ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS OF RAW AND PROCESSED CASHEW NUTS

CHANDRASEKARA MUDIYANSELAGE NEEL RANJITH CHANDRASEKARA

Antioxidant Activity and Phenolic Compounds of Raw and Processed Cashew Nuts

By

© CHANDRASEKARA MUDIYANSELAGE NEEL RANJITH CHANDRASEKARA,
B.Sc., M.Sc.

A thesis submitted to the School of Graduate Studies in partial fulfilment
of the requirements for the degree of
Master of Science

Department of Biology
Memorial University of Newfoundland

December 2010

ST. JOHN'S NEWFOUNDLAND & LABRADOR CANADA

This work is dedicated to
the memory of my late father

Abstract

Roasting of cashew (*Anacardium occidentale* L.) nut is a commonly used processing method to improve their texture, colour, flavour and appearance. The effect of roasting on the content of phenolic compounds, antioxidant activity, and antiradical properties of cashew nuts and testa were studied. Raw whole cashew nuts were roasted at low temperature (LT; 70^0 C for 6 hours) and high temperature (HT; 130^0 C for 33 min). Raw and roasted whole cashew nuts, kernels and recovered testa were used to extract soluble phenolic compounds under reflux conditions with 80% (v/v) ethanol. The residues were used to extract insoluble bound phenolics at room temperature by alkaline hydrolysis under nitrogen. The antioxidant activity was measured using several chemical assays as well as food and biological model systems. Cashew nut oils extracted from raw and roasted whole cashew nuts were examined for their fatty acid composition, colour change and oxidative stability. The results showed that the highest antioxidant activity was achieved when nuts were roasted at HT. The contents of soluble and insoluble bound phenolic of raw, LT and HT roasted cashew nuts and testa ranged from 1.14 ± 0.43 to 348.99 ± 6.88 and 0.03 ± 0.01 to 4.53 ± 0.12 mg of gallic acid equivalents (GAE) per g of defatted meal, respectively. Roasting increased the total phenolic content while decreasing that of the proanthocyanidins. Phenolic acids, namely syringic, gallic and *p*-coumaric acids were identified in soluble extracts of raw, LT and HT roasted cashew nuts and testa by high performance liquid chromatography (HPLC) and amongst which syringic acid was the predominant one. Flavonoids, namely catechin, epicatechin and epigallocatechin were also identified and their contents increased with increasing temperature.

The results of the present study showed that HT roasting effectively enhanced antioxidant activity of cashew nuts and testa. The analysis of fatty acid composition showed that oleic acid was the major monounsaturated (MUFA) fatty acid. Roasting of whole cashew nuts improved the oxidative stability of nut oil during storage. The results suggest that whole cashew and testa could be used as a potential source of natural antioxidants in food applications and for health promotion.

Acknowledgements

I wish to express my sincere gratitude to Dr. F. Shahidi for providing invaluable guidance and financial support throughout my degree programme. My thanks are extended to the supervisory committee members, Dr. R. Hooper and Dr. S. Debnath for their guidance and valuable suggestions. My sincere gratitude goes to the School of Graduate Studies for financial assistance I received throughout my programme. I also wish to extend my thanks to the Department of Biology for the assistance extended. My sincere gratitude goes to Mr. Kapila Wellapuli, General Manager, Green Field Bio Plantation (Pvt.) Ltd., 49 ½, Bray brook Street, Colombo2, Sri Lanka of providing me with cashew samples. I also wish to acknowledge members in Dr. Shahidi's lab for creating a friendly environment to conduct my work. My special thanks are due to colleagues, and my family for their support extended during the completion of this work.

Table of contents

List of figures

List of tables

List of abbreviations

AAPH	2,2`-azobis-(2-methylpropionamidine)dihydrochloride
$ABTS^{\cdot}$	2,2`-azinobis-(3-ethylbenzthiazoline-6-sulphonate) radical anion
ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EPR	Electron paramagnetic resonance
Eq	Equivalents
FL	Fluorescence
hLDL	Human low density lipoprotein
HPLC	High performance liquid chromatography
LDL	Low density lipoprotein cholesterol
MAD	Malonaldehyde
$ORAC_{FL}$	Oxygen radical absorbance capacity
PBS	Phosphate buffered saline
PF	Protection factor
ppm	Parts per million
PUFA	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SAS	Statistical analysis software
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid-reactive substances
TBHQ	*tert*-butylhydroquinone
TEAC	Trolox equivalent antioxidant capacity
TPC	Total phenolic content
USDA	United States Department of Agriculture

Chapter 1

Introduction

Tree nuts are known to serve as a nutritious food source with a high lipid content. They have been shown to lower total and LDL cholesterol levels significantly when replacing half of the daily fat intake in human subjects (Abbey *et al.*, 1994). Many authors have also reported the blood cholesterol lowering effects of tree nuts (Etherton *et al.*, 1999; Kendall *et al.*, 2002; Shahidi *et al.*, 2007). In 2003, the US Food and Drug Administration (FDA) recommended a qualified health claim stating that consumption of 1.5oz (42g) per day of most tree nuts may reduce the risk of heart disease. Dreher *et al.* (1996) also showed a connection between regular nut consumption and the decreased incidence of coronary heart disease. Nuts are rich sources of unsaturated fatty acids, protein, micronutrients, vitamins, and phytochemicals (Rainey & Nyquist, 1997) with different proportions and contents among nuts. Walnuts are a good source of both antioxidants and the omega-3 fatty acid, α-linolenic acid, at a higher level than other nuts such as almonds, pecans, and pistachios (Bravo, 1998). Almonds are considered as a rich source of vitamin E and magnesium (Etherton *et al.*, 2002). Brazil nuts are particularly rich in the antioxidant compounds, while pecans are rich in bone-building manganese (Bravo, 1998; Etherton *et al.*, 2002; John & Shahidi, 2010). Peanuts are good sources of folic acid and contain considerable amount of phytochemicals, including phenolics (tannins and ellagic acid), flavonoids (luteolin, quercetin, myricetin, kaempferol, and resveratrol), isoflavones (genistein and daidzein), terpenes, organosulphur compounds, and vitamin E (Bravo, 1998; Etherton *et al.*, 2002).

Dietary antioxidants provide protection against oxidative attack by decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals, binding of metal ion catalysts, decomposing primary products of oxidation to non-radical compounds, and chain breaking to prevent continuous hydrogen removal from substrates (Shahidi, 1997). A growing interest in biology and medicine has been focused on oxidative stress from the viewpoint of its participation in several diseases such as arteriosclerosis (Carney *et al.,* 1991), cancer (Palinski *et al.,* 1989), and ageing (Ames *et al.,* 1993). Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidation chain reactions (Velioglu *et al.,* 1998) and scavenging of free radicals. Food such as fruits, vegetables, nuts, and grains are reported to contain a wide variety of antioxidant components that include phenolic compounds. The presence and content of these compounds are found to correlate with antioxidant potential (Katalinic *et al.*, 2004). Upon addition to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and extend shelf life (Jadhav *et al.,* 1996).

Synthetic antioxidant such as, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT), are largely used by the food industry. However, possible toxicity as well as general consumer rejection of synthetic products has led to their decreased use. Much work has been done to find safe and potent natural antioxidants from various plant sources (Namiki, 1990). As one potential source, plant phenolics serve as primary antioxidants (Shahidi & Wanasundara, 1992). Dietary components from different plant sources, including phenolic acids, flavonoids, carotenoids and vitamins C and E, are

effective in the prevention of oxidative stress and related diseases (Kaur & Kapoor, 2001; Moure *et al.,* 2001).

Phenolic compounds constitute one of the largest, most abundant and widely distributed group of substances in the plant kingdom. Phenolics are products of the secondary metabolism of amino acids phenylalanine and to a lesser extent tyrosine in plants. External stimuli such as microbial infection, wounding, ultraviolet radiation, and chemical stressors induce their synthesis (Shahidi & Naczk, 2004). They contain aromatic rings bearing one or more hydroxyl groups together with a number of other substituents (Shahidi & Naczk, 2004). In plants, phenolics may act as phytoalexin, antifeedant, attractant for pollinators, and contributor to plant pigmentation, antioxidant, and protective agents against UV light, among others. In food, phenolics may contribute to the bitterness, astringency, colour, flavour, odour, and oxidative stability of foods (Shahidi & Naczk, 2004). In addition to their role in plants, several epidemiological and clinical research findings have demonstrated that phenolic antioxidants occurring in cereals, fruits, nuts, and vegetables are principal contributing factors for the decreased incidence of several chronic diseases (Shahidi, 2000). Earlier interest in phenolic compounds was concentrated on the deleterious effects caused by the ability of certain phenolics to bind and precipitate macromolecules, such as dietary proteins, carbohydrates, and digestive enzymes, thereby reducing food digestibility. Recent interest, however, in food phenolics has increased gradually because of the antioxidant and free radical scavenging abilities associated with some phenolics and their potential effects on human health (Bravo, 1998). Natural phenols can range from simple molecules, such as phenolic acids, to highly polymerized compounds, such as

proanthocyanidins also known as condensed tannins. Depending on their basic chemical structure, phenols can be divided into different classes such as flavonoids, phenolic acids, hydroxycinnamic acid derivatives, and lignans, among others (Bravo, 1998).

Cashew (*Anacardium occidentale* L.) is one of the most important tree nuts and ranks third in the international trade after hazelnut and almond (Mandal, 2000). Cashew nut shell liquid, a byproduct obtained during the processing of cashew nuts, is reported to possess antioxidant activity (Singh *et al.,* 2004). The kernel of cashew nut which is valued in trade is covered with a thin reddish-brown skin called testa. The testa has been reported to serve as a good source of hydrolysable tannins (Pillai *et al.,* 1963) with catechin as a major polyphenol (Mathew & Parpia, 1970).

In processing of cashew nut, they must be roasted or cooked in steam / boiling water to remove the kernel. Subsequently, the kernels are removed manually, and the outer red skin (testa) is discarded. The texture, colour, flavour and appearance of cashew kernels are altered significantly during roasting. Dry roasting of cashew kernels in hot air is generally preferred due to low oil content in the final product. Physical and chemical changes occurring in roasting are time and temperature dependent. The degree of roasting plays a major role in determining the sensory quality parameters, namely aroma, colour, texture and taste of the product (Azam-Ali & Judge, 2001). Therefore, selection of appropriate roasting conditions for optimum product quality is essential in this processing step (Saklar *et al.,* 2001). Several studies have reported textural changes resulting upon roasting of hazelnuts (Saklar *et al.,* 1999; Demir & Cronin, 2004), coffee

beans (Pittia *et al.*, 2001), pecans (Ocon *et al.*, 1995) and peanuts (Hung & Chinnan, 1989).

A close scrutiny of literature on cashew nut and roasting of it shows a wide gap in the available information on phenolic contents and their antioxidant activities under different roasting conditions. Therefore, this study aimed to determine the phenolic content and antioxidant properties of cashew nut and testa subjected to low and high temperature roasting conditions. Several *in vitro* assays, as well as food and biological models were used to assess the effect of roasting on antioxidant and antiradical activity of cashew nuts and testa. In addition, lipid profile of raw and processed cashew oils was determined and the oxidative stability of cashew nut oil as affected by low and high temperature roasting treatments was also investigated. Thus, the objectives of present work was (1) to determine the effects of low and high temperature roasting of cashew nuts and testa on the content of phenolic compounds and their antioxidant properties, (2) to identify and quantify major phenolic acids and flavonoids in cashew nuts and testa, (3) to assess the antioxidative and antiradical efficacy of phenolics from cashew nuts and testa in food and biological model systems, and (4) to determine the oxidative stability of cashew oil as affected by low and high temperature roasting conditions.

Chapter 2

Literature review

2.1 History of nut consumption

Human beings began gathering nuts for food long before the development of agriculture. Recent archeological studies of a 10,000 year old village in Eastern Turkey have discovered the existence of a non-migratory society whose economy centred on the harvesting of almonds and pistachios (Jeor *et al.,* 1995). Recent research suggests that civilizations relied on nuts, even before cereal grains, as a staple food. Nuts were able to play this role because they were a more predictable food source than smaller and less-hearty cereal grains that might have been damaged by severe weather (Fraser *et al.,* 1992; Jeor *et al.,* 1995). They could also be stored through long winters, thus providing a stable food supply throughout the year, and to provide energy, essential fatty acids, protein, and important micronutrients.

Nuts have also been used for centuries as medicine. For example, in Brazil, a tea made from peanuts is thought to have a calming and relaxing effect. As cultures evolved, the role played by nuts in cuisines throughout the world also occurred. Cooks from the Mediterranean region, South America, and Asia have long used tree nuts and peanuts as ingredients in savoury sauces, stuffings, entrees, snacks, appetizers, and desserts (Prineas *et al.*, 1993).

Almonds are related to stone fruits, such as peaches, plums, apricots, and cherries. They originated in either China or Iran and were a valuable trading commodity throughout Asia and the Middle East. Almonds were brought to California by Franciscan priests

from Spain. Today California is the world's major supplier of almonds. Hazelnuts, also called filberts, were described in Chinese manuscripts dating back to 5000 years ago (Sabate *et al.,* 1993). The ancient Greeks and Romans may have used them for medicinal purposes as well as for food. Today hazelnuts are used throughout the world, and are especially popular in Europe and Turkey. Macadamia nuts originated from evergreen trees in the rain forest of Australia and were introduced into Hawaii a century ago. Over the years, agricultural specialists have developed varieties of macadamia trees that have flourished on lands that are too hilly or rocky for other crops (Sabate *et al.,* 1993).

Walnuts are considered to be one of the oldest tree foods known to man. Historical references date back to Persia in 7000 B.C. Today they are commonly called "English" walnuts because English merchants transported them to ports around the world. Franciscan priests are credited with bringing walnuts from Spain to California (Sabate *et al.,* 1993).

Cashew nuts are native to Brazil, and Portuguese took the cashew plant to Goa, India, between the years of 1560 and 1565. Then it was spread throughout Southeast Asia and eventually Africa. The first country to import cashew nuts from India was the United States in 1905 (Sabate *et al.,* 1993). Peanuts are native to Central and South America. Records reflect their use as a food in Peru as early as 2000 B.C. Spanish explorers brought peanuts back to Europe and the Philippines while Portuguese explorers introduced them to East Africa. Peanuts were brought to North America along with the slave trade. Today peanuts are widely cultivated in India, China, and the United States (Sabate *et al.,* 1993).

2.1.1 Nuts and various dietary patterns

Nuts are part of the meat alternate group of USDA's Food Guide Pyramid (USDA, 2005). This means they can be eaten daily, in moderate amounts, as part of a healthful diet. Nuts provide plant protein, fat (most of which is unsaturated), dietary fibre, and several important vitamins and minerals. Since nuts are extremely versatile and can be consumed in many different ways, consumers can easily incorporate them into their diets (USDA National Nutrient Database, 2009). The Mediterranean and, more recently, Asian diet pyramids both place nuts on the same level as fruits, vegetables, and legumes. This placement, close to the foundation of these pyramids, conveys the message that nuts can be eaten frequently. Vegetarians combine grains, such as rice and bread, with nuts as one source of high quality protein. In the past the intake of nuts declined due to the increased concern about their high fat content and possible adverse effects on health. The actual benefit of limiting intake of nuts is now a past concern that has been successfully refuted. Americans consume less than one ounce of nuts (including nut butters, food ingredients, and snacks) per day (USDA National Nutrient Database, 2009) . Nuts account for only about 2.5% of the total fat intake in the United States. In addition, new technologies now allow for production of reduced-fat nuts and nut butters. Interestingly, Mediterranean countries, noted for their traditionally healthful diets, have a per capita consumption of nuts that is almost two fold higher than that of the United States. Menu analyses verify that nuts can fit into a variety of nutritionally sound diets that derive 20 to 37% of calories from fat (USDA National Nutrient Database, 2009).

2.1.2 Benefits of eating nuts

One of the most unexpected nutritional discoveries of the 1990s was that the frequent eating of nuts appears to dramatically improve health (Fraser, 1999). In particular, nut consumption greatly lowers the risk of heart disease (Kris-Etherton *et al.*, 2001). Fraser *et al.* (1992) reported that individuals eating nuts daily had up to 60% fewer heart attacks than individuals who ate nuts less than once per month.

Some epidemiological studies have confirmed the benefits to the heart of nut eating (Fraser *et al.*, 1995; Kushi *et al.*, 1996; Kris-Etherton *et al.*, 2001). In addition to the cardiac benefits of consuming nuts, the risks of having strokes, type 2 diabetes, dementia, macular degeneration, and of gallstones were found to be lowered by eating nuts (Yochum *et al.*, 2000; Jiang *et al.*, 2002; Zhang *et al.*, 2002; Seddon *et al.*,2003; Tsai *et al.*, 2004). Calculations suggest that daily nut eaters gain an extra five to six years of life free of coronary heart disease and that regular nut eating appears to increase longevity by about 2 years (Hu & Stamfer, 1999; Fraser & Shavik, 2001). Adding 30 g/day of nuts to a Mediterranean diet resulted in a significant reversal of the metabolic syndrome (Salas-Salvado *et al.*, 2008). The risk of fatal coronary disease and developing type 2 diabetes decreased steadily as nut consumption increased from less than once a week to once or more per day (Sabate, 1999; Jiang *et al.*, 2002). The above studies suggest that 30 to 60 grams (1-2oz) of nuts should be consumed daily to gain the maximum benefits. Whether even larger amounts confer further benefits is currently unknown.

Nuts are considered as a fatty food and many might worry that they will put on weight by eating more nuts. After all, 30 grams of nuts may contain about, on the average, 190

calories. According to the present evidence, nuts do not seem to cause weight gain (Fraser, 1999; Garcia-Lorda *et al.*, 2003). Nuts appear to satisfy hunger sufficiently and appropriately to reduce the consumption of other foods.

2.1.3 Nutrient content of nuts

Nuts are nutrient dense foods because they have a high total fat content, ranging from 46% in cashews and pistachios to 76% in macadamia nuts, and provide 4.7-7.1 Calories /g nuts (**Table 2.1**). However, the fatty acid composition of nuts is not considered harmful because the saturated fatty acid (SFA) content is low. Nearly one half of the total fat of nuts is made up of unsaturated fat, which includes monounsaturated fatty acids (MUFAs; mainly oleic acid) in most nuts and polyunsaturated fatty acids (PUFAs) including linoleic acid and α-linolenic acid (ALA; 18:3n-3), the latter, in walnuts (Ros & Mataix, 2006). It should be noted that, with 3 g/serving, walnuts are considered as whole foods with the highest content of ALA of all edible plants (Exler & Weihrauch, 1986). The fatty fraction of nuts also contains considerable amounts of plant sterols, with antioxidant and cholesterol lowering properties (Vivancos & Moreno, 2005; Segura *et al.*, 2006). Nuts are rich sources of other bioactive macronutrients. They are an excellent source of protein (25% of energy) and often have a high content of L-arginine, the amino acid precursor of the endogenous vasodilator nitric oxide (Huynh & Chin-Dusting, 2006). Nuts are also a good source of dietary fibre, which ranges from 4 to 11 g/100 g (**Table 2.1**), and standard servings provide 5-10% of daily fibre requirements. Among nut constituents, there are also significant micronutrients. Nuts contain sizeable amounts of folate and are rich sources of antioxidant vitamins and phenolic compounds (Blomhoff *et al.*, 2006; Segura *et al.*, 2006). Almonds in particular are especially rich in α-tocopherol,

whereas walnuts contain significant amounts of γ-tocopherol, increasingly recognized as a relevant antiatherogenic molecule (Wagner *et al.*, 2004). Importantly, most phenolics are located in the outer pellicle of nuts, which means that the peeled product loses much of its antioxidant capacity (Blomhoff *et al.*, 2006). Industrial bleaching, sometimes used to restore a desirable white colour to the hard shells of nuts, destroys most of the antioxidants when the shells are naturally cracked, as shown for pistachios (Seeram *et al.*, 2006). These facts, rarely taken into consideration in prior studies with nuts, should not be overlooked in future feeding trials or when giving advice on nut intake in healthy diets. Compared with other common foodstuffs, nuts have an optimal nutritional density in salutary minerals, such as calcium, magnesium, and potassium. Like most vegetables, the sodium content of nuts is very low, ranging from undetectable in hazelnuts and pecans to 18 mg/100 g in peanuts (Segura *et al.*, 2006). Clearly, this advantage of nuts is lost when they are consumed as a salted product. Whole, unpeeled, and otherwise unprocessed nuts have a unique composition, with unaltered nutrient and non-nutrient bioactive molecules. Most nut constituents have shown beneficial effects when clinically tested, as such or as part of enriched foods, for effects on diverse cardiovascular outcomes, including risk markers (Brown & Hu, 2001; Blomhoff, 2005; Kay *et al.*, 2006).

Table 2.1 Average nutrient composition of tree nuts (per 100 g)

Nuts	Energy kJ	Fat g	SFA g	MUFA g	PUFA g	Protein g	Fiber g	Folate µg	α-Toco. mg	Ca mg	Mg mg	K mg
Almond	2418	50.6	3.9	32.2	12.2	21.3	8.8	29	25.9	248	275	728
Brazil nut	2743	66.4	15.1	24.5	20.6	14.3	8.5	22	5.7	160	376	659
Cashew	2414	46.4	9.2	27.3	7.8	18.2	5.9	25	0.9	37	292	660
Hazelnut	2629	60.8	4.5	45.7	7.9	15.5	40.4	113	15	114	163	680
Macadamica	3004	75.8	12.1	58.9	1.5	7.9	6	11	0.5	85	130	368
Peanut	2448	49.7	6.9	24.6	15.7	23.7	8	145	6.9	54	176	658
Pecan	2889	72	6.2	40.8	21.6	9.2	8.4	22	1.4	70	1321	410
Pine nut	2816	68.4	4.9	18.8	34.1	13.7	3.7	34	9.3	16	251	597
Pistachio	2336	44.4	5.4	23.3	13.5	26.6	9	51	2.3	107	121	1035
Walnut	2738	65.2	6.1	8.9	47.2	15.2	6.4	98	0.7	98	158	441

Abbreviations: SFA, Saturated fatty acid; MUFA, monounsaturated fatty acid; PUSF, polyunsaturated fatty acid; α – Toco, α-tocopherol; Ca, Calcium; Mg, Magnesium; K, Potassium (US Department of Agriculture Nutrient Database. Available at http://www.nal.usda.gov/fnic/cgi-bin/nut_search.pl (2 May2008).

2.2 Antioxidants

Antioxidants are compounds that when present at low concentrations compared to that of an oxidizable substrate retard or prevent the autoxidation process (Halliwell & Gutteridge, 1999). Ingold (1968) classified all antioxidants into two groups, namely primary chain breaking antioxidants, which directly react with lipid radicals converting them to non-radical products, and secondary or preventive antioxidants.

2.2.1 Autoxidation

The oxidative deterioration of food lipids involves, primarily, autoxidative reactions which are accompanied by various secondary reactions having oxidative and non-oxidative character. Autoxidation is a natural process that takes place between molecular oxygen and unsaturated lipids in the environment. Polyunsaturated fatty acids (PUFA) are susceptible to autoxidation and many undergo decomposition; these PUFA could be in the form of free fatty acids, triacylglycerols or phospholipids. An alternative pathway leading to lipid oxidation is photooxidation through excitation of lipids or oxygen in the presence of light and a sensitizer (Gordon, 2001). The main steps of lipid autoxidation are initiation, propagation, and termination.

In the initiation step, the free radical is formed from an unsaturated lipid molecule (RH) at an allylic methylene group, by the action of an initiator. After initiation, propagation reactions occur in which one lipid radical is converted into a different lipid radical. These reactions commonly involve abstraction of a hydrogen atom from a fresh lipid molecule or addition of oxygen to an alkyl radical. In the termination step, two radicals react with one another to yield a product that does not sustain the propagation phase.

Termination is also possible in the presence of antioxidants that possess free radical scavenging activity (St. Angelo, 1996).

2.2.2 Primary antioxidants

Primary antioxidants can be defined as the compounds, which can react with lipid radicals to convert them to more stable products. Antioxidants have the ability to donate a hydrogen atom to lipid radicals and neutralize them.

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

$$RO^{\bullet} + AH \longrightarrow ROH + A^{\bullet}$$

$$ROO^{\bullet} + A^{\bullet} \longrightarrow ROOA$$

$$RO^{\bullet} + A^{\bullet} \longrightarrow ROA$$

$$R^{\bullet} + A^{\bullet} \longrightarrow RA$$

The resulting antioxidant phenoxyl radical ($A^{\bullet}$) does not initiate new free radicals and is not subject to rapid oxidation by a chain reaction. Antioxidant radicals may also participate in termination reactions of $ROO^{\bullet}$, $RO^{\bullet}$ and other antioxidant radicals, thus preventing propagation of chain reactions.

Reactions between antioxidant radicals and lipid molecules and oxygen are exothermic in nature and the activation energy increases with increasing A-H and R-H bond dissociation energies (Shahidi *et al.*, 1992). A molecule will be able to act as a primary

antioxidant if it is able to donate a hydrogen atom to a lipid radical and if the radical derived from the antioxidant is much more stable than the lipid radical, or is converted to other stable products. Phenol itself is inactive as an antioxidant, but substitution of alkyl groups into the *ortho(o), meta (m)* or *para (p)* positions increases the electron density on the hydroxyl group by an inductive effect and this enhances the reaction with lipid radicals (Gordon, 1990).

The presence of bulky substituents in the *ortho* and *para* positions of the phenol ring also reduces the rate of reaction of the phenol with lipid radicals. The steric effect opposes the increased stabilization of the radical and both effects must be considered in assessing the overall activity of an antioxidant (Gordon, 1990). The introduction of a second hydroxyl or methoxyl group at the *ortho* or *para* positions of the hydroxyl group of a phenol increases its antioxidant activity.

Antioxidants are effective in extending the induction period only when added to unoxidized substrates. Antioxidants are virtually ineffective in retarding deterioration, which has already begun. The effect of antioxidants depends on several factors including antioxidant structure, oxidation condition and the nature of the sample being oxidized (Gordon, 1990). The effectiveness of an antioxidant depends on the activation energy, rate constants, oxidation-reduction potential, and solubility properties (Nawar, 1996).

2.2.3 Secondary antioxidants

The preventive antioxidants deactivate possible precursors of ROS by functioning as metal chelators, peroxide decomposers, singlet oxygen quenchers, and inhibitors of lipoxygenase and other related enzymes. Metal chelating agents have a significant effect on preventing lipid oxidation. Generally food lipids contain trace amounts of metal ions, which may arise from the presence of metal activated enzymes or their decomposition products (Gordon, 1990). Furthermore, metal ions may be included as contaminants from food processing and equipment, and storage vessels. Muscle food products contain a high concentration of iron in association with muscle hemoglobin present. Transition metal ions such as Cu, Fe, and Mn reduce the length of the lag time and increase the rate of oxidation of lipids.

Metals act as prooxidants by electron transfer liberating radicals from fatty acids or hydroperoxides. Chelation of metal ions by food components reduces the pro-oxidative effects of these ions and raises the energy of activation of the initiation reactions considerably (Gordon, 2001). Ethylenediaminetetraacetic acid (EDTA), citric acid, polyphosphates, phytates, oxalates, phosphoric acid, tartaric acid, and malic acid, and phospholipids are among the commonly used sequesterants in the food industry. EDTA forms thermodynamically stable complexes with all transition metal ions, thereby making the metal ions unavailable for chemical reactions. Amino acids and peptides also serve as metal chelators (Pokorny, 1987). The metal chelating characteristics of polyphenolic compounds such as flavonoids are also important factors in their antioxidant activities. Carotenoids such as lycopene, zeaxanthin, lutein, and canthaxanthin quench singlet

oxygen by either physical or chemical routes (Yanishlieva-Maslarova, 2001). The neutralization of singlet oxygen by carotenoids is predominantly through physical quenching that involves the transfer of excited energy from singlet oxygen to the carotenoids resulting in the formation of triplet ground state oxygen and triplet excited carotenoid (Stahl & Sies, 1993). Excited carotenoid dissipates absorbed energy through rotational and vibrational interactions converting back to ground state carotenoids (Stahl & Sies, 1993). The protonated phenolic is not a good ligand for metal chelation, but once deprotonated, it serves as a good chelator. In the presence of suitable cations, the proton is displaced at physiological pH levels (Hilder *et al.*, 2001).

2.2.4 Synthetic antioxidants

Most synthetic antioxidants are phenolic derivatives, usually substituted with more than one hydroxy or methoxy groups (Pokorny, 1996). Synthetic food antioxidants currently approved for use in foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ). The Food and Drug Administration (FDA) in the USA governs the application of antioxidants in foods and FDA regulations require that antioxidants and their carriers be declared in the ingredient label of the product in the United States. **Figure 2.1** shows the structures of the frequently used synthetic antioxidants.

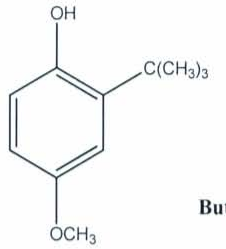
Approximately 40 countries reportedly permit the use of BHT. Food grade BHA, referred to as 2(3)-tert-butyl-4-hydroxyanisole, is generally a mixture of greater than 85% of 3-tert-butyl-4-hydroxyanisole (3-BHA) and 15% or less 2-tert-butyl-4-hydroxyanisole (2-BHA), while food-grade BHT which is 3,5-di-tert-butyl-4-hydroxytoluene is not less

than 99% (w/w) pure (Williams *et al.,* 1999). BHA is metabolized to tert-butylhydroquinone (TBHQ) and tert-butylquinone (TBQ) in the liver. DNA damage has been reported for TBQ, but not for BHA or TBHQ (Morimoto *et al.*, 1991).

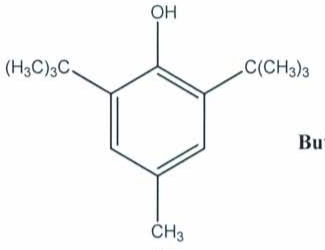
It has been found that BHA at high doses of above 3000 ppm (0.3%) induces forestomach squamous cell carcinoma in rodents, but not glandular cell or other types of neoplasms in the glandular stomach. Humans do not have a forestomach and therefore are less sensitive to exposure to BHA damage than rodents (Williams *et al.,* 1999).

2.2.5 Natural antioxidants

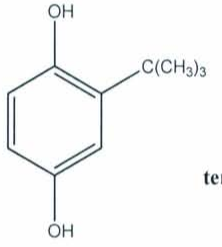
Antioxidative compounds naturally available in foods are well appreciated for both preserving foods and supplying essential antioxidants *in vivo* to combat oxidative stress related conditions. Natural antioxidants include phytochemicals such as flavonoids, isoflavones, phenolic acids, carotenoids, tocols and other low-molecular-weight compounds. Natural antioxidants occurring in foods may be used as components of composite food formulations or may be extracted and subsequently added into foods. Extracts of green tea, sage, and rosemary can be added to a variety of foods, while tocopherols found in plant oils can be used in bulk oil and other commodities to prevent oxidation. Vegetables, spices, herbs, fruits, onions, teas, tomato, and oilseeds are rich in natural antioxidants, among others (Pokorny, 1996; Naczk & Shahidi, 2006).



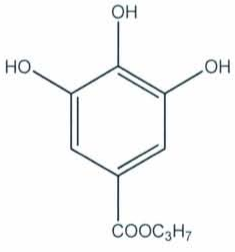
Butylated hydroxyanisole (BHA)



Butylated hydroxytoluene (BHT)



tert-Butylhydroquinone (TBHQ)



Propyl gallate (PG)

Figure 2.1 Chemical structures of commonly used synthetic antioxidants

2.3 Plant phenolics

Plant phenolics are secondary metabolites composed of an aromatic ring bearing one or more hydroxyl substituents together with a number of other side groups (Shahidi & Naczk, 2004). They are derived from a limited pool of biosynthetic precursors such as pyruvate, acetate, a few amino acids, acetyl CoA and malonyl CoA (Robards *et al.,* 1999) following the pentose phosphate, shikimate, and phenylpropanoid metabolism pathways (Randhir *et al.*, 2004). Two main amino acids involved in the synthesis of phenolic compounds in plants are phenylalanine and to a lesser extent tyrosine (Shahidi, 2000; 2002). Phenolic compounds most widely occurring in plants include simple phenolics, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans and lignins (Naczk & Shahidi, 2006).

