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UTILIZATION OF PALM OIL MILL WASTES IN THE PRODUCTION OF NATURAL RUBBER ANTIOXIDANT

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Title

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ABSTRACT

The increasing production of wastes from the fast-growing palm oil industry in Thailand calls for the development of new waste management strategies before its negative impact prevails. To address this issue and, in turn, to give added value to palm oil mill wastes, this study was conducted. The main objective of the study was to investigate the feasibility of using palm oil mill wastes which include the oil palm kernel meal (OPKM) and the palm oil mill effluent (POME), as raw materials for the production of natural rubber (NR) antioxidant.

The possible antioxidant from OPKM was isolated using the ash-anthraquinone pulping process which involved alkaline digestion, removal of polysaccharide degradation products, and precipitation at pH 2. Electrocoagulation followed by subsequent recovery of the electrocoagulated compounds from the coagulum through acid dissolution and solvent extraction was employed to isolate the possible antioxidant from POME. The average yield of the potential antioxidant from the OPKM and POME were 4.1g/100g and 17.1g/L, respectively. Preliminary test on the antioxidant activity of the isolates using the DPPH radical scavenging assay showed strong antioxidant activity for OPKM lignin and weak antioxidant activity for the POME isolate when compared to vitamin E.

Partial structural characterization of the potential antioxidants was conducted using UV-Vis, FTIR, ^{13}C-NMR, GC-MS, LCMS and degradation or derivatization experiments. Results showed that the OPKM lignin has a total phenolic (TP) content of 158 mg gallic acid equivalent per gram OPKM lignin and contains the hydroxyl, methoxyl, carbonyl, aromatic, and alkyl groups. Syringyl units followed by the guiacyl units were the dominant monomers as determined through nitrobenzene oxidation. The molecular weights of the components in an alkaline solution have a range of 200-800 amu. For the POME isolate, characterization studies revealed a lower TP content (27 mg gallic acid equivalent per gram POME isolate) and the presence of hydroxyl,

carbonyl, aromatic and alkyl groups. The POME isolate also has a larger content of long chain of saturated C-atoms relative to unsaturated and aromatic C-atoms based on the ^{13}C-NMR spectrum, which was confirmed also by the abundance of long chain-fatty acids and sugar derivatives in the acetylation products. The molecular weights of the individual components range from 300-800 amu.

The isolated antioxidants were used in the preparation of vulcanized natural rubber (NR). Two types of rubber were prepared: the vulcanized NR from STR 5L and the NR latex films. The concentrations of antioxidants were varied from 1 to 3 parts per hundred of rubber (phr) and the properties of the rubber produced before and after accelerated thermal ageing were evaluated and compared to those treated with commercial antioxidants.

The vulcanized NR from STR 5L before thermal ageing showed better resistance to compressive forces, lower rebound resilience, hardness, and tensile strength, but comparable Modulus 300 and % elongation at break (%EB). Increasing the concentration of antioxidant gave varied effects. The NR latex films before thermal ageing have higher tensile strength, Modulus 300, and %EB than those treated with commercial antioxidant. Increase in the concentration of the POME isolate enhanced the tensile properties. After thermal ageing, hardness and Modulus 300 increased while tensile strength and %EB decreased. Better resistance to thermal ageing was achieved when the concentration of the studied antioxidant was 1 phr for the vulcanized NR from STR 5L, and 3 phr for the vulcanized latex film for both the OPKM lignin and POME isolate.

Swelling experiment was conducted to determine crosslink density. Increase in the concentration of both antioxidants favored chain scission for vulcanized NR from STR 5L as reflected in the decrease of crosslink density, thereby decreased the resistance to accelerated thermal ageing. For vulcanized NR latex films, increase in the concentration of the POME isolate favored crosslinking, thereby increased both the crosslink density and resistance to thermal ageing. The effect of increasing the concentration of OPKM lignin, on the other hand, decreased crosslink density.

The thermal degradation of the vulcanized NR from STR 5L and latex films as monitored by thermogravimetric analysis showed similar degradation patterns to those treated with commercial antioxidants, but provided slightly lower onset temperatures.

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MELISSA BUENAVENTURA AGUSTIN

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INTRODUCTION

1.1. Background of the Study

Palm oil and natural rubber industries are among the major agricultural industries operating in Thailand. Both industries have played an important role in the economy of the country and to the economic welfare of the population as well. Introduced in 1968 (Chungsiriporn, 2004), palm oil industries continue to grow and contribute a significant income to the country mainly through the production of edible palm oil which has found great applications in food manufacturing, and in the chemical, cosmetic and pharmaceutical industries (Wiberg and Bafor, 1995) Similarly, increased production of natural rubber to approximately 3 million tons in 2005 still makes Thailand the world's largest producer and exporter of natural rubber (Suanpraert, 2006).

Despite the continuous development both industries are gaining, problems associated with the various manufacturing processes still persist. For natural rubber industries, the increasing price of imported natural rubber additives particularly antioxidants and accelerators might have resulted to an increase in the production cost. For palm oil industries, a large volume of wastes produced in the manufacturing processes could pose a great problem to environment if not properly managed. It is estimated that in 2003, a total of 2.1 million tons of solid wastes/by-products and 2.5 million cubic metres of wastewater were generated (Chavalparit, et al., 2006). The huge volume of waste produced in palm oil industries calls for proper measures to convert these wastes into useful chemicals or energy. Whereas rubber industries need to look for local raw materials that can be processed and used as natural rubber additive to lessen its dependence to imported additives thereby reducing the cost of production.

Palm oil mill wastes generated during the production of crude palm oil and crude kernel oil are categorized into two. First is the solid residue which comprises the empty fruit bunches (EFB), fruit bunch (FB) fibers, oil palm kernel meal (OPKM), nuts, and shells. Second is the liquid wastes known as the palm oil mill effluent (POME). There are 55 palm oil mill factories in Southern Thailand and receive an approximately 395,017 ton of fresh fruit bunches/ month

(Agsomtramat, et al., 2003). It is estimated that for every 1ton of fresh fruit bunches of oil palm, 230-250 kg EFB, 130-150 kg FB fibers, 55-60 kg OPKM, 60-65 kg shells, and 0.44-1.18 m^3 POME are produced (Kittikun, et al., 2000). It is evident from these data that a large amount of wastes are produced monthly from palm oil mill factories.

Some measures to lessen the impacts of palm oil mill wastes have been employed by the industry before but strategies that focus on using these wastes as a resource for energy (Yusoff, 2006) and chemicals have been of interest to many researchers lately. Some attempts were done such as production of molecular sieving carbon by the pyrolysis of oil palm shell (Hussein, et al., 1995; Tan and Ani, 2004), recovery of useful hydrocarbons using zirconia as catalyst (Masuda, et al, 2001), production of a bio-degradable polymer (Wollerdorfer and Bader, 1998), and extraction of lignin (Sun and Tomkinson, 2001; Mohamad Ibrahim, et al., 2004), polysaccharides (Sun, et al., 1999a), cellulose and hemicellulose (Sun, et al., 1999b).

Of the possible compounds obtainable from palm oil mill wastes, the isolation of lignin and other phenolic compounds was the interest of this research owing to its reported antioxidant activity (Dizhbite, et al., 2004). Although, there are studies that isolated lignin from the oil palm empty fruit bunch fibers (Sun and Tomkinson, 2001; Mohamad Ibrahim, et al., 2004) none so far have utilized the POME and the OPKM in the production of lignin or other compounds as natural rubber antioxidant, one of the additives that prevent oxidative degradation of natural rubber. In addition, the POME has not been subjected to electrocoagulation; a water treatment method which has been used industrially and found to have superior performance in treating effluents (Chen, 2004).

With the idea that lignin or other phenolic compounds are present in palm oil mill wastes and that phenolic compounds are known to be good antioxidants, this study on the isolation of lignin or other phenolic compounds as antioxidant for natural rubber was conducted. Moreover, this study is in response to the problems associated with palm oil mill waste management and increased production cost of natural rubber.

1.2. Objectives of the Study

This study aimed to utilize palm oil mill wastes in the production of natural rubber antioxidants in the form of lignin or related compounds. Specifically, the study attempted to

1. Isolate lignin from the oil palm kernel meal through ash –AQ(anthraquinone) pulping,
2. Isolate the possible antioxidant from palm oil mill effluent (POME) by electrocoagulation,
3. determine the antioxidant activity of the isolated compounds using DPPH radical scavenging assay,
4. Partially characterize the structure of the isolated compounds from the oil palm kernel meal and palm oil mill effluent.
5. Prepare vulcanized natural rubber from dry rubber sheets with varying concentrations of the isolated antioxidants,
6. Prepare vulcanized NR latex films with varying concentrations of the isolated antioxidants,
7. Measure the physico-mechanical properties of the vulcanized natural rubber sheets and latex films before and after thermal ageing,
8. Determine the swelling property and calculate from the swelling parameters the crosslink density of the vulcanized natural rubber sheets and latex films
9. Evaluate the thermal stability of the vulcanized natural rubber sheets and latex films by thermogravimetric analysis,
10. Evaluate the effect of varying concentration of antioxidants in the physico-mechanical properties, swelling behavior and thermal stability of the rubber produced, and
11. Compare the efficiency of lignin as antioxidant for natural rubber with commercially available antioxidants.

1.3. Significance of the Study

Utilization of palm oil mill wastes in the production of antioxidants for natural rubber may answer the problems faced by palm oil industries with regard to waste management and by the rubber industries in terms of importation of synthetic antioxidants for natural rubber. The

production cost for rubber may be lessened because local materials will be harnessed in producing one of the additives in rubber production. Aside from lessening the impact of the problems posed by palm oil mill wastes, collaborative efforts between industries could be created in giving additional value to palm oil mill wastes. Results of this study may also significantly contribute to the sustainability of the two industries with lower production cost and more environment- friendly processing.

The application of electrocoagulation principles in the isolation of natural rubber antioxidants could also provide a new approach for waste management in Thailand at the same time an alternative method in the isolation of lignin and other phenolic compounds. Results on the lignin characterization could also provide additional information in the field of lignin chemistry.

Chapter 2

REVIEW OF RELATAD LITERATURE AND STUDIES

2.1. Palm Oil Mill Wastes

The production of palm oil generates wastes which include solid residues (empty fruit bunches (EFB), fruit bunch (FB) fibers, oil palm kernel meal (OPKM), nuts, and shells) and liquid wastes mainly in the form of palm oil mill effluent (POME). These wastes are mostly generated in palm oil mill factories which processed the oil palm fresh fruit bunch (FFB) to produce crude palm or kernel oil.

2.1.1 The Oil Palm Kernel Meal (OPKM)

Oil palm kernel meal (Figure 1) is a by-product of palm kernel oil extraction from the nut of the palm tree. The global production of OPKM is ever increasing due to the tremendous growth of the oil palm industry in many parts of Asia and Africa. In 2005, it is estimated that the production of OPKM reached 94,000 metric tons (Palm Kernel Meal World Supply and Distribution Database). It is an established feed ingredient for ruminants, supplying valuable dietary sources of protein, energy and fiber (Umunn et al., 1980). It has also been successfully tested in poultry and swine feeds at low levels of incorporation. The low cost and availability of OPKM in many tropical countries where aquaculture is practiced have recently generated much interest in its potential use in fish diets (Wing-Keong, 2003). A study conducted by Kolade et al. (2006) in Nigeria also showed the feasibility of composting OPKM for use as fertilizer.

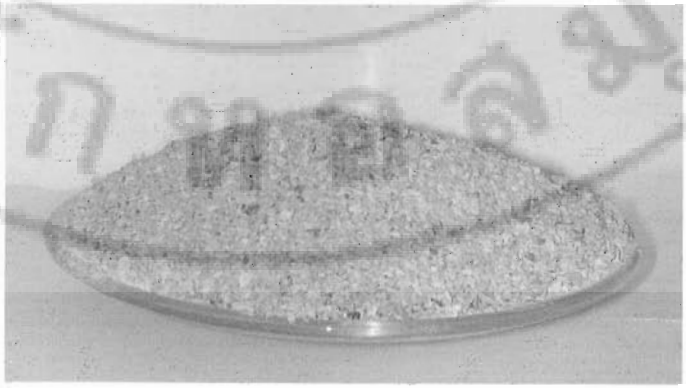


Figure 1. Oil palm kernel meal, the waste left after extracting oil from the palm kernel.

2.1.2 Palm Oil Mill Effluent (POME)

Palm oil mill effluent (Figure 2) is the mixture of high polluted effluent (from sterilizer and oil room) and low polluted effluent (steam condensate, cooling water, boiler discharge and sanitary effluent (Kittikun, et al., 2000). It is a colloidal dispersion of organics with an unpleasant odour and with a total solids content of 5–7% of which a little over half is dissolved solids, and about the other half being a mixture of various forms of organic and inorganic suspended solids (Bhatia et al., 2007). These various suspended components include cell walls, organelles, short fibres, a spectrum of carbohydrates ranging from hemicellulose to simple sugars, a range of nitrogenous compounds from proteins to amino-acids, free organic acids and an assembly of minor organic and mineral constituents (Ugoji, 1997). The POME has high organic load as reflected in the BOD (50,000 mg/L) and COD (80,000 mg/L). In Thailand, it was found out that for every 1 ton of fresh fruit bunches of oil palm, 0.44-1.18 m^3 of POME are produced and the estimated annual production of POME as revealed from the study of Kittikun and Prasertsan (2000) was 1,202,260 tons. It is evident from these figures that a huge volume of the effluent is produced along with the production of palm oil. If not given due attention, POME could pose a great problem to the environment. In fact, one study (Reijnders and Huigbregts, 2008) in Malaysia had looked into the potential of POME as being a green house gas contributor.

Figure 2. Palm oil mill effluent, the wastewater produced in palm oil mill factories.

Problems associated with POME management have been of great interest to researchers owing to the fact that several studies have been conducted to find suitable treatment methods for POME. Biological treatments that employ aerobic or anaerobic microorganisms to digest the highly complex organocompounds in POME have been widely studied and reported by several researchers (Vijayaraghavan, et al., 2007; Najafpour, et al., 2006; and Tjandra, et al., 1996). Bioconversion also has been of great interest. Hipolito, et al. (2008) used POME as a media in the biological production of butanol while Atif, et al. (2005) utilized POME to produce hydrogen gas during anaerobic degradation process. A study conducted by Ali Hassan, et al. (1997) showed also the feasibility of isolating poly-hydroxyalkanoates; polyesters with properties similar to petro-chemical based plastics but biodegradable, from POME. The principle of coagulation, mainly through the use of chemical coagulant has been applied also for POME. Bhatia, et al. (2007) have used *Moringa eleifera* seeds after oil extraction as a coagulant for suspended solids in POME. Another study that is based also on the principles of coagulation was conducted by Ahmad, et al. (2006a). In their study, chitosan as an alternative coagulant to alum and polyaluminium chloride (PAC) was used. Other treatment methods which are based on membrane technology have been of interest too. Ahmad, et al. (2006b) used ultrafiltration to reclaim drinking water from POME while Wu, et al. (2007) employed ultrafiltration as a method for renewing possible resources such as fertilizer from POME.

2.2. Electrocoagulation

2.2.1 Electrocogulation (EC) and its principles

Several definitions of electrocoagulation have been cited in literature throughout the years attributed to its wide applications in wastewater treatment (Khemis, et al., 2006; Kobya, et al., 2006, Nafa and Monser, 2004; Lai and Lin, 2004) and recently in natural products chemistry (Chairungsi, et al., 2006; Jumpatong et al., 2006; Miwa, 1979). Mollah, et al. (2004) provided a comprehensive and detailed discussion of EC.

EC is an electrochemical method that removes dissolved and suspended materials in a solution through a complicated process involving many chemical and physical phenomena that use consumable electrodes to supply ions in the solution (Holt, et al., 2005). In an EC process the coagulating ions are produced 'in situ' and it involves three successive stages: (i) formation of

coagulants by electrolytic oxidation of the 'sacrificial electrode',(ii) destabilization of the contaminants, particulate suspension, and breaking of emulsions and (iii) aggregation of the destabilized phases to form flocs. The destabilization mechanisms of the contaminants, particulate suspension, and breaking of emulsions have been described in broad steps and may be summarized as follows (Mollah, et al., 2004):

- Compression of the diffuse double layer around the charged species by the interactions of ions generated by oxidation of the sacrificial anode.

- Charge neutralization of the ionic species present in wastewater by counter ions produced by the electrochemical dissolution of the sacrificial anode. These counter ions reduce the electrostatic interparticle repulsion to the extent that the van der Waals attraction predominates, thus causing coagulation. A zero net charge results in the process.

- Floc formation; the floc formed as a result of coagulation creates a sludge blanket that entraps and bridges colloidal particles still remaining in the aqueous medium.

The solid oxides, hydroxides and oxyhydroxides provide active surfaces for the adsorption of the polluting species. Electrocoagulation has been successfully employed in removing metals, suspended particles, clay minerals, organic dyes, and oil and greases from a variety of industrial effluents. In this process, a potential is applied to the metal anodes, typically fabricated from either iron or aluminum. (Mollah, et al., 2004)

2.2.2 Reactions during EC

Electrocoagulation involves the generation of coagulants in situ by dissolving electrically either aluminum or iron ions from respectively aluminum or iron electrodes. The typical EC set-up is shown in Figure 3. The metal ions generation takes place at the anode and hydrogen gas is released from the cathode. The hydrogen gas would also help to float the flocculated particles out of the water (Chen, 2004).

The chemical reactions taking place at the anode are given as follows.

For aluminum anode:

$Al \longrightarrow Al^{3+} + 3e^{-}$,

at alkaline conditions; $Al^{3+} + 3OH^{-} \longrightarrow Al(OH)_3$,

at acidic conditions; $Al^{3+} + 3H_2O \longrightarrow Al(OH)_3 + 3H^{+}$.

For iron anode:

$Fe \longrightarrow Fe^{2+} + 2e^-$,

at alkaline conditions; $Fe^{2+} + 3OH^- \longrightarrow Fe(OH)_2$,

at acidic conditions; $4Fe^{2+} + O2 + 2H2O \longrightarrow 4Fe^{3+} + 4OH^-$.

In addition, there is oxygen evolution reaction $2H2O \longrightarrow O2 + 4H^+ + 4e^-$.

The reaction at the cathode is $2H_2O + 2e^- \longrightarrow H_2 + 2OH^-$.

The nascent Al^{3+} or Fe^{2+} ions are very efficient coagulants for particulates flocculating. The hydrolyzed aluminum ions can form large networks of Al–O–Al–OH that can chemically adsorb pollutants (Shen, et al., 2003).

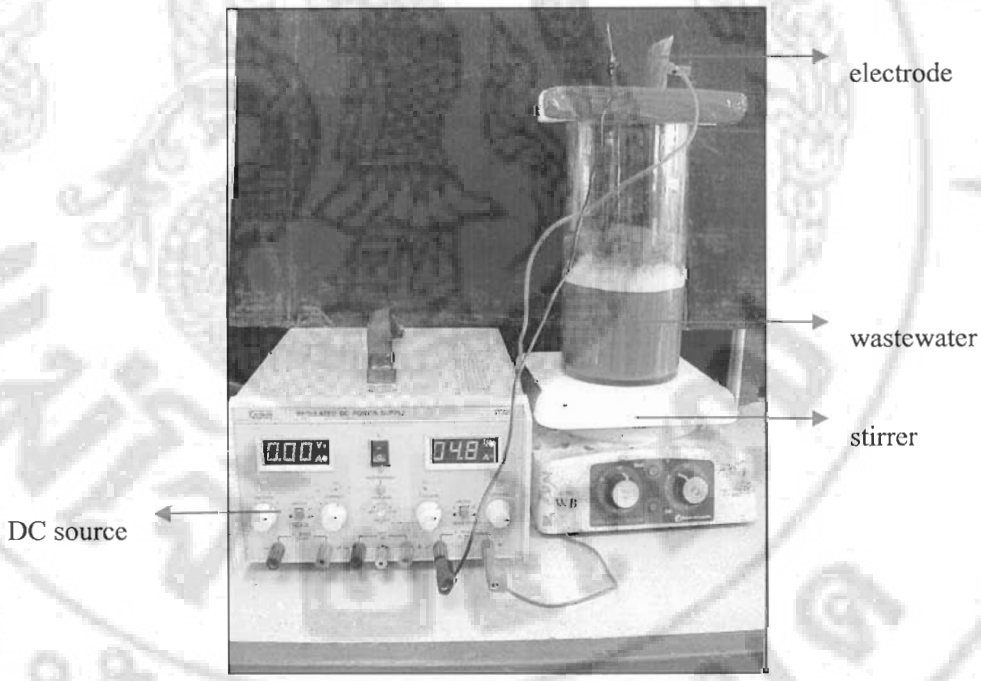


Figure 3. The electrocoagulation set-up with aluminum plates as electrodes.

2.2.3 Advantages and disadvantages of EC

The fact that EC had been widely used as a water treatment method suggests that EC offers a lot of advantages over other methods. But some disadvantages must also be taken into account in considering this method. The advantages and disadvantages of EC are summarized by Mollah, et al. (2001).

Advantages of EC

a. EC requires simple equipment and is easy to operate with sufficient operational latitude to handle most problems encountered on running.

b. Wastewater treated by EC gives palatable, clear, colorless and odorless water.

c. Sludge formed by EC tends to be readily settable and easy to de-water, because it is composed of mainly metallic oxides/hydroxides. Above all, it is a low sludge producing technique.

d. Flocs formed by EC are similar to chemical floc, except that EC floc tends to be much larger, contains less bound water, is acid-resistant and more stable, and therefore, can be separated faster by filtration.

e. EC produces effluent with less total dissolved solids (TDS) content as compared with chemical treatments. If this water is reused, the low TDS level contributes to a lower water recovery cost.

f. The EC process has the advantage of removing the smallest colloidal particles, because the applied electric field sets them in faster motion, thereby facilitating the coagulation.

g. The EC process avoids uses of chemicals, and so there is no problem of neutralizing excess chemicals and no possibility of secondary pollution caused by chemical substances added at high concentration as when chemical coagulation of wastewater is used.

h. The gas bubbles produced during electrolysis can carry the pollutant to the top of the solution where it can be more easily concentrated, collected and removed.

i. The electrolytic processes in the EC cell are controlled electrically with no moving parts, thus requiring less maintenance.

j. The EC technique can be conveniently used in rural areas where electricity is not available, since a solar panel attached to the unit may be sufficient to carry out the process.

Disadvantages of EC

a. The 'sacrificial electrodes' are dissolved into wastewater streams as a result of oxidation, and need to be regularly replaced.

b. The use of electricity may be expensive in many places.

c. An impermeable oxide film may be formed on the cathode leading to loss of efficiency of the EC unit.

d. High conductivity of the wastewater suspension is required.

e. Gelatinous hydroxide may tend to solubilize in some

2.3. Lignin

Second only to cellulose, lignin is among the most abundant renewable resources (Goseelink, et al., 2004). It is estimated that the planet contains $3x10^{11}$ metric tons of lignin with an annual biosynthetic rate of approximately $3x10^{10}$ metric tons (Whittaker and Likens, 1975 cited by Argyropoulos and Menachem, 1997). It is usually insoluble in all solvents and can only be degraded by physical or chemical treatments (Scalbert and Monties, 1986). Lignin occurs in plants and act as a stabilizer against mechanical, biochemical and environmental stresses (Scalbert and Monties, 1986). Lignin composition will be different not only among plants of different genetic origin, but also among different tissues of an individual plant (Glasser and Kelley, 1987).

Lignin attracts present day researches due to its antioxidant properties for various materials (rubber, wood, thermomechanical pulp) as well as medicines and dietary products. The antioxidant effects of lignin are considered mainly derived from the scavenging action of their phenolic structures on oxygen containing reactive free radicals (Dizhbite, et al., 2004). Of the various applications, the incorporation of lignin into different polymer materials has received higher attention. The use of lignin and some lignin derivatives as reinforcing filler for butadiene-styrene rubber (Kosikova and Gregorova, 2005), polyethylene and polypropylene (Alexy, et al., 2000) was established in previous studies. The stabilizing effect of low molecular weight lignin was also confirmed in polypropylene and recycled polypropylene matrices (Gregorova, et al., 2005), and carbon filled natural rubber (Gregorova, et al., 2006). Lignin has also found great applications as curative agent for nitrile rubber (Yang, et al., 2002) and bromobutyl rubber (Wang et al., 2003) and a compatibilizing agent for natural fibers (Thielmans and Wool, 2004).

2.3.1 Structure and biosynthesis

Lignin is a large, cross-linked macromolecule (Figure 4) with molecular mass in excess of 10,000 amu (Wikipedia). The lignin structural elements are linked by carbon-carbon and ether bonds to form tri-dimensional; network associated with the hemicelluloses and polysaccharides inside the cell wall (Akim, et al., 1997). The molecule consists of various types of substructures which appear to repeat in random manner. Differences exist in molecular composition and linkage type between the phenyl propane monomers (Figure 5); p-hydroxyphenyl, guiacyl, and syringyl units, derived from coumaryl, coniferyl and sinapyl alcohol precursors, respectively. The major chemical functional groups in lignin include hydroxyl, methoxyl, carbonyl, and carboxyl groups in various amounts and proportions, depending on genetic origin and applied extraction process (Goseelink, et al., 2004).

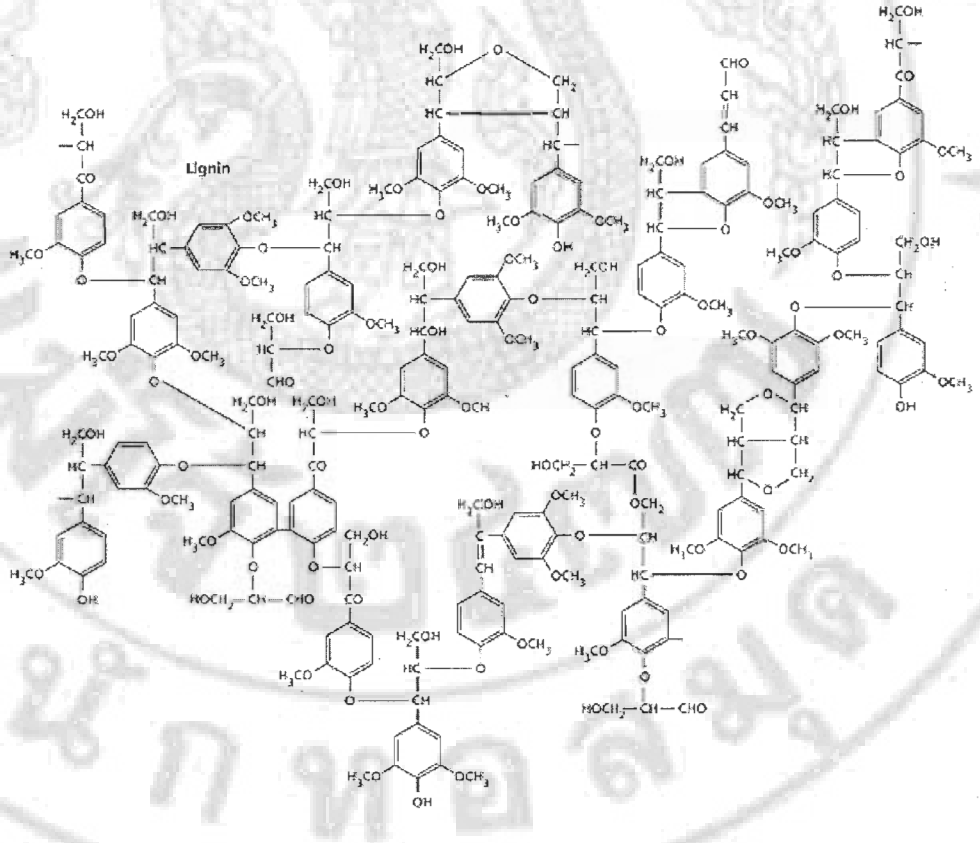


Figure 4. A partial structure of hypothetical lignin molecule (Taiz and Zeiger, 2007).

