Research Article

Screening of antioxidants as stabilisers for *Jatropha curcas* L. oil

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The effect of antioxidants on the oxidation stability of oils extracted from *Jatropha curcas* seeds was measured by the accelerated oxidation test specified in EN 14112 using commercial Rancimat 873 equipment. To find the appropriate antioxidant for jatropha oil, fourteen different antioxidants were screened at concentrations of 500 and 1000 ppm. Pyrogallol (PY) and propyl gallate (PG) significantly improved the oxidative stability of jatropha oil and PY was further studied at concentrations of 50–1000 ppm. Even at concentrations as low as 50 ppm, PY was found to fulfill the specifications set by the DIN 51605 norm for plant oil as biofuels. Mixtures of antioxidants were tested at concentrations varying between 100 and 1000 ppm, showing a synergistic effect for the combination of PY and N,N′-di-sec-butyl-*p*-phenylenediamine at all concentrations and ratios tested. It further was found that the quality, i.e. the history of the oil in terms of processing, age and storage conditions, strongly affects the performance of PY as the antioxidant. PY in particular improved the oxidative stability of oxidized and highly acidic oils. PY was found to be a good antioxidant for both jatropha oil and the derived biodiesel.

Practical applications: Jatropha oil is one of many potential triglyceride feedstocks suitable for the production of biofuel or other consumer products. Finding the best antioxidant for this particular resource is important since the resistance to oxidative degradation depends on the chemical structure of the triglyceride. Pyrogallol was found to be the preferred antioxidant with beneficial results found for oxidized and, as a result, highly acidic oils. The results of this study are useful for companies involved in jatropha oil-derived products such as biodiesel, lubricants or other non-food applications.

Keywords: Antioxidants / *Jatropha curcas* L. oil / Oxidative stability / Synergism

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1 Introduction

In recent decades bio-fuels such as bio-diesel and pure plant oils have received much attention because they can be extracted from renewable resources, are biodegradable and can be used in different end-uses that currently apply hydrocarbon-based fuels [1]. In general, it is used in transportation and agricultural sectors, for electricity generation, and for domestic applications such as cooking, lighting or heating. When it is produced in sustainable ways, bio-fuel also has the potential to reduce net carbon emissions compared to petroleum-derived fuel [2].

Biodiesel is the most widely vegetable oil based bio-fuel to date and is usually produced from edible plant oils – such as palm oil, sunflower oil, rape-seed or soybean oil. However, these resources are not economically attractive for this

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Abbreviations: APE, allylic position equivalent; **BAPE,** bis-allylic position equivalent; **BHA,** butylated hydroxyanisole; **BHT,** butylated hydroxytoluene; **DT,** dimethyl 3,3′-thiodipropionate; **DTBHQ,** 2,5-di-*tert*-butylhydroquinone; **IP,** induction period; **JO,** jatropha oil; **MMBP,** 2,2′-methylenebis-(4-methyl-6-*tert*-butylphenol); **PD,** N,N′-di-sec-butyl-*p*-phenylenediamine; **P-DSC,** pressurized-DSC; **PG,** propyl gallate; **PY,** pyrogallol; **SE,** synergism effect; **TBHQ,** *tert*-butylhydroquinone; **α-T,** α-tocopherol; **TP,** triphenyl phosphite purpose as they are part of the food-chain and more expensive than diesel fuel.[3, 4] In recent years, jatropha oil is launched as a promising raw material for biodiesel production as it is non-edible oil and thus will not impair a food security issue [5, 6]. As a result, jatropha is considered to be a more sustainable feedstock for fuel production than the other above-mentioned food-based crops [5].

Jatropha oil, as pure plant oil, is used as a fossil-based fuel substitute for diesel engines or cookers. It is suitable as fuel for compression ignition diesel engines either as blends with petro-diesel or after chemical conversion into biodiesel [7–9]. It is reported that the use of a blend of 10% v/v jatropha oil with diesel fuel does not cause engine problems [8]. Besides numerous academic studies, many efforts to use plant oil directly as fuel in diesel engines come from industries. ELSBETT Technologie GmbH has been developing diesel engines for pure plant oil since 1970. German companies such as German Deutz AG, Hatz diesel GmbH, ELSBETT Technologie GmbH and Diesel Morten und Gerätebau GmbH (DMS) offer engines that also can be fueled with pure plant oil. The engines were tested in long-term experiments with different plant oils, including jatropha oil [10]. Bosch and Siemens (BSH GMbH) have developed a cooker that can be fueled with plant oil, called Protos [11]. In addition, biodiesel derived from jatropha oil satisfies the specifications of both the EN 14214 and US ASTM D 6751-02 standards [5]. These technical developments and the successful oil quality assessments will stimulate more interest to use jatropha oil as fuel, either as pure plant oil or as biodiesel.

However, there is one major problem associated with the use of pure plant oil and the derived biodiesels. Both substances are more susceptible to oxidation than petroleum-derived fuels. Oxidative stability has become a concern, especially when plant oil or biodiesel is stored for longer periods of time. Plant oils, as well as biodiesel, oxidize and degrade over time which results in peroxides, organic acids and insoluble polymers [12, 13]. When used as fuel, these oxidation products can cause engine problems such as filter plugging, fuel injector deposits, injector coking and corrosion [14, 15]. These operability problems can increase maintenance costs and decrease equipment reliability.

