COMPARISON STUDY ON ISOLATION PROCEDURES OF BIOACTIVE COMPOUNDS FROM PYROLIGNEOUS ACID OF *RHIZOPHORA APICULATA*

NUR HAJJAR BINTI KAMARUDDIN

UNIVERSITI SAINS MALAYSIA

2008

COMPARISON STUDY ON ISOLATION PROCEDURES OF BIOACTIVE COMPOUNDS FROM PYROLIGNEOUS ACID OF *RHIZOPHORA APICULATA*

by

NUR HAJJAR BINTI KAMARUDDIN

Thesis submitted in the fulfillment of the requirements for the degree of Master of Science

October 2008

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim, in the name of Allah the Most Gracious and the Most Merciful. First and foremost, I wish to thank my supervisor Assoc. Prof. Dr. Mohd. Jain Noordin Mohd. Kassim, for his continuous support, patience, guidance, help, supervision and encouragement throughout the progress of this project.

Secondly, my sincere appreciation to Prof. Darah Ibrahim as my co-supervisor for her guidance and support in the biological study of pyroligneous acid at School of Biological Sciences, Universiti Sains Malaysia (USM).

Next, I would like to acknowledge all the technical staffs of the School of Chemical Sciences, USM, especially Mr. Ali Zaini, Mr. Yee Chin Leng, Mr. Aw Yeong, Mr. Abdul Wahab, Mr. Jamal, Mr. Ramlee, Mr. Chow Cheng Por and Mr. Nazeef, for being helpful and cooperative throughout the course of this research.

I am also very grateful to Mr. Hilman from Centre for Drug Research, USM for his support and cooperation in helping me with the GC-MS study of pyroligneous acid. Without his assist, I would never have succeeded in completing this project.

I wish to express my gratitude to USM especially Institute of Graduate Studies (IPS) for funding me an allowance which supports my living expenses during the term of my study.

Not forgetting, to my family members especially my parents, I am so thankful for their unconditional love and support who have always been very encouraging during the completion of my study.

I am blessed to be surrounded by friends who have been very cooperative and helpful during the term of my study, so I wish to thank Mr. Wendy Rusli, Mr. Oo Chuan Wei, Mrs. Suraya, Mr. Muhammad Douh, Ms. Loo Ai Yin, Ms. Phang Pei Kheng, Ms. Siti Nordahliawate, Ms. Noor Kusmawati, Mr. Kong Nein Hing, Mrs. Azrat Khan, Ms. Siti Nurdiana, Mrs. Nur Ariesma, Ms. Marsita and Ms. Mardiana.

Last but certainly not least, I would like to thank all who had helped me directly and indirectly, throughout the progress of my research, too numerous to list but I am sincerely appreciate their support and contributions in my effort to finish my graduate study, most especially to Mr. Mohd. Saiful Anuar bin Che Ibrahim.

Nur Hajjar binti Kamaruddin
October 2008

TABLE OF CONTENTS

Page

CHAPTER THREE – RESULTS AND DISCUSSION 59

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

B1	Aqueous basic layer 1
B2	Aqueous basic layer 2
CC	Column chromatography
$CHCl_3$	Chloroform
$CHCl_3$-$(CH_3CH_2)_2O$	Chloroform-diethyl ether
CH_2Cl_2 A	Dichloromethane extract A
CH_2Cl_2 I	Dichloromethane extract I
CH_2Cl_2 II	Dichloromethane extract II
CH_2Cl_2 III	Dichloromethane extract III
$(CH_3CH_2)_2O$	Diethyl ether extract
$CH_3COOCH_2CH_3$	Ethyl acetate extract
CH_3COOH~100	Acetic acid solution (100 mg/ ml)
CH_3COOH~50	Acetic acid solution (50 mg/ ml)
C_M	Control (methanol)
CPA	Concentrated pyroligneous acid
C_W	Control (water)
C_6H_{14}	Hexane extract
C_6H_{14}-$C_6H_5CH_3$	Hexane-toluene
eV	Electron volt
F1	Fraction F1
F2	Fraction F2
F3	Fraction F3
F4	Fraction F4

FID	Flame Ionization Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography – Mass Spectrometry
m/z	Mass/charge
MS	Mass Spectrometry
NA	Nutrient agar
PA	Pyroligneous acid
R_F	Retention Factor
sp.	Species
S_Y	Syringol
TLC	Thin Layer Chromatography
t_R (min)	Retention time (min)
UV	Ultra Violet light

KAJIAN PERBANDINGAN PROSEDUR PEMISAHAN AGEN BIOAKTIF DARIPADA ASID PIROLIGNEOUS *RHIZOPHORA APICULATA*

ABSTRAK

Asid pirolignenous (PA) yang merupakan cecair yang terhasil daripada kondensasi wap dan gas yang terbebas semasa pembuatan arang kayu di kilang arang di Kuala Sepetang, Perak telah digunakan dalam kajian ini. Asid pirolignenous ini dipekatkan dengan menggunakan alat pemeruap berputar pada 80°C kepada satu per sepuluh (1/10) isipadu asalnya memberikan cecair asid pirolignenous pekat (CPA). Ujian awal aktiviti antimikrobial menunjukkan PA dan CPA mempunyai ciri-ciri antimikrob terhadap 14 spesies mikroorganisma yang diuji iaitu 10 spesies bakteria, 2 spesies yis dan 2 spesies kulat. Pengekstrakan cecair-cecair ke atas PA menghasilkan ekstrak $(CH_3CH_2)_2O$, pengekstrakan diklorometana ke atas CPA memberikan ekstrak CH_2Cl_2 I, pengekstrakan asid-bes ke atas ekstrak CH_2Cl_2 I memberikan ekstrak CH_2Cl_2 III manakala kolum kromatografi-pengkekstrakan beralkali ke atas CPA menghasilkan ekstrak CH_2Cl_2 A. Ketiga-tiga ekstrak $(CH_3CH_2)_2O$, ekstrak CH_2Cl_2 III dan ekstrak CH_2Cl_2 A mempamerkan ciri-ciri antimikrob yang signifikan terhadap 10 spesies bakteria yang diuji dengan ekstrak CH_2Cl_2 A adalah yang paling kuat merencatkan pertumbuhan bakteria yang diuji. Pemisahan dan pengenalpastian bahan bioaktif dalam asid pirolignenous melalui analisa TLC, FT-IR dan GC/ GC-MS mendapati pelbagai sebatian terbitan karbonilik dan fenolik hadir dalam PA. Didapati bahawa asid asetik menjadi sebatian utama yang berperanan dalam aktiviti antimikrobial ekstrak $(CH_3CH_2)_2O$ memandangkan sebatian ini dikesan dalam kelimpahan yang paling tinggi berbanding sebatian lain. Ekstrak CH_2Cl_2 III dan CH_2Cl_2 A menunjukkan campuran sebatian yang lebih kompleks seperti keton, alkohol, asid, ester, terbitan furan dan pyran, terbitan fenol, terbitan guaiacol, terbitan syringol, pyrocatechol, sebatian bernitrogen dan beberapa sebatian lain. Analisis GC-MS bagi ekstrak CH_2Cl_2 A mempamerkan kromatogram yang lebih ringkas dengan bilangan puncak yang berkurangan. Lebih banyak sebatian fenolik dikenalpasti dalam ekstrak CH_2Cl_2 A berbanding dalam ekstrak CH_2Cl_2 III. Sebatian 2,6-bis(1,1-dimetiletil)-4-metilfenol (BHT) turut dikesan buat pertama kali dalam ekstrak CH_2Cl_2 A. Kajian mendapati prosedur pemisahan kombinasi antara kolum kromatografi-pengkestrakan beralkali adalah paling berkesan dalam memberikan ekstrak kaya fenolik berbanding kaedah lain.