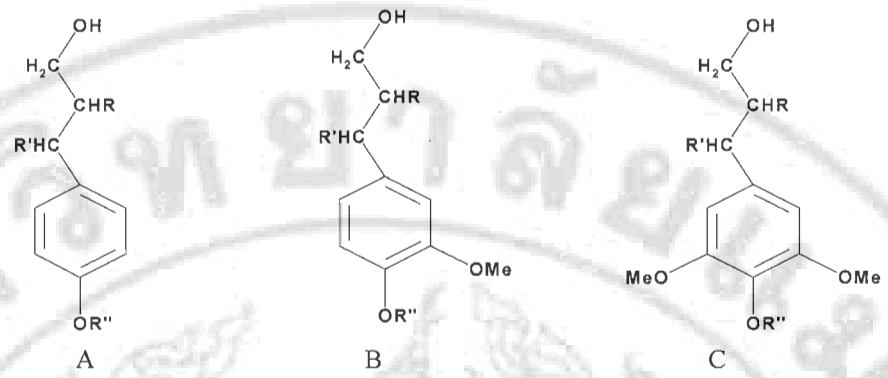


Figure 5. The structure of three phenylpropane monomers (C6-C3 units) of lignin molecule. A) p-hydroxyphenyl B) guiacyl and C) syringyl units.

Lignin biosynthesis (Figure 6) begins with the synthesis of monolignols; precursors for lignin synthesis. The starting material is the amino acid phenylalanine. Lignification proceeds with the conversion of l-phenylalanine to trans-cinnamic acid. This deamination process is catalyzed by l-phenylalanine ammonia lyase (PAL), a key enzyme found only in plants that can synthesisze lignin and some cinnamic acid derivatives. As lignification proceeds, cinnamic acid is hydroxylated to p-coumaric and caffeic acids by specific hydroxylase enzymes. The caffeic acid thus formed is then methylated to ferulic acid, which when acted upon by two different enzymes namely ferulic acid-5-hydroxylase (FAH) or reductase system (RS) produces sinapyl alcohol and coniferl alcohol, respectively (Argyropoulos and Menachem, 1997).

The monolignols formed are of low solubility and are readily oxidized; in the cell wall they are stabilized as glucosides. The glucose is added to the monolignol to make them water soluble and to reduce their toxicity. The glucosides are transported through the cell membrane to the apoplast. The glucose is then removed and the monolignols are polymerized into lignin.The polymerisation step is catalysed by oxidative enzymes. Both peroxidase and laccase enzymes are present in the plant cell walls, and it is not known whether one or both of these groups participates in the polymerisation. The oxidative enzyme catalyses the formation of monolignol radicals by abstracting a proton from the phenolic hydroxyl group of the precursor molecules, creating resonance stabilized free radicals (Figure 7). After the oxidation of the monomeric alcohols to phenoxy radicals, the reaction changes and no longer subjected to enzymatic control,

but to a random polymerization process. The details of this final step are being debated, since it is not known how the abundance of various possible bond types between monolignols is controlled. Some theories favour pure chemical coupling, others have suggested that cellulose and hemicellulose can provide a template (Argyropoulos and Menachem, 1997).

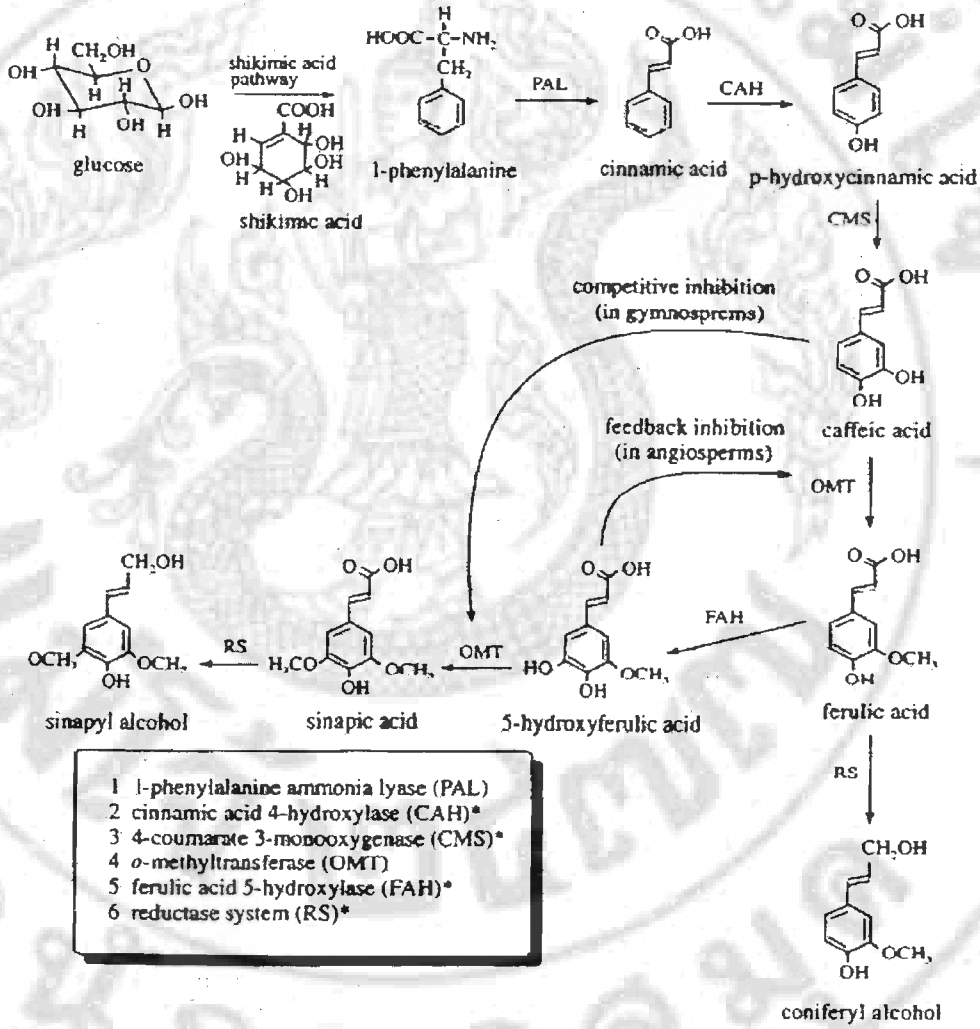


Figure 6. A simplified metabolic pathway of 1-phenylalanine to lignin precursors. (Argyropoulos and Menachem, 1997).

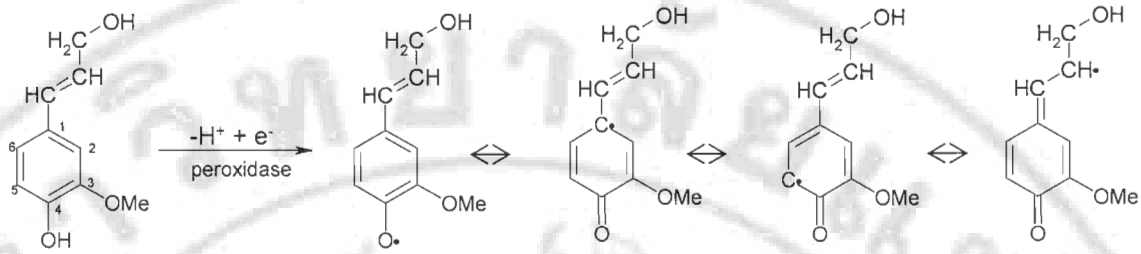


Figure 7. Example of resonance forms of lignin phenoxy radicals.

2.4. Natural Rubber

Natural rubber (NR), possibly the most important polymer produced by plants, is a strategically important raw material used in many thousands of products, including hundreds of medical devices. It is obtained from latex, an aqueous emulsion present in the laticiferous vessels (ducts) or parenchymal (single) cells of rubber-producing plants (Puskas, et al., 2006). The typical composition of fresh NR latex is shown in Table 1. Latex is obtained from the bark of the rubber tree by tapping. The latex tends to oxidise and solidify; it cannot be stored for long in its natural state. It is either stabilised by mixing with liquid ammonia to produce latex, or centrifuged to remove excess water and produce latex concentrate or converted into ribbed smoked sheets (RSS) or solid block rubber (Crumb rubber) or pale latex crepe. Modified natural rubbers are also available, with treatment usually performed at the latex stage. These include epoxidized natural rubber (ENR); deproteinized natural rubber (DPNR), oil extended natural rubber (OENR), into which 10-40% of process oils have been incorporated; Heveaplus MG rubber – natural rubber with grafted poly(methacrylate) side chains; and thermoplastic natural rubber (TPNR) – blends of natural rubber and polypropylene is one example. (Ciullo and Hewitt, 1999)

Table 1. The typical component of fresh natural rubber latex.

Constituent*	% Composition
Rubber particles (cis-1,4-polyisoprene)	30-40%
Protein	2-3%
Water	55-65%
Sterol glycosides	0.1-0.5%
Resins	1.5-3.5%
Ash	0.5-1.0%
Sugars	1.0-2.0%

* Data derived from the website of The PechSiam Group.

Natural rubber is a polyterpene, i.e., it consists of isoprene molecular units linked into loosely twisted chains (Figure 8). The monomer units along the backbone of the carbon chains are in a cis arrangement and it is this spatial configuration that gives rubber its highly elastic character. (Puskas, et al., 2006). Due to its high structural regularity, natural rubber tends to crystallize spontaneously at low temperatures or when it is stretched. Low temperatures crystallization causes stiffening, but is easily reversed by warming. The strain-induced crystallization gives natural rubber high tensile strength and resistance to cutting, tearing and abrasion. (Ciullo and Hewitt, 1999)

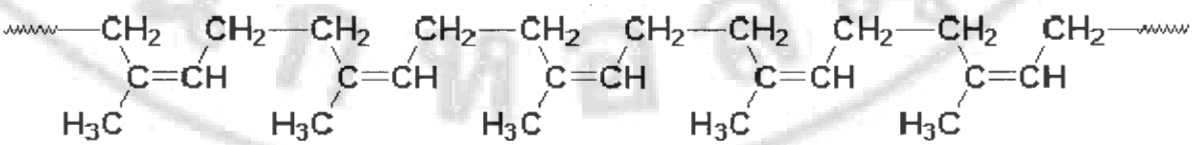


Figure 8. Natural rubber, a biopolymer consisting of isoprene molecular units

2.4.1 Natural rubber technology

The technology of natural rubber can be categorized into two major divisions: the latex and the dry rubber technology. Latex technology involves the processing of NR latex (Figure 9) in the production of various rubber products such as dipped goods, molded and cast articles, foam, rubber-sprayed products, extruded threads, rubberized hair, water-based adhesives and binders and many more. The dry rubber technology on the other hand processed the NR latex to produce dry rubber such as ribbed-smoked sheets, technically specified rubber in block form (Figure 10), pale crepe, rubber crumbs and lumps to be used then as a starting material in the manufacture of various products such as tyre, rubber bearings, etc. The principles involve in both technologies are somewhat similar but the methodologies involved on the other hand are different. Both required various compounding materials such as vulcanizing agent, accelerators, activators, antioxidants, fillers, etc. in different proportions necessary to yield the desired properties for a particular product but the method of compounding; i.e. the incorporation of ingredients and other ancillary substances (Morrel, 1982) are different from each other. For the dry-rubber technology, compounding is usually done using a two-roll mill or internal mixer and the materials are added on the dry basis while for the latex technology compounding is done using a mixer and the ingredients are added in the form of solution, dispersion or emulsion.

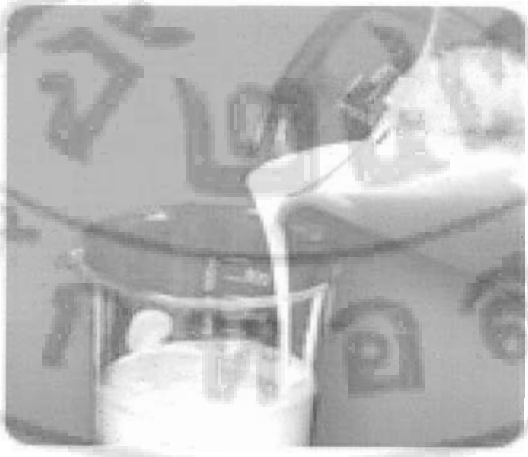


Figure 9. Processed natural rubber latex as a raw material in the preparation of various dipped and molded rubber products

Finally, true to the characteristics of natural rubber, vulcanization or curing is important for both technologies to produce a product having reduced tendency to crystallize, improved elasticity, and substantially constant modulus and hardness over a wide temperature range (Morrel, 1982). A more detailed review on compounding and the chemistry of vulcanization is given on the succeeding sections.

Figure 10. The technically specified rubber in block form as a raw material in the preparation of various rubber products.

2.4.2 Compounding

Natural rubber as already well known is hardly useable in its original form. It has to be mixed with other ingredients ("compounded") to get the required specific properties (Newsletter of the Rubber Foundation Information Center for Natural Rubber). Compounding is the means by which elastomer and additives are combined to ensure efficient manufacture of the best possible product (Ciullo and Hewitt, 1999). Basically a rubber compound contains various groups of compounding-ingredients:1) the elastomer which include synthetic or NR or a blend of NR with one or more polymeric materials; 2) a vulcanizing system (including activators and accelerators); 3) fillers (inorganic and/or organic); 4) softeners (organic and/or synthetic); 5) a protective system (heat stabilizers, antioxidants or antidegradants); 6) processing aids (peptizers, homogenizers and tackyfiers); 7) special purpose ingredients such as blowing agents, flame-retardants, abrasives,

anti-statics, colorants, odorants, fibres, etc. For this review, only those compounding materials used in the study are discussed. These include fillers, vulcanizing system and antioxidants.

Fillers

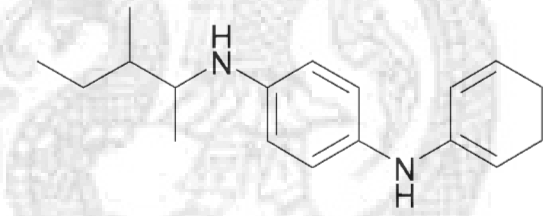
Fillers are used to modify the properties of the rubber. Properties such as tensile and tear strength, fatigue life and abrasion resistance are influenced by the type of filler used. By increasing the amount of fillers, the rubber becomes harder. Depending on particle size fillers can be divided into reinforcing (<0.03 micron), semi-reinforcing (0.03 to 1 micron) and nonreinforcing fillers (>1 micron). Fillers can be either black or white. The most common black filler prepared from crude oil is the carbon black. Most white mineral fillers are silica, $CaCO_3$, clays, whiting, silicates, etc. Reinforcing types of white fillers are silicas prepared by precipitation. Organic fillers are based on high styrene polymeric materials. These resins do not necessarily increase tensile strength, but they greatly harden, or stiffen and improve wearing properties of products (Rubber Foundation Information Center for Natural Rubber, 2005).

Vulcanizing system

Almost all vulcanization systems for natural rubber are based on sulfur and consist of a vulcanizing agent (sulfur), activators and accelerators (and/or sulfur donors). Due to the double bond in the unsaturated hydrocarbon chain, sulfur is the most interesting crosslinking (vulcanization) agent. To activate the sulfur, activators such as metal oxides (zinc oxide) and fatty acids (stearic acid) are commonly used. The vulcanization time depends on and can be adjusted by the type and quantity of accelerators used and the vulcanization temperature. Accelerators are divided in groups ranking from slow to ultra fast. Examples are guanidines, sulphenamides, thiazoles, thiurams, carbamates and xantates. For NR sulfur vulcanization sulphenimides are commonly used, sometimes boosted by secondary accelerators such as thiurams or guanidines to speed up even more. Vulcanization systems can be divided in three classes: high sulfur (conventional), medium sulfur (semi-efficient) and low sulfur (efficient). Conventional sulfur vulcanizing systems for soft rubber products consist of relatively high amounts of elemental sulfur (about 1–3 phr) combined with a low concentration of accelerator(s). If the amount of sulfur is increased to 25-45 phr, a hard rubber, or ebonite, is formed. Other vulcanization (crosslinking) systems are based on peroxides or urethanes (Rubber Foundation Information Center for Natural Rubber, 2005).

Protective system: Antioxidant

Antioxidants, either natural or synthetic are essential additives to prevent oxidative reaction. The addition of antioxidants is vital for the production and use of a polymer. Antioxidants interrupt the degradation process in different ways, depending on their structure. The two major classifications are chain terminating primary antioxidants and hydroperoxide decomposing secondary antioxidants. Primary antioxidants react rapidly with peroxy radicals and are therefore called "radical scavengers". The majority of primary antioxidants for polymers are sterically hindered phenols or amine-based compounds (Bauer, et al., 1997). Secondary antioxidants react with hydroperoxides to yield non-radical products and are therefore frequently called hydroperoxide decomposers. Secondary antioxidants are particularly useful in synergistic combinations with primary antioxidants (Bauer, et al., 1997). Shown in Figure 11 are the structures of two of the common commercial antioxidants.



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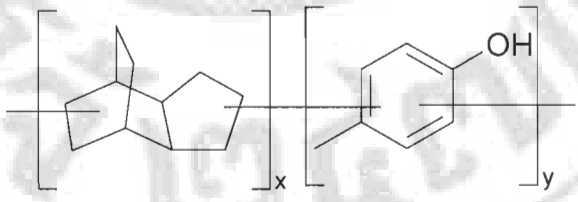


B

Figure 11. Structures of the two commercial antioxidants A) 6-PPD and B) Wingstay-L, used in this study.

Another classification of antioxidants depending on how it interferes with the polymer was given by Al-Malaika (1991) as cited by Abad, et al. (2002) is listed below.

a. The chain breaking donors (CB-D) are those which are capable of competing with the substrate (RH) for the alkylperoxyl radicals. The hindered phenols and aromatic amines such as substituted p-phenylene diamines are the most important of these antioxidants.

b. Chain breaking acceptors (CB-A) are antioxidants which can compete with oxygen for the alkyl radicals. Quinones, nitro compounds and stable oxyl radicals are considered as CB-A antioxidants.

c. Preventive antioxidants decompose hydroperoxides by a process which does not give rise to free radicals or that can stabilize the hydroperoxide. The phosphite esters and a whole family of sulfur-containing compounds are among this set of compounds

2.4.3 Oxidation of natural rubber

Natural rubber like any other polymer is susceptible to oxidative degradation which results in loss of physical properties. Typical signs of oxidation of polymer can be change of appearance and loss of mechanical properties such as elongation, impact strength, tensile strength and flexibility. Oxidation can occur at every stage of the life cycle of a polymer, i.e. during manufacturing and storing of the material or during processing and end-use (Lundback, 2005).

The oxidation of polymer like rubber follows also the mechanism of the basic autoxidation scheme (BAS) of low molecular weight hydrocarbons which was developed in the 1940's (Bolland and Gee, 1946 cited by Lundback, 2005) as shown in Figure 8. Autoxidation is initiated by heat, light (high-energy radiation), mechanical stress, catalysts residues or reaction with impurities to form alkyl radicals (free radicals). The free radical can, in turn react, and result in degradation of the polymer (Al-Malaika, et al., 1993). However for polymers, there are additional factors to consider for the oxidation process, such as restricted mobility of the radicals and morphological variations in semicrystalline polymers (Lundback, 2005).

The BAS scheme is divided into three steps: inititation, propagation and termination. Initiation as the polymer or the hydroxyperoxides with the addition of energy form radicals. The next step is the propagation where a free radical can react with oxygen or a peroxyradical can react with another polymer to form other radicals. The propagation of these free radicals results to

scission reactions of the rubber molecules. The last step, the termination is when radicals react with each other to form non-reactive products (Lundback, 2005).

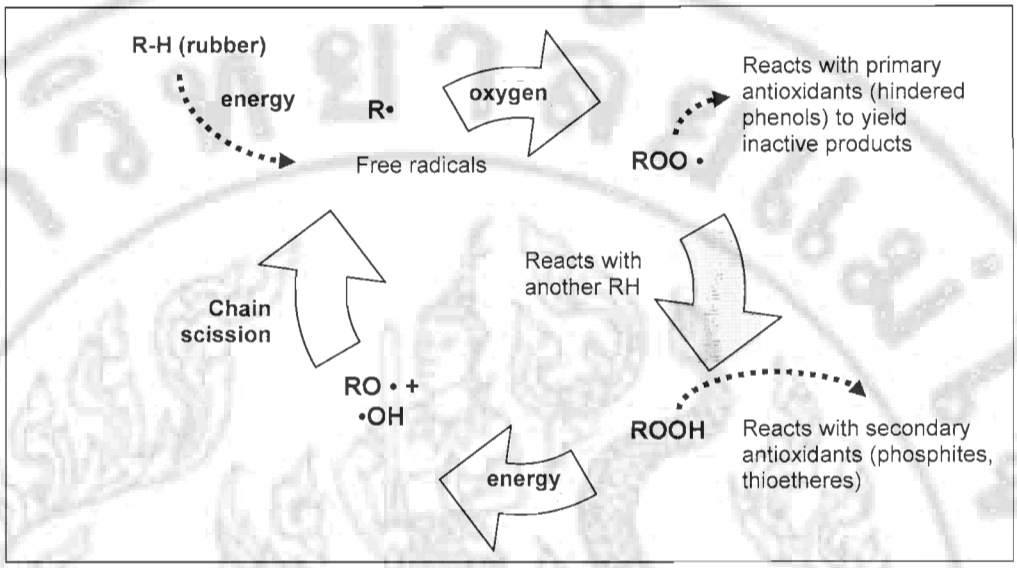


Figure 12. The basic autoxidation scheme (BAS) of natural rubber (Lundback, 2005).

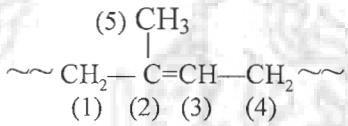
2.4.4 The chemistry of vulcanization

Vulcanization is the introduction of crosslinks into the elastomer's polymer matrix and is a fundamental determinant of rubber properties. It converts substances that are plastic and moldable into one that is flexible and elastic. Vulcanization increases tensile strength, modulus (stiffness), hardness, abrasion resistance, and rebound, and decreases elongation, hysteresis (heat buildup), compression set, and solubility. Vulcanization-induced changes are proportional to the number of crosslinks and their length. Excessive crosslinking can convert the elastomer to a hard, brittle solid. Longer (polysulfide) crosslinks promote better tensile and tear strength and better fatigue properties. Shorter crosslinks provide better oxidative and thermal stability and lower compression set. (Ciullo and Hewitt, 1999)

The initial step in vulcanization seems to be the reaction of sulfur with the zinc salt of the accelerator to give a zinc perthio-salt XS_xZnS_xX, where X is a group derived from the accelerator. This salt reacts with the rubber hydrocarbon RH to give a rubber bound intermediate

$$XS_xZnS_xX + RH \longrightarrow XS_xR + ZnS + HS_{x-1}X$$

and a perthio-accelerator group which, with further zinc oxide will form a zinc perthio-salt of lower sulfur content; this may, nevertheless, again be an active sulfurating agent, forming intermediates $XS_{x-1}R$. In this way, each molecule of accelerator gives rise to a series of intermediates of varying degree of polysulphidity. The hydrogen atom which is removed is likely to be attached to a methylene group in the alpha position to the double bond, i.e. in natural rubber the hydrogen atoms at positions 4 and 5 are the most labile in this type of reaction (Morrel, 1982).



The intermediate XS_xR then reacts with a molecule of rubber hydrocarbon RH to give a crosslink, and more accelerators is regenerated.

$$XS_xR + RH \rightarrow RS_{x-1}R + XSH$$

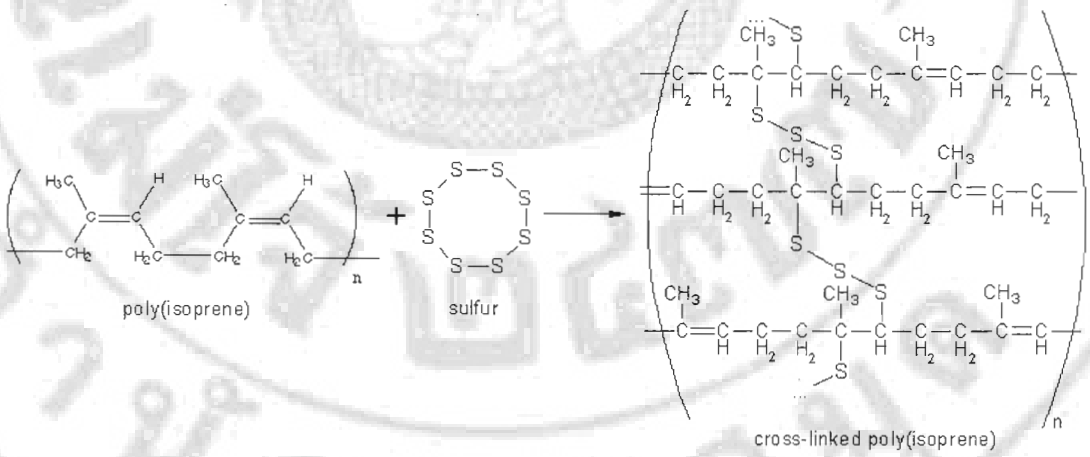


Figure 13. The formation of crosslink between rubber molecules during vulcanization.

Chapter 3

METHODOLOGY

3.1. Research Design

This study utilized two types of palm oil mill wastes in the production of antioxidant for natural rubber. These were the OPKM left after pressing out the crude oil from oil palm kernel and the POME which is the wastewater produced after separator or decanter (Kittikun, et al., 2000). The potential antioxidant from OPKM and POME was isolated by the proposed Ash-AQ pulping method of Sun and Tomkinson (2001) and by electrocoagulation followed by recovery techniques described by Phutdhawong, et al. (2000), respectively. The isolated lignin or lignin-related compounds from the wastes were tested for its antioxidant activity using the DPPH radical scavenging assay before being used as an antioxidant in the preparation of vulcanized natural rubber. Two methods of preparation of vulcanized NR were employed. The first method used dry rubber (technically specified block rubber with an industry code STR 5L) while the second method used NR latex. The physico-mechanical properties before and after ageing of the vulcanized NR were evaluated. The swelling parameters were determined and thermal degradation behavior was also studied. The efficiency of the isolated compounds from wastes as an antioxidant was compared with commercial natural rubber antioxidants. The structure of the isolated antioxidants was partially determined using different spectroscopic and chromatographic methods, as well as some derivatization methods.

The schematic diagram shown in Figure 11 provides the flow of the research experimental procedures. The detailed procedures of each step are given in the succeeding sections.

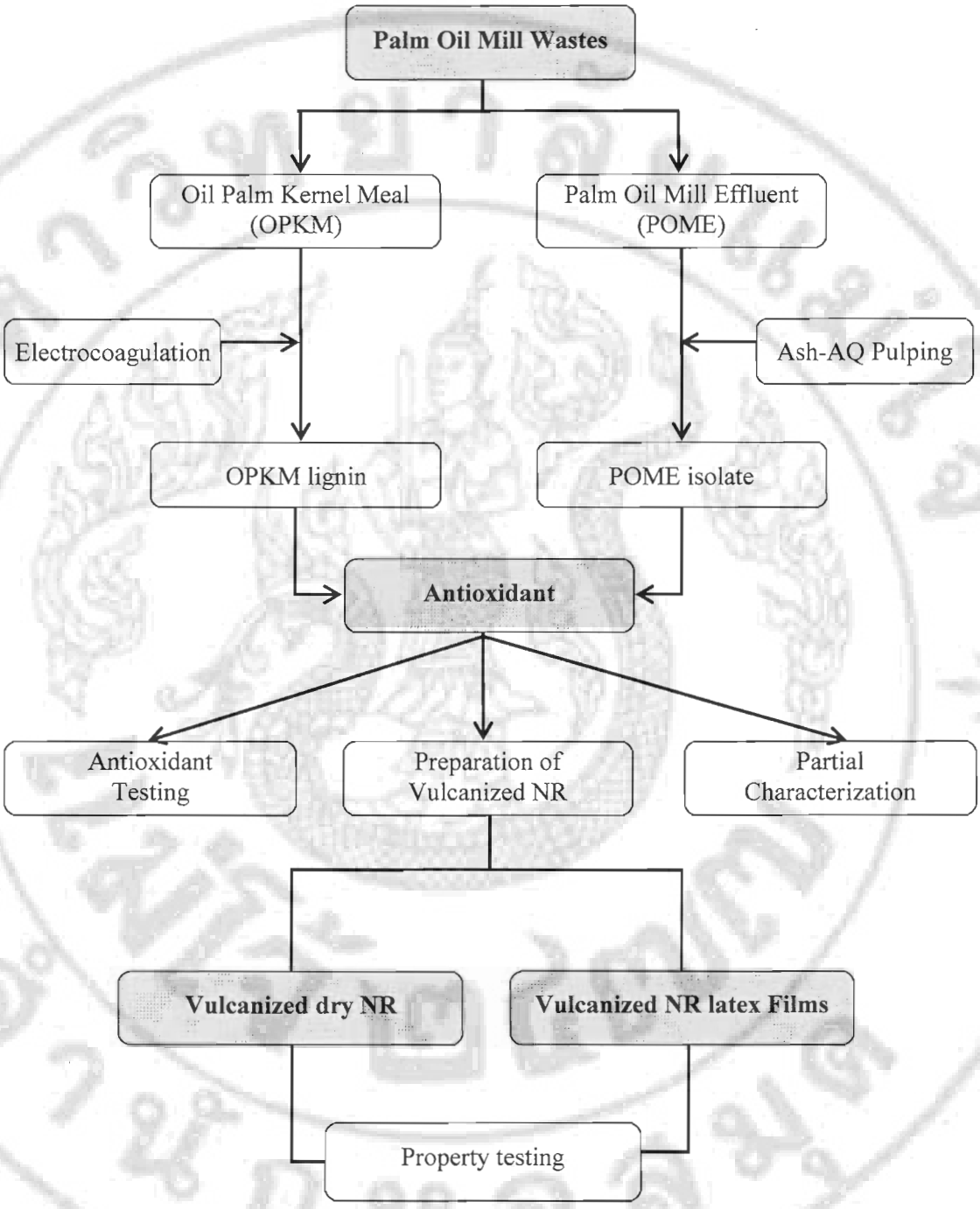


Figure 14. Schematic diagram of the experimental procedure.