Oxidative stability of plant oils is mainly determined by the chemical structure of the fatty acids present in the triglycerides, especially the number and position of unsaturated C–C bonds. The bis-allylic positions in common polyunsaturated fatty acids such as linoleic acid (one bis-allylic position at C-11) and linolenic acid (two bis-allylic positions at C-11 and C-14) are more susceptible to oxidation than allylic positions in mono-unsaturated fatty acids such as oleic acid [16]. Relative rates of oxidation based on oxygen uptake at 37°C are 1, 42 and 98 for the ethyl esters of oleate, linoleate and linolenate [17]. The corresponding induction periods till oxidation as measured by the EN 14112 method for the ethyl esters of oleate, linoleate and linolenate are 3.5, 1 and 0.2 h, respectively [18].

Soybean oil is the main vegetable oil produced in the USA, rapeseed and sunflower oils in Europe, and palm oil in Southeast Asia (mainly Malaysia and Indonesia). They are all being considered as feedstock for biodiesel production [19]. The fatty acid compositions of these oils and jatropha oil are shown in Table 1 (ref.[6, 20, 21]). Palm oil with its high saturated fatty acid content (as indicated by a low APE and BAPE) showed better oxidative stability (measured as IP) than rapeseed and soybean oil. As jatropha oil has a lower APE and BAPE compared to rapeseed oil, it is expected to show a better oxidative stability than this oil.

Besides the fatty acids structure as a major factor influencing oxidative stability, temperature, air and/or light exposure, the presence of metallic compounds, water and other minor components affect the oxidative stability of the plant oils. The minor components are peroxides, free fatty acids, phospholipids, mono and di-glycerides, chlorophylls, carotenoids, sterols, phenolic compounds and tocopherols which are naturally present in plant oils. Some of them act as pro-oxidants while others act as antioxidants [22].

Table 1. Fatty acid composition of *Jatropha curcas* oil

Fatty acid composition	PO	RSO	SFO	SBO	JO
C16:0 palmitic acid	44.1	3.6	6.3	11.0	15.3
C18:0 stearic acid	4.4	1.5	3.7	4.0	6.6
C18:1 oleic acid	39.0	61.6	24.3	23.4	41.0
C18:2 linoleic acid	10.6	21.7	65.3	53.2	35.3
C18:3 linolenic acid	0.3	9.6	–	7.8	0.3
Others/unidentified	1.6	2	0.4	0.6	1.5
APE	100.2	187.4	179.2	168.8	155.4
BAPE	11.2	40.9	65.3	68.8	35.9
IP (h) [21]	19.95	7.14	–	5.24	–

IP, induction period; PO, palm oil; RSO, rapeseed oil (low erucic); SFO, sunflower oil; SBO, soybean oil; APE (allylic position equivalent) – calculated with the following equation = 2 × (% C18:1 + % C18:2 + % C18:3).
BAPE (bis-allylic position equivalent) – calculated with the following equation = 1 × % C18:2 + 2 × % C18:3 [16].

Antioxidants are successfully being used to delay the oxidation reaction of plant oil-derivedproducts. Some of the most common synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), pyrogallol (PY), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ). Some common natural antioxidants are: α-tocopherol (α-T) and ascorbic acid. Despite numerous publications on the effect of natural and synthetic antioxidants on the stability of plant oils or biodiesel, limited work is available on the effect on the oxidative stability of jatropha oil. Likewise, little is known regarding the use of mixtures of antioxidants and potential antioxidant synergism in the stabilization of jatropha oil. Dittmar et al. [23] investigated eleven phenolic antioxidants and one phosphate-based antioxidant in sunflower-oil based biodiesel in a range of 100–10 000 ppm. In their study, PY was found to be the best antioxidant. Dunn et al. [24] evaluated four synthetic antioxidants, TBHQ, BHT, BHA and PG, and one natural type, α-T, for increasing the oxidative stability of soybean-oil based biodiesel using dynamic non-isothermal pressurized-DSC (P-DSC). The results showed antioxidant activity in the following decreasing order: PG ~ BHT > BHA> TBHQ > α–T > none. Mittelbach and Schober [25] investigated the influence of a series of commercial natural and synthetic antioxidants on the oxidative stability of (distilled and crude) biodiesel, prepared from various feedstocks, namely rapeseed, sunflower, tallow and used cooking oils. The oxidative stability could be improved significantly with TBHQ, PY and PG. The effectiveness of antioxidant was found to depend on the fatty acid composition and the purity of the samples. Sarin et al. [26] compared the effectiveness of natural (α-T) and synthetic antioxidants (TBHQ, BHT, *tert*-butylated phenol derivatives (TBP), octylated butylated diphenyl amine (OBPA)) on jatropha-oil based biodiesel. The results showed antioxidant activity in the following order: TBHQ > BHT > tert-butylated phenol derivatives > octylated butylated diphenyl amine > α-T. Jain and Sharma [27] studied the effectiveness of PY, PG, TBHQ, BHT and BHA on the oxidative stability of jatropha biodiesel-diesel blends. The effectiveness of the antioxidants investigated was found to be: PY > PG > TBHQ > BHT > BHA. Jain and Sharma [28] also studied the effect of PY on the oxidative stability of metal contaminated jatropha-oil based biodiesel. They developed several relations for expressing the oxidation stability as a function of antioxidant and metal concentration. Sarin et al. [29] studied the synergism between BHT and a metal deactivator (N,N′-disalicylidene-1,2-diaminopropane) in metal contaminated jatropha-oil based biodiesel. It was found that the usage of antioxidant can be reduced by 30–50%, even with very small amounts of metal deactivator added to jatropha-oil based biodiesel.

