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Industrial & Engineering Chemistry Research
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pubs.acs.org/IECR

Potential of Alligator Fat as Source of Lipids for Biodiesel Production

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ABSTRACT: A large amount of alligator fat (AF) is produced by alligator meat processing industry and disposed in landfills or discarded as waste. The AF can be used as a potential feedstock for biodiesel production due to its high lipid content. In this work, recovery of lipids from the AF tissue was studied by solvent extraction as well as by microwave rendering. Microwave rendering resulted in AF oil recovery of 61% by weight of the frozen AF tissue obtained from producers. The fatty acid profile of the lipid showed that palmitic acid (C16:0), palmitoleic acid (C16:1), and oleic acid (C18:1) were the dominant fatty acids accounting for 89−92% of all lipids by mass; 30% of the fatty acids were saturated and 70% were unsaturated. The biodiesel produced from AF oil was found to meet the ASTM specifications of biodiesel concerning kinematic viscosity, sulfur, free and total glycerin, flash point, cloud point, and acid number.

1. INTRODUCTION

Finite crude oil reserves, rapidly increasing demand, and realization of adverse consequences of the rising concentration of green house gases,[1] have prompted worldwide efforts to produce liquid transportation fuels from renewable resources.[2] The enormity of task, especially regarding biodiesel, is made clear by the 45 billion gallons of diesel consumed in the United States of America in 2008.[3] Soybean is the major biodiesel feedstock in the USA; it is also a significant source of vegetable oil and protein meal. It has been suggested that drawing more than 35% of US soybean production for biodiesel would adversely impact the food and agricultural markets.[4] On the other hand, production of just one billion gallons of biodiesel from soybean oil would consume about 690 million bushels of soybeans or approximately 21% of total soybean production in the USA in 2010.[5] Only 700 million gallons of biodiesel were produced in USA in 2008 (compared to 20 million gallons in 2003), and soybean prices have already risen from $7.34 per bushel in 2003−04 to $10.10 in 2009.[6] As a result, alternative feedstocks produced from nonarable land and wastes are under consideration for production of biodiesel. These include sewage sludge, chinese tallow, used vegetable oil, animal fat wastes (lard), and yeast.[7−10]

A potential biodiesel feedstock in the southeastern United States is alligator fat, which is a byproduct from the alligator meat processing industry. The alligators are harvested from the wild and from alligator farms for their skin and meat. In the USA, Louisiana and Florida account for the highest populations of alligators.[11] It is estimated that around 15 million pounds of alligator fat is produced annually and is disposed in landfills.[12,13] In this work, the potential of alligator fat for production of biodiesel was explored. The specific objectives of research work were (a) to quantify the recovery of lipids from alligator fat tissue left after removing meat and skin, (b) to characterize the fatty acids present in alligator fat oil, and (c) to produce and characterize biodiesel from the fat-oil.

2. MATERIALS AND METHODS

Alligator fat was obtained from the Louisiana USDA Fisheries and Wildlife Department in Abbeville, LA, and from the Prairie Cajun Seafood Wholesale Distributors, Eunice, LA. The fat was received frozen and contained some tissue as well. It was kept frozen at −20 °C until used. No record was available of the origin or age of the alligators that were source of the fat tissue. Reagent grade chemicals (methanol, NaOH, NaCl, $MgCl_2$, $MgSO_4$, H_2SO_4, KOH) and solvents (hexane, chloroform) were obtained from Fisher Scientific, Houston, TX. Deionized (DI) water was produced in our lab using Barnstead Mega Pure System D2 (Dubuque, IA) and it was used throughout this study.

Recovery of lipids from the fat tissue was studied by solvent extraction as well as by rendering. For solvent extraction, the method proposed by Osborne and Voogt[14] using chloroform−methanol was utilized. For rendering, the frozen fat tissue was thawed, blended, rendered, and then filtered to recover the lipids. Several variations of these operations as described with the results were investigated. Fatty acid composition in the fat samples was determined using GC−MS analysis of fatty acid methyl esters prepared according to the method proposed by Busboom et al.[15]

Biodiesel production from rendered alligator oil was carried out in a three-neck round-bottom flask equipped with a reflux condenser, a thermometer, and sampling port. Temperature in the flask was controlled at 60 °C by keeping it in an isothermal water bath. Contents of the flask were mixed with a magnetic stirrer at 700 rpm. Methanol and alkali were mixed separately and added to the alligator oil in the flask once its temperature reached reaction temperature. Alkali (NaOH) was added at a level of 1% by weight of oil. Reaction time (2, 2.5, and 3 h) and molar ratio