3.2. Collection of Samples

The palm oil mill wastes, which include the oil palm kernel meal and the palm oil mill effluent used in this study, were collected at Chumporn Palm Industries.

3.3. Isolation of the Potential Antioxidant from Palm Oil Mill Wastes

3.3.1 Ash-AQ pulping of OPKM

Lignin was extracted by the Ash-AQ pulping process described by Sun and Tomkinson (2001). The method involves digestion of OPKM in a 20% solution of KOH/0.10% Anthraquinone (AQ) in a ratio of 10:1 (alkali solution to OPKM). The mixture was digested to a maximum cooking temperature of 170°C for 3 hours. Isolation of the polysaccharides degradation products and ash from the black liquor produced during digestion was accomplished by adjusting the pH to 6 with 20% H_2SO_4 and precipitation with 3 volumes of ethanol. The resulting solution was filtered and lignin was precipitated by adjusting the pH of the filtrate to 2. The precipitated alkali soluble lignin was filtered and dried in air.

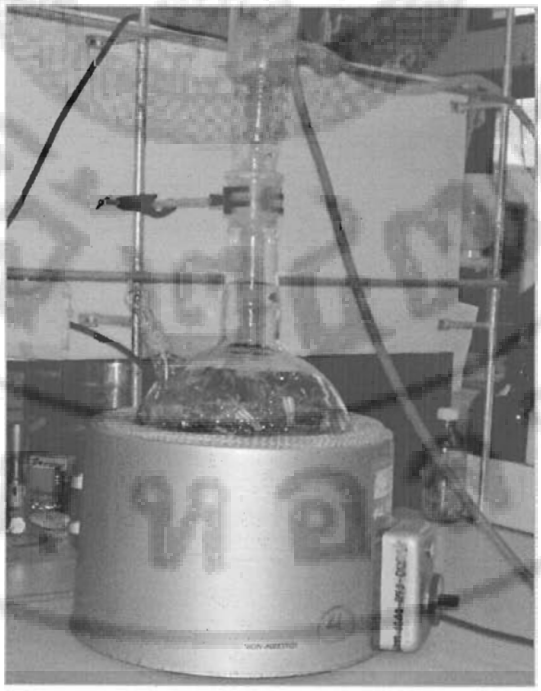


Figure 15. The set up for the Ash-AQ pulping of OPKM.

3.3.2 Electrocoagulation of POME

Prior to EC, residual oils from POME were removed by mixing equal volumes of POME and hexane in a separatory funnel. The mixture was shaken and allowed to separate overnight. POME, being denser settled at the bottom and was drained out from the funnel. One liter of the de-oiled POME was transferred to a cylindrical glass vessel (inner diameter: 12 cm, height: 23 cm) with a special cover to support the pair of aluminium electrodes having a dimension of 30cm x 10cm x 1mm. Sodium chloride (2.0g) was added to the solution as supporting electrolytes. The electrodes were immersed 4 cm apart and 8 cm deep into the solution which was kept stirred throughout the experiment. Crotech (Model ZT3202) was used to supply the solution with a direct current (3.50-12V, 1.40-2.01A). EC was continued for a total of 6 hrs excluding the time consumed for replacing the electrode every hour. After EC, the solution was filtered using vacuum filtration. The filtrate (370ml) obtained was kept in a glass bottle and stored in the refrigerator for further analysis and the coagulum was collected and dried at 40°C. The dried coagulum was pulverized and dissolved with 7% HCl in the ratio 1 g coagulum: 5ml acid. The acid-coagulum mixture was kept stirred to ensure complete dissolution and was then transferred to a separatory funnel. Equal volume of n-butanol (n-BuOH) was added and the mixture was shaken vigorously and allowed to separate overnight. The n-BuOH extract was collected and the remaining aqueous solution was further extracted with n-BuOH twice. All the n-BuOH extracts were combined and washed with water three times to remove any trace of inorganic compounds from the aqueous layer. The n-BuOH was removed using vacuum rota-evaporation which enabled the recovery of the solvent. The isolate was further dried at 40°C in an oven

3.4. Qualitative Test for Phenolic Groups Using $FeCl_3$ Test

The lignin obtained from alkali digestion of OPKM, the POME solution before and after EC, and the POME isolate from the butanol extraction were all tested for the presence of phenolic functional groups. A few drops of 1% $FeCl_3$ were added to a 10ml sample solution (500ppm for the isolates) and the mixture was stirred. The formation of a bluish to black solution confirmed the presence of phenolic groups in the sample.

3.5. COD and BOD_5 Determination

COD and BOD_5 of the palm oil mill effluent before and after EC were determined according to the Standard Methods for Examination of Water and Wastewater. COD was analyzed using the closed reflux titrimetric method. Briefly, the method involves refluxing a known volume of sample with an oxidizing agent ($K_2Cr_2O_7/H_2SO_4$) in a closed ampule at 150°C for two hours, and titrating the excess oxidizing agent with standard ferrous ammonium sulfate using ferroin as indicator. BOD_5 determination involves filling with sample, to overflowing, a BOD bottle of the specified size and incubating it at 20°C for 5 d. Dissolved oxygen (DO) is measured initially and after incubation using titrimetric method and the BOD is computed from the difference between the initial and final DO. All determinations were done in duplicate.

3.6. Analysis of Antioxidant Properties of the Isolates Using DPPH Free Radical Scavenging Assay

Solutions of the isolated compounds from waste were prepared at varying concentrations (1000, 500, 250, 100, 50 ppm) in methanol. To 1 ml of the solution, 3 ml of 0.20μM 1,1-diphenyl-2-picrylhydrazyl (DPPH) methanolic solution were added. For blank analysis, 1 mL of MeOH was used. The decrease in concentration of DPPH as a measure of antioxidant activity was measured via UV-Vis spectrophotometer (ThermoSpectronic) at 517nm. The antioxidant activity was compared with a standard solution of vitamin E. The percentage reduction was calculated using the equation:

$$\% \text{ reduction} = \frac{A_b - A_s}{A_b} \times 100$$

where A_b is the absorbance of the blank solution and A_s is the absorbance of the sample.

3.7. Preparation of Vulcanized Natural Rubber

3.7.1 Materials

The properties of the technically specified dry rubber (STR 5L) used in this study are summarized in Table 2. The commercial antioxidants, butylated reaction product of p-cresol and dicyclopentadiene (Wingstay L) and N-(1,3-dimethylbutyl)-N'-phenyl-paraphenylenediamine (6-PPD); and other rubber additives such as fillers (CaCO3), activators (Stearic acid and ZnO), accelerators (CBS), etc., are all of industrial grade. Concentrated latex (60%) was supplied by Rubber Research Institute of Thailand.

Table 2. Properties of STR 5L used in this study

Properties	**STR 5L**	**Standards**
Dirt content (% by wt.)	0.004	<0.04
Volatile matter (% by wt.)	0.31	<0.80
Nitrogen content (% by wt.)	.39	<0.6
Initial Plasticity (Po)	35	>35
Plasticity Retention Index (PRI)	88.6	>60
Viscosity ML (1+4)	59.4	-
Colour Lovibond Scale	4.0	<6.0

3.7.2 Preparation of NR vulcanizates from STR 5L

Compounding

The components for rubber blends are given in Table 3. Eight treatments were prepared. For easy identification on the succeeding discussions, treatments IDs are named such that the first letter stands for the type of NR used, in this case D for dry rubber; the second letter for the antioxidant used, O for OPKM isolate, P for POME isolate, W for Wingstay L and A for amine-based antioxidant which is 6-PPD; and the number at the end stands for the concentration (in phr) of the antioxidant used.

Mixing was done on a two-roll mill with an outer diameter of 20 inches and with speeds of 20 rpm and 23 rpm for the front and back roll mills, respectively. The order of adding the curing agents and other additives were done uniformly in the order given in ISO 1658 with a constant mixing duration and temperature of 20 minutes and 70°C, respectively. The rubber was vulcanized using a hydraulic press at a pressure of 2000psi, temperature of 150°C, and at a specified cure time (t_{90}) obtained from the rheometer data.

Table 3. Formulation of the dry rubber compounds for each treatment.

Compounding	**Treatments**							
Materials*	**DO1**	**DO2**	**DO3**	**DP1**	**DP2**	**DP3**	**DW1**	**DA1**
STR 5L**	100	100	100	100	100	100	100	100
ZnO	5	5	5	5	5	5	5	5
Stearic acid	2	2	2	2	2	2	2	2
CBS***	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sulfur	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
OPKM lignin	1	2	3	-	-	-	-	-
POME isolate	-	-	-	1	2	3	-	-
Wingstay L	-	-	-	-	-	-	1	-
6PPD	-	-	-	-	-	-	-	1

*Concentration is given in parts per hundred of rubber (phr)

** Standard Thai Rubber

*** *N*-Cyclohexyl-2-benzothiazyl sulfonamide (accelerator)

Determination of the cure characteristics

Prior to vulcanization, the characteristics of the unvulcanized rubber compound were determined using rheometric analysis and Mooney viscosity determination. The rheometric analysis for the unvulcanized rubber compound was done following the procedures given in ASTM D-2084-93 using the Rheotech (TechPro 121105) oscillating disk cure meter. The test temperature and arc of oscillation were set to 150°C and 3°, respectively. The Mooney viscosity of

the unvulcanized material was measured using the Mooney Viscometer (Shimadzu, SMV-201) at a temperature of 100°C and a run time of 4mins as followed from ASTM D1646-03.

3.7.3 Preparation of vulcanized NR films from NR latex

Preparation of antioxidant dispersion

Solid substances to be able to mix with natural rubber latex must be prepared first in the form of dispersion. Thus, the OPKM lignin, the POME isolates, and the commercial antioxidant, Wingstay L, were all prepared as dispersion prior its addition to natural rubber latex. The formulation of a 25% by mass antioxidant dispersion is shown in Table 4. The mixture was poured in a container one-third full of glass ball and milled in a laboratory scale ball mill until the mixture showed a dispersing behavior when dropped in water. Dropping the mixture on water is a rapid qualitative test used to determine the quality of the dispersion. When the mixture is dropped in water and it forms a lump that either floats or sinks in water, the mixture is not yet completely dispersed. However, if the mixture is dropped in water, and it uniformly distributes itself, the mixture is considered a dispersion. After the dispersion is produced, its pH was adjusted to 10 by the addition of 10% KOH solution and milled again for another 24 hours to ensure complete mixing. Basic antioxidant dispersion is needed to avoid coagulation of NR latex. Finally, the prepared dispersion was filtered using aluminum screen and kept in sealed containers.

Table 4. Typical formulation for the preparation of 25% antioxidant dispersion.

Ingredient	Mass (in grams)
Antioxidant*	25
Dispersing agent	1
Bentonite	1
Water	73

* wingstayL, OPKM lignin, or POME isolate

Compounding of the NR latex

Seven treatments (Table 5) for the NR latex compound that vary in the concentrations and types of antioxidants used were prepared. The general formulation of the NR latex compound

in parts by mass used in this study is shown in Table 6. For treatments with antioxidant concentration of more than 1phr, the mass of the dispersion used was adjusted to obtain the desired concentration.

Table 5. Treatments employed in the preparation of NR latex film

Treatment	Treatment ID*	Antioxidant	Concentration (phr)
1	LO1	OPKM lignin	1
2	LO2	OPKM lignin	2
3	LO3	OPKM lignin	3
4	LP1	POME isolate	1
5	LP2	POME isolate	2
6	LP3	POME isolate	3
7	LW1	Wingstay L	1

*Treatments are named in such a way the first letter stands for the type of NR used, in this case L for latex; the second letter for the antioxidant used, O for OPKM isolate, P for POME isolate; W for Wingstay-L and the number at the end stands for the concentration (in phr) of the antioxidant used.

Prevulcanized NR latex compound was prepared accordingly. The designated weight of NR latex was poured in a stainless steel container, set on a hot plate with a temperature maintained at 60°C, and stirred by a mechanical stirrer for about 2 minutes. After which, the remaining ingredients in a form of dispersion were added one at a time into the NR latex with continuous stirring. When the temperature of the mixture reached 60°C, the curing characteristic of the prevulcanize NR latex was assessed every 5 minutes using the chloroform test described in the next section. When the latex compound attained a curing characteristic designated arbitrarily as chloroform number 2, heat was removed, and stirring was stopped. The latex compound was allowed to cool in a water bath, and finally filtered and stored in plastic bottles sealed with caps.

Thin films of the latex compound were prepared by casting approximately 35g of the previously prepared prevulcanized NR latex on a glass plate with a dimension of 15cm x 15cm (Figure 16). It was then allowed to dry at room temperature for two days and later cured at 120°C for 15 minutes.

Table 6. The formulation of NR latex compound used in this study

Compounding materials	Wet part (g)	Dry part (phr)
60% NR latex	167	100
10% KOH	2	0.20
10% Teric 16A	0.2	0.02
50% Sulfur	1.6	0.8
50% ZDEC (zinc diethyldithiocarbamate)	0.8	0.40
50% ZMBT (zinc mercaptobenthiazole)	0.8	0.40
50% TiO_2	2	1
25% Antioxidant	4	1
50% ZnO	2	1
Distilled water	170.5	-

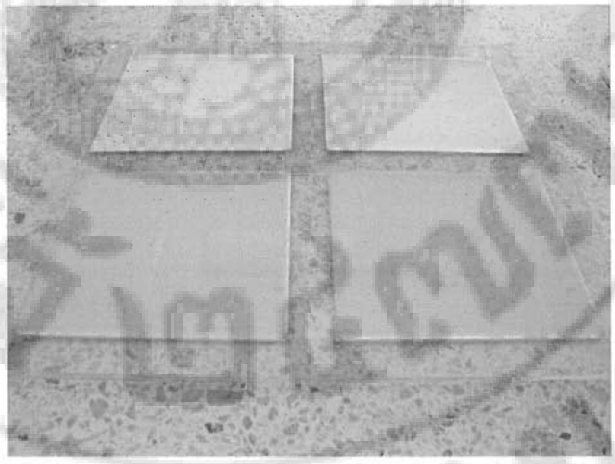


Figure 16. The drying of the compound latex for two days prior to vulcanization.

Chloroform test for prevulcanized natural rubber latex

A small volume (≈10ml) of the latex was mixed with an equal volume of chloroform and the mixture was stirred until coagulation occurs as a consequence of the latex particles having much absorbed of the chloroform. The physical character of the coagulum which depends on the state of vulcanization was then examined. The most highly crosslinked are the particles, the less

readily do they coalesce together to form a coherent coagulum. As the degree of vulcanization increases, the coagulum changes from a soft, plastic, well-integrated mass to a crumbly material. Four stages of prevulcanization assigned with arbitrary numbers, known as chloroform number (listed below), are assigned to the latex on the basis of appearance of coagulum. The higher the chloroform number, the higher the perceived degree of vulcanization (Blackley, 1997).

- chloroform number 1: the coagulum is a tacky mass, breaking in a string manner when stretched;
- chloroform number 2: the coagulum is a weak lump which breaks short when stretched;
- chloroform number 3: the coagulum has the form of non-tacky agglomerates; and
- chloroform number 4: the coagulum has the form of small dry crumbs.

Determination of the total solids content of prevulcanized NR latex compound

The total solids content (TSC) of the prevulcanized NR latex was determined by weighing approximately 2.0±0.5g of the prevulcanized NR latex compound on a pre-weighed circular glass plate. The plate was swirled to spread the latex on the plate, and was then dried in an oven for 16hrs at 70°C. After which, the glass plate was removed out from the oven, cooled in the desiccators and weighed. The plate containing the latex was dried for another hour in an oven at the same temperature, cooled, and weighed again. The TSC of the compound latex was calculated using the equation

$$\% \text{ TSC} = \frac{W_{17} - W_{eg}}{W_s} \text{ X } 100$$

where; W_{17} is the weight of the glass plate and the dried latex after 17 hrs of drying,

W_{eg} is the weight of the empty glass plate

W_s is the weight of the sample

3.8. Analysis of Physico-mechanical Properties of the Vulcanized NR

The physico-mechanical properties determined on the rubber samples produced include specific gravity, hardness, tensile properties such as tensile strength, elongation and Modulus 300,

compression set, and rebound resilience. For rubber film samples, hardness, compression set and rebound resilience were not measured. Brief details for each procedure used in the analysis are described in the succeeding sections.

3.8.1 Specific gravity

Specific gravity was measured using the standard method, ASTM D92. The dry rubber sheet was cut into a square shape and was weighed both in air and in water using Density kit model EW-120SC (Mirage Trading Co. Ltd.). At least five samples were tested and specific gravity values were averaged.

3.8.3 Hardness

Hardness was determined following the methods in ASTM D2240. The measurement was made by pressing the indentor (Shore A durometer) against four samples of 2-mm thick piled together. The hardness of the material was measured along five different points on the rubber samples and the hardness readings on a scale of 0-100 were averaged.

3.8.3 Tensile properties

Tensile properties which include the tensile strength, Modulus 300, and percentage elongation at break were determined using the ASTM D412-98a method. Samples were cut into dumbbell shape (Figure 17) by a die cutter and then stretched by Instron tensometer (Model 5565) using 500N load cell and a crosshead speed of 500mm/min. At least 5 samples per treatment were tested and values were averaged.

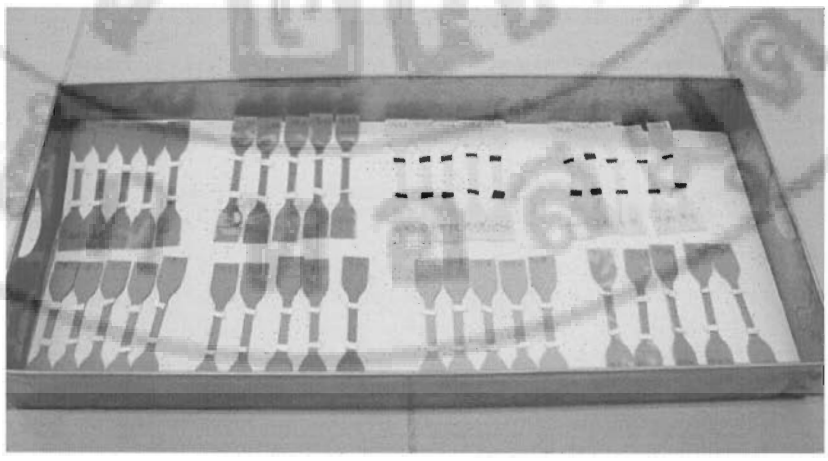


Figure 17. A typical NR latex film ready for tensile test after die cutting, thickness measuring and benchmarking.

3.8.4 Compression set

Compression set is defined as the residual deformation of a material after removal of an applied compressive stress. Resistance to compression set is the ability of an elastomeric material to recover to its original thickness after having been compressed for an extended period. Low values of compression set mean that the material has recovered nearly to its original height, and there is very little deformation (Ciullo and Hewitt, 1999).

Compression set was measured using the test method B of ASTM-D395. In this method, compression set is measured under the condition of constant deflection in air. Briefly, circular specimen of known thickness (t_o), which usually ranges from 11-12mm, are placed between the steel plates of the compression device (Figure 18) with the spacers on each side of known thickness (t_n). The compressive force is maintained at a temperature of 70^{o}C for 22 hours by putting the set-up inside an air circulating oven. After which, the compressive force was removed, the specimen were allowed to cool for 30 mins and the final thickness (t_f) of the sample was measured. Compression set (C_B) was calculated using the equation

$$C_B = \frac{T_o - T_i}{T_o - T_n} \text{ X } 100$$

Figure 18. A compression device set-up.

3.8.5 Rebound Resilience

Resilience is the energy returned by a setup vulcanized elastomer when it is suddenly released from a state of strain or deformation. High resilience is what often causes the bounce or snap often associated with rubber products (Ciullo and Hewitt, 1999). The rebound resilience usually expressed as percentage is the ratio between the returned and applied energies of a moving mass which impacts a test piece. Following the method given in BS-903 , the rebound resilience was measured using a Wallace Dunlop tipsometer (Figure 4). A circular disc sample was placed in the test piece holder and left for 5 minutes to reach the test temperature (23±1°C). The test piece was subjected to a number of impacts of the pendulum from its release position of 45°, in rapid succession, until the reading obtained from three consecutive impacts agrees to within ±1% point of resilience. Test was done in triplicates for each treatment.

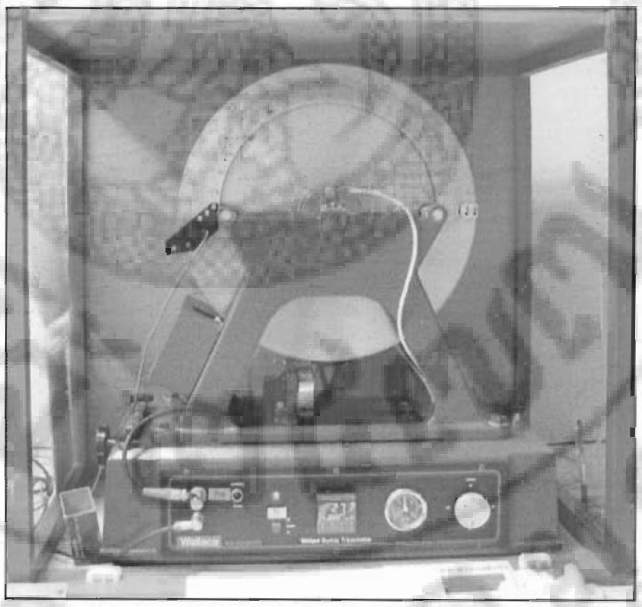


Figure 19. A Dunlop tipsometer to measure rebound resilience.

3.9. Accelerated Thermal Ageing

Accelerated thermal ageing was performed by heating the sample in an air oven for 22 hours at 100°C. Then, changes in the hardness and tensile properties of the rubber were

determined. For the latex films, ageing were done in two conditions: 22 hours and 3 days, both at 100°C.

3.10. Swelling (*Q*) Property Determination

Rubber samples produced from each treatment was subjected for swelling test. For the vulcanized NR from STR 5L, the mass and dimension of the samples used were 0.30±0.001g and 1.5cm x 1.5cm x 2mm, respectively. For the NR latex film, sample mass was 0.10±0.01g and the dimension was approximately 1.5cm x 1.5cm x 0.50mm. Each sample was immersed in a liquid (toluene for this study) to reach equilibrium, 24 hours for NR latex film and 7 days for the vulcanized NR from STR 5L, which are much thicker than the latex film. The mass of the sample before and after immersion in toluene was then determined by an analytical balance enclosed with a clear box. Weight determination was done rapidly at an approximate rate of 30sec/ sample and the sample was contained in a small glass jar while weighing to avoid the escape of solvent from the sample. The weight was then recorded and the degree of swelling for each sample was calculated. Swelling determination was done in triplicates for each treatment.

3.11. Thermogravimetric Analysis

Thermogravimetric analysis was carried out using Perkin Elmer TGA7. Approximately 10 mg of sample was heated from room temperature up to 600°C with a continuous flow of Nitrogen at 20ml/min. The heating rate was 20°C/min. Sample weight lost was continuously measured as a function of temperature.

3.12. Characterization of the Isolated Antioxidants

3.12.1 Qualitative test for phenolic functional group using UV-Vis spectrophotometer

The phenolic group in the isolates was determined qualitatively using UV-Vis spectrophotometer. Five-milligram sample was dissolved into 10ml 90%v/v dioxane-water solution. 1ml of the aliquot was further diluted to 25ml by 50% v/v dioxane-water solution. The absorbance of the sample was scanned from 220nm-350nm using the UV-Vis spectrophotometer (ThermoSpectronic).

3.12.2. Quantitative determination of total phenolic content

Total phenolic content in the OPKM lignin and POME isolate was determined using the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard phenolic compound. Samples were prepared by dissolving 0.10g isolate in 50mL MeOH. Using a pipette, 1 mL of the sample was drawn and mixed with 20mL distilled water in a volumetric flask, then 0.50mL of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 mins, 1.50mL of saturated Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm. Standard solutions of concentrations ranging from 0-10ppm were also prepared. The total phenolic compound in the sample was determined as microgram of gallic acid equivalent by using an equation that was obtained from standard gallic acid calibration curve. All determinations were done in triplicate.

3.12.3. Quantitative determination of tannin content

Tannin content in the isolated antioxidants was determined using the spectrophotometric method given in AOAC Official Methods of Analysis . The colour reagent, Follin-Dennis reagent was prepared by refluxing for 2 hours a mixture containing 175mL distilled water, 25g $Na_2WO_4.2H_2O$, 5 g phosphomolybdic acid, and 12.5mL H_3PO_4. After, the mixture was cooled; and was transferred to a 250-mL volumetric flask and diluted to volume. The sample used in the analysis was obtained from the same solution prepared in the determination of total phenolic content. 1 mL aliquot of the sample was transferred to a 10-ml volumetric flask containing 7 mL distilled water. 0.50 mL of the Follin-Dennis reagent and 1 mL of the saturated Na_2CO_3 were added and the solution was mixed and diluted to volume. After 30 min, the absorbance was measured at 760nm. The tannin content of the sample was determined as microgram of tannic acid equivalent by using an equation that was obtained from standard tannic acid calibration curve. All determinations were done in triplicate.

3.12.4. Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups present in the isolates were determined using FTIR. The KBr pellets were prepared containing 1% fine ground sample. The IR spectra were recorded on Perkin-Elmer 2000 spectrophotometer. Each sample was run in triplicate.

3.12.5 Nitrobenzene oxidation of the OPKM isolate

Nitrobenzene oxidation was carried out by adding 50mg dry lignin into a mixture of 7ml of 2M NaOH and 4mL of nitrobenzene in a screw-capped tube fitted with a teflon gasket. The mixture was heated to 165^{O}C for 3 hours in a pre-heated thermostat oil bath. After the heating period, the tube was cooled with ice water. The mixture was then transferred to a separatory funnel and removal of excess nitrobenzene and nitrobenzene reduction products was achieved by solvent extraction with dichloromethane. The oxidation mixture was acidified with concentrated HCl to pH 3-4 and further extracted with dichloromethane. The solvent from the second dichloromethane solution will be removed by using rotary evaporator under reduced pressure. The mixture was dissolved into DCM to make 10mL stock solution. The nitrobenzene oxidation products were analyzed using gas chromatography-mass spectrometry (GC-MS) tandem. The GC-MS conditions used are summarized below.

