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Influence of Different Extraction Methods on Fatty Acid Composition of Lipid Extracts of *Chlorella vulgaris* Beijerinck from Laguna De Bay, Philippines

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***Chlorella vulgaris* Beijirinck cultures isolated from Laguna de Bay were cultivated and the biomass produced after one month of cultivation was subjected to stepwise selection of solvent system, algae-to-solvent ratio, and extraction method. The optimized procedure for *C. vulgaris* was found to be sonication with 2:1 (v:v) chloroform:methanol as extractant at 1:20 (g:ml) algae:solvent ratio producing 51% crude lipid (dry-weight basis). Using thin layer chromatography, the *C. vulgaris* lipid extract showed the following approximate composition: triglycerides (79%), sterols (14%), and 1,3-diglycerides present in substantial amounts. Gas chromatography – flame ionization detection of the fatty acids as fatty acid methyl esters showed the following components: myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and traces of lauric acid. Analysis of the lipid extracts showed that extraction procedure, along with the solvent system, affects both yield and lipid profile. Total lipid yield and sterol yield increase with increasing polarity of the solvent system. Triglycerides, on the other hand, decreases with the polarity of solvent systems used. A more varied fatty acid class was obtained using more polar solvents (8 fatty acids) compared with the usage of less polar ones (1–3 fatty acids).**

Keywords: algal lipids, fatty acid profile, lipid yield, solvent polarity, sonication

INTRODUCTION

Microalgae are regarded as a promising sustainable energy resource due to their capacity to amass huge amounts of lipids that performs similarly to petroleum (Sheehan *et al.* 1998). This has resulted in researches focusing on the cultivation of algae for lipid production – specifically biodiesel – via lipid transesterification. Lipids can be categorized as polar such as glycolipids and phospholipids and neutral/nonpolar lipids such as mono-, di-, and tri-acylglycerides (Greenwell *et al.* 2010).

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These lipid components are also studied as source of food additives and high-value products such as eicosapentoic acid (C20:5, EPA) and docosahexanoic acid (C22:6, DHA) (Schenk *et al.* 2008). The potential of microalgae as source of food commodities and fatty acids for nutritional efficacy has triggered an interest among researchers for the past decades (Li *et al.* 2012).

One of the microalgae cultivated primarily for biodiesel production is *Chlorella vulgaris*, considered one of the most promising strains among the 3,000 species screened (Sheehan *et al.* 1998). They are easy to cultivate, can grow with little or no attention using water unsuitable

for human consumption, and easy to obtain nutrients (Mata *et al.* 2010). It has been reported that *C. vulgaris* may be a suitable diesel replacement based on the study of calorific values of different *Chlorella* species (Illman *et al.* 2000). In addition, microalgal biomass has the potential to attain higher lipid productivity in comparison to oilseed crops (Schenk *et al.* 2008), can be cultivated in brackish water or non-arable land (Searchinger 2008), and its biochemical composition can be modulated by varying growth conditions so the lipid yield can significantly increase (Qin 2005).

Significant progress has been made in the upstream process of producing biomass for lipid yields (Li *et al.* 2014). The cultivation of *Chlorella* involves growing the algae in a suitable media that are often modified to yield a specific component. Lipid and starch contents increase while biomass decreases during unfavorable growth conditions (Lv *et al.* 2010) such as nitrogen and phosphorus limitation, high CO_2 concentration (Chinnasamy *et al.* 2009), or excess of iron in medium (Liu *et al.* 2008). In the Philippines, studies on optimization of cultivation conditions had been conducted on local strains of freshwater *C. vulgaris* by a team from UPLB and Ateneo de Manila University under the DOST-PCAMRD research project – Biodiesel Feedstock Production from Freshwater Microalgae – from 2009 to 2011. Some studies have been done to optimize the parameters for microalgal growth such as light (Morillo 2010), nutrient starvation (Argete 2012), and carbon dioxide concentration – and on designing and fabricating photobioreactors for its cultivation (Arinduque 2009, Redondo 2009).

On one hand, lipid extraction process is still a challenge for commercial lipid production despite the enormous literature in lipid extraction methods in microalgae such as mechanical pressing and procedures involving supercritical fluid, microwave, osmotic, ultrasound waves, and solvent extraction (Mubarak *et al.* 2015). Solvent extraction entails extraction of lipids by repeated washing and percolation with an organic solvent. This remains the main extraction method used by researchers due to its simplicity and relatively inexpensive requiring almost no investment for equipment (Letellier and Budzinski 1999). Solvent extraction also offers other advantages in terms of its high selectivity and solubility towards lipids (Neto *et al.* 2013). The use of ultrasonication can enhance the extraction process due to the creation of bubbles near the cell wall that produces shock waves and, in the process, releases the lipids in the solvent (dos Santos *et al.* 2015).

