



## COMPARISON OF FATTY ACID COMPOSITIONS AND FUEL CHARACTERISTICS OF BIODIESELS MADE FROM ISOCHRYSIS GALBANA LIPIDS AND FROM USED COOKING OIL

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# COMPARISON OF FATTY ACID COMPOSITIONS AND FUEL CHARACTERISTICS OF BIODIESELS MADE FROM *ISOCHRYSIS GALBANA* LIPIDS AND FROM USED COOKING OIL

Cherng-Yuan Lin and Bo-Yu Lin

Key words: microwave-assisted extraction, *Isochrysis galbana* lipid, fuel characteristics, fatty acid methyl esters, cetane number.

## ABSTRACT

The fatty acid compositions and typical fuel characteristics of the biodiesels produced from *Isochrysis galbana* lipids and used cooking oil were analyzed and compared in this study. The experimental results showed that the major fatty acid compositions of the biodiesel made from the extracted *Isochrysis galbana* lipids were significantly different from those of the biodiesel made from used cooking oil. The biodiesel made from the microalgae lipids primarily comprised 35.34 wt. % myristic acid (C14:0) whereas the major compound of the used cooking-oil biodiesel was 47.51 wt. % oleic acid (C18:1). Docosahexaenoic acid (DHA) at 4.67 wt. % was only found in the microalgae biodiesel. The fuel properties of the microalgae fatty acid methyl esters were more optimal, including a significantly lower iodine number, 71.0, and higher cetane number, 65.3. *Isochrysis galbana* is therefore a promising feedstock source for the production of biodiesel.

## I. INTRODUCTION

The use of algae as a feedstock for biofuels could significantly mitigate the global greenhouse gas effect. Biodiesel is produced from the transesterification of triglycerides contained in animal fats, vegetable oils, or algae lipids with short-chained alcohols such as methanol or ethanol. For example, fatty acid methyl esters (FAME) are made from triglycerides with methanol through transesterification. Used vegetable oil or animal fat can be recycled after being repeatedly used for cooking at high temperatures and/or pressures.

Microwaves, which are electromagnetic waves with a fre-

quency between 200 MHz and 300 GHz, have been widely applied in the communications, food, and manufacturing industries. The application of microwaves is seldom found in biofuel production. In this study, microwave irradiation was used to facilitate the extraction of *Isochrysis galbana* lipids. The lipid extracted from the microalgae with the assistance of microwave irradiation was further transesterified to produce biodiesel. Used cooking oil was transesterified with methanol with the aid of a strong alkaline catalyst.

The lipid content and fatty-acid type of microalgae are the key parameters for producing biodiesel with adequate properties. *Isochrysis galbana* is considered as a potential feedstock for biodiesel production due to its high lipid content, ranging from 7-40 wt. % (Yan et al., 2014). The quantity of FAME made from *Isochrysis galbana* lipid extracted by microwave irradiation with an n-hexane/isopropanol solvent can reach as high as 77.4 wt. % (Lin and Lin, 2015). Microalgae biodiesel is characterized by a higher free fatty acid content, heating value, density and kinematic viscosity than general oil crop biodiesel (non-edible, edible or used cooking oil), although its properties differ from those of microalgae species (Azad and Islam, 2012). Biodiesel produced from used cooking oil can reduce exhaust emissions and improve engine performance. However, the variations in the chemical compositions and fuel characteristics of biodiesels produced from these two feedstocks have not yet been studied. To address this gap in the research, the compositions of the fatty acids and significant fuel properties of the FAME from used cooking oil and *Isochrysis galbana* lipids were analyzed and compared in this study. The potential application of *Isochrysis galbana* as a feedstock of biofuels was also evaluated.

