RAPID COMMUNICATION

Biodiesel potential of rendered fat from avian influenza infected poultry in a burial site

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Abstract−To test the feasibility of biodiesel production using rendered fat from avian influenza (AI) infected poultry in a burial site, we conducted a laboratory-scale transesterification. Each content of fatty acid methyl esters (FAME) was identified to estimate the characteristics of produced biodiesel. Analytical results of the whole FAME profile show that the rendered fat from AI carcass can be a useful source of biodiesel production. The results show 47% of total fat mass can be recovered as biodiesel. Although natural biodegradation in the burial site and soil impurities lowered the yield, the compositional properties of FAMEs confirm that the produced biodiesel presents adequate fuel characteristics, satisfying common biodiesel specifications. These findings provide evidence that our approach can be a viable option to recycle buried carcass sustainably while terminating burial sites.

Keywords: Biodiesel, Fatty Acids Methyl Ester, Avian Influenza, Rendered Fat, Waste to Energy

INTRODUCTION

Highly pathogenic avian influenza (AI) causes a substantial consequence in the poultry industry due to instantaneous burial action [1], leading to severe economic losses. This action is essential because some AI viruses may exhibit a zoonotic potential which may cause fatal diseases in animals, including humans [2]. Thus, a number of burial sites have been generated indiscreetly [3]. Although livestock mortalities have been a major waste stream within agriculture, massive and sudden burial of the carcasses has incurred civil complaints related to agricultural field possession of the site, groundwater pollution by leachate and so on [3]. These complaints make governments move to find appropriate termination procedures of the sites to return them to their agricultural purposes though such actions have been banned in some countries by stringent legislation [1,4]. However, in Korea, current legislation has been generating many burial sites due to frequent outbreaks of AI [3]. At the same time, the governments want to terminate old sites because on-site pollution seems to be inevitable due to natural biodegradation [5,6].

Various technologies may provide a cost-effective, practical and biosecure mechanism for burial site termination while lowering the environmental burden [7]. One of reasonable termination methods is to use the rendering process because it mashes up buried carcasses into small particles under high temperature and pressure condition [4]. Thus, it can almost completely resolve the infection issue while the steamed particles are separated into fat, proteinaceous water, and insoluble inorganics. Since such residues can be

a severe burden to the environment, researchers have suggested the possibility that some proportion of the rendered residues can be utilized as alternative sources of bioenergy [8,9]. For example, fat from AI-infected poultry can be a good source of biodiesel production. The previous study reported that poultry fat methyl ester can be blended with diesel fuel for conventional diesel engines without any major modification [10-12]. If health risk is not a problem via the rendering process, fat from AI-infected poultry may be considered as a cheap raw material that can lower biodiesel production cost [13]. However, the experimental approach is far from optimal and there remains a need to test the feasibility of this approach, mainly due to difficulty in the proper supply of test materials.

We conducted laboratory-scale transesterification of rendered fat from AI-infected poultry in a burial site as a proof of new waste control concept. The main goal was to test the feasibility of biodiesel production while terminating a burial site and to identify the composition of fatty acid methyl ester (FAME). Differences in the FAME characteristics were compared with general biodiesel from vegetables and animals to assure the safe, sustainable recycling of buried carcass.

MATERIALS AND METHODS

1. Substrate

Rendered fat from AI-infected poultry in a burial site was obtained from a pilot plant which conducts rendering of buried carcasses as a test-bed site of the governmental research project. Rendering operation was conducted for 60 min at the high temperature (200 °C) and pressure (18 bars) condition after shredding. Saturated steam generated from a boiler was used to increase the temperature of the reactant instantaneously. Higher pressure than saturated water vapor pressure was applied to maintain the better supply of

Table 1. Chemical oxygen demand (CODcr), total nitrogen (T-N), total phosphorus (T-P) concentrations of rendered lipid from AI-infected poultry in a burial site

Item	Average value (mg/L)	SD (mg/L)	RSD (%)
COD_{cr}	287,879	33,361	12
T-N	6,792	253	4
T-P	532	92	17

the steam. Total 40 kg of rendered and separated fat above water surface was collected for transesterification. Table 1 presents the basic chemical characteristics of the sampled rendered fat. Although initial content of free fatty acids was high (16.9%) due to the physical and chemical condition of rendering as well as biological degradation in a burial site, we tested a base-catalyst transesterification to promote rapid conversion as a feasibility test [13].