The effect of antioxidant addition on the biodiesel quality and its emission characteristics has been evaluated by several researchers. Schober and Mittelbach [30] found that addition of antioxidants did not lead to significant changes in biodiesel quality parameters such as viscosity, density, carbon residues, cold filter plugging point (CFPP) and sulphated ash content. In terms of acidity, a noticeable increase was observed when antioxidant is added at a concentration of 1000 ppm. At lower antioxidant concentrations, this increase was less dramatic, and the values remained within the required limits. Based on a study by Ryu [31], antioxidants had no significant effects on the combustion characteristics and the exhaust emissions of a diesel engine running on biodiesel.

The first objective of this study was to determine the effect of single antioxidants on the oxidative stability of jatropha oil. The second objective was to evaluate potential synergism and its mechanism of binary mixtures of antioxidants on the oxidative stability of jatropha oil. To achieve a maximum efficiency, primary antioxidants are often used in combination with other primary antioxidants or with a secondary antioxidant. The use of synergistic antioxidant mixtures allows for a reduction in the concentration of each antioxidant and also increases the antioxidant effectiveness as compared to the activity of single antioxidant [32]. The third objective was to investigate the effect of various jatropha oil feedstocks including the effect of the acid value on the oxidative stability in the presence of antioxidants. The final aim was to study the use of antioxidant in jatropha-oil based biodiesel since the degradation reaction pathway, for alkyl esters derived from naturally occurring fatty oils, is also determined by the olefinic unsaturation of the fatty acid chain. The chemical structure of the fatty acid chain is not affected by the transesterification of the triglyceride to the alkyl ester [33]. The stability against oxidation is evaluated using the Rancimat test, as recommended in EN 14112. This method is a common procedure which is used to investigate the influence of antioxidants or other extraneous materials on the oxidative stability of biodiesel and/or pure plant oils [34]. The German standard for oil-based fuel, DIN 51605, requires a minimum Rancimat induction period of 6 h at 110°C.

2 Materials and methods

2.1 Materials

The crude jatropha oil (JO) used in this experiment was obtained from Diligent (The Netherlands), termed as JO1. The oil was used in the first exploration stage of this study (Section 3.1 and 3.2). In addition to JO1, several jatropha oils were used to study the effect of various feedstocks to antioxidant activity. JO2 is jatropha oil from Diligent (JO2) which has been stored for one year at 6°C. The other crude jatropha oils were laboratory pressed from hulled or dehulled seeds obtained from Cape Verde, namely JO3 and JO4. Jatropha seed from Cape Verde arrived in February 2009 and was stored at RT and a relative humidity of ±45%. JO3 is oil pressed from jatropha seed with a screw press (BT Bio Presse Type 50, BT biopresser aps, Dybvad, Denmark) in February

Table 2. Oil quality parameters of various jatropha oil feedstocks

Parameters	Method	JO1	JO2	JO3	JO4
Density (15°C; kg/m^3)	Pycnometer	930.1	933.5	923.5	927.8
Kinematic viscosity (40°C; mm^2/s)	Viscometer	38.7	39.3	35.8	37.2
Flash point (°C)	ASTM D 6450	164	156	228	204
Cloud point (°C)	ASTM D 6749	−2	−1	−1	−1
Pour point (°C)	ASTM D 2500	−4	−4	−3	−3
Oxidative stability (h)	DIN EN 14112	1.58	1.22	11.59	5.06
Phosphorus content (ppm)	DIN EN 14107	4.5	4.5	8.3	3.3
Acid value (mg KOH/g oil)	DIN EN 14104	4.95	7.8	1.4	1.3
Water content (ppm)	DIN EN ISO 12937	842	1232	941	730
Peroxide value (meq/kg oil)	AOCS Cd 8-53	14.31	16.22	6.87	7.43
Tocopherols content (ppm)	DGF F-II 4a	7	<5	18	10
Ca (ppm)	DIN 51627-6	1.3	1.3	1.2	0.7
Mg (ppm)	DIN 51627-6	0.9	0.9	0.5	<0.5
Fe (ppm)	ICP OES	0.29	0.29	0.15	0.06
Cu (ppm)	ICP OES	n.d	n.d	n.d	n.d

n.d., not detectable. Phosphorus content is indicating phospholipids.

2009, while JO4 is pressed from dehulled jatropha seeds using a hydraulic press (self-manufactured laboratory scale) at September 2011. The seeds were deshelled directly before pressing. All the oils were stored in a refrigerator at 6°C until further use. The quality of these oils is shown in Table 2. Jatropha biodiesel was made from JO4 according to the transesterification method described in L. Daniel et al. [35]. The antioxidants with a purity of 95–99.9% were bought from Sigma–Aldrich (Amsterdam, The Netherlands) with exception of Ethanox 4702 (4,4′-methylenebis-(2,6-di-*tert*-butylphenol) which was kindly donated by Albemarle (Amsterdam, The Netherlands). Most antioxidants were soluble in jatropha oil at RT up to a concentration of 1000 ppm, with the exception of PY and PG which were heated for 10 min at 60°C for solubilisation. Potassium hydroxide (85%), oxalic acid (>99%), ethanol (96%), diethyl ether (>99%), hydranal solvent and hydranal titrant 5 were bought from Sigma–Aldrich (Amsterdam, The Netherlands).

