
Article:
Alwehaibi, Abdulrahman S., MacQuarrie, Duncan J. orcid.org/0000-0003-2017-7076 and Stark, Moray S. orcid.org/0000-0002-2175-2055 (2016) Effect of spruce-derived phenolics extracted using microwave enhanced pyrolysis on the oxidative stability of biodiesel. Green Chemistry. pp. 2762-2774. ISSN 1463-9262

https://doi.org/10.1039/c5gc02520f

Journal Name

ARTICLE

Effect of Spruce-derived Phenolics Extracted Using Microwave Enhanced Pyrolysis on the Oxidative Stability of Biodiesel

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Abdulrahman S. Alwehaibi,[a] Duncan J. Macquarrie,[a] and Moray S. Stark*[a]

An investigation has been carried out to characterize and evaluate phenolic compounds of bio-oils produced by the microwave enhanced pyrolysis of spruce woodchips (*picea abies*) for their potential application in stabilizing biodiesel from autoxidation. Four extracts were isolated from the bio-oil through multi-fractionation steps using a liquid-liquid extraction method: water-soluble, neutral, phenolic and organic acids extracts. The crude bio-oil and the isolated extracts were characterized by GC-MS, GC-FID, total phenols by Folin-Ciocalteu assay, ATR-IR and ^{13}C NMR. The antioxidative effect of the crude bio-oil, its isolated extracts and two significant phenolic components (eugenol and catechol) of the crude bio-oil were also investigated using methyl linoleate as a biodiesel model by means of a high temperature (120 $^{\circ}$C) oxidation test. The results show that methyl linoleate induction time increased after blending small amounts (1.4 - 5.6% w/w) of either the crude bio-oil or the isolated extracts. However, the crude bio-oil showed higher induction times in comparison with its isolated extracts, which was significant because the crude bio-oil contained a lower concentration of phenolic species (23% w/w), especially in comparison to the phenolic concentration in the phenolic extract (49.6% w/w). Furthermore, catechol was found to be very effective and was similar to crude bio-oil in the inhibition of methyl linoleate autoxidation, unlike eugenol, which was less effective at equivalent molar concentrations. Also, the effect of catechol and the crude bio-oil on methyl linoleate induction time was approximately comparable with a commercial antioxidant (butylated hydroxytoluene) when treated at equivalent molar concentration of phenols.

Introduction

In recent years, there has been increasing concern about the future of petroleum derived fuels. Therefore, there is increased interest in alternative fuels, such as biodiesel, because of what they can offer compared to fuels derived from petroleum. For example, they may help reduce greenhouse gas (GHG) emissions, and encourage the use of renewable and sustainable energy sources, and also the development of secure and local fuel supplies. The European Union (EU) Parliament and Council has adopted an ambitious renewables target in the 2009 EU Renewable Energy Directive (RED) so that by 2020, 20% of energy consumption and 10% of the total transport fuel demand should be based on renewable sources.[1] Therefore, it can be likely that biofuels and non-food biomass will make a major contribution in the bioenergy sector to meet these EU targets.

Within the EU, biodiesel is used as the main biofuel for transport and accounted for approximately 70% of the EU biofuels market on a volume basis in 2012, and over 30% of the UK biofuel market on a volume basis between April 2013 and April 2014.[2,3]

Biodiesel is typically a mixture of methyl esters of saturated and unsaturated long chain fatty acids that are derived either from animal fats or from vegetable oils.[4] Globally, most biodiesel-consumed fuels are derived from rapeseed or soybean oil feedstock, and made *via* transesterification reaction with low molecular weight alcohol, commonly methanol, in the presence of a homogeneous alkali catalyst (usually NaOH or KOH).[4]

Despite the current achievement of biodiesel as a renewable fuel, it suffers from low oxidative stability in comparison with petroleum derived diesel, which is thought to be due to the presence of significant amounts of unsaturated fatty acid methyl esters (FAME), particularly polyunsaturated fatty acids (PUFA).[5] The number and location of PUFA double bonds, as well as their concentration, controls the autoxidation speed in the biodiesel fuel.[5] The autoxidation process of biodiesel can increase its viscosity, as well as causing the formation of polymer insolubles, which can block fuel filters and damage fuel injection systems in car engines.[6] Furthermore, the chain of autoxidised fatty acid methyl esters can break up into shorter acids and aldehydes,[7] which can increase the possibility of corrosion in engine fuel system and lead to the failure of moving parts.[7] In addition, the hydroperoxides formed from the autoxidation reactions of fatty acid methyl esters are also highly unstable and have the ability to attack rubber components.[7]

[a] *Green Chemistry Centre of Excellence, Department of Chemistry, University of York, York, YO10 5DD, UK*

Electronic Supplementary Information (ESI) available: mass spectra of species identified and comparisons with library matches, along with further details of analysis techniques and measured concentrations of components. See DOI: 10.1039/x0xx00000x

The oxidation rates of unsaturated fatty acid methyl esters can differ significantly. The fact that biodiesel fatty acid methyl esters typically exist in a complex mixture makes an understanding of their oxidation more complicated.[8] In general, biodiesel oxidation can be influenced by different aspects, including fatty acid methyl esters composition, the level of total glycerin, natural antioxidant content, status of fuel storage, for instance exposure to air and light, elevated temperature, as well as container makeup material.[9-11] However, all these influencing factors are difficult to control, and hence, the use of chemical intervention by adding antioxidants is the most applicable way to control the autoxidation, as these can delay or reduce autoxidation.[12]

Chain-breaking is one of the most effective ways in which antioxidants can prevent unsaturated fatty acid methyl esters autoxidation pathways. Chain-breaking or radical scavenging antioxidants typically act by reacting with alkyl peroxy radicals ($ROO^{\bullet}$) *via* a hydrogen atom transfer to form alkyl hydroperoxids (ROOH) (Reaction 1), and therefore inhibit oxidation propagation reactions by removing an alkyl peroxy radical.[13]

$$A\text{-}H + ROO^{\bullet} \longrightarrow A^{\bullet} + ROO\text{-}H \qquad (1)$$

This radical scavenging is well known with mono- or poly-hydroxylated phenolic compounds with varying substituents on one or several phenol rings. The phenol's capacity to release a hydrogen atom is well correlated to the O-H bond strength of the phenol, with a lower bond strength typically giving a more effective antioxidant.[14] Moreover, the radical produced from the antioxidant (as in reaction 1) should also be inert towards unsaturated fatty acid methyl esters and oxygen. This can usually be achieved by the ability of the antioxidant molecule to stabilise its unpaired electrons by delocalisation *via* the antioxidant aromatic ring, which enhances the stability of phenol radicals.[14] In order to be even more efficient, antioxidant derived radicals should react with alkyl peroxy radical ($ROO^{\bullet}$) to form non-radical products (as in Reaction 2).[15]

$$A^{\bullet} + ROO^{\bullet} \longrightarrow \text{non-radical products} \qquad (2)$$

In recent years, many published articles have successfully demonstrated that typical synthetic antioxidants, such as butylated hydroxytoluene (BHT), can be effective towards biodiesel by increasing its oxidation stability.[10,16-21] However, on the other hand, limited studies have demonstrated that renewable antioxidants, derived from biomass, can be used to improve biodiesel's poor oxidation stability.[19]

In this work, a detailed study was carried out on a crude spruce bio-oil extracted using microwave-enhanced pyrolysis for its potential use as a biodiesel autoxidation inhibitor. Therefore, to understand the antioxidant activity of the crude bio-oil, a several-step fractionation procedure was performed. The crude bio-oil and its obtained extracts were characterised by GC-MS, GC-FID, total phenols by Folin-Ciocalteu assay, ATR-IR and ^{13}C NMR. Also, the crude bio-oil and the extracts were individually examined for their antioxidative effect to inhibit the autoxidation of methyl linoleate—unsaturated biodiesel component.

Experimental

Materials

Norway spruce woodchips (Latin name *Picea abies*, raw material for pyrolysis crude bio-oil) were harvested in southern Sweden. Ethanol (99.97%) and dichloromethane (DCM, 99.9+ %) were purchased from VWR chemicals. Eugenol (99%), catechol (99+ %), hydrochloric acid (1M), sodium hydroxide (98.8%, in pellets form) and sodium carbonate anhydrous (99.5%) were purchased from Fisher Scientific UK Ltd. Butylated hydroxytoluene (BHT, 99+ %), DMSO-d6 (99.9%), Folin-Ciocalteu reagent, HYDRANAL Composite 5K and HYDRANAL KetoSolver were purchased from Sigma-Aldrich and used as received. Nitrogen (99.9%) and oxygen (99.5+ %) gas cylinders were supplied by BOC. Methyl linoleate (95+ %) was purchased from TCI UK Ltd and used without further purification.

