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Biodiesel Production with Jatropha (*Jatropha curcas*) and Palm Oil (*Elaeis guineensis*) Catalyzed by *Candida antarctica* and *Thermomyces lanuginosus* Lipases

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ABSTRACT

This study evaluated yield and quality of biodiesel produced from Jatropha (*Jatropha curcas*) and palm oil (*Elaeis guineensis*) using free *Thermomyces lanuginosus* (TL) and immobilized *Candida antarctica* (CA) lipases vs. a basic catalyst (sodium hydroxide). Manufacturer recommended reaction conditions were used for each lipase. A completely randomized design was used to evaluate a 2 × 3 factorial arrangement, with three replicates. Biodiesel % Yield (g biodiesel/100g oil), % moisture, % free fatty acids (FFA), peroxide value, oxidative stability index (OSI), color, cetane number and fatty acid methyl esters (FAME) profile were measured according to ASTM D6751. TL lipase yielded 83.4% biodiesel from *Jatropha curcas* oil, statistically similar (P=0.87) to CA lipase (82.8%) and higher (P<0.05) than NaOH (74.7%). Biodiesel from palm oil using CA lipase showed a 75.3% yield statistically similar (P=0.52) to TL lipase (72.7%) and to NaOH (72.6%). Quality was similar for biodiesel produced by basic and enzymatic catalysis according to ASTM D6751 standard. Biodiesel production by enzymatic catalysis with free and immobilized lipases were comparable in yield and quality to basic catalysis for Jatropha and palm oil.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

A completely randomized design with three replications was used to evaluate a 3 (catalysts) x 2 (vegetable oils) factorial. Transesterificaction catalysis of Jatropha (*Jatropha curcas*) and palm (*Elaeis guineensis*) oil into biodiesel with a lipase from *Candida antarctica* (CA) was compared to a lipase from *Thermomyces lanuginosus* (TL) vs. a basic catalyst (NaOH). ANOVA, LSMEANS and Tukey means separation were performed with SAS® v. 9.3.

LIPASE ACTIVITY

Activity of immobilized *CA* (10,000 PLU/g) and free liquid *TL* (100 KLU/g) lipases in oil was monitored 24h by % free fatty acid (FFA) production with AOCS Ca 5a-40. Optimum reaction time (h) was determined.

TRANSESTERIFICATION

Oil was extracted with an expeller and partially refined (filtered, degummed and neutralized) according to Haas and Mittlebach (2000). Lipase assisted transesterification conditions (Table 1) were used according to Su *et al.* (2011) and Cesarini et al. (2013). Transesterification with NaOH was performed according to Baccaro (2007) for palm oil and Gonzalez (2012) for Jatropha oil.

OIL AND BIODIESEL QUALITY AND STABILITY

J. Curcas and E. guineensis oil suitability for biodiesel was evaluated with DIN V 51605 standard, while biodiesel quality was compared with ASTM D6751 standard. AOCS methods were used to measure moisture (Ca 2e-84), fatty acid profile (Ce 2b-11), free fatty acids (Ca 5a-40), peroxides (Cd 8b-90) and Oil Stability Index (OSI, Cd 12b-92).

Table 1. Oil transesterification reaction conditions

Catalyst	Oil	Catalyst Concentration (% w/ow)*	Molar Ratio (MeOH-oil)	Water (% w/ow)*	Temp (°C)	Time (h)	Agitation (rpm)
NaOH	Jatropha	1	6-1	0	60	1	400
	Palm	1	6-1	0	60	1	400
C.antarctica lipase	Jatropha	14	3-1	0	40	14	200
	Palm	14	3-1	0	40	14	200
T. lanuginosus lipase	Jatropha	1	1.5-1	5	40	24	200
	Palm	1	1.5-1	5	40	24	200

*% w/ow = percent based on oil weight.