2.2 Methods

Chemical analyses of the samples were carried out according to the standard test methods: AOCS Cd 8-53, DGF F-II 4a, DIN EN 14104, DIN EN ISO 12937, DIN EN 14112, DIN EN 14107, DIN EN 14538 and ICP OES (Inductively Coupled Plasma Optical Emission Spectrometry) for peroxide value, tocopherol contents, acid value, water content, oxidative stability, phosphorus content, Ca + Mg content and Fe + Cu content, respectively. Density analysis was carried out by measuring the mass of the samples with respect to their volume using a 10 mL pycnometer. The density at 15°C was obtained by extrapolation of data collected from 30 to 100°C, in 10°C increments. Dynamic Viscosity analysis was carried out by using a cone-and-plate viscometer AR 1000-N (40 mm 2° aluminium cone) at 40°C with a shear rate of 40/s for 10 min.Kinematic viscosity was calculated from the corresponding dynamic viscosity and density at corresponding temperature. Most of the chemical property analyses of plant oil samples were conducted in our laboratory with the exception of elements (P, Ca and Mg), metals (Fe and Cu) and tocopherol contents that were conducted by ASG Analytik-Service, Germany. Duplicate measurements were performed for each sample and average values were taken.

2.2.1 Oxidative stability measurement

Oxidative Stability was measured using a Rancimat Model 873 Apparatus (Metrohm, Herisau, Switzerland) in accordance with the Rancimat method EN 14112. In the apparatus, a stream of air is passed through the sample at 10 L/h while the heating block heated the sample at 110°C. The oxidation products – mainly organic acids – are transferred through the vessel containing distilled water where the conductivity is measured continuously. The time till the conductivity begins to increase rapidly is defined as the "induction period."

3 Results and discussion

3.1 Single antioxidant screening

Neat jatropha oil (JO1) showed an IP of 1.58 h, which is below the minimum limit of 6 h IP as required by EN 14112. Thus, an antioxidant must be added to enhance the IP of Jatropha oil. In the first part of the study, fourteen different antioxidants were screened at two addition levels, 500 and 1000 ppm. The best antioxidants were further studied at various concentrations and relationships expressing oxidative stability as function of antioxidant concentration were generated.

For this set of screening experiments, the study was conducted using jatropha oil from Diligent (JO1). Fourteen different types of antioxidants were investigated. The list was generated on the basis of studies performed with different plant oils and the derived biodiesels, and included one natural antioxidant, 11 synthetic chain breaking antioxidants (8 phenolic antioxidants and 3 amine-based antioxidants) and two hydroperoxide decomposers (one sulphur-based and one phosphate-based). Those antioxidants were screened at concentrations of 500 and 1000 ppm; the corresponding molar concentrations and induction periods are shown in Table 3. Most antioxidants had a positive effect on the stability of the jatropha oil. PY gave the highest oxidation stability, followed by PG and N,N′-disec-butyl-p-phenylenediamine (PD), expressed by induction periods at 1000 ppm of 36.15, 29.51 and 10.53 h, respectively. This is in agreement with results obtained for various types of plant oils and biodiesels [23, 25, 36]. The order of effectiveness of antioxidants in this study was in accordance with the results found by Chen et al. [36]. According to these authors, the order of the antioxidants that improved the oxidation stability of the Jatropha biodiesel was PY > PG > PD > 2,2′-methylenebis-(4-methyl-6-tert-butylphenol) (MMBP) > BHA > BHT ~ TBHQ > 2,5-di-*tert*-butylhydroquinone (DTBHQ) > α-T.

Most antioxidants enhanced the oxidative stability of jatropha oil, with the poorest result observed for α-T. This is in line with the limited activity of α-Tin plant oils reported elsewhere [23, 25, 31, 36]. α-Tocopherol is generally regarded as a poor antioxidant for plant oils, especially those containing poly-unsaturated fatty acids. It has been reported that the α-T activity strongly depends on the degree of triglyceride unsaturation, with the highest antioxidant activity of α-T recorded for the less unsaturated oils [37, 38].

TBHQ is known as a potent antioxidant. However, addition of TBHQ in jatropha oil did not give good oxidation stability compared to its effectiveness in other plant oils or biodiesel as reported elsewhere [31, 39–41]. TBHQ has a low activity in jatropha oil compared with BHA and BHT, which is in line with other studies [24, 36]. Several authors have concluded that the variances in antioxidant activity may be attributed to the differences in the fatty acid composition and/or minor components present in the biodiesel feedstock [25, 42–44].

The oxidative stability of the tested oil enhanced when the antioxidant concentration was raised from 500 ppm to 1000 ppm except for α-T which levelled off at higher concentration. However, this positive increase in induction period with increasing antioxidant concentration appeared to be non-linear except for PD, as observed in other study [36]. The reduced activity of α-T at higher concentrations is in good agreement with other studies [45–48]. The latter is due to the abstraction of hydrogen by the α-T radicals from poly-unsaturated fatty acids and their hydroperoxides. These reactions are reported to become more significant at higher concentration of α-T [49].