Microwave pyrolysis of woodchips

The crude bio-oil was obtained by microwave pyrolysis of Norway spruce woodchips, that was carried out in a Milestone ROTO SYNTH Rotative Solid Phase Microwave Reactor (Milestone Srl., Italy), fitted with a vacuum pump (Figure 1). Samples of woodchips (150 g) were exposed to a full microwave power of 1200 W with an operating microwave frequency of 2.45 GHz (wavelength 12.2 cm, multimode) in a 2 dm^3 glass flask within the microwave cavity. Microwave pyrolysis was carried out under vacuum with an initial pressure of approximately 11 mbar absolute, and the maximum pyrolysis temperature was controlled at 200 $^{\circ}C$. During pyrolysis, microwave cavity temperature was monitored *via* infrared detector, and the total average pyrolysis time per run took approximately 10 minutes.

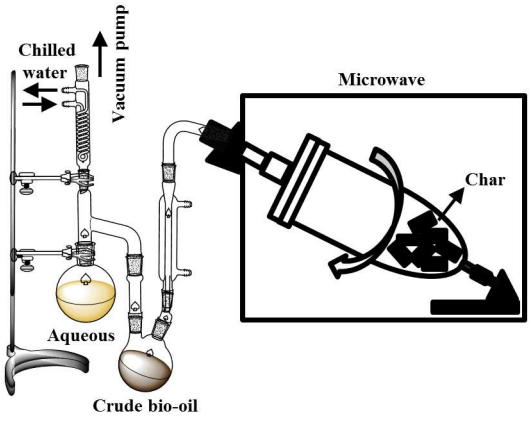


Figure 1 Schematic diagram of the experimental microwave pyrolysis set-up.

Fractionation steps of crude bio-oil

The fractionation procedure of crude bio-oil was performed using a method previously reported to isolate phenols from lignocellulosic materials.[22-24] The crude bio-oil was first mixed with ethanol and filtered to remove solid contents, as shown in Figure 2. After removing ethanol by vacuum distillation, the bio-oil was added to 75 ml distilled water with stirring for 30 min. The upper water-soluble phase formed was separated from the lower water-insoluble phase. Then a solution of 2.5 mol/dm^3 NaOH was added to the water-insoluble phase with stirring. At pH > 12, the water-insoluble phase was mostly dissolved in the NaOH solution, then, 100 ml of dichloromethane (DCM) was used twice for the extraction of neutral extract. The remaining DCM-insoluble alkaline solution was acidified with 1 mol/dm^3 HCl to pH ~ 6, and then, a phenolic-rich extract was extracted with 100 ml DCM twice. The remaining DCM-insoluble acidic solution was further acidified with 1 mol/dm^3 HCl to pH < 2 and organic acids extracted with 100 ml DCM twice. Finally, the remaining acidic solution was filtered to collect the solid precipitate. The water-soluble extract, neutral extract, phenolic extract, and organic acids extract all had the solvent removed using a rotary evaporator under partial vacuum.

Analysis of crude bio-oil and its obtained fractions

The microwave pyrolysis crude bio-oil and the fractions obtained from it were analysed separately. The elemental distribution of carbon, hydrogen and nitrogen content in the crude bio-oil was evaluated using a carbon-hydrogen-nitrogen (CHN) analyser CE440 (Exeter Analytical, Warwick, UK).

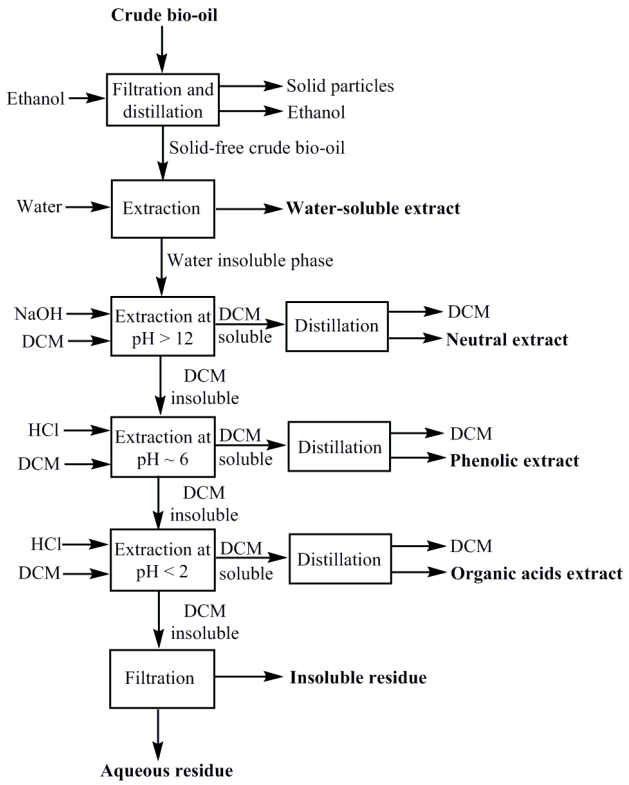


Figure 2 Schematic diagram of fractionation steps of crude bio-oil.[22-24]

The water content determination in the crude bio-oil was based on Karl Fischer (KF) titration using a Metrohm KF titrator (Metrohm 903 Titrando, with an integrated Metrohm 803 Titration Stand and a Reagent Organizer) supplied by Metrohm UK Ltd. (Runcorn, Cheshire, UK). HYDRANAL Composite 5K was used as the titrant, to avoid any possible interference by the bio-oil content of ketones and aldehydes with KF titration, and HYDRANAL KetoSolver was used as the solvent.

The identification of the more volatile components, including phenolic compounds, in the crude bio-oil and the extracts fractions was carried out with a Perkin Elmer Clarus 500 gas chromatograph attached to Perkin Elmer Clarus 560s mass spectrometer (GC-MS). A 5% phenyl – 95% dimethylpolysiloxane column was used (Zebron ZB-5HT-INFERNO 30m x 0.25mm ID x 0.25µm) Phenomenex Torrance, CA, USA. The column temperature was maintained at 60 $^{\circ}$C for 1 minute then to 360 $^{\circ}$C at a rate of 8 $^{\circ}$C per minute and then set to hold 1 minute at 360 $^{\circ}$C. The Perkin Elmer Clarus 560s mass spectrometer operated at ion source temperature of 300 $^{\circ}$C and an electron ionisation mode (EI) at 70 eV. The initial assignment of each phenolic peak was established by comparison to NIST MS Spectral library (v. 2.2) 2008. For quantitative analysis, the same crude bio-oil and the extracted fractions samples used in GC-MS were reanalysed, including BHT as external standards, using gas chromatography- flame ionisation detector (GC-FID) with the same column specifications and similar conditions used in GC-MS. After matching each identified phenolic peak *via* GC-MS with the peaks on GC-FID, the relative response factor (RRF) of each phenolic compound identified was calculated using the effective carbon number (ECN) method,[25-27] which was then used to evaluate concentrations of the identified species by comparison with the reference compound (BHT).[28] A description in more detail is enclosed in the supporting information in SI Tables 1 and 2.

ATR-IR spectra of the crude bio-oil and the extracted bio-oil fractions were obtained using a Bruker Vertex 70 with resolution of 4 cm^{-1} at 128 scans. The spectra were recorded from 4000 to 600 cm^{-1} and analysed using IR Opus 5.5 Software.

^{13}C nuclear magnetic resonance (NMR) spectroscopic analysis was carried out on crude bio-oil and the extracted bio-oil fractions using a JEOL ECS 400 NMR (100 MHz) spectrometer at 25 $^{\circ}$C. Each sample (120-130 mg) was dissolved in 1 ml of DMSO-d_6.