RESULTS

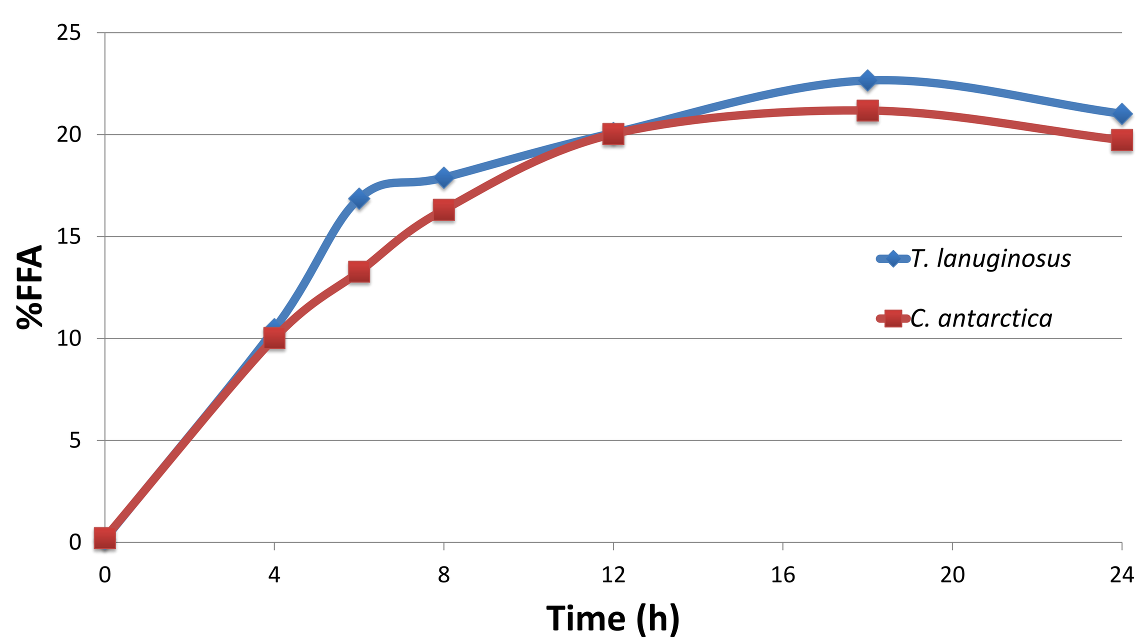

Figure 1. Activity of *Thermomyces lanuginosus* and *Candida antarctica* lipases on Jatropha oil

Table 2. ANOVA summary for transesterification of Jatropha and palm oil with two lipases and NaOH

Source of Variation	Biodiesel Yield		Glycerin Yield		% FAME	
	F	P[1]	F	P	F	P
Oil	9.19	0.02	18.89	0.01	281.59	0.01
Catalyst	2.24	0.17	2.70	0.13	27.43	0.01
Oil × Catalyst	1.29	0.33	0.50	0.63	0.61	0.44
%C.V.[2]	6.15		16.62		9.87	

[1] Statistically significant at P <0.05.
[2]%C.V. = Percent Coefficient of Variation.

Table 3. Biodiesel yield of partially refined Jatropha and palm oil produced by enzymatic transesterification

Catalyst	Biodiesel Source	Biodiesel Yield % ± S.D. [1]	Glycerin Yield % ± S.D.
NaOH	Jatropha	74.7 ± 0.78 ^{abc}	20.1 ± 2.78 ^{ab}
	Palm	72.6 ± 0.27 ^{c}	25.0 ± 0.63 ^{a}
TL Lipase	Jatropha	83.4 ± 0.23 ^{a}	14.0 ± 0.03 ^{c}
	Palm	72.7 ± 1.78 ^{c}	22.7 ± 1.73 ^{a}
CA Lipase	Jatropha	82.8 ± 0.54 ^{ab}	15.7 ± 0.40 ^{bc}
	Palm	75.3 ± 0.86 ^{abc}	22.6 ± 0.73 ^{a}

^{abc}: different letters on the same column indicate significant difference at (P<0.05).
[1]S.D.: standard deviation