Phenolics are synthesized as first line defence chemical compounds against infections (Beckman, 2000), wounding (Hahlbrock & Scheel, 1981), nutritional stress (Graham, 1991), cold stress (Christie *et al.*, 1991) and visible light (Beggs *et al.*, 1987). Furthermore, deficiency of iron, phosphorus and nitrogen and the application of herbicides can also induce the production of secondary metabolites in plants (Weidner *et al.*, 2000; Orsak *et al.*, 2001). They contribute to different attributes in foods such as bitterness, astringency, colour, flavour, odour and stability against lipid oxidation (Shahidi & Naczk, 2004). The proposed mechanisms of antioxidant activity include free radical quenching, transition metal chelation, reducing activity, and stimulation of *in vivo* antioxidative enzyme activities, among others.

Phenolic compounds have the ability to bind and precipitate macromolecules such as proteins, carbohydrates and digestive enzymes, thus imparting deleterious nutritional effects (Lugasi, 2003). However, knowledge about their ability to act as antioxidants and free radical scavengers has drawn the curiosity of researchers to find their association in the prevention of degenerative diseases such as cardiovascular ailments, and certain types of cancer (Duthie *et al.*, 2000; Tapiero *et al.*, 2002).

Typically the phenolic profile of a plant food is species specific (Maillard & Berset, 1995; MeKeehen *et al.*, 1999). The level of phenolic compounds present in a given species of plant material depends on different factors such as cultivar (Harukaze *et al.*, 1999; Yu *et al.*, 2002; Abdel-Aal & Pierre, 2003; Chethan & Malleshi, 2007), environmental conditions (Abdel-Aal & Pierre, 2003), cultural practices (Li & Zhang, 2001), post harvest (Siebenhandl *et al.*, 2007), processing, and storage (Rao & Muralikrishna, 2001; Yang *et al.*, 2001) conditions.

2.3.1 Phenolic acids

Two classes of phenolic acids, hydroxybenzoic acids and hydroxycinnomic acids are found in plant materials (Shahidi & Naczk, 2004). Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, vanillic, syringic, and protocatechuic acids, among others (**Figure 2.2**). The hydroxycinnamic acids include coumaric, caffeic, ferulic, and sinapic acids (**Figure 2.3**) (Shahidi & Naczk, 2004). These latter compounds with a phenyl ring (C_6) and a C_3 side chain are known as phenylpropanoids and serve as precursors for the synthesis of other phenolic compounds. Loss of a two carbon moiety from phenylepropanoids leads to the formation of benzoic acid derivatives and decarboxylation

of benzoic acid derivatives is responsible for the formation of simple phenols (Shahidi & Naczk, 2004).

2.3.2 Flavonoids

Flavonoids are synthesized via condensation of a phenylpropanoid with three molecules of malonyl coenzyme A. This reaction is catalyzed by the enzyme chalcone synthase that leads the formation of chalcones which are subsequently cyclized under acidic conditions to form flavonoids (Shahidi & Naczk, 2004). There are different subclasses of flavonoids, namely flavones, flavonols, flavonones, flavononols, isoflavones, flavans (catechins and anthocyanidins) and flavonols. Flavones and flavonols are present as aglycones in foods (Shahidi & Naczk, 2004). They have similar C ring structures with a double bond at the 2-3 positions. Flavones lack a hydroxyl group at the third position (Shahidi & Naczk, 2004). **Table 2.2** and **Figure 2.4** present different classes of flavonols and flavonoids, respectively, in each class of compounds commonly present in plants.

2.3.3 Lignans

Lignans are compounds that comprise two coupled phenylpropanoid units linked by the central carbons of their side chains (Shahidi & Naczk, 2004). The common plant lignans found in the human diet include secoisolariciresinol (SDG), matairesinol, lariciresinol, pinoresinol and syringaresinol (Liu, 2007). SDG, and matairesinol are readily converted to mammalian lignans, enterodiol and enterolactone, respectively, by intestinal microflora in the human gut and are known to exert strong antioxidant (Thompson *et al.*, 1991; Wang & Murphy, 1994; Thompson *et al.*, 1996) and estrogenic (Glitso *et al.*, 2000) activities.

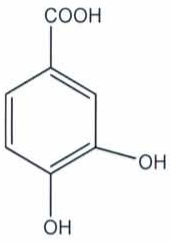
2.3.4 Lignins

Lignins are formed via polymerization of a mixture of the three monolignols, namely *p*-coumaryl, sinapyl and coniferyl alcohols (Lewis & Yamamato, 1990). Additional compounds are incorporated into lignins in small quantities. They include coniferaldehyde (Ralph *et al.*, 2001), sinnamaldehyde (Pillonel *et al.*, 1991), dihydroconiferyl alcohol (Ralph *et al.*, 1997), 5-hydroxyconiferyl alcohol (Ralph *et al.*, 2001; Marita *et al.*, 2003), tyramine ferulate (Ralph *et al.*, 1998) and *p*-hydroxy-3-methoxybenzaldehyde (Kim *et al.*, 2003), among others.

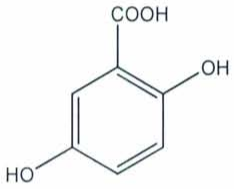
2.3.5 Tannins

Tannins are polyphenolic secondary metabolites of higher plants. However, they have not yet been isolated from the animal kingdom. The polyphenolic structure of the secondary metabolites from higher plants is a necessary but not sufficient requirement for membership in the tannin class. When the structural characteristics of the currently known tannins are analysed, the relatively low occurrence of *C*- and/or *O*- glycosidic derivatives of gallic acid becomes evident. These are composed of a group of compounds with a wide diversity in structure and have the ability to bind and precipitate proteins (Shahidi & Naczk, 2004). Tannins are classified into three groups, namely condensed tannins, hydrolysable tannins and complex tannins (Khanbabaee & Van Ree, 2001).

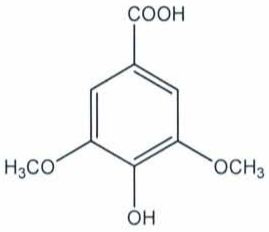
p-Hydroxybenzoic



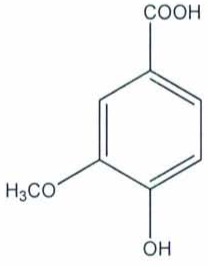
Protocatechuic



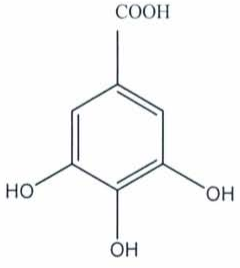
Gentisic



Syringic



Vanillic



Gallic

Figure 2.2 Chemical structures of hydroxybenzoic acids

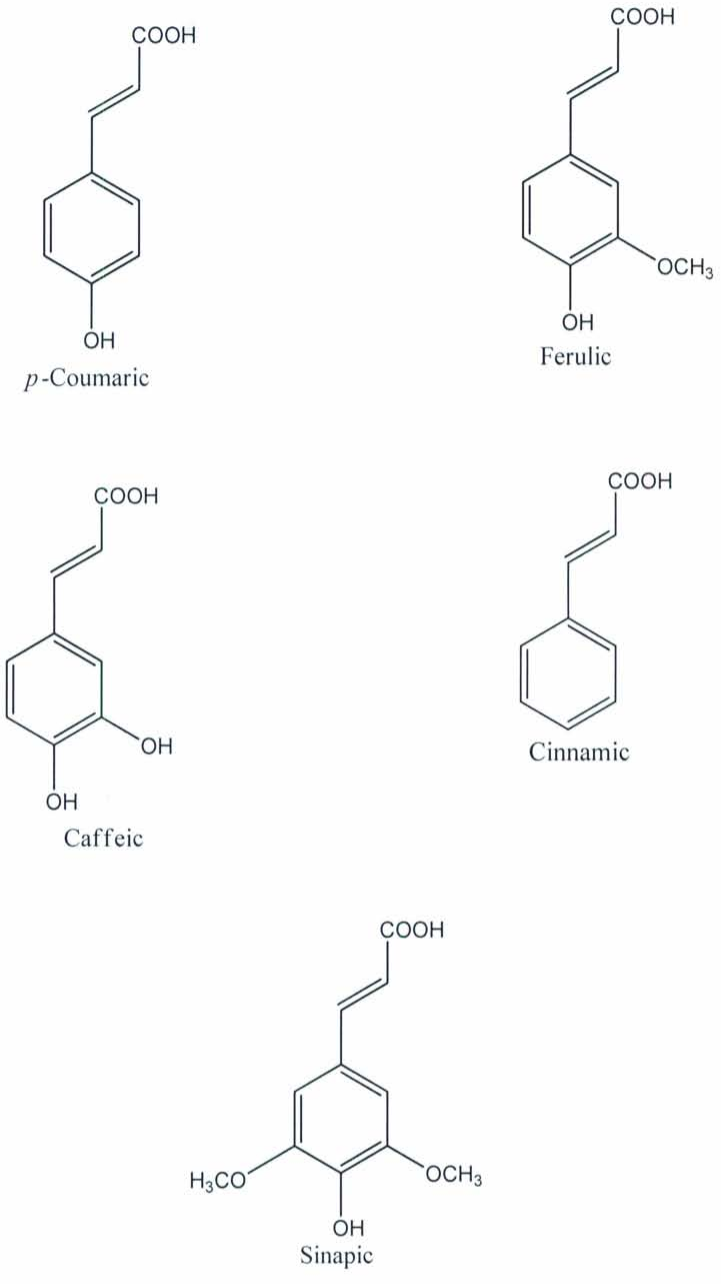
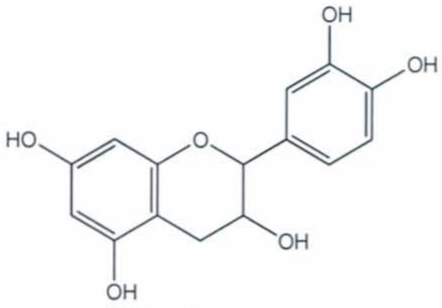
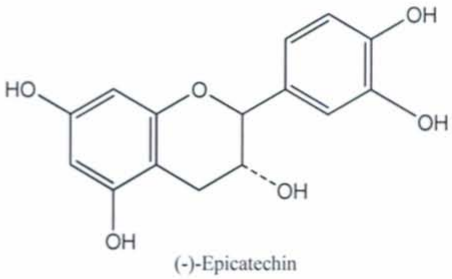


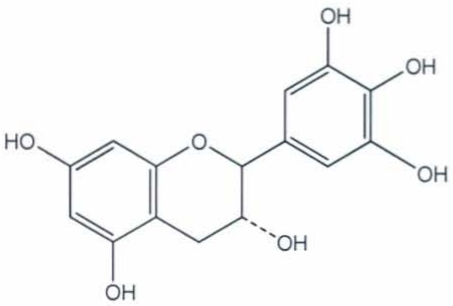
Figure 2.3 Chemical structures of hydroxycinnamic acids



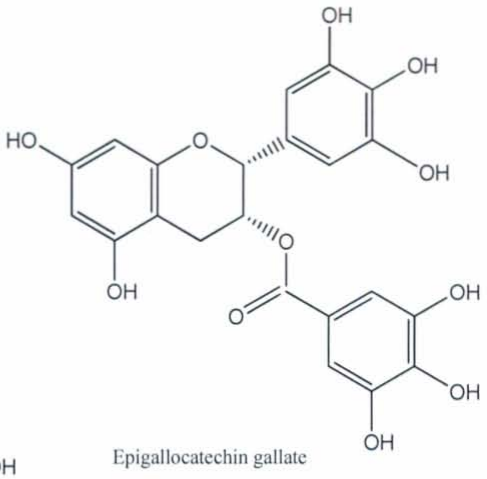
Catechin



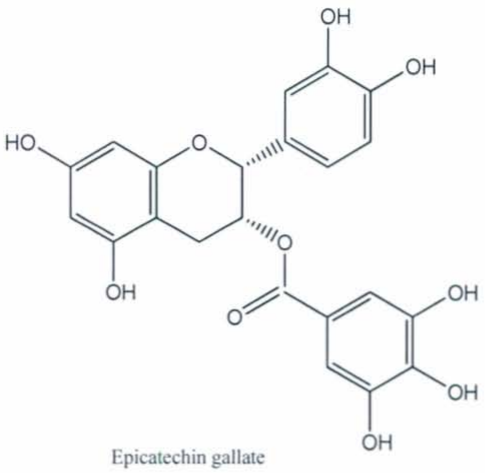
(-)-Epicatechin



(-)-Epigallocatechin



Epigallocatechin gallate



Epicatechin gallate

Figure 2.4 Chemical structures of flavanols (catechins)

Table 2.2 Different classes of flavonoids

Class	Examples of compounds
Flavone	Luteolin, apigenin,rutin
Flavonol	Quercetin, kaempferol, myricetin, isorhamnetin
Flavonone	Hesperidin, naringenin, eriodictyol
Flavononol	Engeletin,genistin, taxifolin
Isoflavone	Daidzein, genistein and glycitein
Flavanol	Catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate
Anthocyanidin	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin
Chalcone	Butein, okanin

2.4 Botanical characteristics of cashew

The cashew (*Anacardium occidentale* L.) tree, belongs to the Anacardiaceae family of plants, which also includes the mango, the pistachio and the poison ivy. The tree is native to Brazil, but has spread to other parts of tropical South and Central America, Mexico and the West Indies. In the 1600s, Portuguese traders introduced the cashew tree into India and Africa to prevent soil erosion. It is now widely cultivated for its nuts and other products in the coastal regions of South Africa, Madagascar and Tanzania and in South Asia, from Sri Lanka to the Philippines (Andrighetti *et al.,* 1989).

The cashew tree is a tropical evergreen, which grows up to 12 metres high and has a symmetrical spread of up to approximately 25 metres. It has leathery oval leaves. Reddish flowers grow in clusters and the pear-shaped fruits, referred to as cashew apples that are red or yellowish in colour. At the end of each fruit is a kidney-shaped ovary, the nut, with a hard double shell (**Figure 2.5**). Between the shell and nut is a black caustic oil, which is difficult to remove and can be used in varnishes and plastics. The cashew tree grows with little care and is easily cultivated. It is usually found from sea level to an altitude of 1000 metres, in regions with annual rainfall as low as 500 mm and as high as 3750 mm. The tree has an extensive root system, which helps it to tolerate a wide range of moisture levels and soil types, but commercial production is only advisable in well drained, sandy loam or red soils. The cashew tree can flourish in the sand of open beaches, but it grows poorly in heavy clay or limestone. Most cashew trees start bearing fruit in the third or fourth year and are likely to reach their mature yield by the seventh year if conditions are favourable. The average yield of nuts of a mature tree is in the

range of 7 to 11 kg per year. Although the cashew tree is capable of living for 50 to 60 years, most trees produce nuts for about 15 to 20 years (Geraldoa *et al.*, 1976).

India is a one of the major cashew exporting countries in the world. Export of cashew kernels from India during 2004-2005 was 126,667 MT valued at US$ 603 million, and these were mostly exported to the USA, the Netherlands and other European countries. Export of cashew nut shell liquid and allied products during 2004-2005 from India was 7,474 MT valued at US$ 35 million (Andrighetti *et al.,* 1989; Cashew Bulletins, India, 2005). According to the estimate of the Directorate of Cashew Nut and Cocoa Development Board of India, the production of raw cashew nut in India during 2004-2005 was 544,000 MT compared to 535,000 MT during 2003-2004, and 506,000 MT during 2002-2003. While the raw nut requirement of the cashew processing industry in India is estimated to be over 1,200,000 MT per annum while the availability from domestic sources is less than half of it. Consequently, import of raw cashew nut into India is necessary, and during 2004-2005, it was 578,884 MT, compared with 452,398 MT during 2003-2004, mostly from the African continent (Andrighetti *et al.,* 1989; CTCS network, 1993; Azam-Ali & Judge, 2001).

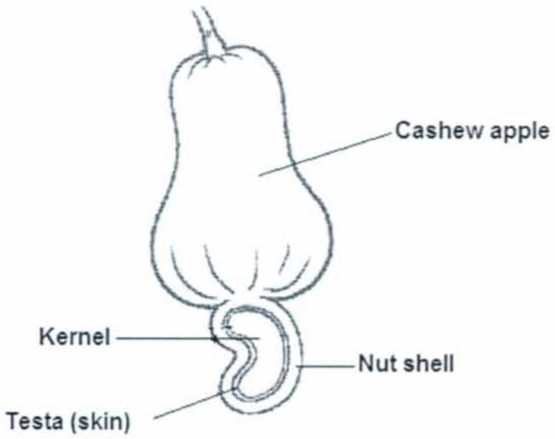
2.4.1 Harvesting of cashew

The harvesting and processing of cashew is very labour intensive. After producing clusters of flowers, cashews produce the edible apple and also a nut encased in a heavy shell, which is the true cashew fruit. The cashew tree flowers for two or three months and fruits mature about two months after the bloom. The cashew nuts form first at the end of the stem. Afterwards, the stem swells to form the "apple" with the nut attached

externally. The cashew nut is 2.5 to 4.0 cm long and kidney shaped. Its shell is about 5 mm thick, with a soft leathery outer skin and a thin hard inner skin (**Figure 2.5**). Cashew fruits are generally left to fall to the ground before being collected, as this is an indication that the kernel is mature. If fruit are picked from the trees, the cashew apple will be ripe, but the kernel will still be immature (Andrighetti *et al.,* 1989). Workers clean the area and detach the nut from the fruit. For the nuts to be easily traced, the surface under the tree has to be free from weeds. In some places, the whole area under the tree is swept free of dry leaves. The nuts are generally collected in baskets or sacks. The quantity of nuts, which can be harvested, depends upon the yield of the trees. Where many nuts fall together, much less time is required for walking in search of them. On the average, each individual can harvest a maximum of 50 kg of cashew nuts per day (Ohler, 1979).

2.4.2 Uses of cashew nuts and byproducts

The cashew tree has been cultivated for food and medicine for 400 years. Cashews have served nutritional, medicinal and wartime needs. More recently, they have been used in the manufacture of adhesives, resins and natural insecticides. During World War II, the cashew tree became highly prized as a source of valuable oil drawn from the shell. The cashew kernel is a rich source of fat and protein and is a good source of calcium, phosphorus and iron (**Table 2.1**). It has a high percentage of polyunsaturated fatty acids, in particular linoleic acid. The apple is a source of vitamin C, calcium and iron (Nagaraja, 2000). The bark, leaves, gum and shell are all used in medicinal applications. The leaves and bark are commonly used to relieve toothache and sore gums and the boiled water extract of the leaves or bark is used as a mouthwash (Wimalsiri *et al.,* 1971).



Unshelled nut

Kernel with testa

Kernel

Figure 2.5 Cashew apple, unshelled nut, and kernel

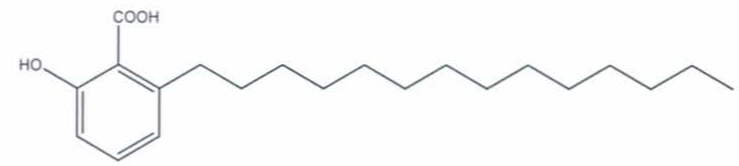
A paste of bark ground in water is used in topical applications for the cure of ringworm; in this form it can however act as an irritant and should not be applied to sensitive skin or to children. The root has been used as a purgative. Fibres from the leaves can be used to strengthen fishing lines and nets and as folk remedies for calcium deficiency and intestinal colic, as well as a vitamin supplement. The water-resistant wood is used for boats and ferries, while the resin, in addition to having industrial uses, is used as an expectorant, cough remedy and insect repellent (Andrighetti *et al.,* 1989; CTCS network, 1993).

2.4.2.1 Cashew nut shell liquid

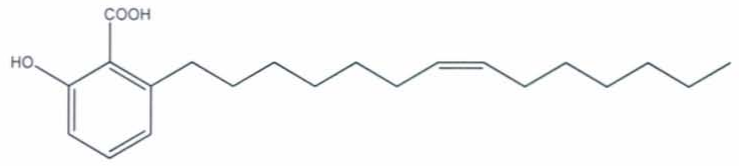
The cashew nut shell contains a viscous and dark liquid, known as cashew nut shell liquid (CNSL). It is contained in the thin honeycomb structure between the soft outer skin of the nut and the harder inner shell. The CSNL content of the raw nut varies between 20 and 25 % (Jain & Kumar, 1997). There are more than 200 patents for its industrial application, in particular, its use as raw material for phenolic resins and friction powder for the automotive industry (brake linings and clutch disks). In drum-brake lining compounds, cashew resins are used as fillers and may also be used as binders (Jain & Kumar, 1997). In disc pads, the role of cashew resin is restricted to the use of friction dust as filler. The advantage of the cashew resins compared with synthetic phenolic resins is that they are more economical and produce a softer material, which gives a quieter braking action (CTCS, 1993). CNSL is also used in mouldings, acid-resistant paints, foundry resins, varnishes, enamels and black lacquers for decorating vases and as

insecticides and fungicides. In tropical medicine, CNSL has been used in treating leprosy, elephantiasis, psoriasis, ringworm, warts and corns (Jain & Kumar, 1997).

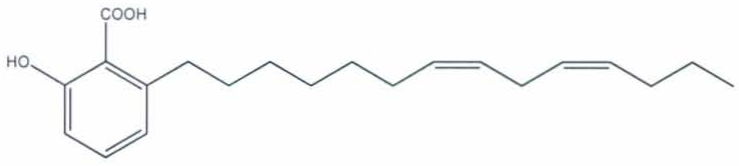
The major phenolic constituents of CNSL are anacardic acids (Kubo *et al.,* 1993a), cardol (Kubo *et al.,* 1994a), and cardanol (Wassermann & Dawson, 1948) (**Figure 2.6**). Anacardic acid inhibits enzymes such as prostaglandin synthase (Grazzini *et al.,* 1991), tyrosinase (Kubo *et al.,* 1994a), and lipoxygenase (Shobha *et al.,* 1994). It is also known to exhibit antitumour, antimicrobial, and antiacne properties (Kubo *et al.,* 1993a; 1993b; Kubo *et al.,* 1994b). Cardanol finds many applications in the form of phenol formaldehyde resins in varnishes, paints, and brake linings (Tyman, 1980). Even though cardol was reported to be toxic (Wassermann &Dawson, 1948), recent studies using rats have demonstrated that there is tolerance of up to 5 g / kg body weight (Suresh & Kaleysa, 1990). Cardol is also active against the filarial parasite of cattle *Setaria digitata* (Suresh & Kaleysa, 1990). In view of its biological and industrial applications it was considered necessary to develop a simple and efficient method for the isolation of all the major phenolic constituents of CNSL. Because of the thermolability of the carboxylic group of anacardic acid (tendency to be converted to cardanol), CNSL constituents cannot be separated by fractional distillation (Tyman *et al.,* 1989). General Foods Corporation (1946) was the first to report a method to isolate anacardic acid as alkaline earth metal salt.



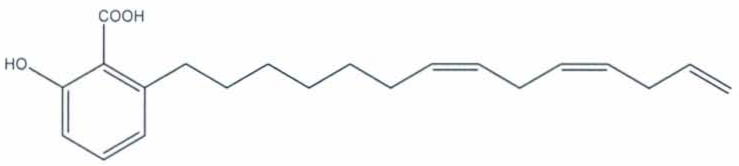
Anacardic acid - 1



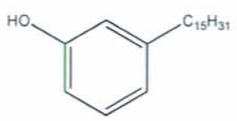
Anacardic acid - 2



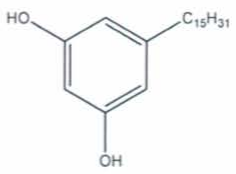
Anacardic acid - 3



Anacardic acid - 4



Cardanols



Cardols

Figure 2.6 Major phenolic constituents of CNSL

2.4.2.2 Cashew apple

The cashew apple is an edible food rich in vitamin C. It can be dried, canned as a preserve or eaten fresh from the tree. It can also be squeezed for fresh juice, which can then be fermented into cashew wine, which is a very popular drink in West Africa. In parts of India, it is used to distil cashew liquor referred to as feni (Maini & Anand 1993). In some parts of South America, local inhabitants regard the apple, rather than the nut kernel, as a delicacy. In Brazil, the apple is used to manufacture jams and soft alcoholic drinks. The cashew apple is between three and five inches long and has a smooth, shiny skin that turns from green to bright red, orange or yellow in colour as it matures (Ogunmoyela 1983). It has a pulpy, juicy structure, with a pleasant but strong astringent flavour. The cashew apple is very rich in vitamin C (203 mg/100 ml of juice) and contains five times more vitamin C than an orange (Akinwale, 2000; Silva *et al.,* 2000). A glass of cashew apple juice meets an adult's daily vitamin C (30 mg) requirement (Silva *et al.,* 2000). The cashew apple is also rich in sugars and contains considerable amounts of tannins and minerals, mainly calcium, iron and phosphorous (Akinwale, 2000). Furthermore, the fruit has medicinal properties like antimicrobial activity (Muroi *et al.,* 1993). It is used for curing scurvy and diarrhoea and it is effective in preventing cholera and also regarded as a first class source of energy. Until recently, the potential of cashew apple had not been investigated due to its highly astringent and acrid taste which is believed to originate from the waxy layer of the skin and which causes tongue and throat irritation after eating (Pimentel, 1992). Cashew fruit can be made suitable for consumption by removing the undesirable tannins and processing the apples into value-added products, such as juices, syrups, canned fruits, pickles, jams, chutneys, candy and

toffee. The recommended methods for removing the astringency of the cashew apple include steaming the fruit for five minutes before washing it in cold water, boiling the fruit in salt water for five minutes or adding a gelatin solution to the expressed juice (Pimentel, 1992).

Cashew apples should be processed within two to three hours of picking, since they undergo rapid deterioration when kept for a longer time (Chempakam, 1983). It is also extremely difficult to use the whole fruit commercially as the apple ripens prior to the nut. The quality of nuts detached from the green fruit, is unacceptable for commercialization (Chempakam, 1983). The development of processing options for the cashew apple has also been limited by its high degree of perishability and consequent difficulties in transportation from growing areas to distant processing plants.

2.4.2.3 Cashew wine

Cashew wine is made in many countries throughout Asia and Latin America. It is a light yellow alcoholic drink, with an alcohol content of 6 to 12 %. Processing Cashew apples includes cutting into slices in order to ensure a rapid rate of juice extraction when they are crushed in the juice press. The fruit juice is sterilized in stainless steel pans at a temperature of 85^{o}C in order to eliminate any wild yeast (Rao, 1985). The juice is filtered and treated with either sodium or potassium metabisulphite, to destroy or inhibit the growth of undesirable types of micro-organisms such as acetic acid bacteria, wild yeast and moulds. Wine yeast (*Saccharomyces cerevisiae*) added and the juice is then thoroughly stirred and allowed to ferment for about two weeks. The wine is separated from the sediment and clarified by mixing fining agents, such as gelatin, pectin or casein,

with the wine. Filtration is carried out with filter-aids such as fullers earth (Maldonado *et al.*, 1975). The filtered wine is transferred to wooden vats. The wine is pasteurized at 50 to 60°C. The temperature should be controlled, so that it does not exceed 70°C, since alcohol vapourizes at a temperature of 75 to 78°C. The wine is then stored in wooden vats and subjected to ageing. At least six months should be allowed for ageing. If necessary, the wine should be clarified again before bottling. During ageing and subsequent maturing in bottles, many reactions, including oxidation, occur. The formation of trace amount of esters and aldehydes, together with the tannin and acids already present enhance the taste, aroma and preservative properties of the wine (Wimalsiri *et al.*, 1971; Maldonado *et al.*, 1975). The product is packaged in glass bottles with corks and should be kept out of direct sunlight.

2.4.2.4 Cashew nut

The cashew nut kernel is made up of three different portions namely the shell, the kernel and the adhering testa. The primary product of cashew nuts is the kernel, which is the edible portion of the nut and is consumed in three ways: directly by consumers, as roasted and salted nuts, in confectionery and bakery products. For example, finely chopped kernels are used in the production of sweets, ice creams, cakes and chocolates, both at home and industrially and as paste to spread on bread (Wimalsiri *et al.*, 1971). The relative importance of these uses varies from year to year and country to country, but it is estimated that at least 60% of cashew kernels are consumed as salted nuts. Separately packed cashew nuts are a good selling line, mainly as an appetizer to cocktail drinks. Salted cashews are part of the snack food market. They compete mainly with other nuts,

although chips, salted popcorn and other savoury snacks can impinge on the nut market. The price of cashew nuts is much higher than the price of peanuts or other snacks so those sales must be based on a strong taste preference by the consumer. Cashew nuts are generally considered a luxury product and an element of their appeal may lie in this status (Wimalsiri *et al.*, 1971).

2.5 Processing of cashew nuts

The processing of cashew is more complicated than that of other nuts because it must be roasted or cooked in steam or boiling water to remove the kernel from the inside of the shell. The kernels are removed manually, followed by drying and removal of the outer red skin. Good-quality raw cashew kernels are low in moisture content (5–6% wet basis) and are slightly off-white in colour (Woodroof, 1979; Azam-Ali & Judge, 2001). Cashew kernels are typically dry or oil roasted like most other nuts and are consumed as a snack or added to confectionary and bakery products. The texture, colour, flavour and appearance of cashew kernels are altered significantly during roasting. The resulting product is crisp and uniquely tasty compared to raw kernels, and is widely enjoyed by consumers. Dry roasting of cashew kernels in hot air is generally preferred because of the lower oil content in the final product. Roasting is a time-temperature-dependent process leading to physical and chemical changes (Saklar *et al.*, 2001). The degree of roasting plays a major role in determining the sensory attributes such as aroma, colour, texture and taste of the product. Thus, the selection of appropriate roasting conditions for optimum product quality is essential in the roasting operation (Saklar *et al.* 2001). The kinetics of colour change have been extensively studied as a means of controlling the

roasting process (Davidson *et al.,* 1999; Ozdemir & Devres, 2000; Demir *et al.*, 2002). Several studies have reported textural changes upon the roasting of hazelnuts (Saklar *et al.,* 1999; Demir & Cronin, 2004), coffee beans (Pittia *et al.,* 2001), pecans (Ocon *et al.,* 1995) and peanuts (Hung & Chinnan, 1989).

2.5.1 Roasting of cashew kernels

The shelled kernel is covered with the testa, and the removal of which is facilitated by drying the shelled kernel, to produce the blanched kernel. Roasting causes shrinkage of the kernel, thereby allowing the testa to be easily removed either mechanically or by hand with a knife. Roasting also protects the kernel from pest and fungal attack at this vulnerable stage. All processors dry the shelled kernels prior to peeling. The moisture content of the kernel is reduced from approximately six percent to three percent by roasting (Woodroof, 1979).

2.5.2 Effect of roasting process

During the roasting process of foods, important chemical reactions including sugar caramelization and Maillard reaction take place, which cause significant changes in product quality (Yousif & Alghzawi, 2000). The Maillard reaction (MR) which is a part of non-enzymatic browning reaction system predominates when components such as reducing sugars and amines (amino acids, peptides or proteins) react with each other during thermal treatments in food processing or the storage of foods. Thus, thermally processed foods generally contain various levels of Maillard reaction products (MRP), which are ideal time-temperature indicators for determining the extent of a thermal process (Friedman, 1996; Giribert & Ribas, 2000).

2.5.2.1 The Maillard reaction

The Maillard reaction (MR) is a general term used to describe a complex series of reactions between reactive carbonyl groups, such as those of reducing sugars, and free amino groups of proteins (Maillard, 1912). It is a non-enzymatic browning reaction and may produce coloured or colourless reaction products depending on the stage of the reaction as well as other factors such as pH, type of reactants, temperature, and water activity, among others. Condensation reactions between amino acids and lipid oxidation products may also form MRP, and the role of lipids in the MR is similar to the role of reducing sugars (Hidalgo & Zamora, 2000). Group of compounds in the final products of the reaction includes high-molecular-weight melanoidins, which are furan ring and nitrogen containing brown compounds. Little is known about their physical, chemical and physiological properties because of their complex structures. This complexity in MRP structures limits the determination of antioxidant activity for each compound in the whole group of MRPs (Hidalgo & Zamora, 2000).