Extensive information on the fatty acid composition of *C. vulgaris* is available. Major fatty acids of *C. vulgaris* are palmitic (16:0), linoleic (18:2), and linolenic (18:3) acids. Myristic (14:0), palmitoleic (16:1), hexadecadienoic (16:2), hexadecatrienoic (16:3), stearic (18:0), and oleic (18:1) acids were also found as minor components (Tsuzuki *et al.* 1990). This agrees with the report of Zhukova and Aizdacher (1995) in their study of the fatty acid composition of marine *Chlorella sp.* This information is used for evaluating the biological value of microalgae and some are used for chemotaxonomic purposes (Volkman 1989).

Though there are numerous reports regarding the lipid extraction in *Chlorella* and its fatty acid composition abroad, there have been no literature on these parameters for the *C. vulgaris* from Laguna de Bay. There are also no local studies conducted relating the different extraction methods and their corresponding fatty acid profile. Thus, the objectives of this study were to optimize lipid extraction from *C. vulgaris* and characterize the fatty acids from the different solvent systems and extraction procedures.

MATERIALS AND METHODS

The general procedure for the cultivation of *C. vulgaris,* optimization of lipid extraction, lipid profiling, and fatty acid analysis is outlined in Figure 1.

Obtaining Microalgae Biomass

The *C. vulgaris* CL1 strain isolated from Laguna de Bay and maintained in the UPLB Museum of Natural History Algal Culture Collection in the Phycology Laboratory I of the Institute of Biological Sciences (IBS), College of Arts and Sciences, University of the Philippines Los Baños, was selected for the study. To obtain the inoculum, cells were grown in 1-L glass bottles containing 500 ml of BG-11 media. The BG-11 media was composed of $NaNO_3$ (150 g L^{-1}), $KH_2PO_4 \cdot 3H_2O$ (8.0 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (15.0 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (7.2 g L^{-1}), citric acid (1.2 g L^{-1}), $FeC_6H_5O_7 \cdot NH_4OH$ (1.2 g L^{-1}), Na_2EDTA (0.2 g L^{-1}), and Na_2CO_3 (4.0 g L^{-1}) plus trace metals such as H_3BO_3 (2.86 g L^{-1}), $MnCl_2 \cdot 4H_2O$ (1.81 g L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.22 g L^{-1}), $Na_2MoO_4 \cdot 2H_2O$ (0.39 g L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.08 g L^{-1}), and $Co(NO_3)_2 \cdot 5H_2O/CoCl_2 \cdot 5H_2O$ (0.05/0.04 g L^{-1}) (Allen & Stainier 1968). The medium was sterilized at 1.02 atm (120 °C) for 15 min in a laboratory pressure cooker.

The inoculum obtained was transferred to autoclavable polyethylene bags (15 cm x 20 cm) containing 1 L of BG-11 medium. These bags were placed under indirect sunlight on the covered rooftop of the Molecular Biology and Biotechnology Building of IBS (Figure 2). Aeration was provided by attachment of aquarium aerators to the polyethylene bags. Culture growth was monitored by cell counting in haematocytometers for one month every four days. The microalgal cells were harvested at stationary phase after four weeks of growing period.