Table 4. Biodiesel moisture and stability

Catalyst	Biodiesel	Moisture % ± S.D.*	FFA£ % ± S.D.	Peroxide Value meq/kg ± S.D.	OSI¥ h ± S.D.
NaOH	Jatropha	0.11 ± 0.01 ^{d}	0.10 ± 0.01 ^{f}	211 ± 14 ^{a}	3.90± 0.50 ^{b}
	Palm	0.09 ± 0.01 ^{d}	0.17 ± 0.01 ^{e}	91 ± 8 ^{c}	22.96 ± 2.40 ^{a}
T. lanuginosus lipase	Jatropha	0.30 ± 0.01 ^{a}	1.63 ± 0.03 ^{a}	145 ± 10 ^{b}	0.10 ± 0.02 ^{c}
	Palm	0.13 ± 0.01 ^{c}	1.28 ± 0.01 ^{b}	76 ± 5 ^{c}	0.48 ± 0.10 ^{c}
C. antarctica lipase	Jatropha	0.29 ± 0.01 ^{a}	0.85 ± 0.05 ^{d}	115 ± 2 ^{c}	0.08 ± 0.01 ^{c}
	Palm	0.15 ± 0.03 ^{b}	0.96 ± 0.05 ^{c}	103 ± 4 ^{c}	0.06 ± 0.02 ^{c}

* S.D. = Standard Deviation.
^{abcd} = Different letters on the same column indicate significant difference at (P<0.05).
£FFA = Free Fatty Acids.
¥OSI = Oil Stability Index.

Table 5. Effect of basic and enzymatic catalysts on *Jatropha* biodiesel FAME* profile

FAME*	NaOH % ± S.D.[1]	*T. lanuginosus* lipase % ± S.D.	*C. antarctica* lipase % ± S.D.
8:0	0.13 ± 0.03 ^{x}	0.05 ± 0.01 ^{y}	0.14 ± 0.01 ^{x}
14:0	0.10 ± 0.00 ^{y}	0.13 ± 0.00 ^{x}	0.13 ± 0.01 ^{x}
15:0	0.06 ± 0.01 ^{y}	0.24 ± 0.11 ^{x}	0.28 ± 0.00 ^{x}
16:0	14.97 ± 0.75 ^{y}	18.12 ± 0.26 ^{x}	18.24 ± 0.99 ^{x}
17:0	0.06 ± 0.00 ^{y}	0.98 ± 0.02 ^{x}	0.96 ± 0.07 ^{x}
18:0	7.63 ± 0.39 ^{y}	8.86 ± 0.12 ^{x}	8.77 ± 0.15 ^{x}
19:0	0.23 ± 0.02 ^{y}	0.26 ± 0.01 ^{x}	0.25 ± 0.02 ^{xy}
15:1 cis-10	0.23 ± 0.27 ^{x}	0.05 ± 0.00 ^{x}	0.04 ± 0.00 ^{x}
18:1 n9c cis-9	41.62 ± 1.14 ^{x}	39.36 ± 0.40 ^{y}	40.18 ± 0.46 ^{y}
20:1 n9 cis-11	0.16 ± 0.01 ^{y}	0.24 ± 0.03 ^{x}	0.20 ± 0.01 ^{y}
18:2 n6 cis-9,12	33.39 ± 2.09 ^{x}	30.91 ± 0.67 ^{y}	30.10 ± 0.89 ^{y}
22:5 cis-7,10,13,16,19	0.00 ± 0.00 ^{z}	0.47 ± 0.06 ^{y}	0.63 ± 0.04 ^{x}
∑ Saturated	23.31 ± 1.08 ^{y}	28.63 ± 0.43 ^{x}	28.77 ± 1.14 ^{x}
∑ Monounsaturated	42.24 ± 1.06 ^{x}	39.65 ± 0.38 ^{y}	40.42 ± 0.45 ^{y}
∑ Polyunsaturated	33.51 ± 2.09 ^{x}	31.72 ± 0.71 ^{xy}	30.73 ± 0.87 ^{y}

*FAME = Fatty Acid Methyl Esters.
[1] S.D. = Standard Deviation.
^{xyz} = Different letters on the same row indicate significant difference at (P<0.05).