Special Issue: Nigam Issue

Received: May 9, 2011
Accepted: July 15, 2011
Revised: July 14, 2011

of oil to methanol (1:4, 1:5, 1:6) were the two variables investigated. At the end of reaction, the biodiesel layer was carefully removed from the cooled reaction mixture and washed twice with DI water. Biodiesel was recovered from residual methanol and solvents using a rotary evaporator at 85 °C and dissolved in hexane for GC−MS analysis.[12] The GC−MS consisted of an Agilent 7890A GC connected to Agilent 5790C VL mass selective detector. The column in GC was HP$^{@}$-5MS, a 30 m × 0.25 mm capillary column. Data analysis was done using W Search 32 and NIST Mass Spectral Database. Analytical protocol involved injector temperature of 250 °C, detector temperature of 280 °C, oven initial temperature of 60 °C, initial time of 3 min, temperature gradient ramp of 10 °C/min, final oven temperature at 250 °C, and final time of 5 min.

Biodiesel samples were analyzed using ASTM methods at the LSU Agricultural Center Research Laboratories and compared with ASTM D6751-09 standards for biodiesel.

Statistical analysis of data from replicate experiments was conducted using Microsoft Excel 2007.

3. RESULTS AND DISCUSSION

3.1. Recovery of Llipids from Alligator Fat Tissue. The 5 g samples of frozen, unthawed alligator fat were subjected to solvent extraction as per the method of Osborne and Voogt.[14] Using this method, the mean weight fraction of extractable lipids in frozen alligator fat tissue was determined to be 46.3% (w/w) with a standard deviation of mean equal to 4.65%. The large standard deviation of the mean is due to several transfers involved in the process of extraction and also possibly due to heterogeneity of the samples.

For recovery using rendering, 100 g samples of frozen AF were subjected to thawing, blending, rendering, and filtering to recover oil. In different experiments, the thawing was conducted in a microwave or under water in a sink at room temperature. Similarly, blending was carried out in the presence of different additives (water, canola oil, alligator oil itself). Rendering was also conducted either on a hot plate or in a microwave. These different rendering methods were attempted in light of observations that methods of rendering impact yield of broiler fat.[16] The method that resulted in the highest yield of oil involved thawing the 100-g sample in a microwave (Galaxy: model number 721.650002400, frequency = 2450 MHz, and 1500 W full power) for 5 min, followed by decanting the liquids, blending the thawed AF tissue for 1 min, and then rendering in the microwave for another 5 min at full power with the decanted oil added back. The results of three repeat experiments using this protocol are presented in Table 1. The average lipid content was 41.3% and it was statistically not different from the results of solvent extraction. Interestingly, Sheu[16] also found the lipid yield from broiler skin to be 47.5% and microwave rendering resulted in highest lipid recovery.

Due to the issues of sample heterogeneity and losses that occurred during transfers, it was decided to scale-up the microwave rendering process to larger samples. The results with five frozen-sample sizes ranging from 100 to 1050 g are presented in Figure 1. As the sample size increased, the total period (thawing + rendering) in the 1500 W microwave was raised from 10 to 70 min. The error bars in this diagram show the range of experimental observations from two or more replicates. The results for 200 g sample reflect eight replicates. As the sample size was increased from 100 to 200 g, the yield of lipids increased from 41% to 61%. With further increase in the sample size from 200 to 1000 g, the lipid yield remained essentially constant. The differences in different experiments reflect losses during transfers from treatment vessels to blender and back, and during filtration. The 61% lipid content in the fat tissue is in agreement with reports of subcutaneous adipose tissue in pig carcass[17] in which the authors reported fat content in the range of 57−68% with lower values in the intermuscular adipose tissue and the higher values in the outer subcutaneous layers.