Lysine is the most important carrier of a free amino group in proteins, and therefore is the most significant amino acid participant in the MR. Beside lysine, arginine, tryptophan, and histidine are also carriers of free amino groups. Cladinin and Robllee (1952) noticed that a peptide chain containing modified lysine was not susceptible to the effects of trypsin and therefore was not utilisable in animal diet. Studying the effect of the MRP on protein digestion, Oste *et al.* (1986) determined that low-molecular-weight compounds developed in the reaction of glucose and lysine inhibited *N*-amino peptidase. This inhibition resulted in reduced protein absorption in the digestive tract. Despite more than 90 years of research work in this area, the molecular mechanisms of MR are still not well

understood, although an increasing number of the MRP have recently been identified (Gerrard *et al.,* 1999). Low-molecular-weight products of the MR play an exceptionally important role in the formation of flavour, aroma, colour and texture in thermally treated foods. The MR partially proceeds even during storage. Melanoid products are also formed in the reaction of amino acids or proteins with oxidised lipids. These products are of structures similar to those developed in the reaction with reducing sugars (Gerrard *et al.,* 1999). MRP may inhibit processes such as growth, protein and carbohydrate digestion, amino acid absorption and activity of intestinal enzymes, including amino peptidases, proteases, and saccharidases, and pancreatic enzymes such as chymotrypsin (Finot, 1990), and induce cellular changes in the kidneys, liver, and stomach cecum. In addition they exert adverse effects on mineral metabolism (Ca, Mg, Cu, and Zn) and render variable effects on allergic responses and cholesterol metabolism. However, the MRPs also showed not only adverse effects but also antioxidative effects, as well as, antimutagenic, antibiotic and antiallergenic effects (Yen & Hsiesh, 1994; Friedman, 1996; Einarsson *et al.,* 1998). Various MRP, obtained under strictly controlled conditions, are used as commercial food additives; such as food aromas and antioxidants (Friedman, 1996).

Chapter 3

Effect of roasting on phenolic content and their antioxidant activities of cashew kernels and testa

3.1 Abstract

The effect of roasting on the content of phenolic compounds and antioxidant properties of cashew nuts and testa was studied. Whole cashew nuts, subjected to low temperature (LT) and high temperature (HT) treatments were used to determine the antioxidant activity of products. Antioxidant activities of cashew nut, kernel and testa phenolic extracts increased as the roasting temperature increased. The highest activity, as determined by the 1,1-dipheny-2-picrylhydrazyl (DPPH) radical scavenging capacity, oxygen radical absorbance capacity (ORAC), hydroxyl radical scavenging capacity, trolox equivalent antioxidant activity (TEAC), and reducing power was achieved when nuts were roasted at 130 ^{0}C for 33 min. Furthermore, roasting increased the total phenolic content while decreased that of the proanthocyanidines. Phenolic acids, namely syringic, gallic and *p*-coumaric acids were identified, amongst which syringic acid was the predominant one. Flavonoids, namely catechin, epicatechin and epigallocatechin were also identified and their contents increased with increasing temperature. The results of the present study suggest that HT short time (HTST) roasting effectively enhanced antioxidant activity of cashew nuts and testa.

Key words: DPPH, ORAC, TEAC, proanthocyanidines, phenolic acids, flavonoids

3.2 Introduction

Regular consumption of fruits, vegetables, grains and nuts are considered beneficial to health and are reported to reduce the incidences of ischemic heart disease and several types of cancer such as those of lungs, stomach, oesophagus, pancreas and colon (Block *et al.,* 1992; Heimendinger *et al.,* 1996; Reddy *et al.,* 2003; Jenab *et al.,* 2004). Studies have shown that phenolics are the major phytochemicals with health benefits in humans. Foods of plant origin, such as fruits and vegetables, tree nuts and whole grain products have been suggested as a natural source of antioxidants (Cao *et al.,* 1996; Sun *et al.,* 2002; Silva *et al.,* 2004, 2008; Giada *et al.,* 2006; Parry *et al.,* 2006; Yang *et al.,* 2009).

Tree nuts have been considered to be a significant component of the Mediterranean diet. In 2003, the U.S. Food and Drug Administration recommended a qualified health claim stating that consumption of 1.5oz (42g) per day of most tree nuts may reduce the risk of heart disease. Since free radicals play a key role in the pathology of diseases, such as cancer, atherosclerosis or inflammatory diseases, the supply of antioxidants via the food chain is of high importance for a healthy life (Scalbert & Williamson, 2000, Alasavar & Shahidi, 2009). In particular, nuts contain protein, unsaturated fatty acids, dietary fibre, sterols, as well as other phytochemicals and micronutrients that may exert health benefits (Hu *et al.,* 1999; Kris-Etherton *et al.,* 2001).

Synthetic antioxidant, like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), are largely used by the food industry and are included in the human diet. However, in recent years the use of natural antioxidants has been promoted because of concerns regarding the safety of synthetic antioxidants (Kaur & Kapoor, 2001).

Dietary components, including polyphenols, carotenoids and vitamins C and E, are considered effective antioxidants useful in the prevention of oxidative stress and related diseases (Kaur & Kapoor, 2001; Moure *et al.,* 2001).

Phenolic compounds contribute to different attributes in foods such as bitterness, astringency, colour, flavour, odour and stability against lipid oxidation. The proposed mechanisms of antioxidant activity include free radical quenching, transition metal chelating, reducing peroxide, and stimulation of *in vivo* antioxidative enzyme activities (Shahidi & Naczk, 2004).

Cashew (*Anacardium occidentale* L.) is one of the most important tree nuts and ranks third in the international trade after hazelnuts and almonds (Mandal, 2000). The processing of cashew nut is more complicated than other nuts. Cashew nut must be roasted or cooked in boiling water (or steam) to remove the kernel. The kernels are removed manually, followed by drying and peeling of the testa which is a thin reddish brown membrane difficult to remove. Good-quality raw cashew kernels are low in moisture content (5-6%) and are slightly off-white in colour (Woodroof, 1979; Azam-Ali & Judge, 2001).

Generally, cashew kernels are consumed as roasted nuts. Roasting is reported as one of the processing conditions that would change the constituents of edible nuts (Durmaz *et al*., 2010). Cashew kernels are consumed as a snack or added to confectionary and bakery products like most other nuts. The texture, colour, flavour and appearance of cashew kernels are altered significantly during roasting. The resulting product is crisp and uniquely tasty compared to raw kernels. The degree of roasting affects the sensory

quality attributes such as aroma, colour, texture and taste of the product. Thus, selection of appropriate roasting conditions for optimum product quality is essential in the roasting operation (Saklar *et al.,* 2001).

The biological activities of cashew nut shell liquid (CNSL) constituents have attracted much interest in the areas of anti-tumour activity (Itokawa *et al.,* 1987 & 89; Kubo *et al.,* 1993a &1993b), antimicrobial activity (Himejima & Kubo, 1991; Kubo *et al.,* 1993a &1993b; Muroi & Kubo, 1993), inhibition of tyrosinase (Kubo *et al.,* 1994), and xanthine oxidase (Masuoka & Kubo, 2004), uncoupling effects of oxidative phosphorylation on liver mitochondria (Toyomizu *et al.,* 2000), and antioxidant activity (Amorati *et al.,* 2001). Cashew apple and their juices are also reported to possess antioxidant potential and antimutagenic activity (Cavalcanate *et al.,* 2003). Very few studies have evaluated the antioxidant activity of phenolics from the edible cashew kernels (Kornsteiner *et al*., 2006; Yang *et al*., 2009).

A close scrutiny of the literature shows lack of information on phenolic content and antioxidant activity of cashew nuts and testa (skin) subjected to different thermal processing conditions. The objective of present study was to determine the effects of low and high temperature thermal processing on the content of phenolic compounds and antioxidant properties of cashew nuts and testa.

3.3 Materials and methods

3.3.1 Materials

Raw shelled cashew with testa was obtained from the Green Field Bio Plantation (Pvt.) Ltd., 49 ½, Braybrook Street, Colombo2, Sri Lanka. Folin Ciocalteu's reagent, gallic acid, vanillin, catechin, 1,1-dipheny-2-picrylhydrazyl (DPPH), 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), fluorescein, trolox, ethylenediaminetetraacetic acid trisodium salt (Na_3EDTA), mono- and dibasic potassium phosphates, sodium chloride, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), hydrogen peroxide, ascorbic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, ferrous chloride, epigallocatechin, epicatechin, syringic acid, and *p*-coumaric acid were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). Hexane, methanol, ethanol, sodium hydroxide, hydrochloric acid, diethyl ether, ethyl acetate, sodium carbonate, formic acid, and acetonitrile were purchased from Fisher Scientific Ltd (Ottawa, ON).

3.3.2 Sample preparation

Two different processing temperatures were used in this study. For low temperature processing (LT) raw whole cashew nuts (kernel with testa) were roasted in a forced hot-air convection oven at 70^0 C for 6 hours. In this, cashew kernels weighing approximately 100 g were spread in a single layer on a stainless steel wire mesh tray placed in the centre of the oven during hot-air roasting. After roasting, the hot cashew kernels were cooled in a dessicator at room temperature, and kept in sealed plastic bags at 4^0C, until further analysis. Under industrial cashew processing operations, both small and large scale cashew producers use these conditions to obtain good quality products (Hebbar *et al.*,

2005). For high temperature processing (HT), raw whole cashew nuts were roasted in a forced air convection oven at 130^0 C for 33 min. This temperature and time combination was the optimum roasting conditions for cashew kernels based on hedonic sensory evaluations according to Wanlapa and Jindal (2006). Raw whole cashew nuts were used as the control to compare the effect of two different roasting conditions.

The raw whole and roasted whole nuts were peeled manually to remove the testa. Raw and roasted whole cashew nuts, kernels and recovered testa were ground separately using a coffee bean grinder (Model CBG5 series, Black & Decker®, Canada Inc. Brockville, ON) to obtain a fine powder which passed through mesh 16 (sieve opening 1mm, Tylor test sieve®, Mentor, OH). Each sample was defatted by blending with hexane (1:5, w/v, 5 min, 3 x) in a Waring blender (Model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT) at ambient temperature (20°C). Defatted samples were air dried for 12 h at room temperature before vacuum packing in polythene pouches and stored at -20°C until used for extraction of phenolics.

3.3.2.1 Extraction of soluble phenolic compounds

Preliminary studies showed that heating of ground samples under reflux conditions with 80% (v/v) ethanol afforded high total phenolic content and antioxidant activity. Therefore, refluxing conditions were used to extract soluble phenolic compounds (Shahidi *et al.,* 2007). Defatted meal (6 g) was mixed with 100 mL of 80% (v/v) ethanol and then placed in a thermostated water bath at 60°C for 40 min. After centrifugation of the resulting slurry for 5 min at 4000 x g (IEC Centra MP4, International Equipment Co®., Needham Heights, MA), the supernatant was collected and extraction was repeated

two more times. Combined supernatants were evaporated in *vaccuo* at 40°C (Buchi, Flawil, Switzerland) and lyophilized for 72 h at -46°C and 34 x 10^{-3} mbar (Freezone, Model 77530, Labconco Co., Kansas City, MO). Residues of samples were air dried for 12 h and stored at -20°C until used to extract bound phenolic compounds within a week.

3.3.2.2 Extraction of bound phenolic compounds

The sample residue obtained after the extraction of soluble phenolics, as explained above, was mixed with 50 mL of 4 M NaOH and hydrolyzed at room temperature for 4 h with stirring under a stream of nitrogen. The resulting slurry was acidified to pH 2 with 6 M HCl and extracted 5 times with hexane to remove fatty acids released. Insoluble bound phenolic compounds were extracted five times with diethyl ether / ethyl acetate (1:1, v/v) and subsequently desolventized to dryness at room temperature using a rotary evaporator. Phenolic compounds were reconstituted in 6 mL of HPLC grade methanol and stored at -20°C until used for further analysis within two weeks.

3.3.3 Methods

3.3.3.1 Determination of total phenolic content (TPC)

The contents of total phenolics of the extracts were determined by Folin Ciocalteu's reagent assay according to a modified version of the procedure described by Singleton and Rossi (1965). The crude extracts of soluble phenolic compounds were dissolved in methanol to obtain a concentration of 0.2mg/mL. Folin Ciocalteu's reagent (0.5 mL) was added to centrifuge tubes containing 0.5 mL of extracts. The contents were mixed thoroughly and 1 mL of a saturated solution of sodium carbonate was added to each tube

to neutralize the reaction mixture. The volume was adjusted to 10 mL with adding distilled water, and the contents were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature in the dark for 35 min followed by centrifugation for 10 min at 4000 x g. Absorbance of the resulting blue colour supernatant was read at 725 nm (Model HP 8452A diode array spectrophotometer, Agilent Technologies, Palo Alto, CA) using appropriate blanks. The content of total phenolics in each extract was determined using a standard curve prepared for gallic acid and expressed as mg gallic acid equivalents (GAE)/g defatted meal.

3.3.3.2 Determination of proanthocyanidins content (PC)

Proanthocyanidins content of crude phenolic extracts of cashew was determined colorimetrically as described by Price *et al.* (1978). To 1 mL methanolic solution of the extract, 5 mL of 0.5% vanillin-HCl reagent were added followed by incubation for 20 min at room temperature. A separate blank for each sample was read with 4% concentrated HCl in methanol. The absorbance was read at 500 nm and the content of proanthocyanidins was expressed as μmol catechin equivalents (CE)/g defatted meal.

3.3.3.3 Determination of 1, 1-dipheny-2-picrylhydrazyl (DPPH) radical scavenging capacity using electron paramagnetic resonance (EPR) spectrometry

The DPPH radical scavenging assay described by Shahidi *et al.* (2007) was adapted with slight modifications. Two millilitres of DPPH in methanol (0.18 mM solution) were added to 500 μL of extracts dissolved in methanol. The contents were mixed well and after 10 min, the mixture was passed through the capillary tubing which guides the sample through the sample cavity of Bruker E-scan EPR spectrometer (Bruker E-scan®,

Bruker Biospin Co. Billercia, MA). The spectrum was recorded using the appropriate softwares (E-Scan analyzer, Bruker Biospin Co. Billercia, MA). The parameters were set as follows; 5.02 x 10^2 receiver gain, 1.86 G modulation amplitude, 2.621 s sweep time, 8 scans, 100.000G sweep width, 3495.258 G centre field, 5.12 ms time constant, 9.795 GHz microwave frequency, 86.00 kHz modulation frequency, and 1.86 G modulation amplitude. DPPH radical scavenging capacities of the extracts were calculated by using the following equation: DPPH radical scavenging capacity, (%) = 100 – {EPR signal intensity for the medium containing the additive / EPR signal intensity for the control medium} X 100.

3.3.3.4 Determination of oxygen radical absorbance capacity (ORAC)

The ORAC was determined using a Fluostar Optima plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps with fluorecsein as the probe and 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH) as the radical generator. The reaction was carried out in 75 mM phosphate buffer (pH 7.0) using a final reaction mixture of 200 µL in a 96-well Costar 2650 black plate. Fluorescein (120 µL; 64 nM, final concentration) was injected into the wells containing the extract using the injector pump. The mixture was incubated for 20 min at 37°C in the built-in incubator and subsequently APPH solution (60 µL; 29 mM final concentration), equilibrated at 37°C, was rapidly injected into the wells. The plate was shaken for 4 s after each addition. To optimize the signal amplification in order to obtain maximum sensitivity, a gain adjustment was performed at the beginning by manually pipetting 200 µL of fluorescein into a designated well. Fluorescence was determined and recorded every

minute for 60 min and the antioxidant activity of the extracts was calculated as trolox equivalents using a standard curve prepared with 1-10 μM (Prior *et al.*, 2003).

3.3.3.5 Determination of hydroxyl radical scavenging capacity

Hydroxyl radicals were generated via the Fe^{2+}-catalyzed Fenton reaction and spin-trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). The resultant 2-hydroxy-5,5-dimethyl-1-pyrrolidinyloxy (DMPO-OH) adduct was detected using a Bruker E-scan EPR. Cashew extracts were dissolved in deionized water and diluted to obtain various concentrations (1.33 – 13.2 mg/mL, final concentrations). Extracts (100 μL) were mixed with 100 μL of 10 mM H_2O_2, 200 μL of 17.6 mM DMPO and 100 μL of 1 mM. After 1 min the mixtures were introduced into the EPR spectrometer and the spectrum was recorded. Hydroxyl radical scavenging capacities of the extracts were calculated by using the following equation; Hydroxyl radical scavenging capacity, (%) = 100 – {EPR signal intensity for the medium containing the additive / EPR signal intensity for the control medium} * 100.

3.3.3.6 Determination of trolox equivalent antioxidant activity (TEAC)

The TEAC assay was performed using a modified version of the method described by Van den Berg *et al.* (1999). The TEAC assay is based on the scavenging of 2, 2′-azinobis-(3-ethylbenzothiazoline -6-sulphonate) radical ($ABTS^{\bullet-}$). A solution of $ABTS^{\bullet-}$ was prepared in 100 mM phosphate buffered saline (pH 7.4, 0.15 M sodium chloride) (PBS) by mixing 2.5 mM 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) with 2.0 mM ABTS solution. The solution was heated for 16 min at 60°C, by covering in a tin foil to protect from light, and used within 2 h as the absorbance of the

radical itself depletes with time. Extracts were dissolved in PBS at a concentration of 1 mg/mL and diluted accordingly to have them fit in the range of values in the trolox standard curve. For measuring antioxidant capacity, 40 µL of the sample were mixed with 1.96 mL of the $ABTS^{\bullet-}$ solution. Absorbance of the above mixture was read at 734 nm at 0 and 6 min. The decrease in absorption at 734 nm after 6 min of addition of cashew extract was used for calculating the TEAC values. A standard curve was prepared by measuring the reduction in the absorbance of the $ABTS^{\bullet-}$ solution at different concentrations of trolox. Appropriate blank measurements (decrease in absorption at 734 nm due to solvent without any compound added) were carried out and the values recorded. TEAC values were expressed as µmol trolox equivalents per gram of defatted material.

3.3.3.7 Determination of reducing power

The reducing power of cashew extracts was determined using the method explained by Chandrasekara and Shahidi (2010). The assay medium contained 2.5 mL of extract (2 mg/mL) in 0.2 M phosphate buffer solution (PBS) (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After incubating for 20 min at 50°C, 2.5 mL of 10% trichloroacetic acid (TCA) were added followed by centrifugation at 1750 x g for 10 min. The supernatant (2.5 mL) was transferred into a tube containing 2.5 mL of deionized water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm and the results were expressed as ascorbic acid equivalents using appropriate standard curves.

3.3.3.8 Analysis of phenolic compounds by high-performance liquid chromatography (HPLC)

The reversed phase HPLC (RP-HPLC) analysis were carried out using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a G1311A quaternary pump, a G1379A degasser and a G1329A ALS automatic sampler, a G1330B ALS Therm, a G1316A Colcom column compartment, a G1315B diode array detector (DAD) and a system controller linked to Chem Station Data handling system (Agilent Technologies, Palo Alto, CA). For analytical work, dilute solutions of freeze-dried crude extracts (10 mg/mL) were passed through 0.45 μm polytetrafluoroethylene (PTFE) membrane syringe filter (Whatman Inc., Florham Park, NJ), and 10 μL aliquots were injected onto a SUPERLCOSIL™ LC-18 column (4.6 × 250 mm, 5 μm; Merck, Darmstad, Germany). A gradient profile using two solvents was applied at room temperature (25^0C), with solvent A (0.05% aqueous formic acid) and solvent B (methanol / acetonitrile - 5:95, v/v) and a flow rate of 0.6 mL/min. Compounds of interest were detected on the basis of characteristic UV-vis spectra (spectral range of 254 - 520 nm) and retention times. To confirm the identity of phenolic compounds HPLC- mass spectrometry (MS) analysis was performed under the HPLC analytical conditions explained above using Agilent 1100 series capillary liquid chromatography/mass selective detector (LC/MSD) ion trap system in electrospray ionization (ESI) negative ion mode. Complete system control and data evaluation were achieved with Agilent LC/MSD Trap software (Agilent Technologies, Palo Alto, CA). An external standard

method with authentic compounds was used for the quantification of identified compounds.

3.3.3.9 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences were estimated by the analysis of variance (ANOVA) followed by Tukey's "Honest Significant Difference" test. Differences were considered significant at $p \leq 0.05$. Correlation analysis was performed between phenolic contents and antioxidant activity of soluble and bound extracts using Pearson correlation. All statistical analyses were performed using the free statistical software SPSS 13.0 version (SPSS Inc., Chicago, IL).

3.4 Results and discussion

The yields of extracts of raw, low temperature (LT), and high temperature (HT) roasted whole cashew nut, cashew nut kernel, and cashew nut testa are shown in **Table 3.1**. The highest yield of the soluble and insoluble bound phenolic extracts of 44.2 ± 1.4 and 9.63 ± 0.1 g/100 g of defatted meal was afforded by the HT processed cashew testa, respectively, whereas the lowest values of 23.1 ± 1.2 and 0.18 ± 0.001 g/100 g of defatted meal were observed, respectively, for raw cashew kernels. Higher yields were obtained for both soluble and bound extracts of the cashew nut testa. These results are in agreement with those of hazelnut kernel and byproducts (Shahidi *et al.,* 2007). To the best of our knowledge this is the first study that determines the soluble and insoluble bound phenolic contents and corresponding antioxidant activities of thermally processed cashew nut and their testa as a byproduct.

3.4.1 The total phenolic contents (TPC)

The contents of total phenolic compounds of soluble and insoluble bound cashew extracts obtained with different processing conditions are shown in **Table 3.1**. The data were expressed as mg of gallic acid equivalents (GAE) per g of defatted meal. The phenolic contents of soluble and insoluble bound extracts of raw, LT and HT treated cashew nuts and testa ranged from 1.14 ± 0.43 to 348.99 ± 6.88 and 0.03 ± 0.01 to 4.53 ± 0.12, respectively.

The results of this study showed that contribution of bound phenolic fraction to the total phenolic content of the cashew nuts and testa was not prominent and the values ranged from 0.5 to 2% and 0.6 to 1.7 % for raw and HT treated samples, respectively. In contrast to the results obtained in this study, Yang *et al.* (2009) reported a high contribution (72%) of insoluble bound form to the total phenolic content of cashew nut kernels.

Raw cashew kernel showed the least TPC whereas HT treated testa showed the highest. In general, raw as well as LT and HT treated cashew testa showed higher TPC than that of kernel (**Table 3.1**). It is reported that the outer layers such as peels, shells, and hulls or skin of plant materials contain higher phenolic content, thus acting as defence substances against pathogens, parasites, and predators, as well as contributing to the colour of plants (Hartley *et al.*, 1990).

In this study, soluble phenolic extracts of HT treated cashew nuts and testa showed a significant ($p \leq 0.05$) increase, which ranged from 29 to 372% in TPC than these of their raw counterparts. Furthermore, HT treated bound phenolic extracts also showed

significantly ($p \leq 0.05$) higher phenolic contents than these of their raw counterparts that ranged from 173 to 234%. The TPC of soluble phenolic extracts of LT treated cashew nuts and testa ranged from 4.89 ± 0.84 to 308.52 ± 9.53 mg of GAE / g of defatted meal. The soluble phenolic extracts of LT treated kernel and testa showed significantly ($p \leq 0.05$) higher TPC that ranged from 14 to 344 % compared to their raw counterparts. In addition, bound phenolic extracts of LT treated cashew nuts and testa showed significantly ($p \leq 0.05$) higher TPC compared to raw counterparts and the increment ranged from 153 to 223 %.

According to the results obtained in the present study, thermal processing increased the TPC of cashew nuts and their testa. These results are in agreement with similar studies conducted using other types of nuts such as peanuts and hazelnuts (Yu *et al.*, 2005; Locatelli *et al.*, 2010). Yu *et al.* (2005) showed that roasting (175^0 C for 5 min) increased the TPC of peanut skin by 40% compared to the raw peanut skin. According to Locatelli *et al.* (2010) high roasting conditions (180^0C 20 min.) brought about higher TPC of the soluble phenolic extract, than that of medium roasting (180^0C 10 min.) of hazelnut skin. Talcott *et al.* (2005) found that the TPC of peanuts (testa removed) increased or decreased depending on the cultivar upon roasting at 175^0 C for 10 min.

In the present study, roasting at low and high temperatures resulted in higher TPC compared to raw cashew nuts and testa. This could be attributed to the liberation of cashew phenolics during roasting, which could be more soluble in ethanol. Jeong *et al.* (2004) showed that the content of phenolic compounds of defatted sesame meal extract

submitted to different roasting temperatures increased, probably due to the release of bound phenolic compounds.

On the other hand, during heat treatment, a reaction between reducing sugars and amino acids, known as the Maillard reaction, can take place, thus leading to the formation of a variety of byproducts, intermediates and brown pigments (melanoidins) which may contribute to the TPC, flavour, antioxidative activity and colour of food. The reaction is favoured by low water activity during roasting of nuts, pulses and seeds. The intermediate Maillard reaction products (MRPs) as well as the resultant melanoidins have high antioxidant activities, which are related to the presence of reductone-type structures (Hayase *et al.,* 1989). Thus, in addition to phenolics, other compounds such as MRPs present in the extracts of roasted samples could interfere with the determination of TPC by Folin Ciocalteu's assay, giving higher values compared to the raw samples in the present study (Sahin *et al.,* 2009). Due to the fact that cashew nuts and their skins contain protein and sugars (Nagaraja, 2000;Venkatachalam & Sathe, 2006), formation of Millard browning products in the cashew skin and kernel during roasting could be possible. It appears that roasting conditions as well as type of nuts affect the TPC of the extracts of the skins. Monagas *et al.* (2009) reported that the TPC of roasted (145^0 C 30 min) peanut, hazelnut, and almond skins were 371, 315 and 134 mg of GAE / g of sample, respectively. In the present study HT treated cashew skin showed 348 mg of GAE / g of defatted meal which is in the range of the values reported by Monogas *et al.* (2009) .

3.4.2 Proanthocyanidins content (PC)

The proanthocyanidins, also known as condensed tannins, are flavan-3-ol oligomers / polymers, occurring in a wide variety of foods including berries, red wines, and nuts (Hammerstone *et al.*, 2000). Venkatachalam and Sathe (2006) reported that cashew contained 40 mg of tannin per 100g of edible nut.

Proanthocyanidin contents of different cashew extracts obtained from raw and samples roasted under different conditions are shown in **Table 3.1.** The PC of soluble and insoluble bound phenolic extracts ranged from 0.11 ± 0.001 to 23.89 ± 0.51 and 0.0016 ± 0.0001 to 0.3077 ± 0.0003 mg catechin equivalents (CE) per g of defatted meal, respectively. In general, roasting significantly ($p \leq 0.05$) decreased the content of PC of soluble and bound phenolic extracts of cashew nuts as well as their testa and this reduction ranged from 6 to 42 and 7 to 34% for soluble and bound extracts, respectively.

The results of the present work showed that heat processing decreased the assayable tannin content and this may partly be due to the degradation / polymerization of tannins. Tan *et al.* (1984) reported that dry heat treatment of winged beans reduced the tannin levels by 56-75%. In accordance with the results of the present study, Sze-Tao *et al.* (2001) also showed that tannin content of walnuts which were thermally processed (204 °C for 5 min) decreased by 14% compared to that of their unroasted counterparts. Furthermore, Gentile *et al.* (2007) showed that roasting of pistachios decreased their proanthocyanidin content by 12 % compared to the raw nuts.

3.4.3 DPPH radical scavenging capacity

DPPH is a synthetic organic radical frequently used to evaluate antiradical properties of bioactive compounds and food extracts. It is more stable than common natural radicals and unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. The assay is based on the measurement of the reducing ability of antioxidants toward DPPH, which can be monitored by measuring the decrease in the absorption intensity of the EPR signal or absorption at 517 nm.

DPPH radical scavenging activity of raw and roasted cashew nuts and testa extracts are presented in **Table 3.2.** DPPH radical scavenging activity of soluble and insoluble bound extracts of raw cashew nuts and testa ranged from 3.17 ± 0.15 to 179.3 ± 1.14 and 0.13 ± 0.01 to 81.16 ± 5.38 mg of GAE per g of defatted meal, respectively. The DPPH radical scavenging activity of soluble phenolic extracts of kernel and testa significantly ($p \leq 0.05$) increased with the roasting temperature whereas bound phenolic extracts generally showed a decrease. The soluble phenolic extracts of HT treated testa showed a higher DPPH radical scavenging activity than that of LT treated testa. However, according to Locatelli *et al.* (2010) there was no significant ($p \leq 0.05$) difference between the soluble phenolic extracts of medium and high temperature treated hazelnut skin. This could be due to the varietal differences of nuts, the content of phenolics therein and processing conditions employed.

The DPPH radical scavenging activity of soluble phenolic extracts of cashew nuts and byproducts highly correlated with TPC ($r^2 = 0.943$; $p<0.0001$) and PC ($r^2 = 0.966$; $p<0.0001$). Furthermore, DPPH radical scavenging activity of bound phenolic extracts

positively and significantly correlated with their corresponding TPC (r^2 = 0.999; $p<0.0001$) and PC (r^2 = 0.997; $p<0.0001$). This study clearly demonstrated that roasting of cashew has a significant effect on the DPPH radical scavenging activity of the extracts of nuts and their testa and this could be attributed to their phenolic contents as well as MRPs present.

3.4.4 Oxygen radical absorbance capacity (ORAC)

The present study showed that oxygen radical absorbance capacity of soluble and insoluble bound phenolic fractions of cashew extracts were different and depended on whether raw or roasted nuts were considered (**Table 3.2).** The ORAC of soluble phenolic extracts of HT treated cashew kernels and testa showed significantly ($p \leq 0.05$) higher values compared to their raw counterparts. The ORAC values obtained in the present study showed the same trend as TPC and PC. There was a strong positive relationship between TPC and ORAC (r^2 = 0.977; $p<0.0001$) as well as PC and ORAC (r^2 = 0.972; $p<0.0001$) in soluble phenolic extracts of cashew nuts and testa. According to Monogas *et al.* (2009) ORAC values of roasted peanuts, hazelnuts and almonds skins were 13.3, 14.5 and 4.03 mmoles of TE/g of sample. However, in the present study ORAC of HT treated cashew testa showed a higher value (74 mmoles of TE/g of defatted meal) than those reported in other studies for roasted nut skins. Davis *et al.* (2010) reported a 14% higher ORAC value for whole roasted blanched peanuts than that of the raw sample whereas findings of the present study showed an 8% higher ORAC value for HT treated whole cashew nuts compared to their raw counterpart.

3.4.5 Hydroxyl radical scavenging capacity

Hydroxyl radical is generated through Fenton reactions in the presence of Fe^{2+} and H_2O_2 and may be spin-trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) due to the very short life of the radical. The DMPO-adduct, a relatively stable free radical, can easily be detected using electron paramagnetic resonance (EPR) spectroscopy.

The hydroxyl radical scavenging capacities of cashew extracts obtained under different processing conditions are shown in **Table 3.2**. Hydroxyl radical scavenging capacity of soluble and insoluble bound phenolic samples ranged from 18.5 ± 1.21 to 109.15 ± 7.17 and 6.89 ± 0.0 to 684.67 ± 1.78 mg of CE per gram of defatted meal, respectively. Interestingly, roasting did not change hydroxyl radical scavenging capacity of whole cashew nut and testa significantly ($p \leq 0.05$) except for the soluble phenolic extract of cashew kernel which showed a 2 fold increase compared to its raw counterpart. The present analysis showed that extracts of whole cashew nut and testa contained a higher amount of flavonoids such as catechin, epicatechin and epigallocatechin which may have prooxidative effects at high concentrations, specially in the presence of transition metal ions (Rodriguez *et al.,* 2001). Thus, although the content of phenolic compounds of roasted cashew extracts increased, as determined by Folin Ciocalteu's assay and HPLC analysis, all phenolics present may not contribute to hydroxyl radical scavenging activity in the extracts. However, as cashew kernel contained comparatively low content of flavonoids this effect may not be prominent and may exert high hydroxyl radical scavenging capacity as observed in the present study. To the best of our knowledge this

is the first study that showed the effect of roasting on the hydroxyl radical scavenging activity of cashew nuts and testa.