2. Transesterification

The catalyst, KOH, was selected as it easily dissolves in alcohol, which was methanol. By applying a mixture of methanol and KOH, we conducted transesterification. Mixing condition of the alcohol and catalyst was adapted from previous studies [14,15]. Initially, 15 ml of a catalyst-alcohol mixture was applied to 3 g of the collected fat in a 50 mL borosilicate glass bottle. The actual weight ratio of lipid : catalyst : alcohol was 15 : 1 : 60 on a mass basis. The contents continuously mixed in a shaking incubator under 37 °C for 1 h, during which ester-linked fatty acids were released and methylated. We followed the procedure of previous reports [16] which used base-catalyst; thus the reaction period is reasonably shorter than that of acid-catalyzed transesterification [17]. Each sample was vortexed every 10-20 min. When the transesterification was over, a 10 ml of n-hexane was injected to the crude biodiesel separated from the upper layer of the final transesterification mixture and was shaken vigorously to dissolve FAMEs into n-hexane layer. The FAME samples were taken from the n-hexane layer and transferred to 2 ml conical tube by using a pipette; we purified it with a centrifuge at 10,000 rpm for 20 minutes. The supernatant was transferred to a gas chromatograph vial for FAME analysis.

3. FAME Profile and Yield

Individual FAME content was analyzed by gas chromatograph with a flame ionization detector (GC-FID) for the centrifuged sample following a previous study [18]. We used a Sigma-Aldrich column (SP-2330, 30 m 0.25 mm I.D., 0.20 μm) with a split ratio of 1 : 10. Using a gas-tight glass syringe, 1 μL of the sample was injected via installed autosampler. The temperature of FID detector was set to 240 °C. Oven temperature was maintained at 140 °C for 1 min and then increased at a rate of 4 °C/min up to 220 °C. Carrier gases were helium (99.999%) and air (99.999%). Hydrogen (99.999%) gas was used for flame ignition. Based on the integrated peak area of the FAMEs identified in the samples, we quantified the amount of each FAME. Calibration curves were prepared by using FAME mix (Sigma-Aldrich) as a reference material.

4. Analytical Procedures

To monitor the characteristics of lipid, chemical oxygen demand (COD), total solids (TS), total nitrogen (TN), total phosphorus (TP), and volatile solids (VS) were determined following standard methods [19]. All other physical, chemical, and biological analyses were

conducted more than in triplicate; thus we used average values with standard deviation (SD) and relative standard deviation (RSD). The content of free fatty acid was measured by following ASTM procedures [20]. The biodiesel yield during the batch transesterification was determined by dividing the total amount of FAME obtained with the total amount of lipid applied for transesterification. It gives the proportion of quantified average FAME among lipid utilized. We calculated the degree of unsaturation and long chain saturated factor (LCSF) following methods described elsewhere [21]. The degree of unsaturation was calculated by summing wt% of mono- and poly-unsaturated FAMEs. LCSF was calculated from the composition of saturated FAME and their corresponding melting point. Based on the information about the molecular weight, the number of double bonds, and the weight proportion of each FAME component, fuel properties of the produced biodiesel were estimated following previous studies [21-25]. The properties were compared with biodiesel specification of EN 14214 and ASTM D6751 to test the adequacy of the produced biodiesel.

RESULTS AND DISCUSSION

1. FAME Yield and Profile

To evaluate biodiesel conversion potentials of rendered fat from AI-infected poultry in a burial site, we conducted a laboratory-scale transesterification experiment. Table 2 presents the quantified concentration of each FAME according to carbon and double bond numbers. Major FAME components were oleic acid methyl ester (C18:1, 227 mg/g lipid), palmitic acid methyl ester (C16:0, 102 mg/g lipid), and linoleic acid methyl ester (C18:2, 101 mg/g lipid). Total biodiesel yield, which was recovered as FAME among fat transesterified, was revealed as 47.4±1.5% based on total mass balance. Compared with other literature, the obtained data present relatively lower yield due to various impurities such as water and soil contents of raw rendered lipid. Previous study has shown a linear decrease in ester conversion efficiency according to water content [11]. In addition, the anaerobic condition of the burial site can convert the carcass in a burial site to free fatty acids, phospholipids, sterols, water, odorants and other impurities via microbial biodegradation [26]. Especially for free fatty acids, previous work has confirmed that increased level of free fatty acids undesirably leads to the loss of biodiesel [27].

Table 2 also compares the compositional profiles of FAME from rendered lipid of AI-infected carcass with other literature which used normal vegetables and animal fat as the transesterification sources. Oleic acid methyl ester (C18:1) was the most abundant (46.1%) FAME, and it was quite similar to reported values of normal poultry-fat transesterification [28]. When comparing the results of C18:1 with literature, it is also consistent with the data obtained in previous studies which used animal based fats [28]. However, a clear difference in the C18:1 composition was observed when compared with those of soybean, sunflower, corn, and cottonseed, respectively [26].