Column: AT-1MS (30m x 0.25mm x 0.25mm

Inlet temperature: 250^{O}C

MS source temperature: 280 ^{O}C

Quadrupole temperature: 150 ^{O}C

Helium flow rate: 1ml/min

Solvent delay: 1.80 min

Temperature Programming: 150 ^{O}C, held for 1 min and later increased at a rate of 10 ^{O}C/min up to 280 ^{O}C and finally held for another 25 mins.

3.12.6 Acetylation of POME isolate

Acetylation of POME isolate was done to introduce a less polar group on the POME isolate. One gram POME isolate was allowed to react to a 20-ml 1:1 mixture of pyridine and acetic anhydride for 24 hours with constant stirring. The acetylated products were then separated by extracting with dichloromethane 3x using a separatory funnel. The DCM extract was further washed with acidified water to remove any excess pyridine. The acetylated products were then analyzed using GC-MS.

A 1-µl acetylated POME sample was injected in a GC-MS (Agilent 6890-HP5975 model). and separation was carried out using helium as the carrier gas maintained at c.a. 16psi

and a flow rate of 1.2ml/min. HP-5MS (5% phenylmethyl siloxane) was used as the capillary column with a nominal diameter of 250μm, length of 30m, and film thickness of 0.25μm. The oven temperature was held initially at 150°C and later ramped at 4 °C/min to 275 °C and was held isothermally at this point for 30 mins. Chromatograms were obtained in scan mode. The mass spectrometer was operated in electron impact ionization mode. The temperatures of the quadrupole, ionization source, and transfer line were 150°C, 230°C and 280°C respectively.

3.12.7 ^{13}Carbon Nuclear Magnetic Resonance (^{13}C -NMR) Spectroscopy

The ^{13}C-NMR spectra were recorded on a Bruker Avance 300 operating in the FT mode at 300 MHz under total proton decoupled conditions. The solvents used were $CDCl_3$ for the OPKM lignin and KOH/MeOD for the POME isolate. The following acquisition parameters were employed:

Number of scans (NS) - 12000

Number of dummy scans (DS) – 4

Spectral width in Hz (SWH) – 18115.9

Acquisition time in seconds (AQ) – 0.90

Dwell time (DW) – 26.6μs

Pre-scan delay (DE)– 20 μs

90° pulse length (P1)– 5.20 μs

Power level for pulse (PL1) – -3.00 dB

Irradiation frequency for channel 1 (^{13}C) – 75MHz

Relaxation delay (D1) – 2.00s

3.12.8 Molecular weight determination by Liquid chromatography-Mass spectroscopy (LC-MS)

Molecular weights of each component in the OPKM lignin and POME isolate were determined by LC-MS. Samples were dissolved in 25% NH_4OH and filtered. About 1 μl was injected to the Agilent 1100 Series HPLC system coupled to diode array detector (Agilent 1100) and equipped with autosampler. The separation was achieved using a C18 (2.1mm x 3cm x 5um) column at a temperature set to 25°C. Solvents were (A) water and (B) MeOH. Gradient elution was employed with a flow rate of 0.20ml/min. The initial mobile phase, 50%A/50%B, was held

for 5 min; then ramped linearly to 100%B at 10 mins and was then reset to the initial solvent system for another 10 min. MS analysis was performed by coupling the Agilent 1100 liquid chromatograph to a MS detector. The mass spectrometer was equipped with an electrospray ionization source which was controlled by the MS Agilent 1100 software. The mass spectrometer was operated in the positive ion mode. The compounds were chemically ionized by proton transfer and the ions generated were introduced into the mass spectrometer and the abundance of selected *m/z* in the range 50-2000 was recorded.

Chapter 4

RESULTS AND DISCUSSION

4.1. Ash-AQ Pulping of OPKM

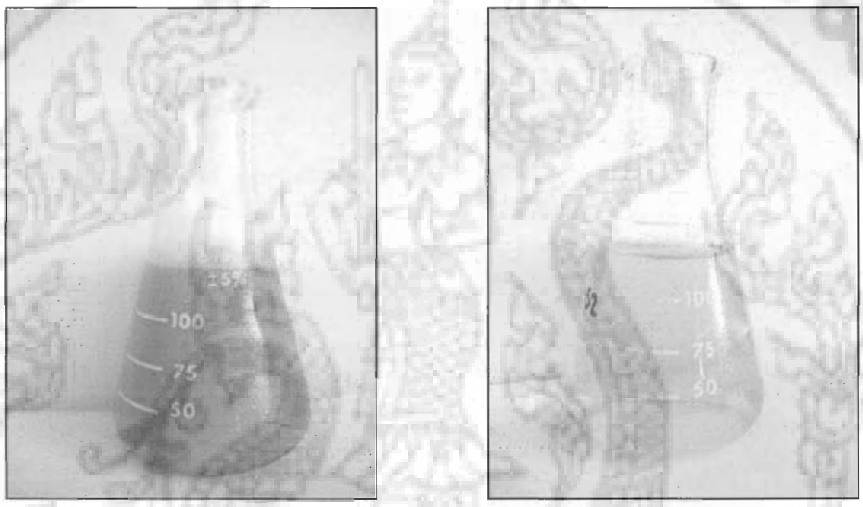
The Ash-AQ pulping of the OPKM produced black liquor with a density of 1.178g/ml and a pH of 12.5. Precipitation at pH 2 gave a yield of 4.12g/100g OPKM. Shown in Figure 20 is the isolated OPKM lignin. The presence of phenolic group in the isolate was confirmed by the formation of bluish-black precipitate with $FeCl_3$. Typical with lignin derived from other plant sources, the OPKM lignin also showed very small solubility in water and other polar or non-polar organic solvents. Thin layer chromatographic tests of the methanol solution of OPKM lignin did not provide good separation with various solvent systems.

Figure 20. The isolated OPKM lignin.

4.2. Electrocoagulation of POME

Figure 21 shows the appearance of POME before and after 6 hrs EC and the comparison is summarized in Table 7. From a dark brown, opaque solution, the POME solution became pale yellow and almost clear. The pH of the solution also increased from 4.30 to 7.63 after EC. This could be explained by the formation of hydroxyl ions when water is reduced at the cathode during

EC. In EC, the coagulant is produced in situ during the oxidation reactions that occur at the anode. And in this study, since aluminum was used as the electrodes, the coagulant was produced from the oxidation of aluminum to Al^{+3} ions. These ions could either react directly to an organic compound that contains negatively charged atoms or form polymeric Al^{+3} hydroxo complexes that can remove pollutants by adsorption to produce charge neutralization, and by enmeshment in a precipitate or coagulum (Mollah et al., 2004).



A B

Figure 21. The POME before (A) and after (B) electrocoagulation.

The reaction of the filtrate obtained from POME after EC with the $FeCl_3$ gave a pale yellow solution indicative of a negative result, which could mean that the dissolved organic compounds especially the phenol-containing substances were almost completely removed by EC. The partial removal of dissolved and suspended organic substances from POME was also confirmed by the decrease in chemical oxygen demand (COD) and biochemical oxygen demand in a 5-d test period (BOD_5) after EC. The COD level in POME before EC was 36 800 mgl^{-1} which was reduced to 25 600 mgl^{-1} registering 30% removal. BOD_5 reduced from 23 400 mgl^{-1} to 14 400 mgl^{-1}, showing 38% removal. The observed percentages of removal for COD and BOD_5 from this study were lower than that determined by the study of Ugurlu, et al. (2008) in the electrocoagulation of paper mill effluent which registered 75 and 70% removal of COD and BOD, respectively. However, it has to be noted that in the study of Ugurlu, et al. (2008), the initial COD and BOD levels of the paper mill effluent were approximately 86 and 900 times

lower than the initial COD and BOD of POME, respectively. The removal of COD and BOD by electrocoagulation could be attributed to the removal of suspended solids and to precipitation of dissolved organic molecules as organometallic compounds (Ugurlu, et al., 2008).

Table 7. Comparison of POME solutions before and after 6-hour electrocoagulation.

Properties/Test	Before EC	After EC
Color	Dark brown	Pale yellow
Turbidity	Opaque	Transparent (clear)
pH	4.30	7.63
$FeCl_3$ test	Positive	Negative
COD	36 800 mgl^{-1}	25600 mgl^{-1}
BOD_5	23 400 mgl^{-1}	14 400 mgl^{-1}

From a liter of POME that was subjected to EC, the yield of the potential antioxidant (Figure 22), which was termed as the POME isolate throughout the succeeding discussions, was 17.10g. Although, initially soluble in butanol during the recovery process, the POME isolate was found to have very little solubility in most polar or non-polar organic solvents after the removal of butanol. It was soluble only in a basic solution. This could imply that the POME isolate may have polymerized during the recovery stage. Similar with the OPKM lignin, chromatographic test showed tailing on various solvent systems employed.

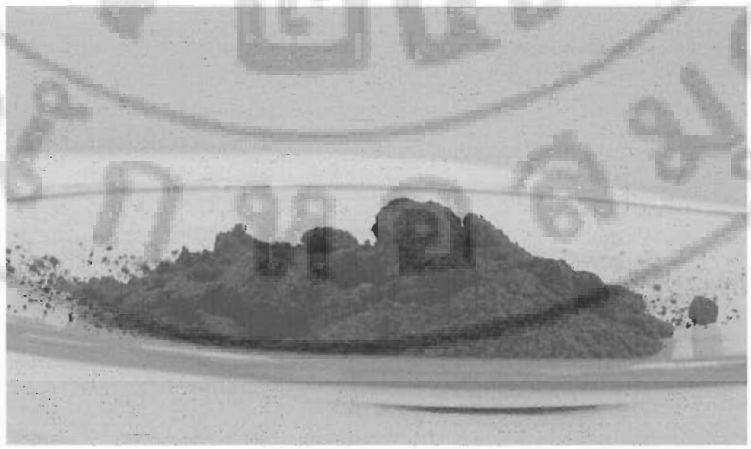
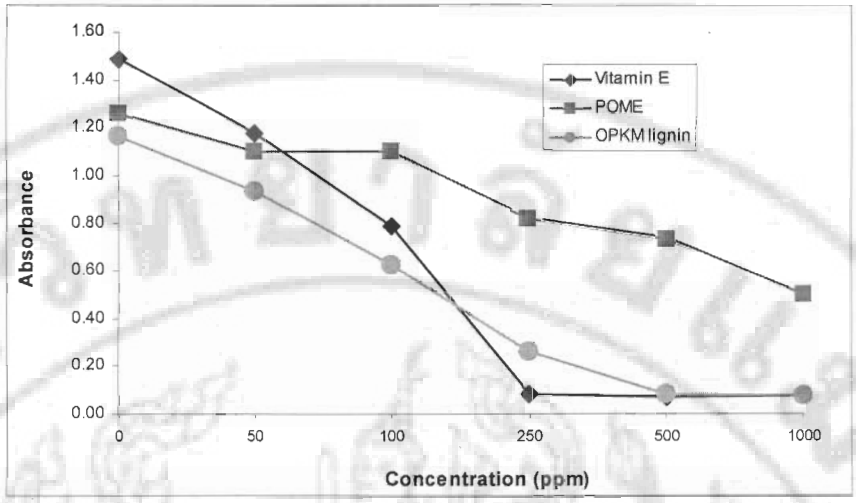


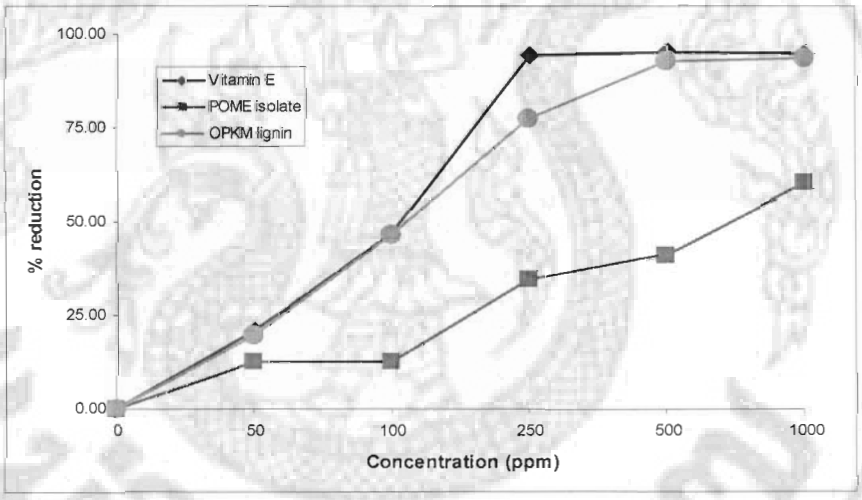
Figure 22. The POME isolate

4.3. DPPH Antioxidant Test

The antioxidant activity of the OPKM lignin and the POME isolate was determined by its ability to reduce the activity of the stable free radical diphenylpicrylhydrazyl (DPPH).The antioxidant activity of OPKM lignin and the POME isolates were compared with Vitamin E, a known antioxidant. It can be seen from Figure 23 that the absorbance values of the DPPH solution decreased with increasing concentration of OPKM lignin and POME isolate. The antioxidant activity of OPKM lignin also showed similar trend with Vitamin E. In contrast the POME isolate showed lower antioxidant activity than OPKM lignin. The % reduction of a 1000ppm OPKM lignin (93.57%) was only slightly lower than the % reduction of Vitamin E (94.68%) of the same concentration. However, POME isolate gave only 60.34 % reduction. This imply that to obtain a percentage reduction similar with Vitamin E, the concentration of POME isolate must be higher while that of the OPKM lignin is almost the same as that of Vitamin E. Extrapolation of points at 50% reduction using the graph in Figure 23B provided the EC50, the effective concentration at which 50% of the DPPH radical activity has been reduced. The EC50 values for Vitamin E, OPKM lignin and POME isolate were 108, 117, and 725 ppm, respectively. It clearly showed that the antioxidant activity of the POME isolate is lower than Vitamin E, while that of the OPKM lignin is almost similar.



A



B

Figure 23. The decrease in absorbance (A) and the percentage reduction (B) of the the DPPH radical solution plotted against the concentration of the isolated antioxidant.

4.4. Characterization of OPKM Lignin

4.4.1 Phenolic content

The UV-absorption spectrum of the OPKM lignin is shown in Figure 24. The maximum absorbance is at 252nm, which is assigned to the phenolate ion compound. Analysis of the total phenolic (TP) content of the OPKM lignin using the Follin-Ciocalteu method revealed a TP content of 158 mg gallic acid equivalent per gram OPKM lignin. This value is higher than the TP

content of milled wood lignin from the tree, *Astronium urundeuva* (Morais, et al., 1999). A high phenolic content for lignin is sometimes attributed to contamination by oxidized tannins. The tannin content of the OPKM lignin was found to be 129 mg tannic acid equivalent per gram OPKM lignin.

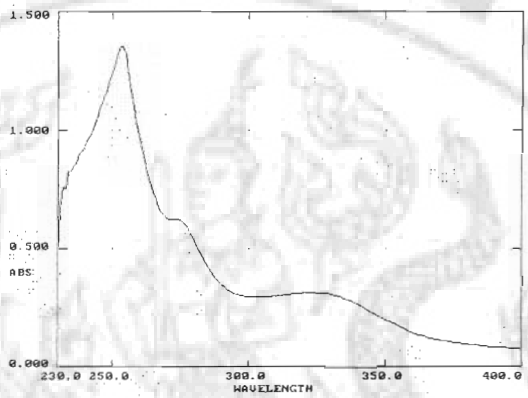


Figure 24. UV spectrum of the OPKM lignin isolated through Ash-AQ pulping.

4.4.2 FTIR analysis

The FTIR spectrum of the OPKM lignin is shown in Figure 25. Table 8 summarizes the assignment of wave numbers to its possible functional group based from the spectrum. As lignin's structural elements are linked by carbon-carbon and ether bonds to form tri-dimensional network (Akim, et al., 1997), the major chemical functional groups in lignin include hydroxyl, methoxyl, carbonyl, and carboxyl groups in various amounts and proportions, depending on genetic origin and applied extraction process (Goseelink, et al., 2004). Indeed, the spectrum of the OPKM lignin showed the above mentioned functional groups and even similar in characteristics with the spectrum of the lignin from the palm empty fruit bunch fibers isolated by Mohamad Ibrahim, et al. (2004) and Sun and Tomkinson (2001) using alkaline digestion. The strong broad band at 3410 cm^{-1} has been assigned to the hydroxyl group. Aromatic skeleton vibrations in the OPKM lignin are assigned at 1593 and 1509 cm^{-1}. Absorption at 1458 cm^{-1} indicates the aromatic methyl group vibrations. The peak at 1284 cm^{-1} has been attributed to C-O stretching. Out of plane bends for the C-H group have been assigned to peaks between 694 -937 cm^{-1}. It is also to

be noted that C-S stretching was observed. This is similar with the result of Mohamad Ibrahim, et al. (2004) and was attributed to the use of sulfuric acid as the precipitating agent for lignin.

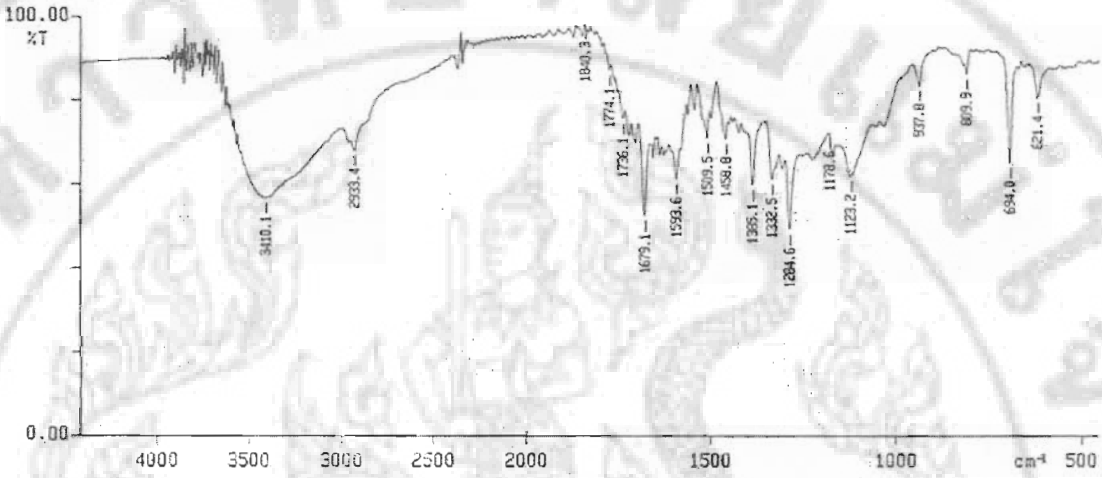


Figure 25. FTIR spectrum of the OPKM lignin isolated through ash-AQ pulping.

Table 8. Assignment of wavenumbers to its possible functional group based from the FTIR spectrum of OPKM lignin.

Wavenumbers (cm^{-1})	**Characteristics**
3410.1	Strong, broad band due to OH stretching
2933.4	C-H stretching
1800-1900	Aromatic overtones
1679.1	Carbonyl group
1593.6 and 1509.5	C=C ring stretch absorptions that occur in pairs
1458.8	CH_3 assymetric bending or sideways ring stretch
1385.1	CH_3 symmetric bending absorption
1284.6	C-O stretching
694. 0 -937.8	C-H out of plane bends
621.4	C-S stretching

4.4.3 Nitrobenzene oxidation

The nitrobenzene oxidation of lignin is one of the standard procedures for analyzing lignin by chemical degradation technique in order to gain information about the composition of the original polymer. The composition of the three monomeric lignin units namely p-hydroxyphenyl, guiacyl, and syringyl (Figure 5) give during oxidation its corresponding aldehydes: p-hydroxybenzaldehyde, vanillin, and syringaldehyde, respectively (Figure 26) (Mohamad Ibrahim, et al., 2004).

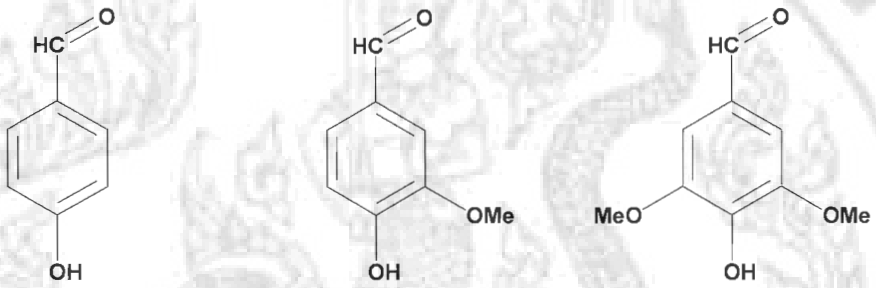


Figure 26. The structures of the nitrobenzene oxidation products, A) p-hydroxybenzaldehyde B) vanillin and C) syringaldehyde.

The gas chromatogram of the oxidation products of lignin is shown in Figure 37. The GC-MS data is tabulated in Table 9. Six peaks from the chromatogram were identified using the Natioanl Institute of Standards and Technology (NIST) database of mass spectra. It can be deduced from the GC-MS data that the most dominant monomer present in OPKM lignin is the syringyl units that correspond to peak #3 which was identified to be syringaldehyde and comprised 51.9% of the oxidation mixture. It was then followed by guiacyl that gives vanillin (peak #1), and made up the 28.3% of the oxidation products. These results are similar with that observed by Mohamad Ibrahim, et al. (2004) and Sun and Tomkinson (2001) in the oxidation products of lignin from oil palm empty fruit bunch fibers which also showed the predominance of syringyl units over the guaicyl units. However, in their study, a small amount of p-hydroxybenzaldehyde, the oxidation product of p-hydroxyphenyl was determined. It cannot be concluded however from these data that p-hydroxyphenyl unit is not one among the monomers of OPKM lignin. The methods of isolation and the sensitivity of the instrument are factors to be considered as well. Other oxidation products of phenolic types were also found out. These

include 3,4-dimethoxy-methylmonoacetal benzaldehyde (peak #2), which is probably a derivative of the oxidation product of veratryl alcohol and the methyl ester of 4-phenoxy benzoic acid (peak #4). Veratryl alcohol has been one of the several aromatic substrates and degradation products of lignin that had been assayed for their ability to increase ligninolytic activity (Cancel, et al., 1993). Some traces of fatty acids and pigment were also isolated during the oxidation process.

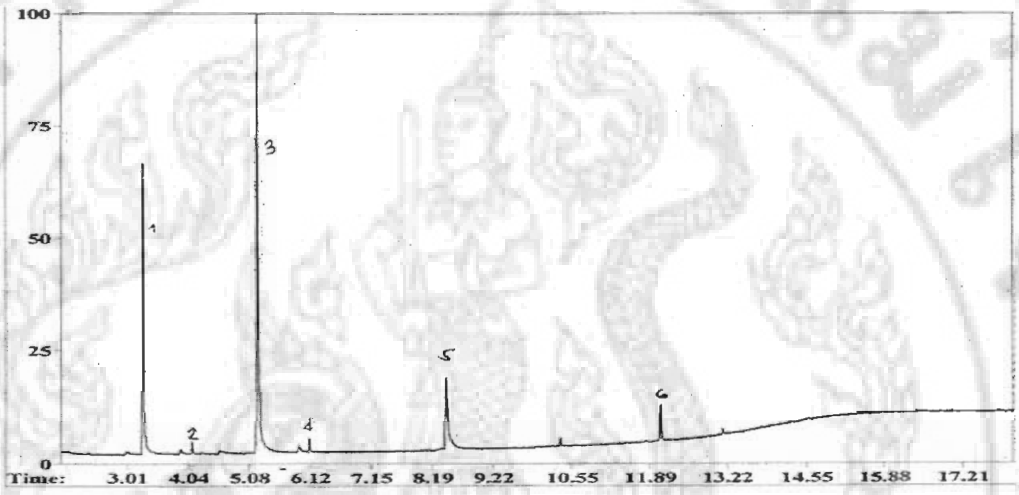


Figure 31. Gas chromatogram of OPKM lignin oxidation products.

Table 18. GC-MS data of the oxidation products of OPKM lignin.

Peak #	RT	% Area	Identification*	Quality
1	3.27	28.3	Vanillin or 4-hydroxy-3-methoxybenzaldehyde	97.1
2	4.10	1.50	3,4-dimethoxy-methylmonoacetal benzaldehyde	81.2
3	5.21	51.9	Syringaldehyde or 4-hydroxy-3,5-dimethoxybenzaldehyde	93.1
4	6.09	1.29	Benzoic acid, 4-phenoxy-, methyl ester	85.5
5	8.41	13.4	4-(Phenylazo)phenol	96
6	12.1	3.66	Hexanedioic acid, bis(2-ethylhexyl) ester	91.3

*Identification used the Natioanl Institute of Standards and Technology (NIST) Database

4.4.4 ^{13}C- NMR analysis

The ^{13}C NMR spectrum of OPKM lignin is shown in Figure 28 and the assignment of its chemical shifts is presented in Table 10. Assignment of peaks was based on the NMR Database (Ralph et al., 2004) of lignin and cell wall model compounds dissolved in $CdCl_3$.

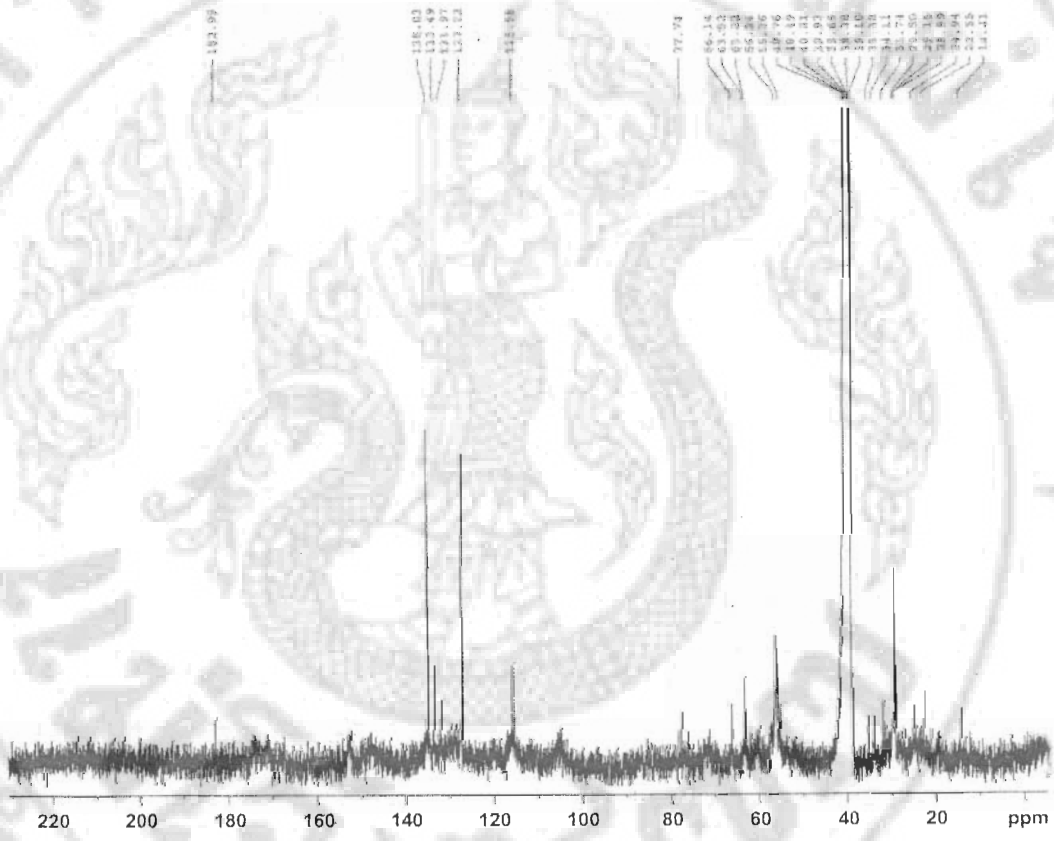


Figure 28. ^{13}C-NMR spectrum of the OPKM lignin isolated through ash-AQ pulping.

The ^{13}C NMR spectrum of OPKM lignin is similar with that of the studies of Mohamad Ibrahim, et al. (2004) and Sun and Tomkinson (2001) and can be divided into 4 distinct zones: 14ppm -40 ppm for alkyl C in the lateral chain, 50-120 ppm for O-alkyl carbon, 110-150ppm for aromatic C groups, and >150ppm for carbonyl carbon.