3.4.6 Trolox equivalent antioxidant activity (TEAC)

The 2,2′-azinobis-(3-ethylbenzothiazoline -6-sulphonate) (ABTS) solution is oxidized by an oxidizing agent, leading to the formation of $ABTS^{\bullet-}$, which is intensely coloured. The antioxidative capacity of test compounds is assessed by measuring their ability to decrease the colour reacting directly with the ABTS radical. The $ABTS^{\bullet-}$ can be generated chemically by oxidizing $ABTS^{2-}$ using ferrylmyoglobin, magnesium oxide, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH), potassium persulphate or through enzymatic reactions. However, the use of oxidizing agents in the assay medium allows the antioxidant compounds to directly react with them, thus leading to erroneous estimations (Prior *et al.*, 2003).

The TEAC values of tested cashew samples are summarized in **Figure 3.1**. The soluble extracts of LT treated cashew testa yielded the highest TEAC value of 880 ± 33 μmoles of TE /g of defatted meal whereas that of raw kernel had the least value of 38.9 ± 1.0 μmoles of TE /g of defatted meal. Consistent with the results obtained for other antioxidant assays employed in this study, the soluble phenolic extracts, showed high TEAC values for whole cashew, kernel and testa which were 15, 57 and 21 times higher than these of their bound counterparts, respectively. Pellegrini *et al.* (2006) reported that TEAC values of soluble phenolic extracts of hazelnuts, pistachios, almonds and walnuts were 1.3, 1.5, 2.2 and 7 times higher than those of their bound extracts, respectively. The analyses in the present study are also in agreement with TEAC values of soluble

extracts reported for thermally processed cashew nuts at 150 °C for 60 min (Acar *et al.*, 2009). In the present study, TEAC of HT treated cashew kernels showed a 1.3 times higher value compared to the raw kernels. Acar *et al.* (2009) also reported a 1.6 times increase in TEAC values of HT treated cashew kernels compared to that of its unroasted counterparts. In addition, Yu *et al.* (2005) reported that roasting increased the TEAC value of peanut skin compared to its raw counterpart due to the increase of phenolic content during thermal processing. The present analysis showed a direct strong relationship between TPC and TEAC ($r^2 = 0.991$; $p<0.0001$) as well as PC and TEAC ($r^2 = 0.993$; $p<0.0001$) of cashew extracts further confirming the findings of others (Jeong *et al.*, 2004, Yu *et al.*, 2005).

3.4.7 Reducing power

The reducing power of an extract serves as a good indicator of its antioxidative activity (Zou *et al.*, 2004). Ascorbic acid was used as a standard in this study and the results were expressed as μmoles of ascorbic acid equivalents (AAE) per g of defatted meal. **Figure 3.2** depicts the reducing power of different cashew extracts examined in this study. Among the soluble phenolic extracts HT treated cashew testa had the highest reducing power of 2394 ± 120 μmoles of AAE per g of defatted meal whereas raw kernel showed the least of 9.5 ± 0.23 μmoles of AAE per g. Insoluble bound phenolic extracts of cashew kernels and by-products showed a lesser reducing power compared to their soluble counterparts. The reducing power of the extracts followed a similar trend as TPC reported in this study. Furthermore, the correlation analysis showed a strong positive association between reducing power and TPC ($r^2 = 0.972$; $p<0.0001$) and reducing power

and PC (r^2 = 0.963; $p<0.0001$) in this study. Thus phenolics present in the extracts demonstrated a substantial reducing power due to their ability to donate electrons or to terminate radical chain reactions by converting free radicals to stable products. In addition, enhancement in the reducing power by roasting could be due to the formation of new reductones during heat treatment.

3.4.8 Phenolic compounds in soluble cashew extracts

Major phenolic acids and flavonoids identified in soluble extracts of raw and roasted cashew nuts and testa are presented in **Table 3.3**. A typical chromatogram obtained for HT treated cashew whole nut is depicted in **Figure 3.3**. In general the predominant phenolic acids identified in cashew whole nuts and testa were syringic, gallic and *p*-coumaric acids. However, this work showed that only trace amounts of syringic and *p*-coumaric acids were present in cashew kernels whereas testa was a rich source of all three phenolic acids identified. The contents of syringic, gallic and *p*-coumaric acids were 2.507, 0.361 and 0.252 mg/g of defatted raw testa meal, respectively. Thermal processing affected the content of phenolic acids present in cashew soluble extracts. Thus, HT treated cashew kernels had a significantly ($p \leq 0.05$) lesser content of gallic acid compared to the raw kernel. Conversely, testa of HT treated cashew nuts had about 3 times higher gallic acid content compared to its raw counterpart suggesting liberation of gallic acid during heat processing. Pillai *et al.* (1963) reported that cashew nut testa contained a considerable amount of hydrolysable tannins. Thus, it is possible that roasting may yield gallic acid from hydrolysable tannins present, leading to a higher content of it in the HT treated testa as shown in the present study. Earlier, Shahidi *et al.*

(2007) reported five phenolic acids, namely gallic, caffeic, *p*-coumaric, ferulic and sinapic acids in hazelnut kernel and its by-products. In almond and its by-products, Wijeratne *et al.* (2006) showed the presence of caffeic, *p*-coumaric, ferulic and sinapic acids. Nevertheless, the results of the present study showed that cashew nuts had syringic acid as a predominant phenolic acid in the samples tested. Senter *et al.* (1983) reported the presence of 0.23 µg of syringic acid per g of extract of pine nuts. Walnuts also contained a considerable amount (34 mg /100g kernel) of syringic acid (Colaric *et al.*, 2005).

Major flavonoids identified in the present study were catechin followed by epicatechin and epigallocatechin. The contents of catechin, epicatechin and epigallocatechin in defatted meals of raw cashew nut kernel and testa were 0.70, 0.09, 1.64 and 47.28, 28.29 2.0 mg/ g, respectively. These results suggest that cashew testa which is a byproduct of cashew processing has a significant nutraceutical importance due to its high content of polyphenolic compounds, including flavonoids. It is well established that flavonoids are effective natural antioxidants (Shahidi & Naczk, 2004). In agreement with the present results, Mathew and Parpia (1970) previously reported the presence of catechin and epicatechin as predominant polyphenolics in cashew testa. In general, HT treated testa had a higher flavonoid content, which showed a 2- 4 fold increase when compared to the raw testa. The results obtained in the HPLC analysis suggest liberation and isomerization of such compounds during heat treatment of cashew nuts and testa. This fact gains support as it corresponds to a significant ($p \leq 0.05$) decrease in tannin content in HT treated cashew testa (**Table 3.1**). Furthermore, Yu *et al.* (2006) showed that roasting

decreased proanthocyanidin (trimers and tetramers) of peanut skin and increased its monomers content when compared to the raw skin.

3.5 Conclusions

The results of the present study indicate that cashew nut kernels and testa constitute phenolic compounds that are responsible for a wide array of antioxidant activities. The contribution of bound fraction is insignificant ($p \leq 0.05$) compared to the soluble phenolic fraction of cashew nuts and testa. The HT treated cashew nuts and testa showed a higher phenolic content and antioxidant activity than LT treated samples. Overall, the findings of this study suggest that thermal processing enhances the antioxidant value of cashew kernels. Furthermore, it is noteworthy that cashew testa, a waste byproduct can be utilized, as a health-promoting and disease-preventing nutraceutical ingredient.

Table 3.1: Extract yield, total phenolic content, and proanthocyanidin content of cashew nut kernel and testa from different processing conditions

Processing condition	Soluble phenolics			Bound phenolics		
	Whole	Kernel	Testa	Whole	Kernel	Testa
Extract yield (g / 100g of defatted meal)						
Raw	27.2±1.10[a]	23.1±1.20[a]	42.9±0.90[a]	5.72±0.02[a]	0.18±0.01[a]	7.32±0.15 [a]
LT treated	26.3±1.20[a]	25.3±0.40[b]	43.9±1.10[a]	0.62±0.01[b]	0.75±0.02[a]	8.98±0.03[b]
HT treated	27.0±2.40[a]	25.1±0.30[b]	44.2±1.40[a]	0.34±0.01[c]	3.59±0.10[a]	9.63±0.12[c]
Total phenolic content (GAE mg / g of defatted meal)						
Raw	07.01±1.20[a]	01.14±0.43[a]	269.05±9.77[a]	0.06±0.01[a]	0.028±0.01[a]	1.36±0.10[a]
LT treated	08.88±0.19[a]	04.89±0.84[b]	308.51±9.35[a]	0.16±0.01[b]	0.082±0.01[b]	4.26±0.15[b]
HT treated	30.24±3.97[b]	05.28±1.00[b]	347.99±6.88[b]	0.18±0.01[b]	0.089±0.01[b]	4.53±0.12[b]
Proanthocyanidins content (CE mg /g of defatted meal)						
Raw	2.58±0.04[a]	0.11±0.01[a]	23.89± 0.50[a]	0.03±0.01[a]	0.01±0.01[a]	0.31±0.01[a]
LT treated	1.53±0.02[b]	0.12±0.01[a]	22.64± 0.36[b]	0.03±0.01[a]	0.01±0.01[a]	0.29±0.01[b]
HT treated	1.50±0.01[b]	0.13±0.01[a]	22.57± 0.45[b]	0.03±0.01[b]	0.01±0.01[b]	0.21±0.04[c]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same superscript letter, within a column are not significantly different ($p > 0.05$). GAE, gallic acid equivalents; CE, catechin equivalents; LT, low temperature treated; and HT, high temperature treated.

Table 3.2: Free radical-scavenging capacities of extracts of cashew nut kernel and testa from different processing conditions

Processing condition	Soluble phenolics			Bound phenolics		
	Whole	Kernel	Testa	Whole	Kernel	Testa
	DPPH scavenging activity (GAE mg/g of defatted meal)					
Raw	65.35±2.24[a]	3.17±0.15[a]	179.29±1.14[a]	5.07±0.32[a]	0.13±0.01[a]	81.16±5.38[a]
LT treated	65.61±1.10[a]	36.92±1.50[b]	640.51±38.20[b]	4.95±0.38[a]	0.27±0.04[b]	73.32±3.04[a]
HT treated	74.86±6.51[a]	58.14±2.84[c]	708.49±6.32[c]	4.68±0.45[a]	0.12±0.00[a]	33.07±1.65[b]
	ORAC activity (TE µmoles per g of defatted meal)					
Raw	14089±1651[a]	3207±209[a]	54171±2900[a]	0.002±0.001[a]	0.012±0.001[a]	0.026±0.003[a]
LT treated	14796±366 [a]	3925±173[a]	62159±1591[b]	0.001±0.001[a]	0.025±0.002[b]	0.023±0.001[a]
HT treated	15207±904[a]	4136±536[b]	74088±2956[c]	0.016±0.006[b]	0.020±0.002[c]	0.046±0.002[b]
	OH radical scavenging (CE µmoles/g of defatted meal)					
Raw	19.69±0.25[a]	23.70±0.88[a]	1091.52±71.7[a]	68.75±0.17[a]	6.89±0.00[a]	679. 14± 5.53[a]
LT treated	18.50±1.21[a]	46.54±3.56[b]	1090.64±72.7[a]	68.97±0.15[a]	6.92±0.02[a]	684.67± 1.78[a]
HT treated	18.73±0.79[a]	44.99±1.17[b]	1021.41±91.7[a]	69.44±1.09[a]	6.90±0.02[a]	684.24±13.65[a]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same superscript letter, within a column are not significantly different ($p > 0.05$). GAE, gallic acid equivalents; CE, catechin equivalents; TE, trolax equivalents; LT, low temperature treated; and HT, high temperature treated.

Table 3.3: Major soluble phenolic compound identified from cashew nut kernel and testa from different processing conditions

Processing condition	Gallic acid	Syringic	*p* - Coumaric	Catechin	Epicatechin	Epigallocatechin
			Cashew whole (mg/g of defatted meal)			
Raw	0.108 ± 0.005[a]	0.613 ± 0.001[a]	0.099 ± 0.012[a]	11.733 ± 0.254[a]	7.429 ± 0.140[a]	4.459 ± 0.123[a]
LT treated	0.098 ± 0.000[b]	0.483 ± 0.011[b]	0.073 ± 0.002[b]	9.608 ± 0.153[a b]	6.083 ± 0.044[b]	4.208 ± 0.087[b]
HT treated	0.251 ± 0.000[c]	0.867 ± 0.001[c]	0.112 ± 0.002[a]	15.646 ± 0.276[c]	8.368 ± 0.001[c]	6.544 ± 0.023[c]
			Cashew kernel (mg/g of defatted meal)			
Raw	0.215 ± 0.002[a]	Trace	Trace	0.702 ± 0.018[a]	0.095 ± 0.007[a]	1.640 ± 0.019[a]
LT treated	0.037 ± 0.001[b]	Trace	Trace	1.888 ± 0.007[b]	0.257 ± 0.002[b]	0.504 ± 0.008[b]
HT treated	0.065 ± 0.002[c]	Trace	Trace	2.912 ± 0.064[c]	0.437 ± 0.009[c]	0.481 ± 0.000[b]
			Cashew testa (mg/g of defatted meal)			
Raw	0.361 ± 0.005[a]	2.507 ± 0.009[a]	0.252 ± 0.000[a]	47.289 ± 3.760[a]	28.291 ± 0.081[a]	2.005 ± 0.061[a]
LT treated	0.437 ± 0.001[b]	2.800 ± 0.009[b]	0.337 ± 0.001[b]	45.235 ± 2.444[a]	28.292 ± 0.086[a]	2.251 ± 0.104[a]
HT treated	0.974 ± 0.030[c]	5.705 ± 0.000[c]	0.693 ± 0.043[c]	109.012 ± 0.932	77.045 ± 2.144[b]	4.065 ± 0.159[b]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same superscript letter, within a column are not significantly different ($p > 0.05$). LT, low temperature treated; and HT, high temperature treated.

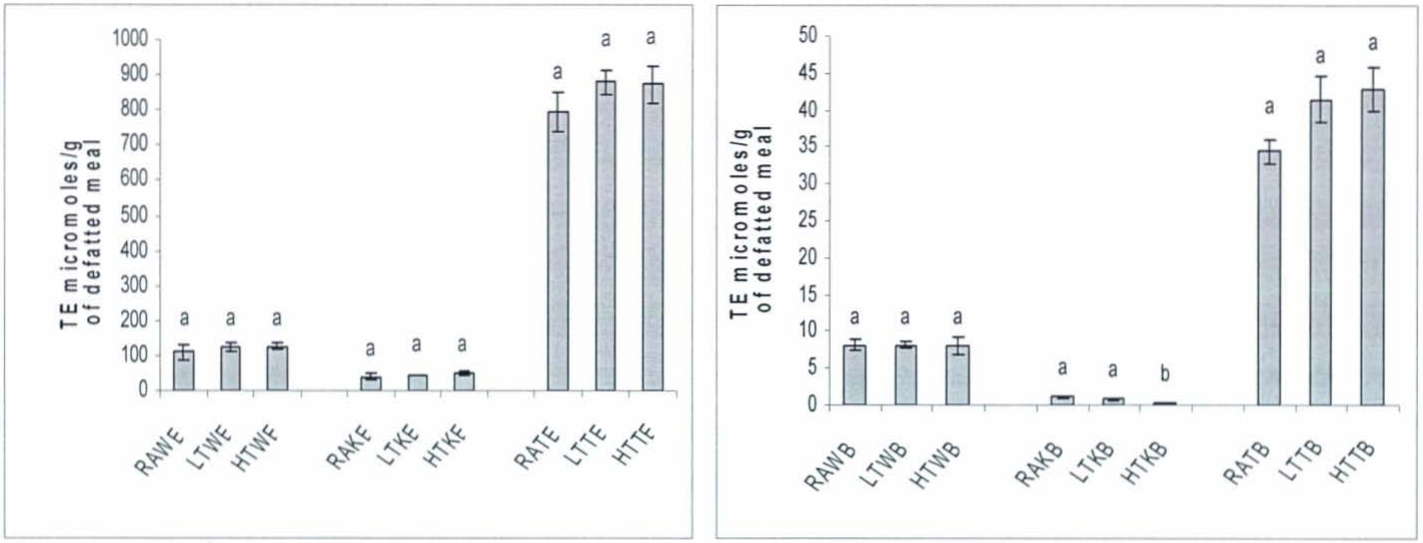


Figure 3.1: Trolox equivalent antioxidant activity of soluble and bound extracts of different cashew products

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). TE, trolox equivalents; RAWE, raw whole nut soluble; RAKE, raw kernel soluble; RATE, raw testa soluble; LTWE,low temperature whole soluble; LTKE, low temperature kernel soluble; LTTE, low temperature testa soluble; HTWE, high temperature whole soluble; HTKE, high temperature kernel soluble; HTTE, high temperature testa soluble; RAWB, raw whole nut bound; RAKB, raw kernel bound; RATB,raw testa bound; LTWB, low temperature whole bound; LTKB, low temperature kernel bound, LTTB, low temperature testa bound; HTWB, high temperature whole bound; HTKB, high temperature kernel bound; and HTTB, high temperature testa bound.

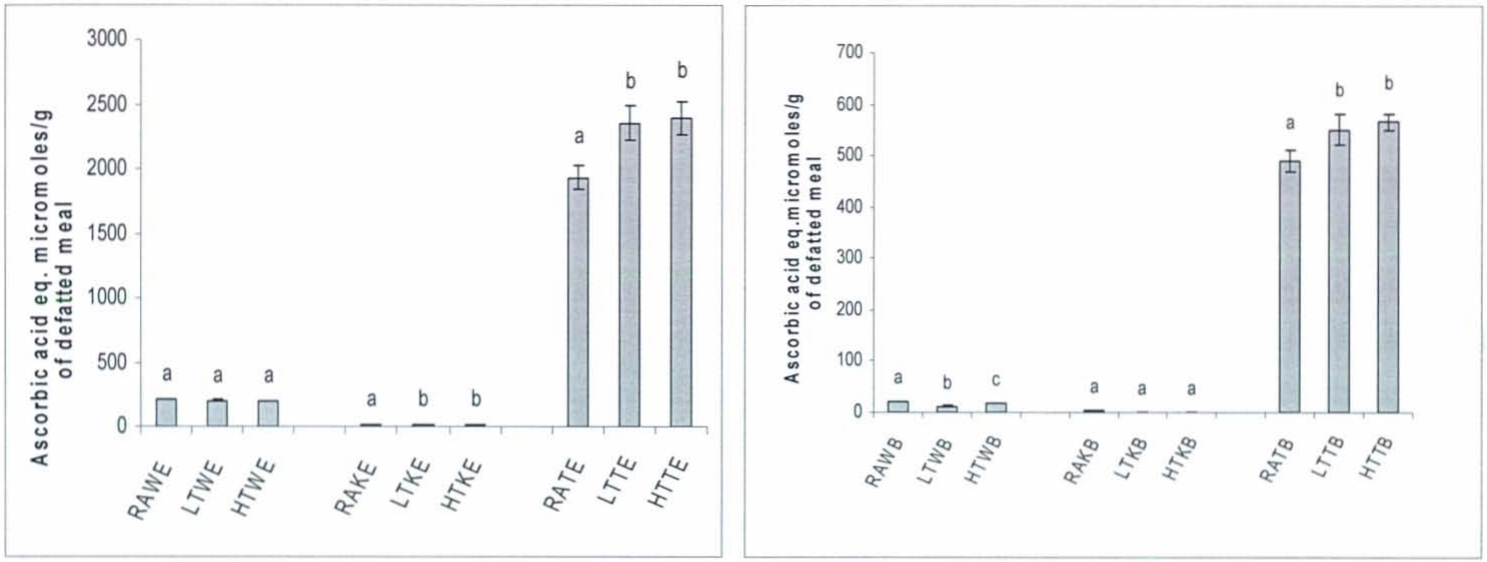


Figure 3.2: Reducing power of soluble and bound extracts of different cashew products

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). RAWE, raw whole nut soluble; RAKE, raw kernel soluble; RATE, raw testa soluble; LTWE, low temperature whole soluble; LTKE, low temperature kernel soluble, LTTE, low temperature testa soluble; HTWE, high temperature whole soluble; HTKE, high temperature kernel soluble; HTTE, high temperature testa soluble; RAWB, raw whole nut bound; RAKB, raw kernel bound; RATB, raw testa bound; LTWB, low temperature whole bound; LTKB, low temperature kernel bound, LTTB, low temperature testa bound; HTWB, high temperature whole bound; HTKB, high temperature kernel bound; and HTTB, high temperature testa bound.

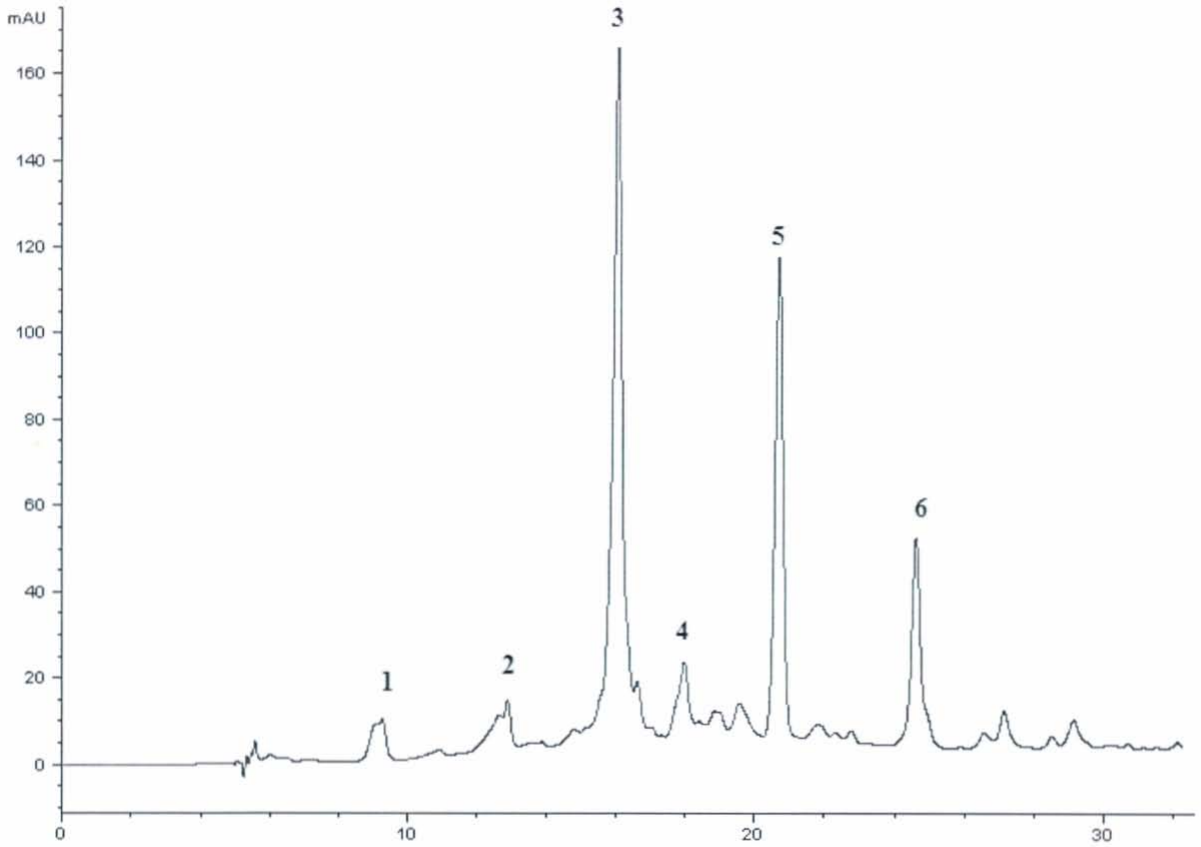


Figure 3.3: HPLC profile of major phenolic compounds identified for soluble extracts of high temperature treated cashew whole nuts

1, gallic acid; 2, epigallocatechin; 3, catechin; 4, epicatechin; 5, syringic acid; and 6, *p*-coumaric acid.

Chapter 4

Antioxidative potential of cashew phenolics in food and biological model systems as affected by roasting

4.1 Abstract

The effect of different roasting conditions on antioxidant capacity of phenolics of cashew nuts and their testa was evaluated using several food and biological model systems. Total phenolic content was determined by Folin Ciocalteu's assay. Accelerated oxidative stability of stripped corn oil in the presence of cashew extracts was determined using the Rancimat method. Antioxidant activity of extracts obtained under high and low temperature roasting conditions was assessed in a β-carotene linoleate and a cooked comminuted pork model system. Inhibition of oxidation of human low density lipoprotein (LDL) cholesterol and stand breaking of supercoiled deoxyribonucleic acid (DNA) was also investigated. In general, whole cashew nuts and testa extracts demonstrated stronger antioxidant activity than that of cashew kernel except for the inhibition of LDL cholesterol oxidation. In general, roasted cashew showed considerable antioxidative efficiency, in model systems employed in this study, however, the effect was not significantly ($P \leq 0.05$) different from that of their raw counterparts, except for the Rancimat assay. The results suggest that whole cashew nut and testa extracts could be used as a potential source of natural antioxidants in certain food applications and for disease risk reduction.

Key words: β-carotene linoleate, cooked comminuted pork model, DNA scission inhibition, LDL oxidation, Rancimat

4.2 Introduction

A general consensus has been reached during the last couple of decades that diet plays a major role in the development or control of chronic diseases, such as cancer, coronary heart disease, obesity, and diabetes. The recommendations, which are mainly based on epidemiological studies, show that fruits, vegetables, grains, nuts, and less processed staple foods provide the best protection against the development of diseases with little or no merit in recommending vitamin or other micronutrient supplements for disease prevention (Jacobs *et al.*, 1998; Willett, 1999; Alasavar & Shahidi, 2009).

Nuts have been part of the human diet for a long time and valued as an alternative source of protein and lipid. Recently, many nuts have been identified as a rich source of antioxidants. Nuts therefore constitute one of the most nutritionally concentrated kind of food available. Most nuts, left in their shell, have a remarkably long shelf-life and can conveniently be stored for winter use (Halvorsen *et al.,* 2002; Shahidi & Naczk, 2004; Wu *et al.,* 2004).

Nuts have favourable effects on cardiovascular diseases through several possible mechanisms. These effects may be mediated by their fatty acid profiles, fibre or antioxidant contents, or by a combination effect of compound presents. Several recent studies have shown that nut antioxidants have interesting biological effects that may be related to their favourable influence on cardiovascular disease (Shahidi & Naczk, 2004; Wijerathne *et al.,* 2006).

Among tropical nuts, cashew (*Anacardium occidentale*, L) plays an important role as an edible nut. The nut is valued due to its kernel which is the principal industrialized

product. The kernels are rich in lipids (42.6%) and proteins (20.0%) and processed kernels are consumed as snacks in the roasted and salted forms (Sathe, 1994). These are also used in the preparation of a variety of food products such as cakes, sweets, ice cream, biscuits and chocolates to incorporate their characteristic taste (Sathe, 1994). Quantitative determination of the major phenolic lipids in cashew apple, kernels, and shells of cashew nut at various stages of development suggested the possibility of fatty acid type biogenesis of these phenolic lipids (Shobha *et al.,* 1992). Ryan *et al.* (2006) reported the presence of unsaturated fatty acids and phytosterols in cashew nuts. Recently, the antioxidant activities of various bioactive compounds such as phenolics, flavonoids, phospholipids, sphingolipids, sterols, and tocopherols were reported in cashew nut samples (Miraliakbari & Shahidi, 2008a; Yang *et al.,* 2009). Furthermore, the ethanolic extract of cashew nut testa exhibited significant antioxidant activity (Kamath & Rajini, 2007); the polyphenolic compounds present in the testa appear to contribute to the observed antioxidant activity (Sajilata & Singhal, 2006).

Dietary antioxidants are believed to play a significant role in human health by prevention of radical damage to biomolecules such as DNA, ribosenucleic acid (RNA), proteins, and cellular organelle. Therefore, there is an increasing interest in identifying and assessing commonly consumed foods that contain bioactives with a potential to inhibit free radical damage. Polyphenolics have been shown to possess free radical-scavenging and metal-chelating activities, in addition to their reported anticarcinogenic properties (Middleton, 1998). These plant-based, non-nutrient phytochemicals may have a protective effect on the susceptibility of LDL cholesterol to oxidative modification and ultimately, on atherosclerosis. The oxidative modification and inflammation hypothesis of

atherogenesis is widely accepted and supported by experimental data in hypercholesterolemic animal models and human epidemiologic studies, as reviewed by Chisolm and Steinberg (2000). Atherogenesis is a multifactorial process that includes oxidatively modified LDL cholesterol, which triggers pathological events through multiple pathways, leading to atherosclerosis (Berliner & Heinecke, 1996).

Even though few reports are available on the presence of some bioactive compounds in cashew nut kernels, to the best of our knowledge there is no information on the effect of various heat processing methods on the levels of bioactive compounds and their antioxidant activity in different food and biological model systems. Hence, the present study was carried out to determine the antioxidative potential of cashew nut and testa in food and biological model systems as affected by low and high temperature roasting treatments to identify suitable and effective heat processing methods, which maximize the health beneficial bioactive compounds.

4.3 Materials and methods

4.3.1 Materials

Raw shelled cashew with testa were obtained from the Green Field Bio Plantation (Pvt.) Ltd., Colombo, Sri Lanka. Folin Ciocalteu's reagent, sodium carbonate, gallic acid, stripped corn oil, butylated hydroxyanisole (BHA), trichloroacetic acid (TCA), thiobarbituric acid (TBA), malonaldehyde, β-carotene, linoleic acid, Tween 40, human low density lipoprotein (LDL) cholesterol, ethylenediaminetetraacetic acid trisodium salt (Na_3EDTA), mono- and dibasic potassium phosphates, sodium chloride (NaCl), PBR 322 Plasmid DNA, 2,2'-azobis(2-methylpropionamidine) dihydrochloride (AAPH), bromophenol blue, xylene cyanol, and sucrose, were purchased from Sigma-Aldrich

Canada Ltd. (Oakville, ON). Hexane, methanol, ethanol, sodium hydroxide, hydrochloric acid, diethyl ether, ethyl acetate, and sodium carbonate, were purchased from Fisher Scientific Ltd. (Ottawa, ON). SYBR safe gel stain was purchased from Probes Invitrogen, Eugene, OR, USA.

4.3.2 Sample preparation

Two different processing temperatures were used in this study. For low temperature processing (LT) raw whole cashew nuts (kernel with testa) were roasted in a forced hot-air convection oven at 70^0 C for 6 hours. In this, cashew kernels weighing approximately 100 g were spread in a single layer on a stainless steel wire mesh tray placed in the centre of the oven during hot-air roasting. After roasting, the hot cashew kernels were cooled in a dessicator at room temperature, and kept in sealed plastic bags at 4^0C, until further analysis. Under industrial cashew processing operations, both small and large scale cashew producers use these conditions to obtain good quality products (Hebbar *et al.*, 2005). For high temperature processing (HT), raw whole cashew nuts were roasted in a forced hot-air convection oven at 130^0 C for 33 min. This temperature and time combination was the optimum roasting conditions for cashew kernels based on hedonic sensory evaluations according to Wanlapa and Jindal (2006). Raw whole cashew nuts were used as the control to compare the effect of two different roasting conditions.

The raw whole and roasted whole nuts were peeled manually to remove the testa. Raw and roasted whole cashew nuts, kernels and recovered testa were ground separately using a coffee bean grinder (Model CBG5 series, Black & Decker, Canada Inc. Brockville, ON) to obtain a fine powder which passed through mesh 16 (sieve opening 1mm, Tylor test

sieve, Mentor, OH). Each sample was defatted by blending with hexane (1:5, w/v, 5 min, 3 x) in a Waring blender (Model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT) at ambient temperature. Defatted samples were vacuum packed in polythene pouches after air drying at room temperature for 12 h and stored at -20°C until used for extraction of phenolics.