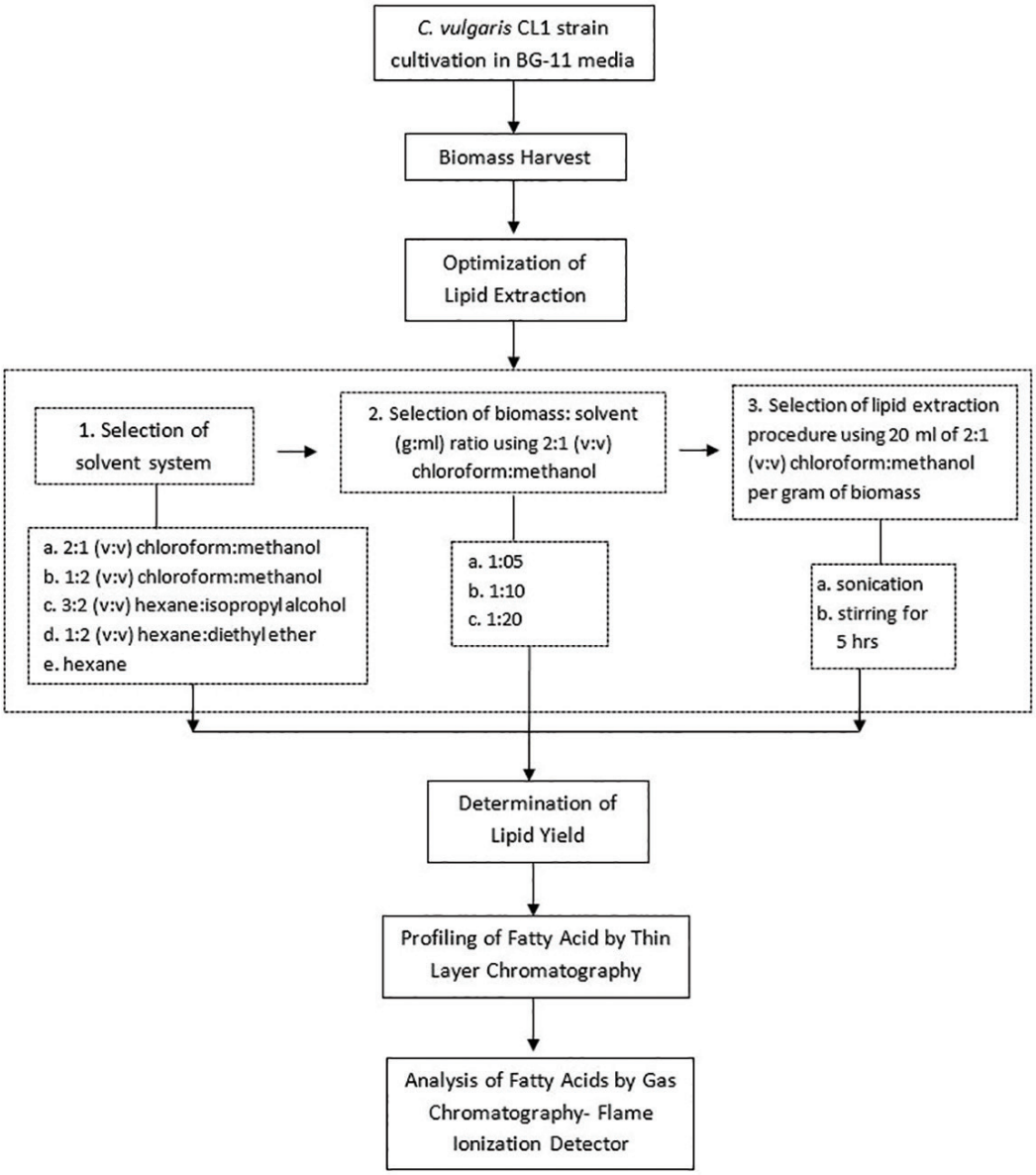

Figure 1. Flow diagram of optimization of lipid extraction and fatty acid analysis of *C. vulgaris* from Laguna de Bay.

Figure 2. Cultivation setup of *C. vulgaris.*

The harvested biomass was collected by centrifugation for 10 minutes at 716xg using Kubota KS-5200C, then placed in petri dishes and oven-dried at 80 °C for 72 hours. The dried microalgae were ground using mortar and pestle, sieved (70 mesh), placed in vials, weighed, and stored in the refrigerator (7 °C) until use.

Optimization of Lipid Extraction

Six solvent systems were tested: 2:1 (v:v) chloroform:methanol, 1:2 (v:v) chloroform:methanol, 3:2 (v:v) hexane:isopropyl alcohol, 1:2 (v:v) hexane:diethyl ether, and pure hexane. All solvents used in the extraction

are of analytical grade and were obtained from standard sources. Lipid was extracted by soaking the biomass for 24 hours using 10 ml of the extractant. The mixture was then filtered and washed with fresh extracting solvent after subsequent filtrations. Extracted lipids were then concentrated by evaporating the solvent in a hot water bath. Based on its highest lipid yield, the 2:1 (v:v) chloroform:methanol solvent system was chosen for subsequent extraction procedure.

Using different volumes (5, 10, and 20 ml) of 2:1 (v:v) chloroform:methanol, a gram of algae was soaked for 24 hours thrice. The extracted lipids were concentrated using RE 47 Buchi Rotary Evaporator and lipid yield was determined for each extraction process. The algae:solvent (g:ml) ratio with the highest lipid yield was 1:20 (v:v). This was then used for the subsequent extraction method.

