI: 0.74-0.96; $P_{\text{trend}} = 0.0009$). Interestingly, separating out the effects of peanuts, peanut butter, and other nuts still revealed a protective effect among all three categories. High intake of peanuts reduced risk of cholecystectomy by 19%, peanut butter by 15%, and other nuts by 35%. These results indicate that high intake of nuts can reduce risk of cholecystectomy in women, suggesting nuts contain components that confer beneficial effects on gallbladder health.

CONCLUSION

The health benefits of edible nuts have been attributed to their composition of MUFAs, PUFAs, vitamins, minerals, and fiber. However, bioactive compounds presents in tree nuts and peanuts play an important role in health promotion and the prevention of chronic disease. These components beneficially alter serum lipid profiles, protect against oxidative stress and inflammation, and improve insulin sensitivity. The beneficial attributes in nuts are ascribed to a number of bioactive compounds that synergistically or/and additively reduce the risk factors for various chronic diseases. It is not surprising, then, to see that higher nut intake protects against such conditions

as cardiovascular disease, diabetes, and gallstones. Research on the effects of nuts on cancer incidence suggests some protective effects in certain cases, but more studies are needed. Importantly, increased nut intake does not lead to weight gain, and may even aide weight loss and contribute to maintenance of a healthy weight. Taken together, these findings demonstrate that nuts can be consumed as part of a healthy diet and highlight the critical role whole foods and dietary patterns play in preventing chronic disease.

Although an array of putative mechanisms may be responsible for the health benefits of bioactive compounds in nuts, the information on this topic is limited and much research is needed to define and demonstrate the roles of these compounds. Complete bioactive component profiles are lacking for most nuts, and information is limited with respect to their bioactivities, bioavailability, bioaccessiblity, metabolism, and elimination in humans. And finally, little is known regarding differences in bioactive components due to species and variety of nuts, as well as cultivation, processing, and storage. Therefore, further research in this area is also warranted.

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Chapter 2

PHYSICAL AND NUTRITIONAL ATTRIBUTES OF THE FRUITS AND NUTS OF THE SHEA TREE (*VITELLARIA PARADOXA* C. F. GAERTN) IN NIGERIA

F. D. Ugesi^{1*}, K. P. Baiyeri² and B. N. Mbah²

¹Department of Crop Production, University of Agriculture,
P M B 2373, Makurdi, Nigeria

²Department of Crop Science, University of Nigeria, Nsukka , Nigeria

ABSTRACT

The shea tree, *Vitellaria paradoxa*, is a tree widely distributed and usually protected in the Northern Nigeria. Both the fruit pulps and the nuts are economically important to the rural poor. There are distinct ecological variations in the fruit and nut physicochemical attributes of Shea in Nigeria. Our studies indicated significant variation in all metric traits of fruits and nuts, except fruit length, fruit shape index and testa weight, across agro-ecological zones. All metric traits except fruit shape index also showed remarkable diversity across accessions (individual locations), with fruit length, nut length, fruit weight and nut weight ranging from 4.3-5.9 cm, 3.1-5.4 cm, 26.8-63.4 g and 8.7-22.0 g, respectively. Fruit pulp nutritional composition is significantly influenced

* Author for correspondence (e-mail: f_ugese@yahoo.com).

by agroecological zone in respect of carbohydrate, protein, fibre, energy, Na, K, Mg and Fe. Fruits from the wetter southern guinea savanna zone have less fibre but higher amount of carbohydrate, energy and Na while those from the drier sudan savanna zone are richer in protein, K, Mg and Fe. The specific locations of fruit collection (accessions) have significant influence on all nutritional traits. The range in energy related proximate traits is 29.3-45.3% carbohydrate, 2.6-7.0% protein and 0.7-1.7% fat. The element Fe has significant positive statistical linkage with Zn, Mg, K and Na. All proximate traits of the shea kernel except ash content vary remarkably across ecological zones. With the exception of moisture and fibre all other proximate traits of the kernel cake are statistically similar across agroecological zones. However, all proximate traits of the shea kernel and kernel cake vary ($P < 0.05$) across sites with shea kernels from Kachia and Jalingo recording highest values for fat. Correlations between kernel and fruit pulp proximate qualities revealed a low number of significant relationships. Fatty acid profile has shown significant influence of agroecology over stearic and oleic acids content while all the four fatty acids (stearic, oleic, linoleic and palmitic acids) are significantly influenced by individual locations. The range in the stearic and oleic acids content is 45.1-49.7% and 37.2-43.4%, respectively. Generally, the fruit pulp and seed of shea have excellent nutritional properties capable of meeting the dietary needs of the rural population. Besides, both physical (metric) and nutritional traits of fruits and nuts of the shea tree have shown considerable variation across the major distribution zones in Nigeria suggesting a possibility of selection for the genetic upgrading of the species in the country.

Keywords: Shea tree, *Vitellaria paradoxa*, physical properties, nutritional content.

INTRODUCTION

The shea tree, *Vitellaria paradoxa*, which belongs to the family sapotaceae, is a species of African origin [Keay, 1989; Opeke, 1987]. The tree, which is small to medium in size, averages a height of 10-15 m, has a spreading canopy and a bark with deep fissures [ICRAF, 2000]. On the of Africa continent, the species cuts across 19 countries, namely Benin, Ghana, Chad, Burkina Faso, Cameroon, Central African Republic, Ethiopia, Guinea Bissau, Cote D'Ivoire, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo Uganda, Zaire and Guinea [Salle *et al.*, 1991]. It thus gracefully adorns a

500-700 km wide and 5000 km long strip of the African savanna landscape extending from Senegal to Ethiopia and Uganda [Umali and Nikiema, 2002]. Although the African savanna is the natural distribution range of the species, it is presently found in Dominica and Honduras through human agency [ICRAF, 2000]. In Nigeria, the tree is mostly distributed in the southern guinea savanna, northern guinea savanna and the sudan savanna zones [Keay, 1989] [Figure1].

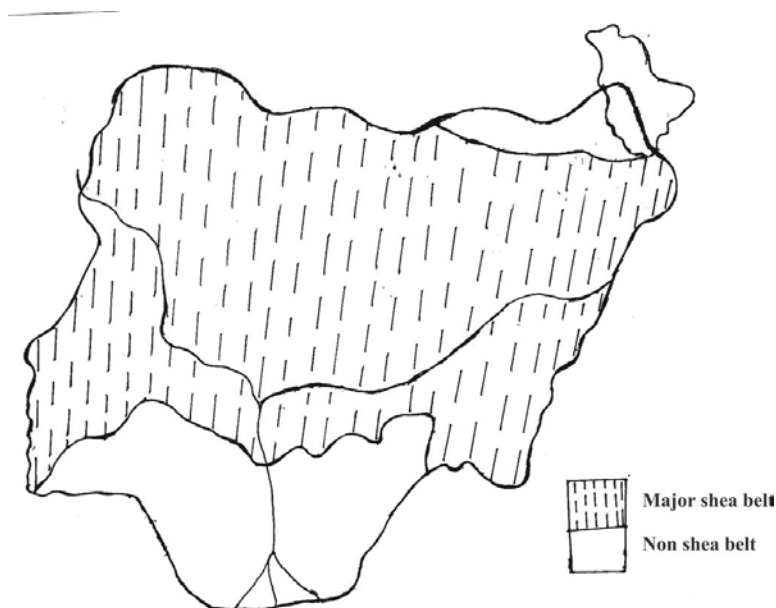


Figure 1. Map of Nigeria showing major shea distribution belt.

The shea tree is useful in numerous ways. However, its global popularity is hinged on the fat extract of its nuts which is used in Japanese and European food and cosmetic industries, but even in Africa, its usefulness in the local cosmetic industry is on the rise [Boffa *et al.*, 1996]. However, the fat is most commonly used in Africa for cooking and is second in importance only to palm oil, becoming much more important in this regard in areas whose climate have precluded them from successful cultivation of the oil palm [ICRAF, 2000]. The fat is also useful in the treatment of cough, minor bone dislocation [Badifu, 1989] and inflammations [Ugese *et al.*, 2009]. This is in addition to its usefulness as illuminant and in candle and pomade preparations [Vickery and Vickery, 1969].

Besides the above, the shea tree produces a fruit which ripens at the beginning of the rainy season. This period is considered critical in the annual food supply chain as it coincides with the period of rigorous farm work and depleted food reserves [Lamien *et al.*, 1996]. It therefore serves to alleviate hunger during the hunger season and provide the much needed energy for the tasking farm operations. Generally, agroforestry species have been known to provide vital nutrients and essential vitamins which help to curb malnutrition among growing children in the rural areas. Thus, rural dwellers who depend on these products for their livelihood attach due value to them [FAO, 2005]. Fortunately, scientific investigations into the nutritional content of shea fruit pulp have confirmed its nutritional competence in meeting the nutritional needs of especially rural dwellers.

Sale of products from the shea tree such as butter, nuts and edible insects have been shown to contribute significantly to household incomes. This is in addition to improvements in the economies of shea nuts exporting countries [Popoola and Tee, 2001]. The role of *Vitellaria* at enhancing sustainable land use has been recognized [Bayala *et al.*, 2005]. The great potential of the shea tree at alleviating poverty, combating hunger and disease at the rural level and ensuring environmental sustainability informed its inclusion among the 17 priority species that were farmer identified for domestication in four tropical areas [Leakey, 1999].

METRIC TRAITS OF FRUITS AND NUTS

Metric traits of fruits and nuts of the shea tree vary widely across agroecological zones and sites in Nigeria (Table 1). For instance, fruits from the sudan savanna have small diameters, low fruit and pulp weights while those from the southern guinea savanna have high percentage pulp weight, implying that they are comparatively more fleshy. Fruit weight of the southern and northern guinea savanna fruits are statistically the same but the nut weight and kernel weights of the latter zone are remarkably superior. Among sites sampled by Ugese *et al.* [2010a], fruit weights of Akwanga provenance are much higher. On the other hand nut weights of the Kachia, Akwanga and Jalingo provenances are much higher than the rest of the accessions or provenances. But generally, variation in some key phenotypic traits of shea fruits and nuts is as follows: fruit length: 4.3-5.9 cm; fruit diameter: 3.2-4.4 cm; fruit weight: 26.8-63.4 g and percentage pulp weight: 54.2-71.1.

Table 1. Metric traits of the fruits and nuts of the shea tree across agroecological zones and sites in Nigeria

Zone/Location	FRLT (cm)	FRDM (cm)	FSI	FRWT (g)	PUPWT (g)	PUPWT (%)	NTWT (g)	NTLT (cm)	NTDM (cm)	NSI	KNWT (g)	TSWT (%)
Agroecological Zone												
Southern guinea savanna	5.2	3.7	1.4	41.7	28.7	68.1	13.1	3.2	1.9	2.0	6.1	28.1
Northern guinea savanna	5.1	3.7	1.4	45.5	24.8	54.5	20.8	4.8	3.0	1.6	12.4	32.1
Sudan savanna	4.8	3.2	1.5	27.6	15.7	56.9	11.9	3.7	2.1	1.8	7.1	26.8
LSD 0.05	NS	0.4	NS	9.7	5.2	3.9	2.4	0.4	0.2	0.2	2.1	NS
Accession												
Ilorin	-	-	-	-	-	-	12.5	3.9	2.2	1.8	-	-
Lokoja	4.3	3.3	1.3	30.1	21.4	71.1	8.7	3.1	1.7	1.9	4.6	28.1
Makurdi	5.4	3.6	1.5	31.7	21.6	68.1	10.1	3.4	1.5	2.3	4.3	27.9
Akwanga	5.9	4.4	1.5	63.4	43.1	68.0	20.3	4.3	2.1	2.1	8.9	18.3
Minna	-	-	-	-	-	-	13.8	3.8	2.2	1.8	6.5	37.9
Kachia	5.5	3.8	1.5	48.0	26.0	54.2	22.0	5.4	3.2	1.7	16.0	35.9
Jalingo	4.8	3.6	1.3	43.0	23.5	54.7	19.5	4.1	2.8	1.5	8.7	28.3
Yola	4.6	3.2	1.4	28.4	16.6	58.5	11.8	3.6	2.2	1.7	5.8	27.4
Kano	5.0	3.2	1.6	26.8	14.8	55.2	12.0	3.7	1.9	1.9	8.4	26.3
LSD 0.05	0.7	0.6	NS	5.6	2.7	5.0	1.3	0.3	0.2	0.1	0.9	5.2

Adapted from Ugese [2009]

FRLT = Fruit length; FRDM = Fruit diameter; FSI = Fruit shape index; FRWT = Fruit weight; PUPWT = Pulp weight;

%PUPWT = Percentage pulp weight; NTWT = Nut weight; NTLT = Nut length; NTDM = Nut diameter;

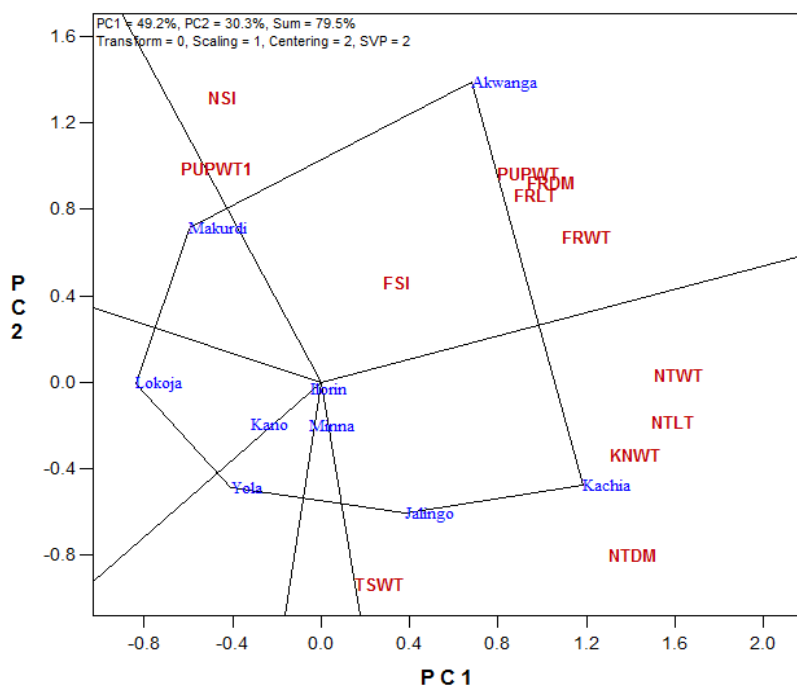
NSI = Nut shape index; KNWT = Kernel weight; %TSWT = Percentage testa weight.

NS – No significant difference.

Other attributes like nut weight, nut length, kernel weight and percentage testa weight are 8.7-22.0g, 3.1-5.4 cm, 4.6-16.0g and 18.3-37.9% respectively. Values of nut length reported from Ghana shows variation of 1.74-3.74 cm [Lovett and Haq, 2000]. Basing on database of nut dry weights, Maranz *et al.* [2004] estimated fruit weights from five African countries to vary between 15.2 and 60.8 g. They also indicated that fruits from the highlands of Cameroon may far be in excess of 60 g – up to 100g – particularly those with more than one seed. Testa weight makes up between 18 and 36% of dry nut weight. This value is large enough and should compel closer attention to the extent this structure is able to influence certain nut properties such as oil yield and speed of germination or emergence.

Generally, phenotypic characteristics of fruits and nuts from Ilorin, Kano and Minna are similar, in contrast to those from the rest of the sites (Figure2). However, the evidence is clear, that fruits and nuts from Kachia and Akwanga have better nut and fruit traits, respectively. Variation in phenotypic traits of a species over a wide distribution zone is common and could be expected. In Mali and Burkina Faso, Maranz and Wiesman [2003] attributed the larger nut weights and diameters of the guinean zone fruits to higher amounts of rainfall while ascribing higher temperatures to the longer and narrower shape of fruits in the sahel zone. In Nigeria, variation in phenotypic traits of fruits and nuts is also strongly influenced by environmental variables particularly altitude. For instance, while fruits at higher altitude and latitude have higher percentage pulp weight, those from areas with higher temperature regimes are characteristically smaller in weight. Similarly Ugese *et al.* [2010a] found a negative correlation between length of dry period and percentage pulp content or fruit fleshiness suggesting that trees in moisture stressed localities may tend to place less priority on pulp formation

Phenotypic variation in populations of a species is not limited to *Vitellaria* and has been reported for two key agroforestry species – *Dacryodes edulis* and *Irvingia gabonensis* – though the source of the differences has been attributed to tree-to-tree variation [Leakey *et al.*, 2003, 2004 and 2005]. In contrast, the reported variation in shea characters in Nigeria is not based on tree-to-tree variation, but on average trait values in a given population [Ugese *et al.*, 2010a]. But this may serve to demonstrate the degree of heterogeneity in trait values of the shea tree across the vast Nigerian shea belt and the ease of finding individuals with defined phenotype in particular locations or zones.



FRLT = Fruit length; FRDM = Fruit diameter; FSI = Fruit shape index; FRWT = Fruit weight; PUPWT = Pulp weight; PUPWT1 = Percent pulp weight; NTWT = Nut weight; NTLT = Nut length; NTDM = Nut diameter; NSI = Nut shape index; KNWT = Kernel weight; TSWT = Percent testa weight.

Figure 2. GGE-Biplot analysis of the fruit and nut traits across the collection sites. Collection sites/accession names are in lower case letters while the traits measured are in upper case. Adapted from Ugehe [2009].

For instance, from the foregoing, it is apparent that one is likely to find genotypes with more fleshy fruit in the southern guinea savanna locations than anywhere else, while one looking for large nuts will more easily locate such genotypes in the northern guinea savanna zone.

Nut weight is a key trait and has shown great diversity across Nigeria's shea zone. The pattern of nut distribution appears clear cut with most sites in the southern guinea savanna and the sudan savanna showing a clear dominance of the smaller nut categories over the bigger ones. In these locations, finding nuts weighing above 30 g is a rare occurrence. Nut samples from sites such as Akwanga, Jalingo and Kachia are manifestly more diverse in weight distribution, with nuts ranging from <10g to about 50 g. This

diversity in nut weight distribution could be attributed to a low level of human intervention in those shea populations. Generally, human selection activity is known to scale down trait diversity in any given population [Maranz and Wiesman, 2003]. However, it has been observed that human selection may not always be detrimental to the perpetuation of trait heterogeneity [Ugese *et al.*, 2010a] given that preference for the shea tree and its fruits and nuts vary widely between men and women [Saint Sauveur, 1999]. Thus if selection in any given community is carried out along such lines of preference, it will hardly be expected to lower the degree of trait diversity in the least. The opinion that human intervention in any given population of a species may not necessarily lead to trait homogenization was found also to be true in two key agroforestry species, *D. edulis* and *I. gabonensis*, in Nigeria and Cameroon – at least at their early stages of domestication [Leakey *et al.*, 2004]. Moreover, the low level of ethno-botanical studies on the shea tree in Nigeria could make definitive conclusions at this point premature.

Another feature that may be of some interest especially to the cause of shea tree domestication is the nature of statistical relationships existing among fruits and nuts. For instance the significant positive correlation between fruit length on one hand and fruit weight, pulp weight and nut diameter on the other hand could be exploited to select for genotypes with desirable pulp attributes under field conditions using fruit length which can be more easily estimated visually [Ugese *et al.*, 2010a].

NUTRITIONAL CONTENT OF FRUIT PULP

Proximate and mineral nutrient composition of shea fruit pulp has shown noticeable variability across Nigeria's vast shea belt. Agroecological zone exerts significant influence over carbohydrate, protein, fibre, energy, Na, K, Mg and Fe. Fruits from higher latitudes have less carbohydrate but higher amounts of protein. Shea fruits from the wetter southern guinea savanna zone have higher concentration of carbohydrate, energy, Na and K, although with the latter, values are not significantly different from those of the sudan savanna zone (Table 2). Interestingly, the drier sudan savanna zone is richer in Mg, Fe and K (even though values of the latter are not statistically different from those of the southern guinea savanna zone). Moisture, fat, ash, P, Ca and Zn do not differ remarkably across the three ecological zones. Proximate and elemental nutrient composition of shea fruits however, has marked variation from one site to another across Nigeria (Table 2).

Table 2. Nutritional composition of the fruit pulp of the shea tree across agroecological zones and sites in Nigeria

Zone/Locati on	Moist ure %	Carbohy d rate %	Protei n %	Fat %	Fibr e %	Ash %	Energy Cal/10 0g	Na (mg/1 00 g)	P (mg/10 0 g)	K (mg/100 g)	Ca (mg/100 g)	Mg (mg/1 00 g)	Fe (mg/10 0 g)	Zn (mg/10 0 g)
Agroecological Zone														
Southern guinea savanna	5.2	3.7	1.4	41.7	28.7	68.1	13.1	27.0	69.8	883.0	2.6	52.1	15.6	2.0
Northern guinea savanna	5.1	3.7	1.4	45.5	24.8	54.5	20.8	13.4	76.5	442.0	2.4	64.5	10.1	1.7
Sudan savanna	4.8	3.2	1.5	27.6	15.7	56.9	11.9	13.6	68.8	976.0	2.5	76.0	20.6	1.8
LSD 0.05	NS	0.4	NS	9.7	5.2	3.9	2.4	10.7	NS	418.3	NS	21.2	5.7	NS
Accession														
Lokoja	8.5	45.3	4.4	1.2	35.5	5.2	209.2	45.0	94.0	147.0	1.4	50.2	20.8	1.9
Makurdi	10.1	40.4	3.5	1.4	38.9	5.9	187.6	18.0	36.9	588.0	4.9	56.7	13.7	2.3
Akwanga	8.8	42.5	3.5	1.7	39.0	4.6	198.8	18.1	78.4	590.0	1.5	49.5	12.2	1.8
Kachia	9.2	29.3	7.0	1.5	48.6	4.5	158.3	17.9	51.7	295.0	ND	75.2	ND	ND
Jalingo	9.7	38.1	2.6	1.3	40.4	8.0	174.5	9.0	101.4	589.0	2.2	53.8	4.7	1.5
Yola	10.0	41.1	6.1	0.7	39.8	3.6	194.7	9.3	55.4	591.0	0.6	44.9	15.2	1.2
Kano	9.0	29.3	5.3	1.6	48.8	6.2	152.2	17.9	82.1	136.0	4.4	107.2	25.9	2.4
LSD (.05)	0.3	0.8	0.2	0.2	1.6	0.2	2.0	1.2	1.5	1.8	0.2	0.9	0.4	0.2

NS – No significant difference

ND – Not determined

Adapted from Ugese [2009] and Ugese et al. [2008a].

Differences in energy related proximate traits of fruit pulp range from 29.3-45.3% carbohydrate, 2.6-7.0% protein and 0.7-1.7% fat. Fruits also have high fibre (35.5-48.8%) and energy (152.2-209.2 Cal 100g⁻¹). Fruits from Makurdi and Yola are noted for their high moisture content while those from Lokoja and Jalingo have the highest carbohydrate and protein contents respectively. The location Kano, has the highest concentration of Mg, Fe and Zn.

The greater quantities of carbohydrate in the wetter southern guinea savanna fruits has been thought to be linked to more optimum rates of photosynthesis under conditions of more optimum water availability [Ugese *et al.*, 2008a]. Similarly, the higher rates of protein and some other nutrient elements in fruits from the drier zones (northern guinea savanna and sudan savanna) is apparently due to the highly restricted leaching rates in those zones. Since soil nitrate nitrogen is mobile and easily leached [Lombin, 1986] fruits from wetter environments, with greater opportunities for leaching losses, are expected to have less protein and other minerals. In any case, variation in nutritional content of fruits may not always be dependent on environmental factors alone. For instance, Maranz *et al.* [2004] found higher amounts of some mineral elements, including Ca, in the drier sahel zone of Mali and Burkina Faso which they ascribed to the lower leaching rates characteristic of such environments. In the report by Ugese *et al.* [2008b], Ca concentration did not show wide variation across the different ecological zones of Nigeria. Besides, Na, which has been grouped together with K, Ca and Mg as the second most easily leached class of chemicals (Ahn, 1970), was found to be more highly concentrated in fruits of the wetter locations, with greater leaching capacity. Thus, to satisfactorily explain these phenomena, soil and genetic factors needs to be more adequately explored [Ugese *et al.*, 2008b].