4.3.2.1 Extraction of soluble phenolic compounds

Preliminary studies showed that heating of ground samples under reflux conditions with 80% (v/v) ethanol afforded high total phenolic content and antioxidant activity. Therefore, reflux conditions were used to extract soluble phenolic compounds (Shahidi *et al.*, 2007). Defatted meal (6 g) was mixed with 100 mL of 80% (v/v) ethanol and then placed in a thermostated water bath at 60°C for 40 min. After centrifugation of the resulting slurry for 5 min at 4000 x g (IEC Centra MP4, International Equipment Co., Needham Heights, MA), the supernatant was collected and extraction was repeated two more times. Combined supernatants were evaporated in *vaccuo* at 40°C (Buchi, Flawil, Switzerland) and lyophilized for 72 h at -46°C and 34 x 10^{-3} mbar (Freezone, Model 77530, Labconco Co., Kansas City, MO).

4.3.3 Methods

4.3.3.1 Determination of total phenolic content (TPC)

The contents of total phenolics of the extracts were determined according to a modified version of the procedure described by Singleton and Rossi (1965). The crude extracts of soluble phenolic compounds were dissolved in methanol to obtain a concentration of 0.2mg/mL. Folin Ciocalteu's reagent (0.5 mL) was added to centrifuge tubes containing

0.5 mL of extracts. The contents were mixed thoroughly and 1 mL of a saturated solution of sodium carbonate was added to each tube to neutralize the reaction. Volume was adjusted to 10 mL with adding distilled water, and the contents were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature in the dark for 35 min followed by centrifugation for 10 min at 4000 x g. Absorbance of the resulting blue colour supernatant was read at 725 nm (Model HP 8452A diode array spectrophotometer, Agilent Technologies, Palo Alto, CA) using appropriate blanks. The content of total phenolics in each extract was determined using a standard curve prepared for gallic acid and expressed as mg gallic acid equivalents (GAE)/g of crude extract.

4.3.3.2 Determination of oxidative stability of stripped corn oil at 100 ^{0} C by Rancimat method

The effectiveness of cashew extracts on delaying oxidation of commertially available stripped corn oil (SCO) was measured under accelerated oxidative conditions using a Rancimat apparatus (Model 743 Rancimat, Metrohm Ion Analysis Ltd., CH-9101, Herisau, Switzerland). The cashew extracts were added into the sample tubes of the Rancimat apparatus containing 3 g of SCO. The volatile oxidation products were collected in the measuring vessels containing 60 mL of deionized water. A constant stream of dry air (20 L/h), obtained by passing laboratory air through molecular sieve, was blown through the samples in the reaction vessel. The oil samples are brought to an elevated temperature (100^{0}C) and maintained these over the course of the experiment. The conductivity of the aqueous solution was monitored continuously and recorded. The inflection point was calculated by the software (PC software version 1.0, 2000, Metrohm Ion Analysis Ltd., CH-9101, Herisau, Switzerland). A blank containing pure SCO devoid

of extracts was used. Results were reported as protection factor (PF). PF = ($IP_{additive}$ / $IP_{control}$), Where, $IP_{additive}$ = inflection point of oil mixture containing the additive; and $IP_{control}$ = inflection point of pure oil.

4.3.3.3 Inhibition of oxidation in comminuted pork model system

The thiobarbituric acid reactive substances (TBARS) in the cooked pork was determined using a modified version of the method described by Wettasinghe and Shahidi (1996). Ground pork was mixed with 20% (w/w) deionized water in Mason jars. Cashew phenolic extracts and butylated hydroxyanisole (BHA) were added separately to meat (100 g) that was then thoroughly homogenized. A control sample containing no extract was also prepared. Samples were cooked in a thermostated water bath at 80 ± 2°C for 40 min while stirring every 5 min with a glass rod. After cooling to room temperature, meat systems were homogenized, transferred into plastic bags, and then stored in a refrigerator at 4°C for 14 days. Samples for the analyses of TBARS were drawn on days 0, 5, 7 and 14 and were analyzed for TBARS according to the method of Siu and Draper (1978) with slight modifications. Two grams of each sample were weighed in a 50 mL centrifuge tube to which 5 mL of a 10% (w/v) solution of trichloroacetic acid (TCA) were added and vortexed (Fisher Vortex Genie 2; Fisher Scientific, Nepean, ON, Canada) at high speed for 2 min. An aqueous solution (0.02 M) of thiobarbituric acid (TBA) (5 mL) was then added to each centrifuge tube, followed by further vortexing for 30 s. The samples were subsequently centrifuged at 3000 x g for 10 min and the supernatants were filtered through a Whatman No. 3 filter paper. Filtrates were heated in a boiling water bath for 45 min, cooled to room temperature in an ice bath, and the absorbance of the resultant pink-coloured chromogen read at 532 nm. A standard curve was prepared using 1,1,3,3-

tetramethoxypropane as a precursor of the malondialdehyde (MDA). The TBARS values were then calculated using the standard curve and expressed as milligrams MDA equivalents per kilogram of sample.

4.3.3.4 Antioxidant activity of cashew soluble phenolic extracts in β-carotene - linoleate model system

The antioxidant activity of extracts was evaluated in a β-carotene-linoleate model system as explained by Chandrasekara and Shahidi (2010) with some modifications based on the ability of the extracts to decrease the oxidative bleaching of β-carotene in a β-carotene / linoleate emulsion. A 10 mg sample of crystalline β-carotene was dissolved in 10 mL of chloroform and 0.5 mL of the solution was pipetted into a 50 mL round bottom flask. After chloroform was removed under vacuum, using a rotary evaporator at room temperature, 20 mg of linoleic acid, 200 mg of Tween 40 emulsifier and 50 mL of aerated distilled water were added to the flask with vigorous shaking. Absorbance at 450 nm was read using a microplate reader equipped with a built-in incubator (FLUOstar OPTIMA, BMG LABTECH GmbH, Offenburg, Germany). Methanolic extracts (20 µL) were manually pipetted into sample wells of a Costar flat bottom 96 well assay plate (Corning Incoperated, Corning, NY) and injector pump was programmed to inject β-carotene-linoleic acid emulsion (200 µL) in each of the well with automatic mixing. The microplate was incubated at 45^0 C and absorbance was read at 450 nm. The microplate reader was programmed to perform additional shaking of the contents in wells before each reading. Readings of samples were recorded immediately at zero time and every 10 min up to 120 min. An equal amount of methanol was used for the control. Blank samples devoid of β-carotene were prepared for background subtraction. Butylated

hydroxyanisole (BHA) (200 ppm) in methanol was used as a reference standard. Antioxidant activity coefficient (AAC) after 120 min of incubation was calculated using the following equation; AAC = $(A_{a\,(120)} - A_{c\,(120)}) / (A_{c\,(0)} - A_{c\,(120)})$ where $A_{a\,(120)}$ and $A_{c\,(120)}$ are the absorbance values measured at 120 min for the sample and the control, respectively, and $A_{c(0)}$ is the absorbance value of the control, at 0 min. The results were expressed as AAC /g extract.

4.3.3.5 Effect of cashew extracts on preventing cupric ion-induced human low density lipoprotein (LDL) cholesterol peroxidation

The method described by Andersen *et al.* (2001) and Hu and Kitts (2000) was used to measure human LDL cholesterol oxidation. Human LDL cholesterol (in PBS, pH 7.4 with 0.01% EDTA) was dialyzed against 10 mM PBS (pH 7.4, 0.15 M NaCl) for 12 h under a flow of nitrogen at 4°C and EDTA-free LDL cholesterol was subsequently diluted to obtain a standard protein concentration of 0.2 mg/mL with PBS. The diluted LDL cholesterol solution (200 µL) was mixed with 1000 µL of PBS and 10 µL of extract (2 mg/mL) in a test tube. Oxidation of LDL cholesterol was initiated by adding 5.1 mM cupric sulphate solution resulting in a 4 µM copper concentration in the reaction mixture. The mixture was incubated at 37°C for 100 min. The initial absorbance (t = 0) was read at 234 nm immediately after mixing and every 5 min thereafter. The pattern of changing absorbance was plotted against time and percentage inhibition of conjugated dines (CD) formation was calculated as follows. Inhibition of formation of CD (%) = $(Abs_{\,oxidative} - Abs_{\,sample}) / (Abs_{\,oxidative} - Abs_{\,native}) \times 100$, Where, $Abs_{oxidative}$ = absorbance of LDL

mixture with $CuSO_4$ only; Abs_{sample} = absorbance of LDL with extract/standard and $CuSO_4$; Abs_{native} = absorbance of LDL without $CuSO_4$.

4.3.3.6 Supercoiled strand DNA scission by peroxyl and hydroxyl radicals

Plasmid supercoiled DNA (pBR 322) was dissolved in 10 mM PBS (pH 7.4, 0.15 mM sodium chloride). Two micro litres of DNA (50µg/mL) were mixed with 2 µL of cashew extracts (0.2 mg/ mL) dissolved in the same PBS. Peroxyl radical was generated using 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) dissolved in PBS to attain a final concentration of 9 mM and mixed with the DNA and the extract mixture at a total volume of 10 µL. The reactants were incubated at 37ºC for 1 h in the dark (Hu *et al.*, 2000). Upon completion of incubation, the loading dye (2 µL), consisting of 0.25% bromophenol blue, 0.25% xylene cyanol and 40% sucrose in distilled water, was added to the sample and loaded to a 0.7% (w/v) agarose gel prepared in Tris-acetic acid-EDTA buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.5) added along with 100 µL/L of SYBR safe gel stain. Horizontal gel electrophoresis was performed at 60 V for 5 h. The bands were visualized under the UV light and images were analyzed using AlphaEase™ stand alone software (Alpha Innotech Co., San Leandro, CA). The protective effect of cashew extracts was calculated based on the following equation. DNA retention % = (Intensity of supercoiled DNA in sample/ Intensity of supercoiled DNA in control) X 100. Normalized DNA percentage inhibition was expressed.

4.3.3.7 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences were estimated by analysis of variance (ANOVA) followed by Tukey's ''Honest Significant Difference" test. Differences were considered significant at

$p < 0.05$. All statistical analyses were performed using the free statistical software SPSS 13.0 version.

4.4 Results and discussion

This study evaluated the antioxidant activity of the phenolic extracts from raw and roasted whole, kernel and testa of cashew nuts in several food and biological model systems. The present analysis showed that 100 g of shelled raw cashew nuts with testa on a dry weight (dw) basis contained 369 mg of gallic acid equivalents (GAE) of total phenolics. In addition roasting at low temperature (LT) and high temperature (HT) significantly ($p \leq 0.05$) increased the total phenolic content from 369 to 536 and 1891 mg GAE/100g nuts (dw), respectively.

Due to the fact that, phenolic extracts were used in all model systems employed in this study, the total phenolic content (TPC) of crude cashew extracts are summarized in **Table 4.1**. Soluble phenolic extracts of HT treated cashew testa yielded the highest TPC value of 790.9 ± 15 mg of GAE per g of extract whereas that of raw kernel had the least TPC of 5.0 ± 1.8 mg of GAE per g of extract. In general, roasting significantly ($p \leq 0.05$) increased the TPC of extracts of cashew nuts as well as their testa and this incensement ranged from 20 to 344%.

4.4.1 Antioxidant activity by the Rancimat method

Oxidation of lipids is a pivotal cause of quality deterioration in many food systems, and leads to off-flavour development and formation of toxic compounds that affect the quality and nutritional value of foods. Moreover, lipid oxidation products have been known to be associated with aging, heart disease, and cancer (Ramarathnam *et al.,* 1995). In the present study, the antioxidant activity of raw and roasted cashew extracts were evaluated

for their potential in inhibiting accelerated autoxidation of commercial stripped corn oil (SCO) at 100° C. The experiment was carried out using a Rancimat® apparatus and the protection factors (PF) were calculated for whole nut, kernel, and testa extracts subjected to different roasting conditions. The higher PF of SCO indicates the better antioxidant activity of the phenolic extract. The PF of different cashew extracts obtained from raw and roasted samples are shown in **Table 4.1.** The PF of phenolic extracts of whole, kernel and testa of cashew increased significantly ($p \leq 0.05$) with increasing roasting temperature at the concentration of 0.5mg/mL of stripped oil and ranged from 1.49 to 1.59, 1.39 to 1.57 and 1.35 to 1.51, respectively. The increasing antioxidant activity with roasting could be due to their compositional changes occurring during heat treatment. As shown in the previous study (**Chapter 3**), the contents of phenolic acids (gallic, syringic, *p*-coumaric) and flavonoids (catechin, epicatechin, epigalocatechin) increased upon roasting. In general, phenolic acids and flavonoids, among others, have been recognized to confer stability to vegetable oils against autoxidation (van Ruth *et al.,* 2001). In the present study, when extracts were used at a concentration of 5 mg/mL in stripped corn oil, phenolic extracts of raw cashew whole, kernel and testa showed higher PF than that of the values obtained at a low concentration (0.5mg/mL). Interestingly, raw cashew testa at 5 mg/mL had 1.8 times higher PF than that at the concentration 0.5 mg/mL. However, at the high concentration (5mg/mL), phenolic extracts of roasted samples had lower PF than those of their raw counterparts (**Table 4.1**). On the other hand, no prooxidative activity was found with extracts obtained for raw or roasted samples at the two concentrations of soluble phenolic extracts employed in this study. Thus, additional experiments at even higher concentrations are needed to ascertain whether the extracts of

roasted cashew samples could become prooxidative at a higher concentration. In the present study, it was found that the PF of butylated hydroxyanisole (BHA), at a concentration of 0.2 mg/mL was 2.48 ± 0.13. This was approximately 1.5 times higher than that of cashew extracts at a concentration of 0.5 mg/mL in SCO. Although synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, tertiary-butylhydroquinone, and propyl gallate have widely been used in controlling lipid oxidation, their safety has recently been questioned. Thus, there is much interest in the development of safer antioxidants using natural extracts from oilseeds, cereals, legumes and other plant materials (van Ruth *et al.*, 2001; Liyana-Pathirana & Shahidi, 2006; Madhujith & Shahidi, 2007).

4.4.2 Cooked comminuted pork model system

Lipid oxidation is a major cause for quality deterioration in muscle foods that leads to a number of products which are responsible for off odours and off flavours (Kanner *et al.*, 1991). The results obtained in this work, using a cooked comminuted pork model system, did not indicate any great difference among phenolic extracts used in lowering of the thiobarbituric acid reactive substances (TBARS) (**Table 4.2**). The extracts were effective in inhibiting the oxidation of cooked pork in comparison with the control which showed the highest MDA equivalent values of TBARS for the entire period of storage. Although there was a significant ($p \leq 0.05$) difference in TBARS contents among the phenolic extracts of whole, kernel and testa, in general no significant ($p \leq 0.05$) trend existed among phenolic extracts as a result of roasting. The different additives inhibited oxidation slightly on tested days of storage. At the end of day 7 of storage, cashew extracts at 0.2 %, inhibited the formation of TBARS by 77.5 to 80.4, 1.0 to 16.5 and 93.2

to 94.6% for whole, kernel and testa, respectively. Furthermore, BHA at 0.02% inhibited oxidation as evaluated by TBARS by 91.2 %. The foregoing results showed that raw as well as roasted testa had the highest effectiveness in inhibition of TBARS formation among others. In agreement with the present results, Wijerathne *et al.* (2006) also showed that phenolic extracts of the brown skin had a higher inhibition (60%) than that of whole seed extract (54%) of almonds on the seventh day of storage.

4.4.3 β-carotene -linoleate model system

The discolouration of β-carotene has widely been used to measure the antioxidant activity of plant extracts (Wijerathne *et al.,* 2006; Madhujith & Shahidi 2007; Shahidi *et al.,* 2007). In a β-carotene-linoleate emulsion system, phenolic antioxidants prevented the loss of colour by neutralizing the linoleic acid free radicals. The greater the potential of the antioxidant, the lesser the depletion of colour in the system that can be determined spectrophotometrically. Thus, this system can be employed to evaluate the efficacy of unknown antioxidative compounds (Wettasinghe & Shahidi, 1999). In the present study, the method was modified using a 96-well microplate for sample incubation and automatic absorbance measurement.

Figure 4.1 presents the changes of corrected absorbance of retained β-carotene with time due to the phenolic extracts of cashew whole, kernel and testa. The linoleic acid free radicals attack the highly conjugated β-carotene molecule, thus reducing the β-carotene content at a faster rate in the absence of extracts. However, in the presence of additives, the loss of β-carotene occurred at a slower rate (**Figure 4.1**). The reference antioxidative compound, BHA, exerted the strongest antioxidative effect, among all samples tested. The descending order of inhibition of β-carotene bleaching was testa>whole>kernel.

Interestingly, as noted in the comminuted pork model system in the present study, roasting did not render a significant ($p \leq 0.05$) differential effect on the inhibition of β-carotene bleaching. **Figure 4.2** shows the antioxidant activity coefficient (AAC) of cashew extracts. HT treated cashew whole, kernel and testa showed significantly ($p \leq 0.05$) lower AAC values than that of the corresponding raw extracts indicating a reduced antioxidant activity. In this study the TPC of phenolic extracts of LT and HT treated cashew showed higher values compared to that of their raw counterpart (**Table 4.1**). This could be due to the liberation of phenolic compounds during heat treatments or/and due to the formation of Maillard reaction products (Hayase *et al.,* 1989). However, reduced antioxidant activity of HT treated extracts in the β-carotene-linoleate emulsion system in this study demonstrates that these compounds may not contribute to the prevention of oxidation in this study.

Furthermore, cashew testa showed a higher AAC values compared to cashew whole and kernel. However, a high AAC value was observed for BHA at 200 ppm, which was comparable to that of the activity of raw cashew testa extract. These results are in agreement with an earlier study on hazelnuts (Shahidi *et al.,* 2007). In the same study it was reported that hazelnut skin possessed 1.3 times higher retention of β-carotene compared to the whole hazelnut. However, in contrast to the results obtained in this study, Wijerathne *et al.* (2006) showed that almond brown skin extract had the lowest activity, but the β-carotene retention percentage was 83%. The variations observed in the present analysis could be due to the extraction differences of the proportion of lipophilic and hydrophilic compounds present in each extract. In the present analysis attempts were not made to distinguish between lipophilic and hydrophilic compounds present.

4.4.4 Cupric ion-induced human low density lipoprotein (LDL) peroxidation

Oxidation of polyunsaturated lipid components of LDL cholesterol by reactive oxygen species (ROS) is an important cause which leads to the pathogenesis of atherosclerosis. Furthermore, it has been shown that transition metal ions may promote oxidative modification of LDL cholesterol through hydroperoxides (Decker *et al.,* 2001). Although physiological significance of copper (Cu^{2+}) induced LDL cholesterol oxidation is controversial (Kontush & Beisiegel, 1999), it has been used as a useful biological *in vitro* model to determine the antioxidant activity of natural plant extracts (Liyana-Pathirana & Shahidi, 2006; Wijerathne *et al.,* 2006). Ziouzenkova *et al.* (1998) showed that binding of redox active Cu^{2+} to LDL cholesterol at both low and high affinity binding sites is necessary to initiate LDL cholesterol oxidation. Furthermore, Giessauf *et al.* (1995) showed that copper-mediated oxidation of tryptophan residues in the LDL-apolipoprotein B is responsible for lipid oxidation in the LDL cholesterol particles. According to Decker *et al.* (2001) both free radical scavenging and copper chelation activity of antioxidants are responsible for inhibition of LDL cholesterol oxidation.

In the present study, the efficacy of cashew phenolic extracts to inhibit LDL cholesterol oxidation was evaluated by monitoring conjugated diene (CD) formation at 37°C. The inhibitory percentages of extracts against CD formation are presented in **Table 4.3**. After incubation for 12 h, extracts of raw as well as roasted cashew kernels exerted the highest inhibition of 69%. In addition, the oxidation control by whole cashew nuts ranged from 35.6 to 51.1% and the HT roasted nuts showed the highest effect. In contrast to other systems employed in this study, cashew testa exhibited the least inhibition which ranged from 41.5 to 46.1% after 12 h of incubation. The observed differences could be due to

the variations in the solubility and partitioning of responsible compounds between aqueous and lipid phases in the LDL cholesterol. According to Frankel *et al.* (1994) the physicochemical properties of antioxidants are known to affect their antioxidant efficacy in complex, multiphase systems. It has been shown that lipophilic antioxidants such as α-tocopherol provide a better protection against LDL cholesterol oxidation than hydrophilic antioxidants (Ziouzenkova *et al.,* 1996). This is due to the fact that lipophilic antioxidants enter LDL cholesterol particles, whereas hydrophilic compounds act on the surface of the LDL cholesterol particles, hence making the latter less effective in the system (Abuja *et al.,* 1998). Recently, Trox *et al.* (2010) reported that 100g of raw cashew nut kernels (on a dry weight basis) contain an appreciable amounts of *β*-carotene (9.57 μg), lutein (30.29 μg), zeaxanthine (0.56 μg), α-tocopherol (2900 μg), and γ-tocopherol (11000 μg). Furthermore, it has been shown that, depending on the flavonoid structure, transition metal ions may serve as catalysts of flavonoid oxidation imparting prooxidative effects in the systems (Sugihara *et al.,* 1999; Rodriguez *et al.,* 2001). Possible prooxidant effect of catechin, epicatechin, and epigallocatechin may originate from their autoxidation which may occur by the formation of superoxide and semiquinone free radicals and accelerated by cupric ions (Mochizuki *et al.,* 2002). HPLC analysis showed that testa had 68, 314 and 1.3 times higher catechin, epicatechin and epigallocatechin, respectively, than that in the cashew kernel (**Chapter 3**). The high concentrations of the aforementioned flavonoids of testa in the cupric induced human LDL cholesterol oxidation system may compensate their antioxidant effect at the concentration (300 ppm) used in the present study.

It is noteworthy that catechin (0.03mg/mL) which was used as a standard compound in the present analysis showed a 40% inhibition against LDL cholesterol oxidation and this was similar to the values obtained for cashew testa (**Table 4.3**). In the present study, raw cashew testa showed a higher inhibition than that of raw whole cashew nuts. In agreement with the results obtained in this study, Wijerathne *et al.* (2006) and Shahidi *et al.* (2007) showed that almond brown skin and hazelnut skin had higher inhibition percentages than those of their corresponding whole nuts.

4.4.5 Supercoiled strand DNA scission

Figure 4.3 shows the percentage of supercoiled DNA strands retained, after incubation with peroxyl radicals generated by AAPH. Both extracts of cashew whole and testa showed a high retention percentage of supercoiled DNA and ranged from 78 to 91 and 84 to 91%, respectively. Peroxyl radicals are known to exert oxidative damage in biological systems due to their comparatively long half-life and thus greater affinity to diffuse into biological fluids in cells (Hu & Kitts, 2001). The phenolic extracts from cashew kernels showed the least effect than those of whole cashew nut and testa. Furthermore, no significant ($p \leq 0.05$) difference was observed between low and high roasting conditions for any of the whole, kernel or testa samples.

Radicals cleave supercoiled plasmid DNA (form I) to nicked circular DNA (form II) as shown in **Figure 4.4.** Lane 1 represents the native supercoiled DNA sample without any additives. The presence of peroxyl radicals resulted in a scission of supercoiled DNA and this was clearly seen in the well, where the reaction mixture did not contain any antioxidant (**Figure 4.4,** Lane 2). The presence of a high intensity form II band and the disappearance of the form I band in lane 2 indicates that supercoiled DNA was

completely nicked. Wells 3 through 8 contained supercoiled DNA, along with the same concentration of radical together with cashew extracts (40 ppm). Wells 3, 4 and 5 contained extracts of raw whole, kernel and testa, respectively, whereas wells 6, 7 and 8 had HT treated whole, kernel and testa, respectively. The intensity of form I band in the lanes containing whole and testa extracts was high, reflecting a high level of retention percentage of supercoiled DNA (**Figure 4.3**).

The inhibitory effects of cashew extracts may be due to their ability to scavenge peroxyl radicals. This is further confirmed by the results obtained through oxygen radical absorbance capacity (ORAC) assay which demonstrated the peroxyl radical inhibition, as reported in **Chapter 3**. The ORAC values of soluble phenolic extracts of cashew whole, kernel and testa followed the same trend as those observed for supercoiled DNA strand scission. Shahidi *et al.* (2007) showed that hazelnut skin extract had the highest inhibition whereas kernel (with skin) showed the least preventive effect on DNA strand scission. However, in the present study both whole cashew nut as well as testa showed a similar effect on inhibition of DNA strand scission (**Figure 3**). The foregoing results indicate that cashew phenolic extracts may inhibit DNA scission by oxygen radicals.

High performance liquid chromatographic (HPLC) analysis, as shown in **Chapter 3,** revealed that extracts of whole cashew and testa were rich sources of both phenolic acids (gallic, syringic, *p*-coumaric) and flavonoids (catechin, epicatechin and epigallocatechin) compared to the kernel. Phenolic acids and flavonoids are known to render antioxidant properties (Shahidi & Naczk, 2004). Furthermore, the extract is a mixture of different compounds that may render synergistic or antagonistic effects. The nature of peroxyl radicals formed depends on the food and biological models used in the study. There are

complex peroxyl radicals such as cholesterol derivatives in LDL model and fatty acid derivatives in β-carotene linoleic acid emulsion system. Thus, the chemistry of these peroxyl radicals and their reactions in biological systems are variable. This may explain the inhibition variability of cashew extracts against oxidation in different model systems employed in the present study. To the best of our knowledge this research reports, for the first time, the antioxidant activity of phenolics of raw and roasted cashew nuts and testa.

4.5 Conclusions

The results of this study revealed that, in general, whole cashew and nuts testa were better sources of antioxidants compared to the kernel as assessed in different food and biological model systems. However, extracts of cashew kernel showed effective inhibition against copper induced human LDL oxidation. Furthermore, roasting did not contribute significantly to enhancing the antioxidant activity of cashew products in food and biological model systems compared to their raw counterparts except under accelerated autoxidation conditions in stripped corn oil.

Table 4.1: Total phenolic content and protection factor as determined by Rancimat assay

Processing conditions	Total phenolic content	Protection factor	
	GAE mg/g of crude extract	0.5mg/mL of oil	5mg/mL of oil
Raw cashew whole	27.0 ± 4.0 [a]	1.49 ± 0.01 [a]	1.95 ± 0.01 [a]
LT treated cashew whole	35.5 ± 0.7 [a]	1.57 ± 0.07 [b]	1.30 ± 0.06 [b]
HT treated cashew whole	120.0 ± 7.0 [b]	1.59 ± 0.04 [b]	1.06 ± 0.01 [c]
Raw cashew kernel	5.0 ± 1.8 [a]	1.39 ± 0.01 [a]	1.68 ± 0.10 [a]
LT treated cashew kernel	19.6 ± 3.3 [b]	1.39 ± 0.07 [a]	1.17 ± 0.02 [b]
HT treated cashew kernel	21.1 ± 4.0 [b]	1.57 ± 0.02 [b]	1.11 ± 0.01 [b]
Raw cashew testa	656.2 ± 23.0 [a]	1.35 ± 0.05 [a]	2.83 ± 0.05 [a]
LT treated cashew testa	701.2 ± 21.1 [a]	1.36 ± 0.05 [a]	1.57 ± 0.02 [b]
HT treated cashew testa	790.9 ± 15.4 [b]	1.51 ± 0.05 [b]	1.48 ± 0.01 [c]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same superscript letter, within a column are not significantly different ($p > 0.05$). GAE, gallic acid equivalents; LT, low temperature treated; and HT, high temperature treated.

Table 4.2: TBARS (as malondialdehyde equivalents / kg of meat) of cashew extracts

Processing conditions	TBARS (μmols MDAE / kg of meat), storage time			
	Day 0	Day 5	Day 7	Day 14
BHA	0.38 ± 0.38	1.42 ± 0.08	2.12 ± 0.15	1.11 ± 0.02
Control	25.54 ±0 .42	30.33 ± 2.73	38.15 ± 2.29	33.32 ± 0.31
Raw cashew whole	5.62 ± 0.64 [a]	5.81 ± 1.43 [a]	7.82 ± 0.34 [a]	9.25 ± 0.43 [a]
LT treated cashew whole	5.46 ± 0.73 [a]	6.70 ± 0.81 [a]	7.66 ± 0.14 [a]	8.61 ± 0.90 [a]
HT treated cashew whole	4.93 ± 0.30 [a]	7.15 ± 0.04 [a]	8.58 ± 1.30 [a]	11.24 ± 0.77 [b]
Raw cashew kernel	26.39 ± 0.96 [a]	27.17 ± 3.01 [a]	38.82 ± 0.65 [a]	41.22 ± 1.35 [a]
LT treated cashew kernel	24.61 ± 1.67 [a]	34.22 ± 0.66 [b]	34.56 ± 2.61 [a]	35.38 ± 0.75 [b]
HT treated cashew kernel	15.53 ± 0.58 [b]	30.62 ±0 .65 [a]	32.85 ± 3.15 [a]	29.90 ± 0.27 [c]
Raw cashew testa	2.46 ± 0.24 [a]	2.60 ± 0.15 [a]	2.75 ± 0.34 [a]	3.38 ± 0.88 [a]
LT treated cashew testa	2.23 ± 0.42 [a]	2.24 ± 0.26 [a]	2.34 ± 0.31 [a]	3.90 ± 0.92 [a]
HT treated cashew testa	2.60 ± 0.33 [a]	2.86 ± 0.22 [b]	2.75 ± 0.18 [a]	3.65 ± 0.56 [a]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same superscript letter, within a column are not significantly different ($p > 0.05$). MDAE, Malondialdehyde equivalents; BHA, Butylated hydroxyanisole; LT, low temperature treated; and HT, high temperature treated.

Table 4.3: Effect of cashew extracts on preventing cupric ion-induced human low density lipoprotein (LDL) cholesterol peroxidation

Processing conditions	Inhibition (%)		
	0h	12h	24h
Catechin	8.97 ± 2.82	40.00 ± 1.52	39.81 ± 0.72
Raw cashew whole	9.45 ± 0.81 [a]	35.60 ±0.44 [a]	34.07 ± 1.83 [a]
LT treated cashew whole	8.98 ± 0.79 [a]	36.97 ± 6.13 [a]	38.40 ± 0.83 [b]
HT treated cashew whole	9.61 ± 1.24 [a]	51.09 ± 4.70 [b]	50.36 ± 1.91 [c]
Raw cashew kernel	9.79 ± 0.61 [a]	69.03 ± 3.41 [a]	69.03 ± 1.81 [a]
LT treated cashew kernel	8.63 ±0.33 [a]	68.35 ± 1.43 [a]	68.49 ± 3.64 [a]
HT treated cashew kernel	8.92 ± 0.23 [a]	68.90 ± 0.62 [a]	68.74 ± 0.83 [a]
Raw cashew testa	8.49 ± 0.94 [a]	46.05 ± 0.24 [a]	56.82 ± 3.22 [a]
LT treated cashew testa	8.92 ± 0.71 [a]	41.51 ± 0.72 [b]	50.42 ± 2.23 [a]
HT treated cashew testa	9.12 ± 0.61 [a]	43.66 ± 2.13 [b]	47.79 ± 3.01 [b]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same superscript letter, within a column are not significantly different ($p > 0.05$). LT, low temperature treated; and HT, high temperature treated.