The next step of the method selection involves the employment of 5-hour stirring and sonication thereafter. Flasks were covered with aluminum foil during the extraction of lipids. The extraction procedures were done simultaneously.

In the five-hour stirring procedure, a gram of *C. vulgaris* was soaked in 20 ml of 2:1 (v:v) chloroform:methanol and stirred for five hours straight using a magnetic stirrer. The temperature was maintained below 60 ºC to avoid lipid oxidation. Lipid yield from each of the three-step extraction procedure was determined and combined for further analysis. Similar parameters were employed for the sonication procedure except that the microalgae-solvent mixture was subjected to ultrasonication for two hours. The residues were stored for further analysis while the extracts were concentrated using RE 47 Rotary Evaporator. Percent lipid yield was calculated using this formula:

$$\% \, lipid = \frac{mass \; of \; lipid}{mass \; of \; dry \; biomass} \; x \; 100 \quad (1)$$

All extractions and analysis were carried out in triplicates and the data are reported as mean ± standard deviation. The determination of fatty acid profile was carried out in all lipid samples obtained using different solvent systems, algae-to-solvent ratio, and extraction procedure.

Thin Layer Chromatography (TLC)

Lipid samples (0.01–0.02 g) were prepared by adding 100 µl of extractant to the microalgal lipid. Using a syringe, 5 µl of the lipid was applied to the plates. Chromatograms were developed with hexane:diethyl ether:formic acid (80:20:2;v:v:v) and visualized with iodine vapor. Developed chromatograms were photographed and the composition of lipids was estimated using Biosoft Quantiscan Software (Figure 3).

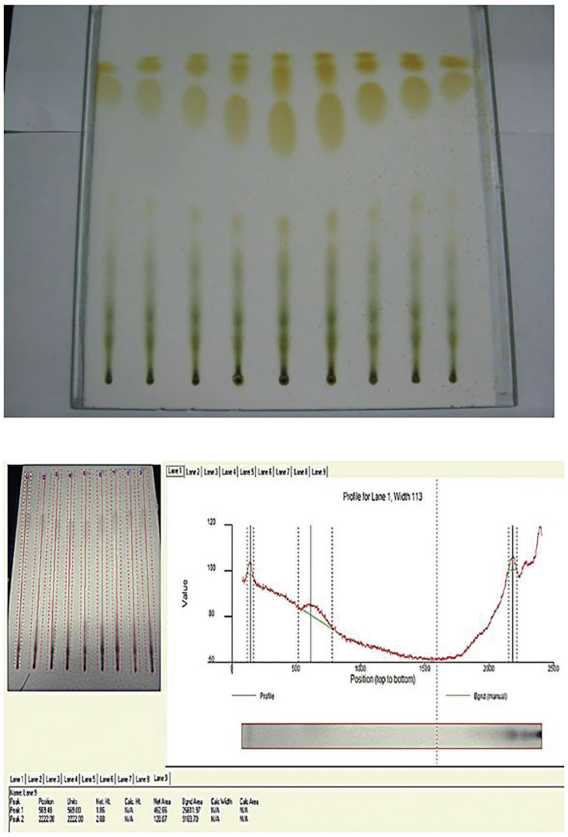

Figure 3. (A) Sample of a developed chromatogram for lipid profiling of *C. vulgaris*; (B) estimation of the composition of lipids using Biosoft Quantiscan Software.

Analysis of Fatty Acid Composition

Ten milligrams (10 mg) of lipids were dissolved in 2 ml hexane followed by the addition of 0.2 ml methanolic KOH. Following light centrifugation, an aliquot of dry hexane layer was collected for GC analysis (Ichihara *et al.* 1996). The fatty acid methyl esters were analyzed by Schimadzu GC-14 Gas Chromatograph equipped with Chromosorb column (1.1 m x 2 mm i.d.) and a Flame Ionization Detector. The injection and detector temperature were set at 260 °C. Column oven temperature was programmed from 90 °C (20 min) to 220 °C at 16 °C/min. Flow rate of nitrogen gas was at 40 ml/min. The fatty acids were identified by comparison with retention time of virgin coconut oil (VCO) fatty acid mixture standards (AOAC 969.33 and 963.22).

Statistical Analysis

The extracted lipid contents were compared according to different parameters using *F*-test in one-way ANOVA and Tukey test. The level of significant difference was at $p < 0.05$.