Shea fruit energy content of 152.2-209.2 Cal 100^{-g} can hardly go unnoticed and serves to explain the significant role played by this species in the seasonal food supply chain and agricultural production. As earlier pointed out, the time of shea fruit drop coincides with the period of seasonal food scarcity and the beginning of the rainy season when land must be prepared and seeds planted [Lamien *et al.* 1996; Maranz *et al.* 2004]. Shea fruit thus becomes a welcome relief, providing the energy and essential nutrients so desperately needed by both adult and children for general well being and performance of the tasking farm operations at this critical time.

The fibre content of *Vitellaria* fruits (35.5-48.8%) is comparatively higher than that of other important agroforestry species such as African star apple (*Chrysophyllum albidum*) and African pear (*Dacryodes edulis*) with values of

4% and 17.9%, respectively [Umoro Umoti and Okiy, 1987; Leahey, 1999]. However, with the numerous health benefits associated with dietary fibre, coupled with reports that daily intake is generally far below recommended rate [ENCARTA, 2009, Wikipedia, 2010], this attribute appears to be a plus for the shea fruit. Such health benefits include lowering the risk of obesity by promoting fullness, regulating blood sugar level, reducing the risk of heart disease, preventing constipation and facilitating bowel movement. The upper limit values of some of the nutrient elements found in Nigerian shea fruits are lower than corresponding values reported by Maranz *et al.* [2004] across five African countries, viz: Mali, Burkina Faso, Ghana, Cameroon and Uganda.

Amarteifio and Mosase [2006] investigated the nutritional contents of four indigenous fruits of Botswana namely, *Adansonia digitata*, *Sclerocarya birrea*, *Strychnos spinosa* and *Vangueria infausta*. Comparing their results with values presented by Ugehe *et al.* [2008b] shows that shea fruit values for Na, P, Fe and Zn are superior to those of the Botswana fruits except *S. spinosa* (for Na) and *V. infausta* (in terms of P). Even in terms of Mg, shea values surpass those of *S. spinosa*. Interestingly, shea fruit mineral values are also higher than the K and Ca values reported by Saka [1994] for *Adansonia digitata*. *Vitellaria* fruit pulp is generally recognized as a rich source of Fe [FAO 1995], which is of critical importance to growing children and whose lack could lead to iron deficiency anaemia [ENCARTA, 2009]. It is noteworthy that with respect to P and Fe, shea fruit is more highly endowed than mango [Ugehe *et al.*, 2008b]. Its protein content range of 2.6-7.0% is high above the zero percent reported for mango [Rice *et al.*, 1987], one of the leading staple tropical fruits. Similarly, all proximate traits of the shea fruit, except the lower carbohydrate values are comparable to those of banana and plantains [Baiyeri and Tenkouano, 2006] which are also highly rated and commonly consumed tropical fruits. With the nutritional credentials of the pulp of the shea tree as highlighted above, it definitely merits a greater pride of place among both tropical mainline and agroforestry fruit tree species.

The mistaken notion by urban dwellers that indigenous species are nutritionally inferior and less prestigious [Hoe and Siong, 1999], results directly from ignorance. This attitude may also be a carry-over effect of the relegation of Africa's indigenous fruits to the background consequent upon the introduction of exotic species in the era of colonialism. Ironically, the indigenous fruits are more adapted to the environment, having withstood unfavourable climatic elements for centuries [FAO, 2005]. With prevailing climate change, this class of plants is likely to maintain their edge over introduced types. Fortunately, modern trends are revealing a shift by

enlightened consumers, to indigenous species since they do not have problem of pesticide residues [Hoe and Siong, 1999]. With nutritional investigations' revealing that indigenous species are not just comparable, but even superior in some aspects, to the conventional species, this shift is bound to go deeper and be sustained.

Widespread appreciation and consumption of shea fruit is demonstrated by the sale of this item throughout the guinea and sudan savanna zones which happen to be the most important zones in citrus and mango production in Nigeria [Olaniyan, 2004]. Evidence for this sale has been reported for Benue State [Ugese *et al.*, 2008b], reputed to be Nigeria's overall leading producer of citrus and mango fruits [Avav and Uza, 2002]. Ugese *et al.* [2008b] had highlighted the more prominent nutritional role played by shea fruit in traditional Tiv society a few decades ago. Normally, in some communities, when a tree with superior fruit quality is identified, there is a scramble by women and children who pluck the fruits even before they are fully ripe. Such fruits are kept to ripen more fully and later consumed at home or taken to the market for sale. Whether the nutritional content of such fruits is comparable to that of fully ripe fruits that voluntarily fall to the ground is another matter. In some cases, pickers will rise earlier than others in the morning to pick the fruits that have naturally fallen under the tree canopy.

It needs to be stated that apart from the fruit, other food related products from this species are also widely sold. Thus, in several places the nuts and the butter are also items of commerce. Another common product from this species is the protein-rich caterpillars of *Cirina butyrospermi* which feed exclusively on the leaves of *Vitellaria*. This food insect is highly cherished by the Yoruba, Nupe [Ande, 2004] and Tiv [Ugese *et al.*, 2005] ethnic groups in Nigeria; although there are indications that many other ethnic groups, particularly in the Middle Belt of Nigeria, consume this insect. The nutritional and economic importance of this caterpillars has been demonstrated by a report by Popoola and Tee [2001] that prospects of the harvest of this insects constitutes the main reason some farmers preserved the shea tree on their farms. As it is common with most protein sources, the price of this insect on the market of recent has been shifting towards the high side.

It is common to link *Vitellaria* with non-nomadic peoples who rely on it for provision of dietary fat [Boffa *et al.*, 1996]. In Nigeria however, the nomadic Fulani are known to consume the fruit pulp even though there is nothing to suggest that they rely on it for provision of cooking fat. It is thus a common fact that ethnic groups inhabiting the shea belt have one form of association or the other with the tree.

Leakey [1999] has made an extensive review of the nutritional properties of several key agroforestry tree products, highlighting their potential as sources of novel food products. The production of shea jam, using shea fruit and honey, by a women's Association in Burkina Faso, which is already an export item to neighbouring African countries, Canada and Europe [Spore, 2008] is heartwarming and raises hopes of possibilities for the production and marketing of more novel products from this important fruit tree. For instance, Lovett and Haq [2000] have mentioned that elsewhere the fruit is dried and stored for later consumption. Thus the processing of the fruit into dry forms (such as biscuits) or powdered forms will not be strange to consumer tastes. Such an effort is praiseworthy when considered that annual production of fresh shea fruits in Africa stands at over 1.5 M mt which is comparable to the 2 M mt of avocado, a well known commercial oil crop [Ferris *et al.*, 2001]. This to Nigeria, has pleasant and challenging implications since she produces the greatest amount of shea nuts (obtained from shea fruit) in Africa, such that in 1999, she contributed 58% of total nut output from the continent [Umali and Nikiema, 2002]

RELATIONSHIP BETWEEN SHEA NUTRIENT ELEMENTS

A number of significant positive statistical linkages exist between various minerals present in shea fruit such as the significant positive correlation between P and K (Table 3). Perhaps, the most outstanding relationship is that demonstrated by Fe which has significant statistical linkage with four other elements namely, K, Mg, Na and Zn. This linkage implies that these mineral nutrients occur together and suggest the likelihood that genetic or agronomic effort geared towards increasing the content of any one of these five elements, will invariably lead to an increase in the other elements as well [Ugese *et al.*, 2008b].

The prevalence of malnutrition among children in many African villages due to lack of essential vitamins and minerals is a cause for concern, prompting scientists to embark on biofortification in order to breed crops with higher levels of essential nutrients. Such efforts have led to the breeding of high vitamin A sweet potato for Uganda and will soon lead to the production of staple crops rich in Fe and Zinc for African and South Asian countries [Spore, 2008b].

In the shea fruit, a good number of these nutrients are naturally present and associated and may require little or no effort to enhance their level.

Table 3. Correlation coefficients between elemental nutrients of shea fruit pulp in Nigeria

	Na	P	K	Ca	Mg	Fe	Zn
Zn	0.321	-0.159	0.380	0.838**	0.650**	0.539*	-
Fe	0.500*	-0.051	0.716**	0.256	0.627**	-	
Mg	-0.068	0.025	0.338	0.630**	-		
Ca	-0.092	-0.322	0.110	-			
K	0.679**	0.532*	-				
P	0.278	-					
Na	-						

*, ** - Correlation is significant at the 5% and 1% levels of probability, respectively
Adapted from Ugese [2009].

This gives additional impetus to the opinion that the shea tree – as well as other indigenous tree species – needs to be given more serious attention than is presently the case, as they hold the key to the realization of some of the Millenium Development Goals especially those concerned with eradication of extreme poverty and hunger, combating of diseases and ensuring environmental sustainability.

NUTRITIONAL CONTENT OF SHEA SEED AND CAKE

All proximate attributes of shea seed except ash content vary remarkably across agroecologies in Nigeria (Table 4). Proximate quality of shea seeds from the northern guinea savanna have high fat, fibre and energy contents but is significantly lower in carbohydrate and protein compared to the other two zones. Maranz and Wiesman (2003), in their study of shea fat content in four African countries found differences in fat concentration across the four climatic zones. The sahelian zone, typically the most dry and hot of the four zones, had higher fat content. The Nigerian case is slightly different as the highest fat content is registered by the northern guinea savanna zone which occupies a middle position relative to the other two zones with respect to the climatic variables of temperature and moisture (Agboola, 1976). This suggests the possibility that the genetic factor could moderate the influence of climatic elements in determining shea fat content of certain *Vitellaria* populations.

Table 4. Proximate traits of the seed of shea across agroecological zones and sites in Nigeria

Zone/Location	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Energy Cal/100g
Agroecological Zone							
Southern guinea savanna	3.2	47.8	8.1	30.2	5.8	4.9	495.4
Northern guinea savanna	2.7	44.1	7.1	34.8	6.8	4.5	518.1
Sudan savanna	3.0	48.1	8.1	30.1	6.1	4.6	495.6
LSD 0.05	0.4	1.7	0.7	1.0	0.3	NS	10.8
Accession							
Lokoja	3.4	47.2	7.6	30.8	6.0	5.0	496.4
Makurdi	3.0	48.7	8.4	30.8	4.6	4.6	505.6
Akwanga	3.4	48.3	7.4	28.6	6.9	5.4	480.2
Minna	2.9	47.2	8.9	30.6	5.8	4.6	499.7
Kachia	3.1	43.4	7.9	34.6	6.4	4.6	516.6
Jalingo	2.2	45.1	6.3	34.9	7.2	4.3	519.7
Yola	3.2	47.6	8.5	30.4	5.9	4.4	498.0
Kano	2.8	48.9	7.6	29.7	6.2	4.8	493.3
LSD (0.05)	0.2	0.9	0.5	1.0	0.4	0.4	14.8

NS – No significant difference

Adapted from Ugese et al. [in press].

Fat percentage varies from site to site in Nigeria, ranging from 28.6-34.9% [Ugese *et al.*, in press]. Maranz *et al.*[2004] have found shea fat values across Africa to range from 20 to 50%, indicating that values less than 20% are possible if immature kernels are used. In the Nigerian case, Kachia and Jalingo nuts have a fat yield of about 35% (Table 4) indicating intermediate performance going by the classification of Maranz *et al.*[2004] that fat values between 20 and 30% are low, those in the mid 30s are intermediate while those above 40% are good. It is however noteworthy that kernel fat values of up to 60% have been reported [Adu Ampomah *et al.*, 1995; Umali and Nikiema, 2002] although Maranz *et al.*[2004] have viewed fat values in excess of 50% as resulting from either elite trees or faulty laboratory procedure.

Other shea seed proximate attributes are protein: 6.3-8.9%; carbohydrate: 43.4-48.9%; fibre: 4.6-7.2 and ash: 4.3-5.4%. The Nigerian protein values compare favourably with those given by Umali and Nikiema [2002], while the carbohydrate values they reported (31-38%) are lower.

The proximate profile of the Nigerian shea seed makes the suggestion for its consideration as possible livestock feed very tempting, notwithstanding the fact that its high fat content will constitute an immediate obstacle to such an attempt. Although this alone may not preclude the use of the kernel in animal feeding as the broken kernels of babacu palm, *Orbignya phalerata*, with 60-70% oil content, are also fed to pigs (FAO, 1995). Such a suggestion becomes justified on two grounds: firstly, of the total shea nut production in Africa, only 35% is collected, implying that 65% goes to waste. Secondly, certain methods of treatment of the nut could reduce the fat content such as fast sun drying [Ferris *et al.*, 2001]. Thus, exploring such other possibilities could ensure successful utilization of the seed in the feed industry and help to curtail the huge annual shea nut wastage. This development, when adopted on large scale, could generate obvious socio-economic advantages including financial empowerment and household food security. The latter point is even more pertinent considering that the large scale use of some staple food items for biofuel production is already impacting negatively on global food supplies [Spore, 2008].

As such the development of dietary-aid products from shea fat fractions for animal and human utilization [Lovett, 2005] is a most welcome innovation. In an experiment with broiler chickens, Dei *et al.* [2006] compared the apparent lipid digestibility coefficient and apparent metabolisable energy (AME) value of shea fat with that of soybean oil and cocoa fat. Results indicated that the mean coefficient of apparent lipid digestibility for shea fat (0.58) and that of cocoa fat (0.54) were similar but lower than that of soybean

oil (0.95). Also at dietary levels of 60 g/kg and below, the AME of shea fat (22.0 MJ/kg DM) and cocoa fat (26.4 MJ/kg DM) was significantly lower than that of soybean oil (39.8 MJ/kg DM). A commendable aspect of such studies as the above, notwithstanding the lower performance of shea fat relative to soybean oil, is the innovative ways they seek to utilize the butter. Such developments could in the long run lead to more significant alternative ways of utilizing the fat apart from the highly demanding and competitive global market for shea nut and fat. This will enhance the greater exploitation of this important savanna resource.

The energy content of shea seed is 480.2-519.7 Cal 100^{-g} which is about twice that of the fruit pulp as reported in this document. This is due principally to the comparatively high fat concentration of the seed. This high energy content is thought to give the growth structures the needed energy to grow and survive in the harsh savanna climate.

Shea seed cake across Nigeria's agroecological zones varies little except in terms of moisture and fibre (Table 5) with samples from the southern guinea savanna and the sudan savanna having greater quantities of moisture and fibre respectively. However, across individual sites variation in shea seed cake proximate qualities is marked [Ugese *et al.*, in press]. Ranges for proximate qualities of the seed cake are carbohydrate: 58.4-71.9%; protein: 7.6-10.1%; fat: 2.9-4.0%; fibre: 9.9-19.3% and ash: 4.9-6.1%. These values are in general agreement with those reported by Umali and Nikiema [2002] except for the slightly higher carbohydrate and fibre content of the former.

The nutritional credentials of the seed cake makes its reported use as a livestock and poultry feed [ICRAF, 2000] unquestionable. Although this usefulness is being challenged by the presence of anti-nutritional factors and low digestibility, the low aflatoxin content of the cake even when mouldy [Umali and Nikiema, 2002] appears to be one of the strong points of this by-product. Apart from the above uses, the shea cake is invaluable as a waterproofing material for filling cracks in mud huts and traditional beehives, as fuel and an organic fertilizer [Anonymous, 2008]. This last is a strong incentive to the expanding and fashionable world of organic agriculture.

It is evident that shea seed cake is grossly underutilized in Nigeria. For instance a nationwide survey of ingredients used in the livestock and other sectors of the economy by the Raw Materials Research and Development Council listed among others, soybean cake, groundnut cake and cotton seed cake [RMRDC, 2003]. Not a single mention is made of shea seed cake. This is disheartening considering that Nigeria produces the highest amount of shea nuts in Africa [Umali and Nikiema, 2002; Umobong, 2006].

Table 5. Proximate traits of shea seed cake across agroecological zones and sites in Nigeria

Zone/Location	Moisture (%)	Carbohydrate (%)	Protein (%)	Fat (%)	Fibre (%)	Ash (%)	Energy Cal/100g
Agroecological Zone							
Southern guinea savanna	3.1	66.2	9.2	3.4	12.6	5.5	332.2
Northern guinea savanna	2.4	68.0	8.8	3.2	12.3	5.3	336.0
Sudan savanna	2.5	64.0	9.3	3.4	15.4	5.4	323.8
LSD 0.05	0.4	NS	NS	NS	1.9	NS	NS
Accession							
Lokoja	3.2	68.9	8.6	3.0	10.5	5.8	337.0
Makurdi	3.0	69.1	9.3	3.1	10.6	4.9	341.5
Akwanga	3.3	58.4	8.9	4.0	19.3	6.1	305.2
Minna	2.8	68.4	10.1	3.6	9.9	5.2	346.4
Kachia	3.0	64.4	9.9	2.9	14.4	5.4	323.3
Jalingo	1.8	71.9	7.6	3.4	10.1	5.2	348.6
Yola	2.2	68.6	9.8	2.8	11.5	5.1	338.8
Kano	2.8	59.6	8.8	3.9	19.3	5.6	308.7
LSD _(0.05)	0.3	1.1	0.6	0.3	0.6	0.3	5.6

NS – No significant difference

Adapted from Ugese et al. [in press].

As it is, the other oilseed cakes currently in use in the Nigerian feed industry have other highly competing end-uses, and are costly. Successful incorporation of shea seed cake into livestock feeds could reduce the cost of feeding; a very significant cost component in livestock production, and make available some of the current raw materials for other uses, including direct human consumption. However, for this to be realized, detoxification options and greater exploitation of the shea seed needs to be pursued. Besides, the Nigerian community in particular needs to be enlightened on the great potentials of the seed cake.

FATTY ACID COMPOSITION

Of the four dominant fatty acids in shea butter, stearic and oleic have shown greater variability across agroecologies. The northern guinea savanna zone has high stearic acid content but least amount of oleic acid. Amounts of these fatty acids do not vary considerably between the southern guinea savanna and the sudan savanna (Table 6). In contrast all the four fatty acids (stearic, oleic, linoleic and palmitic acids) vary significantly across sites in Nigeria with Jalingo having the highest stearic acid content (49.7%) and Lokoja having the least (45.1%). Oleic acid variation is from 37.2 to 43.4% (Ugese *et al.*, 2010b). The relative percentages of the two most dominant shea butter fatty acids reported here are consistent with earlier reports of 46-47.5% stearic and 41-41.8% oleic [Badifu, 1989; Badifu and Abah, 1998]. However, among several African samples examined by Maraz *et al.* [2004], only that from Mossi Plateau, Burkina Faso recorded higher stearic acid content than oleic. Generally, West African shea butter is considered a hard butter in contrast to that from East Africa which is soft and often referred to as 'shea oil' [Ferris *et al.*, 2001].

The degree of hardness or softness of a butter source is a function of the relative composition of stearic and oleic acids. If butter has high amounts of stearic acid, it will have a solid consistency (hard) under ambient conditions. On the other hand if it has a clear dominance of oleic acid, then it will be liquid (soft) under similar conditions. This is so since about 4 times higher temperature is required to melt stearic acid, with a melting point of 69.6°C, than oleic with a significantly lower melting point (16.3°C) [Bailey, 1979]. Butters with high melting points, are normally used as cocoa butter improver (CBI) while those with lower or similar melting point as cocoa butter are used as cocoa butter equivalent (CBE).

Table 6. Fatty acid profile of the fat of shea tree across agroecological zones and sites in Nigeria

Agroecological Zone/ Location	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Palmitic acid (%)
Agroecological Zone				
Southern guinea savanna	46.3b	41.8b	6.6a	4.3a
Northern guinea savanna	48.2a	39.7c	6.9a	4.4a
Sudan savanna	46.1b	42.1ab	6.5a	4.6a
Accession				
Lokoja	45.1d	43.4a	6.5b	4.2ab
Makurdi	47.0abc	41.3d	6.5b	3.7c
Akwanga	46.6abc	42.2bc	6.1bc	3.9bc
Minna	46.5abc	40.2e	7.2a	5.4a
Kachia	46.7abc	42.2bc	6.3b	4.1ab
Jalingo	49.7a	37.2f	7.4a	4.6a
Yola	46.2bc	42.7a	5.8c	4.6a
Kano	46.0bc	41.5bcd	7.2a	4.5a

Adapted from Ugese *et al.* [2010b], with permission

Within a column, means followed by the same letter(s) are not significantly different according to Duncan's New Multiple Range Test at $P = 0.05$

The later are also most suitable as a base for cosmetics and pharmaceutical products. Fatty acid data from Nigeria suggests the suitability of the shea fat mostly as cocoa butter improver owing to the high stearic acid content and hence solid consistency. This does not preclude the existence of butter sources that could qualify as CBEs if more populations or individuals are sampled within the vast Nigerian shea belt. In *Vitellaria*, variability could be so pronounced that individuals standing in close proximity to each other could exhibit different fatty acid profiles [Maranz *et al.*, 2004].

Table 7. Correlations between physical traits of fruits/nuts and proximate traits of kernels of *Vitellaria paradoxa* sourced from the savanna of Nigeria

Proximate Traits of Kernel	Physical Traits of Fruits and Nuts											
	FRLT	FRDM	FSI	FRWT	PUPWT	%PUPWT	NTWT	NTLT	NTDM	NSI	KNWT	%TSWT
Moisture	0.145	0.207	0.161	0.126	0.324	0.639	-0.242	-0.102	-0.369	0.508	-0.129	-0.234
Carbohydrate	0.005	-0.141	0.346	-0.314	-0.069	0.519	-0.654	-0.733	-0.905*	0.698	-0.745	-0.718
Protein	0.063	-0.241	0.398	-0.382	-0.270	0.246	-0.479	-0.174	-0.382	0.485	-0.209	0.158
Fat	-0.129	-0.055	-0.396	0.080	-0.169	-0.575	0.483	0.528	0.777*	-0.678	0.542	0.767*
Fibre	0.058	0.363	-0.248	0.584	0.423	-0.413	0.713	0.482	0.640	-0.655	0.509	-0.182
Ash	0.408	0.553	0.278	0.481	0.671	0.630	0.039	-0.046	-0.379	0.568	-0.047	-0.650
Energy	-0.186	-0.222	-0.334	-0.143	-0.366	-0.546	-0.271	0.359	0.606	-0.551	0.368	0.818*

Adapted from Ugese *et al.* [2009].

FRLT = Fruit length; FRDM = Fruit diameter; FSI = Fruit shape index; FRWT = Fruit weight; PUPWT = Pulp weight;

%PUPWT = Percentage pulp weight; NTWT = Nut weight; NTLT = Nut length; NTDM = Nut diameter;

NSI = Nut shape index; KNWT = Kernel weight; %TSWT = Percentage testa weight.

*, ** - Correlation is significant at the 5% and 1% levels of probability respectively.

**INTER-CORRELLATIONS AMONG FRUITS AND NUTS
PHYSICAL TRAITS AND PROXIMATE TRAITS OF FRUIT
PULP AND KERNEL**

There are a low number of significant relationships between metric traits of fruits and nuts and proximate traits of shea kernel (Table 7). Nut diameter has significant negative correlation with carbohydrate but a positive significant relationship with fat content. Testa weight also exhibits positive and significant relationship with fat and energy. These relationships imply that seeds with wider diameters, while containing high amounts of fat are low in carbohydrates.