## II. EXPERIMENTAL DETAILS

The *Isochrysis galbana* microalgae were provided by High-trust Biotechnology Inc. of Hualien County, Taiwan. Surface seawater was collected from the ocean near Keelung city in northern Taiwan and sterilized in a high-pressure sterilizing caldron (TM-321 model, Hoyu Inc., Taiwan) used for the cultivation of the microalgae. The *Isochrysis galbana* biomass was harvested at

**Table 1. Representative fuel properties of typical FAME compositions (Ramírez-Verduzco et al., 2012; Hoekman et al., 2012).**

FAME compositions		Cetane number	Heating value (kcal/mol)	Kinematic viscosity (mm <sup>2</sup> /s)
C8:0	Caprylic acid	33.6	1313	1.19
C10:0	Capric acid	47.6	1625	1.72
C12:0	Lauric acid	61.4	1940	2.43
C14:0	Myristic acid	66.2	2254	--
C16:0	Palmitic acid	74.5	2550	4.38
C18:0	Stearic acid	86.9	2859	4.74
C18:1	Oleic acid	59.3	2828	4.51
C18:2	Linoleic acid	38.2	--	3.05
C22:1	Erucic acid	--	3454	5.91

**Table 2. Coefficients of iodine values of fatty acids (Ramírez-Verduzco et al., 2012).**

FAME type	Coefficients of iodine value
Saturated fatty acids	0
C16:1	0.950
C18:1	0.860
C18:2	1.732
C18:3	2.616
C20:1	0.785
C22:1	0.723

a stable cultivation stage by separating the wet biomass from the culturing sea water with a high-speed centrifuge (Sorvall Legend Mach 1.6R model, Thermo Scientific Inc., Germany). Deionized water was used to wash the collected *Isochrysis galbana* biomass three times. The collected *Isochrysis galbana* biomass was washed three times with deionized water then dried to powder form in a freeze dryer (Eyela FDU-1200 model, Tokyo Rikakikai Inc., Japan). The *Isochrysis galbana* powder was extracted by microwave irradiation and an organic solvent mixture of n-hexane and isopropanol at a volumetric ratio of 2:1 (v/v). Twice the volume of the solvent mixture of the extracted microalgae dry powder was used. The microwave reactor (Tmo-17Mb model, Tatung Inc., Taiwan) was operated at an input power of 260 W for 25 secs to extract the microalgae lipid.

After the microalgae lipids were absorbed by the solvent mixture, a nitrogen blow-down concentrator (HCG-12A model, Hoya Inc., Taiwan) was used to blow the solvent mixture away from the microalgae lipids. A two-step transesterification reaction was used to convert the *Isochrysis galbana* lipids into FAME. The first step in this reaction was the saponification of the fatty acids using a strong alkaline-catalyst process, and the second step was methyl esterification via a strong acid-catalyst process (Patil et al., 2012). The microalgae lipid was added to 8 ml KOH/methanol. The reaction mixture was heated at 75°C for 20 minutes and then cooled to 25°C, to hydrolyze triglycerides to potassium salts of fatty acids. To begin the second step of the transesterification reaction, 10 ml BF<sub>3</sub>/methanol and 8 ml 0.7 N HCl/methanol were added to the reaction mixture. To complete the methyl esterification, the reaction mixture

was heated at 75°C for 15 minutes then cooled to 25°C, and the FAME extracted using a solvent mixture of n-hexane/isopropanol (2/1 v/v). The fatty acid composition of the FAME obtained were analyzed and compared with a biodiesel made from used cooking oil by strong alkaline transesterification.

A gas chromatograph accompanied by a flame ionization detector (GC-FID; HP-6890 model, Hewlett Packard Inc., U.S.A.) was used to analyze the composition of the FAME made from the extracted *Isochrysis galbana* lipids and used cooking oil. The dimensions of the capillary column were 30 m × 0.25 mm × 0.25 μm. Nitrogen was used as the carrier gas at a velocity of 45 ml/min.

The fuel properties of fatty acids, including the cetane number, heating value, and kinematic viscosity, generally differ according to their compositions, such as C16:0 or C18:1 (Hoekman et al., 2012; Islam et al., 2015). The fuel characteristics, including the cetane number, kinematic viscosity, and heating value per unit weight, for each fatty acid composition are listed in Table 1 (Ramírez-Verduzco et al., 2012). The iodine value represents the percentage of unsaturated fatty acids in FAME (Islam et al., 2015). The iodine coefficients for the typical fatty acids that were used to calculate the combined iodine value of the FAME are shown in Table 2 (Ramírez-Verduzco et al., 2012). The fuel properties of the FAME made from *Isochrysis galbana* lipids and used cooking oil were determined by summing the corresponding fuel characteristics per unit weight of each fatty acid composition, with reference to Tables 1 and 2. Each experiment was carried out three times to obtain the mean measurements.