RESULTS

A total of 46.43 g of microalgal biomass was produced – giving an average yield of 750 mg per liter of medium. This value is higher compared to the yield of cellular dry weight of species commonly used in mariculture, which ranges from 80 to 250 mg dry weight per liter (FAO 1996). However, the yield obtained is lower than when pure carbon dioxide (1–5%) was supplied (Lam and Lee 2013) or when the *C. vulgaris* was grown in a flat panel airlift photobioreactor (0.11 g L^{-1} d^{-1}; Degen *et al.* 2001), and those grown in heterotrophic (0.25 g L^{-1} d^{-1}; Liang *et al.* 2009) and mixotrophic conditions (2–5 g L^{-1} d^{-1}; Safi *et al.* 2014).

Optimization of Lipid extraction

Selection of extractant. The highest lipid yield was obtained from 2:1 chlorofom:methanol (v:v) followed by 1:2 chloroform:methanol (v:v) solvent system with 13.92% and 12.71% yield, respectively (Figure 4A). The yields from the two systems, however, do not differ significantly. Lipid yields from 3:2 hexane:isopropyl alcohol (v:v) and 1:2 hexane:diethyl ether (v:v) of 5.46% and 3.13% compared to lipid yield from 2:1 chloroform:methanols were significantly lower by two- and three-fold, respectively. Using hexane as a solvent to extract lipids gained the lowest yield of 2.45% (Figure 4A). Amount of extracts from hexane and 1:2 (v:v) hexane: diethyl ether are not statistically different as well.

On the basis of highest lipid yield, 2:1 (v:v) chloroform:methanol was chosen for subsequent evaluation of extraction parameters.

Selection of algae to solvent ratio. The highest oil yield of 25.69% was obtained using the biomass to solvent ratio of 1:20 (g:ml) (Figure 4B). This is higher by 161% and 48% compared to lipid yield from 1:05 (g:ml) and 1:10 (g:ml) biomass to solvent ratio, respectively. The oil yield obtained increased with the amount of solvent used per gram of biomass because of the greater solute to solvent interaction. It is assumed that the amount of lipid will increase further if the amount of solvent to be used will be increased. However, Moholkar *et al.* (2010) have shown that using more than 20 ml of solvent does not significantly increase the amount of lipid yield.

Multiple extractions were done to maximize the amount of lipids that can be collected. Yields during the second extraction dropped by 29% (0.015 g), 69% (0.084 g), and 28% (0.04 g) for the algae:solvent ratio of 1:05, 1:10, and 1:20 (g:ml), respectively. The third extraction further lowered the lipid yield, thus rendering it practical to extract the lipid thrice.

Selection of lipid extraction procedure. The 51% lipid yield using sonication is higher by 19.6% compared to stirring for five hours (Figure 4C). This procedure is also a great improvement compared to the 14% oil yield obtained from soaking the algae for 24 hours using 2:1 (v:v) chloroform:methanol (Figure 4A).

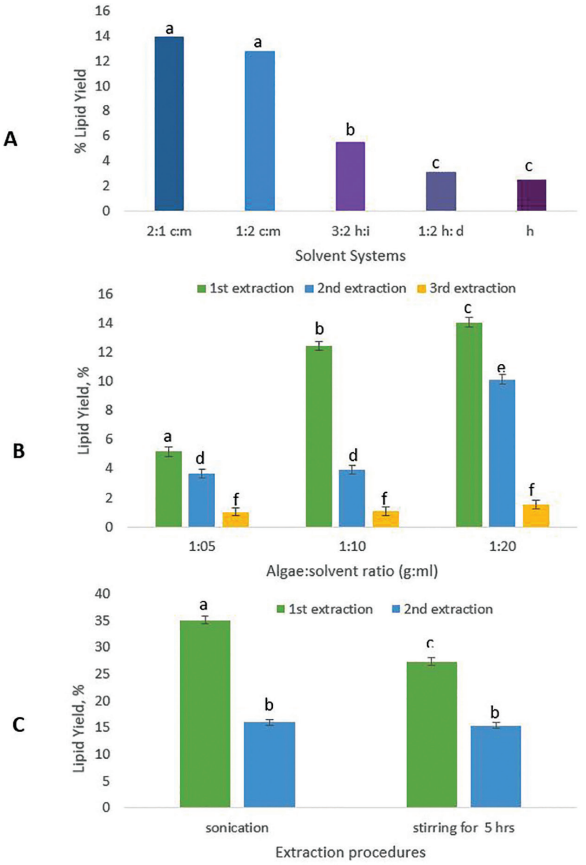

Figure 4. Lipid yield (%) of *C. vulgaris* using (A) different solvent systems, (B) 2:1 c:m with different algae:solvent ratio, and (C) different extraction procedures with 2:1 c:m as extractant. Values with similar letters indicates no significant difference between values.