COMPARISON STUDY ON ISOLATION PROCEDURES OF BIOACTIVE COMPOUNDS FROM PYROLIGNEOUS ACID OF *RHIZOPHORA APICULATA*

ABSTRACT

Pyroligneous acid (PA) a liquid produced from the condensation of vapours and gases escaped during the production of charcoal in charcoal village Kuala Sepetang, Perak was studied in this research. This PA was concentrated to one tenth of its original volume at 80°C using rotary evaporator and resulted in concentrated pyroligneous acid (CPA). Antimicrobial screening of PA and CPA showed that they possessed the antimicrobial properties against 14 microbial species i.e. 10 bacterial species, 2 species of yeasts and 2 species of fungi. Liquid-liquid extraction of PA resulted in $(CH_3CH_2)_2O$ extract, dichloromethane extraction of CPA gave in CH_2Cl_2 I extract, acid-base treatment on CH_2Cl_2 I extract resulted in CH_2Cl_2 III extract while open-column chromatography- alkaline extraction gave rise to CH_2Cl_2 A extract. All three extracts exhibited significant antimicrobial properties against 10 tested bacterial species with CH_2Cl_2 A as the strongest extract to inhibit the growth of bacteria treated. Separation and identification of bioactive compounds in pyroligneous acid using TLC, FT-IR and GC/ GC-MS analysis indicated that PA contains a variety of carbonylic and phenolic derivatives. It was shown that acetic acid played the major role in the antimicrobial activity of $(CH_3CH_2)_2O$ extract since this compound was the major compound identified. CH_2Cl_2 III and CH_2Cl_2 A extract demonstrated a complex mixture of organic derivatives such as ketones, alcohols, acids, esters, furan and pyran derivatives, phenol derivatives, guaiacol derivatives, syringol derivatives, pyrocatechol, nitrogenated compounds and several other compounds. GC-MS study of CH_2Cl_2 A extract revealed a cleaner and simpler chromatogram with lesser number of peaks identified. More phenolic compounds existed in CH_2Cl_2 A extract as compared to CH_2Cl_2 III extract. Compound 2,6-bis(1,1-dimethylethyl)-4-methylphenol (BHT) also detected for the first time in CH_2Cl_2 A extract of pyroligneous acid. This study found that the combination of open-column chromatography-alkaline extraction was the best isolation procedure in giving a rich phenolic extract.

CHAPTER ONE

INTRODUCTION

1.1 Pyroligneous acid

Generally, pyroligneous acid is considered as by-product from the dry distillation of wood (Demirbaş & Güllü, 1998) whereby the process also known as fast pyrolysis of biomass. Generation of pyroligneous acid needs wood as its major sources. The terms ‘pyroligneous’ comes from pyrolysis of wood components i.e. lignin and ‘acid’ from acetic acid (wood vinegar) as its principal constituent in pyroligneous acid. This is a development of the traditional process of charcoal burning whereby the wood feedstock is burnt off in an airless condition so that it is reduced to charcoal, rather than to carbon dioxide, water vapour and ash. As an ancient art, the objective of wood pyrolysis is to produce three major products i.e. charcoal, liquid and gaseous products (Güllü & Demirbaş, 2001).

In the common practice of charcoal production using internal heating of the charged wood by burning a part of it, all the by-product vapours escape into the atmosphere as smoke. Recovery of the vapours can be done by condensing the released smoke through a stainless steel pipe either through air or water to yield the so-called pyroligneous acid and the non-condensable wood gas, which can be used as fuel (Demirbaş & Güllü, 1998). The first distillate obtained from this condensation is almost entirely water and it is not until a few hours later, the liquor slowly darkens and contains increasing amounts of acid and chemicals from the breakdown of wood components. The water content of pyroligneous acid is typically between 15 – 30 wt % depending on the moisture content of the feedstock and pyrolysis conditions. Thus, pyroligneous acid can be defined once more as a liquid, which consist of a complex mixture, derived from chemical breakdown of wood components comprising of cellulose, hemicellulose and lignin through liquefying the condensable gaseous products generated during pyrolysis of wood (Demirbaş & Güllü, 1998).

In addition to be functional for many kinds of uses, the recovery of chemicals from vapours given off when wood is being pyrolysed is very much helpful in reducing the risk of atmospheric pollution (Kjallstrand & Petersson, 2001b). Since pyroligneous acid is considered as a by-product from the charcoal industry, it is rather cheap but has many beneficial values as it is used in several applications such as in industries, agricultures, home applications and pharmaceutical products. Pyroligneous acid was once a commercial source for the production of acetic acid (Nakai *et al.*, 2007).

Pyroligneous acid is referred to by many names including pyroligneous tar, *Eucalyptus* tar, pyrolysis liquid, pyrolysis oil, bio-oil, bio-crude-oil, bio-fuel-oil, wood liquids, wood oil, wood distillate, wood vinegar (due to its high content of acetic acid), wood tar, liquid wood, wood creosote, wood smoke, wood hydrolysates, liquid smoke, smoke condensate, smoke flavouring and in Japan, it is known as “mokusaku-eki” (Bridgwater, 1999; Ikegami *et al.*, 1998; Kartal *et al.*, 2004, Oasmaa & Peacocke, 2001; Araújo & Pasa, 2004).