**Table 3. Comparison of the fatty acid composition (wt. %) of *Isochrysis galbana* lipid FAME and used cooking oil FAME (Lin and Li, 2009).**

Fatty acids	FAME from	
	<i>Isochrysis galbana</i> lipid	used cooking oil
C14:0	35.34	0.54
C14:1	0.84	ND
C16:0	10.82	14.18
C16:1	13.13	0.74
C18:0	0.16	3.77
C18:1	7.04	47.51
C18:2	14.23	24.83
C18:3	10.95	4.97
C20:0	ND	0.8
C20:1	0.12	ND
C20:2	0.15	0.17
C20:3	1.30	ND
C20:4	ND	0.38
C20:5	ND	0.03
C21:0	ND	ND
C22:0	1.00	0.1
C22:1	0.16	ND
C22:2	ND	0.18
C22:4	ND	0.14
C22:5	ND	0.05
C22:6	4.67	0.04
Saturated fatty acids	47.32	19.29
Mono-unsaturated fatty acids	21.29	48.25
Long carbon chain fatty acids (C20-C22)	7.40	1.99

ND: non-detected

### III. RESULTS AND DISCUSSION

#### 1. Comparison of FAME Fatty Acids Composition

Microwave fragmentation, together with a solvent mixture of n-hexane/isopropanol, was used to extract crude microalgae lipids for FAME production. This approach was effective in the reduction of both the lipid extraction time and the production cost of the biodiesel (Dai et al., 2014). Moreover, Terigar et al. (2010) found that the polar isopropanol solvent has excellent absorption properties for microwave energy, and that lipids have superior solubility in the non-polar n-hexane solvent. Microwave irradiation was found to enhance the reaction rate of activated compounds and alter the electrovalence of the molecular bonds (Hernando et al., 2007), resulting in an increase in the quantity of FAME.

The quantity of FAME made from *Isochrysis galbana* lipids and absorbed by the solvent mixture of n-hexane/isopropanol (2:1 v/v) was 77.4 wt. % (Lin and Lin, 2015). The results in Table 3 show that the biodiesel produced from used cooking oil by strong alkaline catalyst transesterification contained five main fatty acids: oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), linolenic acid (C18:3), and stearic acid (C18:0)

(Lin and Li, 2009).

The five major fatty acids in the FAME manufactured from *Isochrysis galbana* lipid were myristic acid (C14:0), linoleic acid (C18:2), hexadecenoic acid (C16:1), linolenic acid (C18:3), and palmitic acid (C16:0). The FAME from the two sources had significantly different fatty acid compositions and weight fractions. The FAME made from *Isochrysis galbana* lipids were primarily composed of 35.34 wt. % C14:0 (myristic acid), whereas the FAME made from used cooking oil (Lin and Li, 2009) were primarily composed of 47.51 wt. % C18:1 (oleic acid). Docosahexaenoic acid (DHA; C22:6) is a highly polyunsaturated fatty acid. The FAME made from *Isochrysis galbana* lipids contained 4.67 wt. % DHA (C22:6). In contrast, the FAME made from used cooking oil contained almost no DHA, as shown in Table 3. Koberg et al. (2011) produced biodiesel from *Nannochloropsis* biomass using microwave and ultrasound radiation. They found that the microalgal FAME were mostly composed of methyl esters with carbon chains consisting of fewer than 18 carbon atoms. They also found that the microwave irradiation method was an efficient way to carry out direct transesterification.

The FAME made from *Isochrysis galbana* lipids contained significantly more saturated fatty acids and long carbon chain

**Table 4. Comparison of the fuel characteristics of FAME made from *Isochrysis galbana* lipids and used cooking oil.**

Fuel characteristics	FAME from	
	<i>Isochrysis galbana</i> lipid	used cooking oil
Iodine value (g I <sub>2</sub> /100g)	71.0	97.6
Kinematic viscosity (mm <sup>2</sup> /s)	4.1	3.8
Cetane number	65.3	56.3

fatty acids (C20 to C22) than the FAME made from used cooking oil, as shown in Table 3. Used cooking oil is often used repeatedly for cooking at high temperatures before it is collected, thus the carbon chain of the fatty acids in cooking oil is prone to breaking. Hence, the long carbon chain fatty acid content was significantly lower (1.99 wt. %) in the FAME from used cooking oil than in the FAME from *Isochrysis galbana* lipids (7.40 wt. %), as shown in Table 3. The *Isochrysis galbana* lipid FAME contained significantly less monounsaturated fatty acids (21.29 wt. %) than the used cooking oil FAME (48.25 wt. %). However, there was no obvious difference in the amount of polyunsaturated fatty acids in the *Isochrysis galbana* lipid FAME and the used cooking oil FAME.