Relationship between proximate traits of seed and those of fruit pulp has shown that only moisture and ash have a significant relationship which is negative (Table 8). The overwhelmingly high level of non-significant relationships goes to demonstrate that accumulation of materials in the two structures (fruit pulp and seed kernel) is largely independent of each other.

Table 8. Correlation coefficients between proximate traits of shea seed and proximate traits of shea fruit pulp from the savanna of Nigeria

Proximate Traits of Fruit Pulp	Proximate Traits of shea kernel						
	Moisture	Carbohydrate	Protein	Fat	Fibre	Ash	Energy
Moisture	-0.423	-0.001	0.297	0.257	-0.418	-0.765*	0.469
Carbohydrate	0.386	0.358	0.038	-0.373	-0.148	0.288	-0.368
Protein	0.373	-0.307	0.575	0.081	-0.118	-0.153	0.068
Fat	-0.053	0.037	-0.344	-0.081	0.221	0.562	-0.180
Fibre	-0.282	-0.311	0.018	0.275	0.173	-0.202	0.252
Ash	-0.859*	-0.085	-0.747	0.419	0.228	-0.309	0.452
Energy	0.556	0.334	0.161	-0.424	-0.174	0.371	-0.437

* - Correlation is significant at the level of probability.

Adapted from Ugese [2009]

CONCLUSION

The main shea belt of Nigeria is vast, ranging from the southern guinea savanna to the sudan savanna zone. Within this belt, physical and chemical traits of fruits and nuts of the tree exhibit considerable variability not just with respect to agroecological zones but more so individual sites. This diversity has interestingly shown that every geographic location has traits that could be desired and none has monopoly of all desirable traits. Thus, the southern guinea savanna, with more fleshy fruits, has a greater concentration of carbohydrates, energy and Na. Fruits of the other two zones, of which the northern guinea savanna types are larger with higher nut weights, are dominated by protein, fibre and Mg. Other proximate traits and minerals – P, Ca and Zn do not vary significantly across zones. Interestingly, out of the seven mineral elements studied, fruits from Kano, a sudan savanna location, have high concentrations of Ca, Mg, Fe and Zn. Similarly, seeds from the northern guinea savanna are comparatively more endowed with fat, fibre and energy while variations in seed cake proximate attributes across zones is only slight. Stearic and oleic acids vary across zones but linoleic and palmitic acids do not. However, all fatty acids vary from site to site. Shea fat in Nigeria is dominated by stearic acid, indicating that it is a ‘hard’ butter with a characteristic solid consistency under ambient conditions. Correlative links between some key traits of the species have revealed interesting results that could be of interest, among others, to the shea tree domestication strategies.

Generally, the shea resource, across its natural range in Nigeria, has proven to be of great social, economic, nutritional and environmental relevance to the inhabitants. It therefore occupies a strategic position in the effort to actualizing many of the Millenium Development Goals (MDGs). An appraisal of the present utilization status reveals gross under-exploitation of this important species. Positive action towards the genetic improvement and utilization of the species will, for Nigeria, hasten the actualization of the opinion by Tappan (1966) that the shea tree has the potential to do for Northern Nigeria what the oil palm had done for the South.

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Chapter 3

**BIOACTIVE COMPOUNDS FROM
ANACARDIUM OCCIDENTALE CASHEW NUT
SHELL LIQUID**

Maria Stasiuk*

University of Wrocław, Faculty of Biotechnology, Department of Lipids
and Liposomes, Wrocław, Poland

ABSTRACT

Anacardium occidentale (cashew), a member of the Anacardiaceae family, is a tropical tree indigenous to Brazil. It is extensively cultivated in India and east Africa for its kernel (the cashew nut). Cashew nut shell liquid (CNSL) is a substance contained between the kernel's inner and outer shells (pericarp) in a honeycomb matrix. It is an important agricultural product of cashew nut cultivation and a unique natural source of unsaturated long-chain phenols. Typically, solvent-extracted CNSL contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%), and traces of 2-methyl cardol. These compounds exhibit antibacterial, antifungal, and antitumor activities and also have molluscicidal, insecticidal, and fungicidal applications. They are known to be uncoupling factors of oxidative phosphorylation in the mitochondria and they show antioxidant activity and inhibitory activity against enzymes

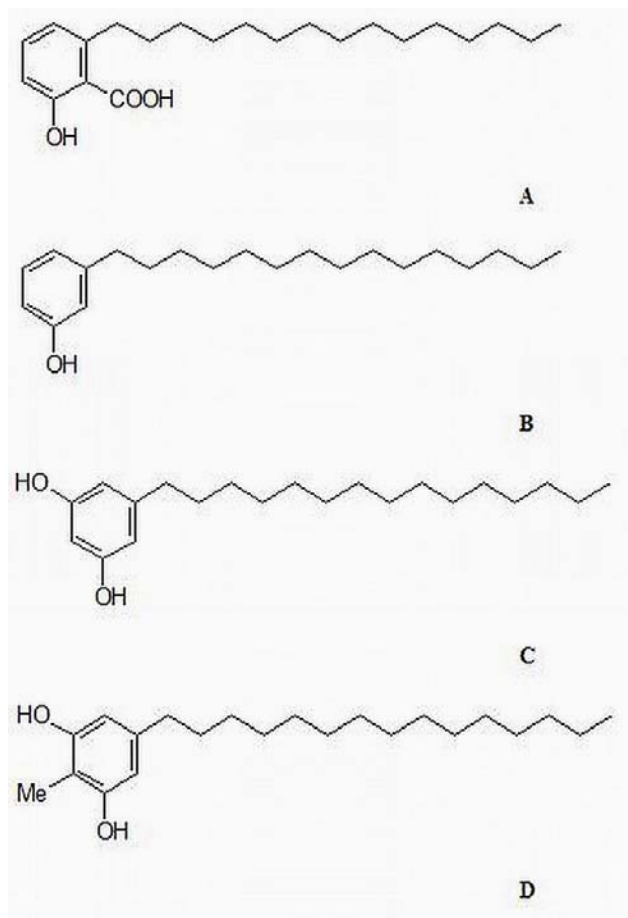
* University of Wrocław, Faculty of Biotechnology, Department of Lipids and Liposomes, ul. Przybyszewskiego 63/77, 51-148 Wrocław, phone: +48 71 3756338; fax: +48 71 3756234
e-mail: stasiuk@ibmb.uni.wroc.pl.

(e.g. α -glucosidase, β -lactamase, lipoxygenase, xanthine oxidase, and tyrosinase). The classes of compounds present in CNSL are also present in other plant extracts. They have identical chemical structures and their biological activities have been very extensively examined. This review focuses on recent data on the biological activities of those bioactive compounds found in both CNSL and other plants with identical chemical structures.

Keywords: alkylphenol, anacardic acids, cardol, Cashew Nut Shell Liquid (CNSL), methylcardol, phenolic lipids.

The cashew (*Anacardium occidentale* L.) belongs to the Anacardiaceae family. It is a native of tropical America, with an area of origin stretching from Mexico to Peru and Brazil and out to the West Indies. It has become naturalised in many tropical countries including Vietnam, India, Nigeria, Tanzania, Ivory Coast, Mozambique, and Bénin. The most important product of the cashew tree is the nut (a true fruit): a world-wide cashew nut production is nearly 500000 tons per year. The cashew nut is embedded in the pearshaped “cashew apple” (a pseudo fruit) [1] which has a shiny, red, orange or yellow skin, depending on cultivar [2]. It is nutritious, juicy and astringent [3]. Part of the cashew apple crop is processed into various products, such as juice, jam, syrup, chutney, beverages, and candy [4]. The cashew nut attached to the cashew apple is grey colored, kidney shaped and 2.5-4.0 cm in length. The nut consists of the kernel (20-25%), the shell liquid (20-25%), and the testa (2%), the rest being the shell. The shell is about 0.3 cm thick, with a soft leathery outer skin and a thin hard inner skin. Between these skins is the honeycomb structure containing the phenolic material popularly called cashew nut shell liquid (CNSL). CNSL occurs as a reddish brown viscous liquid in the soft honeycomb structure of the shell of the cashew nut. It is classified into two types, solvent-extracted CNSL and technical CNSL. A typical solvent-extracted CNSL contains anacardic acid (60-65%), cardol (15-20%), cardanol (10%) and traces of methyl cardol (Figure 1). Technical CNSL is obtained by roasting the shells, and it contains mainly cardanol (60-65%), cardol (15-20%), polymeric material (10%), and traces of methyl cardol [5]. Heating anacardic acid decomposes it into cardanol and carbon dioxide [6]. About 41% of the anacardic acids and cardanols are the tri-unsaturated species (2-hydroxy-6-pentadeca-8,11,14 trienyl benzoic acid and 3-pentadeca-8,11,14 trienyl phenol), while 22% are diunsaturated, 34% monounsaturated and the

remainder saturated. Cardols and methylcardols are 5-n-pentadecylresorcinols with saturated, monoolefinic (8), diolefinic (8, 11) or triolefinic (8, 11, 14) hydrocarbon long side chains [7].



A anacardic acid (saturated homologue); B cardanol (alkylphenol, saturated homologue); C cardol (saturated homologue); D methylcardol (saturated homologue).

Figure 1. The representative structures of CNSL phenolic lipids.

The biological activities of the CNSL components have attracted considerable attention. During the last few years there have been several interesting reviews of the phenolic lipids found in CNSL. There have been reviews of secondary plant metabolites and their analysis and applications

[8,9], a description of the presence, distribution, and biological activities of different phenolic lipids of the family Anacardiaceae [10], a review of the nomenclature, occurrence, chemical structures, biosynthesis and chemical synthesis, isolation and separation, and chemical and biological properties of anacardic acids [11], and a report on some of the industrial applications of phenolic lipids from the Anacardiaceae [12]. This review focuses on recent data on the biological activities of certain bioactive compounds that occur in CNSL and in other plants, and have identical chemical structure.

The compounds present in CNSL have long been used to treat minor health problems. These constituents have many documented biological properties beneficial for human health. Their potential use in the therapy and/or prevention of specific classes of disease increases the interest in them. The phenolic compounds present in cashew exhibited potent antibacterial activity against *Bacillus subtilis* [13,14], *Streptococcus mutans* [15-17], *Propionibacterium acne* [16,18], *Corynebacterium xerosis*, *Escherichia coli* [14], vancomycin-resistant *Enterococcus* [19], and various strains of *Staphylococcus aureus* [14,18], including its methicillin-resistant strains [20,21]. Studies on the relationship between the structure and antibacterial activity of anacardic acids showed that unsaturation in the alkyl side chain is not essential in eliciting this activity, but it is associated with heightened activity [22]. The effects of anacardic acids and methicillin against a strain of *S. aureus* were synergistic [23]. Studies on the mechanisms of the bactericidal activity of anacardic acids against methicillin-resistant *S. aureus* indicated that the observed processes depend less on the biochemical and metabolic interactions described in [24], and more on the ability of anacardic acids to chelate Fe^{2+} and Cu^{2+} [25], which can reduce their bioavailability for bacteria. On the other hand, anacardic acids are factors which inhibit bacterial respiration by inhibiting the respiratory chain [26]. A recent hypothesis about the mechanism of the antibacterial activity of anacardic acids on methicillin-resistant *S. aureus* suggests that the most important effect is that they disorder membrane of the bacteria [27].

Anacardic acids exhibited antibacterial activity against the Gram-negative bacterium *Helicobacter pylori* [28], with a value in the inhibitory range of the reference antibiotics used to test antimicrobial susceptibility and the eradication of *H. pylori* [29].

The antifungal properties of cashew lipids can be shown in the inhibition of *Staphylococcus typhimurium* aflatoxin production [30] and motility inhibition followed by the lysis of zoospores of the phytopathogenic *Aphanomyces cochlioides* [31].

The anacardic acids exhibited antiplasmodial activity against *Plasmodium falciparum* [32]. Lipids isolated from *Anacardium occidentale* have molluscicidal activity against *Biomphalaria glabrata*, a parasite causing schistosomiasis, a serious tropical disease [20], and the *Filaria* class of worms [33]. Phenolic lipids found in CNSL exhibit larvicidal activity. This was demonstrated for cardanol, cardol and their products of hydrogenation against *Aedes aegypti* larvae [34], and for anacardic acid against *Artemia salina* larvae [35] and Colorado potato beetle larvae [36].

The antimicrobial and antiparasitic activities of phenolic compounds found in cashew correspond with their cytotoxic activity. Anacardic acids were found to be moderate cytotoxic agents, affecting the growth of BT-20 breast and HeLa epithelioid cervix carcinoma cells [37], and they exhibited cytotoxic and genotoxic effects on CD1 male mice [24]. Anacardic acids were cytotoxic against Hep-G2 (human hepatocellular carcinoma), MDAMB-231 (human mammary adenocarcinoma), and 5637 (human primary bladder carcinoma) cells [38], and a human breast cancer cell line (SKBR3) [39]. Anacardic acid inhibits cell proliferation, cell cycle progression, and apoptosis in an estrogen receptor α -dependent manner by reducing ER-DNA interaction and inhibiting ER-mediated transcriptional responses [40].

These compounds potentiated the apoptosis induced by tumor necrosis factor and chemotherapeutic agents and down-regulate the expression of NF- κ B-dependent gene products involved in cell proliferation, anti-apoptosis, invasion, and angiogenesis [41,42].

Simultaneously, phenolic lipids found in cashew exhibit an absence of mutagenic, carcinogenic, and cocarcinogenic effects [43], and protect the cells against mutagenesis caused by the direct mutagens methyl methanesulfonate and 4-nitroquinoline-N-oxide and the indirect mutagen benzo[a]pyrene [44].

The protection of cells against carcinogenesis may be the result of the antioxidant properties of phenolic lipids, manifested as the ability to scavenge radicals and inhibit enzymes involved in the generation of free radicals under physiological conditions. Phenolic compounds seem to be efficient nonenzymatic protectors against oxidative stress. They can act as antioxidants in a variety of ways. They can prevent the ions of transition metals from initiating oxidation, quench the intermediates of oxidation (including reactive oxygen species), and inhibit various prooxidant enzymes. Anacardic acids are able to chelate divalent metal ions, such as Fe^{2+} and Cu^{2+} [45,46], and have high selectivity toward transition metal ions, especially Fe^{2+} and Cu^{2+} [47,48]. Compounds present in immature CNSL demonstrate antioxidant activity against radical scavengers that trap peroxy radicals [49], and increase survival rates

after H₂O₂ treatment in strains of *Saccharomyces cerevisiae* that are defective in antioxidant defenses [50]. They also have antioxidant and antimutagenic properties against hydrogen peroxide [51]. It was shown that a mixture of anacardic acids had a higher antioxidant capacity than cardols or cardanols. The antioxidant capacity of anacardic acids is related more to their inhibition of superoxide generation and xanthine oxidase than to scavenging of hydroxyl radicals [52]. Anacardic acid-mediated gastroprotection against ethanol-induced gastric damage in mice is also realized in the manner of an antioxidant mechanism [53]. On the other hand, investigations of oxygen consumption by hydrogenated cardanol and its derivatives during reactions with the peroxy radical showed that cardanol could be a renewable, low-cost, and convenient alternative source for a number of products with good antioxidant properties [54].

The phenolic lipids found in CNSL can modulate the activities of enzymes involved in the formation of free radicals in the human body under physiological conditions. These enzymes include lipoxygenases, cyclooxygenases, and xanthine oxidase. Anacardic acids were found to be effective inhibitors of the leukocytic lipoxygenase (5-LOX) [55] and soybean lipoxygenase isoenzymes [56-58]. This activity is largely dependent on the nature of the alkyl side chains present in the anacardic acid molecules [59]. The LOX inhibition is attributed to the ability of anacardic acids to chelate the iron in the enzyme [46]. Anacardic acid (C15:0) was recognized as a competitive inhibitor and the inhibition was explained by a combination of iron ion chelation and hydrophobic interaction abilities [60].

Hypoxanthine/xanthine oxidase is an enzyme involved in purine metabolism. Xanthine oxidase inhibitors are useful in treating some diseases such as gout and urate calculus, by regulating uric acid formation. Anacardic acids also inhibit the generation of superoxide radicals by xanthine oxidase [52,61] without such radical-scavenging activity [58]. The results indicate that anacardic acid binds to allosteric sites near the xanthine-binding domain in xanthine oxidase [61].

Almost all of the biological activities of anacardic acids, cardol, methylcardol and alkylphenol seem to be mainly non-specific and related to their interaction with membranous structures and the hydrophobic domains of proteins. The amphiphilic properties of these compounds are depend on the presence of separate hydrophilic (a hydroxy- or dihydroxybenzene ring with a carboxylic moiety in the case of anacardic acids) and hydrophobic regions in the lipid molecules. The octanol/water partition coefficient (log Po/w) has been determined for cardanol as 3.15, which is low [62], especially compared

with that of resorcinolic lipid, another compounds that belong to the phenolic lipids [63]. The lipid aggregates of hydrogenated cardanol found in aqueous solution have a micellar character. The structural rearrangement occurring in these aggregates with increasing temperature has a high similarity to a phase transition [64]. Under alkaline conditions, cardol and methylcardol exhibit the ability to self-aggregate spontaneously to a lamellar type of aggregate, and form liposomal structures alone as well as in mixtures with cholesterol, fatty acids, or phosphatidylethanolamine. These vesicular structures show a relatively high level of entrapment of the marker molecules, and a high size stability in comparison with control phospholipid liposomes [65]. Semi-synthetic derivatives of CNSL phenolic lipids also have an amphiphilic character. 2,4-sodium disulfonate-5-n-pentadecylphenol spontaneously forms micellar aggregates, and this process was found to depend on the temperature and potassium chloride concentration in the subphase buffer [66]. The polyetoxylate derivatives of cardol, cardanol, and 3-pentadecylphenol reduce the surface tension of water [67]. Sodium cardanol sulfonate was found to be useful as an alternative anionic surfactant with a CMC of 0.372 M and a relative detergency of 93.7% compared with dodecylbenzene sulfonate [68]. Carboxylate derivatives of cardanol and anacardic acids lower the surface tension, exhibit a critical micelle concentration, and produce microemulsions in mixtures with dodecyl sulfate [69].

Thanks to their amphiphilic properties, ancardic acids, cardol, methylcardol, and cardanol can incorporate into phospholipid bilayers. This process affects phospholipid molecules packaging and can be investigated via various methods. ATR-IR (attenuated total reflection infrared spectroscopy) studies on dry and hydrated film of 3-pentadecylphenol /dipalmitoyl phosphatidylcholine (DPPC) showed a strong intermolecular H-bond between the pentadecylphenol phenol group and the DPPC phosphate group; this bond is maintained during hydration. The addition of 3-pentadecylphenol to the pure dry DPPC bilayers decreased the temperature of the main phase transition [70]. 3-pentadecylphenol, up to a concentration of 50 mol%, formed liposomes in the mixtures with DPPC (dipalmitoylphosphatidylcholine) or MPPC (1-myristoyl-2-palmitoyl-phosphatidylcholine), and the changes in the intrinsic fluorescence of 3-pentadecylphenol indicate that its molecules change the ordering in the head group region of the phospholipid bilayer [64]. ³¹P NMR investigations showed that an equimolar 3-pentadecylphenol/DPPC dispersion adopts a lamellar structure at a temperature below that of the phase transition. At a temperature above the temperature of the observed phase transition this system adopts an isotropic phase. The presence of 3-pentadecylphenol

molecules in the DPPC liposome bilayer caused an increase in T_m proportional to the increase in the 3-pentadecylphenol concentration. At higher 3-pentadecylphenol concentrations the calorimetric transition becomes considerably more complex, which can indicate the coexistence of various phases with different compositions [71]. Investigations using fluorescein-phosphatidylethanolamine as an indicator of surface-associated processes showed that the intensity of fluorescence of this membrane probe increases during the incorporation of anacardic acid [72] into egg lecithin liposomes. Phenolic lipid molecules exhibit different and structurally dependent depths of localization in the liposomal membrane. Cardol affected the properties of the hydrophobic region of the bilayer, but the properties of the subsurface part of the phospholipid bilayer were altered by anacardic acid, methylcardol, and alkylphenol [72]. On the other hand, it was also found that cardanol intensively quenched the 9-anthroyloxystearic acid signal, which suggests a relatively deep location of these molecules within the lipid bilayer (Stasiuk, unpublished data).

When preincorporated into a 1-palmitoyl-2-oleylphosphatidylcholine liposomal bilayer, cardanol decreased the rate constant of leakage of carboxyfluorescein from the liposomes in the absence of Triton X-100. Insertion of this compound had a stabilizing effect on the corresponding liposomes: this was described as a cholesterol-like effect [62].

The phenolic lipids present in CNSL exhibit a strong ability to hemolyse erythrocytes. The EH_{50} (effective concentration resulting in 50% lysis of erythrocytes) value obtained for anacardic acid was 10, for cardol 18, for methylcardol 25, and for cardanol 30 μM [72]. This effect is modulated by the presence of divalent cations that protect erythrocytes against the lysis [72]. All the investigated lipids at sublytic concentrations protect erythrocytes under lysis in hypo-osmotic conditions. Anacardic acid showed the lowest protection, while the most effective antilytic agent was cardol; methylcardol and alkylphenol showed intermediate activity [72].

Interactions of compounds containing the hydroxy- or dihydroxybenzene ring with other cellular membranes may lead to destabilization of electron and proton transport. The possibility of some uncoupler properties, similar to those of the classical uncoupler 2,4-dinitrophenol, was demonstrated for anacardic acids [73]. The uncoupling activity dramatically decreased after the conversion of the anacardic acids to the corresponding cardanols via deprivation of the carboxyl group. This suggests that the C15 alkyl side chain and the carboxyl group may both play an important role in assisting the uncoupling activity of anacardic acids in the liver mitochondria of animals [73]. It was shown that

that anacardic acids could behave as both an electrogenic (negative) charge carrier and a “proton carrier” that dissipating the formed transmembrane proton gradient. It was proposed that the anacardic acid anion, in the same way as the protonated form, is able to permeate the lipid bilayer freely and act as a (negative) charge carrier [74].

The phenolic lipids found in CNSL affect protein structure and activity. Anacardic acids are able to bind to factor VIIa and prevent its binding to sTF [75].

The phenolic lipids from CNSL and their derivatives were also studied as potential candidate acetylcholinesterase inhibitors *in vitro* [50,76] and *in silico* [77,78]. The modulatory properties of these compounds on the activity of acetylcholinesterase may result not only from their direct interaction with a protein molecule, but also from their ability to interact with the phospholipid bilayer surface (Stasiuk, unpublished data).

Anacardic acids are the most frequently investigated phenolic lipids tested for inhibitory activity against many enzymes. These compounds inhibited 3-phosphoglycerate dehydrogenase [79,80] and glyceraldehyde-3-phosphate dehydrogenase [81,82], which is an attractive target for the development of novel chemotherapeutic agents for the treatment of Chagas` disease, which is caused by the flagellate protozoan *Trypanosoma cruzi*. Anacardic acids also inhibited histone acetyltransferase [83-85], the dysfunction of which is often associated with the manifestations of several diseases, primarily cancer, and inhibited p300- and p300/CBP-associated factor histone acetyltransferase activities [86]. Anacardic acid significantly also attenuated the histone acetylation induced by pesticide dieldrin, protein kinase C δ proteolytic activation and DNA fragmentation in dopaminergic cells in primary mesencephalic neuronal cultures [87]. It was suggested that inactivation of histone acetyltransferases by anacardic acid may prevent the degradation of histones [88].

The phenolic lipids found in cashew are also inhibitors of α -glucosidase, invertase and aldolase [89], tyrosinase [90], urease [28], and the Aurora kinases [91]. Binding assays with a fluorescently labeled probes showed that anacardic acid inhibits protein SUMOylation both *in vitro* and *in vivo* without affecting *in vivo* ubiquitination [92]. Anacardic acids were also found to be DNA polymerase β inhibitors and compounds that potentiate the action of DNA-damaging agents such as bleomycin [93-95].