1.2 Pyroligneous acid and its physical properties

The physical state of pyroligneous acid can be a liquid or solid depending on its preparation (Guillén & Ibargoitia, 1996a; Suñen *et al.*, 2001). However, the pyroligneous acid studied in this project is obtained in liquid form.

The physical properties of pyroligneous acid can be explained with the aid of the information on its chemical composition (Sipilä *et al.*, 1998) (**Section 1.3**). After cooling and condensation, pyroligneous acid is a mobile clear reddish-brown liquid which contains oxygenated compounds (Scholze *et al.*, 2001; Nakai *et al.*, 2007). However, the colour might range from a clear yellowish-brown to reddish-brown tone due to the type of wood being pyrolysed and on climatic conditions; it is darker in winter and lighter during summer. The variance in colour though has no effect on the composition of pyroligneous acid and its properties remain the same. It was reported that the carbonyl compounds present in pyroligneous acid are responsible mainly to impart the brown color of pyroligneous acid composition (Moeller, 1997; 2001).

Depicted by its name, pyroligneous acid is acidic with the pH ranging from 2–3 (Sipilä *et al.*, 1998). The increasing acidity of pyroligneous acid is assumed to be caused by the formation of volatile acids such as formic acid, acetic acid and lactic acid which is believed to originate from acetyl group in the hemicelluloses (Kubinsky & Ifju, 1972; Fengel & Wegener, 1984; Yilgor *et al.*, 2001; Sameshima *et al.*, 2002; Yatagai *et al.*, 2002). Low pH value of pyroligneous acid is due to high amounts (8 – 10 wt %) of volatile acids, mainly acetic and formic acid, which with water, these acids are the main reasons for the corrosiveness of pyroligneous acid (Aubin & Roy, 1980; Sipilä *et al.*, 1998; Yatagai *et al.*, 2002).

The flavour chemistry of pyroligneous acid is highly complex as evidenced by the over 400 compounds identified as constituents of pyroligneous acid composition (Underwood, 1991; 1997a; 1997b; 2000; Underwood & Graham, 1991a; 1991b). Pyroligneous acid is well known for its organoleptic properties; it is described to have a pleasant aroma and fragrance. However, its aroma may vary depending on the nature of the material involved in its preparation (Guillén & Manzanos, 1999; 2005); each material will have its own characteristic flavours as the composition of the flavour compounds in pyroligneous acid may be different. Furthermore, pyroligneous acid coming from the same wood and the same smoke generation condition may also create distinctive flavour and composition (Guillén *et al.*, 1995).

Regarding the flavour compounds in pyroligneous acid, it is important to point out that the contribution of each components of flavouring to the global odour depends on its odour threshold, its concentration in the flavouring and also possible synergistic effect (Guillén *et al.*, 2001). All components might be considered as potential contributors to the aroma and flavour of pyroligneous acid (Kostyra & Baryłko-Pikielna, 2006). In general, phenolics are the primary flavouring compounds while acids and carbonyls make a secondary contribution to the flavour of pyroligneous acid (Moeller, 1997; 2001; Smitts & Timmerman, 1982; Underwood, 1991; 1997a; 1997b; 2000; Underwood &

Graham, 1991a; 1991b, Underwood & Rozum, 1998; Kostyra & Baryłko-Pikielna, 2006). The physical properties of pyroligneous acid are summarized in Table 1.1.

Table 1.1 Typical physical properties of pyroligneous acid (Bridgwater, 1999; Oasmaa & Peacocke, 2001).

Physical properties	**Value**
Appearance	Liquid or solid
Acidity	pH 2–3
Density (at 15ºC)	1.2 kg/ dm^3
Viscosity (at 40ºC)	20–100 cSt
Flash point	50–70ºC
Moisture content	15–30%
Water miscibility	15–40 wt%
Boiling curve	Starts boiling below 100ºC, forms 20–50% solid residue
Auto ignition temperature	Approximately above 500ºC
Vapour pressure	Similar to water
Colour	Yellowish-brown to reddish-brown
Transparency	Clear
Odour	Pleasant-smoky aroma

1.3 Pyroligneous acid and its chemical composition

The chemical composition of pyroligneous acid is complicated and varied depends on the nature of the feedstock and concentration of smoke components in the wood (Guillén and Manzanos, 1996; Guillén *et al*. 1995). The high complexity of its composition contributes a variety of reactivity, molecular structures, physical and sensory properties (Jacobson *et al.*, 2000; Kostyra & Baryłko-Pikielna, 2006). The composition of pyroligneous acid is unstable because the components in it may undergo chemical reactions i.e. through polymerization over time. Furthermore, the use of pyroligneous acid does not always meet the expected results. For this reason, knowledge of chemical composition of pyroligneous acid is vital in understanding the relationship between its components and its organoleptic properties, not only to help in improving the process of its production.

In general, pyroligneous acid mainly consists of water, water soluble-organic compounds such as oligomeric material (Oasmaa & Peacocke, 2001). Until late of 1980s, the compounds in pyroligneous acid have been gathered in several reviews by different authors (Knowles *et al.*, 1975; Toth & Potthast, 1984). The identification of compounds in pyroligneous acid is continuously studied and more recent reports on composition of pyroligneous acid came from Guillén, *et al.*, 1995; 2001; Guillén & Ibargoitia, 1996a; 1996b; 1998; 1999; Guillén & Manzanos, 1996; 1997; 1999; 2002; 2005. It is evidenced that numerous and different functional group of compounds, among them are acids, alcohol, aldehydes, ketones, esters, furan and pyran, phenolics, ethers, hydrocarbons and nitrogenated derivatives.

In general, there are two ways in categorizing the pyroligneous acid in accordance with the proportions of carbonylic and phenolic derivatives in the blend. The first group is characterized by a higher proportion of carbonylic derivatives than phenolic derivatives while the second one is characterized by an overall proportion of phenolic derivatives than carbonylic derivatives (Guillén & Ibargoitia, 1996a; b). The differences in both proportions of carbonyls and phenols may be due to the type of woods either softwood or hardwood used in the production.