Table 1. Recovery of Oil from Alligator Fat Tissue Using Microwave Thawing and Rendering Process

initial weight of alligator fat (g)	oil recovered after thawing (g)	total oil recovered after rendering (g)/ (% initial wt)		final tissue weight (g)/ (% initial wt)	
101.7	32.5	41.1	40.4%	18.1	17.8%
101.5	36.5	40.5	39.9%	17.9	17.6%
101.3	34.4	44.3	43.7%	16.7	16.5%
mean			41.3%		17.3%
standard deviation of mean			1.2%		0.40%

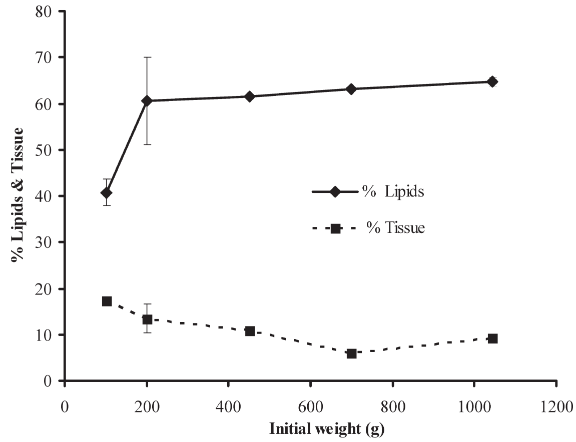

Figure 1. Lipids and tissue yields from microwave rendering of frozen alligator fat. The symbols represent the means and the error bars denote the standard deviation of the mean.

Table 2. Fatty Acid Composition of Alligator Oil

	mass %	
fatty acid	mean	std. dev. of mean
C14:0	1.27	0.02
C16:0	23.30	0.23
C16:1	12.11	0.30
C18:0	5.30	0.09
C18:1	55.54	0.66
C20:1	1.56	0.04
C20:3	0.28	0.13
C20:4	0.64	0.06

3.2. Fatty Acid Profile of Alligator Fat Lipids. The composition of different fatty acids in the alligator fat lipids is presented in Table 2. The data presented are averages of six measurements and have been normalized to 100%. The major fatty acids present are palmitic acid (C16:0), palmitoleic acid (C16:1), and oleic acid (C18:1), accounting for 89−92% of all lipids by mass. The composition presented in Table 2 corresponds to 30% saturated and 70% unsaturated fatty acids. This fatty acid composition is in agreement with the composition reported in the literature[18−20] for Louisiana alligator fat lipids. The fatty acid profile in the animal fats is strongly dependent on animal health and also on animal diet.[18] Relatively small amounts of fatty acids with 20+ carbons were present, suggesting that the alligators may have been fed more on beef than on fish.[21]

Fatty acid profiles of some common vegetable and plant oils are presented Table 3 and a comparison shows that the fatty acid profile found in the alligator fat has characteristics similar to that of very commonly used vegetable-based biodiesel feedstocks. This finding also is interesting in that many animal fats typically contain fatty acid profiles with the degree of saturation in the 50% range.[22,23] In fact, with an unsaturated fatty acid profile percentage in excess of 70%, alligator fat very likely will not exhibit poor cold flow characteristics, indicating a high potential for its use as a biodiesel feedstock.

Table 3. Lipid Profiles of Some Common Plant and Vegetable Oils[28]

	C16:0	C18:0	C20:0	C18:1	C18:2	C18:3	% unsaturation
palm	44.3	4.6	0.00	38.7	10.5	0.00	49.20
corn	11.67	1.85	0.24	25.16	60.60	0.48	86.24
soybean	11.75	3.15	0.00	23.26	55.53	6.31	85.10
sunflower	6.08	3.26	0.00	16.93	73.73	0.00	90.66

3.3. Alkali-Catalyzed Transesterification of Alligator Oil. Biodiesel (fatty acid alky ester) is most often produced via the transesterification reaction using caustic and methanol.[8] The kinetics of producing biodiesel from alligator fat via transesterification was investigated at 60 °C and in the presence of 1% NaOH by weight of oil. The experiments were conducted in a round-bottom flask fitted with a reflux condenser. Temperature was controlled by immersing the flask in a temperature controlled water bath. Reaction times were varied between 1.5 and 3 h. Molar ratio of methanol-to-oil was varied between 4 and 6. The final samples were analyzed for total and free glycerol, and for mono-, di-, and triglycerides. These data are presented in Table 4. The total glycerides are a measure of the extent of the transesterification reaction. According to ASTM standards for biodiesel, a transesterification reaction is complete and good quality biodiesel is produced if the total glycerides are less than 2400 ppm and free glycerides are less than 200 ppm. In all the experiments, the maximum concentration of total glycerides was 633 ppm and the free glycerides were 8.5 ppm. Given the densities of oil and methanol, the initial glyceride content in the reaction vessels would be of the order of 700 000 ppm. Hence, the maximum observed value of the final concentration of total glycerides was less than 0.1% of the starting value. In other words, the reaction was substantially complete in all the flasks. Another experiment, in which the reaction was terminated after 1.5 h, also showed complete reaction. These results indicate that production of biodiesel from alligator oil can be accomplished at 60 °C with 1:4 molar ratio of oil and methanol and reaction time of 1.5 h. This observation is in agreement with the conditions