The total amount of phenolics in the crude bio-oil and the bio-oil extracted fractions were estimated according to a literature method using the Folin-Ciocalteu reagent,[29,30] with the variation that mono-phenol 4-allyl-2-methoxyphenol (eugenol) was used instead of the poly-phenol gallic acid as a standard for calibration as most species identified by GC-MS were mono-phenols (e.g., 81% (w/w) in crude bio-oil, see Table 3). Crude samples were all diluted in ethanol to a final concentration of 1 mg/ml, and calibration standards of eugenol were also diluted with ethanol to final concentrations of 0.05, 0.1, 0.25, 0.5 and 1 mg/ml. Then, 0.1 ml crude bio-oil or its extracts dilution, a blank (distilled water), and eugenol

calibration standard dilutions were individually placed in 10 ml volumetric flasks. Distilled water (7 ml) was added, followed by 0.5 ml FC reagent. The solution in each volumetric flask was mixed, and before reaching 8 min, 1.5 ml sodium carbonate (20% w/v) solution was added, and finally water to the 10-ml volumetric flask line, mixed, and incubated for 2 hours at room temperature. Each sample absorbance was measured at 758 nm with 1 cm matching quartz cells *via* a Jasco model V–550 double beam UV-VIS spectrophotometer operated by Spectra Manager software. The total phenol molar concentration of crude bio-oil, water-soluble, neutral, phenolic and organic acids extracts using this method were calculated assuming all phenolic species present were mono-phenols, and where w/w concentrations are given, these assume the phenolic species have a mass equivalent eugenol.

Testing antioxidancy of extracts

The antioxidant effects of the crude bio-oil, isolated extracts, two components of the bio-oil (eugenol and catechol) and, for comparison, a commercial antioxidant BHT (2, 6-bis(1, 1-dimethylethyl)-4-methylphenol) in preventing the autoxidation of methyl linoleate were examined using an accelerated oxidation test. The reaction of each mixture with oxygen was carried out at 120 $^{\circ}$C, which is a similar temperature to that used in the industry standard Rancimat test (EN 14112) which uses a temperature of 110 $^{\circ}$C for testing the oxidation stability of biodiesel. This work shares with the Rancimat test the assumption that conclusions drawn (for instance on ranking of antioxidants) at the elevated temperatures of 110-120 $^{\circ}$C are applicable at lower temperatures more appropriate for biodiesel storage. The benefits of using a higher temperature to study antioxidancy is that the duration of each run is considerably shorter, so many more candidate antioxidant mixtures can be examined in a given time.

The stainless steel (BS 304) reactor of internal volume of 42 cm^3 was preheated to 120 $^{\circ}$C, then nitrogen gas was allowed to flow inside the reactor at a rate of 1.7 cm^3 s^{-1} to remove atmospheric oxygen, and 2 ml of prepared methyl linoleate sample was then injected into the reactor with a syringe through the rubber septum.[31,32] The temperature was monitored by inserting a stainless steel coated, 0.5 mm diameter x 250 mm long, Type K thermocouple directly into the liquid in the reactor. When the sample reached the desired temperature, the nitrogen gas flow was switched to oxygen and after ca. 5 minutes (to allow the oxygen sensor to reach 100% in the system) the gas inlet and outlet were sealed to trap oxygen inside at 1 bar absolute. The magnetic stirrer was then switched on at a rate of 250 rpm to ensure the headspace gas was well mixed into the liquid. The internal pressure was recorded every two seconds during the reaction on a PC using an analogue to digital converter (Picotec ADC-20). The pressure dropped during the reaction as oxygen is consumed, until a minimum point was reached, which indicated that the oxygen had been consumed. Finally, the reaction was stopped when the pressure started rising noticeably.

Results and Discussion

Solvent fractionation of crude bio-oil

The microwave pyrolysis process of 150 g spruce woodchips generated 27.7 g crude bio-oil, 44.3 g aqueous fraction, 55.9 g char (and 22.1 g gas by difference), see Table 1. The set-up used for the microwave pyrolysis under vacuum helped to separate (*in-situ*) the pyrolysis liquid into aqueous fraction and into crude bio-oil fraction in two collection points based on their boiling point differences (see Figure 1).

The crude bio-oil fraction was dark brown, homogenous, and typically is a mixture of sugars and phenolics.[33] However, on the other hand, the aqueous fraction was light brown and typically contains water, acids and aldehydes.[33] Hence, the crude bio-oil fraction was the only fraction used for the investigation in this study.

Table 1 also shows the carbon-hydrogen-nitrogen (CHN) distribution and the water content results of the microwave-assisted pyrolysis fractions.

Further fractionation of the crude bio-oil was carried out for the isolation of phenolics according to a previous method,[22-24] and described in the experiment section which produced a water-soluble extract, a neutral extract, a phenolic extract and an organic acids extract, as well as an insoluble and an aqueous residues that was not studied further.

Approximately 57% (w/w) of the crude bio-oil was soluble in water and described as water-soluble extract after removing water by distillation. A total of 16.6% (w/w of the crude bio-oil) was recovered from the remaining water-insoluble phase by DCM extraction at three different pH levels. The phenolic extract extracted at pH ~ 6 had the highest extract amount of 11.2% (w/w) of the crude bio-oil, which was approximately equivalent to 2.1% (w/w) of the original woodchips. The quantities recovered of each fraction are shown in Table 2.

Table 1 Fractions distribution obtained by the microwave-assisted pyrolysis of 150 g spruce woodchips, including their CHN microanalytical and water content results.

	Crude bio-oil	Aqueous	Char	Gas	Total
Total recovered with 6 runs (g)	166.2	265.7	335.8	132.3	900
Aver. recovered per run (g)	27.7±0.4[d]	44.3±0.5	55.9±1.2	22.1±0.3	150
Fraction Recovery (% w/w)[e]	18.5	29.5	37.3	14.7	100
C (% w/w)	55.6±0.0	20.3±1.6	61.9±4.4	/[c]	
H (% w/w)	6.6±0.0	6.8±1.3	4.9±0.2	/	
N (% w/w)	0.1±0.0	0	0	/	
O (% w/w)[b]	37.7±0.0	72.9±2.9	33.2±4.2	/	
H_2O (% w/w)	4.7±0.1	/	/	/	

[a,b] Calculated by difference. [c] Not available. [d] ± Standard deviation. [e] of the total recovery.

Table 2 Fractions weight distribution recovered from crude bio-oil fractionation experiment and its equivalent recovery percentage from the original woodchips.

Fraction	Recovery (% w/w) of crude bio-oil	Recovery (% w/w) of woodchips
Water-soluble extract	57	10.5
Neutral extract	2.7	0.5
Phenolic extract	11.2	2.1
Organic acids extract	2.7	0.5
Insolubles & aqueous residue [a]	26.4	4.9
Total (crude bio-oil)	100	18.5

[a] Calculated by difference.

Identification and quantification of phenolic compounds

Figure 3 shows the total identified phenolic peaks in GC traces. The analysis by GC-MS identified 18 phenolic compounds in the crude bio-oil. For the isolated fractions, 8 phenolic compounds were identified in the water-soluble extract, 12 phenolic compounds in the neutral extract, 18 phenolic compounds in the phenolic extract, and 15 phenolic compounds in the organic acids extract. The mass spectra and assignments are provided in the supporting information. Some new phenolic compounds were detected after the fractionation of the crude bio-oil on the GC-MS. The likely explanation for this could be the low phenolic compound concentration in the crude bio-oil or overlap with other peaks in GC-MS chromatogram.

The quantification results by GC-FID of the identified phenolics by GC-MS are presented in Table 3 in terms of w/w of component (the equivalent molar concentrations are given in the supporting information in SI Table 5).