As various woods differ in their chemical composition, the components of pyroligneous acid are naturally variable. Hardwood and softwood are different in their lignin structure. As reported by Fengel & Wegener (1983), there is more lignin in softwood than hardwood. Lignin, a high molecular mass randomly cross-linked polymer, consists of an aromatic system composed of phenylpropane units substituted with hydroxy and methoxy group (Fengel & Wegener, 1983; Goldstein, 1983). During, the pyrolysis of wood, competing thermal degradation reaction takes place to generate different bond cleavage to give phenol derivatives (Guillén & Ibargoitia, 1999). Due to the high structural diversity of lignin, its pyrolysis should provide a high number of products (Guillén & Ibargoitia, 1998).

The difference in structure of lignin in wood gives rise to another two different categories of pyroligneous acid. Pyroligneous acid can also be divided in terms of the ratio of methoxyphenols i.e. 2-methoxyphenol (guaiacol) and 2,6-dimethoxyphenol (syringol), two of the specific methoxyphenols in pyroligneous acid. The higher proportion of guaiacol than syringol characterizes the first type while the higher proportion of syringol than guaiacol characterizes the second type. According to Kjällstrand & Petersson (2001b), syringol is characteristic of hardwood smoke whereas guaiacol is characteristic of softwood smoke. At higher temperatures, the emission of methoxyphenols decreases rapidly and the proportion of polyaromatic hydrocarbons increases instantly (Kjällstrand & Petersson, 2001b).

Some authors also conducted investigation on wood pyrolysis using a variety type of wood. Kjällstrand *et al*. (1998; 2000) studied the methoxyphenols proportion derived from the pyrolysis of lignin from spruce, birchwood, conifer and fern plants and angiosperm (hardwood) plants. Their study revealed that spruce, conifer and fern liquid smoke contains guaiacol only while the angiosperm species including birchwood liquid smoke contains both guaiacol and syringol. Furthermore, more studies on lignin-derived methoxyphenols came from Olsson *et al.* (2003a; 2003b), Olsson & Kjällstrand (2004) and Kjällstrand & Olsson (2004). Their studies focused on pyrolysis of commercial wood pellets made of softwood and fuel wood as starting material. They reported that softwood lignin degradation produced guaiacol as major product whereas hardwood liquid smoke contains syringol as the prominent products with lower proportion of guaiacol. These findings are due to the fact that lignin of softwood consists of guaiacylpropane units while syringylpropane units found in hardwood lignin (Kjällstrand & Olsson, 2004). Therefore, it is evidenced that methoxyphenols from thermal degradation of wood stand out as particularly specific and predominant combustion-formed compounds (Kjällstrand *et al*., 1998) as they are believed to demonstrate high antioxidant activity (Barclay, et al., 1997; Ogata et al., 1997) and antimicrobial as well as anticarcinogenic, antimutagenic and antiviral properties (Guillén & Ibargoitia, 1999). This should provide a reason to why the term ‘ligneous’ in pyroligneous acid comes from lignin instead of cellulose or hemicellulose of which account to approximately 25 - 35 % of the dry weight wood (van der Hage, 1995).

Instead of lignin, wood which is a lignocellulosic material composed of another two major components i.e. cellulose and hemicelluloses (Meier & Faix, 1999; Ronghou *et al.*, 2007). Therefore, numerous other compounds with different functional group arising from wood carbohydrate (cellulose and hemicellulose) thermal degradation (Fengel & Wegener, 1983; Shafizadeh, 1984) such as aldehydes, ketones, diketones, esters, alcohols, acids, furan and pyran derivatives detected in pyroligneous acid. In addition, it also contains a significant number of components arising from lignin thermal degradation (Fengel & Wegener, 1983; Shafizadeh, 1984) such as phenol, guaiacol, syringol, pyrocatechol and their derivatives and some other compounds. Table 1.2 gives some of the detected compounds in pyroligneous acid, grouped in their homologues series.

Table 1.2 Some of compounds detected in pyroligneous acid (Guillén & Manzanos, 1999; 2002; Guillén *et al.*, 2001).

Families	**Compounds**
Aldehydes	Ethanal (acetaldehyde) 3-Methylbutanal (isovaleraldehyde) Pentanal (valeraldehyde) 2-Ethylbutanal
Ketones	2-Propanone 2-Butanone 3-Methyl-2-butanone 4-Methyl-2-pentanone 2-Methyl-3-pentanone 3-Hexanone Cyclopentanone 1-(2,4-Dihydroxyphenyl)-ethanone

Table 1.2 continued

Families	**Compounds**
Diketones	2,3-Butanedione (diacetyl) 2,5-Hexanedione 3-Methyl-1,2-cyclopentanedione (cyclotene) 3,5-Dimethyl-1,2-cyclopentanedione 3-Ethyl-1,2-cyclopentanedione (3-ethylcyclopentenolone)
Furan and pyran derivatives	2-Furancarboxaldehyde (furfural) 2-Furanmethanol (furfuryl alcohol) 2,5-Dimethoxytetrahydrofuran 2-Ethylfuran 3-Methyl-2,5-furandione 5-Methyl-2-furancarboxaldehyde (5-methylfurfural)
Alcohols, esters and acids	Ethyl alcohol Acetic acid Ethyl acetate 2-Pentanol 3-Methyl-1-butanol 2-Hexanol Butyric acid Hexanol Ethyleneglycol monoacetate Pentanoic acid 4-Oxo-pentanoic acid (levulinic acid) Methyl nonanoate
Phenol and derivatives	Phenol 2-Methylphenol 3-Methylphenol 2,6-Dimethylphenol 2-Ethylphenol 3,5-Dimethylphenol 2,3-Dimethylphenol 2,4,6-Trimethylphenol 2,3,5-Trimethylphenol 2-Isopropyl-5-methylphenol (thymol) 4-Propenylphenol
Guaiacol and derivatives	2-Methoxyphenol (guaiacol) 4-Methyl-2-methoxyphenol (4-methylguaiacol) 4-Ethyl-2-methoxyphenol (4-ethylguaiacol) 4-Vinyl-2-methoxyphenol (4-vinylguaiacol) 1-(3-Hydroxy-2-methoxyphenyl)-ethanone 4-(2-Propenyl)-2-methoxyphenol (eugenol) 4-Hydroxy-3-methoxybenzaldehyde (vanillin) 1-(4-Hydroxy-3-methoxyphenyl)-2-propanone (guaiacylacetone) 4-Hydroxy-3-methoxybenzoic acid (vanillic acid) 1-(4-Hydroxy-3-methoxyphenyl)-2-propen-1-al (coniferyl aldehyde)

