Analytical Methods – Electronic Supplementary Information

Feasibility of Dispersive Liquid-Liquid Microextraction to Determine Ca, Mg, K, and Na in Biodiesel by Atomic Spectrometry

Kássia Roberta Nogueira da Silva[a], Anderson dos Santos Greco[b], Marcela Zanetti Corazza[b], Jorge Luiz Raposo Jr[a,b*]

[a]Institute of Chemistry, Federal University of Mato Grosso do Sul, PO Box 549, Zip Code 79070-900, Campo Grande, MS, Brazil.

[b]Faculty of Exact Sciences and Technology, Federal University of Grande Dourados, Highway Dourados-Itahum, km 12, PO Box 364, Zip Code 79804-970, Dourados, MS, Brazil.

[*]Corresponding author: *jorgeluizraposo@gmail.com*

S.1. Production and characterization of biodiesel

The *Helianthus annuus, Brassica napus* L., *Jatropha curcas and Crambe Hochst abyssinica* oilseed provided by Embrapa Agropecuária Oeste (Dourados, MS, Brazil) were firstly thoroughly washed with running tap water and after with deionized water. An amount of 2000 g of cleaned samples were dried at room temperature for 24 h and after at 70 °C for 60 h in a forced air oven. All amounts of the seeds were ground in a stainless steel mill and stored in individual plastic bags.

For extraction of the oil, an amount of 1320 g *Jatropha curcas*, 1653 g *Helianthus annuus*, 1767 g *Crambe Hochst abyssinica*, and 1680 g *Brassica napus* L. powdered seed sample were placed in a Soxhlet system, and the crude vegetable oils were extracted during 6 h at 70 °C using 600 mL of hexane. The oils were separated from the hexane under reduced temperature and pressure by refluxing at 60 °C. The crude vegetable oils were submitted to degumming process, and stored in a 500 mL-amber glass at 4 °C.

Using the *Jatropha curcas*, *Helianthus annuus*, *Crambe Hochst abyssinica*, and *Brassica napus* L. crude oils, the biodiesel were produced by alkali-catalyzed (KOH) transesterification route.[1] The mixtures were kept at 45 °C for 90 min under stirring. The produced biodiesels were transferred into a separation funnel to complete separation of biodiesel/glycerol during 12h. The layers contained glycerol were appropriated discarded. The final biodiesel were washed with sodium chloride,[2] and dried with sodium sulfate before storage.[3-4] To avoid oxidation of the samples, an amount of propyl gallate was added in order to contain 500 mg kg^{-1}.[5]

Preliminary, physicochemical analyses were done according to standards methods, such as kinematic viscosity at 40 °C (ASTM D445),[6] specific mass at 20 °C (ASTM D4052),[7] acid value (ASTM EN 14104),[8] and water content (ASTM D6304).[9]

S.2. Biodiesel preliminary laboratorial analysis

According to the quantity of *Jatropha curcas* (1320 g), *Helianthus annuus* (1653 g), *Crambe Hochst abyssinica* (1767 g), and *Brassica napus* L. (1680 g) oilseed weighed as raw matter, an amount of 514.4 and 255.3 g, 644.8 and 376.2 g, 671.6 and 329.6 g, and 571.1 and 240.5 g of vegetable oil and biodiesel were obtained, respectively. It is important to point out that the amount of vegetable oil extracted from the selected oilseed is higher than those observed in the literature for soybean and cotton.[10-12]

Some physicochemical properties are performed to evaluate the quality of each biodiesel based on the values recommended by National Agency of Petroleum, Natural Gas and Biofuels (ANP) in ANP Resolution No. 45/2014.[13] The following properties, density, acid number, water content and kinematic viscosity of the biodiesel samples are shown in Table S1. According to ANP Resolution No. 45/2014,[13] all alternative biodiesel samples presented density and viscosity in the rages of 850-900 kg m^{-3} and 3.0-6.0 mm^2 s^{-1}, respectively. About acid number, and water content, the values obtained were lower than 0.5 mg KOH g^{-1}, and

200 mg kg^{-1}, respectively. The problems related to higher densities of the biodiesel,[14] high acidity levels,[15] high water content,[10] and high viscosity[16] are best described in specific literature.

Table S1 Physicochemical parameters obtained for the renewable biodiesel samples

Sample	Physicochemical parameters			
	Density ($kg\ m^{-3}$)	Acid number ($mg\ KOH\ g^{-1}$)	Water content ($mg\ kg^{-1}$)	Kinematic viscosity* ($mm^2\ s^{-1}$)
Jatropha curcas	852	0.4	150	3.3
Helianthus annuus	859	0.2	100	4.8
Crambe Hochst abyssinica	858	0.4	100	3.3
Brassica napus L.	855	0.2	150	3.2
ANP 45/2014[13]	850-900	<0.5	<200	3.0-6.0

*Kinematic viscosity at 40 °C.

Table S2. Factors, levels and recoveries of 2^4 full factorial design used to evaluate the DLLME sample preparation procedure.