Table 10. Carbon-13 chemical shift values (δ) and signal assignment of OPKM lignin

δ ppm	Intensity	Assignment
14.41	w	$-CH_3$
22.55-35.38	w	γ -methyl and λ and β – methylene groups in the lateral chain
55.36	w	OCH_3 in lateral chain
56.34	s	OCH_3 in a ring
63.22 -63.52	s	γ C attached to βC in β-O-4
66.14	w	λC attached to βC in β-O-4
77.74	w	βC in β-O-4 linkage or esterfied λC
115.58	s	C_5 of G, C_3/C_5 of H or βC adjacent to carbonyl group
127.22	vs	λC or βC with C=C, C_1 with λ-C=C or λ -C=O, C_5 of G that forms a βC-C_5 linkage,
131.97	w	C_1 in G with λ-C-OH, C_1 in S with esterified C_4, C_5 in veratryl (etherified)
133.49	s	C_1 in S with λ -C=O, esterified C_4 in S, C_4 of S that forms β-O-4 link, C_1 or veratryl with λ-C-OH, or C_1 in G etherified at β-O-4 or linked with β-1
135.03	vs	C_1 of G or S etherified at βC and esterified at C4
182.99	w	C=O

The lowest signal at 14.41 ppm was assigned to the methyl groups of the n-propyl side chain while weak signals ranging from 22-36ppm were assigned to γ -methyl and λ and β – methylene groups in the lateral chain. The methoxy C in the lateral chain gave a signal at 55.36 ppm while the methoxy C attached to the aromatic ring gave a more downfield signal at 56.34 ppm at a higher intensity typical of lignin compounds. The signals of λ and γ C attached to βC in β-O-4 bond appeared at 63.22 and 66.14, respectively. The etherified βC in β-O-4 bond was assigned the signal at 77.74ppm. Signals for the monomeric residues of syringyl (S), guiacyl (G) and p-hydroxyphenyl (H) were observed at different chemical shifts with which some assignments overlapped each other. Signal observed at 115.58 was assigned to C_5 of G or βC adjacent to carbonyl group. A very strong signal at 127.22ppm was assigned for C_1 or C_2/C_6 in G

or S with λ-C=C or λ -C=O, C_5 of G that forms a βC-C_5 linkage, and λC or βC with C=C. Other signals were observed at 131.97 ppm for C_1 in G with λ-C-OH, C_1 in S with esterified C_4, C_5 in veratryl (etherified), 133.49ppm for C_1 in S with λ -C=O, esterified C_4 in S, C_4 of S that forms β-O-4 link, C_1 of veratryl with λ-C-OH, C_1 in G etherified at β-O-4 or linked with β-1 and 135.03ppm for C_1 in S with λ -C=O, esterified C_4 in S, C_4 of S that forms β-O-4 link, C_1 or veratryl with λ-C-OH, C_1 in G etherified at β-O-4 or linked with β-1. The weak signal observed at 183ppm was assigned to carbons in C=O. Although, the monomer p-hydroxyphenyl was not observed in the nitrobenzene oxidation products, specific signals observed at 115.58 and 128.7ppm were also assigned to C_3/C_5, and C_2/C_6 in H, respectively.

4.4.5 Molecular weight

Shown in Figure 29 is the LC chromatogram of the alkaline solution of the OPKM lignin. Table 11 provides the data obtained from the chromatogram. The separation revealed nine components with molecular weights ranging from 200-800 amu. The highest value being observed at 11.7 min retention time and which also comprised 2.2% of the total components. The compound that constitutes the largest percentage (73.5%) in the OPKM lignin was observed at 13.7 min retention time with a molecular weight of 753.5 amu having a base peak at m/z = 736.5. The molecular weight of OPKM lignin determined in this method is much lower compared to the molecular weights of lignin obtained using gel permeation chromatography. Mohamad Ibrahim, et al. (2004) obtained a weight-average of 2444-3279 for the lignin of oil palm empty fruit bunch fibers isolated by various mineral acids. Sun and Tomkinson (2001) also obtained a weight-average of 1980-2160 for the lignin isolated from the same material but at different precipitation pH of the polysaccharides degradation products. The molecular weight obtained from this method is not typical of a lignin compound, although other spectroscopic methods as well as the nitrobenzene oxidation experiment done earlier revealed lignin characteristics for the OPKM isolate. It is probable that the OPKM lignin may have undergone some depolymerization reaction when dissolved in NH_4OH. In the study of Adler, et al. (1968) as cited by Argyropoulos and Menachem (1997) a decrease in the molecular weight of soluble lignosulfonates was observed upon a second exposure to the reaction conditions. Moreover, one study revealed that lignin in aqueous ammonia undergoes transformations connected with oxidation, demethoxylation, and the

formation of amide and amino compounds (Koval'chuk and Forostyan, 1974). Although, no direct evidence, the said transformations could possibly result to bond breaking along the polymer chain which may have resulted to lower molecular weight.

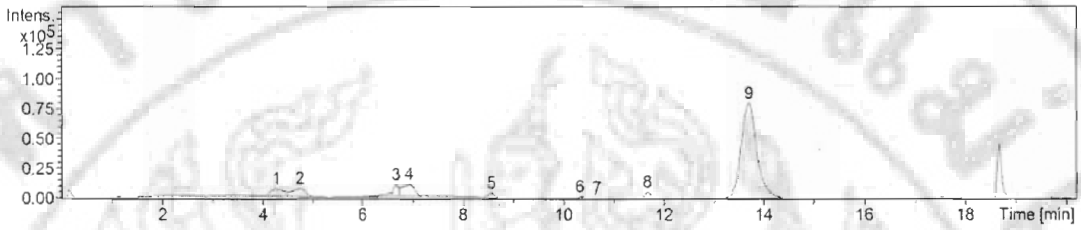


Figure 29. The LC chromatogram of OPKM lignin

Table 11. Molecular ion mass for each peak in the chromatogram of OPKM lignin

Peak number	Retention Time (min)	Percent Area	Base peak (m/z)	Molecular ion mass (m/z)
1	4.3	4.4	208.1	209.1
2	4.7	5.6	208.1	209.1
3	6.7	4.7	213.1	351.0
4	6.9	7.3	209.1	210.1
5	8.6	1.6	301.1	317.1
6	10.3	0.6	412.3	413.3
7	10.7	0.1	282.3	353.3
8	11.7	2.2	413.3	804.5
9	13.7	73.5	736.5	753.5

4.5. Characterization of the POME Isolate

4.5.1 Phenolic content

Shown in Figure 30 is the UV-absorption spectrum of the POME isolate. Similar with the OPKM lignin's UV spectrum, the maximum absorbance is at 250nm, which is assigned to the phenolate ion compound. Analysis of the total phenolic (TP) content of the OPKM lignin using the Follin-Ciocalteu method revealed a TP content of 27 mg gallic acid equivalent per gram POME isolate. This value is much lower than the TP content of milled wood lignin from the tree, *Astronium urundeuva* (Morais, et al., 1999). This could explain the low antioxidant activity of the POME isolate when assayed using the DPPH radical. The tannin content of the POME isolate was found to be 29 mg tannic acid equivalent per gram OPKM lignin.

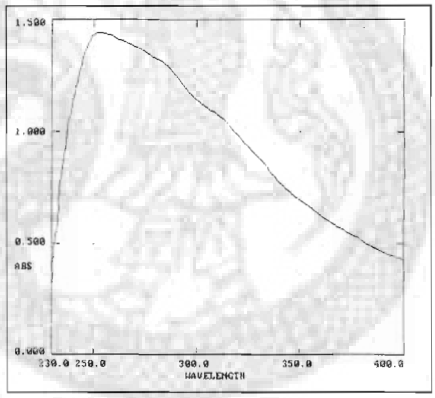


Figure 30. UV spectrum of the POME isolate

4.5.2 FTIR Analysis

The FTIR spectrum of POME isolate is given in Figure 31 and the summary is tabulated in Table 12. The broad band at 3349.5cm^{-1} was assigned to OH group stretching. Absorption bands were mostly attributed to C-H groups and the presence of phenolic group can be inferred from the OH absorption, the aromatic overtones at 1700-1900 cm^{-1}, and the possible C=C ring stretching at 1647.2 cm^{-1}.

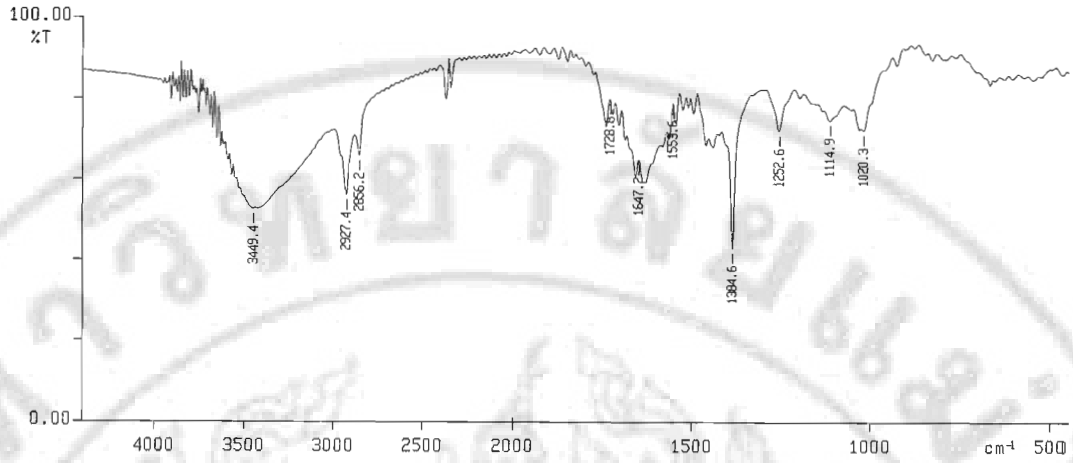


Figure 31. FTIR spectrum of POME isolate obtained through electrocoagulation.

Table 12. Assignment of wave numbers to its possible functional group based from the FTIR spectrum of POME isolate.

Wave numbers (cm^{-1})	Characteristics
3449.4	Strong, broad band due to OH stretching
2927.4	C-H stretching
2856.2	CH2 symmetric stretching
1728.6	Aromatic overtones
1647.2	C=C ring stretch absorptions
1458.9	CH2 bending
1384.6	CH3 umbrella bend
1252.6	C-O stretching
1020.3	C-H out of plane bends

4.5.3 Acetylation

Acetylation of POME isolate was done to increase its solubility to dichloromethane by introducing a less polar functional group for easier analysis on GC-MS. The gas chromatogram of the acetylation products of the POME isolate is shown in Figure 32. Twenty three prominent peaks were observed and the identification and characteristics of each peak is given in Table 13. The acetylation products revealed the presence of sugar derivatives and fatty acids. The only phenolic compound determined was 2-n-hexylphenol (peak#19).

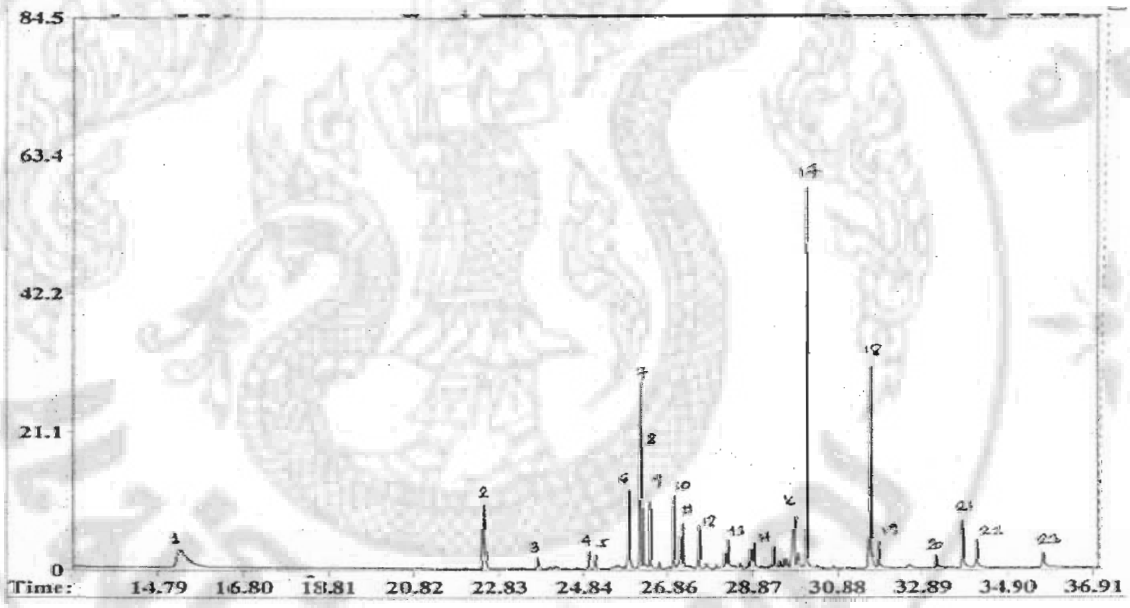


Figure 32. Gas chromatogram of acetylated POME isolate.

Table 13. GC-MS data of the acetylated POME isolate

Peak #	RT	%Area	Identification*	Quality
1	15.3	8.12	1,2,3-Propanetriol, triacetate	93.9
2	22.4	7.96	Butanedioic acid, dibutyl ester	94.2
3	23.7	0.97	1,2,3,4-Butanetetrol, tetraacetate, (R*,S*)-	87.8
4	24.6	1.24	Methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside	90.3
5	25.1	0.91	d-Xylitol, 2,3,4,5-tetraacetyl-1-O-methyl-	86.8
6	25.9	4.89	α-D-Ribopyranose, tetraacetate	94.4
7	26.3	11.4	α-D-Ribopyranose, tetraacetate	92.2
8	26.2	2.79	1,2,3,5-tetra-O-Acetyl-α-D-ribofuranose	91.3
9	26.4	3.43	1,2,3,5-tetra-O-Acetyl-α-D-ribofuranose	90.8
10	26.9	3.60	Methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside	89.8
11	27.1	1.64	Methyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside	85.4
12	27.6	2.30	1-Iodo-2-methylundecane	75.0
13	28.2	1.60	Hexadecanoic acid, ethyl ester	87.6
14	28.8	1.23	D-Glucose, 2,3,4,5,6-pentaacetate	91.3
15	29.3	1.46	Myo-inositol, hexaacetate	91.6
16	29.8	5.89	Myo-inositol, hexaacetate	95.6
17	30.1	18.9	Hexadecanoic acid, butyl ester	92.8
18	31.6	12.6	(Z)-9-Octadecenoic acid butyl ester	90.1
19	31.8	1.19	2-n-Hexylphenol	99.9
20	33.2	0.60	1,2-Benzenedicarboxylic acid, diisooctyl ester	93.6
21	33.8	3.47	(Z)-9-Octadecenoic acid butyl ester	74
22	34.1	2.06	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	91.2
23	35.7	1.65	9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester	85

*Identification used the NIST Database

4.5.4 ^{13}C-NMR analysis

The ^{13}C-NMR spectrum of the POME isolate is shown in Figure 33. The ^{13}C-NMR spectrum of the POME isolate can be divided into four distinct zones: 14-60 ppm for alkyl C, 61-100 ppm for carbon atoms with a neighboring electronegative element such as O, 101-170 ppm for unsaturated and aromatic C atoms, and >170ppm for carbonyl carbon. Compared with the ^{13}C-NMR spectrum of OPKM lignin and other spectra of lignin from the studies of Mohamad Ibrahim, et al. (2004) and Sun and Tomkinson (2001) the ^{13}C-NMR of the POME isolate is not typical of a lignin compound. It lacks the strong signals from the methoxy groups and βC in β-O-4 bonds of lignin. Moreover, the stronger intensity of the peaks assigned to the alkyl carbon (14-50 ppm) relative to the peaks for aromatic/unsaturated C atoms (101-170 ppm) indicates the presence of long chain structures consisting of saturated C-atoms and not of lignin structure which contain mostly aromatic rings. This confirmed the results of the acetylation products of the POME isolate which showed mostly long chain fatty acids and some sugar derivatives.

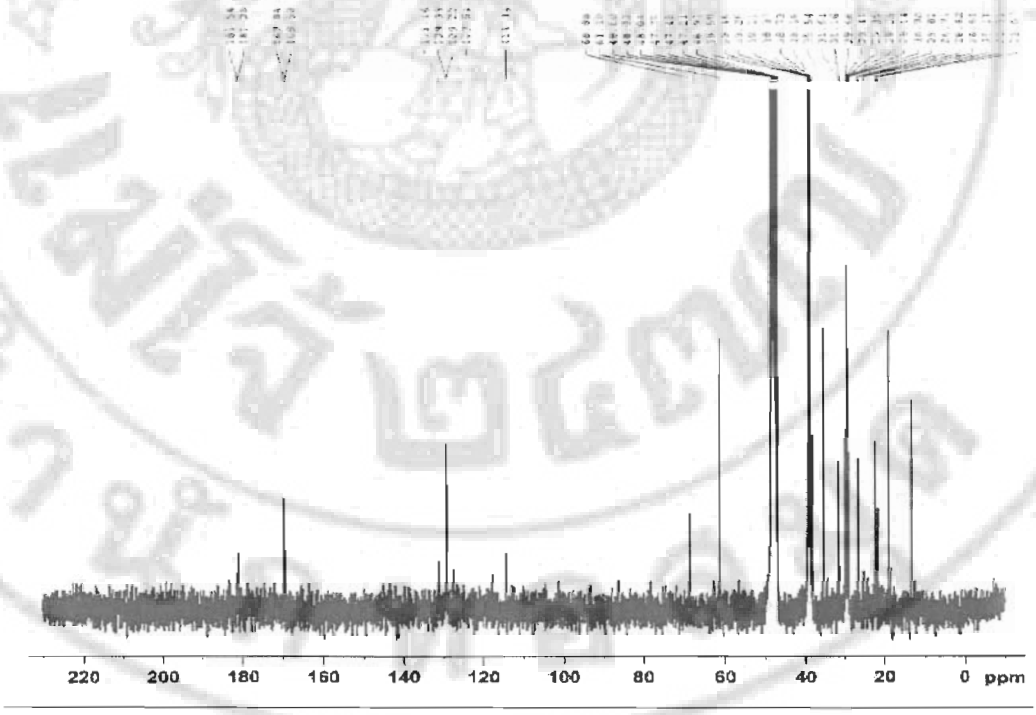


Figure 33. ^{13}C-NMR spectrum of the POME isolate obtained by EC

4.5.5 Molecular weight

The LC chromatogram of the alkaline solution of the POME isolate is shown in Figure 34 and the data obtained from the chromatogram are presented in Table 14. The separation revealed 15 components with molecular ion mass ranging from 300-800 amu. The compound that gave the highest percentage was observed at 8.8 min retention time and has a molecular ion mass of 579.3 with a base peak of m/z=301.1. The highest molecular ion mass (804.5) comprised 7.2% of the total components.

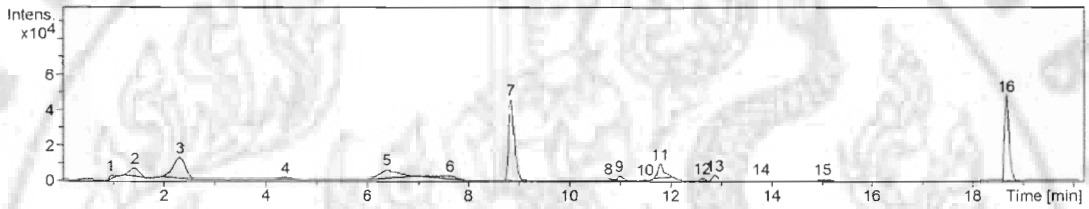


Figure 34. The LC Ccromatogram of the POME isolate

Table 14. Molecular ion mass for each peak in the chromatogram of POME isolate

Peak number	Retention Time	Percent Area	Base peak	Molecular ion mass
1	0.9	1.4	453.3	679.5
2	1.4	6.4	453.3	724.6
3	2.3	20.9	679.5	724.6
4	4.4	1.8	453.3	454.3
5	6.4	12.9	213.1	403.3
6	7.6	4.6	213.1	309.2
7	8.8	36.3	301.1	579.3
8	10.8	0.7	282.3	413.3
9	11.0	1.9	413.3	584.5
10	11.5	0.3	427.3	554.5
11	11.8	7.2	413.3	804.5
12	12.6	1.0	293.2	736.5
13	12.9	2.6	319.3	736.5
14	13.8	0.7	359.3	522.6
15	15.0	1.5	335.3	336.3

4.6. Properties of NR Vulcanizates from STR 5L

4.6.1 Cure characteristics

The cure characteristics of the different rubber blends are given in Table 15. The highest Mooney Viscosity was observed in treatment DA1 and lowest in DP2. Generally, the scorch time and cure time of the rubber blends treated with the studied antioxidants are higher than that of the commercial antioxidant 6PPD and almost similar with that of Wingstay L. Most likely, this is due to the phenolic nature of the studied antioxidants. Wingstay L is a phenolic antioxidant while 6PPD belongs to amine-based antioxidants. Shortening of the scorch time associated with amine-based antioxidant has been attributed to the ability of the amine group to enhance the formation of more active sulfurating agent (Poh and Teh, 1999). It can be seen also that increasing the concentration of the studied antioxidants from 1 to 3 phr, did not significantly affect the cure and scorch times of the compound rubbers. This result is similar with the study of Poh and Teh (1999) which showed that increasing the phenol-based antioxidant concentration in natural rubber (SMR-L) has no significant effect on its scorch time.

Table 15. The cure characteristics of the different natural rubber blends from STR 5L.

Treatment ID	Mooney Viscosity(ML 1+4)	Scorch time,t_{s2} (min)	Cure time, t_{90} (min)
DO1	75.77	6.38	11.00
DO2	79.46	6.21	12.00
DO3	81.09	6.30	11.36
DP1	77.91	5.63	10.58
DP2	75.31	5.58	11.38
DP3	76.74	6.21	11.53
DW1	84.58	6.13	11.04
DA1	97.35	5.29	9.83

4.6.2 Physico-mechanical properties before thermal ageing

The physico-mechanical properties of the vulcanized dry natural rubber are summarized in Table 16. Not much variation on the specific gravity was observed. Although, compression set and rebound resilience are properties without a direct link to the efficiency of an antioxidant but more of the filler effects, these were measured to determine if the incorporation of the antioxidant to the rubber blends affected these parameters. It was observed that compression sets of all treatments containing the OPKM and POME isolates were lower than that of the samples with commercial antioxidants. For the treatments containing OPKM lignin (DO1, DO2, and DO3), increasing the concentration of the antioxidant, lowered compression set. As noted earlier, low values of compression set mean better resistance to compressive forces (Ciullo and Hewitt, 1999). It could be inferred that that addition of the studied antioxidants could enhance the filler-filler interaction or the filler-elastomer adhesion, thereby decreasing the compression set. As to rebound resilience, lower values were observed mostly in the sample containing the studied antioxidant at a concentration of 1 phr. At higher concentrations, rebound resilience values are comparable with that of the commercial antioxidants.

Table 16. Physico-mechanical properties of the NR vulcanizates treated with different antioxidants before thermal ageing.

	Treatments							
Properties	**DO1**	**DO2**	**DO3**	**DP1**	**DP2**	**DP3**	**DW1**	**DA1**
Specific Gravity	1.12	1.11	1.13	1.14	1.13	1.13	1.12	1.11
Compression Set (%)	34.1	29.7	28.7	33.3	29.4	33.5	38.5	35.9
Rebound Resilience (%)	60.5	85.0	84.4	64.8	80.2	78.9	81.1	83.0
Hardness (Shore A)	41.4	44.2	44.6	44.2	43.8	42.8	47.4	47.3
300% Modulus (MPa)	2.8	2.5	2.5	2.7	2.5	2.4	2.7	3.5
Tensile Strength (MPa)	17.2	18.6	19.1	18.2	16.6	16.0	20.3	19.0
Elongation (%)	574.0	641.3	647.0	637.0	636.2	643.2	652.4	554.7

Hardness values of dry rubber treated with the studied antioxidants are lower than that of the commercial antioxidants. Different trends were observed when the concentration of the

antioxidant was increased. For treatments containing OPKM, increasing concentration, slightly increased hardness. For POME-treated dry rubbers, increased in concentration caused a decrease in hardness.

The tensile properties that were measured include the Modulus 300, tensile strength and percentage elongation at break (%EB). As can be seen from Table 9, not much variation in Modulus 300 was observed among different treatments. Values of Modulus 300 of treatments with the studied antioxidant were also similar with the rubber treated with Wingstay L (DW1). The highest Modulus 300 was observed in DA1. For the tensile strength, it was observed that lower tensile strength was exhibited by samples containing the studied antioxidants. It can be seen also that increasing the concentration of OPKM isolate from 1 to 3 phr increased the tensile strength. However, an opposite trend was observed with the POME isolate-treated rubbers. As to the %EB, not much variation was also observed. Surprisingly, the lowest value was observed in treatment DA1.

4.6.3 Physico-mechanical properties after thermal ageing

Percent change on the tensile properties and hardness of the vulcanized dry rubber after thermal ageing is summarized in Table 17. Profiles for hardness, Modulus 300, tensile strength, and elongation at break (EB) before and after thermal ageing are shown in Figure 35. From these profiles it can be deduced that the larger the gap between the two plots (before and after ageing), the higher the change in properties. Hardness and Modulus 300 in general increased after ageing with some exceptions observed in treatment DP2 for hardness and in treatment DA1 for Modulus 300. On the other hand, the tensile strength and %EB decreased after ageing. But, it is interesting to note that the smallest change (-7.3%) in tensile strength after ageing was observed in the rubber treated with 1 phr OPKM isolate. Moreover, the percent change of the vulcanizate with 1phr POME was also lower than with that of Wingstay L. As the concentration of the studied antioxidant is increased, the higher the deviation from the original tensile strength and %EB was also observed. This effect is contrary to the hypothesis that increasing the concentration of the antioxidant may stabilize the vulcanizate towards thermo-oxidation and to the study of Gregorova, et al.(2005) which showed that lignin concentration of 4-8 phr was found to be the optimum concentration for used as antioxidant for carbon black-filled natural rubber. It could be

in this case, that the antioxidant effect was superseded by the negative effects of the antioxidant particles towards cross-linking.

Table 17. Percent changes in hardness and tensile properties of the NR vulcanizates from STR 5L after thermal ageing.