Nuts are an important part of the human diet in many countries. This food items has been associated with protective effects against degenerative diseases such as cardiovascular diseases and cancers. Nutritionally, cashew nuts are a

good source of nutrients, including protein, fiber, selenium (Se), magnesium, phosphorus and thiamin. They also contain niacin, vitamin E, vitamin B6, calcium, iron, potassium, zinc and copper [96]. The phenolic lipids present in CNSL are classified as plant secondary metabolites, and they may be a significant source of new leads and drug candidates for a wide variety of diseases. Further investigation into the various aspects of their biology may uncover new opportunities to exploit their properties as, for example, chemopreventive and anti-tumor agents, and to develop pharmaceuticals based on the phenolic lipid constituents.

Table 1. The names of the CNSL phenolic lipids

Common Name	Chemical Name
Anacardic acid:	6[8'(Z)-pentadecyl]salicylic acid (*)
Cardanol (alkylphenol)	3- <i>n</i> -pentadecylphenol (*)
Cardol	1,3-dihydroxy-5- <i>n</i> -pentadecylbenzene (*)
Methylcardol	1,3-dihydroxy-2-methyl-5- <i>n</i> -pentadecylbenzene (*)

* The long aliphatic side-chain can be saturated, mono-olefinic (8), diolefinic (8, 11), or tri-olefinic (8, 11, 14).

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Chapter 4

**EFFECT OF PACKAGING MATERIAL O₂
PERMEABILITY, LIGHT, TEMPERATURE
AND STORAGE TIME ON QUALITY
RETENTION OF RAW GROUND ALMOND
(*PRUNUS DULCIS*) AND WALNUT (*JUGLANS
REGIA* L.) KERNELS**

S.F. Mexis, A.V. Badeka, and M.G. Kontominas*

Laboratory of Food Chemistry and Technology
Department of Chemistry, University of Ioannina, Ioannina 45110, Greece

ABSTRACT

The present study investigated the effect of packaging material O₂ permeability, light, temperature and storage time on quality of raw ground walnuts and almonds. Samples were packaged in a) PET//LDPE, 70 µm in thickness and b) PET-SiOx//LDPE pouches, 62 µm in thickness under nitrogen. Samples were stored either under fluorescent light or in the dark at 4 or 20 °C for a period of 12 months. Quality parameters monitored were peroxide value (PV), hexanal, and the sensory attributes: odor and taste of product.

* Corresponding author email mkontomi@cc.uoi.gr. tel. +3026510-08342.

PV ranged between 0.3 meq O₂/kg oil for fresh ground walnuts and 30.0 meq O₂/kg oil for samples packaged in PET//LDPE pouches under N₂, exposed to light at 20 °C after 12 months of storage. Respective values for ground almonds were 0.3 and 20.0 meq O₂/kg oil. Hexanal ranged under 28.5 µg/kg (method detection limit) for fresh ground walnuts and 34.0 mg/kg for samples packaged in PET//LDPE exposed to light at 20 °C after 12 months of storage. Respective values for ground almonds were < 28.5 µg/kg and 9.0 mg/kg. Values for odor ranged between 8.6 (scale 9-1) for fresh walnut kernels and 1.4 for walnut kernels packaged in PET//LDPE exposed to light after 12 months of storage at 20 °C. Respective values for taste were 7.8 and 1.3. Odor values for ground almonds ranged between 8.9 for fresh products and 4 for products packaged in PET//LDPE exposed to light after 12 months of storage. Respective values for taste were 8.9 and 2.2. Taste proved to be a more sensitive attribute than odor. Based mainly on sensory analysis, ground walnuts retained acceptable quality for ca. 6 months in PET//LDPE-N₂ and at least 12 months in PET-SiOx//LDPE-N₂ pouches at 20 °C, with samples stored in the dark retaining higher quality than those exposed to light. Respective shelf lives at 4 °C were 6-7 and at least 12 months. Shelf life of ground almonds were ca. 6-7 months packaged in PET//LDPE and 8 months packaged in PET-SiOx//LDPE pouches under N₂ irrespective of lighting conditions at 20 °C while at 4 °C shelf life was extended by an additional month as compared to storage at 20 °C. PET-SiOx//LDPE proved to be an effective oxygen barrier for the protection of ground walnut and almonds sensory quality.

Keywords: raw ground almonds and walnuts, shelf life, quality, lipid oxidation

INTRODUCTION

Nuts are consumed for their sensory, nutritional and health promoting attributes (Savage et al., 1999). Almonds and walnuts are consumed as shelled, peeled or unpeeled raw or roasted whole or ground kernels, being used as ingredients of many foodstuffs such as bakery products and confectionery as well as flavoring agents in beverages and ice-cream (Rosengarten, 1984). Proximate analysis of almonds and walnuts shows these products to be a rich source of oil (49.42 and 65.21%, respectively) with a high content of unsaturated fatty acids (43.95 and 56.10%, respectively). They are also a good source of protein (21.22 and 15.23%, respectively) (USDA, 2009).

Epidemiological studies indicated that consumption of nuts, as compared to other foodstuffs rich in fat, decreases oxidative stress, improves total cholesterol and high density lipoprotein levels, thereby decreasing risk of coronary diseases. Consumption of nuts also helps to control weight (Sanders et al., 2000; Alper et al., 2002; Kocyigit et al., 2006).

High levels of unsaturated fatty acids however, render nuts prone to oxidation. Oxygen concentration is one of the most important extrinsic factors affecting nuts' quality through lipid oxidation. Oxidation may be enhanced by exposure to light (photo-oxidation), high storage temperatures and the grinding process due to the increased surface to weight ratio of ground nuts as documented in our previous work (Mexis et al., 2009a; Mexis et al., 2009b).

Packaging can directly influence the development of off-flavors in dried nuts by protecting the product from both oxygen and light (Mexis et al., 2009a; Mexis et al., 2009b). For this reason barrier materials, including polyethylene terephthalate (PET), polyamide (PA) and high barrier materials including ethylene vinylalcohol (EVOH), polyvinylidenechloride (PVDC) and/or vacuum coated films (Aluminum, SiO_x) with or without light barriers are being used to extend product shelf life (Jensen et al., 2003; Mexis et al., 2009; Mexis et al., 2010). These materials are normally used in combination with active or modified atmosphere for the protection of the product with respect to lipid oxidation (Mexis et al., 2009a; Mexis et al., 2009b; Garcia-Pascual et al., 2003).

Based on the above, the objective of the present study was to investigate the effect of 1) packaging material oxygen and light transmission and 2) storage temperature on quality retention of shelled raw ground almond and walnut kernels during long term storage.

2. MATERIAL AND METHODS

2.1. Materials

Almonds and walnuts were supplied by a local supplier (Zdoukos SA, Ioannina, Greece). According to the supplier, samples were harvested in the fall of 2008, mechanically shelled and packed in fiberboard cartons with an inner LDPE film, 10kg per carton.

2.2. Experimental Design

Almonds and walnuts were ground in a home type blender and packed in two different packaging materials: 1) barrier [polyethylene terephthalate // low density polyethylene (PET//LDPE) pouches, 75 μm in thickness and 103 mL/(m² day atm) in oxygen permeability] and 2) high barrier [polyethylene terephthalate-SiOx//low density polyethylene (PET-SiOx/LDPE)] pouches, 62 μm in thickness, having an oxygen permeability of 1.4 mL/(m² d atm). Oxygen permeability was measured using the Oxtran 2-20 permeability tester (MOCON Co. Minneapolis, MN, USA) at 75% RH and 25 °C. Pouches (PET//LDPE and PET-SiOx//LDPE) containing ground almonds or walnuts (100g) were first evacuated and then immediately injected with nitrogen produced by a PBI Dansensor Mix 9000 Gas mixer (Dansensor, Denmark). Pouches were immediately heat-sealed using a BOSS model NE 48 thermal sealer (BOSS, Bad Homburg, Germany) and stored either under fluorescent light or in the dark at either 4 or 20 °C in commercial temperature and light (825+50 lux) controlled cabinets. Control samples were prepared by packaging ground almond and walnut kernels in glass jars flushed with N₂ and stored at –18°C for up to 12 months. After 0, 2, 4, 6, 8, 10 and 12 months of storage, three separate identical samples were withdrawn from each treatment for chemical and sensory analysis. Duplicate measurements were carried out on each of three replicate samples (n=2x3=6).

2.3. Methods

2.3.1. Gas composition

At each sampling day, the headspace gas composition in each pouch was determined using a Dansensor CheckMate 9900 gas space analyzer (PBI, Ringsted, Denmark). Gas analysis was performed by drawing a headspace gas sample by piercing a syringe needle through a rubber septum glued on the surface of the PET//LDPE and PET-SiOx//LDPE pouches.

2.2 Oil Extraction

Almond and walnut oil was extracted using the Welmann method (G.S.C.L. 1976). Ground samples (5 g) were transferred into a separatory funnel with 100 ml of diethyl ether and 10 ml of distilled water. The

separatory funnel was agitated for a few minutes and subsequently left to equilibrate for 24 hours. 50 ml of the sample were transferred to a crystallizing dish and diethyl ether evaporated in a water bath at 40 °C. The extracted oil was dried in an oven at 105 °C for 3 min and the residue was used to determine the peroxide value.

2.3 Peroxide Value (PV)

The PV was determined according to the official EC (2568/91) (1991) method for the measurement of the characteristics of olive oil.

2.4 Hexanal Determination

2.4.1 SPME Procedure

Ground nut samples (0.1 g), along with 1 mL of distilled water and a micro-stirring bar were placed in a 10mL glass serum vial sealed with an aluminum crimp cap provided with a needle-pierceable polytetrafluoroethylene/silicone septum. Solid-phase microextraction (SPME) was performed with a 75-mm Carboxen/Polydimethylsiloxane (CAR/PDMS) fiber mounted to a SPME manual holder assembly (Supelco, Bellefonte, USA). The sample vial was placed in a 60 °C water bath and stirred at high speed. After allowing 10 min for the sample to equilibrate at 60°C, the needle of the SPME device was inserted into the vial through the septum, and the plunger of the SPME apparatus was pushed down to expose the Carboxen/PDMS fiber to the vial head space. After 10 min of exposure time with constant stirring, the fiber was retracted into the needle assembly, removed from the vial, and transferred to the injection port of the GC unit. The whole SPME procedure was optimized in preliminary work, testing the following variables: sample size, fiber type, extraction temperature, extraction time and sample agitation (Pastorelli et al., 2006). Blank runs were carried out prior to sample analysis to make sure that there was no contamination that could cause memory effects and misinterpretation of findings (Carasek and Pawliszyn, 2006).

2.4.1.1. GC-FID Analysis Conditions

GC analysis of hexanal adsorbed onto the SPME fiber was carried out on a Hewlett Packard HP 5890 series II GC unit (Wilmington, DE. USA)

equipped with a FID detector. A non-polar capillary column (HP-5, J. and W. Scientific, Folsom, USA) 30 m long, 0.32 mm in internal diameter and 0.25 μm in thickness was used. The GC oven was programmed as follows: temperature was initially set at 40 °C for 5 min, and then raised at the rate of 15 °C per min to 230°C. The injector and detector temperatures were set to 270 °C and 330 °C. Flow rate of the Helium carrier gas was 0.8mL per min. The injector was operated in the split mode (1:2 split ratio) at 330 °C. For thermal desorption, the SPME fiber was kept in the injector for 10 min. Data recording and analysis was performed using the HP GC Chemstation software for Windows (Hewlett-Packard).

2.5. Sensory Evaluation

Sensory evaluation (acceptability test) was carried out by a 51 member untrained panel (20 females and 31 males) consisting of faculty and graduate students of the Department of Chemistry of the University of Ioannina. Panelists were chosen using the following criteria: ages between 22 and 60, non smokers, without reported cases of food allergies who consume dried nut products regularly. Approximately 20 g of ground nuts were placed in small plastic containers coded with 3-digit random numbers and tightly capped. The samples were allowed to stand for 1/2h prior to the evaluation to allow equilibration of volatiles in the headspace. The sensory evaluation of almonds and walnuts was carried out on separate days. Panelists were served a set of 8 treated almond or walnuts samples [2 films x 2 lighting conditions x 2 temperatures x 1 atmosphere (N_2)] along with a control reference sample (stored at -18 °C); they were instructed to consume the whole sample and rinse mouth with sparkling water (room temperature), in between sample evaluation. Sensory attributes evaluated included color, texture and taste. Scoring was carried out on paper ballots using a 9 point hedonic scale where: 9=like extremely and 1=dislike extremely for the evaluation of color and taste and 9=very crispy and 1=very soft for evaluation of texture. A score of 5 was taken as the lower limit of acceptability for color, taste and texture.

2.6. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the software SPSS 16 for windows. Means and standard deviations were calculated, and,

when F-values were significant at the $p < 0.05$, mean differences were separated by the least significant difference procedure.

3. RESULTS AND DISCUSSION

3.1. Gas Composition

Results showed that samples packaged in the barrier film (PET//LDPE) the N₂ concentration fell below 97.5% after 2 months of storage in all treatments regardless of temperature, creating favorable conditions for lipid oxidation (data not shown). It has been documented that oxygen concentrations as low as 2% in the headspace of the packaged product enhance lipid oxidation producing rancid taste in walnuts (Jensen et al. 2003). On the other hand almonds and walnuts packaged in the high barrier material (PET-SiOx//LDPE), even after 12 months of storage, retained a N₂ concentration above or equal to 99.8%. Reduction of the N₂ concentration was accompanied by a respective increase in O₂ concentration. Present results are in good agreement with those of Jensen et al. (2003) and Mexis et al. (2009b) regarding preservation of whole walnuts using modified atmosphere packaging.

3.2. Lipid Oxidation

Lipid oxidation of raw ground almonds and walnuts was evaluated by measuring a) peroxide value (PV) for primary oxidation products and b) hexanal for secondary oxidation products. Hexanal is the main oxidation product of linoleic acid.

3.2.1. Peroxide Value

Changes in PV of ground almonds and walnuts as a function of storage time, packaging material oxygen and light barrier at 20 and 4° C are shown in Figures 1-4. The initial PV of fresh raw ground almonds and walnuts exhibited a very low peroxide value (0.17 meq O₂ / kg almond oil and 0.3 meq O₂/kg walnut oil, respectively), indicative of good product quality in terms of degree of lipid oxidation. Figure 1 shows the effect of the packaging material oxygen barrier, lighting conditions and storage time on PV of ground almonds at 20 °C.

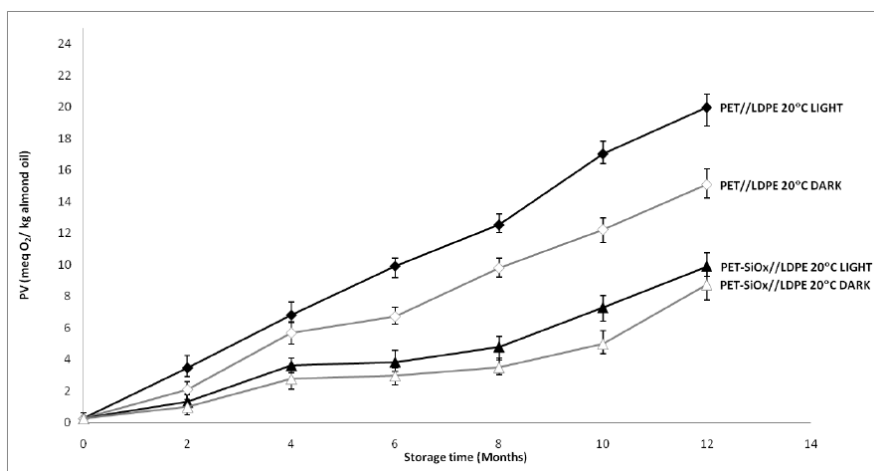


Figure 1. Changes in peroxide value of raw ground almonds as a function of packaging material oxygen barrier, lighting conditions and storage time at 20°C.

PV increased with storage time and exposure to light. After 12 months of storage at 20 °C, almonds packaged in PET-SiOx/LDPE under N₂, irrespective of lighting conditions, had a PV ca. 8.8-9.9 meq O₂ / kg almond oil. Respective PV for almonds packaged in PET//LDPE pouches, in the dark under N₂, was 15.1 meq O₂ / kg almond oil and under light exposure ca. 20.0 meq O₂ / kg almond oil ($p < 0.05$). Thus the use of the high barrier material PET-SiOx//LDPE results in PV values ca. 50% lower than those of the barrier material PET//LDPE. Light, in turn, had a statistically significant but a less pronounced effect on almond oxidation. Figure 2 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on PV of ground walnuts at 20 °C.

After 12 months of storage ground walnuts packaged in the PET-SiOx/LDPE pouches had a low PV ca. 3.6 meq O₂/kg walnut oil when exposed to light and 3.3 meq O₂/kg walnut oil stored in dark at 20 °C. In contrast, samples packaged in PET//LDPE in N₂ under light, had a PV of ca. 30.0 meq O₂/kg walnut oil and ca. 28.1 meq O₂/kg walnut oil in the dark. Thus, in the case of PET-SiOx//LDPE lighting conditions did not substantially affect PV. In the case of PET//LDPE light had a small but statistically significant effect ($p < 0.05$) on PV. Comparison of Figures 1 and 2 shows that under identical experimental conditions, ground walnuts packaged in PET//LDPE suffer a longer degree of oxidation than ground almonds. On the contrary, ground walnuts packaged in PET-SiOx//LDPE show a lower degree

of oxidation as compared to ground almonds. This finding should be further investigated. Figure 3 shows the effect of pouch oxygen barrier, lighting conditions and storage time on PV of ground almond at 4 °C.

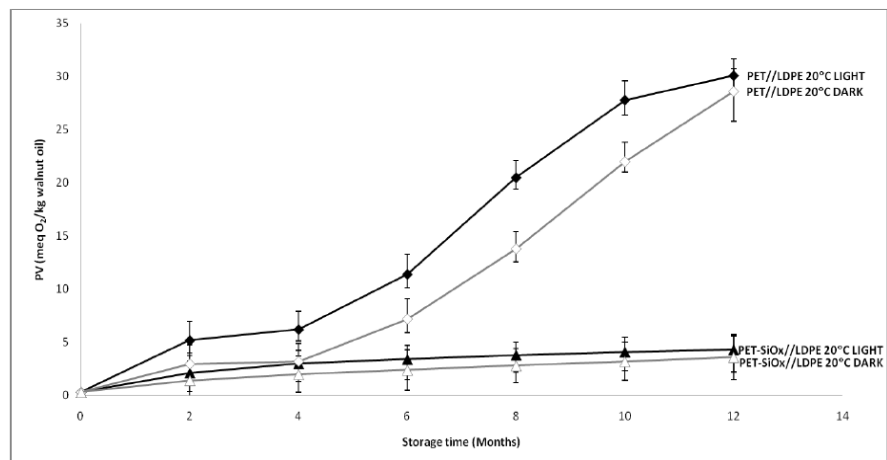


Figure 2. Changes in peroxide value of ground walnuts as a function of packaging material oxygen barrier, lighting conditions and storage time at 20°C.

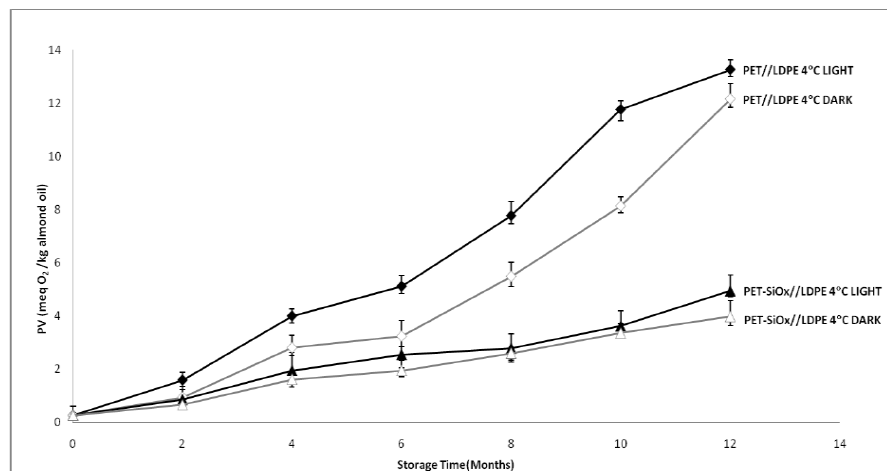


Figure 3. Changes in peroxide value of raw ground almonds as a function of packaging material oxygen barrier, lighting conditions and storage time at 4°C.

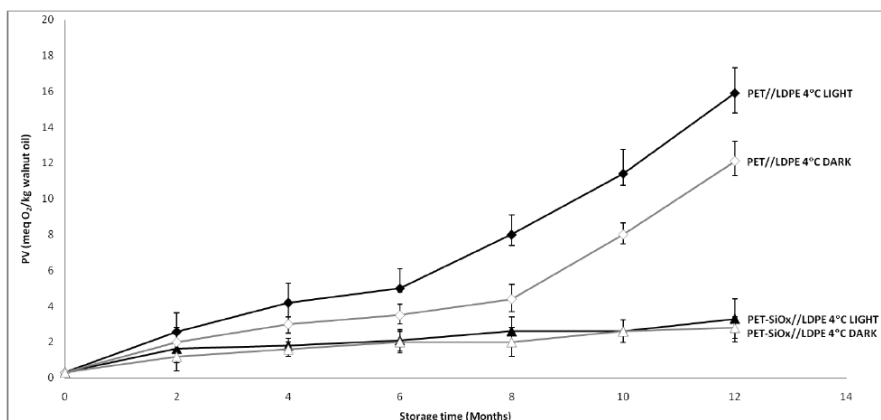


Figure 4. Changes in peroxide value of ground walnuts as a function of packaging material oxygen barrier, lighting conditions and storage time at 4°C.

After 12 months of storage at 4 °C, almonds packaged in PET-SiOx//LDPE in the dark under N₂ had a PV ca. 4.0 meq O₂ / kg almond oil while under light exposure a PV ca. 4.9 meq O₂ / kg almond oil. Respective PV for almonds packaged in PET//LDPE pouches in the dark under N₂ was ca. 12.2 meq O₂ / kg almond oil and under light exposure ca. 13.3 meq O₂ / kg almond oil. Comparison of data in Figures 1 and 3 shows that storage temperature substantially affected lipid oxidation of ground almonds resulting to twice the amount of peroxides as temperature increased from 4 to 20 °C for a given substrate, packaging material and lighting conditions. Similarly, Figure 4 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on PV of ground walnuts at 4 °C

After 12 months of storage at 4 °C, ground walnuts packed in PET-SiOx//LDPE pouches had 3.0-3.2 meq O₂/kg walnut oil irrespective of lighting conditions. Thus, in case of use of the high barrier film, lighting conditions did not affect ($p>0.05$) PV of ground walnuts. Respective PV values for ground walnuts packaged in PET//LDPE in the dark under N₂ was ca. 12.0 meq O₂ /kg walnut oil and under light ca. 16.0 meq O₂ /kg walnut oil. Comparison of data in Figures 2 and 4 shows that storage temperature substantially affected oxidation in the same manner as that for ground almonds.

Comparison of data in Figures 1-4 leads to the conclusion that as the O₂ barrier of the packaging material increases, the effect of temperature becomes less significant. Also, storage temperature had a more pronounced effect than light.