Table 4. Total and Free Glycerides at Different Molar Ratios of Oil to Methanol and Reaction Times of 2, 2.5, and 3 h

	molar ratio = 1:4			molar ratio = 1:5			molar ratio = 1:6		
	2 h	2.5 h	3 h	2 h	2.5 h	3 h	2 h	2.5 h	3 h
total free glycerol (ppm)			6.06						8.45
monoglycerides (ppm)	41.96	41.96	59.19	73.66	64.86	90.69	18.78	18.17	48.68
diglycerides (ppm)	20.87	20.88	26.94	25.59	7.13	27.07	5.10	3.33	
triglycerides (ppm)	158.77	158.77	540.82	46.37	46.41	65.88	7.78	6.61	319.52
total glycerides (ppm)	221.61	221.61	626.95	145.63	118.41	183.64	31.67	28.13	368.20
total and free glycerides (ppm)	221.61	221.61	633.01	145.63	118.41	183.64	31.67	28.13	376.65

Table 5. Comparison of Alligator Fat Biodiesel Characteristics with ASTM6751 Biodiesel Specifications

property	units	ASTM method no.	alligator fat biodiesel	biodiesel ASTM6751 limits
Ca and Mg combined	ppm (μg/g)	EN 14538	10.5*	5 max
flash point (closed cup)	°C	D 93	126	93 min
water and sediment	% volume	D 2709	0.16*	0.05 max
kinematic viscosity, 40 °C	mm^2/sec	D 445	4.68	1.9−6.0
sulfated ash	% mass	D 874	not detected	0.02 max
sulfur	ppm	D 5453	4.9	15 max
cloud point	°C	D 2500	8	−3 to 12
carbon residue 100% sample	% mass	D 4530	0.0005	0.05 max
acid number	mg KOH/g	D664	0.4	0.50 max
free glycerin	% mass	D 6584	not detected	0.020 max
total glycerin	% mass	D 6584	0.03	0.240 max
phosphorus	% mass	D 4951	not detected	0.001 max
Na/K combined	ppm	EN 14538	not detected	5 max

Asterisk indicates value outside standard limits.

reported for alkali-catalyzed transesterification of oils. Bradshaw and Meuly[24] reported a desirable oil-to-methanol molar ratio between 1:3.3 and 1:5.25 depending on the quality of oil. Marinkovich and Tomasevic[25] reported that transesterification of soybean oil with 1% KOH gives best yields of biodiesel. Cheryan and Darnoko[26] have recommended a reaction temperature between 50 and 65 °C for transesterification reactions forming fatty acid methyl esters (FAME). Campbell[27] suggests that the reaction is completed in 90 min when temperature is maintained at 60 °C.

3.4. Quality of Biodiesel Produced from Alligator Fat. The quality of biodiesel produced by transesterification of alligator oil by methanol was determined by appropriate ASTM methods and compared to ASTM6751 biodiesel standards. The results are presented in Table 5. The alligator oil biodiesel met all the ASTM specifications except for the contents of Ca and Mg and water and sediment in the sample prepared. These failures suggest that the biodiesel production process needs improvements in washing and drying of the organic phase. It is suspected that the higher Ca/Mg content values were due to use of $MgSO_4$ as the drying agent. The material quality properties such as flash point, kinematic viscosity, sulfur, cloud point, carbon residue, acid number, and phosphorus were all within the limits of the ASTM standard. The operational properties such as free and total glycerin content, Na/K were also within specifications.

4. CONCLUSION

Rendering was found to be an adequate method for recovery of lipids from waste alligator fat. The samples obtained from different vendors gave an AF oil recovery of 61%. The lipids had primarily the palmitic acid (C16:0), palmitoleic acid (C16:1), and oleic acid (C18:1). The ratio of unsaturated to saturated fatty acids in the AF oil was 70:30. The AF oil could be transesterified into fatty acid methyl esters in the presence of 1% NaOH as catalyst at 60 °C with a 1:4 molar ratio of oil and methanol and the reaction was complete in 1.5 h. The quality of biodiesel produced by transesterification was compatible with ASTM specifications. These observations clearly show that alligator oil can be used as a potential biodiesel feed stock and that given that this feedstock is traditionally a waste product, its use should result in reduced processing costs.

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