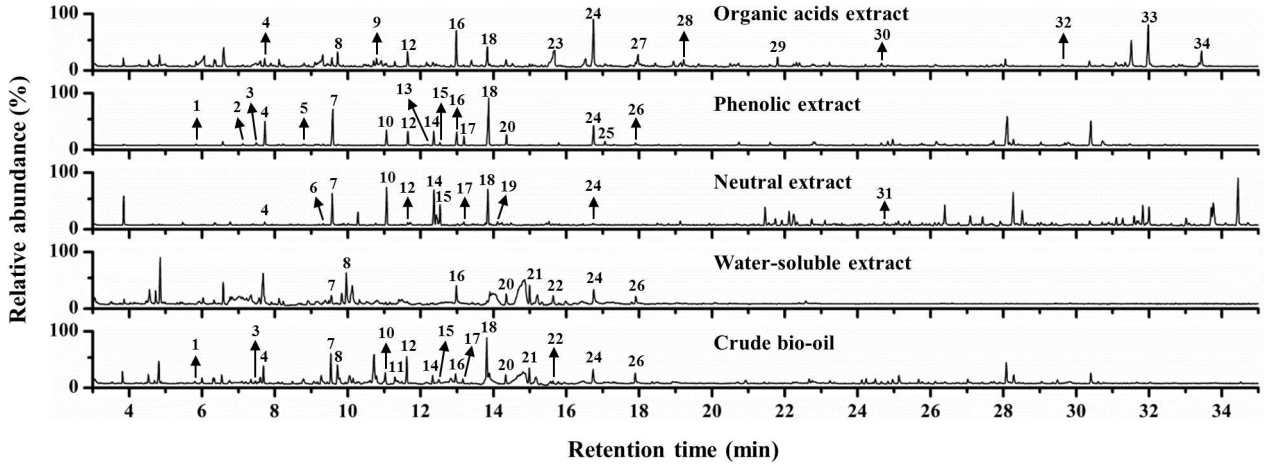
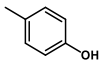
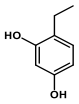
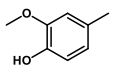
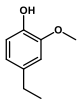
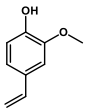
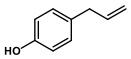
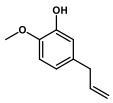
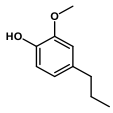
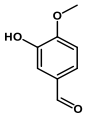
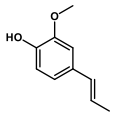
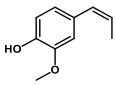
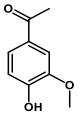
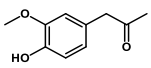
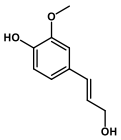
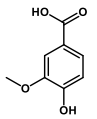
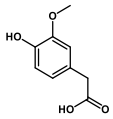
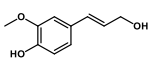
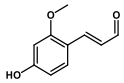
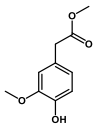
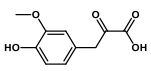
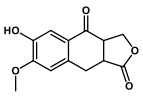
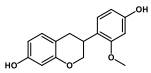
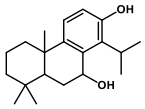
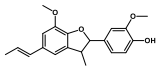
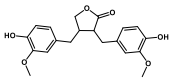
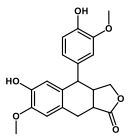


Figure 3 The GC-MS chromatograms of the identified phenolic compounds in crude bio-oil and in its isolated fractions.

Table 3 Identification and quantification of phenolic compounds in crude bio-oil and in its extracted fractions.

Compound	Structure	Peak Num.	Ret. time (min)[a]	Quantity (mg/g)				
				Crude bio-oil	Water-soluble extract	Neutral extract	Phenolic extract	Organic acids extract
Phenol		1	5.81	0[b]	/[c]	/	0.91	/
Phenol, 2-methyl-		2	7.12	/	/	/	0.55	/
Phenol, 4-methyl-		3	7.47	0	/	/	1.66	/
Phenol, 2-methoxy-		4	7.69	7.5	/	0.98	21.3	3.4
Phenol, 2,5-dimethyl-		5	8.79	/	/	/	0.15	/

1,3-Benzenediol, 4-ethyl-		6	9.33	/	/	0.5	/	/
Phenol, 2-methoxy-4-methyl-		7	9.53	6.27	0.7	14	35.2	/
1,2-Benzenediol		8	9.72	9.35	7.21	/	/	4.65
1,2-Benzenediol, 3-methyl-		9	10.79	/	/	/	/	0.93
Phenol, 4-ethyl-2-methoxy-		10	11.03	1.8	/	13.54	8.4	/
1,2-Benzenediol, 4-methyl-		11	11.30	2.36	/	/	/	/
2-Methoxy-4-vinylphenol		12	11.62	4.6	/	0.35	8.73	1.61
Phenol, 4-(2-propenyl)-		13	12.10	/	/	/	0.5	/
3-Allyl-6-methoxyphenol		14	12.33	1.3	/	15	8.57	/
Phenol, 2-methoxy-4-propyl-		15	12.50	0.21	/	6.13	1.16	/
Benzaldehyde, 3-hydroxy-4-methoxy-		16	12.96	2.82	2.2	/	13.58	7.82
Phenol, 2-methoxy-4-(1-propenyl)-		17	13.16	0.38	/	0.82	5.5	/
Phenol, 2-methoxy-4-(1-propenyl)-, (Z)-		18	13.82	8.74	/	15.7	37.9	2.25
3,7-Benzofurandiol, 2,3-dihydro-2,2-dimethyl-		19	14.12	/	/	1.4	/	/
Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-		20	14.34	2.04	0.6	/	8.09	/
2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)-		21	14.98	6.46	1.45	/	/	/

Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-		22	15.63	0.79	0.23	/	/	/
Benzoic acid, 4-hydroxy-3-methoxy-		23	15.68	/	/	/	/	9.64
Benzeneacetic acid, 4-hydroxy-3-methoxy-		24	16.74	4.1	1.6	0	17.42	9.94
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol		25	17.06	/	/	/	2.76	/
4-Hydroxy-2-methoxycinnamaldehyde		26	17.89	2.54	0.6	/	1.27	/
Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester		27	17.97	/	/	/	/	2.6
Phenylacetylformic acid, 4-hydroxy-3-methoxy-		28	19.22	/	/	/	/	1.33
Naphtho[2,3-c]furan-1,4-dione, 3,3a,9,9a-tetrahydro-6-hydroxy-7-methoxy-		29	21.80	/	/	/	/	1.55
2H-1-Benzopyran-7-ol, 3,4-dihydro-3-(4-hydroxy-2-methoxyphenyl)-		30	24.66	/	/	/	/	0
Podocarpa-8,11,13-triene-7β,13-diol, 14-isopropyl-		31	24.73	/	/	0.5	/	/
Phenol, 4-[2,3-dihydro-7-methoxy-3-methyl-5-(1-propenyl)-2-benzofuranyl]-2-methoxy-		32	29.63	/	/	/	/	0
2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]-, (3R-trans)-		33	31.98	/	/	/	/	4.5
Naphtho[2,3-c]furan-1(3H)-one, 3a,4,9,9a-tetrahydro-6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-7-methoxy-, [3aR-(3aα,4α,9aβ)]-		34	33.44	/	/	/	/	1.36

[a] Retention times according to detection in crude bio-oil GC-MS spectrum, some according to GC-MS detection in the other extracts. [b] Detected but too small to quantify reliably. [c] Not detected.

1,2-Benzenediol and (Z)-2-methoxy-4-(1-propenyl)phenol, are the most abundant phenolic components in crude bio-oil present at 9.35 and 8.74 mg/g, respectively. The total content of phenols in crude bio-oil, as determined by GC-FID, was 6.13% (w/w), while of the extracts, the phenolic had the highest total phenolic content of 17.4% (w/w), with (Z)-2-Methoxy-4-(1-propenyl)phenol and 2-methoxy-4-methylphenol being the most abundant phenolic components at 37.9 and 35.2 mg/g, respectively. In comparison with their quantity before fractionation, they are 4.3 and 5.6 times higher, respectively.

The largest phenolic species identified by GC had a mass of ca. 350 Da ($C_{20}O_6H_{18}$), however, as it was suspected that larger polyphenolic species could be in the samples, but were not volatile enough to pass through the GC column, another phenolic quantification method was also carried

out by Folin-Ciocalteu (FC) assay. The total phenolic determination by means of Folin-Ciocalteu (FC) assay showed a higher phenolic content for the crude bio-oil and the extracted fractions in comparison with GC-FID results. Table 4 shows the total phenolic content estimated by GC-FID and by Folin-Ciocalteu (FC) assay. In the phenolic extract, the phenolic content quantified by GC-FID was 17.4% (w/w), whereas by FC assay was 49.6% (w/w of mono-phenol equivalent). The large difference between the two methods could be consistent with the presence of phenolic compounds with high molecular weights, such as phenolic dimers, trimers or other larger phenolics, being too large to be detectable by GC, but however detectable by the FC method.