Kazantzis et al. (2003) packaged early and late harvest whole almonds, both in-shell and shelled at 5 °C (80 % RH) and 20 °C (60 % RH) in polyethylene (PE) bags for 6 months. They found that after 6 months of storage shelled whole almond oil had a lower K232 absorption coefficient than that of in-shell almonds tested. García-Pascual et al. (2003) stored four varieties of almonds, three Spanish and one imported from California, and evaluated the effect of storage temperature (8 and 36°C) and packaging atmosphere (air and N₂) on quality of raw, roasted, shelled and in shell whole almonds for a period of 9 months. In contrast to our results, they reported that packaging atmosphere did not affect the peroxide value for any almond variety. The main increase in PV occurred in the roasted shelled almonds stored at 36°C, since roasting accelerated product deterioration. Finally, results on PV of walnuts regarding the use of high barrier PET-SiOx//LDPE of the present study are in good agreement with those of Jensen et al. (2001) for whole walnuts, considering the differences in product initial PV (2.2 meq O₂/kg walnut oil vs. 0.3 meq O₂/kg walnut oil in the present study).

3.2.2. Hexanal Content

Changes in hexanal content of ground almonds and walnuts as a function of storage time, packaging material oxygen and light barrier at 20 and 4 °C are shown in Figures 5-8. The initial hexanal content of fresh raw ground almonds and walnuts was lower than the method detection limit (28.5 µg / kg). Hexanal is directly related to the development of oxidative off-flavors; it has a low odor threshold 5ng/g (Buttery et al., 1988) and is thus considered as an indicator of oil quality. Figure 5 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on hexanal content at 20°C for ground almonds.

After 12 months of storage almonds packaged in PET-SiOx//LDPE under N₂ in the dark had a hexanal content of ca. 3.7 mg hexanal / kg almonds while almonds exposed to light had a hexanal content of ca. 4.3 mg hexanal / kg almonds ($p < 0.05$). Respective hexanal content for almonds packaged in PET//LDPE pouches under N₂ in the dark was: ca. 6.6 mg hexanal / kg almonds and under light exposure 9.0 mg hexanal / kg almonds ($p < 0.05$). Thus, as for PV, lighting conditions had only a small effect on hexanal formation in the case of the high barrier material PET-SiOx//LDPE. On contrary, light had a substantial effect on hexanal formation in the case of the barrier material PET//LDPE. Figure 6 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on hexanal of ground walnuts at 20 °C.

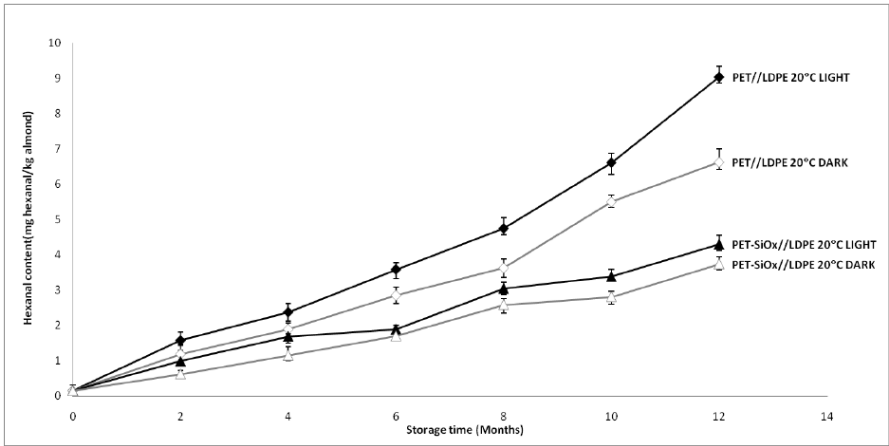


Figure 5. Changes in hexanal content of raw ground almonds as a function of packaging material oxygen barrier, lighting conditions and storage time at 20°C.

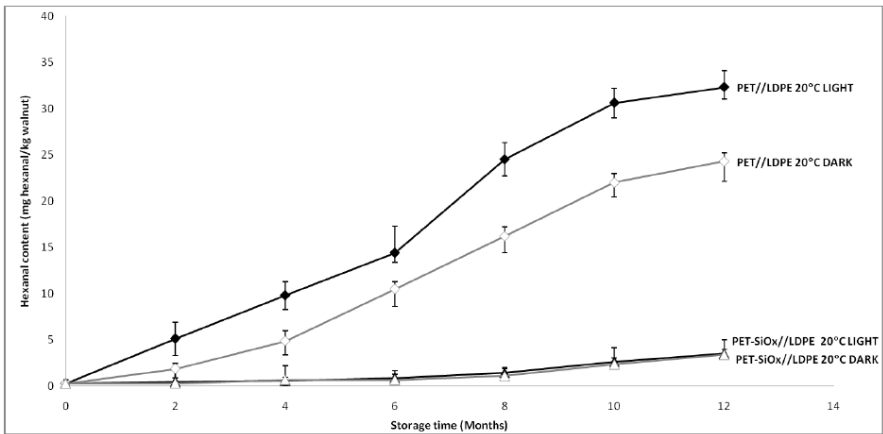


Figure 6. Changes in hexanal content of ground walnuts as a function of packaging material oxygen barrier, lighting conditions and storage time at 20°C.

After 12 months of storage, walnuts packaged in PET-SiOx//LDPE pouches had very low ca. 3.4 mg hexanal / kg walnut at 20 °C irrespective of lighting conditions. Hexanal values at 20 °C were ca. 24.5 and 32.3 mg hexanal / kg walnuts for walnuts packaged in PET//LDPE stored in dark and light, respectively. An observation to be made is that storage time and packaging material oxygen barrier significantly ($p<0.05$) affected hexanal

content. Lighting conditions affected hexanal formation only for the barrier materials (PET-LDPE) but not for the high barrier material (PET-SiOx/LDPE). Finally the effect of packaging material oxygen transmission rate and storage time was substantially higher than that of lighting conditions. Comparison of data in Figures 5 and 6 shows the same trend observed for PV of two substrates.

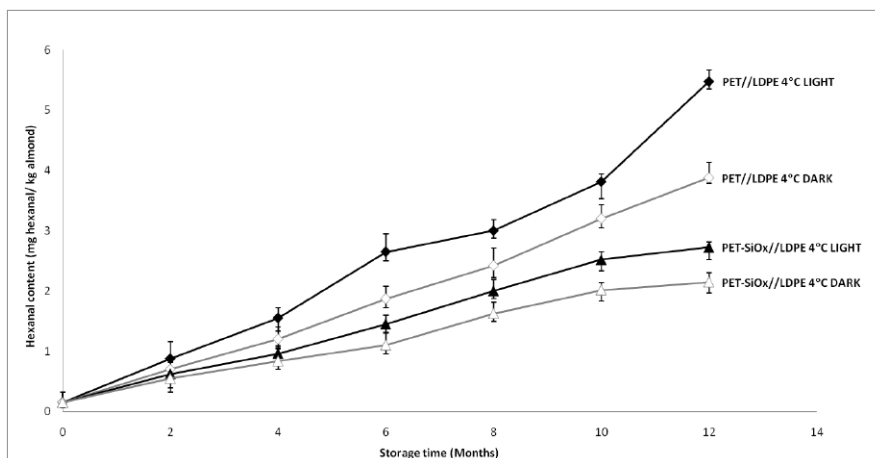


Figure 7. Changes in hexanal content of raw ground almonds as a function packaging material oxygen barrier, lighting conditions and storage time at 4°C.

Figure 7 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on hexanal content of ground almonds stored at 4 °C. After 12 months of storage at 4 °C, ground almonds packaged in PET-SiOx/LDPE under N₂ in the dark had a low hexanal content ca. 2.1 mg hexanal / kg almonds and under light exposure 2.7 mg hexanal / kg almonds ($p < 0.05$). Respective hexanal content for almonds packaged in PET//LDPE under N₂ in the dark was ca. 3.9 mg hexanal / kg almonds and under light exposure ca. 5.4 mg hexanal / kg almonds ($p < 0.05$). It is observed that for a given temperature, exposure to light resulted in higher hexanal values as compared to storage in the dark ($p < 0.05$). Comparison of data in Figures 5 and 7 shows that storage temperatures substantially affected hexanal formation, resulting to ca. a 50% increase of the amount of hexanal content with an increase in temperature from 4 to 20 °C for a given substrate, packaging material and lighting conditions.

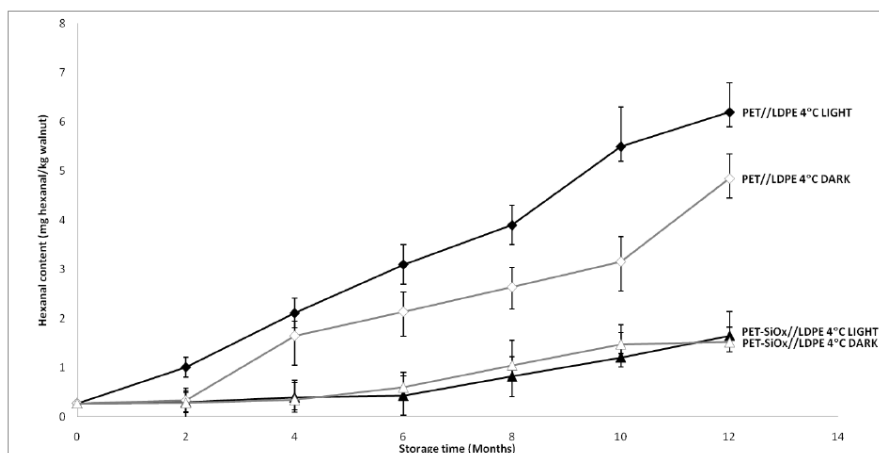


Figure 8. Changes in hexanal content of ground walnuts as a function of packaging material oxygen barrier, lighting conditions and storage time at 4°C.

Finally, Figure 8 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on hexanal content of ground walnuts stored at 4 °C. After 12 months of storage, ground walnuts packaged in PET-SiOx//LDPE pouches had very low ca. 1.5 mg hexanal / kg walnut at 4 °C irrespective of lighting conditions. Hexanal values at 4 °C were ca. 4.8 and 6.2 mg hexanal / kg walnuts for walnuts packaged in PET//LDPE stored in dark and light respectively. Comparison of Figures 6 and 8 shows that storage temperature substantially affected hexanal formation, resulting to a fivefold increase in content of hexanal as temperature increased from 4 to 20°C for PET//LDPE and a tenfold increase for PET-SiOx//LDPE.

Comparison of data in Figures 5-8 leads to the conclusion that as the O₂ barrier of the packaging material increases, the effect of temperature becomes less significant. Also, storage temperature had a more pronounced effect than light.

Kazantzis et al. (2003) found that after 6 months of storage, shelled almonds retained a constant K₂₇₀ value irrespective of lighting conditions. This is in contrast to results of the present study, according to which, secondary oxidation products increased with storage time. Similar to our results, Zacheo et al. (2000) reported that hydroperoxide production gave rise to the formation of malondialdehyde (MDA) during storage, which increased by 200% as compared to fresh harvest almonds. Finally, Jensen et al. (2001) packaged whole walnut kernels in high barrier transparent and aluminum coated plastic laminates and reported hexanal values of ca. 5.0 mg hexanal / kg for products

stored at 5 °C in the dark, 25.0 mg hexanal / kg for products stored at 5 °C under light, 70.0 mg/kg for products stored at 21°C in the dark and finally 185.0 mg/kg for products stored at 21 °C under light after 25 weeks of storage. These values are higher than those of the present work. Differences in values may be attributed to the high concentration of oxygen (50%) used in the former study to achieve accelerated storage conditions.

3.3. Sensory Evaluation

No significant changes ($p>0.05$) were observed in texture of samples after 12 months of storage (data not shown). Products remained crisp probably due to adequate protection from environmental humidity, provided by the PE layer(s) of both packaging materials tested. In contrast to texture, statistically significant ($p<0.05$) changes were recorded for color of both raw ground almonds and walnuts stored at 20 °C and exposed to light during 12 months of storage (data not shown). Most profound changes in color were recorded for samples packaged in PET//LDPE while respective changes for samples packaged in PET-SiOx//LDPE were small but statistically significant ($p<0.05$) Exposure of samples to light, showed more intense darkening. In general, higher temperature and oxygen concentrations seem to accelerate product darkening, attributed to browning reactions due to phenols' oxidation (Ryan and Roberts, 1998). Whole almond kernels' darkening during storage have also been reported by several authors (Ledbetter and Palquest 2006; Sandez-Bel et al. 2008; Mexis and Kontominas, 2010). According to sensory panel comments, darkening of samples was accompanied by the development of rancid flavors in the product. Taste proved to be a more sensitive attribute than odor. Taste changes in almonds and walnuts are shown in Figures 9-12.

As shown in Figure 9, after 12 months of storage of ground almonds, the lowest scores were recorded for almonds packaged in PET//LDPE under N₂ and stored at 20°C under light (score 2.19) while the most protected samples (PET-SiOx//LDPE in the dark) showed the highest scores (score 3.5). Based on taste, the shelf life of ground almonds at 20°C was ca. 6 months for the PET//LDPE under N₂ packaged samples under light, 7 months for the PET//LDPE under N₂ packaged samples in the dark, 8 months for the PET-SiOx//LDPE packaged samples under light and 9 months for the PET-SiOx//LDPE packaged samples in the dark.

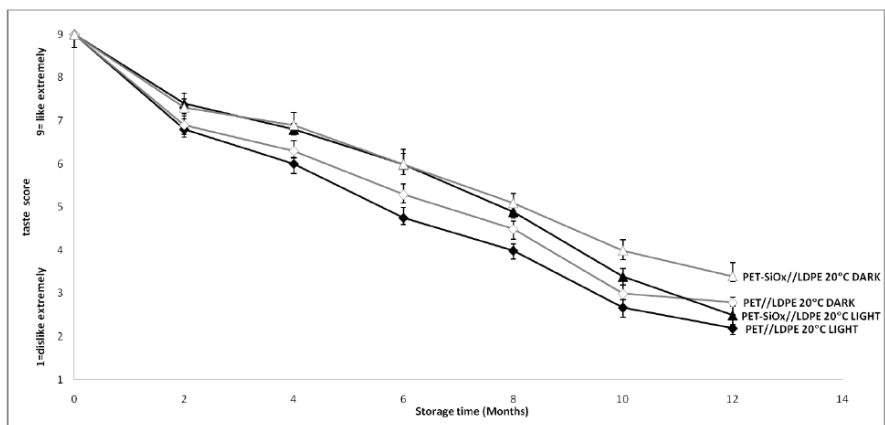


Figure 9. Changes in taste of raw ground almonds as a function of packaging material oxygen barrier, lighting conditions and storage time at 20°C.

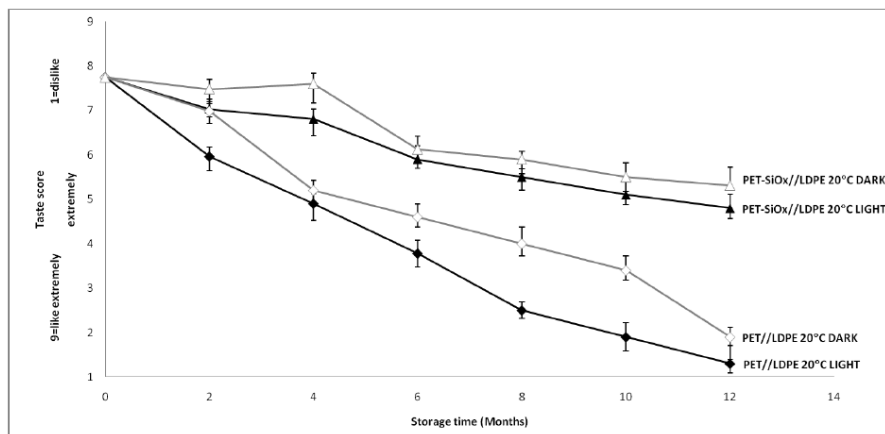


Figure 10. Changes in taste of ground walnuts as a function of packaging material oxygen barrier, lighting conditions and storage time at 20°C.

Respectively for ground walnuts, after 12 months of storage at 20°C, the most pronounced changes in taste were recorded in products packaged in PET//LDPE exposed to light (score 1.3) while samples packaged in PET-SiOx//LDPE under N₂ in the dark retained their quality at acceptable levels (score 5.2). Based on taste, the shelf life of ground walnuts at 20°C was ca. 4 months for the PET//LDPE packaged samples under light; 5-6 months for the PET//LDPE packaged samples in the dark; 11-12 months for the PET-

SiOx//LDPE packaged samples under light and at least 12 months for PET-SiOx//LDPE packaged samples stored in the dark.

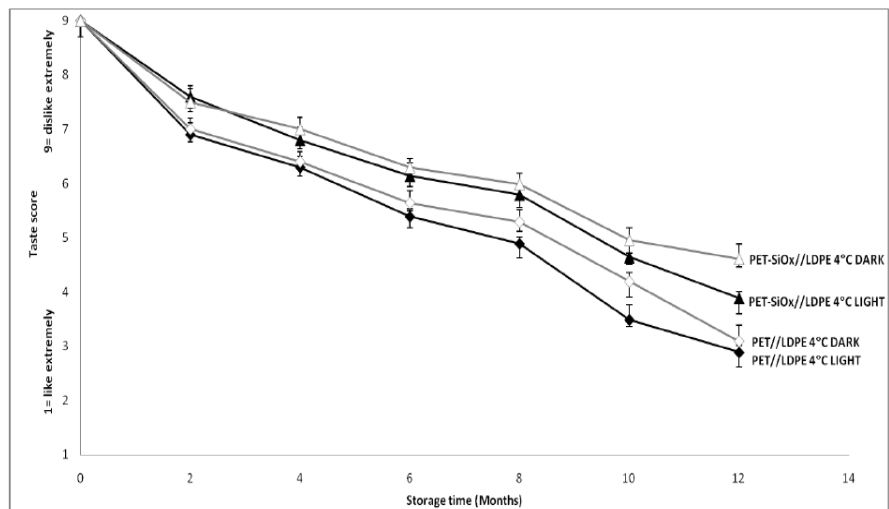


Figure 11. Changes in taste of raw ground almonds as a function of packaging material oxygen barrier, lighting conditions and storage time at 4°C.

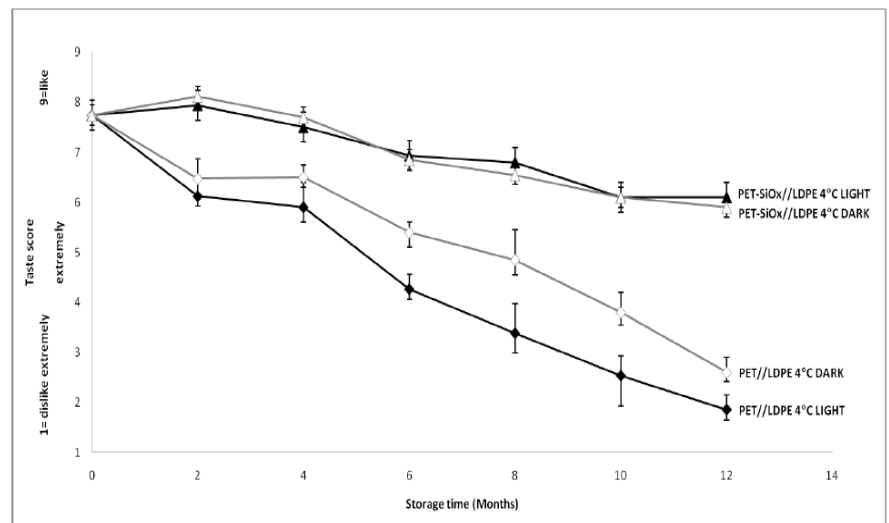


Figure 12. Changes in taste of ground walnuts as a function of packaging material oxygen barrier, lighting conditions and storage time at 4°C.

A similar trend in taste deterioration was recorded in the case of samples stored at 4°C (Figure 11 and 12) but to a lower extent as compared to storage at 20°C. Based on taste shelf life of ground almonds at 4°C (Figure 11) was ca. 8 months for the PET//LDPE packaged samples under light; 9 months for the PET//LDPE packaged samples stored in the dark; 10 months for PET-SiOx//LDPE packaged samples under light and 11 months for the PET-SiOx//LDPE packaged samples stored in the dark. Figure 12 shows the effect of packaging material oxygen barrier, lighting conditions and storage time on taste scores of ground walnuts at 4 °C.

For ground walnuts stored at 4 °C, taste scores were 5.9-6.1 for PET-SiOx//LDPE irrespective of lighting conditions and 1.9 and 2.7 for PET//LDPE under light and in the dark, respectively.

Based on taste evaluation, shelf life of ground walnuts at 4 °C was 5 months in PET//LDPE exposed to light, 8-9 months in PET//LDPE stored in the dark and at least 12 months when packaged in PET-SiOx//LDPE irrespective of lighting conditions. Comparison of data in Figures 9-12 leads to the following observations: a) variation in shelf life of ground almonds is less than that of ground walnuts as a function of packaging material barrier to O₂ and storage temperature, b) at a given temperature, the effect of packaging material barrier to O₂ is more pronounced in the case of ground walnuts vs. ground almonds, c) temperature had a stronger effect on shelf life than light and d) the higher the packaging material barrier to O₂ the less was the effect of light.

Sanchez-Bel et al. (2005) and (2008) reported that almonds reached the limit of overall acceptability score of 3 on a scale of 5 (very pleasant) to 1 (very unpleasant) after 6 months of storage. Zacheo et al., (2000) reported no significant changes in rancidity of almonds, after 18 months of storage in the dark at 20 °C by sensory testing, while after 36 months of storage, moderate rancid flavors were recorded. Similarly, Jensen et al. (2001) reported a positive correlation between sensory attributes (rancidity) and hexanal content of walnuts. Mexis et al. (2009b) reported that whole walnut kernels retained acceptable quality for ca. 2 months in PE - air, 4-5 months in PET//PE-N₂ and at least 12 months in PET-SiOx//PE - N₂ pouches at 20 °C, with samples stored in the dark retaining slightly higher quality than those exposed to light. Finally, Mexis and Kontominas (2010) reported that whole raw unpeeled almonds retained acceptable quality for ca. 7-8 months packaged in PET//LDPE and ca. 9 months packaged in PE/EVOH/PE pouches under N₂ irrespective of lighting conditions at 20°C while at 4°C shelf life was extended by 1-2 months as compared to storage at 20°C.

3.4 Conclusion

Based on present data, the optimum conditions for packaging and storage of raw ground almonds and walnuts include the use of a high barrier material such as PET-SiO_x//LDPE in combination with modified atmosphere irrespective of lighting conditions and storage temperature. Lowering of storage temperature substantially increases product shelf life.

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Chapter 5

**PHYSICAL PROPERTIES OF SHEA
(*VITELLARIA PARADOXA* GAERTN.) FRUITS,
NUTS AND KERNELS FROM DIFFERENT
LOCALITIES OF CAMEROON**

***Bup Nde Divine¹, Diarrassouba Nafan²,
Charles Fon Abi³, Tenin Dzudie⁴, Kapseu César⁴,
and Clergé Tchiegang^{*}***

¹Department of Textile, Clothing and Leather Engineering, Higher Institute of the Sahel, University of Maroua, Cameroon

²Unité Régionale de l'Enseignement Supérieur de Korhogo, Côte d'Ivoire.