## 2. Comparison of FAME Fuel Characteristics

The fuel characteristics of the *Isochrysis galbana* lipid and used cooking oil FAME are compared in Table 4. The iodine value is a representative indicator of the degree of unsaturation of the fatty acids in lipids or FAME. The iodine value of *Isochrysis galbana* biodiesel was 71.0, which was significantly lower than the value of 97.6 for the used cooking oil biodiesel and implies greater oxidation stability in the former. This difference is attributable to the higher unsaturated fatty acid content in the used cooking oil biodiesel.

The FAME of the *Isochrysis galbana* lipid had a higher kinematic viscosity (4.1 mm<sup>2</sup>/s) than the used cooking oil FAME (3.8 mm<sup>2</sup>/s), as shown in Table 4, as a result of its significantly higher saturated fatty acid content. Saturated fatty acids, such as stearic acid (C18:0) and palmitic acid (C16:0), frequently have significantly higher pour points than unsaturated fatty acids. Similarly, unsaturated fatty acids are often less viscous and have superior fluidity at low temperatures than saturated fatty acids (Moser et al., 2015). Hence, the FAME made from *Isochrysis galbana* lipids contained a much higher proportion of saturated fatty acids (47.32 wt. %) (Table 3) and had higher kinematic viscosity than FAME made from used cooking oil (Table 4). The kinematic viscosities of both FAMEs were well within the EN 14214 European biodiesel specifications of between 3.5 mm<sup>2</sup>/s and 5.0 mm<sup>2</sup>/s. Du et al. (2011) produced algal bio-oil through the pyrolysis of *Chlorella sp.* in a microwave oven with a power rating of 750 W. The kinematic viscosity of this bio-oil was 61.2 mm<sup>2</sup>/s; considerably higher than that of the microalgal biodiesel produced in this study.

The cetane number is the most important parameter for evaluating the compression-ignition quality of liquid fuels for use in diesel engines. A higher cetane number indicates a shorter

ignition delay, which implies a shorter time interval between the start of fuel injection and the beginning of combustion. Saturated fatty acids usually have higher cetane numbers than unsaturated fatty acids (Miraboutalebi et al., 2016). The *Isochrysis galbana* FAME comprised 47.32 wt. % saturated fatty acids, as shown in Table 3. In contrast, the used cooking oil biodiesel comprised only 19.23 wt. % saturated fatty acids. Hence, the cetane number of the microalgae biodiesel (65.3) was significantly higher than that of the used cooking oil biodiesel (56.3).

## IV. CONCLUSIONS

1. The compositions of the five major fatty acids in the biodiesels produced from *Isochrysis galbana* lipids and from used cooking oil were obviously different. The microalgae FAME comprised primarily 35.34 wt. % myristic acid (C14:0), whereas the used cooking oil FAME comprised primarily 47.51 wt. % oleic acid (C18:1). Only the microalgae FAME contained 4.67 wt. % DHA.
2. The *Isochrysis galbana* FAME contained 47.32 wt. % saturated fatty acids and 7.40 wt. % long carbon chain fatty acids (C20-C22), which was much higher than the contents of the used cooking oil biodiesel, 19.23 wt. % and 1.99 wt. %, respectively. The microalgae biodiesel also had a significantly lower monounsaturated fatty acids content, 21.29 wt. %, than the used cooking oil FAME, 48.25 wt. %.
3. The microalgae FAME had superior oxidation stability and ignition quality than the used cooking oil FAME, due to its significantly lower iodine value (71.0) and higher cetane number (65.3). The kinematic viscosity of both FAMEs met the EN 14214 specification, which implies adequate fluidity at lower temperatures. *Isochrysis galbana* is thus considered to be a more adequate feedstock for the production of biodiesel than used cooking oil.

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