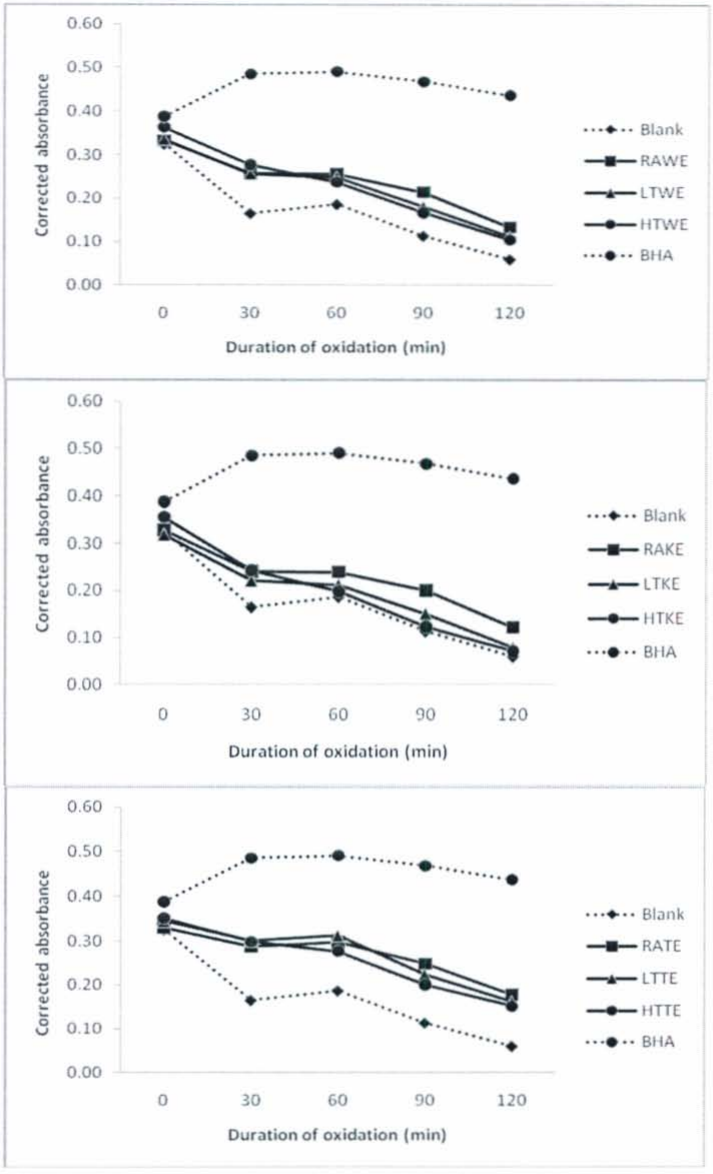


Figure 4.1: Retention of *β*-carotene by cashew extracts in a *β*-carotene -linoleate model system

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). RAWE, raw whole nut extract; LTWE, low temperature treated whole nut extract; HTWE-high temperature treated whole nut extract; RAKE, raw kernel extract; LTKE, low temperature treated kernel extract; HTKE, high temperature treated kernel extract; RATE, raw testa extract; LTTE, low temperature treated testa extract; HTTE, high temperature treated testa extract; BHA, Butylated hydroxyanisole, 200ppm; Extracts are used at a concentration of 500 ppm.

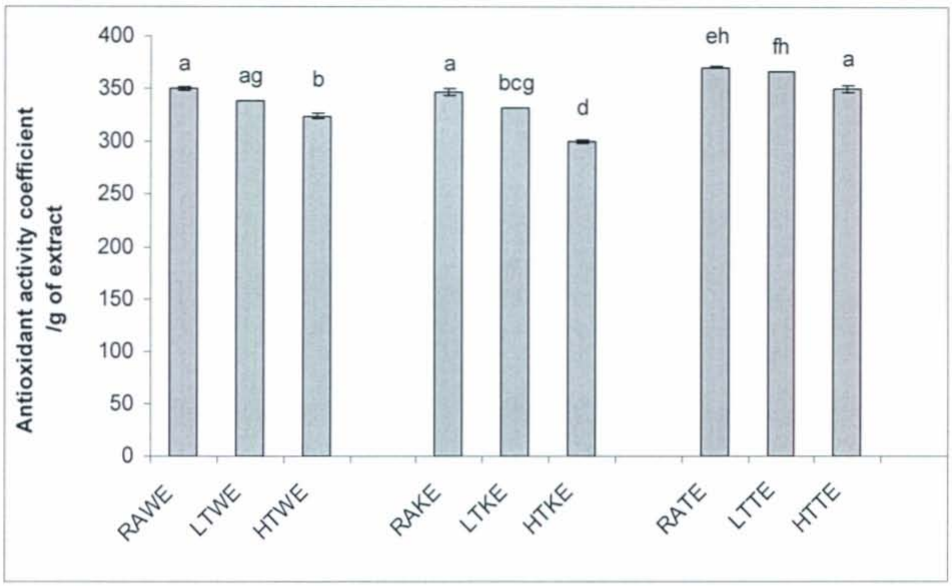


Figure 4.2: Antioxidant activity coefficient (AAC) at 120 min for cashew extracts in a *β*-carotene-linoleate model system

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). RAWE, raw whole nut extract; LTWE, low temperature treated whole nut extract; HTWE, high temperature treated whole nut extract; RAKE, raw kernel extract; LTKE, low temperature treated kernel extract; HTKE, high temperature treated kernel extract; RATE, raw testa extract; LTTE, low temperature treated testa extract; and HTTE, high temperature treated testa extract.

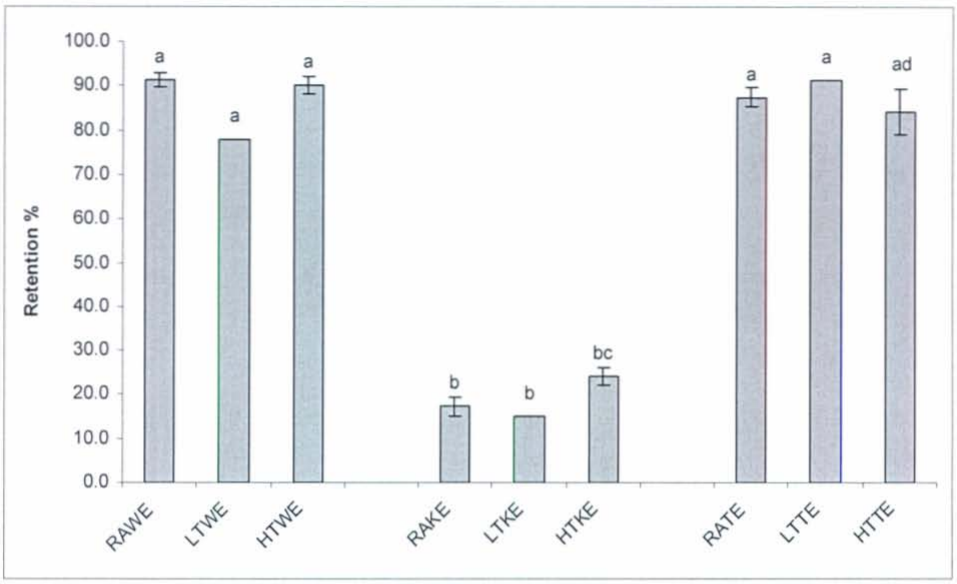


Figure 4.3: Retention percentage of supercoiled DNA by cashew extracts against peroxyl radical induced DNA scission

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). RAWE, raw whole nut extract; LTWE, low temperature treated whole nut extract; HTWE, high temperature treated whole nut extract; RAKE, raw kernel extract; LTKE, low temperature treated kernel extract; HTKE, high temperature treated kernel extract; RATE, raw testa extract; LTTE, low temperature treated testa extract; and HTTE, high temperature treated testa extract.

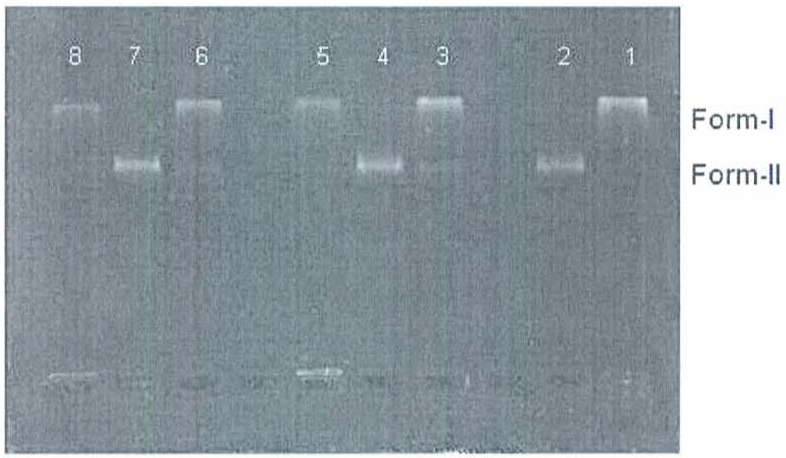


Figure 4.4: Effect of cashew extracts on preventing peroxyl radical induced DNA scission

Lane 1, DNA; Lane 2, DNA + peroxyl radical; Lane 3, DNA + peroxyl radical + raw whole; Lane 4, DNA + peroxyl radical + raw kernel; Lane 5, DNA + peroxyl radical + raw testa; Lane 6, DNA + peroxyl radical + high temperature treated whole; Lane 7, DNA + peroxyl radical + high temperature treated kernel; and Lane 8, DNA + peroxyl radical + high temperature treated testa.

Chapter 5

Oxidative stability of cashew nut oil as affected by different roasting conditions

5.1 Abstract

Cashew nut oil extracted from raw and roasted whole cashew nuts were examined for their fatty acid composition, colour change and oxidative stability. Fatty acids were analyzed using gas chromatography (GC). A spectrphotometric method was used to determine the colour changes of the resultant oil. Oxidative stability was monitored under accelerated oxidation conditions by employing conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS) assays. The contents of monounsaturated (MUFA), polyunsaturated (PUFA) and saturated (SAFA) fatty acids were 61, 17 and 21%, respectively. Oleic acid was the major MUFA whereas linoleic acid was the main PUFA in cashew nut oils. Oxidative stability as determined by CD values at 72 h of storage was 1.08 and 0.65 for the raw and high temperature roasted cashew nut oil, respectively. The TBARS values, expressed as malondialdehyde (MDA) equivalents decreased with increasing roasting temperatures. Roasting of whole cashew nuts improved the oxidative stability of nut oil during storage.

Key words: Colour, GC, SAFA, MUFA, PUFA, CD, TBARS

5.2 Introduction

Tree nuts and their oils are known to contain bioactive and health promoting substances and as such have long been considered an important component of the human diet. Epidemiological evidences indicate that the consumption of tree nuts may exert several cardioprotective effects, which are speculated to arise from their lipid component that includes unsaturated fatty acids, particularly oleic acid, and phytosterols, among others (Hu & Stampfer, 1999). Miraliakbari and Shahidi (2008 a,b) have reported the compositional characteristics of nut oils and the antioxidant activity of their minor components.

Fats, oils, and lipid containing foods are oxidized at different rates, resulting in changes in their sensory and nutritional characteristics. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of fatty acids involved. The presence of natural compounds having different chemical structures that exhibit antioxidant activity may also affect the rate of oxidation (Gutfinger, 1981; Montedoro *et al.*, 1992; Akasbl *et al.*, 1993; Montedoro *et al.*, 1993). Another lipid alteration is lipid hydrolysis, with consequent free fatty acid (FFA) generation, by chemical or enzymic action. This phenomenon is of particular interest in water containing lipid matrices, such as butter and virgin olive oil during olive processing. Although the original causes and the consequences of oxidative and hydrolytic degradation processes are quite different, they seem to interact with each other and contribute to the reduction of shelf life of edible oils.

Oxidative stability is an important parameter for the quality assessment of fats and oils. Autoxidation is affected by atmospheric oxygen and the oxidation process is initiated by free radical reactions involving unsaturated fatty acids (Loury *et al.,* 1965; Frankel, 1984; Gunstone & Amer, 1984). The primary products formed are hydroperoxides, which then break down in a series of complex reactions, to yield secondary products including alcohols and carbonyl compounds (Loury *et al.,* 1965; Frankel, 1984; Gunstone, 1984). These can be oxidized further to carboxylic acids (Loury *et al.,* 1965).

Tree nut oils are primarily composed of triacylglycerols. They also contain diacylglycerols, monoacylglycerols, free fatty acids, and other minor components, including natural antioxidants and fat soluble vitamins. Generally, tree nut oils are somewhat similar to peanut oil and are rich in monounsaturated fatty acids, predominantly oleic acid, but contain much lower amounts of polyunsaturated fatty acids, such as linoleic acid, and small amounts of saturated lipids (USDA, 2005).

During the roasting process, a pleasant aroma and a taste that transfers to the oil and present during extraction is developed. The conventional method for the preparation of condiment oils, such as sesame and red pepper oils, involves cleaning, roasting and pressing but not refining (Kim *et al.,* 2002). The roasting process is the key step for making condiment oil, since the colour, flavour, composition, and quality of the oil are influenced by the process. Some studies have shown that the chemical composition of oil is independent of the roasting temperature used for its preparation (Jung *et al.,* 1999; Kim *et al.*, 2002; Yen, 1990; Yoshida & Takagi, 1997). However, there are no published reports on lipid class compositions of roasted cashew nut oils, and their oxidative stabilities. Therefore, this study was conducted to investigate the changes in colour, fatty

acid composition and oxidative stability of the oil extracted from cashew whole nut roasted at different temperatures.

5.3 Materials and methods

5.3.1 Materials

Raw shelled cashew with testa was obtained from the Green Field Bio Plantation (Pvt.) Ltd., Colombo, Sri Lanka. Fatty acid standards, anhydrous sodium sulphate, 2,2,4-trimethylpentane, 1,1,3,3-tetramethoxypropane, and 2-thiobarbituric acid (2-TBA) were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON). Hexane, chloroform, methanol, sulphuric acid, and 1-butanol were purchased from Fisher Scientific Ltd (Ottawa, ON).

5.3.2 Sample preparation

Roasting was done using two different processing temperatures in this study. For low temperature processing (LT) raw whole cashew nuts were roasted in a forced convection hot-air oven at 70^0 C for 6 hours. In this, cashew kernels weighing approximately 100 g were spread in a single layer on a stainless steel wire mesh tray placed in the center of the oven during hot-air roasting. After roasting, the hot cashew kernels were cooled in a dessicator at room temperature, and kept in sealed plastic bags at 4^0C, until further analysis. Under industrial cashew processing operations, both small and large scale cashew producers use these conditions to obtain good quality products (Hebbar *et al.*, 2005). For high temperature processing (HT), raw whole cashew nuts were roasted in a forced air convection oven at 130^0 C for 33 min. This temperature and time combination was the optimum roasting conditions for cashew kernels based on hedonic sensory

evaluations according to Wanlapa and Jindal (2006). Raw whole cashew nuts were used as the control to compare the effect of two different roasting conditions.

Raw and roasted whole cashew nuts, were ground separately using a coffee bean grinder (Model CBG5 series, Black & Decker, Canada Inc. Brockville, ON) to obtain a fine powder which passed through mesh 16 (sieve opening 1mm, Tylor test sieve, Mentor, OH). Each sample was defatted by blending with hexane (1:5, w/v, 5 min, 3 x) in a Waring blender (Model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT) at ambient temperature (20°C). The resulting oil and hexane mixture was filtered through a Whatman no. 4 filter paper using a Buchner funnel. The residue was re-extracted with hexane twice and the filtrates from the three extractions were combined, and solvent was evaporated in *vaccuo* (Rotavapor model 461, Buchi, Flawil, Switzerland) at 40 °C to reduce the volume. The hexane-oil mixture so obtained was then passed through anhydrous sodium sulphate placed over a filter paper in a funnel, followed by evaporation of the remaining solvent in *vaccuo* at 40 °C. The resulting oil was weighed and transferred into glass bottles, capped with nitrogen, and stored at -80 °C until used for further analysis.

5.3.3 Methods

5.3.3.1 Determination of colour development

The colour of raw, low temperature (LT) and high temperature (HT) treated whole cashew nut oil was determined. As an index of colour development, the absorbance at 420 nm of 5% (w/v) solutions of oils in chloroform as recommended by Yoshida *et al.* (1999) was measured spectrophotometricaly.

5.3.3.2 Fatty acid (FA) composition analysis

Fatty acid methyl esters (FAMEs) were prepared for each oil sample and were analyzed using gas chromatography (GC), as described by Wang and Shahidi (2010). In brief, the FAMEs were prepared using methanol and 6% sulphuric acid. Oils were esterified according to official AOCS (1990) method. Methyl esters of FA were extracted with hexane and 1 μL aliquots of the extracts were auto injected for GC analysis (Agilent Technologies Canada Inc., Mississauga, ON, Canada) equipped with a flame ionization detector (FID). The column used was a Supelcowax-10 fused-silica capillary column (30 m X 0.25 mm diameter, 0.25 μm film thickness; Supelco Canada Ltd., Oakville, ON, Canada). The carrier gas was helium, and the total gas flow rate was 20 mL/min. Identification and quantification was done using known standards.

5.3.3.3 Determination of oxidative stability

The oxidative stability of cashew nut oils were studied using accelerated autoxidation conditions. Two grams of cashew nut oil samples was accurately weighed into 10 mL clear glass sample vials and loosely capped before being placed in a Schaal oven condition (Thelco, Model 2, Precision Scientific Co., Chicago, IL) in the dark and heated to 60 °C. For each sample, six vials were loaded into the oven, and samples were removed after 0, 6, 12, 24, 36, 48, and 72 hours and stored at -80 °C until used for conjugated diene (CD), and thiobarbituric acid reactive substances (TBARS) analysis. Determination for each sample was carried out in triplicate.

5.3.3.4 Determination of conjugated diene (CD) content

The CD contents were determined according to the method explained by Wang and Shahidi (2010). In brief, A specified amount of oil (0.02–0.03 g) was weighed into a 25 mL volumetric flask, and made up to the mark with 2,2,4-trimethylpentane. The solution was thoroughly mixed before reading its absorbance at 234 nm (Model HP 8452A diode array spectrophotometer, Agilent Technologies, Palo Alto, CA). Pure 2,2,4-trimethylpentane was used as a reference. Conjugated diene values were calculated using the following equation: CD = Absorbance of solution at 234 nm /C*l where C= concentration of oil in g per 100 mL, l= length of the cuvette in cm (IUPAC, 1987)

5.3.3.5 Analysis of thiobarbituric acid reactive substances (TBARS)

TBARS values were determined according to the official AOCS (1995) method. The oil (50-100 mg) was weighed into a 25 mL volumetric flask and made up to volume with 1-butanol. Aliquot (5 mL) of this solution was transferred into a screw capped test tube to which freshly prepared 2-TBA reagent (500 mg 2-TBA in 250 mL 1-butanol) was added. Contents were thoroughly mixed and heated in a thermostated water bath at 95°C. After 2 h the samples were removed from the water bath and cooled in an ice bath. The absorbance was then read at 532 nm. A standard curve was prepared using 1,1,3,3-tetramethoxypropane as the malondialdehyde (MDA) precursor and the results were expressed as mmol MDA equivalents/g oil.

5.3.3.6 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences were estimated by the analysis of variance (ANOVA) followed by Tukey's "Honest Significant Difference" test. Differences were considered significant at $p \leq 0.05$. All statistical analyses were performed using the free statistical software SPSS 13.0 version (SPSS Inc., Chicago, IL).

5.4 Results and discussion

5.4.1 Oil yield

The oil yield of cashew nuts roasted at different conditions ranged from 41.30 ± 0.40 to 42.58 ± 0.38 % (**Figure 5.1**). The roasted cashew nut yielded a significantly ($P < 0.05$) higher percentage of oil than that of raw cashew nut. The oil yields obtained in this study are in the range of those reported by others for tree nuts such as cashew nut (40.4 ± 2.0%), almond (50.6 ± 2.1%), Brazil nut (60.8 ± 1.1 %), pecan (58.1 ± 2.8 %), pine nut (58.8 ± 1.9 %), and pistachio (51.2 ± 1.0 %) on a weight basis (Shahidi & Miraliakbari, 2005; Ryan *et al,* 2006).

5.4.2 Colour development of cashew nut oil

Figure 5.2 presents the absorbance values as indices for colour development of cashew nut oil obtained under different roasting conditions. With the roasting process, absorbance of cashew oil at 420 nm increased significantly ($P < 0.05$) and the colour of cashew nut oils changed gradually from light yellow to deep brown. The absorbance values ranged from 0.1563 ± 0.001 to 0.2282 ± 0.015. The results in this study showed

that the colour formation in the roasted oil was influenced by the temperatures employed. The Maillard reaction products (MRPs) are formed in thermally processed foods due to non-enzymatic reactions between reducing sugars and free amino acids (Koechler & Odell, 1970). The increase in colour of oils with increasing roasting temperature seemed to be due to non enzymatic browning and MRP formed at elevated roasting temperatures. Previous studies have also reported an increase in the colour of oils with increasing roasting temperature of seeds, such as rice germ and sesame seed which are consistent with the results of this study (Yen, 1990; Yosida, 1994; Kim *et al.*, 2002).

5.4.3 Fatty acid composition

Fatty acid composition of an oil can be used as an indicator of its stability, physical properties, and nutritional value. Fatty acid profiles of the raw and roasted cashew nut oils determined by GC are presented in **Table 5.1**. The major monounsaturated fatty acid (MUFA) present in cashew nuts was oleic acid (C18:1 n-9), with a content ranging from 60.57 ± 0.11 to 61.33 ± 0.04%. Linoleic acid (C18:2 n-6) was the most abundant polyunsaturated fatty acid (PUFA) present at 16.79 ± 0.07 to 17.03 ± 0.11 % with a lesser amount of linolenic acid (C18:3 n-3). The primary saturated fatty acids (SFA) identified in cashew nuts were palmitic acid (C16:0) and stearic acid (C18:0) present at 10.21 ± 0.01 to 10.31 ± 0.05 % and 9.57 ± 0.05 to 10.14 ± 0.01%, respectively. In accordance with the present study, Venkatachalam and Sathe (2006) reported oleic, linolenic, palmetic, and stearic acid contents of raw cashew oil at 61.15 ± 0.03, 16.88 ± 0.07, 10.70 ± 0.02, 9.33 ± 0.03%, respectively. In addition, Toschi *et al.* (1993) and Ryan *et al.* (2006) also reported similar values for the above mentioned fatty acids found in raw cashew nut oils.

In general, the ratio of total SFA to MUFA to PUFA of cashew nut oils was 1.2: 3.6: 1.0. This ratio remained the same for oils extracted from raw, LT, and HT treated whole cashew nuts, suggesting that roasting had little or no effect on the fatty acid profiles of cashew nut oils. Several authors have reported that FA compositions of rice germ, sesame seed and safflower seed oils prepared under different roasting temperatures and time combinations remained unchanged (Yen, 1990; Yosida, 1994; Kim *et al.*, 2002; Lee *et al.*, 2004).

5.4.4 Oxidative stability of cashew nut oils

The oxidative stability of cashew nut oils were tested by determining the contents of CD and TBARS. The results clearly showed greater oxidative stability of cashew nut oil under accelerated autoxidation conditions with increased roasting temperature. **Table 5.2** shows the changes in the contents of CD and TBARS (mmoles MDA equivalents /g of oil) in cashew nut oils during storage at 60°C. Generally, the CD values of oils extracted from roasted cashew nuts were significantly ($P < 0.05$) lower than that extracted from raw cashew nut suggesting their high oxidative stability.

CD show the degree of formation of primary products of lipid oxidation due to a shift in double bond positions upon oxidation of methylene interrupted lipid diene or polyenes (Salunkhe *et al.*, 1991). CD contents of cashew nut oils increased gradually as the storage time increased. Oxidative stability of cashew nut oils, based on changes of CD contents, followed the same order as those evaluated by the TBARS method in this study. Therefore, the oils from cashew nut roasted at high temperatures had a significantly ($P<0.05$) higher oxidative stability than oils from raw cashew nuts or those roasted at a

low temperature. These results are in agreement with those previously reported for sesame oils that indicate a better oxidative stability for sesame oil that was procured from seed subjected to a higher roasting temperature (Yen & Shyu, 1989). Lee *et al.* (2004) also reported that oxidative stability of safflower oil prepared from seeds roasted at different temperatures (140-180□C) were increased with increasing roasting temperature.

TBARS values provide a measure of the secondary oxidation products in the oil. The TBARS values, expressed as mmoles MDA equivalents per g of oil extracted from raw or roasted cashew nut increased slowly during the storage period for all samples. Moreover, TBA values of oils (**Table 5.2**) from raw, LT, and HT treated cashew nut oil were 0.105 ± 0.02, 0.120 ± 0.01, and 0.113 ± 0.01 mmoles MDA equivalents per g of oil respectively for oil samples after 72 h of storage under Schall oven condition. These results were in agreement of with those of Abou-Gharbia *et al.* (1997) who studied on the effect of processing on oxidative stability of sesame oil extracted from intact and dehulled seeds.

The better antioxidative stability of cashew nut oil prepared from cashew nut roasted at high temperature was possibly due to the formation of Maillard browning reaction products during the roasting process which are known to positively influence products, shelf life (Hayase *et al.*, 1989).

5.5 Conclusions

Cashew nut oil extracted from raw and roasted whole cashew nuts were examined for their fatty acid composition, colour change and oxidative stability. In general fatty acid composition remained unchanged for cashew nut oils extracted from raw as well as roasted whole nuts. Oleic was the major fatty acid followed by linoleic, palmitic, and stearic acids in all oil samples tested. Oxidative stability, as determined by the contents of CD and TBARS, was increased with increasing roasting temperature. The colour of oil extracted from roasted whole cashew nuts exhibited a higher absorbance value compared to that from raw whole cashew nut, possibly due to the formation of MRPs which are known to render antioxidant effect. Thus, roasting of whole cashew nuts improved the stability of their oil components against autoxidation. Further studies are warranted to isolate the active components in cashew nut oil samples and to positively identify their contribution to the antioxidant activity of oils extracted from roasted whole cashew nuts.

Table 5.1: Fatty acid composition of cashew nut oil extracted under different roasting conditions

Fatty acid %	Roasting conditions		
	RAW	LTW	HTW
16 : 0	10.31 ± 0.05 [a]	10.21 ± 0.00 [a]	10.28 ± 0.00 [a]
16 : 1 n - 9	0.34 ± 0.01 [a]	0.34 ± 0.00 [a]	0.33 ± 0.00 [a]
16 : 3 n - 4	0.06 ± 0.01 [a]	0.03 ± 0.00 [a]	0.11 ± 0.00 [b]
17 : 0	0.13 ± 0.00 [a]	0.12 ± 0.00 [a]	0.14 ± 0.00 [a]
18 : 0	9.83 ± 0.06 [a]	9.57 ± 0.05 [a]	10.14 ± 0.01 [a]
18 : 1 n - 9	60.57 ± 0.11 [a]	61.33 ± 0.04 [a]	60.68 ± 0.00 [a]
18 : 2 n - 6	17.03 ± 0.11 [a]	16.79 ± 0.07 [a]	16.79 ± 0.00 [a]
18 : 3 n - 3	0.21 ± 0.01 [a]	0.22 ± 0.00 [a]	0.19 ± 0.00 [a]
20 : 0	0.74 ± 0.01 [a]	0.67 ± 0.01 [a]	0.64 ± 0.01 [ab]
21 : 1 n - 9	0.19 ± 0.01 [a]	0.19 ± 0.01 [a]	0.17 ± 0.00 [a]
22 : 0	0.14 ± 0.00 [a]	0.12 ± 0.01 [a]	0.11 ± 0.01 [ab]
24 : 0	0.11 ± 0.01 [a]	0.09 ± 0.01 [a]	0.09 ± 0.00 [a]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letters, on raw are not significantly different ($p > 0.05$). RAW, raw whole cashew nut oil; LTW, low temperature roasted whole cashew nut oil; and HTW, high temperature roasted whole cashew nut oil.

Table 5.2: Oxidative stability of cashew nut oil extracted under different roasting conditions

Storage time (h)	CD values			TBARS (mmoles MDAE/g of oil)		
	RAW	LTW	HTW	RAW	LTW	HTW
0	0.91 ± 0.00[a]	0.90 ± 0.10[a]	0.81 ± 0.01[a]	0.10 ± 0.08[a]	0.12 ± 0.01[a]	0.02 ± 0.06[a]
6	0.93 ± 0.01[a]	0.90 ± 0.05[a]	0.57 ± 0.13[b]	0.21 ± 0.01[a]	0.21 ± 0.01[a]	0.17 ± 0.02[b]
12	1.23 ± 0.08[a]	0.99 ± 0.00[b]	0.83 ± 0.04[b]	0.30 ± 0.01[a]	0.24 ± 0.04[a]	0.18 ± 0.06[b]
24	1.14 ± 0.23[a]	0.84 ± 0.02[a]	0.68 ± 0.17[b]	0.25 ± 0.01[a]	0.23 ± 0.04[a]	0.09 ± 0.06[b]
36	1.31 ± 0.19[a]	0.83 ± 0.10[b]	0.72 ± 0.03[b]	0.17 ± 0.03[a]	0.13 ± 0.04[a]	0.08 ± 0.04[b]
48	1.34 ± 0.51[a]	0.89 ± 0.08[a]	0.69 ± 0.08[b]	0.15 ± 0.04[a]	0.12 ± 0.01[a]	0.11 ± 0.01[a]
72	1.37 ±0.29[a]	0.74 ± 0.12[b]	0.66 ± 0.02[b]	0.11 ± 0.01[a]	0.12 ± 0.01[a]	0.11 ± 0.01[a]

Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letters, in each row are not significantly different ($p > 0.05$). RAW, raw whole cashew nut oil; LTW, low temperature roasted whole cashew nut oil; HTW, high temperature roasted whole cashew nut oil; CD, conjugated diene; TBARS, thiobarbituric acid reactive substances; and MDAE, malondialdehyde equivalents.

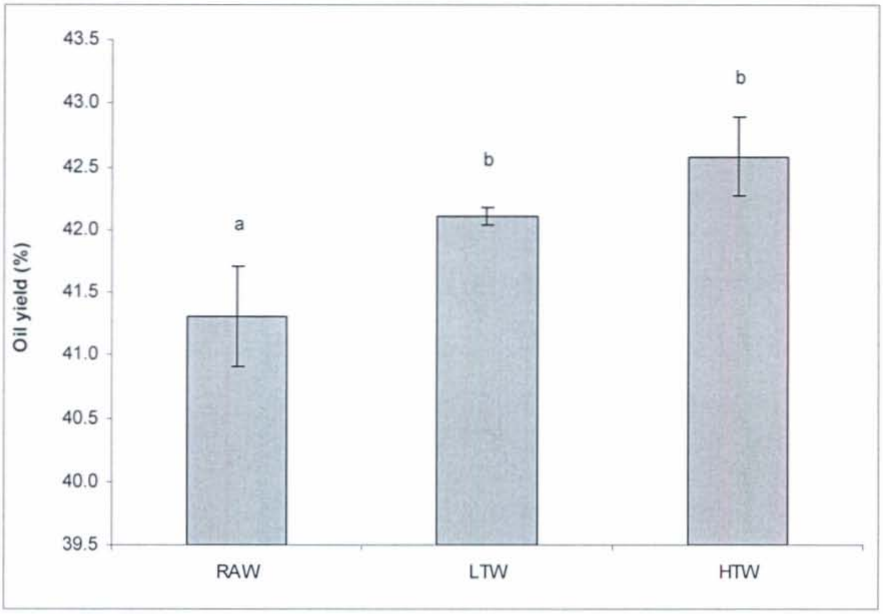


Figure 5.1: Yield of cashew oil at different roasting conditions. Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). RAW, raw whole cashew nut oil; LTW, low temperature roasted whole cashew nut oil; and HTW, high temperature roasted whole cashew nut oil.

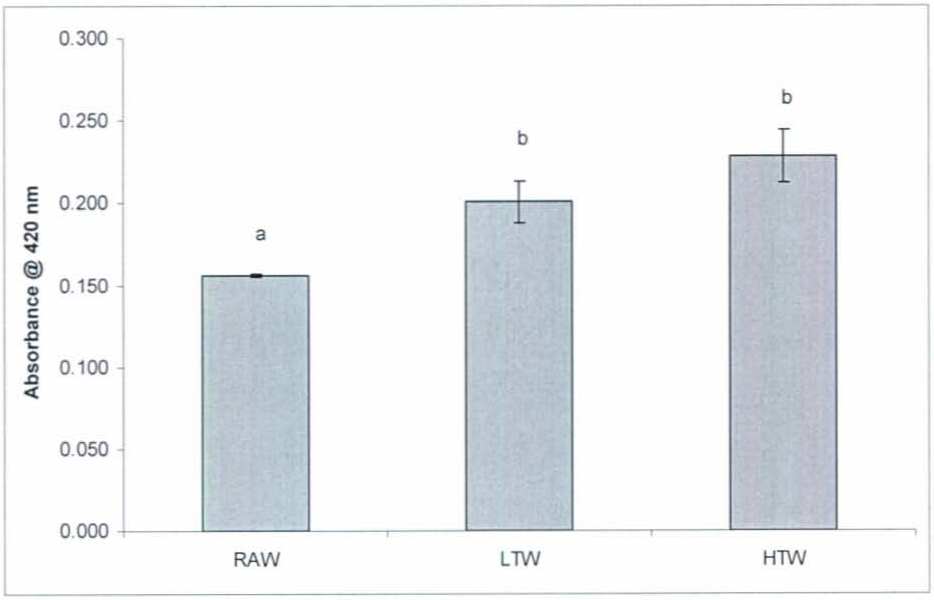


Figure 5.2: Colour development (absorbance at 420 nm) of cashew nut oil at different roasting conditions. Data are expressed as means ± SD (n = 3). Means ± SD followed by the same letter, on bars are not significantly different ($p > 0.05$). RAW, raw whole cashew nut oil; LTW, low temperature roasted whole cashew nut oil; and HTW, high temperature roasted whole cashew nut oil.