The second and third largest compositions were palmitic acid methyl ester (C16:0) and linoleic acid methyl ester (C18:2), which had 20.9% and 21.1%, respectively. In the case of C16:0, no big difference was found between animal fats and the vegetable-based bio-

Table 2. Compositions of FAME obtained from rendered lipid of AI-infected carcass and its comparisons with those from other animal fat and vegetable oils

FAME	Lipid number	[21]	[29]		[14]	This study
		Soybean oil	Lard	Beef tallow	Poultry	AI-infected poultry
		wt%	wt%	wt%	wt%	wt%
Caprylic	C8:0	-	0.03	-	-	0.00
Decanoic	C10:0	-	0.06	0.06	0.12	0.00
Lauric	C12:0	-	0.09	0.08	0.15	0.13
Myristic	C14:0	-	1.48	1.86	1.32	0.80
Palmitic	C16:0	11.3	22.6	23.3	22.43	21.10
Palmitoleic	C16:1	0.1	2.5	3.27	2.64	4.03
Stearic	C18:0	3.6	12.24	12.15	9.31	5.66
Oleic	C18:1	24.9	38.77	43.5	47.63	46.07
Linoleic	C18:2	53	2.51	2.92	14.82	20.94
Linolenic	C18:3	6.1	16.05	8.61	0.78	0.57
Arachidic	C20:0	0.3	1.21	0.47	0.21	0.29
Behenic	C22:0	0.3	0.56	0.64	0.22	0.12
Erucic	C22:1	-	-	-	0.38	0.29
Lignoceric	C24:0	0.1	-	-	-	0.00

Table 3. Compositions of FAME obtained from rendered lipid of AI-infected carcass and its comparisons with those from other animal fat and vegetable oils

Division	[21]	[29]		[36]		[37]	[38]	[14]	This study
	Soybean oil (wt%)	Lard (wt%)	Beef tallow (wt%)	Lard (wt%)	Tallow (wt%)	Poultry (wt%)	Poultry (wt%)	Poultry (wt%)	AI-infected poultry (wt%)
Saturated FAME	15.3	38.1	38.3	39.3	45.6	33.8	34.0	31.6	28.1
Unsaturated FAME	84.7	60.4	58.9	55.3	46.2	66.2	66.0	68.3	71.9
Monounsaturated FAME	25.0	41.3	46.8	44.2	42.4	50.7	40.3	47.3	50.4
Polyunsaturated FAME	59.1	18.6	11.5	11.1	3.8	15.6	25.7	20.9	21.5
Degree of unsaturation	143.2	78.4	69.8	66.4	50.0	81.9	91.7	89.2	93.4
Long-chain saturated factor	45.9	39.4	37.7	37.1	35.1	39.9	38.	37.6	40.0

diesel except palm-based biodiesel [29]. On the other hand, FAME from rendered fat of AI-infected carcass clearly showed higher wt% of C18:2 than other biodiesels. According to [30], high C18:2 presented increased stability to autoxidation. Among minor FAMEs, stearic (C18:0) and palmitoleic (C16:1) acid methyl esters presented 5.7% and 4.0%, respectively. And all other FAMEs showed less than 1 wt%.

2. Unsaturation Characteristics of FAME

Table 3 presents macro compositions of FAMEs. FAMEs were categorized according to the number of double bonds as saturated (0 double bonds), unsaturated (≥1 double bonds), monounsaturated (one double bond), and polyunsaturated (≥2 double bonds). With this categorization, the degree of unsaturation, as well as LCSF, were computed. Results show that monounsaturated FAME from AI-infected poultry was 50.4% and oleic acid methyl ester constitute approximately 91.4% of it. Compared to other poultry, our result shows similar content with the waste cooking grease of poultry, possibly due to exposure to high temperature and pressure. Also, AI-infected poultry demonstrated 14-22% and 6-25% higher monounsaturated FAMEs than those of lard and beef tallow, respec-

tively. Since the content of monounsaturated FAMEs presents a positive relationship with the fluidic behavior of biodiesel under low-temperature condition [21], these results suggest that biodiesel obtained in this study may support better fluidity at low temperature. Also, the higher proportion of oleic acid methyl ester than other cases of animal origin fat also indicates that biodiesel from AI-infected poultry may have enhanced oxidation stability under ambient temperature condition [31].