ATR-IR and ^{13}C NMR analysis

ATR-IR spectra of the crude bio-oil and the extracted fractions are shown in Figure 4, along with the band assignments of the major absorption peaks, also summarized in Table 5. The absorbed broad peak at ca. 3380 cm^{-1} was due to hydroxyl groups (-OH) presence in the crude bio-oil and the extracted fractions. After fractionation, most of hydroxyl-containing molecules remained in the water-soluble phase, which is consistent with the crude bio-oil containing a high content of alcohol groups for instance, sugars. Furthermore, the strong absorbance peak at ca. 1043 cm^{-1} in the water-soluble phase spectrum is consistent with a C-O stretching of primary alcohols, which reinforces the suggestion that most of the alcohols stayed in the water-soluble phase. The neutral extract spectrum shows the lowest hydroxyl absorbance band of the hydroxyl groups suggesting that it has the lowest hydroxyl-containing molecules.

According to the GC-FID results, the phenolic extract contains the highest amount of mono-phenolics, and hence, the hydroxyl absorbance band in the phenolic extract might be from phenols. The sharp absorbance peak at ca. 1517 cm^{-1} in the phenolic extract was attributed to aromatic C=C ring stretching, which also supports the presence of phenols. The C=O stretching band at the position of ca. 1716 cm^{-1} was due to carbonyl and/or carboxyl groups. This peak was intense in the water-soluble phase and in the organic acids extract. The appearance of this band in the water-soluble phase could be from aldehydes, ketones, carboxylic acids and esters. However, in the organic acids extract, the majority of the absorbance could be from carboxylic acids and esters rather than aldehydes and ketones due to the low pH extraction level (< 2) when this fraction was extracted.

^{13}C NMR spectroscopy was also employed for the characterization of the crude bio-oil and the extracted fractions, and their spectra are shown in Figure 5. The typical ^{13}C assignments relative to their chemical shift regions are summarized in Table 6 and also provide information on the typical chemical functional groups that appeared in the spectra.[34] From comparing the extracted fractions spectra to the unfractionated crude bio-oil spectrum, it was obvious that the multi-solvent extraction interestingly fractionated the crude bio-oil into two major

Table 4 Summary of the phenols content estimated by GC-FID and by Folin-Ciocalteu (FC) assay, and the ratio of these.

Sample ID	Phenolic content by GC-FID (% w/w)[a]	Total phenols by FC assay (% w/w)[b]	% of phenolic species detected by GC-FID
Crude bio-oil	6.1	23	26.5
Water-soluble extract	1.5	13.7	10.9
Neutral extract	6.9	11.3	61.1
Phenolic extract	17.4	49.6	35.1
Organic acids extract	5.2	38.3	13.6

[a] Mass ratio of phenolics to the mass of total sample detected by GC-FID. [b] Mass ratio of phenolics to the mass of total sample detected by Folin-Ciocalteu reagent, using eugenol.

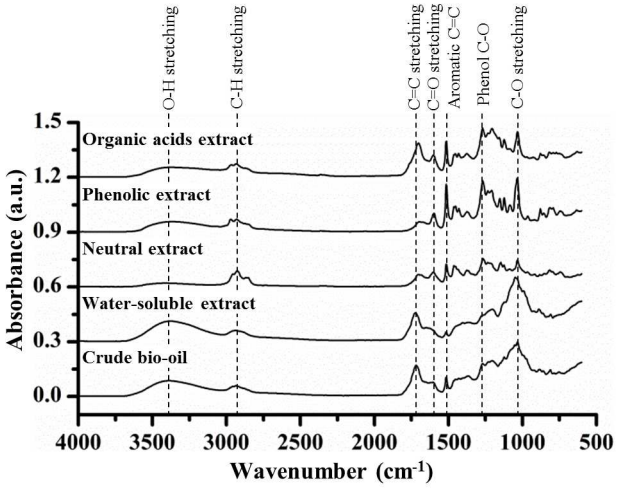


Figure 4 ATR-IR spectra of crude bio-oil and its isolated extracts (spectra offset vertically for clarity).

Table 5 IR absorption assigments to functional groups[24]

Wave no. (cm-1)	Functional groups	Compound class
3380	O-H stretching vibration	phenol, alcohols, water, carboxylic acids
2940	C-H stretching vibration	alkanes
1716	Carbonyl/carboxyl C=O stretching	Aldehydes, ketones, carboxylic acids, esters
1601	C=C stretching vibration	aromatics
1517	Aromatic C=C ring stretching	aromatics
1272	Phenol C-O	phenol
1043	Aliphatic ether C-O and alcohol C-O stretching	Alcohols, ethers

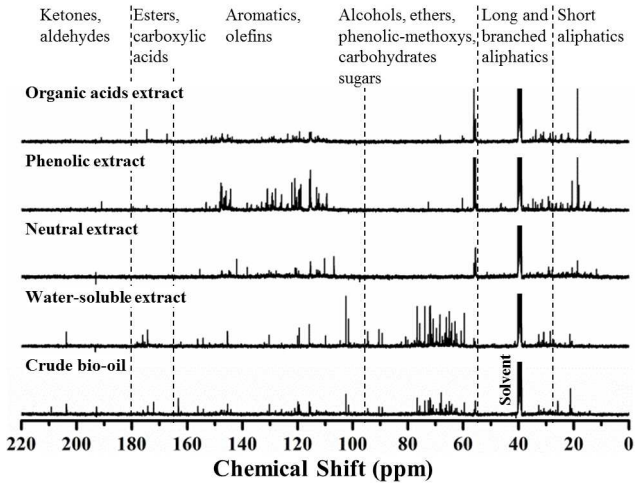


Figure 5 ^{13}C NMR spectra of crude bio-oil and its isolated extracts (spectra normalized and offset for clarity).

Table 6 Peak assignments for ^{13}C NMR spectra.[34]

Chemical Shifts (ppm)	Carbon assignments
0-28	Short aliphatics
28-55	Long and branched aliphatics
55-95	Alcohols, ethers, phenolic-methoxys, carbohydrates sugars
95-165	Aromatics, olefins
165-180	Esters, carboxylic acids
180-215	Ketones, aldehydes

families: sugars, and phenols. Carbohydrates sugars typically appear from 55 to 95 ppm on ^{13}C NMR spectra, which mostly appeared in the water-soluble phase. On the other hand, phenolic extract contains the most phenols fraction that usually lay between 95 to 165 ppm. The majority of these phenols in the phenolic extract might have a methoxy (-OCH_3) substitution due to the sharp peak appearance at ~ 56 ppm.

Effect of bio-oil & extracts on methyl linoleate autoxidation

To examine the effect of the crude bio-oil and the four isolated extracts in preventing the autoxidation of methyl linoleate, a number of high-temperature oxidation tests were carried out. The exposure of methyl linoleate to high temperature (120 $^{\circ}C$) and oxygen pressure at 1 bar absolute gives rapid methyl linoleate autoxidation, which could be delayed by adding an antioxidant, see for example Figure 6.

The effect of the crude bio-oil in the inhibition of methyl linoleate antioxidation is given in Figure 6, where the antioxidant concentrations quoted correspond to the amount of total phenolics added to the methyl linoleate, which is the equivalent molar concentration of a mono-phenol as determined by the Folin-Ciocalteu (FC) assay.

Figure 7 shows the oxidation pressure traces of methyl linoleate with concentrations of butylated hydroxytoluene

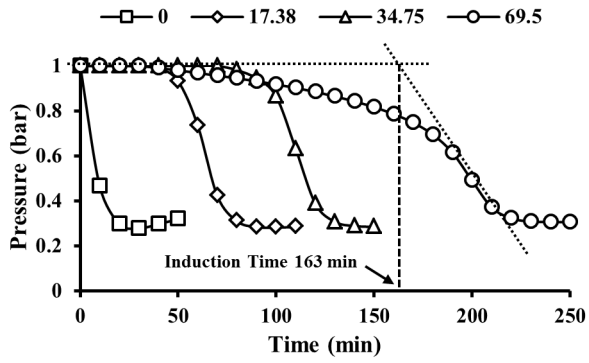


Figure 6 Oxidation pressure traces of 2 ml methyl linoleate with concentrations of crude bio-oil from 0 to 69.5 x 10^{-3} (mol/dm^3) at 120 $^{\circ}C$ and 1 bar of oxygen. (phenolic concentrations determined by FC assay assume only mono-phenolic present)

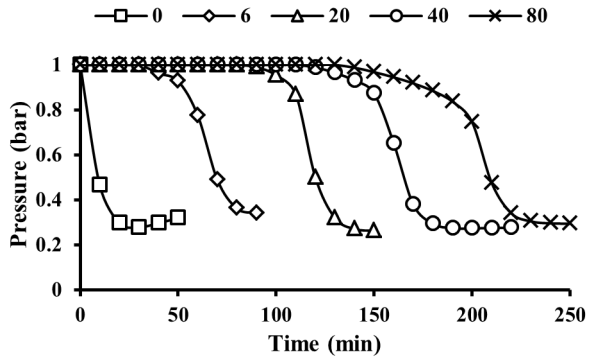


Figure 7 Oxidation pressure traces of 2 ml methyl linoleate with concentrations of BHT from 0 to 80 x 10^{-3} (mol/dm^3) at 120 $^{\circ}C$ and 1 bar of oxygen.