Properties	Treatments							
	DO1	**DO2**	**DO3**	**DP1**	**DP2**	**DP3**	**DW1**	**DA1**
Hardness, points change	1.7	1.7	2.4	2.0	-1.2	0.3	0.1	0.5
300% Modulus (% change)	24.5	61.0	34.4	13.3	11.9	19.4	9.6	-11.2
Tensile strength (% change)	-7.3	-36.4	-30.6	-8.6	-39.3	-43.5	-13.1	-7.7
Elongation (%change)	-9.4	-28.0	-20.0	-6.4	-19.1	-21.6	-8.8	-0.7

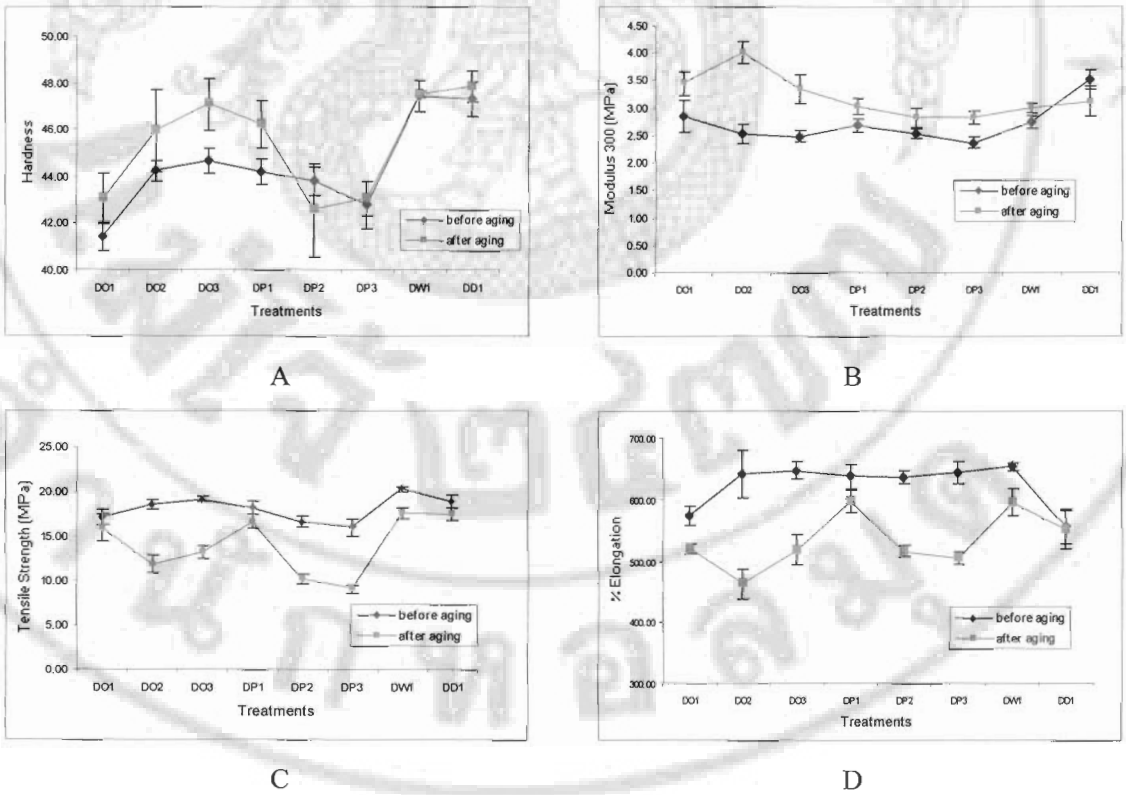


Figure 35. Comparisons of hardness (A), Modulus 300 (B), tensile strength (C) and percentage elongation at break (D) of the NR vulcanizates from STR 5L before and after thermal ageing at 100°C for 22 hours.

4.7. Properties of the Vulcanized NR Latex Films

The total solid content (TSC) of the latex after mixing with the compounding ingredients has an average of 31.10±0.16% typical of the other compound latex preparations. The compound latex after vulcanization produced a film with an average thickness of 0.54±0.08mm and density of 0.95 ± 0.02.

4.7.1 Tensile properties before thermal ageing

The tensile properties of the vulcanized latex film before thermal ageing are shown in Table 18. In general, the tensile properties of the film treated with the studied antioxidants before ageing are comparable with and even higher than that with commercial antioxidant, Wingstay L (Treatment LW1). It would be noteworthy that all treatments containing the POME isolate (LP1, LP2, and LP3) have higher tensile strength and Modulus 300 than treatment LW1. In fact, among the 7 treatments, the highest tensile strength and Modulus 300 was observed in treatment LP3. Moreover, it was found out that increasing the concentration of POME isolate enhanced tensile properties of the films before ageing. But, this was not observed with those treated with OPKM lignin. For treatments containing OPKM lignin, LO2 gave the highest tensile strength and %EB, even better than LW1.

Table 18. Tensile properties before thermal ageing of the vulcanized NR latex films treated with different antioxidants.

Properties	LO1	LO2	LO3	LP1	LP2	LP3	LW1
Tensile Strength	17.26	20.60	14.96	21.02	24.61	26.16	17.63
300% Modulus	1.04	0.91	0.94	1.14	1.39	1.45	1.08
% Elongation	767.12	886.31	832.78	787.30	790.83	769.82	777.61

4.7.2. Tensile properties after thermal ageing

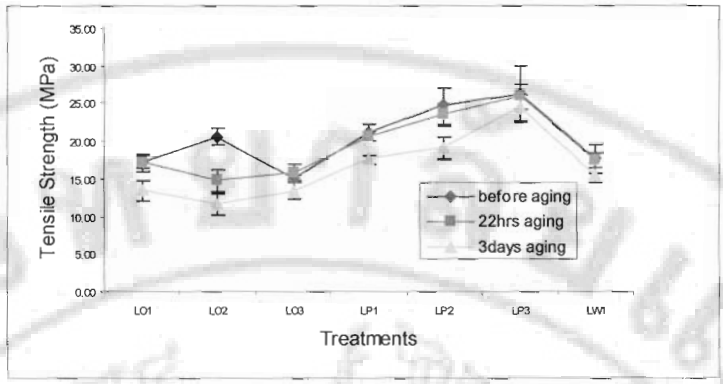
The tensile properties of the NR latex films after thermal ageing are presented in Table 12 and their profiles are shown in Figure 25. Accelerated ageing was initially done for 22 hours at 100°C. Unexpectedly, the treatment containing 3phr OPKM lignin showed an increase in its tensile strength and %EB as can be seen from the positive values of its percent change. This could

be explained from the theory that during ageing, scission of the main chain, crosslink formation or crosslink breakage can take place. Sometimes, existing crosslinks maybe replaced by new more stable crosslinks (Mathew, et al., 2001; Varghese, et al., 1994).

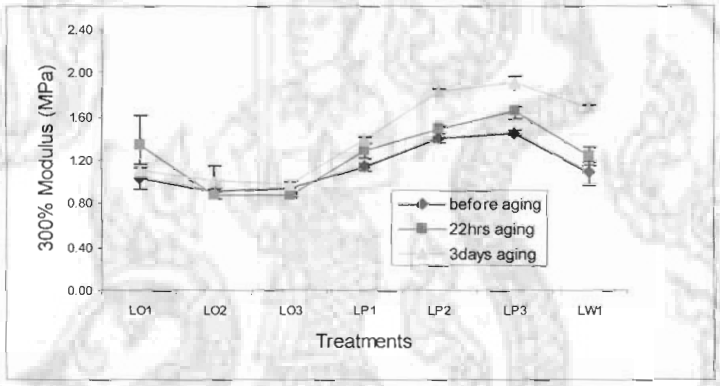
To further see the effect of thermal ageing, it was decided to increase the duration to 3 days at the same temperature. Results showed that Modulus 300 generally increased with ageing while tensile strength drastically decreased after 3 days ageing. It was also observed that among the 3 different concentrations (1-3phr) of the studied antioxidants used in the preparation of NR latex films, 2 phr gave the lowest resistance to thermal ageing as can be seen from the highest percent changes in its tensile properties. However, treatments containing 3 phr of the studied antioxidants (LO3 and LP3) have lower percent changes in tensile strength than that treated with Wingstay L. The percent changes for LO3 and LP3 were -10.53% and -7.06%, respectively; while that of LW1 was -12.68%. This is in contrast to earlier results on the vulcanizate from ribbed smoke sheets. With the treated NR latex film, increasing the concentration of antioxidant seemed to enhance its resistance to thermo-oxidation. This could be that the ability of the studied antioxidants to diffuse through the rubber matrix is not hindered since these were prepared as dispersion; thereby its antioxidant activity was not offset.

Table 19. The percent changes in tensile properties of the vulcanized NR latex films after thermal ageing.

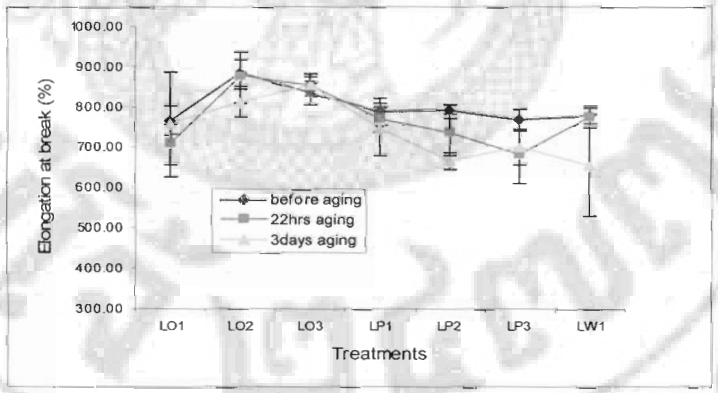
Properties	**LO1**	**LO2**	**LO3**	**LP1**	**LP2**	**LP3**	**LW1**
			After 22 hours , 100°C				
Tensile Strength (% change)	-1.19	-28.33	5.40	-2.13	-4.53	-1.45	-1.67
300% Modulus (% change)	27.92	-4.04	-7.01	12.04	6.14	13.10	14.25
Elongation (% change)	-7.60	-1.19	2.74	-2.18	-7.33	-11.69	-0.37
			After 3 days ageing, 100°C				
Tensile Strength (% change)	-22.36	-43.99	-10.53	-16.57	-22.33	-7.06	-12.68
300% Modulus (% change)	5.89	10.29	2.97	21.62	31.32	31.03	55.84
Elongation (% change)	-1.19	-8.35	2.36	-5.35	-15.77	-8.87	-15.84



A



B



C

Figure 36. Comparisons of tensile strength (A), Modulus 300 (B) and percent elongation at break (C) of vulcanized NR latex films before and after thermal ageing.

4.8. Swelling Properties

The action of a liquid on rubber may result in the absorption of the liquid, extraction of soluble constituents, and chemical reaction. The volume change is a good general measure of the resistance of a rubber to a given liquid (Gwaily, et al., 2003). The degree of swelling (Q) can be determined from the equation,

$$Q = \frac{m_s - m_o}{m_o}$$

where m_s is the mass of the swollen rubber and m_o is the original mass of the rubber before the swelling test. A high degree of Q indicates that the rubber is not suitable for use in that environment (Mathew, et al., 2002). From the swelling test, the volume fraction of the rubber in the swollen network; Φ, can also be determined using the equation,

$$\phi = \frac{W_r / \rho_r}{(W_r / \rho_r) + (W_s / \rho_s)}$$

where W_r is the original weight of the rubber, ρ_r the density of the rubber, ρ_s the density of the solvent, and W_s the weight of the absorbed solvent. A high value of ϕ is an indication of high extent of crosslinking in the system (Mathew, et al., 2002).

Another structural parameter characterizing a crosslinked polymer that can be obtained from the swelling test is M_c, the average molecular weight between crosslinks which is directly related to the crosslink density. M_c can be calculated using the Flory-Rehner equation. According to the theory of Flory, for a perfect network (Mathew, et al., 2006; Flory, 1950),

$$M_c = -V\rho_r \frac{\left(\phi^{1/3} - \frac{\phi}{2} \right)}{\left[\ln(1-\phi) + \phi + \chi\phi^2 \right]}$$

Where:

Mc – the average molecular weight of the polymer between crosslinks

V – the molar volume of the solvent

ρr – the specific gravity of the rubber

ϕ – the volume fraction of the rubber

χ – Florry-Huggins solvent-rubber interaction parameter which has a value of 0.43 for toluene and natural rubber (Magaraphan and Yatae, 2007)

Knowing the *Mc* enabled the calculation of crosslink density, v_e, and for a perfect network (Flory, 1950),

$$v_e = \frac{\rho_r N_A}{M_c}$$

Where:

v_e – the crosslink density, the number of elastically effective chains totally included in a perfect network per unit volume

N_A – Avogadros number

M_c – average molecular weight between crosslinks.

Results of the swelling test of the vulcanized dry NR from STR 5L are summarized in Table 20. Q values of the vulcanized dry rubber containing 1 phr antioxidants are almost similar. The swelling behavior of treatment DO1 is almost similar with that of the commercial antioxidants while that of DP1 is slightly higher. It was also observed that as the concentration of the studied antioxidants was increased, Q values increased. On the other hand, the crosslink density of the rubber was found to decrease as the concentration of the antioxidant was increased. This result conforms to the study of Gal, et al. (1985) on the effects of the nature of the antioxidants on the irradiation crosslinking of another polymer, polyethylene. On their study, it was found out that with increasing antioxidant content, the probability of scission relative to crosslinking is increased. This result in turn validated the earlier assumption that the lower resistance to thermal ageing of vulcanized NR from STR 5L of higher antioxidant concentration could be attributed to the lower crosslinking.

Table 20. Parameters obtained from the swelling test of NR vulcanizates from STR 5L

Treatments	Q	Φ	Mc	v_e
DO1	2.71	0.22	7490.41	$9.01x10^{19}$
DO2	2.89	0.21	8265.96	$8.09x10^{19}$
DO3	2.80	0.22	8208.20	$8.30x10^{19}$
DP1	2.78	0.21	8316.29	$8.26x10^{19}$
DP2	2.97	0.21	9202.68	$7.40x10^{19}$
DP3	2.94	0.21	9006.78	$7.57x10^{19}$
DW1	2.71	0.22	7518.37	$8.97x10^{19}$
DA1	2.73	0.22	7418.84	$9.01x10^{19}$

Table 21 shows the results from the swelling test of the NR latex films. Lower Q values were observed in NR latex films with POME isolate as the antioxidant, which are even lower than that with Wingstay L. It was observed also that as the concentration of the POME isolate was increased, the resistance of the film to swell also increased as deduced from the decreasing Q values. On the other hand, the opposite was observed with NR latex films containing the OPKM lignin. High Q values were determined and the values increased with increasing concentration of the OPKM lignin. For the crosslink density, the highest value was found in treatment LP3, the same treatment which gave the highest tensile properties and lowest percent changes brought by thermal ageing. Moreover, the crosslink density of POME treated NR latex films were all higher than that with Wingstay L. It was also observed that as the concentration of POME isolate is increased, the crosslink density also increases. It can be deduced that crosslinking was favored for latex films even at higher antioxidant concentration.

For the OPKM treated NR latex films, the crosslink density was found lowest in LO3, the same treatment that gave the lowest tensile strength. It was also observed that the crosslink density of NR latex film with OPKM lignin decreased when the concentration of OPKM lignin is increased from 1 to 3 phr. This finding on the other hand conflicted with the results obtained with POME isolate treated films. Increasing the concentration of OPKM isolate in NR latex film

seemed to favor chain scission while increasing the concentration of POME isolate seemed to favor crosslinking as depicted from the crosslink density values obtained in this study.

Table 21. Parameters obtained from the swelling test of vulcanized NR latex films

Treatments	Q	Φ	M_c	v_e
LO1	4.95	0.16	1.54×10^{4}	3.85×10^{19}
LO2	5.12	0.15	1.60×10^{4}	3.59×10^{19}
LO3	5.89	0.13	2.14×10^{4}	2.74×10^{19}
LP1	4.37	0.17	1.16×10^{4}	4.89×10^{19}
LP2	3.96	0.19	1.01×10^{4}	5.91×10^{19}
LP3	3.90	0.19	9.35×10^{3}	6.10×10^{19}
LW1	4.45	0.17	1.04×10^{4}	4.78×10^{19}

4.9. Thermogravimetric Analysis

The thermograms of all the treatments for vulcanized NR from STR 5L and NR latex films are given in Figures 26 and 27, respectively. It can be seen that the obtained thermograms are typical of the degradation patterns observed in natural rubber as observed in other studies (Radhakrishnan, et al., 2007; Menon, et al., 1996), i.e. the thermograms showed a single stage degradation that occurred at a temperature range of 250-480°C. Fragmentation of polyisoprene chains must occur during the degradation yielding volatile fragments such as isoprene and dipentene (McNeil and Guptha, 1985).

The thermal decomposition characteristics for the vulcanized NR from STR 5L and NR latex films obtained from the thermograms are summarized in Table 15 and 16, respectively. Onset temperatures were obtained at a point at which 1% of the sample weight was lost. The % residue was obtained at 500 °C, which is included at the temperature range at which the weight after the peak of degradation is almost constant. The temperature at the peak of degradation was obtained from the derivative weight curves and designated as T_{max}.

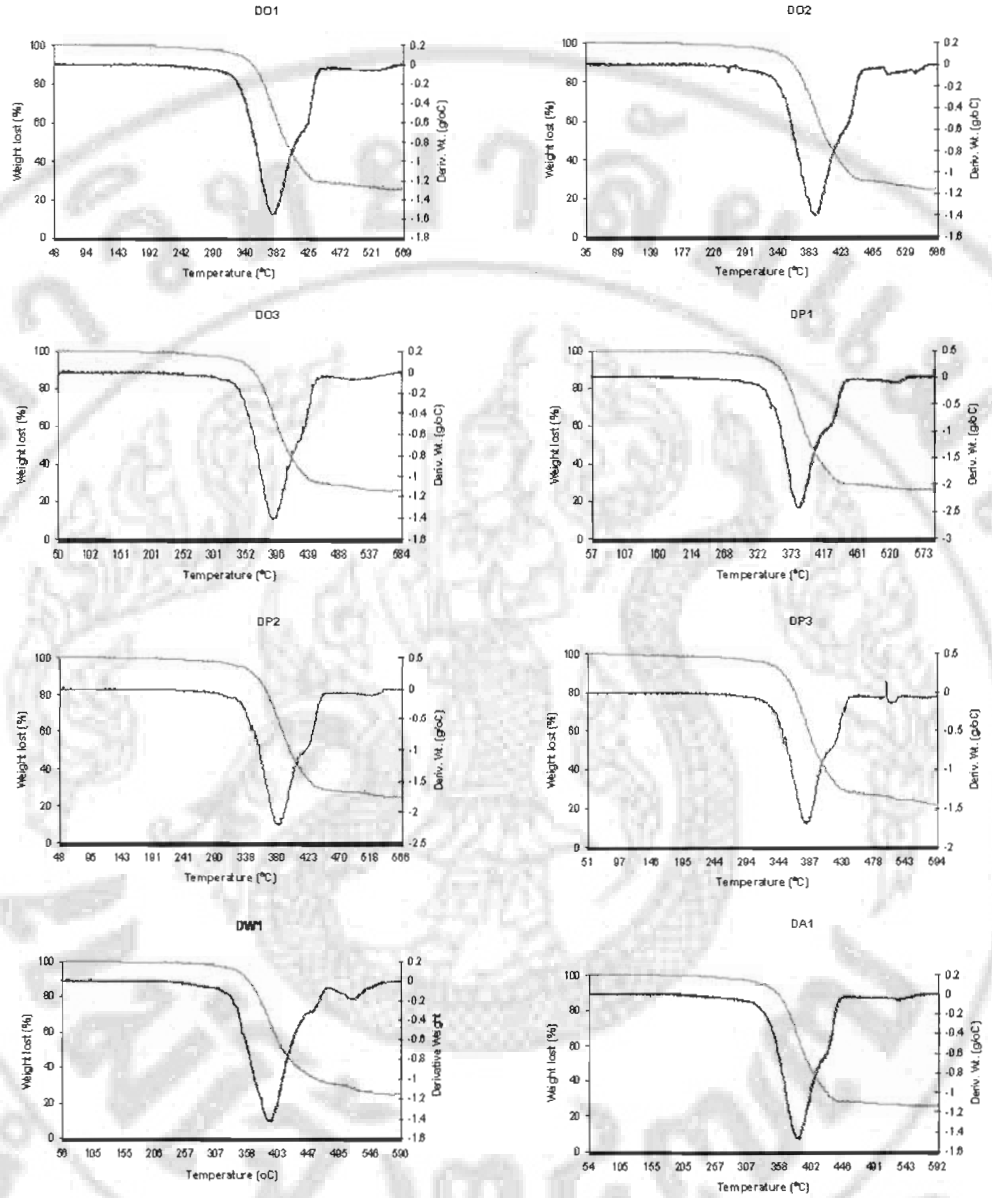


Figure 37. Thermograms of NR vulcanizates from STR 5L treated with different antioxidants

For the vulcanized NR from STR 5L, onset temperatures ranged from 162-256°C, the highest being observed in treatment DP1. Moreover, it was observed that as the concentration of the studied antioxidants was increased, the onset of degradation decreased; i.e., degradation begins at a lower temperature. This could be attributed to the decrease in crosslinking at higher antioxidant concentration as observed from the swelling test. Percent residue ranged from 26-30% which corresponds to 70-74% weight lost. T_{max} ranged from 379-391 °C. For treatments

containing the OPKM lignin, peak temperatures were higher at higher antioxidant concentration and were similar with that containing Wingstay L. On the other hand, for treatments containing the POME isolate, peak temperatures seemed not to vary with the increase in antioxidant concentration and values were a bit lower from the commercial antioxidant. However, the percentages of weight lost at T_{max} for the POME isolate treated rubber were also lower, with the lowest value observed in DP1.

Table 22. Thermogravimetric data of NR v ulcanizates from STR 5L

Treatment	Onset (T1; °C)	T50	Temperature Range (°C)	Peak Temp (Tmax; °C)	Weight Lost at Tmax	Residue at T500 (%)
DO1	223	394	245 - 452	379	35.3	27.3
DO2	223	405	267 - 480	391	35.6	27.9
DO3	216	407	271 - 464	390	33.4	28.2
DP1	256	399	277 - 451	382	30.9	27.4
DP2	223	396	272 - 462	382	34.7	27.3
DP3	162	394	275 - 456	381	35.5	26.3
DW1	253	405	288-472	391	37.0	29.7
DA1	241	398	274 - 456	386	37.6	26.6

For the vulcanized NR latex films, onset temperatures ranged from 194-252 °C, with the highest value determined in LP2. Treatment LP3 which gave the highest crosslink density, however, gave an onset temperature, 230°C, that is lower than that with Wingstay L. Unlike other analyses, no clear trend on the onset temperatures was observed when the concentration of the antioxidant was increased. Percent residue ranged from 2.0-5.4% corresponding to a weight lost of 94.6 -98%. This range is almost similar to the results of Matthew, et al. (2001) on the thermal degradation of liquid natural rubber and epoxidized natural rubber. It was also observed that the % residue increased with increasing concentration of antioxidant. The temperature at the peak of degradation has a range of 381-395°C. Treatments LO3 and LP2 both have T_{max} of 394°C which is near the T_{max} of LW1, 395°C.

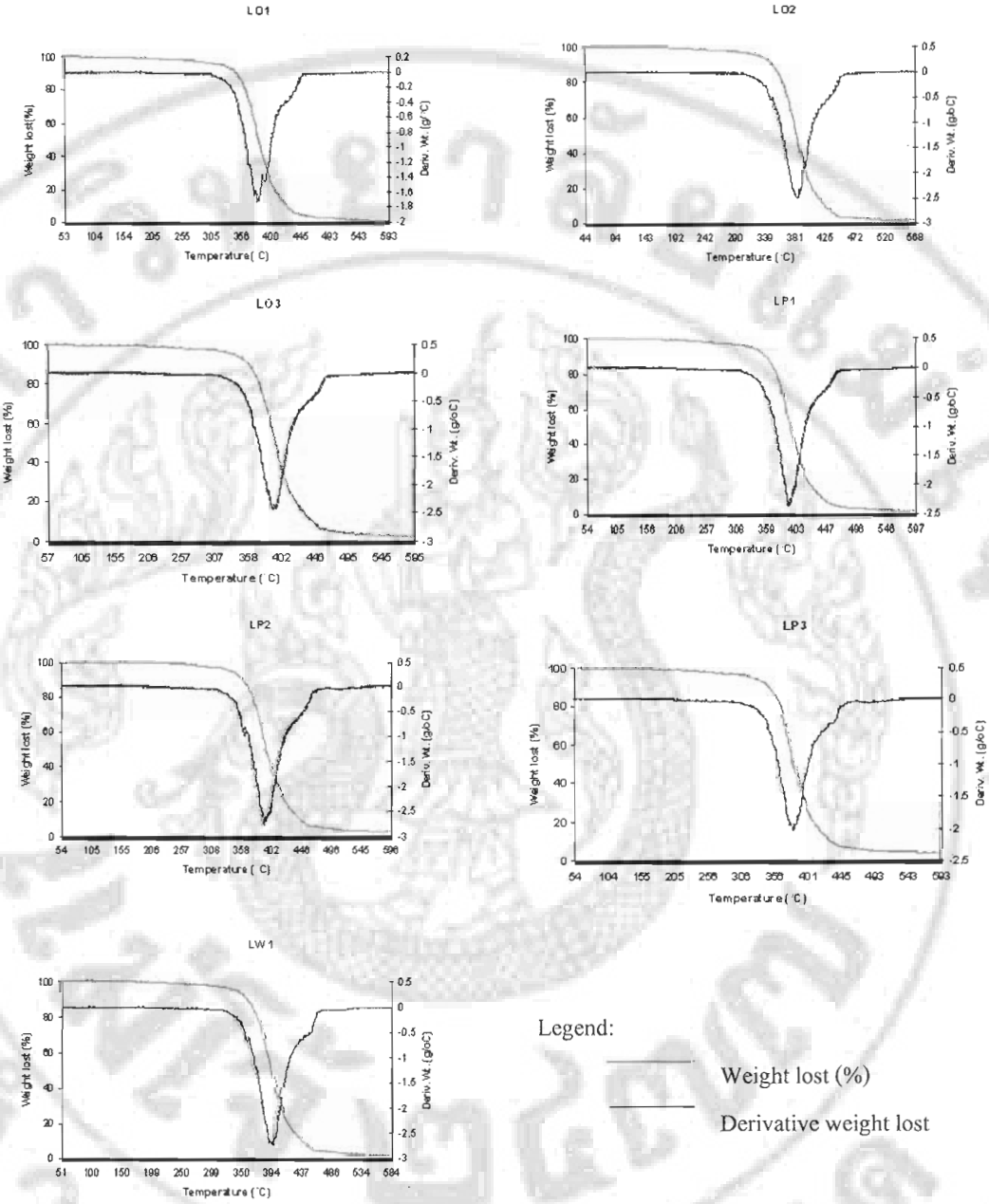


Figure 38. Thermograms of vulcanized NR latex films treated with different antioxidants

Table 23. Thermogravimetric data of vulcanized NR latex films

Treatment	Onset (T1; °C)	T50	Temperature Range (0C)	Peak Temp (Tmax; °C)	Weight Lost at Tmax	Residue at T500 (%)
LO1	194	382	294 - 455	381	49.3	2.0
LO2	232	384	308 - 455	386	52.7	2.5
LO3	222	394	312 - 484	394	50.4	3.9
LP1	238	396	306 – 487	390	40.5	3.8
LP2	252	397	307 – 478	394	43.7	4.4
LP3	230	384	295 - 455	384	49.7	5.4
LW1	240	396	317 - 469	395	50.5	3.2

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In the search for alternative method for the treatment of palm oil mill wastes, this study has presented a new approach of waste management by demonstrating the potential of palm oil mill wastes which include the oil palm kernel meal (OPKM) and the palm oil mill effluent (POME) as raw materials for the production of natural rubber antioxidant. Based on the findings of this study, the following conclusions are drawn:

1. Electrocoagulation and ash-AQ pulping are two methods that can be applied for the treatment of palm oil mill effluent and oil palm kernel meal, respectively, with a subsequent production of antioxidant for natural rubber. EC reduces the turbidity, acidity, COD and BOD of POME. Isolating lignin from OPKM through ash-AQ pulping gives added value to OPKM which has been previously limited as feed supplement or fertilizer.

2. Both isolates exhibited antioxidant activity. However, based on the DPPH test the antioxidant activity of the OPKM lignin is comparable to that of vitamin E and higher than that of the POME isolate.

3. Characterization studies show that the OPKM lignin has a high total phenolic content which explained its high antioxidant activity; contains hydroxyl, methoxyl, carbonyl, aromatic, and alkyl groups; composes primarily of syringyl monomer followed by guiacyl; and consists of molecules in a basic solution with molecular ion mass ranging from 200-800 amu.

4. Characterization studies reveal that the POME isolate has lower total phenolic content compared to OPKM lignin; contains hydroxyl, carbonyl, aromatic and alkyl groups containing saturated and unsaturated C-atoms groups; has a larger content of long chain of saturated C-atoms relative to unsaturated and aromatic C-atoms; contains essentially some fatty acids and sugar derivatives; and consists of molecules with molecular ion mass ranging from 330-800 amu.

5. The isolated antioxidants can be incorporated in the preparation of vulcanized NR. The vulcanized NR can be prepared in the form of NR sheets from smoked-ribbed natural rubber

sheets or as NR latex film from 67% NR latex, with the OPKM lignin or POME isolate as antioxidants. The studied antioxidants disperse homogeneously in the compound rubber when prepared using the NR latex.