Table 3. Oxidative stability of JO1 treated with various antioxidants

	500 ppm		1000 ppm	
Antioxidants	IP (h)	$M \times 10^3$	IP (h)	$M \times 10^3$
JO1 without antioxidant treatment has IP = 1.58 h				
Primary antioxidant (phenolic antioxidant)				
Pyrogallol – (PY)	27.34	3.6	36.15	7.2
Propyl gallate – (PG)	22.49	2.2	29.51	4.3
2,2′-Methylenebis(4-methyl-6-*tert*-butylphenol) – (MMBP)	4.51	1.3	5.98	2.7
4,4′-Methylenebis(2,6-di-*tert*-butylphenol) – (E4702)	3.35	1.1	4.58	2.2
Butylated hydroxyanisole – (BHA)	3.70	2.5	4.82	5.1
Butylated hydroxytoluene – (BHT)	3.05	2.1	4.00	4.1
tert-Butylhydroquinone – (TBHQ)	2.47	2.7	3.19	5.5
2,5-Di-*tert*-butylhydroquinone – (DTBHQ)	2.22	2.1	2.71	4.1
α – Tocopherol – (α-T)	1.91	1.1	1.80	2.1
Primary antioxidant (aromatic amines antioxidant)				
N, N′-Di-sec-butyl-p-phenylenediamine – (PD)	5.80	2.1	10.53	4.2
N-Phenyl-1-naphthylamine – (NN)	3.68	2.1	5.43	4.2
Ethoxyquin – (EQ)	2.66	2.1	3.37	4.2
Secondary antioxidant/hydroperoxide decomposers				
Dimethyl 3,3′-thiodipropionate – (DT)	3.47	1.5	5.64	3.0
Triphenyl phosphite – (TP)	2.76	2.2	3.34	4.4

M, molar concentration.

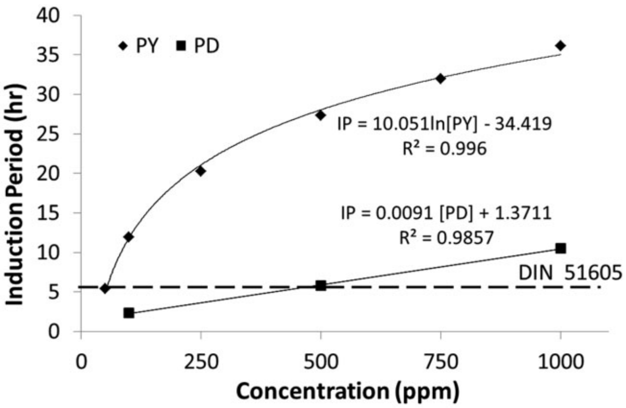

Figure 1. Variation of PY and PD concentrations in *Jatropha curcas* oil (JO1).

3.2 Effect of concentration

The data show that PY addition results in the longest induction time. Therefore PY was added to the jatropha oil at different concentration ranging from 50 to 1000 ppm. The goal of the measurements was to study the dependence of antioxidant concentration on jatropha oil oxidative stability and to determine the corresponding antioxidant concentration that leads to an induction period of at least 6 h, which is the minimum requirement according to the DIN 51605 German fuel standard. Antioxidant activity increased with increasing concentration (see Fig. 1). The induction periods of jatropha oil formulated with PY shows a logarithmic correlation with antioxidant concentration. As the PY concentration increases, further addition of PY did not give significant improvement in oxidative stability of jatropha oil.

Of the non-phenolic primary antioxidants, PD is the best performer in the list of antioxidants screened. In contrast to the logarithmic relationship found for PY, PD showed a linear relationship with increasing concentrations. This suggests that addition of PD beyond 1000 ppm can further increase the oxidative stability of jatropha oil. These antioxidant concentration profiles are in accordance with the results reported by Chen and Luo [50] who studied various antioxidants including PY and PD.

The oxidative stability relationships for PY or PD as function of concentration are shown by the following equations:

$$\text{Induction period (PY)} = 10.051 \ln C_{\text{PY}} - 34.419 \quad (C_{\text{PY}} \text{ in ppm})$$
$$\text{Induction period (PD)} = 0.0091 C_{\text{PD}} + 1.3711 \quad (C_{\text{PD}} \text{ in ppm})$$

The data clearly suggest the use of PY as antioxidant for jatropha oil because oil containing 50 ppm PY exhibited better stability than oils containing commercial antioxidants such as BHA, BHT and TBHQ added at concentrations of 1000 ppm (see Table 2). Even a low concentration of PY (50–100 ppm) is sufficient to achieve the minimum requirement of 6 h induction demanded by the DIN 51605 German fuel standard for pure plant oil.

According to Chen et al. [36], Loh et al. [51] and Cuvilier et al. [52], PY with three adjacent active electron-donating hydroxyl groups in the quinone oxidative state can release the greatest number of hydrogen atoms and thereby, effectively prevent oxidation. Besides the contribution of three adjacent hydroxyl groups, the hydrophilicity of PY also has a significant effect on the efficiency of PY. Based on solubility parameters, PY is the least soluble among the antioxidants studied (calculated using Molecular Modelling Pro Plus software for Windows released by Chem SW @ Inc). Hydrophilic antioxidants are more effective antioxidants than lipophilic types for bulk oil systems. The hydrophilic antioxidant is apparently more protective for oil system by being more oriented towards the air–oil interface, the location where oil oxidation reactions would be expected owing to the high concentration of oxygen and pro-oxidants. As expected, lipophilic antioxidants provide better protection in water-oil emulsion systems [53, 54]. In addition, it is well known that gallols (including pyrogallol) are effective metal chelators [55].

3.3 Screening of antioxidant binary mixtures

To achieve maximum efficiency, primary antioxidants are frequently used in combination with other primary antioxidants or secondary ones [56]. This second part of the study aims to evaluate potential synergism of antioxidant mixtures.