Factor	Level	
	Low (-)	High (+)
Mass (g)	1.0	3.0
Volume (μL)	300	1000
Nitric acid:isopropyl alcohol (v/v)	3:1	1:3
HNO_3 (%)	0.5	5.0

Exp.	Mass	Volume	Nitric acid:isopropyl alcohol	HNO_3	Recovery (%)			
					Ca	Mg	Na	K
1	-	-	-	-	20.3	70.8	103.0	62.4
2	+	-	-	-	44.6	82.8	96.6	86.5
3	-	+	-	-	39.5	87.7	108.8	79.0
4	+	+	-	-	93.0	112.4	122.8	113.3
5	-	-	+	-	14.0	36.7	68.0	33.2
6	+	-	+	-	5.5	24.0	46.7	38.8
7	-	+	+	-	56.6	103.2	115.5	90.5
8	+	+	+	-	70.4	92.5	100.6	94.0
9	-	-	-	+	46.1	92.1	103.8	82.4
10	+	-	-	+	78.2	92.3	91.7	87.0
11	-	+	-	+	90.2	113.8	130.8	101.8
12	+	+	-	+	104.6	119.0	121.6	112.7
13	-	-	+	+	36.2	76.7	86.1	67.3
14	+	-	+	+	47.1	52.2	51.2	48.9
15	-	+	+	+	66.0	105.9	124.3	97.0
16	+	+	+	+	98.5	106.6	108.1	101.9

Conditions: 5 min of thermostatic bath at 80 °C, 30 s of vortex shaking, 3 min of ultrasound bath and 15 min of centrifugation.

Table S3. Recoveries obtained for the Doehlert matrix design used to establish the volume of extraction solution and nitric acid concentration in DLLME

Exp.	Volume (µL)	HNO_3 (%)	Recovery (%)			
			Ca	Mg	Na	K
1	800 (0)	5.0 (0)	106.0	102.1	106.1	101.9
2	800 (0)	5.0 (0)	106.6	102.9	109.5	103.5
3	800 (0)	5.0 (0)	107.2	102.4	107.9	103.6
4	1200 (1,0)	5.0 (0)	60.5	99.2	106.2	97.4
5	1000 (0.5)	7.0 (0.866)	62.0	95.8	101.4	97.2
6	400 (-1.0)	5.0 (0)	65.7	72.0	96.6	87.1
7	600 (-0.5)	3.0 (-0.866)	65.6	87.4	94.0	87.1
8	1000 (0.5)	3.0 (-0.866)	58.4	93.6	96.7	83.7
9	600 (-0.5)	7.0 (0.866)	65.3	78.2	95.4	76.1

The numbers in parentheses represents the Doehlert matrix coded values. Conditions used in this experiment: 3.0 g of sample mass, 3:1 (v/v) of nitric acid:isopropyl alcohol, 5 min of thermostatic bath at 80 °C, 30 s of vortex shaking, 3 min of ultrasound bath and 15 min of centrifugation.

Table S4. ANOVA for linear model fit from the Doehlert matrix design for the results showed for Ca in Table S3

	SS	DF	MS	$F_{calculated}$	P_{value}
(1) Volume (L)	36.855	1	36.855	107.481	0.009176
Volume (Q)	2268.091	1	2268.091	6614.438	0.000151
(2) [HNO_3] (L)	2.608	1	2.608	7.606	0.110166
[HNO_3] (Q)	2313.179	1	2313.179	6745.928	0.000148
1L *by* 2L	3.861	1	3.861	11.260	0.078492
Lack of fit	4.753	1	4.753	13.860	0.065176
Pure error	0.686	2	0.343		
Total	3866.534	8			

SS = sum of square, DF = degree of freedom, MS = means of square.

Table S5. ANOVA for linear model fit from the Doehlert matrix design for the results showed for Mg in Table S3

	SS	DF	MS	$F_{calculated}$	P_{value}
(1) Volume (L)	509.734	1	509.7337	2673.896	0.000374
Volume (Q)	342.259	1	342.2590	1795.378	0.000557
(2) [HNO_3] (L)	12.006	1	12.0062	62.981	0.015509
[HNO_3] (Q)	192.685	1	192.6854	1010.764	0.000988
1L by 2L	32.661	1	32.6612	171.330	0.005786
Lack of fit	1.882	1	1.8816	9.870	0.088127
Pure error	0.381	2	0.1906		
Total	1006.896	8			

SS = sum of square, DF = degree of freedom, MS = means of square.

Table S6. ANOVA for linear model fit from the Doehlert matrix design for the results showed for Na in Table S3

	SS	DF	MS	$F_{calculated}$	P_{value}
(1) Volume (L)	64.8210	1	64.8210	22.15876	0.042287
Volume (Q)	49.1520	1	49.1520	16.80238	0.054680
(2) [HNO_3] (L)	9.5172	1	9.5172	3.25342	0.2113047
[HNO_3] (Q)	186.6011	1	186.6011	63.78870	0.015317
1L by 2L	2.8056	1	2.8056	0.95909	0.430688
Lack of fit	0.1380	1	0.1380	0.04718	0.848189
Pure error	5.8506	2	2.9253		
Total	288.8046	8			

SS = sum of square, DF = degree of freedom, MS = means of square.

Table S7. ANOVA for linear model fit from the Doehlert matrix design for the results showed for K in Table S3

	SS	DF	MS	$F_{calculated}$	P_{value}
(1) Volume (L)	121.7944	1	121.7944	140.6998	0.007032
Volume (Q)	137.6021	1	137.6021	158.9612	0.006232
(2) [HNO_3] (L)	1.6512	1	1.6512	1.9075	0.301310
[HNO_3] (Q)	435.8641	1	435.8641	503.5204	0.001980
1L by 2L	149.4506	1	149.4506	172.6489	0.005742
Lack of fit	8.8817	1	8.8817	10.2603	0.085193
Pure error	1.7313	2	0.8656		
Total	778.8283	8			

SS = sum of square, DF = degree of freedom, MS = means of square.

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