Table 1.2 continued

Families	**Compounds**
Syringol and derivatives	2,6-Dimethoxyphenol (syringol) 3,4-Dimethoxyphenol 4-Methyl-2,6-dimethoxyphenol (4-methylsyringol) * 3-Ethyl-2,6-dimethoxyphenol (3-ethylsyringol) 4-Vinyl-2,6-dimethoxyphenol (4-vinylsyringol) 4-(2-Propenyl)-2,6-dimethoxyphenol (4-allylsyringol) 4-Hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde) 4-(1-Propenyl)-2,6-dimethoxyphenol 1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanal (homosyringaldehyde) 1-(4-Hydroxy-3,5-dimethoxyphenyl)-ethanone (acetosyringone) 1-(4-Hydroxy-3,5-dimethoxyphenyl)-2-propanone (syringylacetone)
Pyrocatechol and derivatives	1,2-Benzenediol (pyrocatechol) 1,4-Benzenediol (hydroquinone) 3-Methoxy-1,2-benzenediol (3-methoxypyrocatechol) 3-Methyl-1,2-benzenediol (3-methylpyrocatechol) 4-Methyl-1,2-benzenediol (4-methylpyrocatechol)
Carbohydrate derivatives	1,6-Anhydro-α-d-galactopyranose 1,6-Anhydro-β-d-mannopyranose 1,6-Anhydro-β-d-glucopyranose (levoglucosan) 1,6-Anhydro-β-d-glucofuranose 1,6-Anhydro-α-d-galactofuranose etc.
Nitrogenated compounds and others	2-Methylpyridine 3-Methylpyridine 3-Methoxypyridine 1,3-dimethyl-1H-pyrazole 2-ethyl-6-methylpyridine 2-methyl-3-pyrinidol
Terpenic compounds	Camphor Linalool Terpinen-4-ol 5-hydroxy-1,8-cineole etc.

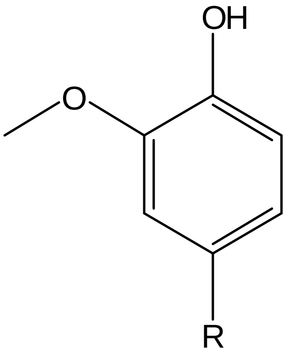
In relation to the organoleptic properties, all identified components in pyroligneous acid might be considered as potential contributors to the global odour of pyroligneous acid (Kostyra & Baryłko-Pikielna, 2006). In general, phenol and methoxyphenols derivatives are the major contributors to the smoke aroma of the pyroligneous acid while furan and pyran derivatives soften the heavy aroma associated with phenolic compounds (Kim *et al.*, 1974; Radecki *et al.*, Kjällstrand & Petersson, 2001a). According to Smits and Timmermans (1982), the specific smoke flavour of pyroligneous acid was about 66 % attributable to phenolics, 14 % for carbonyl compounds and 20 % for the remainder of the constituents. The odour characteristic to profile the organoleptic properties of pyroligneous acid is described in Table 1.3.

Table 1.3 Odour characteristic definitions for profiling of pyroligneous acid.

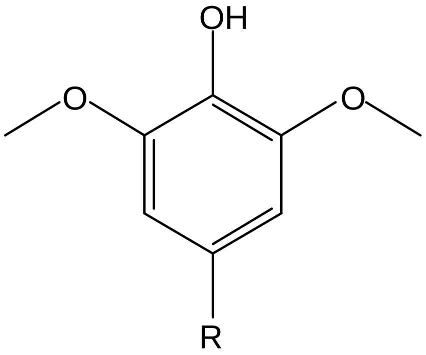
Odour attributes	**Definition of the attribute**
Sharp	Feeling of irritation of nose mucosa when sniffing
Acidic	Sharp-acidic note, characteristic for acetic acid
Smoky	Smell of the smoke from bonfire
Woody	Odour characteristic for fresh, wet wood
Burned wood	Odour characteristic for heated, slightly burned wood
Smoked meat	Odour of freshly smoked meat product
Maggi	Instant broth-like odour
Caramel	Maltol-type odour (reference sample: maltol)
Died bonfire	Odour of ‘‘just died out (extinct) bonfire’’
Almond	Odour of bitter almond
Chemical	Odour of phenolic type disinfectant (reference sample: p-cresol)
Medicinal	Odour of gargle medicine (reference sample: chinoxysol)

Generally, phenolic derivatives from lignin pyrolysis have been described to have pungent, cresolic, heavy, burnt and smokey notes (Maga, 1988) whereas carbonyl compounds is said to give caramel or burnt sugar aromas (Fiddler *et al.*, 1970).

As a conclusion regarding the chemical composition of pyroligneous acid, numerous compounds have been positively identified. The thermal degradation of wood in limited oxygen supply enables the recovery of fine chemical by-products such as acids, alcohols, phenols and etc (Amen-Chen *et al.*, 1997) consists of highly oxygenated compounds both aromatic and aliphatic in nature (Graham *et al.*, 2002). The differences in the composition of smoke are influenced by the lignin structure of hardwood and softwood and of course of the type of wood used (Guillen & Manzanos, 1999; Kjallstrand *et al.*, 2000). The most frequent studied methoxyphenols in pyroligneous acid are methoxyphenols bearing the guaiacyl unit (1) i.e. from softwood lignin and syringyl unit (2), usually from hardwood lignin (Kjällstrand & Petersson, 2001b).



(1)



(2)

1.4 Production of pyroligneous acid

1.4.1 Wood and its composition

Wood is a very old raw material when thousand years ago primitive man used them for fuel and tools. Because wood is natural in origin, serving to strengthen stems, branches and roots of tree and other plants, it returns to the natural cycle after having done its task and is degraded into its basic elements (Fengel & Wegener, 1983).

In other words, wood or biomass is a lignocellulosic material, which mainly composed of biopolymers consisting of various types of cells and the cell walls, are built of cellulose, hemicellulose and lignin (Meier & Faix, 1999). As far as the chemical components of wood are concerned, a distinction must be made between the main macromolecular cell wall components cellulose, hemicelluloses (polyoses) and lignin, which are present in all woods and the minor low-molecular-weight components (extractives and mineral substances), which are generally more related to special wood species in kind and amount. A general scheme of the wood chemical composition is illustrated in Fig. 1.1 with their percentage calculated based on dry weight of wood (d.w.w) (Fengel & Wegener, 1983; Petterson, 1984).