(BHT) from 0 to 80 x 10^{-3} (mol/dm^3). This common commercial petroleum derived antioxidant was used as a reference species to which the bio-derived extract could be compared.

It is noteworthy that the crude bio-oil is clearly an effective antioxidant at comparable molar concentration to the commercial antioxidant BHT.

Figure 8 shows the effect on methyl linoleate antioxidant of four extracts from the crude bio-oil, the water soluble, neutral, phenolic and organic acids extracts. The concentrations quoted again correspond to the total phenolics added to the methyl linoleate, which is the equivalent molar concentration of a mono-phenol as determined by the Folin-Ciocalteu (FC) assay.

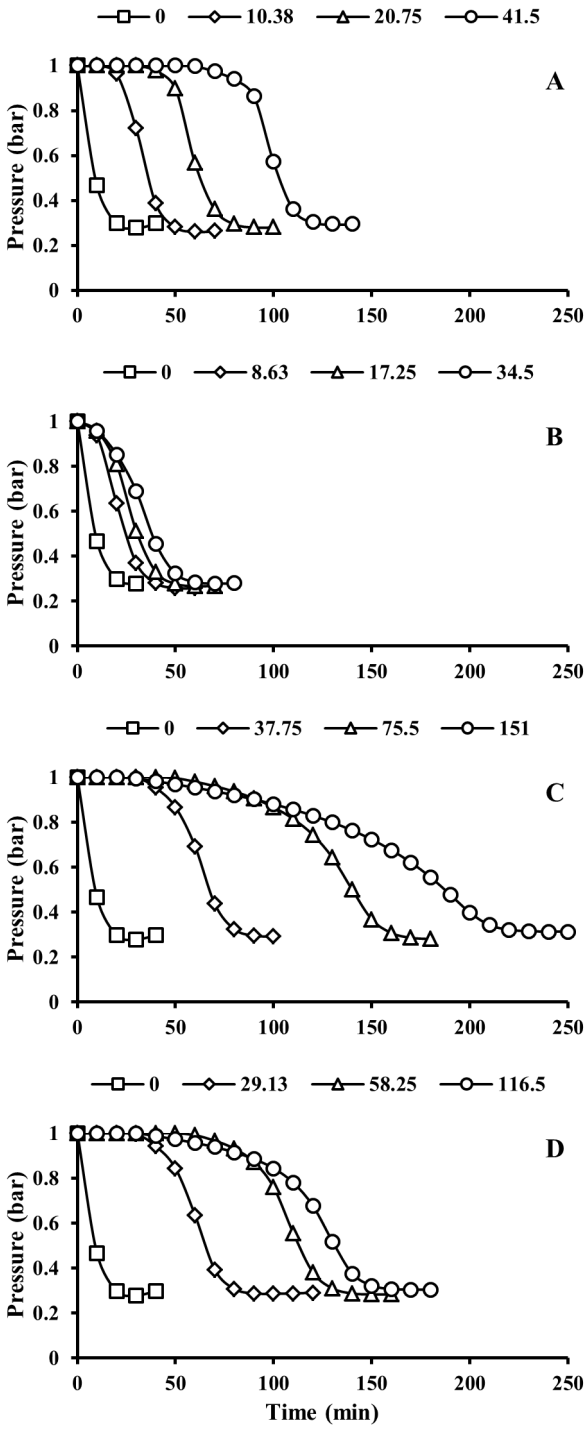


Figure 8 Oxidation pressure traces of 2 ml methyl linoleate with concentrations of: A) water-soluble extract from 0 to 41.5 x 10^{-3} (mol/dm^3); B) neutral extract from 0 to 34.5 x 10^{-3} (mol/dm^3); C) phenolic extract from 0 to 151 x 10^{-3} (mol/dm^3); D) organic acids extract from 0 to 116.5 x 10^{-3} (mol/dm^3), at 120 $^{\circ}$C and 1 bar of oxygen. (phenolic concentrations determined by FC assay assume only mono-phenolic present)

The antioxidancy of two significant phenolic components of the crude bio-oil and extracts was also examined, and Figures 9 and 10 shows the effect of eugenol and catechol.

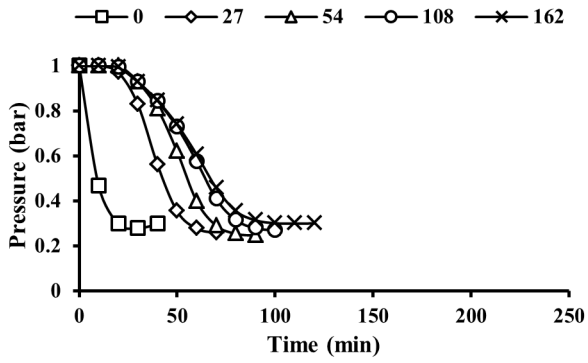


Figure 9 Oxidation pressure traces of 2 ml methyl linoleate with concentrations of 4-allyl-2-methoxyphenol (eugenol) from 0 to 162 x 10^{-3} (mol/dm^3) at 120 $^{\circ}$C and 1 bar of oxygen.

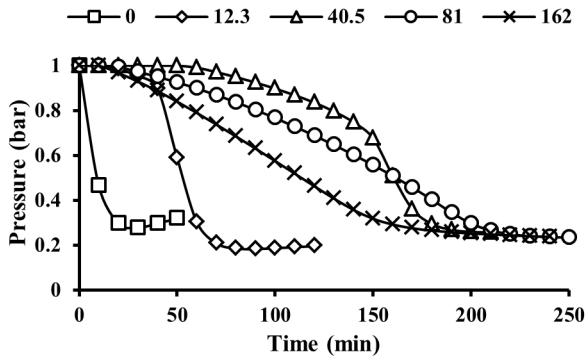


Figure 10 Oxidation pressure traces of 2 ml methyl linoleate with concentrations of 1, 2-benzenediol (catechol) from 0 to 162 x 10^{-3} (mol/dm^3) at 120 $^{\circ}$C and 1 bar of oxygen.

To allow a comparison of the antioxidancy examined in Figures 6-10, the induction times determined for the above Figures are given in Figures 11 and 12.

Induction time is a measure of the effectiveness of an antioxidant, and this was evaluated for this work by determining the time at which the tangent at the point of maximum gradient crosses the initial pressure, see for example Figure 6.