Chapter 6

Conclusions and future studies

6.1 Conclusions

The results of the present study indicate that cashew nut kernels and testa contain phenolic compounds that are responsible for a wide array of antioxidant activities. The contribution of insoluble bound phenolic fraction to the total antioxidant activity was insignificant ($p \leq 0.05$) compared to the soluble phenolic fraction of cashew nuts and testa. The high temperature (HT) roasted cashew nuts and testa showed a higher phenolic content and antioxidant activity than raw and low temperature (LT) roasted samples. In general, the findings of this study suggest that thermal processing enhances the value of cashew kernels and testa as natural antioxidants and cashew testa, a waste byproduct can be utilized, as a health promoting and disease preventing nutraceutical ingredient.

Extracts of cashew kernel showed effective inhibition against copper induced human low density lipoprotein (LDL) cholesterol. However, roasting did not contribute significantly to enhancing the antioxidant activity in food and biological model systems compared to the raw counterparts except under accelerated autoxidation conditions of stripped corn oil and extract system as assed by the Rancimat method.

Fatty acid composition of cashew nut oils extracted from raw as well as roasted whole nuts remained unchanged. Oleic was the major fatty acid followed by linoleic, palmitic, and stearic acids in raw, LT and HT roasted whole cashew oil samples tested. Oxidative stability, as determined by the contents of CD and TBARS, was increased with increasing roasting temperature. The colour of oil extracted from roasted whole cashew nuts

exhibited a higher absorbance value compared to that from raw whole cashew nut, possibly due to the formation of MRPs which are known to render antioxidant effect. Thus, roasting of whole cashew nuts improved the stability of oil extracted from them against autoxidation.

6.2 Future studies

In the present study chemical assays and *in vitro* food and biological models were used to assess the antioxidant activity of raw and roasted cashew nuts and testa. However, it is important to determine the effects of extracts in *in vivo* systems. The available information on the absorption and bioavailability of active components of cashew phenolic extracts is limited, thus such studies are necessary. Furthermore, the effects of storage of raw and roasted cashew nuts on the antioxidant activity under different conditions need to be examined. In addition, identification of active components that contribute to the antioxidant activity of oils extracted from raw and roasted whole cashew nuts is also necessary.

References

Abbey, M., Noakes, M., Belling, G. B., Nestel, P. J. Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low density lipoprotein cholesterol. American Journal of Clinical Nutrition, 1994, 59, 995-999.

Abdel-Aal, E. S. M., Pierre, H. Composition and stability of anthocyanins in blue grained wheat. Journal of Agricultural and Food Chemistry, 2003, 51, 2174-2180.

Abou-Gharbia, H. A., Shahidi, F., Shehata, A. A. Y., Youssef, M. M. Effect of processing on oxidative stability of sesame oil extracted from intact and de hulled seeds. Journal of the American Oil Chemists' Society, 1997, 74, 215-221.

Abuja, P. M., Murkovic, M., Pfannhauser, W. Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. Journal of Agricultural and Food Chemistry, 1998, 46, 4091-4096.

Acar, O. C., Gokmen, V., Pellegrini, N., Fogliano, V. Direct evaluation of the total antioxidant capacity of raw and roasted pulses, nuts and seeds. European Food Research and Technology, 2009, 229, 961-969.

Akasbl, M., Shoeman, D.W., Saari Csallany, A. High performance liquid chromatography of selected phenolic compounds in olive oils. Journal of the American Oil Chemists' Society, 1993, 70, 367-370.

Akinwale, T. O. Cashew apple juice: its use in fortifying the nutritional quality of some tropical fruits. Journal of European Food Research and Technology, 2000, 211, 205-207.

Alasavar, C., Karamac, M., Amarowicz, R., Shahidi, F. Antioxidant and antiradical activities in axtracts of hazelnut kernel (*Corylus avellana* L.) and hazelnut green leafy cover. Journal of Agriculture and Food Chemistry, 2006, 54, 4826-4832.

Alasavar, C., Shahidi, F. Tree nuts: composition, phytochemicals, and health effects: an overview. In: Alasavar, C., Shahidi, F. (Ed.), Tree nuts composition, phytochemicals, and health effects. CRC Press, Boca Raton, FL, 2009, pp 1-10.

American Oil Chemists' Society, Official methods and recommended practices of the American Oil Chemists' Society, 4th ed., edited by D. Firestone, American Oil Chemists' Society, Champaign, IL, 1990.

Ames, B. N., Shigenaga, M. K., Hagen, T. M. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America, 1993, 90, 7915-7922.

Amorati, R., Pedulli, G. F., Valgimgli, L., Attanasi, O. A., Filippone, P., Fiorucci, C., Saladino, R. Absolute rate constants for the reaction of peroxyl radicals with cardanol derivatives. Journal of the Chemistry Society, Perkin Transactions 2, 2001, 2, 2142-2146.

Andersen, O. M., Petersen, H. H., Jacobsen, C., Moestrup, S. K., Etzerodt, M., Andreasen, P. A., Thogersen, H. C. Analysis of a two-domain binding site for the urokinase-type plasminogen activator-plasminogen activator inhibitor-1 complex in low-density-lipoprotein-receptor-related protein. Biochemical Journal, 2001, 357, 289-296.

Andrighetti, L., Bassi, G. F., Capella, P., de Logu, A. M., Deolalikar, A. B., Haeusler, G., Malorgio, G. A., Franca, F. M. C., Rivoira, G., Vannini, L., Deserti R. The world cashew economy. L. Inchiostroblu, Italy, 1989.

AOCS. Official methods and recommended practices of the American Oil Chemists' Society, 4th ed.; AOCS: Champaign, IL, 1995.

Aremu, M. O., Olonisakin, A., Bako, D. A., Madu, P. C. Compositional Studies and Physicochemical Characteristics of Cashew Nut (*Anarcadium occidentale*) Flour. Pakistan Journal of Nutrition, 2006, 5, 328-333.

Azam-Ali, S.H., Judge, E.C. Small scale Cashew Nut Processing. FAO, Rome, Italy, 2001.

Basu, A., Devaraj, S., Jialal, I. Dietary factors that promote or retard inflammation. Arteriosclerosis, Thrombosis and Vascular Biology, 2006, 26, 995-1001.

Beckman, C. H. Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defense responses in plants? Physiological and Molecular Plant Pathology, 2000, 57, 101-110.

Beggs, C. J., Khun, K., Bocker, R., Wellmann, E. Phytochrome induced flavonoid biosynthesis in mustard (*Sinapsis alba* L) cotyledons: enzymatic control and differential regulation of anthocyanin and quercitin formation. Planta, 1987, 172, 121-126.

Berliner, J. A., Heinecke, J. W. The role of oxidized lipoproteins in atherogenesis. Free Radical Biology and Medicine, 1996, 20, 707-727.

Block, G., Patterson, B., Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutrition and Cancer, 1992, 18, 1-29.

Blomhoff, R. Dietary antioxidants and cardiovascular disease. Current Opinion in Lipidology, 2005, 16, 47-54.

Blomhoff, R., Carlsen, M. H., Andersen, F. L., Jacobs, D. R. Healthbenefits of nuts: potential role of antioxidants. British Journal of Nutrition, 2006, 96 S, 52-60.

Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews, 1998, 56, 317-333.

Brown, A. A., Hu, F. Dietary modulation of endothelial function: implications for cardiovascular disease. American Journal of Clinical Nutrition, 2001, 73, 673-686.

Cao, G., Sofic, E., Prior, R. Antioxidant capacity of tea and common vegetables. Journal of Agriculture and Food Chemistry, 1996, 44, 3426-3431.

Caribbean Technological Consultancy Services (CTCS) Network. Information package on cultivation, processing and marketing of cashews. St Michael, Barbados, Caribbean Technological Consultancy Services Network, 1993.

Carney, J. M., Starke-Reed, P. E., Oliver, C. N., Landum, R. W., Cheng, M. S., Wu, J. F., Floyd, R. A. Reversal of age related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tertbutylphenylnitrone. Proceedings of the National Academy of Sciences of the United States of America, 1991, 88, 3633-3636.

Cashew Bulletins, Council calls for urgent need to step up indigenous production of raw cashew nuts in India. Cashew Bulletins, India, 2005, 10, 3-4.

Cavalcante, A. A. M., Rubensam, G., Picada, J. N., Silva, E. G., Moreira, J. C. F., Henriques, J. A. P. Mutagenicity, antioxidant potential, and antimutagenic activity against hydrogen peroxide of cashew (*Anacardium occidentale*) apple juice and cajuina. Environmental and Molecular Mutagenesis, 2003, 41, 360-369.

Chandrasekara, A., Shahidi, F. The content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. Journal of Agricultural and Food Chemistry, 2010, 58, 6706 - 6714.

Chempakam, B. Distribution of ascorbic acid and ascorbic acid oxidase activity in developing cashew apple (*Anarcadium occidentale*, L.). Journal of Horticultural Science, 1983, 58, 447-448.

Chethan, S., Malleshi, N. G. Finger millet Polyphenols: Optimization of extraction and the effect of pH on their stability. Food Chemistry, 2007, 105, 862-870.

Chisolm, G. M., Steinberg, D. The oxidative modification hypothesis of atherogenesis: an overview. Free Radical Biology and Medicine, 2000, 28, 1815-1826.

Christie, P. J., Alfenito, M. R., Walbot, V. Impact of low temperature stress on general phenylpropanoid and anthocyanin pathways: enhancement of transcript abundance and anthocyanin pigmentation in maize seedlings. Planta, 1991, 194, 541-549.

Cladinin, D. R., Robllee, A. R. The effect of processing on the enzymatic liberation of lysine and arginine from soybean oil meal. Journal of Nutrition, 1952, 46, 525-530.

Colaric, M., Veberic, R., Solar, A., Hudina, M., Stampar, F. Phenolic acids, syringaldehyde, and juglone in fruits of different cultivars of *Juglans regia* L. Journal of Agriculture and Food Chemistry, 2005, 53, 6390-6396.

Cuvelier, M. E., Richard, H., Berset, C. Comparison of the antioxidant activity of some acid phenols: structure-activity relationship. Bioscience, Biotechnology and Biochemistry, 1992, 56, 324-325.

Dantas, T. N. C., Dantas, M. S. G., Neto, A. A. D., D'Ornellas, C. V., Queiroz, L.R. Novel antioxidants from cashew nut shell liquid applied to gasoline stabilization. Fuel, 2003, 82, 1465-1469.

Davidson, V. J., Brown, R. B., Landman, J. J. Fuzzy control system for peanut roasting. Journal of Food Engineering, 1999, 41, 141-146.

Davis, J. P., Dean, L. L., Price, K. M., Sanders, T. H. Roast effects on the hydrophilic and lipophilic antioxidant capacities of peanut flours, blanched peanut seed and peanut skins, Food Chemistry, 2010, 119, 539-547.

Decker, E. A., Ivanov, V., Zhu, B. Z., Frei, B. Inhibition of low-density lipoprotein-oxidation by carnosine and histidine. Journal of Agricultural and Food Chemistry, 2001, 49, 511-516.

Demir, A. D., Celayeta, J. M. F., Cronin, K., Abodayeh, K. Modelling of the kinetics of colour change in hazelnuts during air roasting. Journal of Food Engineering, 2002, 55, 283-292.

Demir, A. D., Cronin, K. The thermal kinetics of texture change and the analysis of texture variability for raw and roasted hazelnuts. International Journal of Food Science and Technology, 2004, 39, 371-383.

Diaz, A. C., Analia, V., Gimenez, F., Mendiara, S. N. and Fenucci, J. L. Antioxidant activity of hepatopancreas of the shrimp (*Pleoticus muelleri*) by electron paramagnetic spin resonance spectrometry. Journal of Agriculture and Food Chemistry, 2004, 52, 3189-3193.

Dreher, M. L., Maher, C. V., Kearney, P. The traditional and emerging role of nuts in healthful diets. Nutrition Reviews, 1996, 54, 241-245.

Durmaz, G., Karabulut, I., Topcu, A., Asilturk, M., Kutlu, T. Roasting-related changes in oxidative stability and antioxidant capacity of apricot kernel oil. Journal of the American Oil Chemists' Society, 2010, 87, 401-409.

Duthie, G. G., Duthie, S. J., Kyle, J. A. M. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutrition Research Reviews, 2000, 13, 79-106.

Einarsson, H., Eklund, T., Nes, I. F. Inhibitory mechanisms of Maillard reaction Products. Microbios, 1988, 53, 27-36.

Etherton, P. M. K., Hecker, K. D., Bonanome, A., Coval, M. S., Binkoski, A. E., Hilpert, K. F., Griel, A. E., Etherton, T. D. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine, 2002, 113, 71S-88S.

Etherton, P. M. K., Yu-Poth, S., Sabate, J., Ratcliffe, H. E., Zhao, G., Etherton, T. D. Nuts and their bioactive constituents: Effects on serum lipids and other factors that affect disease risk. American Journal of Clinical Nutrition, 1999, 70, 504-511.

Exler, J., Weihrauch, J. L. Provisional table on the content of omega-3 fatty acids and other fat components in selected foods. Washington, DC: US Department of Agriculture, 1986. (Publication HNIS/PT-103).

Finot, P.A. Metabolism and physiological effects of Maillard reaction products, In The Maillard Reaction, In: Finot, P.A., Aeschbacher, H.U., Hurrell, R.F., Liardon, R. Food Processing, Human Nutrition and Physiology, Birkhauser, Basel, Switzerland, 1990, pp. 259-271.

Frankel, E. N., Huang, S. W., Kanner, J., German, J. B. Interfacial phenomena in the evaluation of antioxidants: bulk oil versus emulsions. Journal of Agricultural and Food Chemistry, 1994, 42 1054-1059.

Frankel, E.N. Lipid oxidation: Mechanisms, products and biological significance. Journal of the American Oil Chemists' Society, 1984, 61, 1908-1917.

Fraser, G. E. Nut consumption, lipids, and risk of a coronary event. Clinical Cardiology, 1999, 22, 1-5.

Fraser, G. E., Lindsted, K. D., Beeson, W. L. Effect of risk factor values on lifetime risk of and age at first coronary event. The Adventist Health Study. American Journal of Epidemiology, 1995, 142, 746-758.

Fraser, G. E., Sabate, J., Beeson, W. L., Strahan, T. M. A possible protective effect of nut consumption on risk of coronary heart disease. Archives of Internal Medicine, 1992, 152, 1416-1424.

Fraser, G. E., Shavik, D. J. Ten years of life: is it a matter of choice? Archives of Internal Medicine, 2001, 161, 1645-1652.

Friedman, M. Food browning and its prevention: An overview. Journal of Agricultural and Food Chemistry, 1996, 44, 631-653.

Garcia-Lorda, P., Rangil, M. I., Salas-Salvado, J. Nut consumption, body weight and insulin resistance. European Journal of Nutrition, 2003, 57, S8-11.

General Foods Corporation (Rye, NY). Improved process for the separation of acid constituent from Cashew nut shell liquid or hydrogenated cashew nut shell liquid. Indian Patent 34671, 1946.

Gentile, C., Tesoriere, L., Butera, D., Fazzari, M., Monastero, M., Allegra, M., Livrea, M. A. Antioxidant activity of Sicilian pistachio (*Pistacia vera* L. var. Bronte) nut extract and its bio-active components. Journal of Agriculture and Food Chemistry, 2007, 55, 643-648.

Geraldoa, M., Brown, W. H., Whiting, F. M., Stull, J. W. Cashew nut unsaponifiable matter, Journal of Food Science, 1976, 41, 190-194.

Gerrard, J.A., Fayle, S.E., Sutton, K.H. Protein product formation and protein cross-linking resulting from Maillard reaction between cyclotene and s model food protein. Journal of Agricultural and Food Chemistry, 1999, 47, 1183-1188.

Giada, M. L., Filho, J. M. The importance of dietary phenolic compounds in the promotion of human health. Biology and Health Science, 2006, 12, 7-15.

Giessauf, A., Steiner, E., Esterbauer, H. Early destruction of tryptophan residues of apolipoprotein B is a vitamin E independent process during copper-mediated oxidation of LDL. International Journal of Biochemistry, Biophysics and Molecular Biology, 1995, 1256, 221-232.

Giribert, M. C., Ribas, A. I. Kinetics of colour development in aqueous glucose systems at high temperatures. Journal of Food Engineering, 2000, 44, 181-189.

Glitso, L. V., Mazur, W. M., Adlercreutz, H., Wahala, K., Makela, T., Sandstroom, B., Knudsen, K. E. B. Intestinal metabolism of rye lignans in pigs. British Journal of Nutrition, 2000, 84, 429-437.

Gordon, M. The development of oxidative rancidity in foods. In Antioxidants in Food. Eds. Pokorny, J., Yanihslieva, N. and Gordon, M. Woodhead Publishing Ltd., Cambridge, UK, 2001, pp.7-21.

Gordon, M. H. The mechanisms of antioxidant action *in vitro*. In Food Antioxidants. Eds. Hudson, B. J. F. Elsevier Applied Science, New York, NY, 1990, pp. 1-18.

Graham, T. L. Flavonoid and isoflavonoid distribution in developing soybean seedling tissue and in seed root exudates. Plant Physiology, 1991, 95, 594-603.

Grazzini, R., Hesk, D., Heininger, E., Hildenbrandt, G., Reddy, C. C., Cox-Foster, D., Medferd, J., Craig, R., Mumma, R. O. Inhibition of lipoxygenase and prostaglandin endoperoxide synthase by anacardic acids. Biochemical and Biophysical Research Communications, 1991, 176, 775-780.

Gunstone, F.D., Reaction of oxygen and unsaturated fatty acids. Journal of the American Oil Chemists' Society, 1984, 61, 441- 445.

Gutfinger, T. Polyphenols in olive oils. Journal of the American Oil Chemists' Society, 1981, 58, 966-968.

Hahlbrock, K., Scheel, D. Physiology and molecular biology of phynylpropaniod metabolism. Plant Molecular Biology, 1989, 40, 347-369.

Halliwell, B. and Gutteridge, J. M. C. The antioxidants of human extracellularfluids. Archives of Biochemistry and Biophysics, 1999, 280, 1-8.

Halvorsen, B. L., Holte, K., Myhrstad, M. C. W.,Barikmo, I., Hvattum, E., Remberg, S. F., Wold, A. B., Haffner, K., Baugerod, H., Andersen, L. F., Moskaug, O., Jacobs, D. R., Blomhoff, R. A systematic screening of total antioxidants in dietary plants. The Journal of Nutrition, 2002, 132, 461-471.

Hammerstone, J. F., Lazarus, S. A., Schmitz, H. H. Procyanidin content and variation in some commonly consumed foods. Journal of Nutrition, 2000, 130, 2086-2092.

Hartley, R. D., Morrison, W. H., Himmelsbach, D. S., Borneman, N. S. Cross-linking of cell wall phenolics to arabinoxylans in graminaceous plants. Phytochemistry, 1990, 29, 3701-3709.

Harukaze, A., Masatsune, M., Homma, S. Analysis of free and bound phenolics in rice. Food Science and Technology Research, 1999, 5, 74-79.

Hayase, F., Hirashima, S., Okamoto, G., Kato, H. Scavenging of active oxygen by melanoidins. Agriculture and Biological Chemistry, 1989, 53, 3383-3385.

Hebbar, U. N., Ramesh, M. N. Optimisation of processing conditions for infrared drying of cashew kernels with testa. Journal of the Science of Food and Agriculture, 2005, 85, 865-871.

Heimendinger, J., Van Duyn, M.A., Chapelsky, D., Foerster, S., Stables, G. The national 5 A Day for Better Health Program: a large-scale nutrition intervention. Journal of Public Health Management and Practice, 1996, 2, 27-35.

Hidalgo, F. J., Zamora, R. The role of lipids in nonenzymatic browning. Grasas y Aceites, 2000, 51, 35-49.

Hilder, R. C., Liu, Z. D., and Khodr, H. H. Metal chelation of polyphenols. Methods in Enzymology, 2001, 335, 190-203.

Himejima, M., Kubo, I. Antibacterial agents from the cashew, *Anacardium occidentale*, nut shell oil. Journal of Agricultural Food Chemistry, 1991, 39, 418-421.

Hu, C., Kitts, D. D. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems *in vitro*. Molecular and Cellular Biochemistry, 2001, 218, 147-155.

Hu, F. B., Stamfer, M. J. Nut consumption and risk of coronary heart disease: a review of the epidemiologic evidence. Current Atherosclerosis Reports, 1999, 1, 204-209.

Huang, D., Ou, B., Prior, R.L. The chemistry behind antioxidant capacity assays. Journal of Agriculture and Food Chemistry, 2005, 53, 1841-1856.

Hung, Y. C., Chinnan, M. S. Mechanical texture measurement of whole and chopped peanuts. Peanut Science, 1989, 16, 32-37.

Huynh, N. N., Chin-Dusting, J. Amino acids, arginase and nitric oxide in vascular health. Clinical and Experimental Pharmacology and Physiology, 2006, 33, 1-8.

Hwang, J. Y., Shue, Y. S., Chang, H, M. Antioxidative activity of roasted and defatted peanut kernels. Food Research International, 2001, 34, 639-647.

Ingold, K. U. Inhibition of autoxidation. Advance in Chemistry, 1968, 75, 296-305.

Itokawa, H., Totsuka, N., Nakahara, K., Maezuru, M., Takeya, K., Kondo, M., Inamatsu, M., Morita, H. A quantitative structure-activity relationship for antitumor activity of long-chain phenols from *Ginkgo biloba* L. Chemistry Pharmacy Bulletin, 1989, 37, 1619-1621.

Itokawa, H., Totsuka, N., Nakahara, K., Takeya, K., Lepoittevin, J. P., Asakawa, Y. Antitumor principles from *Ginkgo biloba* L. Chemistry Pharmacy Bulletin, 1987, 35, 3016-3020.

Jacobs, D. R., Marquart, L., Slavin, J., Kushi, L. H. Whole-grain intake and cancer: an expanded review and meta-analysis. Nutrition and Cancer, 1998, 30, 85-96.

Jadhav, S. J., Nimbalkar, S. S., Kulkarni, A. D., Madhavi, D. L. (1996). Lipid oxidation in biological and food systems. In Madhavi, D. L., Deshpande, S. S., Salunkhe, D. K. (Eds.), Food antioxidants. Dekker, NY, 1996, pp. 5-60.

Jain, R. K., Kumar, S. Development of a cashew nut sheller. Journal of Food Engineering, 1997, 32, 339-345.

Jayaprakasha, G. K., Singh, R. P., Sakariah, K. K.Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chemistry, 2001, 73, 285-290.

Jenab, M., Ferrari, P., Slimani, N., Norat, T., Casagrande, C., Overad, K. Association of nut and seed intake with colorectal cancer risk in the European Prospective investigation into cancer and nutrition. Cancer Epidemiology, 2004, 13, 1595-1603.

Jeong, S. M., Kim, S, Y., Kim, D. R., Nam, K. C., Ahn, D. U., Lee, S. C. Effect of seed roasting conditions on the antioxidant activity of defatted sesame meal extracts. Journal of Food Science, 2004, 69, 377-381.

Jeor, S. T., Brunner, R. L., Harrington, M. E., Scott, B. J., Cutter, G. R., Brownell, K. D., Dyer, A. R., Foreyt, J. P. Who are the weight maintainers? Obesity Research, 1995, 3, 2249s-2259s.

Jiang, R., Manson, J. E., Stampfer, M. J., Liu, S., Willet, W. C., Hu, F. B. Nut and peanut butter consumption and risk of type 2 diabetes in women. Journal of the American Medical Association, 2002, 288, 2554-2560.

John, J. A., Shahidi, F. Phenolic compounds and antioxidant activity of Brazil nut (*Bertholletia excelsa*). Journal of Functional Foods, 2010, 2, 196-209.

Jung, M. Y., Bock, J. Y., Baik, S. O., Lee, J. H., Lee, T. K. Effects of roasting on pyrazine contents and oxidative stability of red pepper seed oil prior to its extraction. Journal of Agricultural and Food Chemistry, 1999, 47, 1700-1704.

Kamath, V., Rajini, P. S. The efficiency of cashew-nut (*Anacardium occidentale* L.) skin extract as a free radical scavenger. Food Chemistry, 2007, 103, 428-433.

Kanner, J., Rosenthal, I. An assessment of lipid oxidation in foods. Pure and Applied Chemistry, 1992, 64, 1959-1964.

Kanner, S. B., Reynolds, A. B., Parsons, J. T. Tyrosine phosphorylation of a 120-kilodalton pp 60 src substrate upon epidermal growth factor and platelet-derived growth factor receptor stimulation and in polyomavirus middle-T-antigen-transformed cells. Molicular Cell Biology, 1991, 11, 713-720.

Katalinic, V., Milos, M., Modun, D., Music, I., Boban, M. Antioxidant effectiveness of selected wines in comparison with (+) catechin. Food Chemistry, 2004, 86, 593-600.

Kaur, C., Kapoor, H. C. Antioxidants in fruits and vegetables - The millenium's health. International Journal of Food Science and Technology, 2001, 36, 703-725.

Kay, C. D., Kris-Etherton, P. M., West, S. G. Effects of antioxidant-rich foods on vascular reactivity: review of the clinical evidence. Current Atherosclerosis Reports, 2006, 8, 510-522.

Kendall, C. W. C., Jenkins, D. J. A., Marchie, A., Parker, T., Connelly, P. W. Dose response to almonds in hyperlipidemia: A randomized controlled crossover trial. American Journal of Clinical Nutrition, 2002, 75, 384-385.

Khanbabaee, K., Van Ree, T. Tannins: classification and definition. Natural Product Reports, 2001, 18, 641-649.

Kim, H., Ralph, J., Lu, F., Ralph, S.A., Boudet, A-M., MacKay, J.J., Sederoff, R. R., Ito, T., Kawai, S., Ohashi, H., Higuchi, T. NMR analysis of lignins in CAD- deficient plants. Part 1. Incorporation of hydroxycynnamaldehydes and hydroxybenzaldehydes into lignins. Organic and Biomolecular Chemistry, 2003, 1, 158-281.

Kim, I. H., Kim, C. J., You, J. M., Lee, K. W., Kim, C. T., Chung, S. H., Tae, B. S. Effect of roasting temperature and time on the chemical composition of rice germ oil. Journal of The American Oil Chemists' Society, 2002, 79, 413-418.

Kiritsakis, A., Tsipeli, A. Relationship of the acidity of olive oil to its resistance to oxidation. Rivista Italiana delle Sostanze Grasse, 1992, 69, 513-515.

Koechler, P. F., Odell, G. V. Factors affecting the formation of pyrazine compounds in sugar-amine reaction. Journal of Agricultural and Food Chemistry, 1970, 18, 895-898.

Kontush, A., Beisiegel, U. Measurement of oxidizability of blood plasma. Methods in Enzymology, 1999, 299, 35-49.

Kornsteiner, M., Wagner, K. H., Elmadfa, I. Tocopherols and total phenolics in 10 different nut types. Food Chemistry, 2006, 98, 381-387.

Kris-Etherton, P. M., Zhao, G., Binkoski, A. E., Coval, S. M., Etherton, T. D. The effect of nuts on coronary heart disease risk. Nutrition Reviews, 2001, 59, 103-111.

Kubo, I., Kinst-Hori, I., Yokokawa, Y. Tyrosinase inhibitors from *Anacardium occidentale* fruits. Journal of Natural Products, 1994a, 57, 545-551.

Kubo, I., Muroi, H., Himejima, M., Yamagiwa, Y., Mera, H., Tokushima, K., Ohta, S., Kamikawa, T. Structure-antibacterial activity relationships of anacardic acids. Journal of Agricultural Food Chemistry, 1993a, 41, 1016-1019.

Kubo, I., Muroi, H., Kubo, A. Naturally occurring anti acne agents. Journal of Natural Products, 1994b, 57, 9-17.

Kubo, I., Ochi, M., Vieira, P. C., Komatsu, S. Antitumor agents from the cashew (Anacardium occidentale) apple juice. Journal of Agricultural Food Chemistry, 1993b, 41, 1012-1015.

Kushi, L., Folsom, A. R., Prineas, R. J., Mink, P. J., Wu, Y., Bostick, R. M. Dietary antioxidant vitamins and death from coronary heart disease in post-menopausal women. New England Journal of Medicine, 1996, 334, 1156-1162.

Lee, S. C. Kim, J. H. Jeong, S. M. Kim, D. R. Ha, J. U. Nam, K. C., Ahn, D. U. Effect of far-infrared radiation on the antioxidant activity of rice hulls. Journal of Agriculture and Food Chemistry, 2003, 51, 4400-4403.

Lee, Y. C., Oh, S. W., Chang, J., Kim, I. H. Chemical composition and oxidative stability of safflower oil prepared from safflower seed roasted with different temperatures. Food Chemistry, 2004, 84, 1-6.

Lewis, N., Yamamato, E. Lignins: Occurrence biosynthesis and biodegradation. Annual Reviews of Plant Physiology, 1990, 41, 455-496.

Li, S., Zhang, Q. H. Advances in the development of functional foods from buckwheat. Critical Reviews in Food Science and Nutrition, 2001, 41, 451-464.

Liu, R. H. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. Journal of Nutrition, 2004, 134, 3479S-3485S.

Liu, R. H. Whole grain phytochemicals and health. Journal of Cereal Science, 2007, 46, 207-219.

Liyana-Pathirana, C., Shahidi, F. Importance of insoluble-bound phenolics to antioxidant properties of wheat. Journal of Agricultural and Food Chemistry, 2006, 54, 1256-1264.

Locatelli, M., Travaglia, F., Coisson, J. D., Martelli, A., Stevigny, C., Arlorio, M. Total antioxidant activity of hazelnut skin (*Nocciola piemonte* PGI): Impact of different roasting conditions. Food Chemistry, 2010, 119, 1647-1655.

Loliger, J. The use of antioxidants in food. In Free Radicals and Food AdditiVes; Aruoma, O. I., Halliwell, B., Eds.; Taylor and Francis: London, UK, 1991, pp 129-150.

Loury, M., Lercharlier, G., Fomey, M. Identification of volatile products resulting of autoxidation of oleic acid by thin layer and paper chromatography, Revue Francaise des Corps Gras, 1965,12:253-262.

Lugasi, A., Hovari, J., Sagi, K. V., Biro, L. The role of antioxidant phytonutrients in the prevention of diseases. Biologica Szegediensis, 2003, 47, 119-125.

Lundberg, W.O. Lipids and Their Oxidation, AVI Publishing Co., Westport, CT, 1962, pp 20-59.

Madhujith, T., Shahidi, F. Antioxidative and antiproliferative properties of selected barley (*Hordeum vulgarae* L.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. Journal of Agricultural and Food Chemistry, 2007, 55, 5018-5024.

Maillard, L. C. Action des acides amides sucres; formation des melanoidies per voie Methodique. Central European Journal of Biology, 1912, 2, 66-69.

Maillard, M. N., Berset, C. Evolution of antioxidant activity during kilning. Role of insoluble bound phenolic acids of barley and malt. Journal of Agricultural and Food Chemistry, 1995, 43, 1789-1793.

Maillard, M. N., Soum, M. H., Boivia, P., Berset, C. Antioxidant activity of barley and malt: relationship with phenolic content. Lebensmittel-Wissenschaft und Technologie, 1996, 3, 238-244.

Maini, S. B., Anand, J. C. Utilization of fruit wastes. In Advances in Horticulture, Vol 4, Fruit Crops (Chadha, K. L. and Pareek, O.P. Eds.), Malhotra Publishing House, New Delhi, India, 1993, pp. 1967-1992.

Maldonado, O., Rolz, C., Cabrera, S. S. D. Wine and vinegar production from tropical fruits. Journal of Food Science, 1975, 40, 262-265.