Legends:
2:1 c:m = 2:1 (v:v) chloroform:methanol, 1:2 c:m = 1:2 (v:v)chloroform:methanol, 3:2 h:i = 3:2 (v:v) hexane:isopropyl alcohol, 1:2 h:d = 1:2 (v:v) hexane:diethyl ether, h = hexane.

Lipid Profile of *C. vulgaris* by Thin Layer Chromatography

Triglycerides constitute majority of the lipid molecular species in *C. vulgaris* – ranging from 70 to 89% regardless of the solvent used (Table 1A). They are by far the most abundant single lipid class and constitute almost all the commercially important fats and oils. Although the use of 1:2 (v:v) chloroform:methanol extracted the highest amount of triglycerides among solvent systems, the percentage composition of triglycerides

Table 1. Relative mass of lipids extracted from *C. vulgaris* in different extraction parameters using thin layer chromatography.

Parameters	Mass of Lipids, (mg/g algae)					
A. Solvents (v:v)[a]	Triglycerides		Sterol		1,3 -Diglycerides	
2:1 chloroform:methanol	98.40		28.95		12.23	
1:2 chloroform:methanol	105.40		12.20		9.62	
3:2 hexane:isopropyl alcohol	42.71		2.38		9.61	
1:2 hexane:diethyl ether	74.22		4.23		15.55	
hexane	65.87		1.28		6.54	
B. Biomass to Solvent[b] Ratio (g:ml)	1st Extraction	2nd Extraction	1st Extraction	2nd Extraction	1st Extraction	2nd Extraction
1:05	32.66	14.74	7.53	21.88	11.44	n.d.
1:10	88.36	23.78	24.39	15.23	11.03	n.d.
1:20	105.70	73.33	28.19	9.24	12.80	n.d.
C. Extraction Procedures[a] using 20 ml 2:1 Chloroform:Methanol (v:v)	Triglycerides		Sterol		1,3 -Diglycerides	
Sonication	278.07		48.45		24.61	
Stirring for 5 h	118.13		18.98		22.61	

Note:
[a]Extracts are from first extraction only.
[b]2:1 chloroform:methanol (v:v)
n.d. – not detected

in the lipid is highest in hexane and lowest in 2:1 (v:v) chloroform:methanol.

Sterol yield, ranging from 1.74 to 20.7%, decreased with decreasing polarity of the solvent. For 1,3-diglycerides, the highest amount was obtained using 3:2 (v:v) hexane:isopropyl alcohol (17.5%) and lowest was detected in hexane and 2:1 (v:v) chloroform:methanol extracts.

Fatty Acid Composition of *C. vulgaris* by GC-FID

Overall, the results show that different extraction methods lead not only to different lipid yields but also influence the fatty acid profile to a great degree (Figure 5). Lipid profile of *C. vulgaris* consists mainly of fatty acids of long chain length (>C14) – of which the major fatty acid is linolenic acid (C18:3 $^{\Delta 9Z,12Z,15Z}$), which ranges from 33 to 68% of the lipid extract. Oleic acid (C18:1 $^{\Delta 9Z}$) and linoleic acid (C18:2 $^{\Delta 9E,12E}$) are also present in substantial amounts. Other extracts obtained through other solvents consists only of lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0). These fatty acids were also present in chloroform:methanol extracts but in very small amounts.

Common fatty acids identified in 2:1 chloroform:methanol extract with algae:solvent (g:ml) ratio of 1:5, 1:10, and 1:20 were palmitic, linolenic, and linoleic constituting 34%, 31%, and 12% of the lipid composition on the average, respectively. Fatty acid composition of extracts between different algae to solvent ratio do not significantly differ.