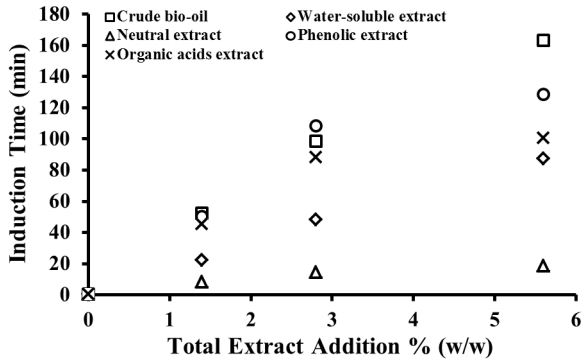


Figure 11 The induction times of 2 ml methyl linoleate at three total addition % (w/w) of crude bio-oil, water-soluble extract, neutral extract, phenolic extract and organic acids extract at 120 $^{\circ}$C and 1 bar oxygen

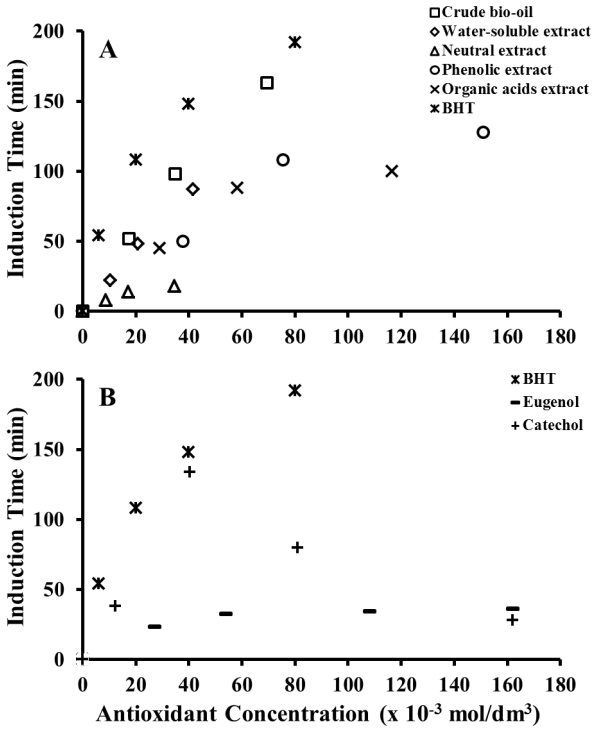


Figure 12 The induction times of 2 ml methyl linoleate with increasing concentrations of: A) crude bio-oil, water-soluble, neutral, phenolic and organic acids extracts; B) BHT, eugenol and catechol at 120 °C and 1 bar oxygen.

Figure 11 shows methyl linoleate induction times with total addition percentages (w/w) of the crude bio-oil and its extracts. The results illustrate the variation of inhibition activity by each extract and the crude bio-oil at three total addition percentages (1.4, 2.8 and 5.6% w/w). The crude bio-oil, phenolic and organic acids extracts gave approximately similar induction times with the total addition of 1.4 and 2.8% (w/w). However, with the total addition of 5.6%, crude bio-oil gave the highest induction time (163 min) in comparison with the other extracts. Moreover, neutral extract gave the lowest induction times at the three investigated addition percentages (w/w) followed by the water-soluble extract.

The results from Figure 12 indicated that the addition of the commercial phenolic antioxidant BHT increased methyl linoleate induction time from 0 to 192 minutes at a concentration of 80 x 10^{-3} mol/dm^3. BHT, along with the other antioxidants show "saturation" effect whereby it is less effective the more is added, most strikingly for eugenol where the induction time was essentially unchanged (~34 minutes) when its concentration increased threefold from 54 to 162 x 10^{-3} mol/dm^3.

The inhibition difference between BHT and eugenol could be attributed to many factors. However, generally, phenolic antioxidants can be evaluated for their antioxidant power by the bond dissociation energy (BDE) of the phenol O-H bond and by the kinetic rate constant for inhibition (k_{inh}), and from a thermodynamic point of view, the O-H BDE value of the phenolic antioxidant has to be lower than the O-H BDE value of the ROO-H (368.2 kJ/mol) formed in the inhibition mechanism in order to give a more favourable reaction.[35] Therefore, comparison of the O-H BDE values for BHT (339.32 kJ/mol) and eugenol (351.5 kJ/mol), as presented in Table 7, indicate that BHT provide more exothermic reactions towards peroxy radicals (ROO$^\bullet$) than eugenol, thus better antioxidant power.

For the crude bio-oil and its extracts, the results from Figure 12 clearly show that the crude bio-oil addition to methyl linoleate also increased its induction time from 0 to 163 minutes at increasing concentrations from 0 to 69.5 x 10^{-3} mol/dm^3 (phenolic concentrations determined by FC assay assume only mono-phenolic present). In comparison with the other extracts, crude bio-oil has the best inhibition performance, and the overall ranking order was as follows: crude bio-oil>water-soluble extract>organic acids extract>phenolic extract>neutral extract. Surprisingly, the water-soluble extract showed comparable induction times to the crude bio-oil at similar molar concentration, and neither showed any saturation effect when increasing their concentration in methyl linoleate. Strangely, the phenolic extract did not show a better inhibition performance than the crude bio-oil, as had been expected.

However, in comparison with eugenol, phenolic extract was better at the inhibition performance than eugenol, especially, at high molar concentrations (> 27 x 10^{-3} mol/dm^3, phenolic concentrations determined by FC assay assume only mono-phenolic present). The inhibition difference between the crude bio-oil and the phenolic extract could be attributed to the absence of other phenolic components which are either better antioxidants or able to create a synergistic effect for stronger inhibition performance.

From the GC-FID quantification results presented in Table 3, 1,2-benzenediol (catechol) has the highest phenolic concentration among the other phenols in the water-soluble extract, and also in the crude bio-oil. Consequently, catechol could be a key phenolic component that plays a significant role as a powerful inhibitor among the other identified phenols in spruce crude bio-oil. Figure 12 also shows the induction times of catechol towards methyl linoleate autoxidation at increasing molar concentrations.

Table 7 Bond dissociation energies (BDE) of some selected phenols O-H bond (kJ/mol) and their rate constant (M^{-1} s^{-1}) at 30 °C for H abstraction by peroxy radicals (ROO$^\bullet$).

Compound	BDE / kJ/mol	Ref.	k_{inh} x 10^4 / M^{-1} s^{-1}	Ref.
Phenol	369 ± 3	36	0.6	37
BHT	339.0 ± 0.5	36	1.4	38
Catechol	342.25	39	55	35
2-Methoxyphenol	354.40 ± 0.8	37	0.47	37
2-Methoxy-4-methylphenol	346.4 ± 0.9	37	1.2	37
Eugenol	351.5	40	\[a]	\

[a] Not available.

Remarkably, the results indicated that catechol showed similar induction times with the crude bio-oil at below 40.5 x 10^{-3} mol/dm^3. However, unexpectedly, catechol at concentrations greater than 40 x 10^{-3} mol/dm^3 showed a noticeably reduced antioxidant effect.

The antioxidant activity of catechol is known to be very strongly affected by the polarity of the medium in which it functions, for instance, 3,5-di-tert-butylcatechol at 30 °C, is 1.2x10^3 times less active in acetone than hexane (by contrast, BHT is comparatively unaffected by the polarity of the medium, being just 7 times less effective in acetone than hexane).[41]

The polarity of methyl linoleate is low, in comparison with the antioxidants used for this work. As the initial antioxidant concentration is increased this will give a corresponding (relatively small) increase in polarity of the linoleate-antioxidant medium. From the work of Barclay *et al*[41] this would be expected to have little effect on the activity of BHT, but it is suggested here that the small amount of added catechol could be increasing the polarity of the medium sufficient to have a substantial effect on the activity of the catechol, due to the exceedingly high sensitivity of catechol to the polarity of the medium.

This effect could also be exacerbated by the catechol used in this work having no alkyl groups to aid with solubilizing the antioxidant (by comparison, Barclay *et al*[41] used the di-alkyl substituted di-tert-butylcatechol, which would be much more soluble in non-polar media than unsubstituted catechol).

By contrast, the unsubstituted catechol used in this work would be expected to be less soluble, and that above a threshold saturation concentration further addition of catechol would results in the catechol molecule aggregating together, so that they are no longer homogeneously distributed throughout the medium. This would have two effects, the bulk of the medium would have a lower catechol concentration than expected from the amount added, and further, where catechol molecules have aggregated to an extent, these would be in a much more polar surroundings, so, from the work of Barclay *et al*[41] these molecules would be substantially less effective and would contribute little antioxidancy. This explanation would be a topic for future investigation.

Comparing crude bio-oil inhibition performance with BHT, crude bio-oil did not show a saturation effect like BHT, and the crude bio-oil induction times were approximately comparable with BHT at an approximately similar molar concentration in methyl linoleate. Remarkably, BHT and catechol inhibition performances were similar at the molar concentration of 40 x 10^{-3} mol/dm^3. The inhibition performance agreement between BHT and catechol at low concentrations could be attributed to the comparable O-H BDE value for catechol (341.41 kJ/mol) and BHT (339.32 kJ/mol).

Conclusions

The microwave-enhanced pyrolysis of spruce woodchips generates a significant amount of crude bio-oil (18.5% w/w) that was rich in phenols and found to be 23% (w/w) by Folin-Ciocalteu (FC) assay.