Several antioxidants were tested as mixtures of primary antioxidants and as combinations of primary with secondary antioxidants. Combination of antioxidants is aimed to take advantage of their different properties. PY has shown excellent activity among phenolic antioxidants, but it has poor solubility in oil. PD was chosen as it showed the highest activity among amine-based antioxidants and good solubility. MMBP is chosen instead of PG since the former shows a better solubility. Triphenyl phosphite (TP) and dimethyl 3,3′-thiodipropionate (DT) are secondary antioxidants (hydroperoxide decomposer), the rest are primary antioxidants. The oxidative stability of the jatropha oil treated with various binary antioxidant mixtures is presented in Table 4.

To evaluate the occurrence of synergistic effects, so-called synergism equivalents (% SE) are calculated based on the following equation [57].

$$\%\,\text{SE} = \frac{(\text{IP}_{\text{mixture}} - \text{IP}_0) - [(\text{IP}_1 - \text{IP}_0) + (\text{IP}_2 - \text{IP}_0)]}{(\text{IP}_1 - \text{IP}_0) + (\text{IP}_2 - \text{IP}_0)} \times 100\% \tag{1}$$

where $\text{IP}_{\text{mixture}}$, IP_0, IP_1 and IP_2 correspond to the induction periods of the samples containing the mixture of antioxidants, of the control sample, and of the samples containing the individual antioxidants, respectively. A positive value defines a synergistic effect between antioxidants, while a negative value corresponds to an antagonistic effect.

Table 4. Induction period of binary antioxidants on Jatropha oil (JO1)

	Concentration		Ratio			
Antioxidant	ppm	$M \times 10^{-3}$	Weight	Mol[a)]	IP (h)	SE (%)
PY	500	3.6			27.34	
PD	500	2.1			5.80	
MMBP	500	1.3			4.51	
DT	500	2.2			3.47	
TP	500	1.5			2.76	
PY:PD	1000	5.7	1:1	2:1	46.65	50.32
PY:TP	1000	5.1	1:1	5:2	28.20	−1.21
PY:DT	1000	5.8	1:1	2:1	23.98	−18.99
MMBP:PD	1000	3.4	1:1	2:3	6.77	−27.48

[a)] Rounded value based on molar concentration.
JO1 (without antioxidant) has IP = 1.58 h, M, molar concentration, for abbreviations see Table 3.

The combination of PY and PD gave a higher induction period than the oil formulated with each as the single antioxidant. The combination of 500 ppm PD and 500 ppm PY was found to be the most effective of those evaluated in the Rancimat methods with a synergism equivalent of 50.32% and an induction period of 46.65 h. The induction period was higher than the induction period observed for pure PY (36.15 h) at an equal total concentration of 1000 ppm. Negative equivalents were found for MMBP-PD, PY-DT and PY-TP combinations (−27.48%, −18.99% and −1.21%, respectively). The induction period for the PY-DT combination (23.98 h) was even lower than the one found for PY as the single additive (27.34 h).

The improved stability measured in the presence of the PY–PD combination confirmed the existence of antioxidant interaction, suggesting a more effective inhibition of lipid peroxidation. Similar results were reported by de Guzman et al. [58] when they studied the synergistic effect for binary formulations of TBHQ with BHA, PG, or PY on soybean based biodiesel and by Marinova et al. [59] in their study with the combination of tocopherol and myricetin in sunflower oil.

The combination of primary antioxidant (PY) with secondary antioxidants (DT and TP) gave only small improvements or even reduced activity. This indicated poor interaction between primary and secondary antioxidants. The binary mixture of PY + TP was more efficient than PY + DT compared at equal concentration, despite the fact that DT was more efficient than TP as single additives.

3.3.1 Effect of ratio

Combinations of PY and PD were studied further at different ratios and concentrations (see Fig. 2). The positive synergisms were found at all PY/PD weight ratios from 1:3 to 3:1, with corresponding mol ratios of 1:2 to 5:1. As a general observation, a mol ratio of 1:2 and 2:1 showed a more or less equal synergism percentage. However, shifting the mol ratio to 5:1, leads to a lower synergism percentage. This observation suggests PY regeneration by PD. Based on previous studies [58, 59], the highest degree of synergy between PY and PD is probably related to the regeneration of one antioxidant by the other. In this study, PY is the more effective antioxidant, which can readily donate a hydrogen radical to a lipid radical. PD then transfers a hydrogen radical to the formed PY radical, and regenerates PY. Through this mechanism, PD is consumed while PY can propagate as oxidation inhibitor (see Fig. 3).

3.3.2 Effect of concentration

As can be seen in Table 5, all mixtures of PY and PD cause a longer IP compared to the sum of the IP determined in the presence of each single antioxidant. This observation indicates a synergistic effect at all concentrations investigated.

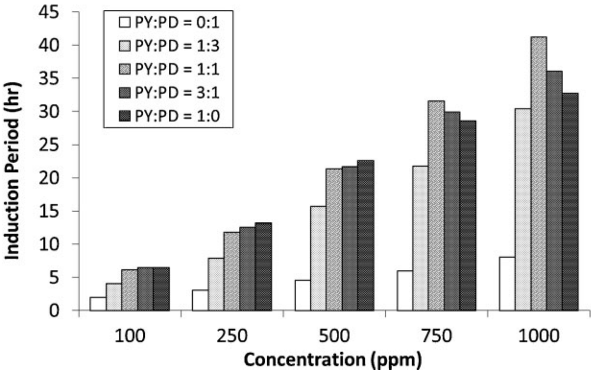

Figure 2. Variation of PD and PY concentrations and ratios in *Jatropha curcas* oil (JO1; PY values in solid black bars based on relation shown in Figure 1).