Table 6. Effect of catalyst on *palm* biodiesel FAME* profile

FAME*	NaOH % ± S.D.[1]	*T. lanuginosus* lipase % ± S.D.	*C. antarctica* lipase % ± S.D.
8:0	0.03 ± 0.00 ^{y}	0.00 ± 0.00 ^{z}	0.04 ± 0.00 ^{x}
12:0	0.43 ± 0.00 ^{z}	0.82 ± 0.04 ^{x}	0.62 ± 0.06 ^{y}
14:0	0.84 ± 0.05 ^{z}	1.24 ± 0.02 ^{x}	1.09 ± 0.02 ^{y}
15:0	0.10 ± 0.00 ^{y}	0.00 ± 0.00 ^{z}	0.14 ± 0.00 ^{x}
16:0	40.60 ± 0.22 ^{z}	52.3 ± 0.24 ^{x}	49.82 ± 0.01 ^{y}
17:0	0.11 ± 0.00 ^{y}	0.13 ± 0.01 ^{y}	0.16 ± 0.00 ^{x}
18:0	4.86 ± 0.06 ^{y}	5.61 ± 0.05 ^{x}	5.63 ± 0.01 ^{x}
20:0	0.40 ± 0.00 ^{z}	0.49 ± 0.01 ^{x}	0.44 ± 0.00 ^{y}
16:1 cis-9	0.16 ± 0.00 ^{y}	0.19 ± 0.02 ^{x}	0.18 ± 0.01 ^{xy}
18:1 n9 cis-9	42.40 ± 0.30 ^{x}	32.52 ± 0.27 ^{z}	34.93 ± 0.02 ^{y}
20:1 n9 cis-11	0.06 ± 0.00 ^{y}	0.00 ± 0.00 ^{z}	0.30 ± 0.00 ^{x}
18:2 n6 cis - 9,12	9.55 ± 0.09 ^{x}	6.37 ± 0.08 ^{z}	6.67 ± 0.01 ^{y}
18:3 n3 cis - 9,12,15	0.31 ± 0.03 ^{x}	0.35 ± 0.04 ^{x}	0.31 ± 0.01 ^{x}
22:5 cis - 7,10,13,16,19	0.00 ± 0.00 ^{z}	0.47 ± 0.06 ^{y}	0.63 ± 0.04 ^{x}
∑ Saturated	47.44 ± 0.32 ^{z}	60.57 ± 0.33 ^{y}	57.92 ± 0.01 ^{y}
∑ Monounsaturated	42.63 ± 0.30 ^{x}	32.71 ± 0.28 ^{z}	35.41 ± 0.02 ^{y}
∑ Polyunsaturated	9.94 ± 0.08 ^{x}	6.72 ± 0.10 ^{y}	6.67 ± 0.01 ^{y}

*FAME = Fatty Acid Methyl Esters.
[1] S.D. = Standard Deviation.
^{xyz} = Different letters on the same row indicate significant difference at (P<0.05).

DISCUSSION

LIPASE ACTIVITY

Figure 1 shows %FFA release by CA lipase peaked at 14-16h, faster than TL (18-20h). Triglyceride hydrolisis into FFA took longer than the 3-5 h reported by Cesarini *et al.* (2013). FFAs decreased after peak production as they were sterified with methanol into FAMEs by lipases, but not below 15%.

BIODIESEL YIELD

Oil source had a significant effect (P=0.01) on biodiesel yield, while the catalyst did not (Table 2). Biodiesel yields with Jatropha oil were above 80% when transesterification was catalyzed by TL and CA lipases, comparable with NaOH (Table 3). The same tendency was observed with palm oil biodiesel yields, although below 75%. Cesarini *et al.* (2013) and Su *et al.* (2011) have reported yields over 90% with TL and CA lipases, respectively. Incomplete transesterification at 24h (Table 2) may have caused this reduction.

BIODIESEL QUALITY

Biodiesel produced by enzymatic catalysis (TL and CA) did not comply with ASTM D6751 stability (Table 4). Optimization of process variables (higher reaction time and temperature) may overcome this. FAME profiles of biodiesel from Jatropha and palm oil were significantly altered by TL and CA lipases (Tables 5 and 6) compared to NaOH. Saturated FAME (16:0 and 18:0) increased, while unsaturated (oleic and linoleic) decreased. TL and CA 1,3 position specificity when hydrolyzing triglycerides (Azocar *et al.* 2010) and incomplete transesterification at 24 h (Table 2) may have caused this.

CONCLUSIONS

- Jatropha and palm oil transesterification catalyzed with TL and CA enzymes showed potential for biodiesel production vs. commercial NaOH catalyst.
- Biodiesel produced by enzymatic catalysis did not comply with ASTM D6751 quality standard. Further process optimization to increase transesterification and biodiesel stability is required.

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