The potential use of the crude bio-oil phenols as an antioxidant for protecting biodiesel from autoxidation has been investigated. Blending small concentrations of crude bio-oil (1.4-5.6% w/w) with methyl linoleate could significantly increase the methyl linoleate induction time, and the induction time of 163 minutes has been achieved when methyl linoleate was blended with 5.6% (w/w) of crude bio-oil.

In comparison to the commercial antioxidant BHT, crude bio-oil gave approximately comparable induction times to BHT when blended with methyl linoleate at approximately similar molar concentrations of total phenols.

To understand and to maximize the antioxidant power of the crude bio-oil phenols, four extracts were isolated from the crude bio-oil: water-soluble, neutral, phenolic and organic acids extracts. Successfully, the phenolic extract had the highest phenolic concentration (49.6% w/w) between the other extracts.

From testing antioxidancy of these extracts, it has been revealed that the extracts have lower antioxidative effects than the crude bio-oil on methyl linoleate undergo oxidation, which was significant especially when the crude bio-oil contained a lower phenolic concentration (23% w/w) than the phenolic extract (49.6% w/w). This finding suggests that the phenolic species in the crude bio-oil vary in their antioxidant power, which was further confirmed by examining eugenol and catechol—two significant phenolic components of the crude bio-oil.

Remarkably, catechol was found to be very effective, like the crude bio-oil, in hindering methyl linoleate oxidation cycle. However, on the other hand, eugenol was less effective than either catechol or crude bio-oil when treated at equivalent molar concentrations.

Acknowledgements

The authors wish to thank Vitali Budarin for advice and assistance with the microwave pyrolysis and Thomas Dugmore for discussion on the Folin-Ciocalteu assay and A. S. Alwehaibi thanks the Ministry of Education of the Kingdom of Saudi Arabia for financial support.

References

1 Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC, http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009L0028&from=EN, (accessed June 2014).

2 B. Flach, K. Bendz, R. Krautgartner and S. Lieberz, *EU-27 biofuels annual*, NL3034, USDA-FAS GAIN Report, The Hague, 2013.

3 Renewable Transport Fuel Obligation statistics: obligation period 6, 2013/14, report 3, https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/306399/rtfo-2013-14-year-6-report-3.pdf, (accessed June 2014).
4 O. Herbinet, W. J. Pitz and C. K. Westbrook, *Combustion and Flame*, 2008, **154**, 507-528.
5 B. Moser, *Journal of the American Oil Chemists' Society*, 2009, **86**, 699-706.
6 S. J. Clark, L. Wagner, M. D. Schrock and P. G. Piennaar, *J. Am. Oil Chem. Soc.*, 1984, **61**, 1632-1638.
7 A. Monyem and J. H. Van Gerpen, *Biomass and Bioenergy*, 2001, **20**, 317-325.
8 G. Knothe, *Fuel Processing Technology*, 2007, **88**, 669-677.
9 R. L. McCormick, M. Ratcliff, L. Moens and R. Lawrence, *Fuel Processing Technology*, 2007, **88**, 651-657.
10 E. Sendzikiene, V. Makareviciene and P. Janulis, *Polish Journal of Environmental Studies*, 2005, **14**, 335-339.
11 S. Schober and M. Mittelbach, *European Journal of Lipid Science and Technology*, 2004, **106**, 382-389.
12 Z. Yaakob, B. N. Narayanan, S. Padikkaparambil, S. Unni K and M. Akbar P, *Renewable and Sustainable Energy Reviews*, 2014, **35**, 136-153.
13 G. Aguilar, G. Mazzamaro and M. Rasberger, in *Chemistry and Technology of Lubricants*, ed. R. M. Mortier, M. F. Fox and S. T. Orszulik, Springer, Netherlands, 3rd edn., 2010, ch. 4, pp. 107-152.
14 M. Laguerre, J. Lecomte and P. Villeneuve, *Prog. Lipid Res.*, 2007, **46**, 244-282.
15 N. Singh, P. J. O'Malley and P. L. A. Popelier, *Physical Chemistry Chemical Physics*, 2005, **7**, 614-619.
16 A. K. Domingos, E. B. Saad, W. W. D. Vechiatto, H. M. Wilhelm and L. P. Ramos, *Journal of the Brazilian Chemical Society*, 2007, **18**, 416-423.
17 C. Liang and K. Schwarzer, *J Amer Oil Chem Soc*, 1998, **75**, 1441-1443.
18 Y. C. Liang, C. Y. May, C. S. Foon, M. A. Ngan, C. C. Hock and Y. Basiron, *Fuel*, 2006, **85**, 867-870.
19 H. Tang, A. Wang, S. Salley and K. Y. S. Ng, *Journal of the American Oil Chemists' Society*, 2008, **85**, 373-382.
20 Supriyono, H. Sulistyo, M. F. Almeida and J. M. Dias, *Fuel Processing Technology*, 2015, **132**, 133-138.
21 A. Ingendoh, *Lipid Technology*, 2010, **22**, 83-86.
22 *US Pat.*, US4209647, 1980.
23 A. Effendi, H. Gerhauser and A. V. Bridgwater, *Renewable and Sustainable Energy Reviews*, 2008, **12**, 2092-2116.
24 S. Wang, Y. Wang, Q. Cai, X. Wang, H. Jin and Z. Luo, *Separation and Purification Technology*, 2014, **122**, 248-255.
25 J.C. Sternberg, W.S. Gallaway, and D.T.L. Jones, In *Gas* Chromatography, ed. N. Brenner, J.E. Callen, and M.D. Weiss, Academic Press, New York, 1962, ch. XVIII, pp. 231-267.
26 J. T. Scanlon and D. E. Willis, *J. Chromatogr. Sci.*, 1985, **23**, 333-340.
27 A. D. Jorgensen, K. C. Picel and V. C. Stamoudis, *Anal. Chem.*, 1990, **62**, 683-689.
28 S.-H. Jung, W.-M. Koo and J.-S. Kim, *Energy*, 2013, **53**, 33-39.
29 V. L. Singleton, R. Orthofer and R. M. Lamuela-Raventos, *Methods Enzymol.*, 1999, **299**, 152-178.
30 A. L. Waterhouse, in *Current Protocols in Food Analytical Chemistry*, ed. R. E. Wrolstad, John Wiley & Sons, Inc., New York, 2002, ch. I1, units I1.1.1-I1.1.8.
31 M. S. Stark, J. J. Wilkinson, J. R. L. Smith, A. Alfadhl and B. A. Pochopien, *Industrial & Engineering Chemistry Research*, 2010, **50**, 817-823.
32 T. I. J. Dugmore and M. S. Stark, *Fuel*, 2014, **124**, 91-96.
33 V. L. Budarin, P. S. Shuttleworth, M. De Bruyn, T. J. Farmer, M. J. Gronnow, L. Pfaltzgraff, D. J. Macquarrie and J. H. Clark, *Catalysis Today*, 2015, **239**, 80-89.
34 C. A. Mullen, G. D. Strahan and A. A. Boateng, *Energy & Fuels*, 2009, **23**, 2707-2718.
35 M. Lucarini and G. F. Pedulli, *Chemical Society Reviews*, 2010, **39**, 2106-2119.
36 M. Lucarini, P. Pedrielli, G. F. Pedulli, S. Cabiddu and C. Fattuoni, *The Journal of Organic Chemistry*, 1996, **61**, 9259-9263.
37 R. Amorati, S. Menichetti, E. Mileo, G. F. Pedulli and C. Viglianisi, *Chemistry-A European Journal*, 2009, **15**, 4402-4410.
38 G. W. Burton, T. Doba, E. Gabe, L. Hughes, F. Lee, L. Prasad and K. U. Ingold, *J. Am. Chem. Soc.*, 1985, **107**, 7053-7065.
39 M. Lucarini, G. F. Pedulli and M. Guerra, *Chemistry-A European Journal*, 2004, **10**, 933-939.
40 Y. Murakami, S. Ito, T. Atsumi and S. Fujisawa, *In Vivo*, 2005, **19**, 1039-1043.
41 L. R. C. Barclay, C. E. Edwards and M. R. Vinqvist, *J. Am. Chem. Soc.*, 1999, **121**, 6226-6231.