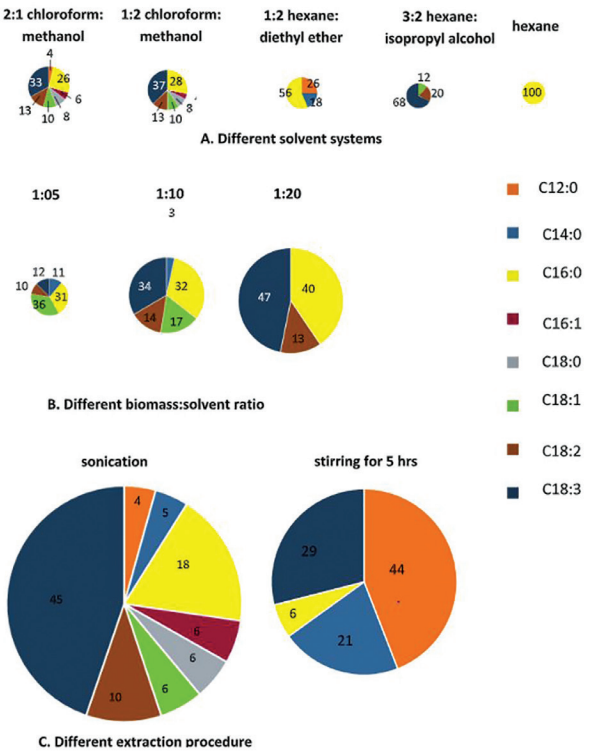

Figure 5. Fatty acid profiles of *C. vulgaris* lipid extract analyzed by GC -FID from (A) different solvent systems, (B) different biomass:solvent (g:ml) ratio, and (C) different extraction procedures. Chart areas are proportional to the lipid yield for each parameter. Each pie chart also includes the percentages of fatty acids detected.

There were eight fatty acids identified from extracts obtained from sonication. In comparison, extracts from stirring procedure contain only four fatty acids. These are lauric acid, myristic acid, palmitic acid, and linolenic acid. However, these were not calculated for their relative weights. Linolenic acid is the most abundant fatty acid (45%) in the extract obtained through sonication, whereas lauric acid is preeminent for extracts obtained through stirring (44%).

It was also observed that, despite the range of fatty acids (C12 to C18:3) extracted using 2:1 (v:v) chloroform:methanol, not all types were obtained when extracted using different ratio of algae:solvent (g:ml). Lauric acid, palmitoleic acid, and stearic acid were not detected using 5 and 10 ml of 2:1 (v: v) chloroform: methanol. Similarly, these fatty acids – in addition to myristic acid and oleic acid – were not detected using 20 ml of the said solvent system. This may be a result of the low sensitivity of the GC-FID used, failure of the solvent system to extract the fatty acids, or both.

DISCUSSION

Optimization of Lipid Extraction

Selection of extractant. The average lipid yield using different solvents tends to decrease with the extractant's decrease in polarity. The highest yields were obtained from the chloroform:methanol systems with polarity indices of 14.30 and 13.30 for 2:1 (v:v) chloroform:methanol and 1:2 (v:v) chloroform:methanol, respectively. This was followed by 3:2 hexane:isopropyl alcohol, 1:2 (v:v) hexane:diethyl ether – with polarity indices of 8.10 and 5.70, respectively – and lastly, pure hexane with 0.10.

The solubility of lipids in organic solvent is based on the principle "like dissolves like." When nonpolar organic solvents such as chloroform and hexane are used as extractants, neutral lipids (*i.e.*, triglycerides) are extracted from microalgae by complexing with these solvents. However, chloroform and hexane are unable to extract polar lipids due to the solvent's inability to separate these lipids from its strong linkage with proteins. Polar solvents such as methanol is therefore necessary to extract polar lipids such as phospholipids, glycolipids, sterols, and carotenoids (Halim *et al.* 2012).

The solvent system of chloroform:methanol takes advantage of different polarities to extract both neutral and polar lipids – in addition to a myriad of other compounds such as waxes, aldehydes, fatty acids, and chlorophyll (dos Santos *et al.* 2015, Ryckebosch *et al.* 2012, Lewis *et al.* 2000). This ability to extract lipids of varying polarities explains why chloroform:methanol obtained the highest lipid yield among the solvent systems used in this study. Lower yields were obtained using low-polarity solvents (hexane:isopropanol, hexane:diethyl ether, and hexane) because of their selectivity toward neutral lipids (Halim *et al.* 2012).

Lipid yields are not always higher using solvent systems (Shen *et al.* 2009). Hexane obtained higher lipid yield from *Scenedesmus dimorphus* and *Chlorella protothecoides* than 1:1 (v:v) hexane:ethanol. However, Ryckebosh *et al.* (2012) disagreed with the results – explaining that this is due to different extraction efficiencies depending on different type of solvents used.

Selection of extraction procedure. The lipids can either be extracted by diffusion or disruption of the cell wall. Soaking the algae for 24 hours and five-hour stirring procedure do not involve shear stress and the main mechanism of extraction is likely to be diffusion across the cell wall. Because the cell walls of microalgae are thick, the intralipids get blocked resulting to lesser yields if use of solvents will be used.