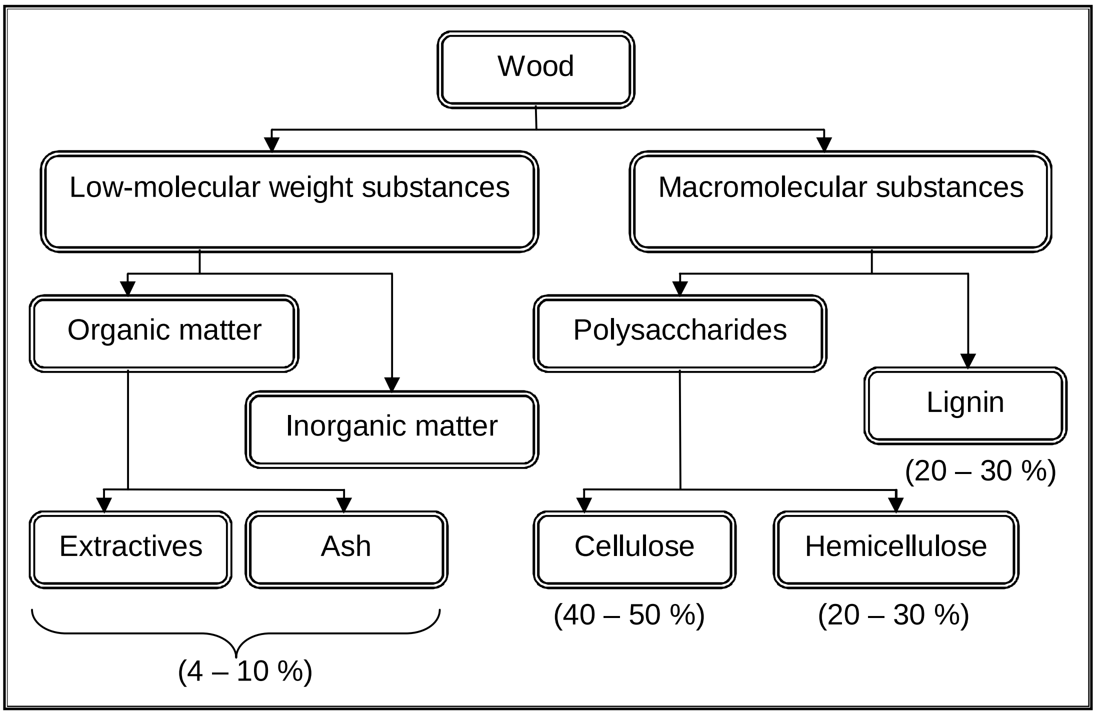
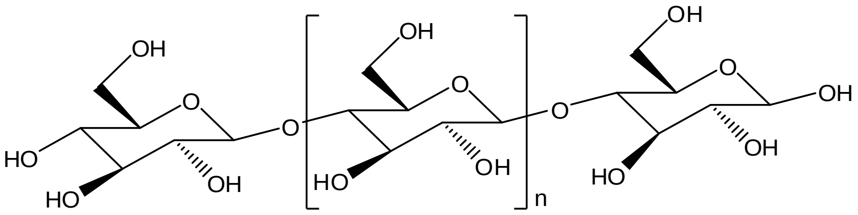


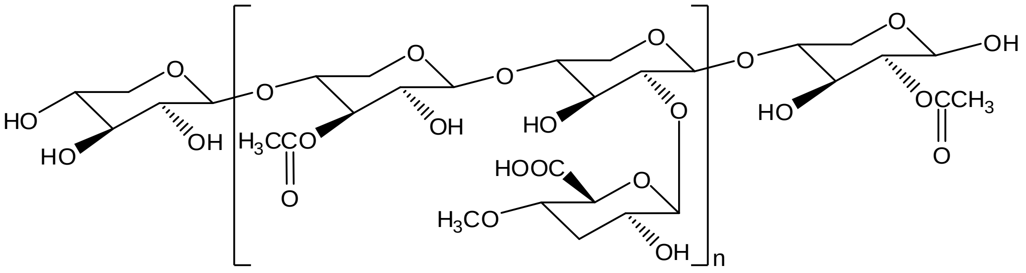
Fig. 1.1. Typical chemical composition of wood and their percentage (Fengel & Wegener, 1983; Petterson, 1984).

Cellulose (3) is a glucan polysugar which has colloidal characteristics consisting of linear long-chain polymer made up of 7000 – 12 000 β(1,4)D-glucopyranose units (Serio *et al.*, 1995; Simoneit, 2002). Aggregation of these linear chains with micro fibrils provides a crystalline structure that is highly inert and inaccessible to chemical reagents (Sinha *et al.*, 2000).



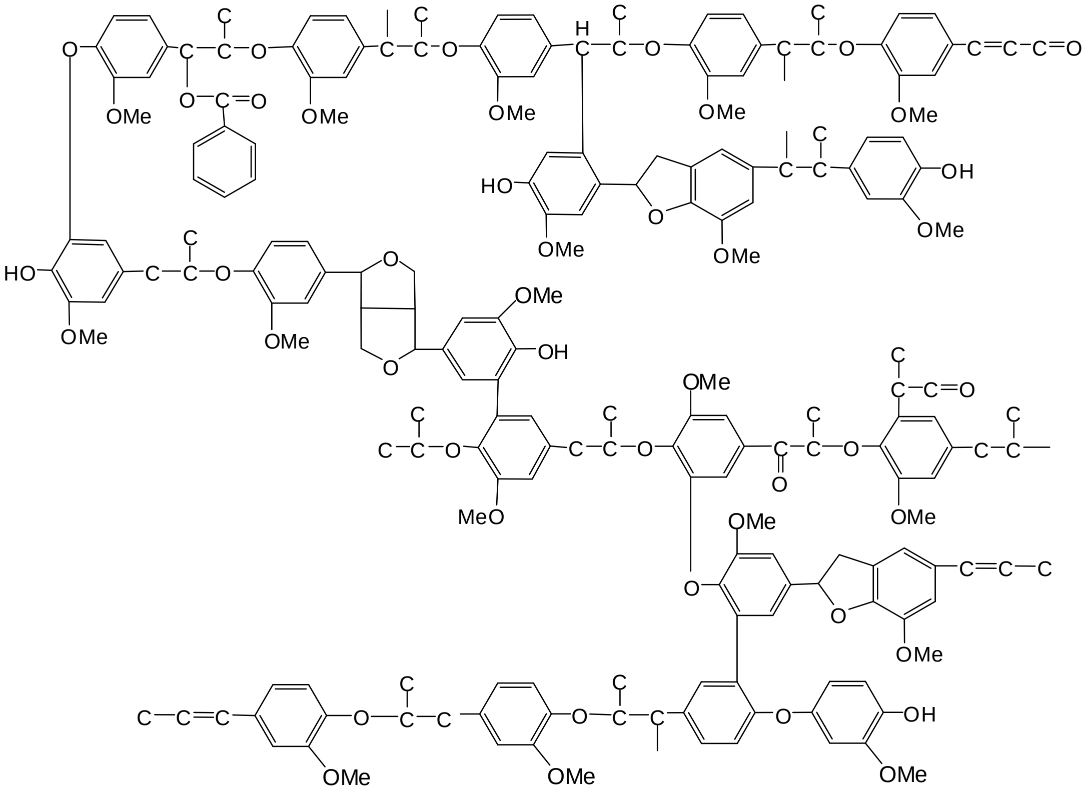
(3)