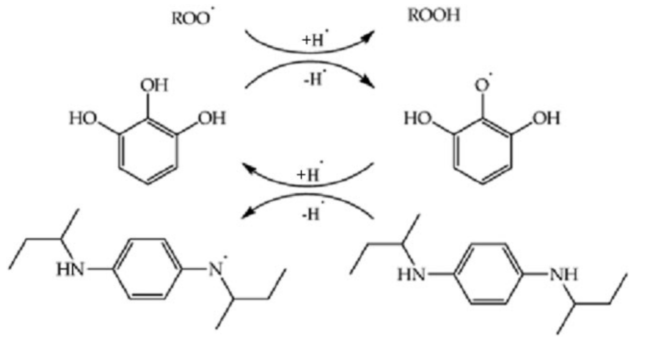

Figure 3. Proposed mechanism for synergistic interaction between PY and PD.

The improved induction period shows different profiles at different concentrations. At concentration of 100 ppm, the induction period increases with an increase in PY/PD ratio. At high concentrations ($\geq$500 ppm), the weight ratios PY/PD of 1:1 resulted in induction periods greater than the ones observed for the individual antioxidants at the same concentration levels. The results indicated that the ratio was not the only parameter influencing the improved induction period, but that the total concentration in the mixture also plays a role. The highest induction period of 46.65 h was observed for the 1:1 weight ratio at a concentration of 1000 ppm with a synergism percentage of 50.32% (see Table 5).

Although the best stabilization improvement is shown at concentrations higher than 500 ppm at a ratio of 1:1, all antioxidants mixtures showed positive synergism (see Table 5). This suggests that regeneration of PY with PD takes place in all formulations. The results also showed a different extent of synergism for the different formulations. This is accordance with the study of Marinova et al. [59] who investigated the synergistic effect of α-T and myricetin on sunflower oil.

3.4 Effect of jatropha oil feedstocks

The potential impact of jatropha oil processing and storage was tested by evaluating PY and the binary mixture of PY–PD in jatropha oils with a different processing and storage history. The degree of effectiveness, however, was found to vary for the different samples (see Table 6). PY showed good activity with all oil samples, including jatropha oil that was oxidized and showed a high acidity level (see JO1 and JO2 in Table 2).

The observed differences in stability between the jatropha oils may be due to the different quantities of natural antioxidants (tocopherols and phospholipids) or pro-oxidants (free fatty acid, hydroperoxides and metal content) present in the oils. Various quality parameters of jatropha oil are shown in Table 2. The metal content of these jatropha oils are far below the acceptable limit required by Codex Alimetarius (1.5 ppm of Fe and 0.2 ppm of Cu for refined oils) [60]. The peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. According to Shahidi [61] fresh oil reveals peroxide values below 10 meq/kg oil. The acid value is a measure of the oil acidity which can be attributed by free fatty acid and/or acidic oxidation products. Both high peroxide numbers and high

Table 5. Percentage of synergism and induction period at various concentrations and ratios of PY:PD on JO1

	Total Concentration		Ratio PY:PD			
MIX	ppm	$M \times 10^{-3}$	Weight	Mol[a)]	IP (h)	SE (%)
MIX-1	100	0.49	1:3	1:2	5.46	61.42
MIX-2	100	0.57	1:1	2:1	8.29	63.42
MIX-3	100	0.65	3:1	5:1	10.21	21.40
MIX-4	250	1.23	1:3	1:2	12.21	52.46
MIX-5	250	1.42	1:1	2:1	21.87	50.75
MIX-6	250	1.62	3:1	5:1	22.47	23.13
MIX-7	500	2.46	1:3	1:2	24.82	47.71
MIX-8	500	2.85	1:1	2:1	32.74	50.31
MIX-9	500	3.24	3:1	5:1	31.09	20.44
MIX-10	750	3.69	1:3	1:2	33.94	50.40
MIX-11	750	4.27	1:1	2:1	41.45	48.90
MIX-12	750	4.85	3:1	5:1	37.49	23.21
MIX-13	1000	4.92	1:3	1:2	41.31	50.24
MIX-14	1000	5.70	1:1	2:1	46.65	50.32
MIX-15	1000	6.47	3:1	5:1	42.20	25.23

[a)] Rounded value based on molar concentration.
JO1 (without antioxidant) has IP = 1.58 h, M, molar concentration, for abbreviations, see Table 3.

Table 6. Activity of antioxidants at various jatropha oil feedstocks

Parameters	JO1	JO2	JO3	JO4
Control	1.58	1.22	11.59	5.06
PD 500 ppm	5.80	4.58	19.37	10.16
PD 1000 ppm	10.53	8.02	n.d.	14.13
PY 500 ppm	27.34	22.61	33.45	31.44
PY 1000 ppm	36.15	32.75	34.81	33.53
PY: PD 1:1 1000 ppm (total)	46.65	41.24	36.70	33.48
%SE of PY: PD 1:1 1000 ppm	50.32	61.70	−15.28	−9.72

n.d., not determined.

acid values lower the oxidative stability of JO2 which is indicated by the induction period of 1.22 h. To obtain the same oxidative stability level for JO2 and JO3, twice the amount of stabilizer is needed for JO2: 1000 ppm of PY for JO2 versus 500 ppm for JO3. The higher degree of oil deterioration indicated by a higher peroxide content and a higher acid value clearly requires a higher level of antioxidant. Further addition of PY antioxidant to JO3 and JO4 did not lead to significant improvements in oxidative stability improvement. JO1, JO2, JO3 and JO4 showed different degrees of synergy when treated with PY/PD at ratio 1:1. The results showed that the effectiveness of antioxidants depends on the processing and storage history of the oil, which is in line with reported data.