Saturated FAME was 28% and unsaturated FAME was 72% (Table 3). Considering weight, the degree of unsaturation from AI-infected poultry was as high as 93%, which was the highest value among compared in Table 3. It is known that the degree of unsaturation higher than 137 could not meet the European Standard for the cetane number since low cetane numbers have been associated with more highly unsaturated components [21]. Based on EU biodiesel standard, linolenic acid methyl ester should be lower than 12%, and our result shows only 0.57%. In addition, the fact that unsaturated FAME showed 2.6-fold higher proportion than saturated FAME indicates biodiesel from AI-infected poultry may contain sufficient oxidation stability and appropriate fluidity.
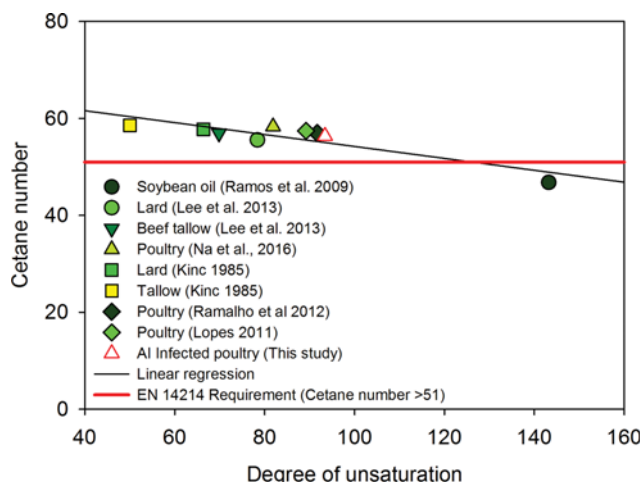
Fig. 1. Correlation between the degree of unsaturation and the cetane number calculated based on the weight percentage of FAMEs. The cetane number was estimated based on the lowest reported value of each FAME [32].

3. Fuel Characteristics of the Biodiesel Product According to Degree of Unsaturation

Fig. 1 demonstrates the relationships between the degree of saturation and the cetane number, which was calculated based on the weight percentage of each FAME. Following biodiesel specifications of EN14214 and ASTM D6751, FAME (biodiesel) for diesel engines should meet the cetane number requirements. Our results indicate that the obtained cetane number was 56.4, which was higher than the requirement 51 (EN) and 47 (ASTM). Because the cetane number plays an indicator role for the ignition and combustion quality of diesel fuel [11], it was verified that the biodiesel from AI-infected carcass can similarly satisfy one of the prime quality aspects as the fuel. Although some literature data for individual

cetane number of FAME may vary depending on the measurement methods and conditions [32,33], our prediction could meet the requirements. Fig. 1 also demonstrates that data obtained in this study was comparably clustered with other poultry results, while lard and tallow presented a relatively low degree of unsaturation. Although high unsaturation of biodiesel may lead to relatively low cetane numbers as the regression shows negative relationships between the degree of saturation and the cetane number [34], the results evidence that biodiesel from rendered fat of AI-infected poultry reasonably satisfy the biodiesel specification.

Table 4 tabulates various fuel properties of the produced biodiesel and compares the characteristic values with the EU and Korea standards. Determined cold filter plugging point satisfied both standards, though it was close to maximum value. Estimated cloud point and pour point were 6.1 $^{\circ}$C and -0.2 $^{\circ}$C, which present reasonable low-temperature properties. Since the oxidative stability was revealed as 8.1 hr (>6 hr), allylic position equivalent and bis-allylic position equivalent, estimated as 89.1 and 22.7, means susceptible induction period of the produced biodiesel [35]. The calorific value of the biodiesel was 39.2 MJ/kg as a high heat value, which is over 12% higher than the EU standard. Kinematic viscosity and density successfully satisfy the specifications listed in both standards (Table 4).

These results indicate the feasibility of the produced biodiesel even though it originated from the AI-infected poultry in a burial site, which opens the premise of problematic waste into value-added energy strategy.

CONCLUSIONS

This study demonstrates that rendered fat originating from AI-infected poultry in a burial site can be a viable source of biodiesel that can contribute to the termination of the burial site. It confirms that the produced biodiesel satisfies fluidity, oxidation stability, and

Table 4. Fuel characteristics of the produced biodiesel

Division	Unit	Biodiesel from AI carcass	EU[a]	KOR	ASTM[b]
Saponification value	mg KOH/g	202.9			
Iodine value		84.9	<120		
Cetane number		54.1	>51	>49	>47
Long chain saturated factor		5.2			
Cold filter plugging point	$^{\circ}$C	0.0	$-15\sim0$	0	
Cloud point	$^{\circ}$C	6.1			
Pour point	$^{\circ}$C	-0.2			
Allylic position equivalent		89.1			
Bis-allylic position equivalent		22.7			
Oxidation stability	hr	8.1	>6	>6	>6
Higher heating value	MJ/kg	39.2	>35		
Kinematic viscosity	mm^2/s	3.8	3.5-5.0	1.9>5.0	
Density	g/cm^3	0.9	0.86-9	0.86-9	0.86-9
FAME recovery from lipid	%	47[c]			

[a][22]

[b][35]

[c]Standard error was 0.59 for the six times of replicate experiment (n=6)

cetane number based on the compositional properties of FAMEs. This satisfaction to biodiesel specifications verifies the potential of biodiesel from AI-infected poultry comparable to conventional biodiesels. We believe that this approach offers one of the practical ways to obtain a sustainable bioresource while terminating problematic burial sites.

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