In contrast, hemicelluloses (4) are a mixture of polysaccharides derived mainly from glucose, mannose, galactose, xylose, arabinose and galacturonic acid residues (Parham & Gray, 1984; Serio *et al.*, 1995). The molecular chain of hemicellulose is much shorter than in cellulose, having side groups and being branched in some cases (Fengel & Wegener, 1983). Hemicellulose is amorphous in structure (Sinha, *et al.*, 2000), thermally more sensitive and decomposes at the earlier stage of pyrolysis (LeVan 1989; Morf, 2001).



(4)

Lastly, lignin (5) is a complex, naturally occurring phenolic phenylpropanoid polymer or ‘protolignin’ (Vuory, 1986; Alèn, 1991). The molecules of lignin are built quite differently from those of polysaccharides, as they consist of an aromatic system composed of phenylpropane units (Fengel & Wegener, 1983). Lignin was constructed biosynthetically by free radical copolymerization of three phenylpropanoid monomers, namely coumaryl-, coniferyl- and sinapyl- alcohols (Monties, 1989) and contains mainly anisyl, vanillyl (guaiacyl) and syringyl nuclei (Simoneit *et al.*, 1993; Rogge *et al.*, 1998).



(5)

In general, lignin polymers can be classified as guaiacyl, guaiacyl-syringyl and guaiacyl-syringyl-p-hydroxyphenyl lignins, according to whether they are derived from gymnosperms (softwood), angiosperms (hardwood) or graminaceae (grasses) (van der Hage, 1995). There is more lignin in softwood than in hardwood and of course there are structural differences between their lignin structures (Fengel & Wegener, 1983). In nature, lignin is amorphous and can be processed to yield aromatic compounds (Sinha *et al.*, 2000). During wood pyrolysis, lignin is the last component to degrade (Güllü & Demirbaş, 2001); this behaviour is due to the wide variety of functional groups and consequently, bond strengths present in lignin (Morf, 2001).

1.4.2 Wood pyrolysis

Wood pyrolysis is essentially the thermal breakdown of complex carbonaceous materials like wood by the action of heat alone, with the absence of externally supplied oxidizing agent i.e. oxygen so that gasification does not occur (Sinha *et al.*, 2000; Demirbaş, 2001, Ronghou *et al.*, 2007). Wood is thermally degradable and combustible material (White and Dietenberger, 2001). Via pyrolysis, wood can be converted into solid residue (charcoal) and liquids as well as gaseous products (Güllü & Demirbaş, 2001).

In wood pyrolysis the only and major feedstock involved is wood itself. Therefore, pyrolysis of wood is strongly influenced by the chemical composition and condition of the fuel (wood). As various woods differ from each other, Table 1.4 lists the average values obtained from ultimate analysis of two broad classes of wood (hardwood and softwood) and Table 1.5 gives the proximate analysis by percentage, % of weight of the major products of pyrolysis (volatiles and char).

Table 1.4. Typical ultimate analysis by dry weight of wood (%) (Krishna *et al.*, 1985)

Type of wood	**Percentage by dry wood weight (d.w.w)**				
	C (%)	**H (%)**	**O (%)**	**N (%)**	**Ash (%)**
Hardwood	50.8	6.4	41.8	0.4	0.9
Softwood	52.9	6.3	39.7	0.1	1.0

Table 1.5. Typical proximate analysis by dry weight of wood (%) (Krishna *et al.*, 1985)

Type of wood	**Percentage by dry wood weight (d.w.w)**		
	Volatile matter (%)	**Fixed carbon (%)**	**Ash (%)**
Hardwood	77.3	19.4	3.2
Softwood	77.2	22.0	1.6

From Table 1.4 and Table 1.5, it can be seen that hardwood has a higher content of H, O and N whereas softwood is higher in terms of C content. It is also noted that volatiles matter from both type of wood are almost 77 % by dry wood weight. Thus a weight loss of this order may be expected after pyrolysis is completed and all the volatile matter is released (Krishna *et al.*, 1985).

Pyrolysis of wood involves the combination of all three main components in wood. When heating wood, its constituents start to hydrolyze, oxidize, dehydrate and pyrolyze with increasing temperature forming combustible volatiles, tarry substances, and highly reactive carbonaceous char (Alger, 1972; Roberts, 1970; Shafizadeh, 1984). This type of pyrolysis is called primary pyrolysis i.e. the simultaneous degradation of hemicellulose, cellulose and lignin (Sinha *et al.*, 2000). The hot volatile products when in contact with unpyrolyzed sawdust or wood, will initiate the secondary pyrolysis reactions, which involve the decomposition of products of primary reactions (Sinha *et al.*, 2000). It is widely accepted that primary decomposition of wood (< 400ºC) consists of a degradative process, whereas the secondary thermolysis (>

400ºC) involves an aromatization process (Fisher *et al.*, 2002; Sharma *et al.*, 2004).

The overall thermal degradation of wood proceeds at different stages of increasing temperature regimes in the charcoal kiln. The hemicellulose is said to decompose first, largely between 200°C and 300°C, followed by the cellulose at 300°C to 350°C, and finally by the lignin at 280°C to 450°C. (Graham & Mattila, 1971; Levan, 1989;; Güllü & Demirbaş, 2001; White & Dietenberger, 2001; Dietenberger, 2002; Simko, 2005; Stolyhwo & Sikorski, 2005, Ronghou *et al.*, 2007). The stages in wood pyrolysis process are Zone A (≤ 200ºC), Zone B (200 – 280ºC), Zone C (280 – 500ºC) and Zone D (≥ 500ºC).