3.4.1 Influence of acid value

As the data in Table 6 show that the PY–PD synergy is mainly observed for the aged oil, the influence of free acids was further studied (see Table 7). The jatropha oil used in this experiment was freshly prepared oil, JO4. Addition of oleic acid to a non-stabilised jatropha oil control sample reduced its induction period, as expected. Free fatty acid is found to oxidize faster than the corresponding triglycerides. Triolein and oleic acid have induction periods (measured at 90°C) of 17.4 and 6.63 h, respectively [62]. The pro-oxidant activity of free fatty acid is widely known [63, 64]. The data found for the 1000 ppm PY show a small increase of the induction period which suggests a positive interaction between free fatty acid (oleic acid) and PY. Likewise, the synergy between PY and PD is influenced by the presence of free fatty acid (oleic acid), a higher free fatty acid content resulted in a higher synergy level. The physical location of antioxidant in oil systems is known to play an important role [54]. Since the higher polarity of free fatty acids (FFA) compared to oil, PY and PD are being more soluble in free fatty acids than in oil. They are being oriented towards the oil–air interface thus protecting the oil system from oxidation. Despite these positive results, it should be noted that a specified maximum acid value corresponding to 2 mg KOH/g oil applies for fuel. At higher acid values, the oil should be refined to reduce the acidity before further application.

Table 7. Influence of oleic acid addition on activity of antioxidants

	Induction period (h)		
Sample	0% OA	3% OA	6% OA
JO4	5.06	3.03	2.53
PY 500 ppm	31.44	29.86	28.27
PY 1000 ppm	33.53	35.58	37.76
PD 500 ppm	10.16	7.31	5.88
PD 1000 ppm	14.13	12.09	12.05
PY:PD 1:1 1000 ppm (total)	33.48	38.43	39.15
%SE of PY:PD 1:1 1000 ppm	−9.72	13.79	25.89

% OA is indicating percentage oleic acid added to the oil. The experiment was using JO4 which has originally 0.65% of free fatty acid (see Table 2. % free fatty acid is equal to half of the acid value).

3.5 Use of antioxidant in jatropha biodiesel

As most of the jatropha oil is used to manufacture biodiesel, an experiment was conducted to compare the effectivity of PY in both jatropha biodiesel and jatropha oil (see Table 8). JO4 was used as a feedstock to prepare jatropha-oil based biodiesel (JO4 biodiesel). The oxidative stability of JO4 biodiesel was found to be reduced compared to the original jatropha oil (JO4). This is due to the transformation of the triglycerides and the loss of natural antioxidants during processing. This structural change gave a lower viscosity biodiesel compared to the oil [62]. Knothe and Dunn [62] stated that the lower viscosity of biodiesel compared to oil can affect the rate of air bubble transport across the sample, the size of the bubbles, the rate at which oxygen contained in the bubbles dissolves into the oil and the mass transfer of oxidation products at the air–oil interface. This result corresponded to the result obtained by Knothe and Dunn [62] that triolein has a higher oxidative stability than methyl oleate.

When compared at more or less the same control value (1.6 h), JO4 biodiesel treated with PY (18.88 h) showed a

Table 8. Antioxidant use in jatropha biodiesel

	Induction period (h)	
Sample	Control	500 ppm PY
JO1	1.58	27.34
JO4	5.06	31.44
JO4 biodiesel	1.65	18.88
Jatropha-oil based biodiesel[a)]	3.27	19.65[b)]

[a)] Result from ref. 27.

[b)] Addition of PY at 600 ppm.

lower oxidative stability than JO1 treated with PY (27.34 h). This result is in accordance with results reported by Yanishlieva and Marinova [65] who compared the effect of phenolic antioxidants in sunflower oil and sunflower-oil based biodiesel. The effectiveness of these antioxidants was higher in the oil than in the biodiesel. This was attributed to a lower contribution of the antioxidant radicals and molecules to chain initiation and propagation in the oxidation of the oil compared to biodiesel oxidation. This is possibly due to mass transfer limitation by the higher viscosity of the oil compared to biodiesel. The oxidative stability of jatropha-oil based biodiesel treated with PY in this study was in line with the data reported by Jain and Sharma [27].

4 Conclusions

Of a series of synthetic and natural antioxidants, pyrogallol was found to have the best antioxidant activity in freshly prepared oil, and in highly acidic and oxidized jatropha oils. It showed a good activity even when used at a concentration as low as 50 ppm in highly acidic and oxidized oil. The high activity of PY compared to other primary phenolic antioxidants can be attributed to the presence of three adjacent hydroxyl groups on the aromatic moiety. The combination of PY with another primary antioxidant, PD, revealed a positive synergism. This synergism between PY and PD on jatropha oil can be understood if one assumes that PY is regenerated by proton exchange with PD. This synergism is however very feedstock dependent; it depends on minor compounds contained in the oil samples which gave either positive or negative synergism with binary antioxidants PY–PD. PY may prove to be the best antioxidant for jatropha oil either used alone or in combination with other primary antioxidants. PY can also be used as antioxidants in other jatropha oil-derived products for non-food application such as in biodiesel.

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