Mandal, R. C. Cashew: production and processing technology. Agrobios (India), 2000, 165-166.

Marita, J., Vermerris, W., Ralph, J., Hatfield, R. D. Variation in the cell wall composition of maize brown midrib mutants. Journal of Agricultural and Food Chemistry, 2003, 51, 1313-1321.

Masuoka, N., Kubo, I. Characterization of xanthine oxidase inhibition by anacardic acids. Biochimica et Biophysica Acta, 2004, 1688, 245-249.

Mathew, A. G., Parpia, H. A. B. Polyphenols of cashew kernel testa. Journal of Food Science, 1970, 35, 140-143.

Mekeehen, J. D., Busch, R. H., Fulcher, R. G. Evaluation of wheat (*Triticum aestieum* L.) phenolic acids during grain development and their contribution to *Fusarium* resistence. Journal of Agricultural and Food Chemistry, 1999, 47, 1476-1482.

Menon, A. R. R., Pillai, C. K. S., Sudha, J. D., Mathew, A. G. Cashew nut shell liquid-its polymeric and otherindustrial products. Journal of Scientific and Industrial Research, 1985, 44, 324-338.

Meyer, A. S., Heinonen, M., Frankel, E. N. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin and ellagic acid on human LDL oxidation. Food Chemistry, 1998, 61, 71-75.

Middleton, E. Effect of plant flavonoids on immune and inflammatory cell function. Advances Experimental Medicine and Biology, 1998, 439, 175-182.

Miraliakbari, H., Shahidi, F. Antioxidant activity of minor components of tree nut oils. Food Chemistry, 2008b, 111, 421-427.

Miraliakbari, H., Shahidi, F. Lipid class composition, tocopherols and sterols of tree nut oils extracted with different solvents. Journal of Food Lipids, 2008a, 15, 81-96.

Miraliakbari, H., Shahidi, F. Oxidative stability of tree nut oils. Journal of Agricultural and Food Chemistry, 2008c, 56, 4751-4759.

Miyashita, K., Takagi, T. Study on the oxidative rate and prooxidant activity of free fatty acids. Journal of Agricultural and Food Chemistry, 1986, 63, 1380-1384.

Mochizuki, M., Yamazaki, S., Kano, K., Ikeda, T. Kinetic analysis and mechanistic aspects of autoxidation of catehins. Biochemica et Biophysica Acta, 2002, 1596, 35-44.

Monagas, M., Garrido, I., Aguilar, R. L., Cordoves, M. C. G., Rybarczyk, A., Amarowicz, R. Bartome, B. Comparative flavn-3-ol profile and antioxidant capacity of roasted peanut, hazelnut, and almond skins. Journal of Agriculture and Food Chemistry, 2009, 57, 10590-10599.

Montedoro, G., Servili, M., Baldioli, M., Miniati, E. Simple and hydrolyzable phenolic compounds in virgin olive oil: Initial characterization of the hydrolyzable fraction. Journal of Agricultural and Food Chemistry, 1992, 40, 1577-1580.

Montedoro, G., Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., Macchioni, A. Simple and hydrolyzable phenolic compounds in virgin olive oil. Spectroscopic characterizations of the secoiridoid derivatives. Journal of Agricultural and Food Chemistry, 1993, 41, 2228-2234.

Morimoto, K., Tsuji, K., Ito, T., Miyata, N., Uchida, A., Osawa, R., Kitsutakah, H. and Takahashi, A. DNA damage in forestomach epithelium from male rats following oral administration of tert-butylhydroxyquinone, one of the fore-stomach metabolites of 3-BHA. Carcinogenesis 1991, 12, 703-708.

Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., Nunez, M. J., Parajo, J. C. Natural antioxidants from residual sources. Food Chemistry, 2001, 72, 145-171.

Muroi, H., Kubo, A., Kubo, I. Antimicrobial Activity of Cashew Apple Flavor Compounds. Journal of Agricultural and Food Chemistry, 1993, 41, 1106-1109.

Muroi, H., Kubo, I. Bactericidal activity of anacardic acids against *Streptococcus mutans* and their potentiation. Journal of Agricultural Food Chemistry, 1993, 41, 1780-1783.

Nagaraja, K. V. Biochemical composition of cashew (*Anacardium occidentale* L.) Kernel Testa. Journal of Food Science and Technology, 2000, 37, 554-556.

Namiki, M. Antioxidants / antimutagens in food. Critical Reviews in Food Science and Nutrition, 1990, 29, 273-300.

Nawar, W. W. In Lipids: O. R. Fennema Eds., Marcel Dekker, NY, 1996, pp. 225-313.

Nayar, K. G. Cashew: A versatile nut for health. Proceedings of International Cashew and Coconut Conference, Dar es Salaam, Tanzania, 1995, 195-199.

Nazck, M., Shahidi, F. Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis, 2006, 41, 1523-1542.

Niwa, Y., Kanoh, T., Kasama, T., Neigishi, M. Activation of antioxidant activity in natural medicinal products by heating, brewing and lipophilization. A new drug delivery system, Drugs Under Experimental and Clinical Research, 1988, 14, 361-372.

Niwa, Y., Miyachi, Y. Antioxidant action of natural health products and Chinese herbs. Inflammation, 1986, 10, 79-97.

Ocon, A., Anzaldua-Morales, A., Quintero, A., Gastelum, G.. Texture of pecans measured by sensory and instrumental means. Journal of Food Science, 1995, 60, 1333-1336.

Ogunmoyela, O. A. Prospects for cashew "apple" processing and utilization in Nigeria. Process Biochemistry, 1983, 6-7.

Ohler, J. G. Cashew. Communication No. 71, Royal Tropical Institute, Amsterdam, 1979, 260-281.

Orsak, M., Lachman, J., Vejdova, M., Pivec, V., Hhamouz, K. Changes of selected secondary metabolites in potatoes and buckwheat caused by UV, γ- and microwave irradiation. Rostlinna Vyroba, 2001, 47, 493-500.

Osawa, T. Novel natural antioxidants for utilization in food and biological systems. In PostharVest Biochemistry of Plant Food- Materials in the Tropics; Uritani, I., Garcia, V.

V., Mendoza, E. M., Eds.; Japan Scientific Societies Press: Tokyo, Japan, 1994, pp 241-251.

Oste, R. E., Dehlqvist, A., Sjostrom, H., Noren, O., Miller, R. Effect of Millard reaction products in protein digestion, *In vivo* studies. Journal of Agricultural and Food Chemistry, 1986, 34, 355-358.

Ou, B., Hampsch-Woodill, M., Flanagan, J., Deemer, E. K., Prior, R. L., Huang, D. Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. Journal of Agriculture and Food Chemistry, 2002, 50, 2772-2777.

Oyaiza, M. Studies on products of browning reactions. Antioxidative activity of prodcuts of browning reaction prepared from glucosamine. Japanese Journal of Nutrition, 1986, 44, 307-315.

Ozdemir, M., Devres, O. Kinetics of colour changes of hazelnuts during roasting. Journal of Food Engineering, 2000, 44, 31-38.

Palinski, W., Rosenfeld, M. E., Yla-Herttuala, S., Gurtner, G. C., Socher, S. S., Butler, S. W., Parthasarathy, S., Carew, T. E., Steinberg, D., Witztum, J. L. Low density lipoprotein undergoes oxidative modification in vivo. Proceedings of the National Academy of Sciences of the United States of America, 1989, 86, 1372-1376.

Parry, J., Su, L., Moore, J., Cheng, Z., Luther, M., Rao, J. N., Wang, J.Y., Yu, L. Chemical compositions, antioxidant capacities, and antiproliferative activities of selected seed flours. Journal of Agriculture and Food Chemistry, 2006, 54, 3773–3778.

Pellegrini, N., Serafini, M., Salvatore, S., Rio, D. D., Bianchi, M., Brighenti, F. Total antioxidant capacity of spices, dried fruits, nuts, pulses, cereals and sweet consumed in Italy assessed by three different *in vitro* assays. Molecular Nutrition and Food Research, 2006, 50, 1030-1038.

Pillai, M. K. S., Kedlaya, K. J., Selvarangan, R. Cashew seed skin as a tanning material. Leather Science, 1963, 10, 317-318.

Pillonel, C., Mudler, M. M., Boon, J. J., Forster, B., Binder, A. Involvement of cinnamyl-alcohol dehydrogenase in the control of lignin formation in *Sorghum bicolor* L Monech. Planta, 1991, 185, 538-544.

Pittia, P., Rosa, M. D., Lerici, C. R. Textural changes of coffee beans as affected by roasting conditions. Lebensmittle Wissenschaft und Technologie, 2001, 34, 168-175.

Pokorny, J. Antioxidants in food preservation. In Handbook of Food Preservation. Eds. Rahman, S. Marcel Dekker Inc., New York, NY, 1996, pp.309-337.

Pokorny, J. Major factors affecting the autoxidation of lipids. In Autoxidation of Unsaturated Lipids. Eds. Chan, H. W. S. Academic Press, London, UK, 1987, pp.141-206.

Price, A. B. Overlap in the spectrum of non-specific inflammatory bowel disease – colitis indeterminate, Journal of Clinical Pathology, 1978, 31, 567-577.

Prior, R. L., Hoang, H., Gu, L., Wu, X., Bacchiocca, M., Howard, L., Woodill, M. H., Huang, D., Ou, B., Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity ($ORAC_{FL}$)) of plasma and other biological and food samples, Journal of the Science of Food and Agriculture, 2003, 51, 3273-3279.

Prineas, R. J., Kushi, L. H., Folsom, A. R., Bostick, R. M., Wu, Y. Walnuts and serum lipids. New England Journal of Medicine, 1993, 329-359.

Rainey, C., Nyquist, L. Nuts – nutrition and health benefits of daily use. Nutrition Today, 1997, 32, 157-163.

Ralph, J., Hatfield, R. D., Piquemal, J., Yahiaoui, N., Pean, M., Lapierre, C., Boudet, A. M. NMR characterization of altered lignins extracted from tobacco plants down regulated for lignification enzymes cinnamyl alcohol dehydrogenase and cinnamyl-CoA reductase. Proceedings of Natural Academy of Science. USA, 1998, 95, 12803-12808.

Ralph, J., Lapierre, C., Marita, J. M., Kim, H., Lu, F., Hatfield, R. D., Ralph, S., Chapple, C., Franke, R., Hemm, M.R., Van Doorsselaere, J., Sederoff, R.R., O'Malley, D.M., Scott, J.T., Makay, J. J., Yahiaoui, N., Boudet, A. M., Pean, M., Pilate, G., Jouanin, L., Boerjan, W. Elucidation of new strctures in lignins of CAD- and COMT-deficient plants by NMR. Phytochemistry , 2001, 57, 993-1003.

Ralph, J., MacKay, J. J., Hatfield, R. D., O'Malley, D. M., Whetten, R. W., Sederoff, R. R. Abnormal lignin in a loblolly pine mutant, Science, 1997, 277, 235-238.

Ramarathnam, N., Osawa, T., Ochi, H., Kawakishi, S. The contribution of plant food antioxidants to human health. Trends in Food Science and Technology, 1995, 6, 75-82.

Randhir, R., Lin, Y. T., Shetty, K. Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors, Asia Pacific Journal of Clinical Nutrition, 2004, 13, 295-307.

Rao, M. S. S. Scope for development of alcoholic beverage from cashew apple. Acta Horticulture, 1985, 108, 164-171.

Rao, M. V. S. S. T. S., Muralikrishna, G. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted finger millet (Ragi, *Elucine coracana* Indaf-15). Journal of the Science of Food and Agriculture, 2001, 50, 889-892.

Reddy, L., Odhav, B., Bhoola, K. D. Natural products for cancer prevention: a global perspective. Pharmacology and Therapeutics, 2003, 99, 1-13.

Robards, K., Prentler, P. D., Tucker, G., Swatsitang, P. and Glover, W. Phenolic compounds and their role in oxidative processes in fruits. Food Chemistry, 1999, 66, 401-436.

Rodriguez, R. J., Miranda, C. L., Stevens, J. F., Deinzer, M. L., Buhler, D. R. Influence of prenylated and non-prenylated flavonoids on liver microsomal lipid peroxidation and oxidative injury in rat hepatocytes. Food and Chemical Toxicology, 2001, 39, 437-445.

Rodriguez, R. J., Miranda, C. L., Stevens, J. F., Deinzer, M. L., Buhler, D. R. Influence of prenylated and non-prenylated flavonoids on liver microsomal lipid peroxidation and oxidative injury in rat hepatocytes. Food and Chemical Toxicology, 2001, 39, 437-445.

Ros, E., Mataix, J. Fatty acid composition of nuts. Implications for cardiovascular health. British Journal of Nutrition, 2006, 96, S29-35.

Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R., O'Brien, N. M. Fatty acid profile, tocopherol, squalene and phytosterol content of Brazil, pecan, pine, pistachio and cashew nuts. International Journal of Food Science and Nutrition, 2006, 57, 219-228.

Sabate, J. Nut consumption, vegetarian diets, ischemic heart disease risk, and all-cause mortality: evidence from epidemiologic studies. American Journal of Clinical Nutrition, 1999, 70, 500S-503S.

Sabate, J., Fraser, G. E., Burke, K., Knutsen, S., Bennett, H., Linsted, K. D. Effects of walnuts on serum lipid levels and blood pressure in normal men. New England Journal of Medicine, 1993, 328, 603-607.

Sahin, H., Topuz, A., Pischetsrieder, M., Ozdemir, F. Effect of roasting process on phenolic, antioxidant and browning properties of carob powder, European Food Research and Technology, 2009, 230, 155-161.

Sajilata, M. G., Singhal, R. S. Effect of irradiation and storage on the antioxidative activity of cashew nuts. Radiation Physics and Chemistry, 2006, 75, 297-300.

Saklar, S., Katnas, S., Ungan, S. Determination of optimum hazelnut roasting conditions. International Journal of Food Science and Technology, 2001, 36, 271-281.

Saklar, S., Ungan, S., Katnas, S. Instrumental crispness and crunchiness of roasted hazelnuts and correlations with sensory assessment. Journal of Food Science, 1999, 64, 1015-1019.

Salas-Salvado, J., Ballart, J. F., Ros, E., Gonzalez, M. A. M., Fito, M., Estruch, R., Corella, D., Fiol, M., Gracia, E. G., Aros, F., Flores, G., Lapetra, J., Raventos, R. L., Gutierrez, V. R., Bullo, M., Basora, J., Covas, M. I. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Archives of Internal Medicine, 2008, 168, 2449-2458.

Salunkhe, D. K., Chavan, J. K., Adsule, R. N., Kadam, S. S. Sesame, in world oil seeds: Chemistry, Technology and Utilization, Van Nostrand Reinhold, NY, 1991, pp. 371-402.

Santiago, L.A., Hiramatsu, M., Mori, A. Japanese soybean miso scavenges free radicals and inhibits lipid peroxidation, Journal of Nutrition Science and Vitaminology, 1992, 38, 297-304.

Sathe, S. K. Solubilization and electrophoretic characterization of cashew nut (*Anacardium occidentale*) proteins. Food Chemistry, 1994, 51, 319-324.

Scalbert, A., Williamson, G. Dietary intake and bioavailability of polyphenols. Journal of Nutrition, 2000, 130, 2073S-2085S.

Scott, G. Atmospheric Oxidation and Antioxidants, Elsevier Publishing Co., NY, 1965, pp 56 - 78.

Seddon, J. M., Cote, J., Rosner, B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts and fish intake. Archives of Ophthalmology, 2003, 121, 1728-1737.

Seeram, N. P., Zhang, Y., Henning, S. M., Lee, R., Niu, Y., Lin, G., Heber, D. Pistachio skin phenolics are destroyed by bleaching resulting in reduced antioxidative capacities. Journal of Agriculture and Food Chemistry, 2006, 54, 7036-7040.

Segura, R., Javierre, C., Lizarraga, M. A., Ros, E. Other relevant components of nuts: phytosterols, folates and minerals. British Journal of Nutrition, 2006, 96S, 36-44.

Senter, S. D., Horvat, R. J. Forbus, W. R. Comparative GLC_MS analysis of phenolic acids of selected nuts. Journal of Food Science, 1983, 48, 798-799.

Shahidi, F. Antioxidants in food and food antioxidants, Nahrung / Food, 2000, 44, 158-163.

Shahidi, F. Antioxidants in plants and oleaginous seeds, in Free Radicals in Food: Chemistry, Nutrition, and Health Effects, Morello, M. J., Shahidi, F., and Ho, C. T., Eds., ACS symposium series 807. American Chemical Society, Washington, DC, 2002, 162-175.

Shahidi, F. Natural antioxidants, an overview. In F. Shahidi (Ed.), Natural antioxidants chemistry, health effects and applications, Champaign, IL: AOCS Press, 1997, pp. 1-11.

Shahidi, F., Alasalvar, C., Liyana-Pathirana, C. M. Antioxidant phytochemicals in hazelnut kernel (Corylus avellana L.) and in hazelnut byproducts. Journal of Agricultural and Food Chemistry, 2007, 55, 1212-1220.

Shahidi, F., Naczk, M. Phenolics in Food and Nutraceuticals. CRC Press, Boca Raton, FL, 2004, 1-442.

Shahidi, F., Janitha, P. K., Wanasundara, P. D. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition, 1992, 32, 67-103.

Shahidi, F., Miraliakbari, H. Tree nut oils. In Bailey's industrial oil and fat products, 6th ed.; Shahidi, F., Ed.; Wiley-Interscience: Hoboken, NJ, 2005; pp 175-193.

Shahidi, F., Naczk, M. Phenolics in Food and Nutraceuticals. CRC press, Boca Raton, FL, 2004, pp1-155.

Shobha, S. V., Krishnaswamy, P. R., Ravindranath, B. Phenolic lipid composition during development of cashew. Phytochemistry, 1992, 31, 2295-2297.

Shobha, S. V., Ramadoss, C. S., Ravindranath, B. Inhibition of soybean lipoxygenase-1 by anacardic acids, cardols and cardanols. Journal of Natural Products, 1994, 57, 1755-1757.

Siebenhandl, S., Grausgruber, H., Pellegrini, N., Rio, D. D., Fogliano, V., Pernice, R., Berghofer, E. Phytochemical profile of main antioxidants in different fractions of purple and blue wheat, and black barley. Journal of Agricultural and Food Chemistry, 2007, 55, 8541-8547.

Silva, B. M., Andrade, P. B., Valentao, P., Ferreres, F., Seabra, R. M., Ferreira, M. A. Quince (*Cydonia oblonga* Miller) seed (pulp, peel, and seed) and jam: antioxidant activity. Journal of Agriculture and Food Chemistry, 2004, 52, 4405-4712.

Silva, B. M., Valentao, P., Seabra, R. M., Andrade, P. B. Quince (*Cydonia oblonga* Miller): an interesting dietary source of bioactive compounds. In: Papadopoulos, K.N.

(Ed.), Food Chemistry Research Developments. Nova Science Publishers, Inc., New York, NY, 2008, pp. 243-266.

Silva, K. D., Collares, F. P., Finzer, J. R. D. A simple and rapid method for estimating the content of solids in industrialized cashew juice. Food Chemistry, 2000, 50, 247-250.

Singh, B., Kale, R. K., Rao, A. R. Modulation of antioxidant potential in liver of mice by kernel oil of cashew nut (*Anacardium occidentale* L.) and its lack of tumour promoting ability in DMBA induced skin papillomagenesis. Indian Journal of Experimental Biology, 2004, 42, 372-377.

Singleton, V.L., Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents, American Journal of Enology and Viticulture, 1965, 16, 144-158.

Siriwardhana, S. S. K.W., Shahidi, F. Antiradical Activity of Extracts of Almond and Its By-products, Journal of the American Oil Chemists' Society, 2002, 79, 903-908.

Siu, G. M., Draper, H. H. A Survey of the malonaldehyde content of retail meats and fish, Journal of Food Science, 1978, 43, 1147-1149.

Sklar, S., Katna, S., Ungan, S. Determination of optimum hazelnut roasting conditions. International Journal of Food Science and Technology, 2001, 36, 271-281.

St. Angelo, A. J. Lipid oxidation in foods. Critical Reviews of Food Science and Nutrition, 1996, 36, 175 - 224.

Stahl, W. and Sies, H. Physical quenching of singlet oxygen in cis-trans isomerization of carotenoids. In Carotenoids in Human Health. Eds. Academy of Sciences, New York, NY, 1993, pp.10-19.

Sugihara, N., Arakawa, T., Ohnishi, M., Furuno, K. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with α-linolenic acid. Free Radical Biology and Medicine, 1999, 27, 1313-1323.

Sun, J., Chu, Y. F., Wu, X., Liu, R. Antioxidant and antiproliferative activities of common seeds. Journal of Agriculture and Food Chemistry, 2002, 50, 7449-7454.

Suresh, M., Kaleysa Raj, R. Cardol: The antifilarial principle from *Anacardium occidentale*. Current Science, 1990, 59, 477-479.

Sze-Tao, K. W. C., Schrimpf, J. E., Teuber, S. S., Roux, K. H., Sathe, S. K. Effects of processing and storage on walnut (*Juglans regia* L) tannins. Journal of the Science of Food and Agriculture, 2001, 81, 1215-1222.

Talcott, S. T., Passeretti, S., Duncan, C. E., Gorbet, D. W. Polyphenolic content and sensory properties of normal and high oleic acid peanuts. Food Chemistry, 2005, 90, 379-388.

Tan, N. H., Wong, K. C., Lumen, B. D. Relationship of tannin levels and trypsin inhibitor activity with the *in Vitro* protein digestibilities of raw and heat-treated winged bean *(Psophocarpus tetragonolobus*). Journal of Agriculture and Food Chemistry, 1984, 32, 819-822.

Tapiero, H., Tew, K. D., Ba, N., Mathe, G. Polyphenols: Do they play a role in the prevención of human pathologies? Biomedecine and Parmacotherapy, 2002, 56, 200-207.

Thompson, L. U., Seidl, M. M., Rickard, S. E., Orcheson, L. J., Fong, H. H. Antitumorigenic effect of a mammalian lignan precursor from flaxseed. Nutrition and Cancer, 1996, 26, 159-165.

Thompson, L.U., Robb, P., Serraino, M., Cheung, F. Mammalian lignan production from various foods. Nutrition and Cancer, 1991, 16, 43-52.

Toschi, G. T., Caboni, M. F., Penazzi, G., Lercker, G., Capella, P. A study on cashew nut oil composition. Journal of American Oil Chemists' Society, 1993, 70, 1017-1020.

Toyomizu, M., Okamoto, K., Ishibashi, T., Chen, Z., Nakatsu, T. Uncoupling effect of anacardic acids from cashew nut shell oil on oxidative phosphorylation of rat liver mitochondria. Life Sciences, 2000, 66, 229-234.

Trox, J., Vadivel, V., Vetter, W., Stuetz, W., Scherbaum, V., Gola, U., Nohr, D., Biesalski, H. K. Bioactive compounds in cashew nut (*Anacardium occidentale* L.) kernels: effect of different shelling methods. Journal of Agricultural and Food Chemistry, 2010, 58, 5341-5346.

Tsai, C. J., Leitzmann, M. F., Hu, F. B., Willett, W. C., Giovannucci, E. L. Frequent nut consumption and decreased risk of cholecystectomy in women. American Journal of Clinical Nutrition, 2004, 80, 76-81.

Tyman, J. H. P. Cultivation, processing and utilization of the cashew. Chemical Industry, (London) 1980, 59-62.

Tyman, J. H. P., Jhonson, R. A., Muir, M., Rokhgar, R. The extraction of natural cashew nut shell liquid from the cashew nut (*Anacardium occidentale*). Journal of American Oil Chemists' Society, 1989, 68, 553-557.

Tyman, J. H. P., Tychopoulos, V., Chan, P. Quantitative analysis of natural cashew nut-shell liquid (*Anacardium occidentale*) by highperformance liquid chromatography. Journal of Chromatography A, 1984, 303, 137-150.

U.S. Department of Agriculture (USDA) Nutrient Database version 15; www.nal.usda.gov/foodcomp, 2003.

U.S. Department of Agriculture (USDA) Nutrient Database version 17; www.nal.usda.gov/foodcomp, 2005.

U.S. Department of Agriculture (USDA) National Nutrient Database, Food and Nutrition, Release 18, 2009, published on www.usda.gov/wps/portal/!ut/p/

Valentine, V. J., Doucette, P. A., Potter, Z. S. Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis. Annual Review of Biochemistry, 2005, 74, 563-593.

Van den Berg, R., Haenen, G. R. M. M., van den Berg, H. and Bast, A. Applicability of an improved Trolox equivalent antioxidative capacity (TEAC) assay for evaluation of antioxidant capacity measurement of mixtures. Food Chemistry, 1999, 66, 511-517.

Van Ruth, S. M., Shaker, E. S., Morrissey, P. A. Influence of methanolic extracts of soybean seeds and soybean oil on lipid oxidation in linseed oil. Food Chemistry, 2001, *75*, 177-184.

Velioglu, Y. S., Mazza, G., Gao, L., Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agriculture and Food Chemistry, 1998, 46, 4113-4117.

Venkatachalam, M., Sathe, S. K. Chemical composition of selected edible nut seeds. Journal of Agricultural and Food Chemistry, 2006, 54, 4705-4714.

Vivancos, M., Moreno, J. J. β-Sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. Free Radical Biology and Medicine, 2005, 39, 91-97.

Wagner, K. H., Kamal-Eldin, A., Elmadfa, I. U. Gamma-tocopherol an underestimated vitamin? Annals of Nutrition and Metabolism, 2004, 48, 169-188.

Wang, H. J., Murphy, P. A. Isoflavone Content in Commercial Soybean Foods. Journal of Agricultural and Food Chemistry, 1994, 42, 1666-1673.

Wang, J., Shahidi, F. Stability characteristics of omega - 3 oil and their randomized counterparts. In recent advances in food and flavour chemistry: Eds.; Ho, C. T., Mussinan, J. C., Shahidi, F., Contis, E. T.; RSC Publishing, Cambridge, UK, 2010, pp 297-307.

Wanlapa, A., Jindal, V. K. Instrumental and sensory evaluation of textural changes during roasting of cashew kernels. Journal of Texture Studies, 2006, 37, 263-275.

Wassermann, D., Dawson, C. R. Cashew nut shell liquid. III. The cardol component of Indian cashew nut shell liquid with reference to the liquid's vesicant activity. Journal of American Oil Chemists' Society, 1948, 70, 3675.

Weidner, S., Amarowicz, R., Karamac, M., Fraczek, E. Changes in endogenous phenolic acids during development of *Secale cereale* caryopses and after dehydration treatment of unripe rye grains. Plant physiology and Biochemistry, 2000, 38, 595-602.

Wettasinghe, M., Shahidi, F. Evening primrose meal: A Source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. Journal of Agricultural and Food Chemistry, 1999, 47, 1801-1812.

Wettasinghe, M., Shahidi, F. Oxidative stability of cooked comminuted lean pork as affected by alkali and alkali–earth halides. Journal of Food Science, 1996, 61, 1160-1164.

Wijerathne, S. S. K., Amarowicz, R., Shahidi, F. Antioxidant activity of Almonds and their byproducts in food model systems. Journal of the American Oil Chemists' Society, 2006, 83, 223-230.

Wijerathne, S. S. K., Abou-zaid, M. M., Shahidi, F. Antioxidant polyphenols in almond and its coproducts. Journal of Agriculture and Food Chemistry, 2006, 54, 312-318.

Willett, W. C. Goals for nutrition in the year 2000. Cancer Journal for Clinicians, 1999, 49, 331-352.

Williams, G. M., Iatropoulos, M. J. and Whysner, J. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food Chemistry and Toxicology, 1999, 37, 1027-1038.

Wimalsiri, P., Sinnatamby, A., Samaranayake, S., Samarasinghe, C. R. Cashew Apple Wine. Industry Prospect Report, 44. Industrial Development Board, Colombo, Sri Lanka, 1971.

Woodroof, J. G. Tree Nuts: Production, Processing, Products. AVI Publishing, Westport, CT, 1979, 150-231.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agricultural and Food Chemistry, 2004, 52, 4026-4037.

Yamaguchi, N., Koyama, Y., Fujimaki, M. Fractionation and antioxidative activity of browning reaction products between D-xylose and glycine. In Progress in Food Nutrition Science, C. Eriksson, (Eds), Pergammon press, Oxford, UK, 1981, 5, 429-439.

Yang, F., Basu, T. K., Ooraikul, B. Studies on germination conditions and antioxidant contents of wheat grain. International Journal of Food Science and Nutrition, 2001, 52, 319-330.

Yang, J., Liu, R. H., Halim, L. Antioxidant and antiproliferative activities of common edible nut seeds. LWT - Food Science and Technology, 2009, 42, 1-8.

Yanishlieva-Maslarova, N. V. Inhibiting oxidation. In Antioxidants in Foods: Practical Applications. Eds. Pokorny, J., Yanihslieva, N., and Gordon, M. Woodhead Publishing Ltd., Cambridge, UK, 2001, pp.22-70.

Yen, G. C. Influence of seed roasting process on the changes in composition and quality of sesame (*Sesame indicum*) oil. Journal of the Science of Food and Agriculture, 1990, 50, 563-570.

Yen, G.C., Hsiesh, P.P. Possible mechanisms of antimutagenic effect of Maillard reaction products prepared from xylose and lysine. Journal of Agricultural and Food Chemistry, 1994, 42, 133-137.

Yen, G. C., Shyu, S. L. Oxidative stability of sesame oil prepared from sesame seed with different roasting temperatures, Food Chemistry, 1989, 31, 215-224.

Yochum, L. A., Folsom, A. R., Kushi, L. H. Intake of antioxidant vitamins and risk of death from stroke in post-menopausal women. American Journal of Clinical Nutrition, 2000, 72, 476-483.

Yoshida, H., Takagi, S. Effects of seed roasting temperature and time on the quality characteristics of sesame (*Sesamum indicum*) oil. Journal of the Science of Food and Agriculture, 1997, 75, 19-26.

Yoshida, H., Takagi, S., Mitsuhashi, S. Tocopherol distribution and oxidative stability of oils prepared from the hypocotyls of soybeans roasted in microwave oven. Journal of the American Oil Chemists' Society, 1999, 76, 915-920.

Yosida, H. Composition and quality characteristic of sesame seed (*Sesame indicum*) oil roasted at different temperature in an electric oven. Journal of the Science of Food and Agriculture, 1994, 65, 331-336.

Yousif, A. K., Alghzawi, H. M. Processing and characterizing of carob powder. Food Chemistry, 2000, 69, 283-287.

Yu, J., Ahmedna, M., Goktepe, I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chemistry, 2005, 90, 199-206.

Yu, J., Ahmedna, M., Goktepe, I., Dia, J. peanut skin procyanidins: Composition and antioxidant activities as affected by processing. Journal of Food Composition and Analysis, 2006, 19, 364-371.

Yu, P., Maenz, D. D., McKinnon, J. J., Racz, V. J., Christensen. Release of ferulic acid from oat hulls by *Aspergillus* ferulic acid esterase and *Trichoderma xylanase*. Journal of Agricultural and Food Chemistry, 2002, 50, 1625-1630.

Zhang, S. M., Hernan, M. A., Chen, H., Spiegelman, D., Willett, W. C., Ascherio, A. Intakes of vitamins E and C, carotenoids, vitamin supplements, and PD risk. Neurology, 2002, 59, 1161-1169.

Zhu, B. Z., Kitrosky, N., Chevion, M. Evidence for production of hydroxyl radicals by pentachloro phenol metabolites and hydrogen peroxides: A metal-independent organic Fenton reaction. Biochemical and Biophysical Research Communication, 2000, 270, 942-946.

Ziouzenkova, O., Giesg, S. P., Ramos, P., Esterbauer, H. Factors affecting resistance of low-density lipoproteins to oxidation. Lipids, 1996, 31, S71-S76.

Ziouzenkova, O., Sevanian, A., Abuja, P. M., Ramos, P., Esterbauer, H. Copper can promote oxidation of LDL by markedly different mechanisms. Free Radical Biology and Medicine, 1998, 24, 607-623.

Zou, Y., Lu, Y., and Wei, D. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. Journal of Agriculture and Food Chemistry, 2004, 52, 5033-5039.