At the first stage (**Zone A**) (easily degrading zone, ≤ 200ºC), first, heat is transferred to the surface of the wood by conduction and continued to the inner part of it. As temperature reaches 100ºC, wood becomes dehydrated and generates water vapor and other non-combustible gases and liquids including CO_2, formic acid, acetic acid and H_2O. When the temperature reaches **Zone B** (200 – 280ºC), the first wood component to be decomposed is hemicellulose, yielding predominantly volatile products such as CO_2, CO, H_2O, acetic acid and high-boiling tar. Acetic acid liberated at this stage is attributed to the deacetylation of hemicellulose. Although cellulose remains unpyrolyzed, however as the temperature increases, the degree of polymerization of cellulose occurs to generate carbonyl and carboxyl groups of compounds. This stage of pyrolysis is an endothermic reaction and wood starts to char. During this “low-temperature pathway” of pyrolysis, exothermic reactions of exposed char and volatiles with atmospheric oxygen are manifested as glowing combustion. If temperature 280 – 500ºC is attained, the wood is said to be in **Zone C**. The thermolysis of cellulose picks up and reaches maximum at around 320ºC. The products are again predominantly volatiles. At 320 – 500ºC lignin starts to degrade. In this temperature range, β – β and C – C linkages between lignin monomeric units cleave to form radicals, which undergo recombination reactions to yield the phenolic compounds. This exothermic process continues until only the carbonized residue called charcoal remains. Additionally, CO, CH_4, formaldehyde, formic acid, acetic acid, methanol and H_2 are also being liberated. If the temperature continues to rise above 500ºC, the process now enters **Zone D** (afterglow). This is where the secondary pyrolysis reactions take place. The remaining wood residues undergo further decomposition by being oxidized to CO_2, CO and H_2O. Table 1.6 summarises all processes involved in the pyrolysis of wood.

Table 1.6. The overall process of wood pyrolysis based on the degradation of wood components when exposed to increasing temperature (Graham & Mattila, 1971; Levan, 1989;; Güllü & Demirbaş, 2001; White & Dietenberger, 2001; Dietenberger, 2002; Simko, 2005; Stolyhwo & Sikorski, 2005, Ronghou *et al.*, 2007).

Temperature regime	**Degraded wood components**	**Products**
Zone A (≤ 200ºC)	Moisture in wood (dehydration)	CO_2, formic acid, acetic acid and H_2O.
Zone B (200 – 280ºC)	Hemicellulose	CO_2, CO, H_2O, acetic acid, high boiling tar and some carbonyl and carboxyl compounds.

Table 1.6. continued

Temperature regime	**Degraded wood components**	**Products**
Zone C (280 – 500ºC)	Cellulose at 200 – 320ºC Lignin at 320 – 500ºC	CO, CH_4, H_2, formaldehyde, formic acid, acetic acid, methanol, carbonyl and phenolic compounds and charcoal.
Zone D (≥ 500ºC).	Wood residue and primary products	CO_2, CO, H_2O and charcoal.

From Table 1.6, charcoal is being produced in the temperature Zone C and D. It is believed that good commercial charcoal should have a fixed carbon content of about 75 % and this can be achieved by carbonizing the wood up to 500ºC i.e. at Zone C. It is well known that during wood pyrolysis, several parameters influence the yield and composition of products (Murwanashyaka *et al.*, 2001a). These parameters are moisture content in wood, wood composition, wood size, heating rate, temperature, pressure and also configuration of pyrolysis reactor (Samolada *et al.*, 1990; Shafizadeh, 1984; Pakdel *et al.*, 1997). According to Güllü & Demirbaş (2001), a low temperature and low heating rate is desirable in order to obtain a high yield of charcoal product. Additionally, woods for pyrolysis purposes should be as dry as possible as any moisture in wood will extend the pyrolysis process and decrease the product percent yield (Zaror & Pyle, 1984), thus increase the cost of operation. The moisture content of wood varies considerably and wood with lower moisture content is favourable as water has a large heat of vaporization thus suppresses pyrolysis temperature elevation (Lipinsky, 1985). To accomplish a slow heating condition, large pieces of wood are normally used as large wood pieces carbonize slowly since the transfer of heat into the interior of wood is a relatively slow process. Lastly, the choice of hardwood or softwood will also affect the quality of charcoal produced due to the variability in their lignin structure. Dense wood like hardwood tends to give a dense and strong charcoal and softwood generally produce softer and more friable charcoal.

1.4.3 The yield of products from wood pyrolysis

Valuable chemicals begin to yield when wood components start to decompose at temperature 200°C in the Zone B as described in **Section 1.4.2**. Therefore, it is believed that pyroligneous acid starts to condense from this stage until the end of the process.

Wood pyrolysis of mangrove wood in the charcoal village, Kuala Sepetang, Perak is considered as carbonization as it occur under atmospheric pressure with long vapor residence time. Wood carbonization produces three main products namely charcoal, liquids, and gas with yields of 37-50% char, 4-l 1% wood tar, 30-36% aqueous phase and 14-29% by weight of non-condensable gases Soltes & Elder (1980).

The yield of important chemicals such as acetic acid, methanol, acetone, phenolic derivatives etc has a strong economic value for pyroligneous acid. The product yield of those essential chemicals has resulted in the further processing of pyroligneous acid into fine products for example the production of acetic acid (Nakai *et al*., 2007). The yield of pyroligneous acid varies greatly with the types of wood, the kind of wood distilled and the efficiency of by-products condensation system. Wood with high pentosan content such as European beech (*Fagus spp.*) gives a high yield of acid; eucalyptus wood meanwhile gives low to intermediate yield of acids (FAO, 1987). Table 1.7 gives the yield of pyroligneous acid from the typical northern hemisphere deciduous hardwood as guidance.

Table 1.7. Yield of pyroligneous acid per ton (1000 kg) of air-dried wood (FAO, 1987).

Components	**Yield (kg)**
Acetic acid	50
Methanol, acetone etc.	24
Aqueous components	190
Non-aqueous components	50

According to Demirbaş (2002), the yield of liquid products from wood pyrolysis can be maximized by using a low temperature, high heating rate and short gas residence time in the charcoal kiln. However the parameters can be altered depending on the preferred products i.e. charcoal, liquid or gaseous products.