³Department of Chemistry, Higher Teachers' Training College Yaounde, University of Yaounde 1

⁴Department of Process Engineering, National School of Agro-Industrial Sciences (ENSAI) Ngaoundere, Cameroun

ABSTRACT

Vitellaria paradoxa Gaertn or the shea tree produces kernels which have a fat content of about 35-60% usually referred to as shea butter. This

1 Email : bupdiv@yahoo.fr; Tel 237 75 41 77 24.

2 PO. Box 1328, Côte d'Ivoire.

* Corresponding author

butter is used traditionally in foods and medicines while on an industrial scale it is used in the cosmetics and chocolate industries. The processing of fruits to obtain butter involves collection of the fruits, depulping to give nuts, cooking of the nuts, dehusking to give the kernels, drying of kernels and oil extraction. The cooking and drying of sheanuts are critical steps in the traditional processing of shea kernels which largely determine butter quality. This work presents results on the physical properties of shea fruits and nuts which affect these critical steps and consequently butter quality. Shea fruits from 7 localities (Gashiga, Rabingha, Hina, Tchabal, Deone, Fouban and Bangoua) which cut across four ecological zones of Cameroon were harvested and their physical properties determined. The major diameters of the fruits and nuts ranged from 43.8 ± 6.3 to 69.62 ± 10.57 mm and 32.80 ± 2.91 to 44.29 ± 5.09 mm respectively. The sizes of the shea fruits and nuts analysed were highly dependent on the altitude of the sampling site. The sphericities of the fruits and nuts lay between 0.7 and 1 indicating that they are essentially spherical in shape. Larger fruits were found at altitudes greater than 1200 m while smaller fruits and nuts grew generally at altitudes ranging from 200-600 m. More than 77 % of the nuts from all the sampling sites had major diameters ranging from 40-45 mm. Significant differences were equally observed in the physical properties of the fruits and nuts obtained from different trees within and between sampling sites. An empirical relation was established and validated for inter-converting between the major diameter of the fruits and nuts. This relation can be used to estimate major diameters of the fruits from the nuts given that most often only the nut is available due to the highly perishable nature of the fruit pulp. Sheanut kernels are large (34-45 mm in diameter) and therefore have to be dried as thin slices in order to fasten drying times. Results on some physical properties of the kernels are also reported.

Keywords: shea fruits, nuts, kernels, slices, physical properties.

1. INTRODUCTION

The physical properties of a material are important to design the equipment for its processing, transportation, sorting, separation and storing. Designing such equipment without taking these into consideration may yield poor results. Therefore the determination and consideration of these properties has an important role. Henderson and Perry (1981) specified sorting, cleaning and grading or classification of agricultural products as being based on their physical properties. The physical properties are also needed to define and

quantify heat transfer problems during heat processing of the seeds (Mohesenin, 1986). The physical properties of shea kernels and nuts from Borno state of Nigeria have been reported by Olajide *et al.* (2000) and Avira *et al.* (2005) respectively. Meanwhile the shape factors (major and minor diameter and masses of shea fruits, nuts and kernels have been reported for some specific localities of Cameroon (Bup Nde, 2003 and Womeni, 2004). Womeni (2004) mentioned the large variations that existed in the physical properties of shea fruits, nuts and kernels from the same locality and suggested that these differences could be due to tree to tree variation within the same locality. However, no studies have taken into consideration this tree to tree variation of these physical properties within sites and between localities in Cameroon. Such studies could permit the calibration of shea fruits and nuts in order to define the range of physical properties for use in design of processing equipments shea fruits and nuts by researchers. Providing this information is important given the fact that research on shea fruits and related fields is expected to rise (Ugehe *et al.* 2008) due to the increasing demand of shea nuts and butter in the local and international markets (Mbetid-Bessane, 2005; Umobong 2006).

The objective was therefore to calibrate shea fruits and nuts using some physical properties from different shea producing areas of Cameroon.

2. MATERIALS AND METHODS

2.1. Sampling Sites

Four shea fruit producing regions (North, Far North, Adamawa and West) which cut across different ecological zones were chosen and sampled from 12 June to 3rd July 2006. In each region 1-2 sites located at least 50 km apart were selected for sampling. These sites included: Gashiga and Rabingha (North region), Hina (Far North region), Tchabal and Deone (Adamawa region) and Founmban and Banguoa (West region).

2.2. Sampling Protocol

According to Palmberg (1985), when the variation of the properties of a particular species over a given surface area is to be studied for the first time, the sampling sites should be chosen over a large surface area as a function of

the ecological gradient of the area in order to take into account differences that may arise due to climate or ecology. This protocol of Palmberg (1985), modified by Masters (2006) as detailed below was used in this field work. At the chosen site, the geographical coordinates (latitude, longitude and height of the site above sea level) were taken using a geographical positioning system apparatus (*GPS, Siemens 60, GARMINI, Taiwan*). These geographical coordinates were used to locate the sampling sites on the map of Cameroon (figure 1). In each locality, 10 shea trees (Palmberg, 1985; Kama-Niamayoua, 2006) located at a distance of about 25 m from each other were then randomly sampled. From each tree 10-15 mature fruits and/or nuts (Diarrassouba, 2000; Diarrassouba *et al.*, 2007a and b ; Kama-Niamayoua, 2006) that had fallen to the ground were sampled.

2.3. Determination of the Physical Properties of Fruits, Nuts and Kernels

The dimensions (major, (x), intermediate, (y), and minor diameter, (z)) as shown on figure 2 of the fruits, nuts and kernels were taken using a digital vernier callipers (Model *SV-03-150*, SCHLENKER enterprises LTD, USA) having a precision of 0.01mm. The measurements were done at the site. The kernels were then put in tissue bags tied and transported to the laboratory for further analyses.

The geometric mean diameter D_e of the kernel was then calculated from the relationship given by Mohsenin (1986).

$$D_e = (xyz)^{1/3} \quad [1]$$

The sphericity ω of the kernels was given by

$$\omega = \frac{(xyz)^{1/3}}{x} \quad [2]$$

2.4. Determination of Bulk Density of the Kernels

To determine the true or solid density of the shea kernel slices, an analytical balance (*model Scout Pro SPU402*, OHAUS, USA) adapted for this purpose was used. The balance was set to the specific gravity mode. A spring

was attached to the balance from which the sample tied to a string of negligible weight was hung. The weight of the sample was taken in air. The sample was then immersed into a beaker of water placed on the balance and the new weight taken. The specific gravity of the sample was then determined from the relation (as indicated in the User's Manual of the balance).

$$\text{Specific gravity} = \frac{\text{weight in air}}{\text{weight in air} - \text{weight in water}} \quad [3]$$

This was then converted to solid density given that the density of water at 22°C is 1 g/cm³. At each moisture content, five kernel slices were used for each determination. The experiment was replicated thrice at each moisture content. These studies were carried out on 5, 10 and 15 mm thick sheanut kernel slices. Different levels of moisture were obtained by drying the sheanut kernel slices in an indirect solar dryer for predefined periods of time and measuring the moisture content by the oven method.

2.5. Determination of Bulk Density of the Kernels

The bulk density was determined using the AOAC (1980) method. This involved the filling of a 500 ml cylinder with kennels from a height of 15 cm and weighing the contents. The bulk density ρ_b in kg/m³ was given by

$$\rho_b = \frac{m_b}{V_b} \quad [4]$$

where V_b is the bulk volume. Each experiment was replicated four times.

2.4. Data Analyses Methods

Statistical analysis (ANOVA) of the physical properties was carried out on Statgraphics Plus Version 5.0 (*Statistical graphic corp. (1994-2000) USA*) and the Duncan's multiple range test was used to detect the differences between means. The data collected during this survey was equally subjected to principal component analysis in order to determine the variables associated with each other. The PCA was performed on average values per tree in the

localities studied. One important aspect of PCA includes determination of the number of fundamentally different properties called Principal Components (PC) exhibited by the data set (Njintang, Mbofung and Kesteloot, 2007). In a next step the PCA factor scores of each sample were correlated with the traits using Pearson rank correlation coefficient. All these analyses were achieved using the StatBox Version 6.40 (Grimmer Logiciel (1999-2002) Paris, France).

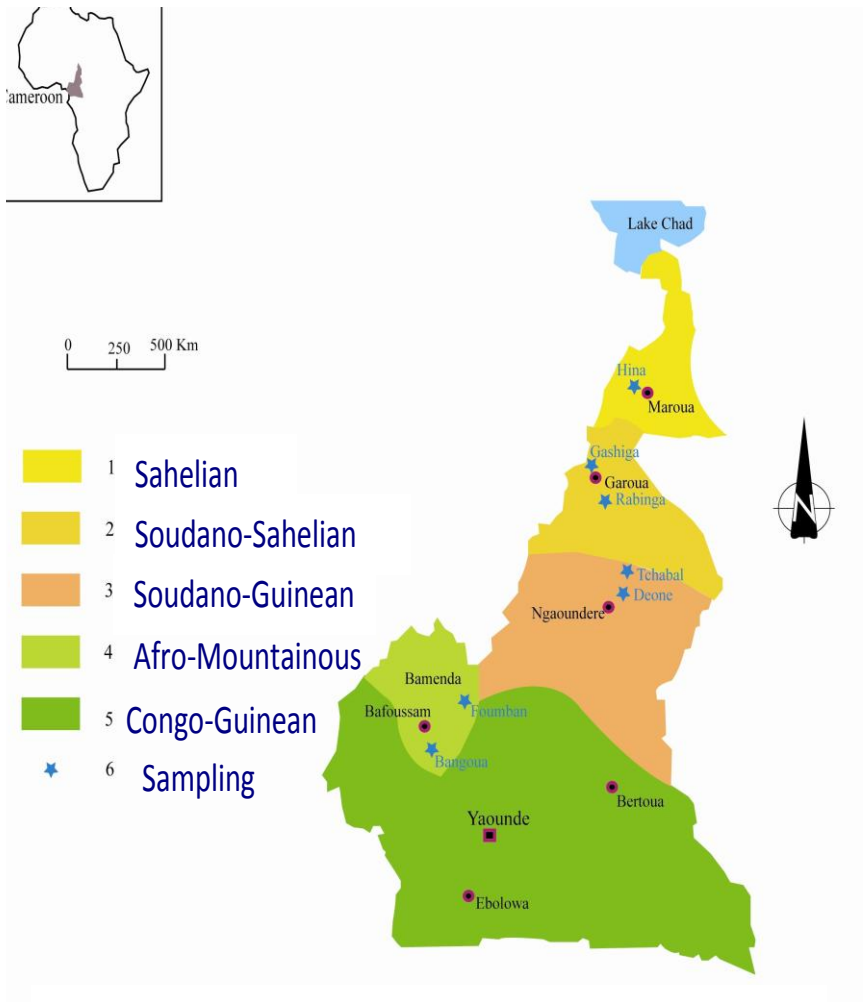


Figure 1. Map of Cameroon showing the sampling.

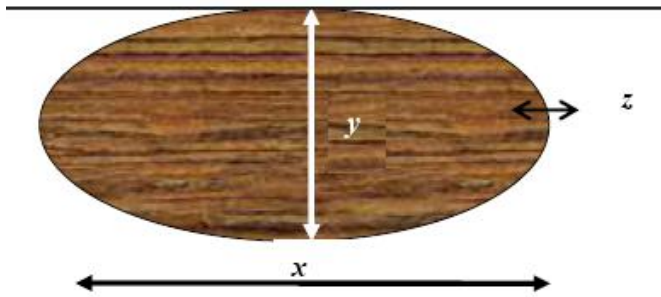


Figure 2. Sketch of shea fruit showing the three different dimensions measured.

3. RESULTS AND DISCUSSION

3.1. Inter Tree Variation Within the Same Site

The coefficient of variation (CV) obtained using equation 3 for major diameter, geometric mean and sphericities for more than 80% of fruits from each tree was less than 10% at all the sampling sites. The corresponding CV values for more than 85% of the nuts were equally less than 10%.

$$CV (\%) = (100\sigma)/ \quad [3]$$

where M and σ are respectively the mean and the standard deviation of the physical property under consideration. These low coefficients of variation in the physical properties of shea fruits and nuts suggested that the fruits and nuts from the same tree were homogenous (Kama-Niamayoua, 2006). This was probably due to the existence of some sort of natural calibration on each tree. Kama-Niamayoua (2006), reported the homogeneity of safou fruits from the same tree and claimed that this was obviously due to natural calibration of the fruits on the same tree. The variation of the major diameter of the fruits and nuts with the sampling site are presented in figures 3 and 4 respectively. The average values of the major diameters used to generate these figures as well as the other shape factors of the fruits and nuts measured are presented in table 1. It was observed that apart from Gashiga (where there was no significant difference between the values of the geometric mean of the fruits) there existed a significant difference in the dimensions (major, intermediate and minor diameters), the geometric mean diameter, sphericity and area of the fruits and nuts in all the sites studied from one tree to the other.

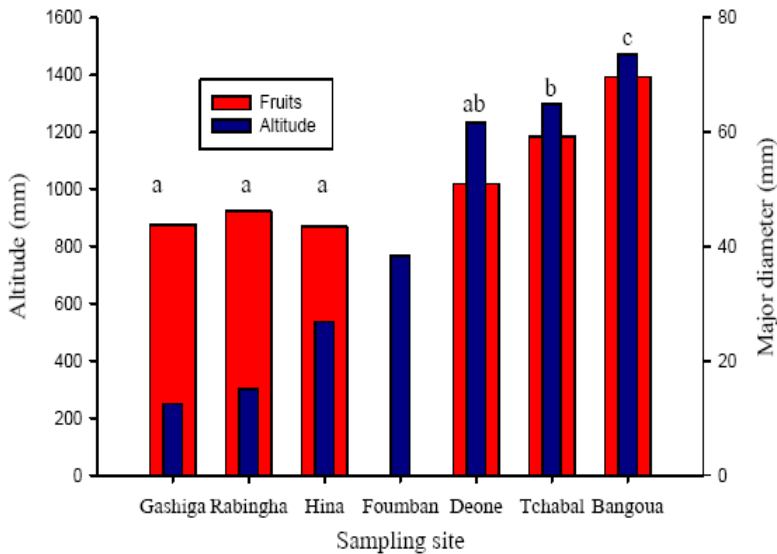
Table 1. Some physical properties (average values) of sheafruits and nuts from different localities of Cameroon

Site	FRUITS						NUTS						
	<i>x</i>	<i>Y</i>	<i>z</i>	G.M	ω	Area	<i>x</i>	<i>Y</i>	<i>Z</i>	G.M	ω	Area	Alt
Gashiga	43.8 ^{a*} (6.25)**	37.69 ^{bc} (3.13)	37.28 ^{bc} (3.02)	39.44 ^a (3.32)	0.91 ^d (0.08)	4919.49 ^{ab} (868.27)	36.44 ^b (3.87)	27.11 ^b (2.40)	25.32 ^{ab} (2.61)	29.21 ^b (2.53)	0.80 ^b (0.05)	2698.28 ^b (460.35)	250
Foumban	nf	nf	nf	nf	nf	nf	41.21 ^c (5.94)	32.69 ^d (5.16)	28.05 ^{de} (4.58)	33.51 ^{cd} (4.87)	0.81 ^b (0.04)	3599.38 ^{cd} (954.75)	768
Banguoa	69.62 ^d (10.57)	48.26 ^e (7.74)	45.34 ^d (6.71)	53.24 ^c (7.08)	0.77 ^a (0.08)	9056.53 ^d (2527.97)	42.80 ^c (4.32)	30.20 ^c (3.25)	26.77 ^{cd} (3.35)	32.52 ^c (2.99)	0.76 ^a (0.04)	3348.25 ^c (619.76)	1471
Deone	50.91 ^{ab} (4.49)	42.24 ^{cd} (1.91)	41.32 ^{cd} (3.23)	44.62 ^b (3.10)	0.88 ^{bcd} (0.02)	6273.45 ^{ab} (872.31)	42.47 ^c (4.84)	32.54 ^d (2.86)	29.1 ^{ef} (3.64)	34.22 ^d (3.43)	0.81 ^b (0.04)	3714.19 ^d (737.98)	1234
Rabingha	46.12 ^a (6.63)	34.89 ^a (3.51)	34.34 ^a (4.10)	38.02 ^a (3.94)	0.83 ^b (0.08)	4588.36 ^a (951.27)	35.8 ^b (4.07)	27.42 ^b (3.21)	26.13 ^{bc} (3.25)	29.46 ^b (3.19)	0.83 ^b (0.06)	2756.48 ^b (586.72)	300
Hina	43.41 ^a (4.66)	35.49 ^{ab} (3.18)	35.05 ^{ab} (3.46)	37.77 ^a (3.44)	0.87 ^{cd} (0.04)	4516.60 ^a (807.12)	32.80 ^a (2.91)	24.15 ^a (2.48)	23.77 ^a (3.76)	26.56 ^a (2.70)	0.81 ^b (0.05)	2238.4 ^a (457.50)	536
Tchabal	59.17 ^c (8.32)	49.59 ^e (4.59)	49.62 ^e (4.56)	52.91 ^c (5.28)	0.88 ^{bcd} (0.04)	8876.53 ^d (1858.34)	44.29 ^c (5.01)	32.10 ^d (2.52)	30.45 ^f (2.54)	34.48 ^d (2.85)	0.82 ^b (0.03)	3759.01 ^d (638.02)	1260

*Means within columns with the same superscript are not significantly different

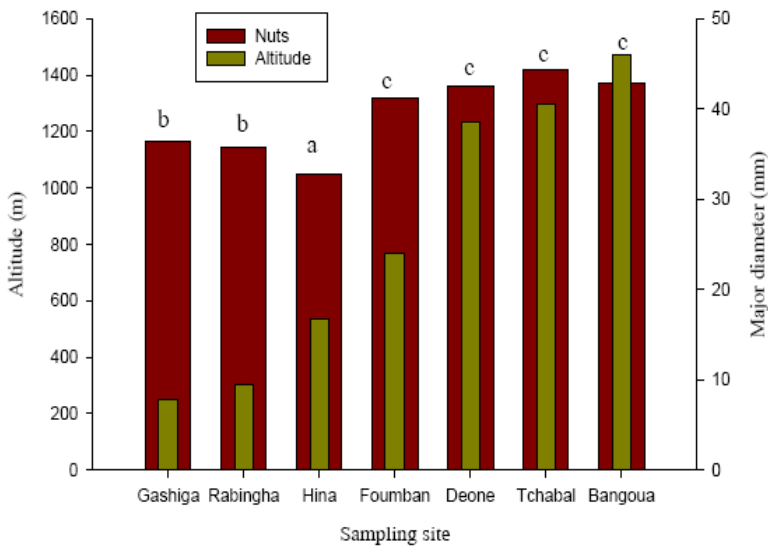
**Values in parentheses are standard deviations

nf = no fruits available during sampling



Bars with different letters are significantly different $P < 0.05$.

Figure 3. Variation of major diameter of fruits with the altitude of the sampling site



Bars with different letters are significantly different $P < 0.05$.

Figure 4. Variation of major diameter of nuts with the altitude of the sampling site.

The geometric mean diameter is a physical property that embodies all the three dimensions (major, intermediate and minor diameters) of a fruit, nut or kernel. It can therefore be used to better represent the average size of a product. Sphericity is used to describe the shape of a particle (spherical, ellipsoidal, round). The major diameter is easily measured compared to the geometric mean diameter and in this work the two diameters were found to be highly and positively correlated ($r^2 = 0.91$) at the 95% confidence interval. The properties (major diameter, geometric mean diameter and sphericity) are very important for the design and construction of equipments for mechanically opening up the pulp and cracking the nuts. Such parameters will be of interest to researchers and potential investors in the field. These parameters (major diameter, geometric mean diameter and sphericity) amongst the six different parameters measured were retained to describe the physical properties of the fruits and nuts. For simplicity, the major diameter and size of the fruit were used synonymously in this work.

3.2. Inter Site Variation of Shea Fruits and Nuts

Figures 3 and 4 summarise some of the physical properties of the samples from 7 different localities of Cameroon carried out on 68 trees. With respect to the physical properties significant differences ($p < 0.001$) were observed between the sampling sites. It was noted that, there existed a positive and significant correlation ($r^2 = 0.79$) between the altitude of the sampling site and the fruit diameters. Aboubakar Dandjouma *et al.* (2009) observed that the major diameter of shea fruits from Lainde Massa (a mountainous and rainy area south of Garoua) was significantly higher than those from the low land areas (Rabingha and Gashiga) in the Garoua neighbourhoods. This buttressed the fact that this parameter varied with the height of the locality above sea level. Infact the average value of the major diameter at Lainde Massa (61.50 ± 0.57 mm) reported by Aboubakar Dandjouma *et al.* (2009) was close to those obtained in this work for Tchabal (60.27 ± 7.51 mm), Tchabal (57.06 ± 8.97 mm) and Banguoa (69.62 ± 10.57 mm) which are all highland areas. This correlation value (< 1) however, suggested that apart from altitude, the physical properties could also be influenced by other factors not taken into consideration in this work such as: the orientation of the fruits on the tree, distance of the fruit from the ground, the degree of sunshine, age of the tree and soil factors. The highest values of the major diameter (69.62 ± 10.57 mm) and geometric mean diameter (53.24 mm) were obtained at Banguoa in west

Cameroon where the altitude was highest (close to 1500 m) while the corresponding lowest values (43.40-46.12 mm) and (37.77-39.44 mm) were obtained at Gashiga, Rabingha and Hina in the northern regions of Cameroon where the altitude ranged from about 250 to 600 m. However, the sphericity of the fruits did not depend on the altitude of the sample site. The average sphericities of the fruits ranged from 0.77-0.91 with the lowest and highest values obtained at Bangoua and Gashiga respectively.

Unlike the fruits, the physical properties of the nuts (figure 4) were not highly dependent on the altitude of the sample site. This could be as a result of the varying average percentages of the pulp from one sampling site to the other. For example, Bangoua had the highest value of the average geometric mean diameter for fruits, but its average geometric mean diameter (42.80 mm) for the nut was lower than that obtained from Tchabal (44.29 mm). The varying percentages of the pulps between trees and between localities properly explained the low correlation coefficient observed between the geometric mean of the fruits and nuts. Apart from Bangoua, there was no significant difference (at the 95% confidence level) between the sphericities of the nuts harvested from the other 6 sites. The varying average percentages of the pulp from one locality to the other could still be used to explain this observation. The sphericity of the nuts was greater than 0.7 for more than 96% of the samples, so the nuts were essentially spherical in shape.

Looking closely at the data obtained from the field, it was observed that a relation could be defined between the major diameter of the fruit and the nut. Such a relation in future will serve in predicting the major diameter of the fruit when that of the nut is known. Thus a ratio of the major diameter of the nut to that of the fruit (x_{fn}) was empirically defined as

$$x_{fn} = \frac{x_f}{x_n} \quad [4]$$

where x_f and x_n are the major diameters of the fruits and nuts respectively. From field results it was observed that, an empirical relation between the major diameters of the fruits and nuts could be expressed in the form

$$x_f = (\mu + 1) x_n \quad [5]$$

μ was called the major diameter factor; a constant for converting from the major diameter of the nut to that of the fruit and vice versa. Substituting equation 5 in 4, then, μ is given by

$$\mu = x_{fn} - 1 \quad [6]$$

Equation 7 was therefore established to calculate the average μ value (μ_m) for all the samples from all the sampling sites where n_s and n_f were the total number of sampling sites and fruits respectively.

$$\mu_m = \frac{1}{n_s} \sum_{i=1}^{n_s} \left[\frac{1}{n_f} \sum_{i=1}^{n_f} (x_{fn} - 1) \right] \quad [7]$$

In this work, μ_m was found to be 0.33. When this value of μ_m was applied to equation 5 to estimate x_f from x_n , an average value of the Standard Relative Error (SRE) of deviation of the calculated from the experimental results of 10.74 % was obtained. An SRE value in the neighbourhood of 10 indicated that, equation 5 could be used to estimate x_f when x_n is known. Most often only the nuts are available due to the highly perishable nature of the pulp. Hence, the equation can be used to obtain major diameters of the fruits when that of the nut is known. This result might be of interest to stakeholders in the field for the design of processing equipments for opening up the pulp to obtain the nuts.