On the other hand, sonication involves both diffusive and disruptive lipid extraction. Cell walls are broken mechanically, which extracts more lipids. Yield are enhanced by 50–500% and extraction time is lower (Suali & Sarbatly 2012). However, Moholkar *et al.* (2010) disagreed stating that the prominent mechanism for lipid extraction was diffusion across the cell wall. He also added that the selectivity of the solvent is a far more dominant factor in overall extraction by diffusion. This supports the results of Lewis *et al.* (2000) that although an appropriate method is necessary for disrupting the cell wall, an efficient lipid extraction is highly dependent on the polarity of organic solvents or solvent mixture used.

Based on the lipid yield, the optimized procedure for the extraction of lipid from *C. vulgaris* is sonication with 2:1 (v:v) chloroform:methanol as extractant in the algae:solvent ratio of 1:20 (g:ml).

Lipid Profile of *C. vulgaris* by TLC

The choice of solvent, therefore, depends on the researcher's objective. If one's goal is biodiesel-oriented, then hexane is a more suitable option. On the other hand, chloroform:methanol (v:v) is more suitable for those who are interested in obtaining other valuable compounds (*e.g.*, unsaponifiable lipids).

The lipid profile for extracts obtained by 2:1 (v:v) chloroform:methanol in different algae:solvent ratio showed that triglycerides were still the largest component of the lipids detected (Table 1A). Levels of triglycerides and sterols increased while 1,3-diglycerides remained the

same with increasing algae:solvent ratio (g:mL) during the first extraction. High levels of triglycerides and sterol were detected in extracts obtained by algae:solvent ratio of 1:20 (v:v).

Quantification of lipids detected during the second extraction using 2:1 (v:v) chloroform:methanol showed different results. Levels of triglycerides increased while that of sterol decreased as the algae:solvent (g:mL) ratio increased. Another significant difference is that 1,3-diglycerides were not detected during the second extraction.

Lipids of the extracts obtained during the third extraction were also quantified but the results were not significant (data not shown). Similar relative composition was observed in the extracts obtained by sonication and stirring procedure (Table 1C). Triglycerides were still largest component among the lipids followed by sterols and 1,3-diglycerides.

Fatty Acid Composition of *C. vulgaris* GC-FID

Results of this study show that *C. vulgaris* has a simple fatty acid composition as compared to almost all green algae. Results of the fatty acid composition agree with studies published through the years (*i.e.*,Zhukova and Aizdaicher 1994, Li *et al.* 2014), which have established the fatty acid composition of *C. vulgaris*. The composition C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, and C18:3 is confirmed for *Chlorella.* Lauric acid were also detected in trace amounts (Petkov and Garcia 2007). This composition is independent of the algae's growth conditions, strains, and species.

Previous reports suggested that *Chlorella* cultures heavily contaminated with bacteria contain fatty acids with odd number of carbons – C15:0, C17:0, and C17:1 (DeMort *et al.* 1972, Wacker *et al.* 2002). Lack of these fatty acids in the composition of the culture used indicates that fatty acids identified in this study came from *C. vulgaris* only.

With regards to requirements of oil for biodiesel production, lipids with fatty acids of 16-18 carbon atoms are preferred for such purpose (Huang *et al.* 2010). In this study, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid constitutes more than 90% of the total fatty acids from chloroform:methanol extracts. Using hexane can generate biodiesel too as it generates palmitic acid only. Other solvents used in this study are not recommended as they generate more of fatty acids with 12–14 carbon atoms.

CONCLUSION

Fresh water microalgae *C. vulgaris* from Laguna de Bay was chosen as the subject in optimization of lipid extraction due to its easy cultivation methods and significant lipid content. Solvent systems, varying ratio of biomass to solvent, and extraction procedures were tested to optimize the amount of lipids that can be extracted from *Chlorella.*

The solvent system 2:1 (v:v) chloroform:methanol obtained the highest yield of lipid and extracted more varied fatty acids. Hexane, on one hand, produced the lowest amount of lipid yield containing mostly medium-chain fatty acids, which are most suitable for biodiesel production. A more polar solvent system extracted more varied types of fatty acids compared with the use of less polar ones. For all solvent systems used, the lipid profile of *C. vulgaris* is primarily composed of triglycerides and the major fatty acid is linolenic acid.

It is important to note that in selecting the method for extracting lipids from *C. vulgaris*, not only the lipid yield but also the desired compounds must be considered.

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