6. The vulcanized NR from STR 5L containing the studied antioxidants when compared with those treated with commercial antioxidants before accelerated thermal ageing generally have better resistance to compressive forces; have lower rebound resilience, hardness and tensile strength but increased in the concentration of antioxidant was found to increase rebound resilience and tensile strength; and have no variation in Modulus 300 and % elongation at break (%EB)

7. The vulcanized NR latex films treated with varying concentrations of the studied antioxidants before thermal ageing have higher tensile strength, Modulus 300, and %EB than those treated with commercial antioxidant, Wingstay L. Increasing the concentration of the POME isolate enhances tensile properties.

8. After thermal ageing, hardness and Modulus 300 generally increase while tensile strength and %EB decrease. Better resistance to thermal ageing is achieved if the concentration of the studied antioxidant is 1phr for the vulcanized NR from STR 5L while 3phr for the vulcanized latex film.

9. For vulcanized NR from STR 5L, increasing the concentration of both the studied antioxidants favors chain scission which in turn lowers both the crosslink density and resistance to accelerated thermal ageing.

10. For vulcanized NR latex films, the effect of increasing the concentration of POME isolate is different from that of the OPKM lignin. Increasing the concentration of the POME isolate favors crosslinking which results in increasing both the crosslink density and resistance to thermal ageing. The effect of increasing the concentration of OPKM lignin on the other hand, was opposite to that of the POME isolate.

11. The thermal degradation of the vulcanized NR from STR 5L and latex films as monitored by thermogravimetric analysis shows similar degradation pattern to that using commercial antioxidants but have slightly lower onset temperatures.

5.2. Recommendations for Future Research Work

Some issues and questions stemmed out as this research was being conducted. The conclusions drawn not only answered some questions regarding the utilization of palm oil mill wastes in the production of natural rubber antioxidant but also generated new questions which could be directed for future research work.

First, in the application of EC as a new treatment method for POME, optimization study is recommended. It is encouraged to determine the optimum condition that will yield the maximum efficiency of EC in treating POME and at the same time to make it more economical in terms of electricity consumption. Some issues regarding the effects of dilution, initial pH, duration of EC, current density, electrode distance and other factors have to be monitored in the optimization study.

In isolating lignin from OPKM, other methods of isolation could be applied. Lignin being a complex molecule is known to have different properties. Pearl (1967) as cited by Argyropoulos and Menachem (1997) once wrote that it is impractically impossible to isolate two lignin preparations with identical properties, even by the same procedure. Thus, a different isolation method of lignin from OPKM would definitely provide new lignin of different properties which could have a different effect as antioxidant for natural rubber.

In this study, the application of the isolates has been limited as antioxidant for natural rubber. Thus, it is recommended to test the feasibility of using the isolates as antioxidant for synthetic elastomer. Moreover, other compounding ingredients may also be varied especially the filler in the compounding of the dry rubber.

With regard to the structure of the isolates, other methods of characterization will be useful towards the identification of the studied antioxidants. Although in general there is a lack of agreement on the ultimate structure of lignin (Argyropoulos and Menachem, 1997), the characterization of OPKM lignin presented in the study still needs further research in terms of the determination of its exact molecular weight and the quantification of its individual monomers by using standard solutions. For the POME isolate, separation of the individual components with concurrent testing of the antioxidant activity using DPPH test would be useful to identify the component that could give the highest antioxidant activity.

Finally, if the palm oil mill waste treatment method presented in this study is applied in large scale, several factors regarding its economics and other practical issues need to be studied and considered as well.

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APPENDICES

APPENDIX A

Information on Chumporn Palm Oil Industry Public Company Limited

The Chumporn Palm Oil Industry Public Company Limited[1]

The Company's principal activity is the production and distribution of refined palm oil to the related industry and consumer markets. The Company's products include Crude Palm Oil, RBD Palm Oil, RBD Palm Olein in bottle, tin and soft pack, and other by-products such as RBD Palm Stearin, Palm Fatty Acid Distillate and Kernel Meal with other Palm Product as Palm Seedling. The Company operates mainly in Thailand. The Company's plantation covers 20,916 rais of land from Pathiu estate, Huaysak estate, Kao Chai Rat estate, Bangson estate, and Klong Wang Chang estate. Its consumer brands are Leela, Rio, Waree, Parichart, and Lobster.

Figure A1. Oil palm fruit bunches at Churmporn Pal Oil Industry.

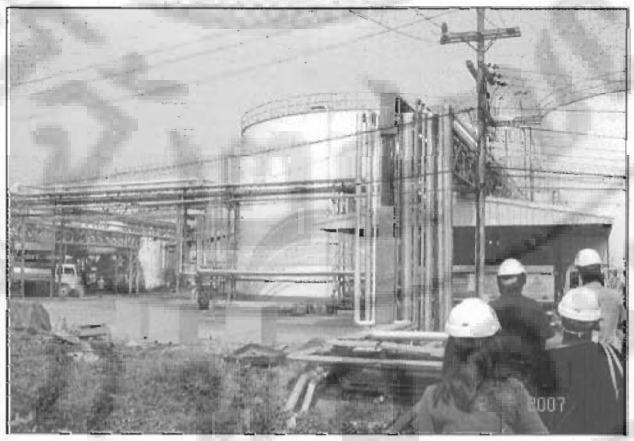


Figure A2. The researcher and other Maejo students during the visit at Churmporn Palm Oil Industry.

[1]Churmporn Palm Oil Industry Public Company Ltd. [Online] Available: http://wrightreports.ecnext.com/ coms2/reportdesc_COMPANY_C76475330

APPENDIX B

List of Natural Rubber Technology Terminology

Natural rubber technology terminology

The following is a list of rubber terminology used in this study and their definitions.

1. *Compression set* – the residual deformation of a material after removal of an applied compressive stress

2. *Cure time* - The amount of time required for a rubber compound to reach maximum viscosity or modulus at a given temperatureElongation at break – the elongation at which rupture occurs in the application of continued tensile stress

4. *Hardness* – the relative resistance of the surface to indentation under specified conditions

5. *Modulus 300* – the stress required to stretch the uniform cross section of a test speciment to 300% elongation.

6. *Mooney viscosity*- a measure of the viscosity of a rubber compound determined in a Mooney shearing disk viscometer, viscosity is indicated by the torque required to rotate a disk embedded in a rubber specimen and enclosed in the die cavity under specified conditions

7. *Oscillating disc rheometer* – measures the complete curing characteristics of an elastomer compound from a green (uncured) stock to a fully cured vulcanizate, at a specified temperature.

8. *Rebound resilience* – the ratio between the returned and applied energies of a moving mass which impacts a test piece which is usually expressed as a percentage.

9. *Scorch time* - the time during which a rubber compound can be worked at a given temperature before curing begins

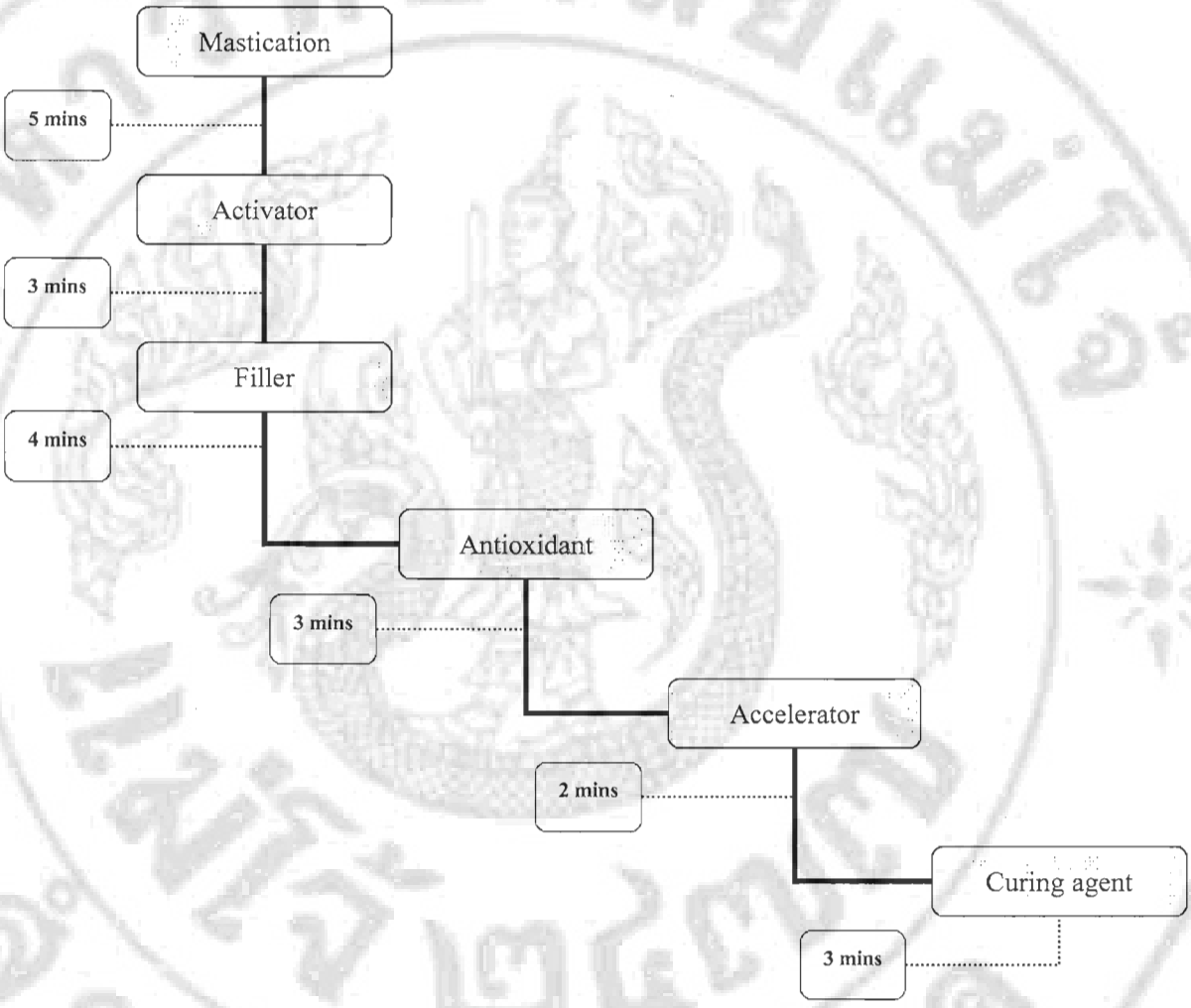
10. *Tensile strength* – the maximum tensile stress applied in stretching a specimen to rupture

11. *Tensile stress* – a stress applied to stretch a test piece

APPENDIX C

Flow Diagram of the Compounding Procedure for the Dry Rubber

The flow diagram employed in the compounding of materials for the dry rubber



APPENDIX D

List of Additional Plates

Figure D1. Weighing of the compounding ingredients.

Figure D2. Compounding of natural rubber using the two-roll mill.

Figure D3. Cutting and forming the compounded rubber based on the type of analysis to be done.

Figure D4. Vulcanizing or curing the compound rubber using a hydraulic press.

Figure D5. Compounding of natural rubber latex.

Figure D6. Hardness determination using a duorometer.

Figure D7. Tensile property determination using a tensometer.

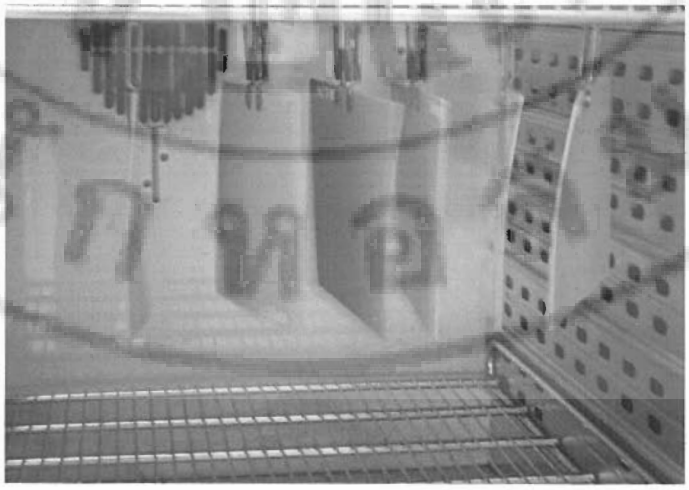


Figure D8. Set up of accelerated thermal ageing.

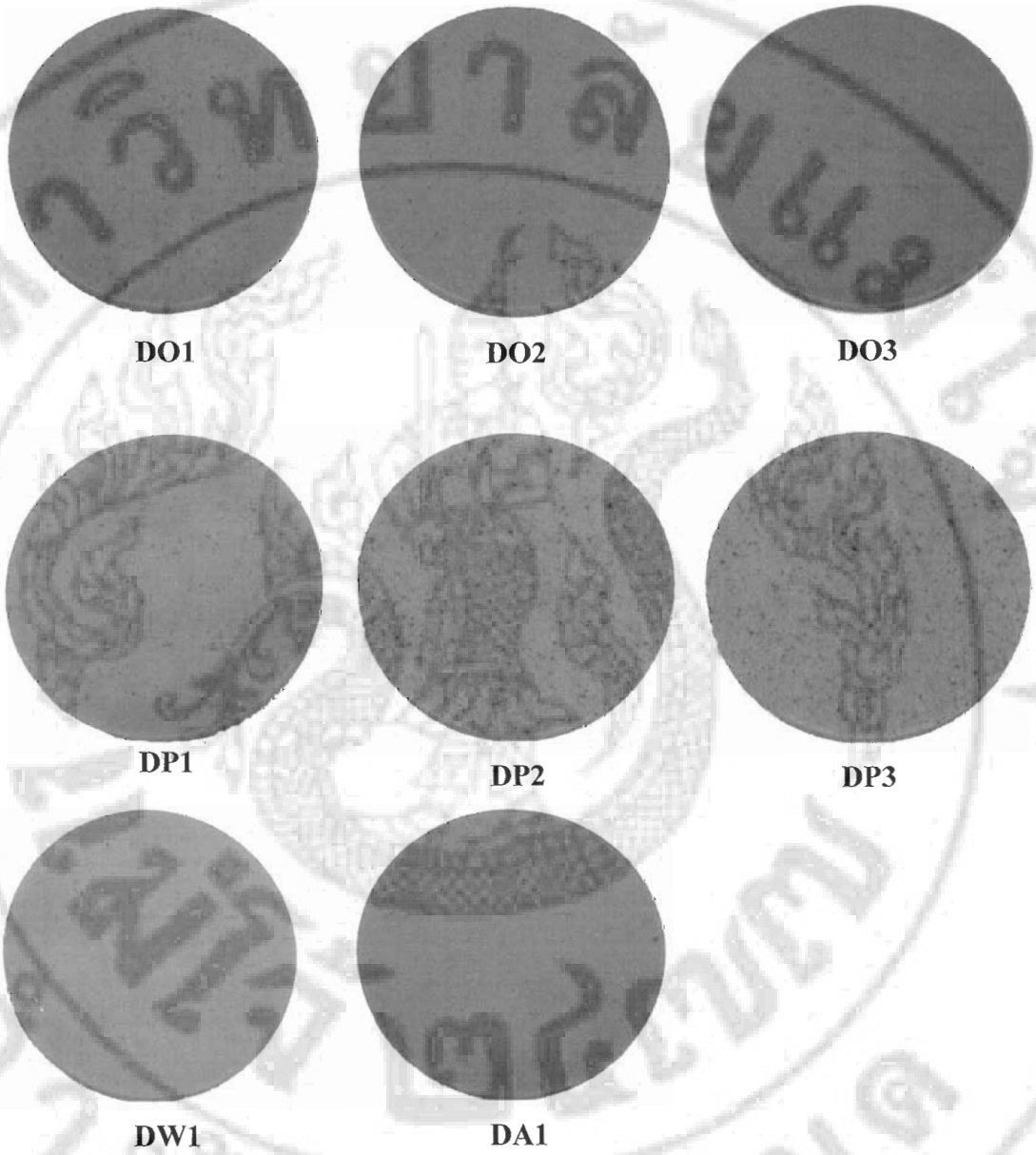


Figure D9. Representative sample of the vulcanized rubber from STR 5L for each treatment.

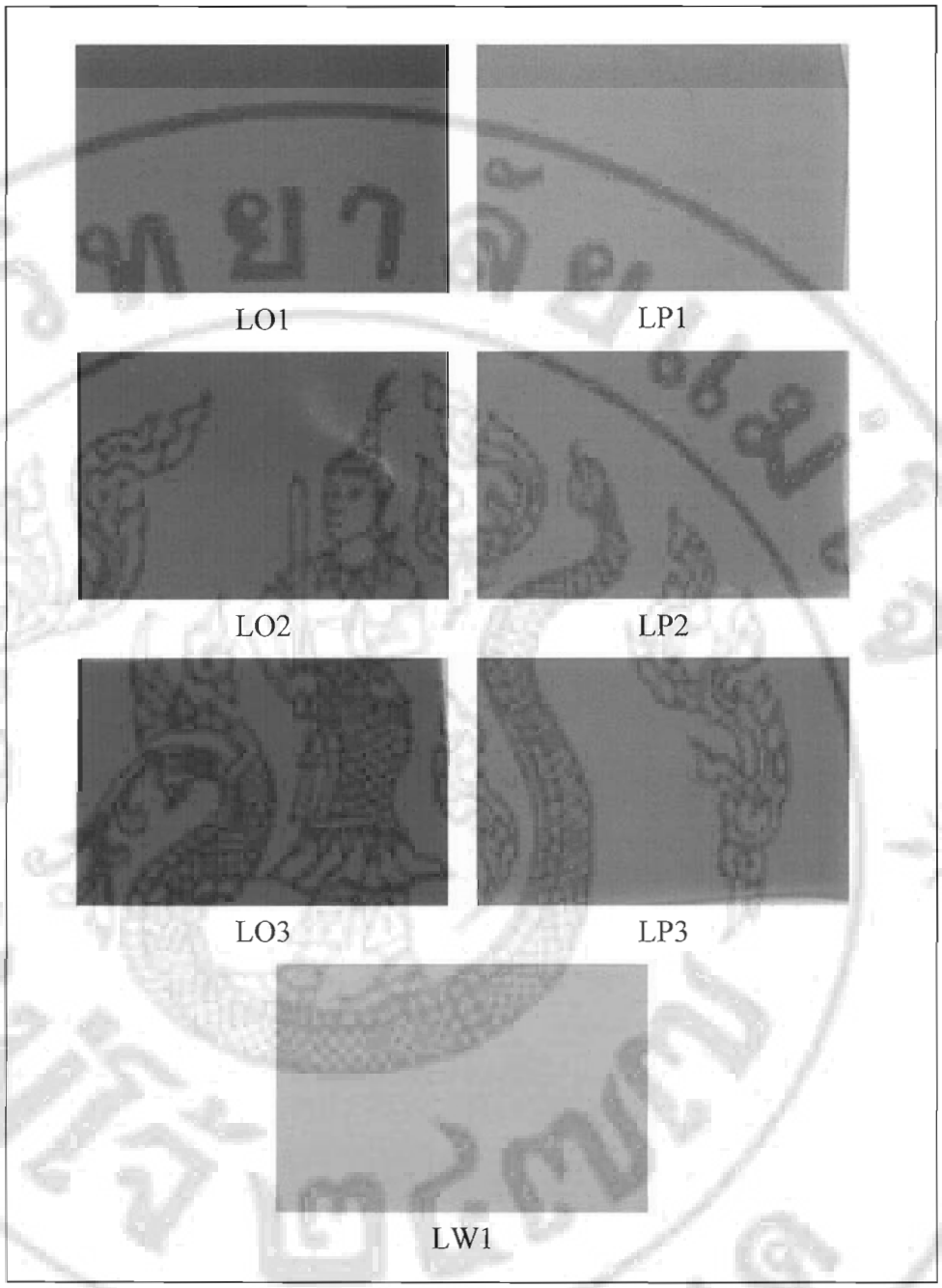


Figure D10. Representative sample of the vulcanized NR latex films for each treatment.

APPENDIX E

List of Additional Experiments Conducted

Metal Analysis of POME

The concentrations of the following metals: Cu, Cr, Mn, Fe and Pb in the POME before and after EC were analysed in the filtered samples using Flame-Atomic Absorption Spectrophotometry (Perkin Elmer Analyst 100). Standard solutions of each metal were prepared from its corresponding salts. The detection limit was estimated as the standard deviation of the concentrations of blank samples multiplied by three.

Table E1. Metal content of POME before and after EC

Metal	Before EC (mgl^{-1})	After EC (mgl^{-1})	Detection Limit (mgl^{-1})
Cu	nd	nd	0.027
Cr	0.059	nd	0.036
Fe	40.57	0.008	-
Mn	10.44	nd	0.042
Pb	nd	nd	0.366

Note: nd = not detected

Thermal Degradation Study Monitored by Fourier Transform Infrared Spectroscopy

To monitor the change in the functional groups of the rubber during thermal degradation, FTIR study was conducted. Since FTIR analysis requires thin films to obtain good spectra, the samples used in this study were the latex film prepared by mixing 20% NR latex and antioxidant only, to discard also the interfering factors associated with the addition of other rubber additives on the functional groups present in rubber. Three treatments of 1 phr antioxidant concentration were prepared. Films were air dried and small rectangular dried samples were then mounted on improvised sample card holders for FTIR study. FTIR spectra were monitored before and after thermal ageing in an air oven at 70°C for 21 days at a 7-day interval. Each treatment was analyzed in duplicates.

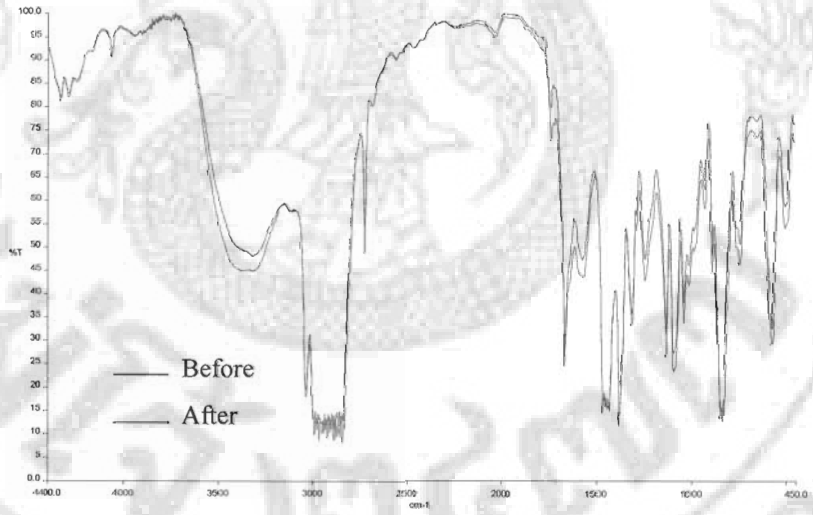


Figure E1. FTIR spectra of latex film containing 1phr OPKM lignin before and after ageing at 70°C for 21 days.

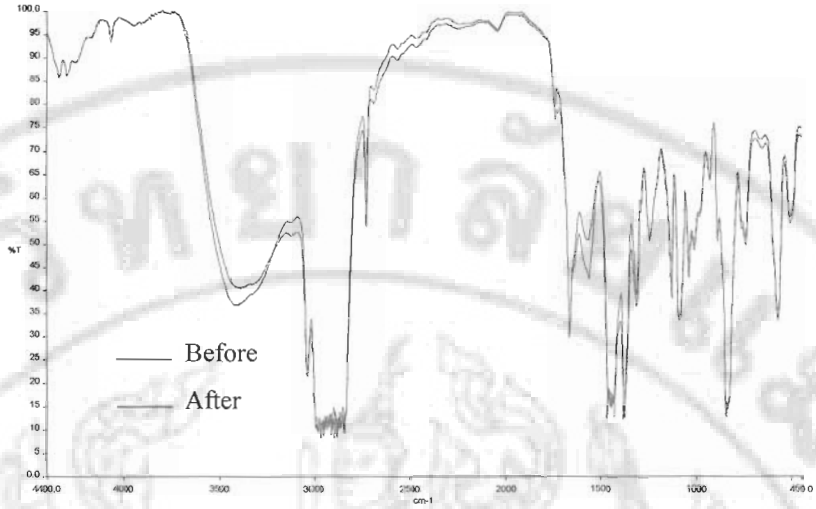


Figure E2. FTIR spectra of latex film containing 1phr POME isolate before and after ageing at 70°C for 21 days.

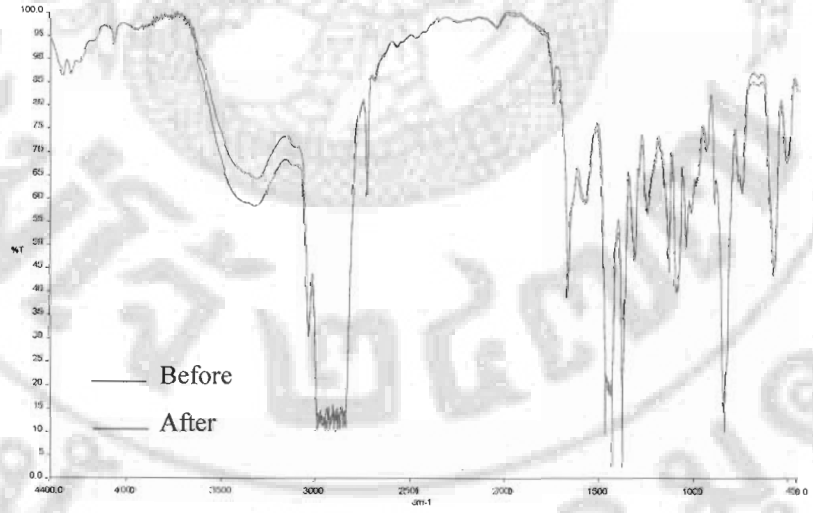


Figure E3. FTIR spectra of latex film containing 1phr Wingstay L before and after ageing at 70°C for 21 days.

Differential scanning calorimetry analysis of the rubber samples

Differential scanning calorimetry was used to determine the thermal stability of the rubber produced. A Perkin-Elmer (Diamond Series) calorimeter with a continuous flow of nitrogen gas or air at 100ml/min, at a heating rate of 20°C/min was employed to study the thermo-oxidation stability. The temperature ranged from 55 to 550 °C. Samples of 2-4 mg were placed in crimped standard aluminium pans, where the lid of each pan was perforated with 4 pinholes. The starting temperature of oxidation was determined as the onset temperature of the oxidation peak.