3.3. Calibration of the Fruits and Nuts

Using information from the literature (Olajide *et al.*, 2000; Tchankou Leudeu, 2002; Nkouam, 2002; Bup Nde, 2003; Womeni, 2004; Aviara *et al.*, 2005) ranges of some parameters for shea fruits and nuts that were encountered frequently were set as follows:

Fruits

$$35 < x \text{ (mm)} < 85; 30 < y \text{ (mm)} < 70; 0.57 < \omega < 1.00$$

Nuts

$$28 < x \text{ (mm)} < 57; 20 < y \text{ (mm)} < 46; 0.68 < \omega < 0.94$$

Given the wide ranges observed in the physical properties of the fruits and nuts as explained above, three classes were proposed (table 2) to describe three sizes of the fruits which protagonists in the field usually refer to as big, average and small fruits or nuts respectively. These sizes were referred to as Size I, Size II and Size III in this study. Through field discussions with local women and traders we were able to define the average class of the fruits and nuts from the sampling study.

Table 2. Proposed classification of shea fruits and nuts according to size

Fruits				Nuts		
Size	Major diameter (mm)	Geometric mean (mm)	Sphericity	Major diameter (mm)	Geometric mean (mm)	Sphericity
I	<35	<33	<0.70	<33	<25	<0.70
II	35-75	35-65	0.70-1.00	35-50	25-40	0.70-1.00
III	>75	>75		>50	>40	

Table 3 gives the classification of the nuts from various sampling sites according to the major diameters of the nuts. It was observed that, apart from Hina, each locality contained more than 68% of size II nuts. Size I nuts were practically absent in the highland areas (Deone, Tchabal and Banguoa) except at Foumban which contained 14.29% of size I nuts. Size III nuts were absent at Gashiga, Rabinga, Hina and Foumban. The highest proportion (15.94%) of size III nuts came from Tchabal. All these analyses suggested that size II nuts were predominant (77.25) in 6 of the 7 localities under study. From this analysis, it can be maintained that, while size II nuts can be found in all the sampling sites, size I fruits are common in Gashiga, Rabinga, Hina and to an extent Foumban and Size III nuts in Deone, Tchabal and Banguoa. Hence this group was considered for the continuation of the work.

Table 3. Proportions of major diameters of nuts from different sampling sites

Size	Gashiga	Rabinga	Hina	Foumban	Deone	Tchabal	Banguoa	Average
I	22.20	31.71	66	14.29	0.00	0.00	2.00	19.39
II	77.80	68.29	34	85.71	96.43	84.06	94.00	77.25
III	0.00	0.00	0.00	0.00	3.57	15.94	4.00	3.36

Table 3 shows the summary of the proportions (%) recorded for the proposed sizes (table 2) from all the sampling sites. It was observed that, considering the reported parameters, more than 93% of the fruits and 77% of nut were of size II. About 95% of the fruits and nuts had sphericities in the range 0.70-1.00 indicating that the fruits and nuts were essentially spherical in shape.

Table 4 Summary of the average proportions (%) of the various sizes of shea fruits and nuts from all the sampling sites

	Fruits			Nuts		
Size	Major diameter (mm)	Geometric mean (mm)	Sphericity	Major diameter (mm)	Geometric mean (mm)	Sphericity
I	0.73	3.01	3.76	19.39	7.14	3.10
II	93.43	95.00	96.24	77.25	85.35	96.90
III	5.84	1.99		3.46	7.51	

3.4. Principal Component Analysis of the Physical Properties

Principal Component Analysis (PCA) was carried out on the major (x), intermediate (y) and minor (z) diameters of the shea fruits and nuts and the altitude (Alt) of the sampling site (figure 5) to assess the validity of some of the assertions earlier stated above. The geometric mean, sphericity and area were left out because they were calculated from the former and as such were directly related. The PCA was performed on average values per tree in the localities studied. One important aspect of PCA includes determination of the number of fundamentally different properties called Principal Components (PC) exhibited by the data set (Njintang, Mbofung and Kesteloot, 2007) (Method?). As the first two PC (F1 and F2) generated from this analysis had eigenvalues >1 and accounted for 88 % of the total variance in the data set, these two axes were retained for describing the data. Figure 6 shows the similarity between the diameters of the fruits and nuts with the altitude. From this figure, it was clearly observed that the physical properties of the fruits and nuts from the same locality were regrouped together indicating some uniformity according to the sampling site. This was particularly true for sampling sites from the Adamawa and West regions. From figure 7, the properties of the fruits and nuts were divided into two larger groups irrespective of the locality. This consisted of the first group in which the trees

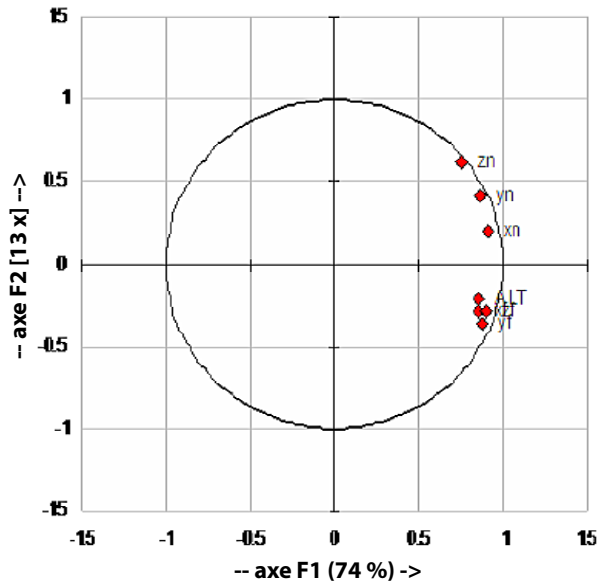
grew at high altitudes (1200-1500 mm) characterised by larger fruits and nuts and the second found at low altitudes (250-600 mm) characterised by small sizes of their fruits and nuts. The Pearson correlation coefficients table 5 obtained from the PCA analysis using the StatBox Version 6.40 (*Grimmer Logiciel (1999-2002) Paris, France*) between the major diameters of the fruits and nuts to the altitude were significant and positive confirming the assertion made earlier that there was a likely hood of finding larger fruits at high altitudes and vice versa. Hence altitude can be used as an indicator of the size of the fruit when planning for sampling.

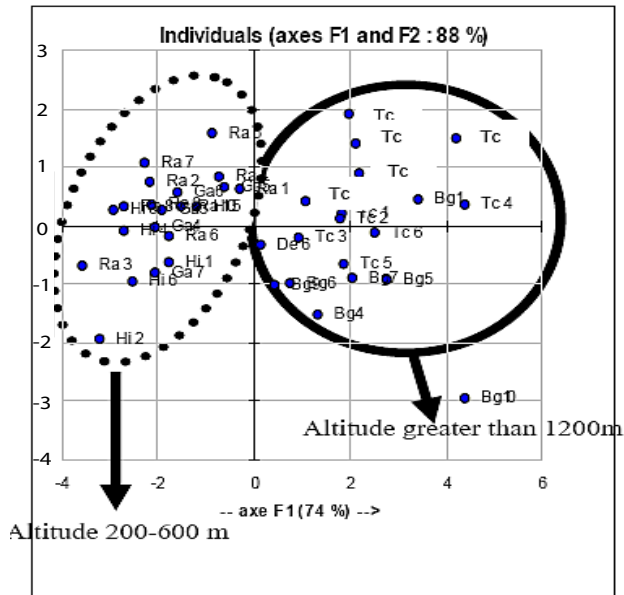
Table 5. Correlation coefficients between some parameters of shea fruits and nuts obtained from PCA analysis

	x _f	y _f	z _f	Altitude	x _n	y _n	z _n
x _f	1	0.77	0.76	0.75	0.83	0.56	0.45
y _f	0.77	1	0.97	0.76	0.65	0.65	0.46
z _f	0.76	0.97	1	0.77	0.70	0.69	0.54
Alt	0.75	0.76	0.77	1	0.71	0.67	0.50
x _n	0.83	0.65	0.70	0.71	1	0.84	0.76
y _n	0.56	0.65	0.69	0.67	0.84	1	0.88
z _n	0.45	0.46	0.54	0.50	0.76	0.88	1

3.4. Mass, Size and Shape of Fresh Sheanut Kernels

The results of the mass and dimensions of the fresh sheanut kernels from different trees harvested from Banguoa (West Region) and Tchabal (Adamawa Region) villages are presented on figures 8 and 9 (only results of some representative trees are shown). Analysis of variance indicated that there existed a significant difference ($p < 0.05$) in the mass and dimensions of the kernels from different trees. The mass of the fresh kernels, major, intermediate and minor diameters ranged from 10.2 ± 2.1 to 28 ± 6.1 g, 33.9 ± 3.2 to 45 ± 3.6 mm, 22.64 ± 1.9 to 33.9 ± 4.9 mm and 18.8 ± 2.4 to 30.5 ± 3.1 mm respectively. The Duncan multiple range tests grouped the physical properties of the kernels from different trees into at least five homogenous groups irrespective of the origin of the kernels. A similar observation was made with the geometric mean and sphericity of the kernels from the different trees studied. These parameters ranged from 24.7 ± 1.9 to 36.1 ± 3.5 mm and from 0.70 ± 0.05 to 0.841 ± 0.05 respectively.





Ra = Rabigha ; Hi = Hina, Ga = Gashiga, Tc= Tchabal, Bg= Banguoa, De = Deone.

Figure 7. Similarities by altitude (57 trees with seven parameters) as given by the PC.

The three diameters and the geometric mean were all positively correlated to the mass of the kernels at the 95% confidence limit (figure 8). The porosity, bulk density and true densities of the fresh kernels equally varied significantly between trees and between localities. From figure 9, the sphericity and the kernel densities were significantly ($P < 0.05$) and positively correlated to the porosities of the kernels. It is therefore established here that the mass, size and shape of the fresh kernel varied significantly between trees and between localities. These are important points to consider when planning experiments or when designing process equipments.

3.6. Solid Density and Bulk Density of Sheanut Kernel Slices

Sheanut kernels are large in diameter (up to 45 mm) and can only be effectively dried when divided into thin slices. The influence of moisture content on the solid and bulk density of sheanut kernel slices were determined so that they could subsequently be used in modelling the drying process of sheanut kernels slices dried as thin layers.

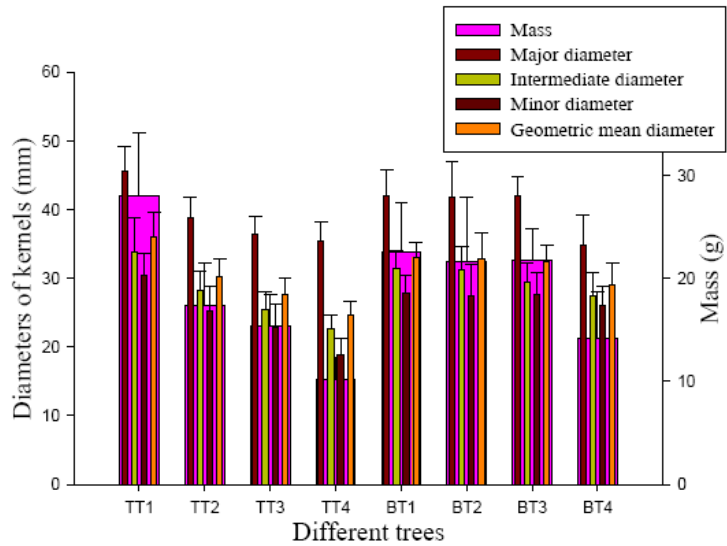
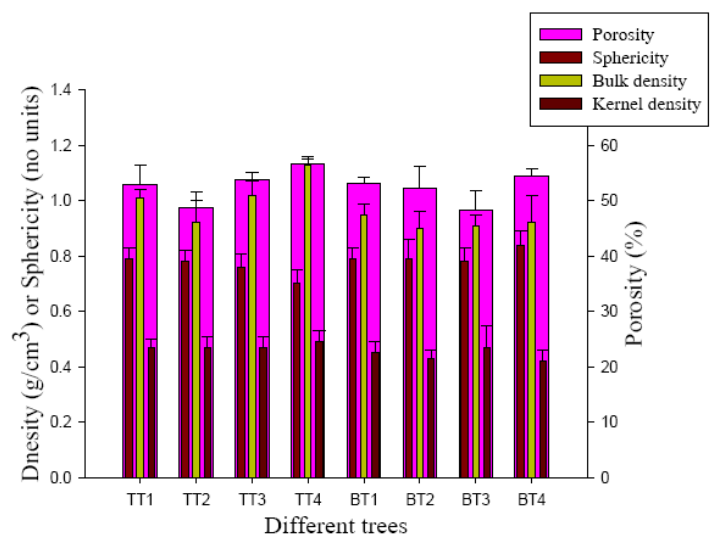


Figure 8. Diameters and mass of fresh sheanut kernels harvested from selected trees in Tchabal and Bangoua.



TTi and BTi represent trees from tree i in Tchabal and Bangoua respectively

Figure 9. Porosity, sphericity and densities of fresh sheanut kernels harvested from selected trees in Tchabal and Bangoua

3.6.1. Solid Density of Sheanut Kernel Slices

The solid density increased linearly during the drying period as the moisture content decreased from 150 to 5 % d.b. (figure 10). The solid density increased from 1.086- 1.160 g/cm³, 1.098-1.131 g/cm³ and 1.086-1.163 g/cm³ for the 5 mm, 10 mm and 15 mm slices respectively in the moisture range studied. The solid density for all the slices at all moisture contents was greater than unity, implying that the slices are heavier than water. This property can be very useful in the design of cleaning and separation equipments for the slices. No clear relation was established between solid density and particle size as the solid density was higher for the 10 mm thick slices at higher moisture content (150-100 % d.b.) compared to the 5 and 15 mm thick slices. The trends were reversed as the moisture content decreased below 100 % d.b. The variation of solid density with moisture content was modelled with a linear equation with the regression coefficients greater than 0.955 % and the SRE less than 5 %.

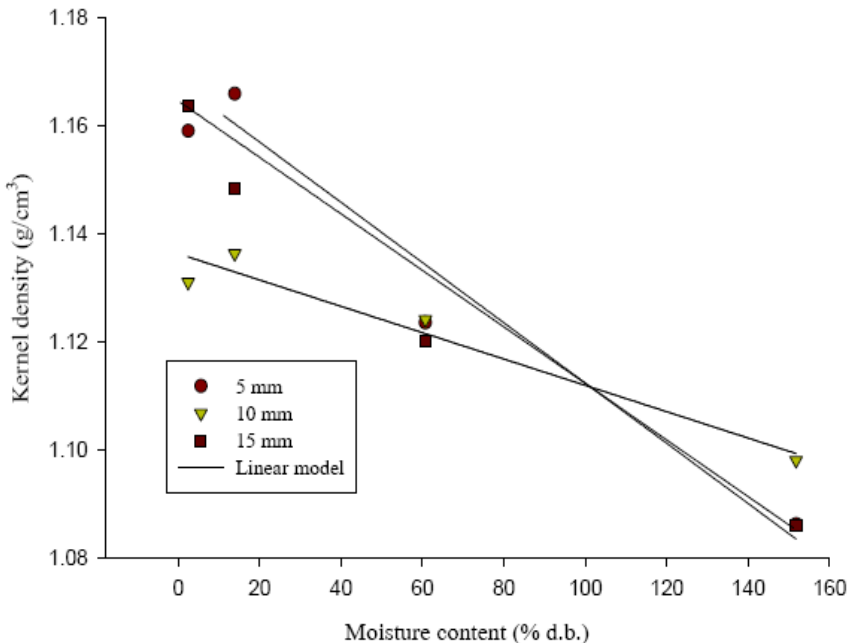


Figure 10. Influence of moisture content on the particle density of sheanut kernel slices.

3.6.1. Bulk Density of Sheanut Kernel Slices

Figure 11 shows the influence of moisture content on the bulk density of sheanut kernels slices. The bulk density of the sheanut kernel slices decreased non-linearly as the moisture content decreased from 150 to 5 % d.b. In the final stages of drying the bulk density slightly increased. The non uniform decrease of bulk density with moisture content could be due to the fact that the bulk volume and mass of the kernels might not have changed uniformly in the course of drying. The bulk density decreased from 0.435 to 0.374 g/cm³, 0.472 to 0.399 g/cm³ and 0.468 to 0.429 g/cm³ for the 5 mm, 10 mm and 15 mm slices respectively in the moisture range studied. From figure 10, it was equally observed that the bulk density was generally dependent on the particle size, increasing as the particle size increased.

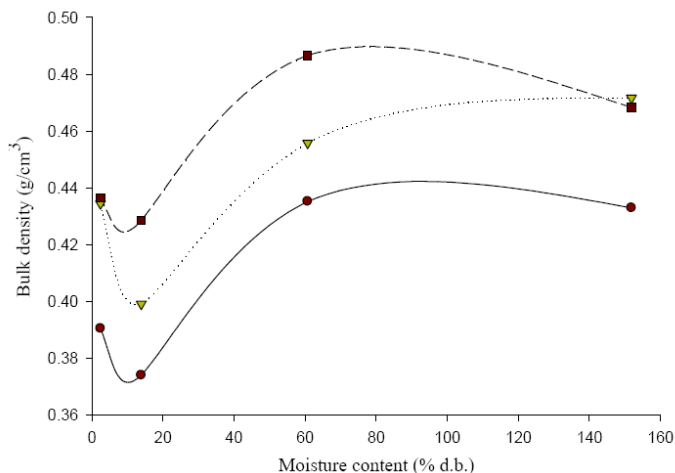


Figure 11. Influence of moisture content on the bulk density of sheanut kernel slices.

CONCLUSION

The objective to calibrate shea fruits and nuts using some physical properties from different shea producing areas of Cameroon was attained as it was found that size II nuts were predominant in six of the seven sampling sites studied. More than 77 % of the nuts from all the sampling sites had major diameters ranging from 40-45 mm. An empirical relation was established and validated for inter-converting between the major diameter of the fruits and nuts. This relation can be used to estimate major diameters of the fruits from

the nuts given that most often only the nut is available due to the highly perishable nature of the fruit pulp. Fruit size was dependent on altitude in which larger fruits were found at higher altitudes and smaller fruits at lower altitudes. Some physical properties of the kernel slices were influenced by moisture content and kernel thickness.

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Chapter 6

GROWTH, YIELD, HEAVY METALS, AND MICROORGANISMS IN SOIL AND FRUIT OF PECANS FERTILIZED WITH BIOSOLIDS

S.H. Tarang^{1,2} o Rivero and E. Orrantia Borunda^{*1}

¹Centro de Investigación en Materiales Avanzados

²Campo Experimental Delicias-INIFAP

Miguel de Cervantes #120, Complejo Industrial Chihuahua;
Chihuahua, México

ABSTRACT

The application of anaerobically digested biosolids as a nutrient source for the pecan *Carya illinoensis* (Wangh.) K. Koch, cultivar Western, during three years was evaluated. The bearing shoot grew 16% more and nut production per tree was 11.3% higher in the biosolid treatment, on a three-year average. The accumulation of As, Cd, Cr, Hg, Ni and Pb in soil due to biosolids was very low and according to the U.S. standard, the maximum allowable concentration would be reached in 34 years. Quantities of Cd, Cr, Ni and Pb in the kernel were below detection limits. As and Hg were found in very small quantities, and were below the limits allowed for nuts in the United Kingdom. During the preharvest, in soil fertilized with biosolids and in nuts which had contact with

* erasmo.orrantia@cimav.edu.mx.

biosolids, the presence of *Escherichia coli* and *Salmonella* sp. were not detected.

Keywords: *Carya illinoensis*, heavy metals, *Escherichia*, *Salmonella*.

INTRODUCTION

The pecan *Carya illinoensis* (Wangh.) K. Koch, cultivar Western, is the most important deciduous fruit tree in México, where 72,748 ha plantations are cultivated (SAGARPA 2007). During cultivation, fertilizer is the most expensive input to production and constitutes 20% of cultivation costs (Sparks 1991, FIRA 2005). In order for a pecan to grow and produce appropriately, it should be supplied with a balanced set of N, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn, B, Cl and Na nutrients (Sparks 1989, Smith 1991). In pecan orchards, conventional NPK fertilizer (CF) can be partially or completely replaced by biosolids (BS), a very low-cost by-product of wastewater treatment plants. BS are a valuable source of N, P, Zn, Cu, Mn, and organic matter. Incorporation of BS in soil improves the physical properties, biological activity, and fertility of the soil (Tester 1990, Sullivan 1998).

In Mexico, once the clean water regulations are fulfilled at a national level, around 650,000 t of dry BS will be produced each year (Jurado et al. 2004). In the state of Chihuahua, four wastewater treatment plants produce 2,930 t of dry BS per month (Flores 2007). If this by-product is not taken advantage of or recycled, depositing it in landfills will occupy space, attract rodents and vector insects; pollute the soil and subsurface mantles with organic compounds, pathogenic microbes, heavy metals, nitrates and/or salts, and the air with methane. The incineration of BS contributes to atmospheric pollution by emitting CO₂, dioxins, and metals (USEPA 1995, 1999). Nevertheless, applying BS to agricultural soils has risks which include the accumulation of heavy metals, nitrates and salts, and contamination by pathogenic organisms and parasites (McBride 1995, Chaney et al. 1992). All of this depends to a great extent on the quality of the BS; high-quality BS do not typically cause such problems (USEPA 1995). In the present study, BS were evaluated as an alternative nutrient source to inorganic fertilizer for pecans, which objective to determine the effect of biosolid application on heavy metal content and microorganisms in calcareous soil and in two tissues of the pecan fruit.

MATERIALS AND METHODS

This study was carried out in Delicias, Chihuahua; during 2004, 2005, and 2006, in the Rancho Trincheras orchard. The plant material was the cultivar Western of pecan, in production and eight years old at the beginning of the study. The trees are planted at a distance of 12x12 m and with each one having a 100 liters per hour microsprinkler for watering. The soil is of a crumbly, sandy texture, very poor in organic matter (0.34%), pH= 8.4 and with low salinity (CE= 0.87 dS m⁻¹).

Experimental design. A completely random design was used with four replicates per treatment. Each pecan tree was a repetition and each tree was assigned to each treatment according to the similarity of trunk diameter. The following treatments were evaluated:

- 1) 1). Conventional inorganic fertilizing. The formula 45-15-15 g/cm of trunk diameter was used. On March 15, 50% of the N and the entire P y K, and on May 15 the remaining 50% of the N were applied. Ammonium nitrate, monoammonium phosphate, and potassium nitrate were used as the source.
- 2) 2). Fertilized with BS. Biosolid material from the Northern plant of Chihuahua City was used. In order to calculate a biosolid dose equal to 45 g of N/cm of trunk diameter, the nutrient content and humidity were taken into account and a mineralization of 50% N was assumed the first year. The biosolid was spread in the sprinkling area and incorporated with a rake. Heavy metal analysis of the biosolids are given in Table 1. During the three years, the biosolid fertilizer dose was comparatively low. Trees of 8, 9, and 10 years of age received a dry-base dose of 2.41, 2.76 and 3.10 t/ha, respectively. The BS were classified as an “excellent type” for their low heavy metal concentration and as “class C” for their microorganism content the first and second years; they were classified as “class A” the third year (USEPA 1995, SEMARNAT 2002).

Variables: Growth and yield. The length of the bearing shoots was evaluated in June (one shoot per quadrant of pecan at a height of 1.5 m). In October, the yield was weighed (kg of nuts/tree).

Heavy metals (HM) concentration in soil. Each year and at the end of the vegetative cycle, soil samples were analyzed in order to measure its HM concentration.

Table 1. Heavy metals and microorganisms concentration of biosolids¹ used in this study, in three years

Element	2004	2005	2006
As mg/kg	12.0	15.4	nd
Cd	0.79	2.20	1.99
Cr	69.4	129.0	32.0
Hg	1.17	0.67	1.51
Ni	16.9	16.7	14.9
Pb	109.1	67.4	42.7
FC ² NMP/10 g	352,000	150,000	580
<i>Salmonella</i>	57	nd ⁴	nd
HE ³ org/10 g	0	0.7	0

¹From the northern treatment plant of Chihuahua City.

²FC= fecal coliform.