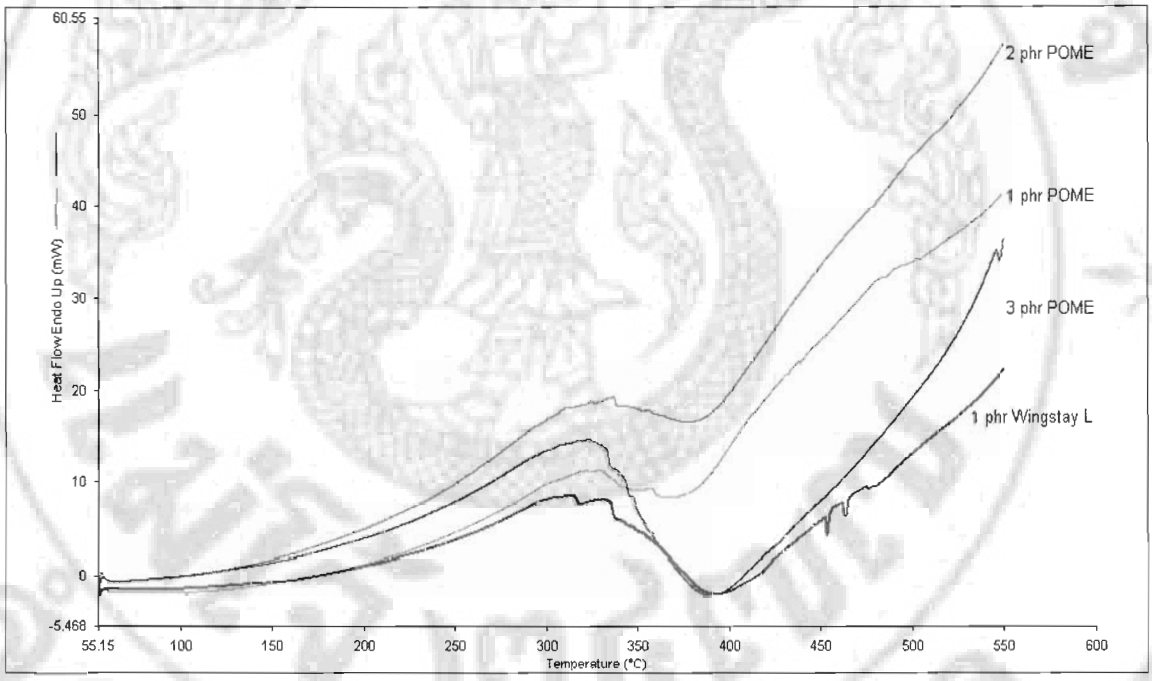
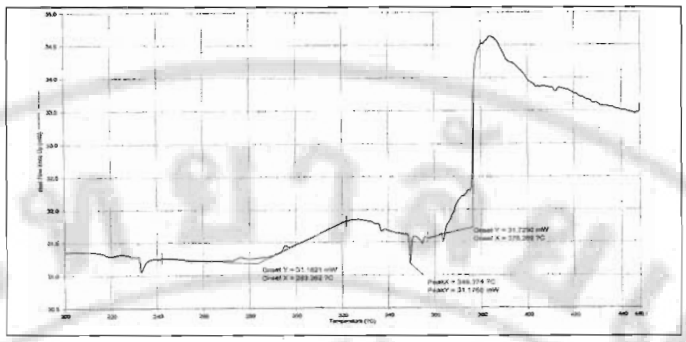
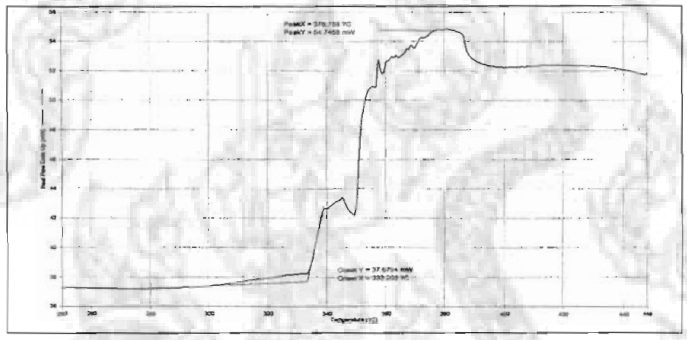


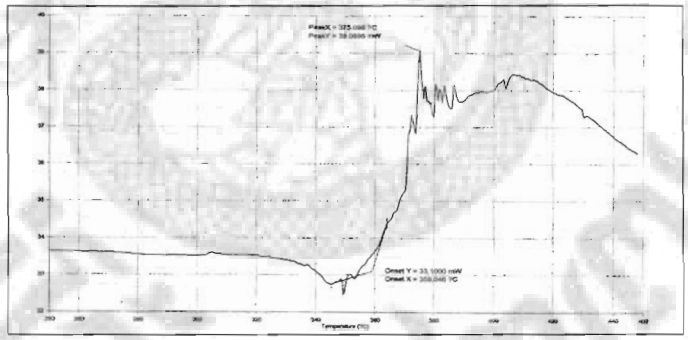
Figure E4. DSC thermograms of vulcanized NR latex films obtained under air condition.



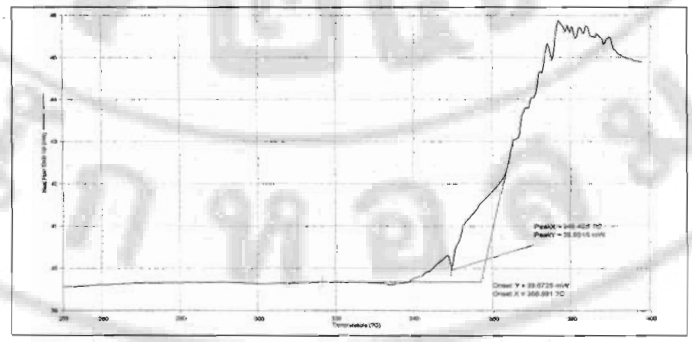
DO1



DP1



DW1



DA1

Figure E5. DSC thermograms obtained under nitrogen gas of vulcanized rubber from STR 5L at 1 phr concentration of antioxidant.

Derivatization Followed by Reductive Cleavage (DFRC) of the OPKM Lignin

DFRC is another degradation technique for the analysis of lignin monomer which was proposed by Lu and Ralph[2] (1997). Derivatization, accompanied by cell wall solubilization, is accomplished with acetyl bromide (acetyl chloride was used in this experiment) in acetic acid. Reductive cleavage of resulting β-bromo ethers (chloro ethers) utilizes zinc in acidic medium. The reduced and cleaved products underwent acetylation and the monomers are analyzed by GC-MS using the same condition stated in the nitrobenzene oxidation.

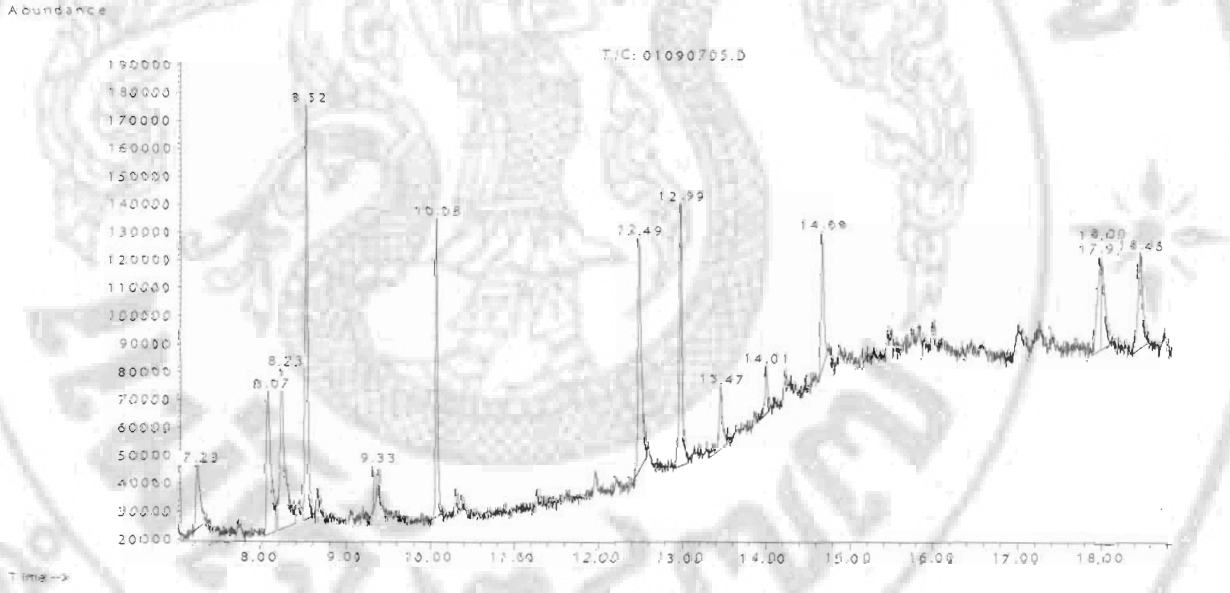


Figure E6. The GC-MS spectrum of the degradation monomers after DFRC.

[2] Lu, F. and Ralph, J. 1997. "Derivatization followed by reductive cleavage (DFRC method), a new method for lignin analysis: Protocol for analysis of DFRC monomers". **J. Agric. Food Chem.**, 45: 2590-2592.

Table E2. Peak identification of the degradation products of DFRC.

Peak #	RT	%Area	Identification*	Quality
1	7.23	4.06	cis-2,5-Dimethoxy-4-ethoxy-á-methylstyrene	78.7
2	8.07	8.88	3-Phenyl-benzofuran	88.0
3	8.23	12.06	9,10-Anthracenedione	92.5
4	8.52	15.53	Phenol, 4-[3-(acetyloxy)-1-propenyl]-2-methoxy-, acetate	96.7
5	9.33	1.20	Cinnoline, 3-phenyl-	90.1
6	10.08	9.16	Pendimethalin	67.4
7	12.49	12.44	.9,10-Diacetoxyanthracene	74.8
8	12.99	10.07	2-Phenyl-1,2-dihydro-3H-indazol-3-one	72.9
9	13.47	3.36	1,4,6-Trimethyl-9H-indeno[2,1-c]pyridine	65.1
10	14.02	1.69	Octamethylcyclotetrasiloxane	61.8
11	14.68	5.37	5-Phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one	69.5
12	17.99	4.55	Diethyl bis(trimethylsilyl) orthosilicate	70.3
13	18.00	5.19	Diethyl bis(trimethylsilyl) orthosilicate	68.2
14	18.46	6.44	9,12-Octadecadienoic acid (Z,Z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester	61.8

GCMS Analysis of the Methanol Solution of POME Isolate

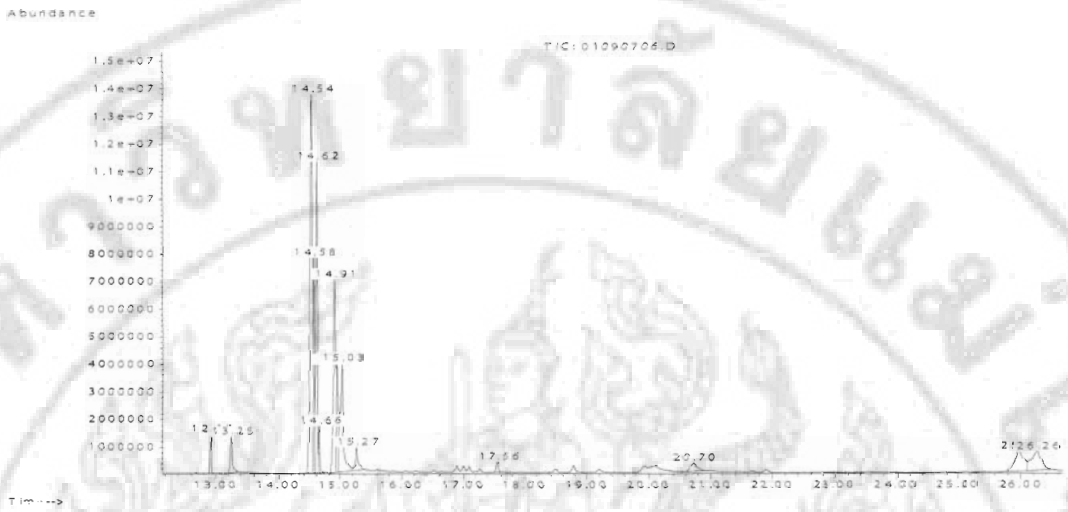


Figure E7. Gas chromatogram of the POME isolate in methanol.

Table E3. Peak identification of the gas chromatogram of the methanolic solution of POME.

Peak #	RT	% Area	Identification*	Quality
1	12.92	1.60	Hexadecanoic acid methyl ester	92.6
2	13.25	2.28	Hexadecanoic acid	89.9
3	14.54	27.03	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	95.4
4	14.58	7.74	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	89.2
5	14.63	14.72	9-Octadecenoic acid, methyl ester, (E)-	93.3
6	14.66	1.75	9-Octadecenoic acid (Z)-, methyl ester	92.2
7	14.91	13.28	Octadecanoic acid, methyl ester	83.6
8	14.95	5.57	9,12-Octadecadienoic acid (Z,Z)-	89.4
9	15.03	10.73	Octadec-9-enoic acid	89.8
10	15.27	1.90	Octadecanoic acid	84.2
11	17.56	0.76	Eicosanoic acid, methyl ester	82.1
12	20.70	1.61	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	83.1
13	25.98	5.66	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	89.7
14	26.26	5.39	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	86.8

APPENDIX F

Principle of the DPPH Radical Scavenging Assay

The DPPH Radical Scavenging Assay: Measure of Antioxidant Activity [3]

The molecule of 1,1-diphenyl-2-picrylhydrazyl (Figure 9) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520 nm.

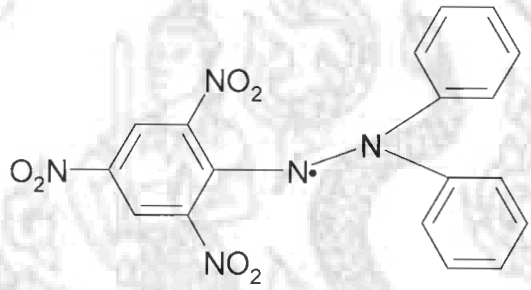


Figure F1. Diphenylpicrylhydrazyl (free radical)

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Figure 10) with the loss of this violet color (although there would be expected to be a residual pale yellow color from the picryl group still present).

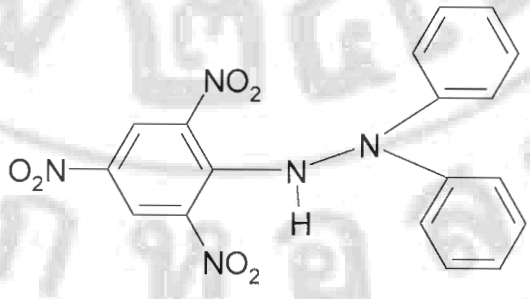


Figure F2. Diphenylpicrylhydrazine (nonradical)

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[3] Molyneux, P. 2004. "The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity". **Songklanarin J. Sci.Technol.** 26(2): 211-218.

Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is

$$Z\bullet + AH = ZH + A\bullet$$

where ZH is the reduced form and A• is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant. The reaction is therefore intended to provide the link with the reactions taking place in an oxidizing system, such as the autoxidation of a lipid or other unsaturated substance; the DPPH molecule Z• is thus intended to represent the free radicals formed in the system whose activity is to be suppressed by the substance AH.

APPENDIX G

Principle of Thermogravimetric Analysis

Thermogravimetric analysis [4]

General Description

Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated. The measurement is normally carried out in air or in an inert atmosphere, such as Helium or Argon, and the weight is recorded as a function of increasing temperature. Sometimes, the measurement is performed in a lean oxygen atmosphere (1 to 5% O2 in N2 or He) to slow down oxidation. In addition to weight changes, some instruments also record the temperature difference between the specimen and one or more reference pans (differential thermal analysis, or DTA) or the heat flow into the specimen pan compared to that of the reference pan (differential scanning calorimetry, or DSC). The latter can be used to monitor the energy released or absorbed via chemical reactions during the heating process. In the particular case of carbon nanotubes, the weight change in an air atmosphere is typically a superposition of the weight loss due to oxidation of carbon into gaseous carbon dioxide and the weight gain due to oxidation of residual metal catalyst into solid oxides.

Operating Principle and Definitions

In most cases, TGA analysis is performed in an oxidative atmosphere (air or oxygen and inert gas mixtures) with a linear temperature ramp. The maximum temperature is selected so that the specimen weight is stable at the end of the experiment, implying that all chemical reactions are completed (i.e., all of the carbon is burnt off leaving behind metal oxides). This approach rovides two important numerical pieces of information: ash content (residual mass, M_{res}) and oxidation temperature (To) (Figure 1). While the definition of ash content is unambiguous, oxidation temperature can be defined in many ways, including the temperature of the maximum in the weight loss rate (dm/dT_{max}) and the weight loss onset temperature (T_{onset}). The former refers to the temperature of the maximum rate of oxidation, while the latter refers to the temperature when oxidation just begins. The use of the former definition, $To = dm/dT_{max}$, is preferred for two reasons.

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[4] Thermogravimetric Analysis [Online] Available: http://www.msel.nist.gov/Nanotube2/Practice%20Guide_Section%202_TGA.pdf

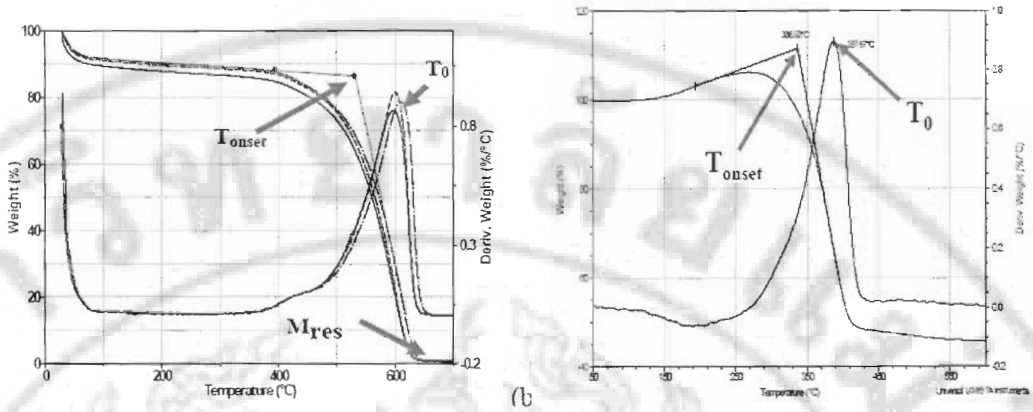

Figure G1. A sample TGA thermogram and the respective identification of important peaks.

First, due to the gradual initiation of transition (sometimes up to 100 °C) it may be difficult to determine T_{onset} precisely. Gradual onset is believed to be due to nanotubes being contaminated with amorphous carbon and other types of carbonaceous impurities that oxidize at temperatures lower than that of nanotubes. In these cases, Tonset describes the properties of the impurities rather than the nanotubes. Second, weight loss due to carbon oxidation is often superimposed on the weight increase due to catalyst oxidation at low temperatures. In some cases this leads to an upward swing of the TGA curve prior to the bulk of the weight loss, which makes the definition of Tonset even more difficult and ambiguous. However, determining dm/dTmax is relatively straightforward. Therefore, oxidation temperature is herein defined as To = dm/dTmax.

TGA measurement of "as-produced" nanotube material in air usually produces only one peak in the dm/dT curve, as "fluffy" raw nanotubes oxidize rapidly in an oxygen-rich environment. However, analysis of purified nanotube material in air may produce more than one peak. These additional peaks are likely due to the fact that purified material contains a fraction of nanotubes with damage and/or with functional groups (i.e., the material is oxidized at lower temperatures) or because purified material is more compacted after drying. The position of each peak is also strongly affected by the amount and morphology of the metal catalyst particles and other carbonbased impurities, as well as their distribution within a specimen. A lean oxygen environment can be used to better separate these peaks. In addition, these peaks have also been attributed to various components in the nanotube material (amorphous carbon, nanotubes,

graphitic particles), and it may be possible to quantify these components by deconvolution of peaks.

Oxidation temperature, To, is basically a measure of the thermal stability of nanotubes in air and depends on a number of parameters. For example, smaller diameter nanotubes are believed to oxidize at lower temperature due to a higher curvature strain. Defects and derivatization moiety in nanotube walls can also lower the thermal stability. Active metal particles present in the nanotube specimens may catalyze carbon oxidation, so the amount of metal impurity in the sample can have a considerable influence on the thermal stability. It is impossible to distinguish these contributions, but, nevertheless, thermal stability is a good measure of the overall quality of a given nanotube sample. Higher oxidation temperature is always associated with purer, less defective samples.

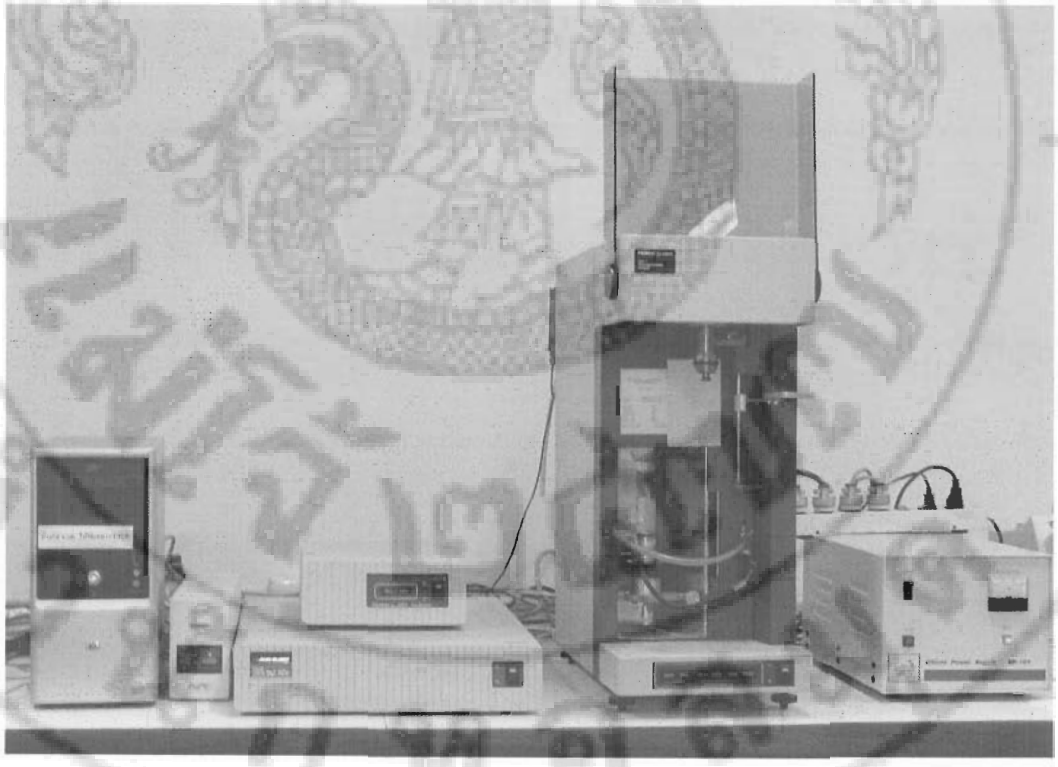

Figure G2. The TGA instrument used in Chiang Mai University.

APPENDIX H

List of Abstracts of Papers Published or Presented

Abstract of the paper published at KMITL Science Journal (ISSN: 1685-2044) Vol. 6, No. 2B and presented as poster during the KMITL International Conference in Science and Applied Science held at Swissotel Le Concorde in Bangkok from 8-10 March, 2006.

A Survey on the Fatty Acid Composition of Commercial Palm Oil in Thailand

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[2] Department of Chemistry, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecjia, Philippines, 3120

ABSTRACT

Seven commercially available palm oils in Thailand were studied to evaluate their fatty acid composition using the GC-MS tandem. Seven to eight different fatty acids distributed in varying proportion were identified in each sample. Saturated fatty acids accounted for 23.20 to 45.60% of the total fatty acids. Hexadecanoic acid contributed the highest percentage among the saturated fatty acids identified. This was followed by octanoic acid. Dodecanoic, tetradecanoic, and eicosanoic acids were also observed in trace amount. The percentage distribution of unsaturated fatty acids with a range of 56.30-76.80% was determined to be higher than the percentage of saturated fatty acids. 9-octadecenoic acid largely contributed to the high percentage of unsaturated fatty acids in the palm oil samples. This was followed by 9, 12-octadecadienoic acid. A trace amount of 9,12,15-octadecatrienoic acid was also detected but only in two palm oil samples.

Abstract of the paper presented as oral presentation during the 32nd Congress on Science and Technology of Thailand held at Queen Sirikit National Convention Center in Bangkok from 10-12 October, 2006.

Electrocoagulation of Palm Oil Mill's Effluent in the Production of Natural Rubber Antioxidant

Melissa B. Agustin[1], Weerachai Phutdhawong[1]

[1]Department of Chemistry, Faculty of Science, Maejo University, Sansai, Chiang Mai, Thailand, 50290

A new approach in the management of palm oil mill's effluent (POME) with an emphasis on giving added value to wastes was demonstrated in this study. Electrocoagulation (EC), an electrochemical technique used in water purification technology was employed to remove dissolved organic substances from the POME at the same time utilized the recovered substance as antioxidant for natural rubber. The presence of phenolic groups, a characteristic functional group of most antioxidants, was confirmed by the formation of black precipitate upon mixing POME with $FeCl_3$. Removal of these phenolic compounds was done by electrocoagulating (2.0 A, 4.6-8.1 V) a liter of POME using aluminum as electrodes for 6 hours. The process afforded a clear solution (pH=7.86) and a coagulum. Recovery of the phenolic compounds from the coagulum was done by dissolution with 7% HCl followed by solvent extraction with butanol. The dried residue from the butanol extract was further extracted with hexane using a Soxhlet to remove oils and waxes. The overall process afforded a yield of 17.1g/L POME. The antioxidant property of the isolate was confirmed by the decolorization of DPPH on the TLC plate using a spray method. The isolate was incorporated into the rubber preparation using NR latex and its effects on the physico-mechanical properties of the rubber were evaluated and compared with the commercial antioxidant.

Abstract of the paper presented as poster during the 6th Princess Chulabhorn International Science Congress held at the Shangri-La Hotel in Bangkok from November 25-29, 2007.

Lignin from Oil Palm Kernel Meal as Antioxidant for Natural Rubber

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[3]Department of Chemistry, Faculty of Science, Silpakron University, Nakhonpathom 73000, Thailand

Oil palm kernel meal (OPKM) is one of the solid wastes produced in palm oil mills. To give added value to this waste, the OPKM in this study was utilized to isolate a possible antioxidant; in the form of lignin, for natural rubber (NR). The lignin from OPKM was isolated using alkali digestion. The isolation involved digestion with KOH-AQ solution for 3 hours at 170°C, removal of polysaccharide degradation products, and precipitation of the desired antioxidant at pH 2. The overall process afforded an average yield of 4.1 g /100 g OPKM. Preliminary test for its antioxidant property using the DPPH radical scavenging assay showed similar activity with Vitamin E. Partial characterization of the OPKM lignin was done using FTIR and nitrobenzene oxidation method. The OPKM lignin was incorporated as antioxidant in the preparation of vulcanized NR sheets. Compounding of NR was done using a two-roll mill and vulcanization was carried out at a specified cure time obtained from rheometer data. The physico-mechanical properties, chemical properties were determined from the samples prepared with different concentrations of OPKM lignin. The efficiency of OPKM lignin as antioxidant for NR was determined by measuring the tensile properties before and after accelerated thermal aging and its stability towards thermal degradation was monitored using thermogravimetric analysis. Results showed that a concentration of 1phr OPKM lignin is found comparable with the commercial phenolic antioxidant, Wingstay L.

Abstract of the paper accepted for publication in the *International Journal of Environmental Research and Public Health* (ISSN 1660-4601, CODEN: IJERGQ, Journal DOI: 10.3390/ijerph).

Electrocoagulation of Palm Oil Mill Effluent

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[2] Department of Chemistry, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120, Philippines

[3] Department of Chemistry, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand

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Electrocoagulation (EC) is an electrochemical technique which has been employed in the treatment of various kinds of wastewater. In this work the potential use of EC for the treatment of palm oil mill effluent (POME) was investigated. In a laboratory scale, POME from a factory site in Chumporn Province (Thailand) was subjected to EC using aluminum as electrodes and sodium chloride as supporting electrolyte. Results show that EC can reduce the turbidity, acidity, COD, and BOD of the POME as well as some of its heavy metal contents. Phenolic compounds are also removed from the effluent. Recovery techniques were employed in the coagulated fraction and the recovered compounds was analysed for antioxidant activity by DPPH method. The isolate was found to have a moderate antioxidant activity. From this investigation, it can be concluded that EC is an efficient method for the treatment of POME.

APPENDIX I

Curriculum Vitae

Curriculum Vitae

Name	**Melissa Buenaventura Agustin**	
Date of Birth	May 5, 1978	
Place of Birth:	Palagay, Cabanatuan City, Philippines	
Academic background	1995	Cabanatuan City Science High School, Secondary education *6th Honorable Mention*
	1999	Central Luzon State University – B.S. Chemistry, *Cum Laude*
	2001	University of the Philippines-Diliman – M.S. Marine Science 24 units course work
Work /Research Experience	1999-2002	University of the Philippines-Marine Science Institute *Research assistant* – worked on the chemistry of porewater and sediment of Manila Bay in relation to red tide occurrences; job includes technical report writing, fieldwork, water and sediment analyses
	2002 - 2005	Central Luzon State University *Instructor* – handled general chemistry laboratory classes for B.S. Chemistry students
	2005- 2008	Maejo University *Graduate student* – worked on the isolation and partial characterization of phenolic compounds from palm waste, studied the application of the isolates as antioxidant for natural rubber and obtained a training on rubber technology
Awards Received	1999	3rd placer- Licensure Examination for Chemists
	2006	Recipient – Thailand Research Fund – Master's Thesis Grant