³HE= helmilth egg.

⁴nd= not detected.

Soil samples were taken at a depth of 0-30 cm at the center of the dripping zone of each tree; three compound samples were formed per treatment. The dry samples, sieved and homogenized at 100 mesh, were digested with HNO₃. In the case of Al, which is an inert element in its oxidized form, microwave assisted digestion was used. Al, Cd, Cr, Ni and Pb were determined by atomic absorption spectrometry (AAS). As and Hg were determined by the same technique and by using a hydride generator (AA-HG) as a sample introduction method.

HM concentration in plant tissues. Each year, during the harvest, 10 nuts per tree were gathered, and the shuck and its kernel were separated from each nut. Three samples composed of 40 kernels and 40 shucks per treatment were made. The shucks were dried in the shade and their outside surfaces were cleaned with a plastic bristle brush. They were ground with a Moulinex® grinder with stainless steel arms and a plastic cup and stored in wax-paper bags. The kernels were finely chopped with a stainless steel knife on a bond-paper-lined board and stored in wax-paper bags. The shucks and kernels were digested with HNO₃. Detection limits were estimated and Al, Cd, Pb, Hg, Cr, Ni, and As concentrations were determined by AAS. For the As and Hg analysis, the samples were introduced by the AA-HG method.

Microorganism determination in nut and soil. Each year, in October (before the harvest), two nuts from each tree were placed in the soil for two days, and put into sterilized glass jars (in an autoclave for 30 min at 120 °C) and hermitically sealed. The presence of *Escherichia coli* and *Salmonella* sp. affixed to the nut's shell and in the soil fertilized by BS was determined. The soil sample was taken at a depth of 0-3 cm in the center of the dripping zone of each tree. In both cases, three samples composed by treatment, in an area of the orchard 60 m away from the sites where biosolids were applied, were made. The analysis was done in accordance to the Official Mexican Standard NOM-114-SSA1-1994. For the preparation of each sample, 25 g of soil was weighed and placed in 225 mL of pre-enrichment medium (lactose broth); the nuts were washing directly in the lactose broth. They were incubated for 24 h at 35 °C. From the pre-enrichment broth, 1 mL was inoculated into test tubes with 10 mL of enrichment broth for *Salmonella* (tetrathionate broth, Rappaport-Vassilidis, and selenite-cystine). A solution of iodine-iodide and brilliant green at 0.1% was added to the tetrathionate broth. The solution was incubated for 24 h at 35 °C. Afterwards, the tubes were shaken and the broth was streaked by cross stria into selective media plates (brilliant green agar, Hektoen enteric agar, *Salmonella*-*Shigella* agar, bismuth sulfite agar, and XLD agar). From each plate two suspicious colonies were selected and were put through biochemical tests (TSI, MIO, LIA, and urea broth) and were incubated for 24 h at 35 °C. At the same time, a tube with blood agar base (BAB) was inoculated. Serologic identification was done by using polyvalent *Salmonella* anti-serum in tests that showed characteristic reactions. For *E. coli*, an aliquot of the pre-enrichment broth was streaked by cross stria with Eosin methylene blue agar (EMB) with MacConkey medium, and then were incubated for 24 h at 35 °C. Two suspicious colonies were chosen from each plate and were inocuated in biochemical tests (TSI, MIO, LIA, urea broth, Simmons citrate, malonate, and RM-VP), and were incubated for 24 h at 35 °C, except for the RM-VP which was incubated for 48 h. Colonies that showed biochemical reaction characteristic of *Escherichia coli* were identified.

Statistical analysis. The data were analyzed by a t-test. When the heavy metals analysis in plant tissue and soil determined that an element was "not detected", it was given a value of zero for the variable analysis. Data of elements with values less than 1 and those which included zeros were transformed through the equation $\sqrt{X+0.5}$ before statistical analysis (Steel y Torrie 1985). The statistical packet SAS 8.2 was used (SAS Institute 2001).

RESULTS AND DISCUSSION

Growth and Yield

In two out of three years and in the final average, pecans amended with BS had a bearing shoot size significantly higher than that of the fertilized trees; on the average, the shoot of pecans under the organic amendment grew 16% more (Table 2). Because the shoot growth phase is very short on bearing pecans, adequate supply of nutrients has a determinant effect on their vigor (Marquard 1980). This means that the biosolid mineralization is enough to provide the nutrients demanded by the short but intense elongation period of the bearing shoot. The organic amendment favored shoot growth within the range for maximum productivity in the Western variety, which is between 15 and 30 cm length (Storey 1990).

Nut production was statistically alike between pecans amended with BS and those receiving fertilizer (Table 3); nonetheless, yield was always bigger in the first group, a 11.3% more on the annual average. In an important way, annual productivity of a pecan tree depends on adequate N supply during the phenological phases of shoot growth and kernel filling (Wood 2002). From the above it can be deduced that BS incorporated into a orchard soil can provide enough nutrients, particularly N, during the critical stages of pecan development to sustain an adequate production of pecan nuts every year.

HEAVY METALS IN SOIL

Table 4 shows the degree of heavy metals accumulation with the most potential for toxicity to plants and herbivores after 3 years of applying BS. It was found that the accumulation of HM was a slow process, due to the fact that the BS used had a low HM content (Table 1) and the relatively low dose at which it was applied. The annual median accumulation rate (mg/kg of soil) due to the biosolid fertilizer was: As 1.75, Cd 0.04, Cr 8.1, Hg 0.05, Ni 0.06 and Pb 4.3. With this data and using the strictest standard as a reference, which is from the European Community (McGrath et al. 1995), the number of years that it would take to reach the maximum allowable concentration (MAC) in agricultural soils for each HM would be: Cd 25-75, Cr 12.3-18.5, Hg 20-30, Ni 500-1,250 and Pb 11.6-69.7.

Table 2. Bearing shoot length (BSL) of pecans fertilized with NPK fertilizers and with biosolids during three years. Delicias, Chihuahua

Treatment	BSL (cm)			Average ¹
	2004	2005	2006	
Fertilizers	16.7	19.3	12.7	16.2
Biosolids	22.7	23.4	11.9	19.3
Pr>F	0.006	0.012	0.519	0.016

¹Of all observations in three years.**Table 3. Nut yield of pecans fertilized with NPK fertilizers and biosolids during three years. Delicias, Chihuahua**

Treatment	kg/tree			Average ¹
	2004	2005	2006	
Fertilizers	4.39	11.15	9.56	8.37
Biosolids	4.99	12.91	10.41	9.44
Pr>F	0.597	0.349	0.682	0.504

¹Of all observations in three years.**Table 4. Heavy metals concentration in soil (0-30 cm) of pecans fertilized with NPK fertilizers and biosolids during three years**

Treatment	mg/kg					
	As	Cd	Cr	Hg	Ni	Pb
<i>2004¹</i>						
Fertilizers	2.6	nd ²	24.5	0.39	6.8	22.0
Biosolids	2.7	nd	25.6	0.23	7.2	23.9
Pr>F	0.886	--	0.870	0.096	0.662	0.627
<i>2005</i>						
Fertilizers	0.74	0.44	6.9	0.01	6.6	10.6
Biosolids	0.80	0.56	8.1	0	8.0	16.6
Pr>F	0.013	0.105	0.057	0.237	0.0002	0.129
<i>2006</i>						
Fertilizers	5.2	nd	39.4	nd	nd	4.7
Biosolids	10.1	nd	61.4	nd	nd	9.8
Pr>F	0.095	--	0.027	--	--	0.332

¹At the end of each cultivation cycle.²nd= not detected.

If the reference were the U.S. standard, the time needed to get to the MAC is much longer: Cd 500, Cr 185, Hg 160, Ni 3,500 and Pb 34.8.

In accordance to the European standard, Cr and Pb are the elements that would limit safe use of BS in the short-term (12 years). However, in the alkaline soils of Chihuahua's pecan region both metals would precipitate as barely-soluble compounds and would be adsorbed by clay and organic matter (Davies and Jones 1992, Rostagno and Sosebee 2001). Furthermore, Pb is practically immobile in the root (Sommers and Barbarick 1990). Since only a third of the pecan's feeding roots are localized in the cultivable layer in truly-textured soil (Worley et al. 1974), and even fewer in sandy soil (Brison 1976), such conditions would reduce the absorption and effect of these HM in pecan trees.

Based on the results for 2004, Hg and Cd could accumulate to their MAC in the medium-term (20 to 25 years); however, since these metals were detected only in one out of the three years and since in a calcareous soil, like the orchard in the present study, Hg would precipitate as a barely-soluble hydroxide or carbonate (Davies and Jones 1992), which would reduce its availability to plants; moreover, this element practically does not move in the root (Sommers and Barbarick 1990). Cd is very mobile in soil and is easily absorbed by plants (Breckle 1991, Menzer 1991), although this process is antagonized by Mn, Fe, organic matter, and most importantly Zn and phosphate content (Mengel and Kirkby 1979, Allaway 1986).

Niquel was a common element in the soil, even in treatments with CF. BS hardly contributed this HM. It is the metal that would require the longest in order to reach a concentration limit in soil. In regards to As, U.S. and European standards do not establish a MAC, although in Argentina the limit is 20 mg/kg of soil (Lavado and Taboada 2002). This metalloid was also common in the soil, and because of its contribution by BS, the element's limit would be reached in just 11.4 years, according to the Argentinean standard. In calcareous soils, Precipitates in barely-soluble forms (Davies and Jones 1992).

Treatments with CF also show the presence of HM in the soil, which occurs because of the contribution of synthetic fertilizers (especially Cd, Ni, and Pb) and most of all, phosphates (Colomer and Sánchez 2000).

HEAVY METALS IN PLANT

HM accumulation in edible parts of plants is important because they are the entrance into the food chain (Chaney and Giordano 1986). HM content in

pecan fruit tissues fertilized with CF and BS are shown in Tables 5 and 6. During the first year of the study, it was found that CF and BS furnish HM to the shuck and kernel. In general, BS tend to increase HM concentration in both tissues, although a statistically significant difference only occurred in the shuck for Ni and Pb (Table 5).

Table 5. Heavy metals concentration in pecan fruit shuck fertilized with NPK fertilizers and biosolids during three years

Treatment	mg/kg					
	As	Cd	Cr	Hg	Ni	Pb
			<i>2004¹</i>			
Fertilizers	0.07	0.16	2.0	0.2	0.22	0
Biosolids	0.32	0.08	16.0	0.43	11.8	0.49
Pr>F	0.167	0.512	0.072	0.612	0.010	0.004
			<i>2005</i>			
Fertilizers	0.05	nd	0	0.39	nd	nd
Biosolids	0	nd	0.63	0.28	nd	nd
Pr>F	0.373	--	0.373	0.587	--	--
			<i>2006</i>			
Fertilizers	0.11	nd	0.16	0.42	0.22	nd
Biosolids	0	nd	0	0.67	0.93	nd
Pr>F	0.373	--	0.373	0.602	0.281	--

¹At the end of each cultivation cycle.

²nd= not detected.

Apparently, the shuck does not function as a tissue-filter, which accumulates metals to reduce their passage into the kernel. In fact, a high As content in the edible part of the nut was determined. Cd was similar in both tissues and Cr and Hg higher in the shuck.

During the first year, As concentration was unchanged and Cr almost unchanged in the kernel when fertilized with CF and BS, while Hg was practically not detected in both treatments. Cd in treatments with BS was 0.23 mg/kg, much below the maximum 5 mg/kg that a plant food can have in order to be considered safe (Chaney *et al.* 2001).

Table 6. Heavy metals concentration in the kernel of pecan fruit fertilized with NPK fertilizers and biosolids during three years

Treatment	mg/kg					
	As	Cd	Cr	Hg	Ni	Pb
<i>2004¹</i>						
Fertilizers	1.02	0.01	0.21	0.01	3.6	8.4
Biosolids	1.07	0.23	0.34	0	5.3	12.0
Pr>F	0.956	0.202	0.706	0.373	0.521	0.609
<i>2005</i>						
Fertilizers	0.04	Nd	nd	0.75	nd	nd
Biosolids	0.24	Nd	nd	0.81	nd	nd
Pr>F	0.107	--	--	0.683	--	--
<i>2006</i>						
Fertilizers	0.37	Nd	0	0.11	nd	nd
Biosolids	0.18	Nd	0.22	0	nd	nd
Pr>F	0.445	--	0.373	0.373	--	--

¹At the end of each cultivation cycle.

²nd= not detected.

Ni reached 5.3 mg/kg when BS were applied, 47% more than with CF. Pb was 12 mg/kg for treatments with BS, barely 3.6 mg/kg more than CF. In the United Kingdom (FSA 2007), the MAC for fruits, vegetables, and nuts is (in mg/kg): As 1.0, Cd 0.05, and Pb 0.1-0.2. According to these levels, As concentration in the kernel was within the U.K. limit with CF as well as BS. Cd concentration was high with CF and very high with BS, and with Pb it was very high with both fertilizers.

A significant accumulation of Cd in plant tissue has been found to occur when high doses of BS are used (100 to 224 t/ha); which is equivalent to 10-25 times more fertilizer than what is applied to a orchard of pecan trees in production. In several studies in the medium-term, addition of biosolids has not increased Hg concentration in corn tissues *Zea mays* L. turnip *Brassica napus* L., and carrot *Daucus carota* L., but it has increased it in tomato fruit *Lycopersicon esculentum* Mill. (Allaway 1986, Chaney and Giordano 1986). Applying BS at 10 t/ha for 5 years (Oliveira *et al.* 2005) or incorporating BS at 30 and 60 t/ha (Cuevas and Walter 2004) did not significantly increase HM concentration in corn shoots and grains. This suggests that the low dose of the “excellent type” of BS applied to pecans in the first year, equivalent to 2.41 t/ha, should not increase HM concentration compared to treatments with CF.

During the second year, when the dose applied was 2.76 t/ha, the Cd, Cr, Ni, and Pb concentration in the shuck and kernel were below detection limits. As was only detected in the kernel, in treatments with CF as well as BS, in a quantity that was lower than the limit allowed by the FSA (2007). Hg was found in both tissues, and in the kernel the median concentration of treatments with BS barely was 0.06 mg/kg higher than with CF. In all cases there was no statistical difference (Tables 5 and 6). During the third year, the amount of BS applied was equivalent to 3.10 t/ha, and again Cd, Cr, Ni, and Pb were not detected in the shuck or the kernel; As was found in the kernel, but in higher concentrations in treatments with CF. The 0.18 mg/kg of As in treatments with BS are very much under the 1.0 mg/kg that is allowed by the FSA (2007) for nuts. Hg was not detected in the kernel, and in the shuck it was almost equal in both types of fertilizer. The results of the second and third years, in an accumulative effect condition due to the repeated application of BS, indicate that the heavy metal concentrations that biosolid fertilizers contribute to the kernel are not higher than what CF contribute; in the short-term.

MICROORGANISMS IN SOIL AND NUT

During the first two years, soil analysis before the nut harvest did not detect the presence of *Escherichia coli* or *Salomonella* sp. During the third year, with both treatments, the presence of *E. coli* was found, but not *Salmonella* sp. It is considered that *E. coli* did not come from the BS, but that it is due to an external contamination problem, given that it was also detected in the CF treatment and in a separate point 60 m from the trees that were treated with BS (Table 7). This illustrates how other activities in pecan orchards, like the defecation of workers among the trees or the presence of wild fauna (Zaleski *et al.* 2005), can cause soil contamination with microbial pathogens. This fact has been reported by Montes *et al.* (2004), who found coliform bacteria on the order of 300 NMP/g in different agricultural soils without the contribution from wastewater or BS.

To a great extent, not detecting pathogenic bacteria in the preharvest can be explained. When fertilizing with biosolids, most survival of such microbes (most importantly *Salmonella* and fecal coliform) on the surface soil is short-term, given that the microbes are destroyed by heat, drying, ultraviolet light, and antagonistic microorganisms (Menzies 1986, Sommers and Barbarick 1990, Epstein 2001). The antagonism between microbial groups is especially intense.

Table 7. Presence of bacteria on the surface of soil (0-3 cm) and in nut's shell of pecans fertilized with NPK fertilizers and biosolids during three years, and also during the preharvest, seven months after biosolid application

Treatment	DTB ¹ (m)	<i>Escherichia coli</i>		<i>Salmonella</i> sp.	
		Soil	Shell	Soil	Shell
<i>2004</i>					
Biosolids	0	- ²	na ⁴	-	na
Fertilizers	12	-	na	-	na
Orchard ³	60	-	na	-	na
<i>2005</i>					
Biosolids	0	-	-	-	-
Fertilizers	12	-	-	-	-
Orchard	60	-	-	-	-
<i>2006</i>					
Biosolids	0	+	-	-	-
Fertilizers	12	+	-	-	-
Orchard	60	+	-	-	-

¹DTB= distance of trees fertilized with biosolids.

² -Not detected, +Detected.

³Orchard= trees from another zone of the orchard, as a reference.

⁴na= not available.

An *E. coli* population is greatly reduced by the soil's native microflora (Jiang *et al.* 2002), were it can be eliminated quickly by the endoparasitic bacteria *Bdellovibrio bacteriovorus* (Martin y Focht 1986). Upon incorporating BS into the soil, in the first two weeks the density of heterotrophic bacteria show a vigorous increase and *Salmonella* spp. and *E. coli* are significantly reduced (Zaleski *et al.* 2005). In their own right, the soil's native fungi can eliminate the fungi that come from BS (Kinsbursky *et al.* 1989).

The presence of bacteria in the nut's shell after two days in contact with the soil and BS residues was also analyzed (simulating the process of a commercial harvest). This is an important variable given that the main infection pathway by biosolid pathogens is through ingestion, upon consuming fruits in contact with the fertilizer (Epstein 2001). During the two sample

years, *E. coli* or *Salmonella* sp. associated with the nut's shell was not detected in any treatment (Table 7). A valid answer is the fact that, at least on the surface of the soil, there is no evidence that pathogens that come from BS survive after seven months of application. Through this mechanism, in a orchard where BS are applied, the nut should not have microbial contamination problems from the fertilizer.

CONCLUSIONS

The application of biosolids in doses of 2.41, 2.76, and 3.10 t/ha in dry base, to pecan 8, 9, and 10 years old, respectively, allowed the trees to grow and produce as the fertilizer-treated trees. The biosolids did not increase toxic heavy metals content of soil or fruit. No coliforms and *Salmonella* sp. were detected in the soil and nut shell at fall harvest time when biosolids application was done in the spring.

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Chapter 7

ARECA NUT MAY KILL CELLS IN A DIFFERENT WAY

***Mei-Huei Lin,^{1,2,*} Shyun-Yeu Liu,^{2,3,4,#}
and Young-Chau Liu^{2,5,†}***

¹Department of Biotechnology, Chia Nan University of Pharmacy, Tainan;

²Department of Medical Research, Chi Mei Medical Center, Tainan;

³Department of Dentistry, National Defense Medical Center, Taipei;

⁴Department of Dentistry, Taipei Medical University, Taipei;

⁵Division of Natural Science, College of Liberal Education, Shu-Te
University, Kaohsiung County, Taiwan, ROC

ABSTRACT

Areca nut (AN, *Areca catechu* L.) is a popular but carcinogenic chewing material used by approximately 200–600 million people

* The first author: Dr. Mei-Huei Lin, 60, Erh-Jen RD., Sec.1, Jen-Te, Department of Biotechnology, Chia Nan University of Pharmacy and Science, Tainan, Taiwan, ROC.
E-mail: mhlin3224@mail.chna.edu.tw

Dr. Shyun-Yeu Liu, Chi Mei Medical Center, No. 901, Chung Hwa Road, Yong Kang City, Tainan, Taiwan (R.O.C.); Tel: 886-6-2812811, ext. 52669; Fax: 886-6-2832639; E-mail: syliu@mail.chimei.org.tw.

† Correspondence to: 1. Dr. Young-Chau Liu, Division of Natural Science, College of Liberal Education, Shu-Te University, No. 59, Hengshan Road, Yanchao, Kaohsiung County, 82445 Taiwan (R.O.C.); Tel: 886-7-6158000, ext. 4210; Fax: 886-7-6158999; E-mail: god15539@stu.edu.tw.

worldwide. In the past few decades, AN has been discovered to possess genotoxic, cytostatic, and cytotoxic effects on cells. Some ingredients of AN, such as AN extract (ANE), arecoline, hydroxychavicol, and oligomeric procyanidins were demonstrated to stimulate apoptotic and/or growth arresting phenotypes in treated cells. However, our recent studies showed that ANE predominantly induces the autophagic responses, albeit the simultaneous initiation of apoptotic pathway. This finding may renew the knowledge about the cytotoxic effects of AN on oral cells in physiological conditions.

Betel quid (BQ) is a psychoactive and addictive substance with about 200–600 million chewers worldwide[1,2]. Constituents of BQ include areca nut (AN, *Areca catechu* L.), lime, inflorescence of *Piper betle* Linn., and *Piper betle* leaf. The recipe of BQ varies among different countries or areas; however, areca nut (AN) is the major and unexpendable component. This popular chewing material commonly gives the user a sense of euphoria, warm sensation, and increased alertness, and stimulates sweating, salivation, palpitation, and higher working capacity[3]. However, both BQ and AN are thought to cause oral diseases including oral submucous fibrosis, premalignant lesions, and oral cancer[4]. After decades of study, they have been recognized as group I human carcinogens by the International Agency for Research on Cancer[5]. Therefore, unraveling the biological effects of BQ components on cells is worthwhile and important.

ANE and arecoline were firstly noticed to arrest the progression of cell cycle in oral KB epithelial cells; however, longer exposure to arecoline stimulates the apoptotic program[6-8]. Arecoline-induced growth arrest is subsequently shown to be p53-dependent in rat hepatocytes[9], whereas ANE-induced apoptosis of murine splenocytes is mitochondrial pathway- and oxidative stress-dependent[10]. It is also shown that ANE induces G1 phase arrests and senescence-associated phenotypes in normal human oral keratinocyte, whereas arecoline arrests cells at prometaphase by deregulating mitotic spindle assembly and spindle assembly checkpoint[11,12].

Similar cytostasis- and apoptosis-inducing effects of another AN ingredient, hydroxychavicol, on KB cells was also observed, and reactive oxygen species (ROS) are further shown to be required for the cytotoxicity of hydroxychavicol[13,14]. Recently, highly oligomeric procyanidins from AN is found to induce lymphocyte apoptosis through the depletion of intracellular thiols[15].

As more apoptosis-inducing AN ingredients are being found, it is reasonable to speculate that chewing AN may cause oral cell apoptosis. However, when we treated cells with ANE, they became swollen and contained nearly empty cytoplasm and shrunken nucleus. These features are quite different from those of apoptosis, and proven to be autophagy by showing the cleavage of type I microtubule-associated protein 1 light chain 3 (LC3-I) into LC3-II and the emergence of acidic vesicles and autophagic vacuoles. Furthermore, this autophagy-inducing AN ingredient (AIAI) is traced to the partially purified 30–100 kDa fraction of ANE (designated as ANE 30–100K), which contains mainly carbohydrates with less than 2% proteins[16, 17]. Both ANE and ANE 30–100K stimulate about 90% increase of acidic vesicle-containing cells in oral carcinoma cells[16], as well as in peripheral blood lymphocytes from healthy donors (our unpublished data). These results suggest that AIAI may exert a dominant autophagy-inducing effect in the presence of apoptosis-inducing ingredients in AN. Note that although most, if not all, ANE- and ANE 30–100K-treated cells die in an autophagic manner, execution of apoptotic pathway, such as caspase-3 activation and micronucleation, is also observed[16].

A recent research has further illustrated that ANE may stimulate autophagy through the induction of oxidative stress and upregulation of hypoxia inducing factor[18]. Collectively, the identification of autophagy-inducing capacity of AN provides a new insight into the AN-mediated cytopathology.

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