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Short Communication

Proximate composition, total phenolics content and antioxidant activities of microalgal residue from biodiesel production

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Abstract: In biodiesel production, lipid is extracted from algal biomass but some beneficial substances may remain in its residue. In this study proximate composition, total phenolics content and antioxidant activities of a microalgal residue after lipid extraction were determined. It was found that the residue has a high protein content and the hot aqueous extract of the residue is high in both the phenolics content and the level of antioxidant activities.

Keywords: biodiesel production, microalgal residue, proximate composition, antioxidant activities, total phenolics content, ferric reducing power, Trolox equivalent antioxidant capacity

INTRODUCTION

Microalgae are interesting as the third generation of biofuel production, especially biodiesel. Lipid is extracted from microalgal biomass and converted into biodiesel by chemical methods [1]. However, microalgal residue is produced after lipid extraction. A good use of this by-product will certainly be beneficial to the solving of both the economic and environmental issues involved in biodiesel production from microalgal biomass [2].

Generally, microalgae are used as feed or feed supplement due to their high nutritional value [3]. In addition, microalgae have been known for their production of bio-active substances, especially antioxidants. For example, the extracts of *Spirulina platensis* and *Dunaliella salina* show high antioxidant activity when analysed by Trolox equivalent antioxidant capacity (TEAC) assay [4]. Natrah et al. [5] reported that extracts from many microalgal strains including *Isochrysis galbana*,

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Chaetoceros calcitrans, *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Nannochloropsis oculata* and *Tetraselmis tetrathele* show inhibitory activity against lipid peroxidation of linoleic acid. Among the microalgae tested, *I. galbana* and *C. calcitrans* exhibit the highest antioxidant activity in the ferric thiocyanate and thiobarbituric acid assays. Recently, some commercial applications of microalgae, such as nutritional supplements, natural dyes and skin care products, were being promoted [3]. However, there seem to have been no reports on the antioxidant activities of lipid-extracted microalgal biomass (microalgal residue) or its extract. Antioxidants could be used in biodiesel production as oil protectant against oxidative deterioration [6]. Thus, antioxidant activities (including phenolics content) and nutritional content of the microalgal residue are worth investigating. The results could lead to applications in biodiesel production or animal feed supplement, and the residue would be used completely and worthily.

MATERIALS AND METHODS

Preparation of Microalgal Residue and Proximate Analysis

A microalgal consortium consisting of *Golenkinia* sp. (45.9%), *Acutodesmus (Scenedesmus) dimorphus* (Turpin) Tsarenkom (34.6%), *Chlorella* sp. (13.4%), *Micractinium* sp. (3.3%) and *Scenedesmus acutus* Meyen (2.7%) was cultivated in a 4×8 m open pond using 10,000 L of CMU03 medium [7] at the Faculty of Fisheries Technology and Aquatic Resources, Maejo University. After 1 month the cells were harvested by spontaneous flocculation and dried with solar heat. Lipid was extracted from the microalgal biomass by Soxhlet extraction with hexane: chloroform: methanol 4:1:1 (v/v) at 80°C for 10 hr [8]. The microalgal residue was filtered and dried at 40°C.

The proximate chemical composition and energy of the dry microalgal residue were analysed at Central Laboratory (Thailand) Co. Ltd. by using the AOAC methods [8] and a bomb calorimeter according to a standard method [9] respectively.

Extraction and Analysis of Microalgal Residue

The dry microalgal residue was ground into powder in a mortar. One hundred grams of the powdered microalgal residue was soaked in 95% ethanol or distilled water at 55°C for 15 hr. The cold aqueous extract was prepared separately by extracting the same amount of material three times with distilled water at ambient temperature with intermittent shaking for 3 days each time. All solutions from the extraction were concentrated with a rotary evaporator at 40^{o}C and the extracts finally dried in a lyophiliser.

Total phenolics content was determined using the Folin-Ciocalteu method as described by Chandler and Dodds [10]. The total phenolics content values were standardised with gallic acid and reported as mg of gallic acid equivalent per gram of sample.

The ferric reducing power of the microalgal residue was evaluated according to the method of Oyaizu [11]. The reducing power was calculated and expressed as mg of gallic acid equivalent per gram of sample.

The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical anion scavenging assay was executed by the method given by Re et al. [12] with some modifications and was expressed as Trolox equivalent antioxidant capacity (TEAC), defined as mg of Trolox equivalent per gram of sample.

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Statistical Analysis

The data were expressed as mean ± standard deviation of three replicates. Statistical comparison between the groups was analysed using one-way analysis of variance (ANOVA) and post-hoc Tukey's b-test using SPSS version 14.0. *p*-Values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

From the proximate analysis, the microalgal residue was found to possess a high nutritional value, especially with respect to protein and carbohydrate (Table 1). The yields of the hot aqueous extract, cold aqueous extract and ethanolic extract of the microalgal residue were 9.46%, 9.36%, and 1.562% respectively. The low yield of the ethanolic extract was expected because most of the non-polar substances in the residue, such as lipids and carotenoids, had been previously extracted, which also corresponds to the low fat content of the residue, as shown in Table 1.

Table 1. Proximate composition of microalgal residue after lipid extraction

Nutrient	%
Protein (N x 6.5)	27.26
Fat	1.36
Ash	26.20
Fibre	9.22
Carbohydrate	35.93
Energy	265.00 (cal.g^{-1})

From Figure 1, the hot aqueous extract of the microalgal residue shows the highest total phenolic content. Phenolic compounds have been noted for their positive correlation with antioxidant capacity [13]. Therefore, the determination of phenolics content is an indirect method of evaluating the antioxidant capacity of a sample.

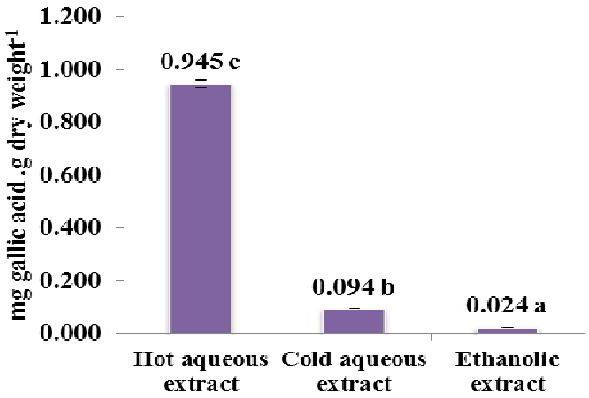

Figure 1. Total phenolics content of extracts of microalgal residue
Note: Values with the different letters are significantly different at $p>0.05$.

The hot aqueous extract of the microalgal residue shows a significantly high ferric reducing power, the highest among the three extracts (Figure 2). The reducing power of an antioxidant is the ability to provide electrons to free radicals, thus stopping the damages incurred by the radical chain

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reactions [11]. The hot aqueous extract of the microalgal residue also shows the highest and most significant scavenging ability against ABTS radical (Figure 3). Scavenging activity is the ability to give electrons or hydrogen atoms to free radicals via single electron transfer or hydrogen atom transfer [12].

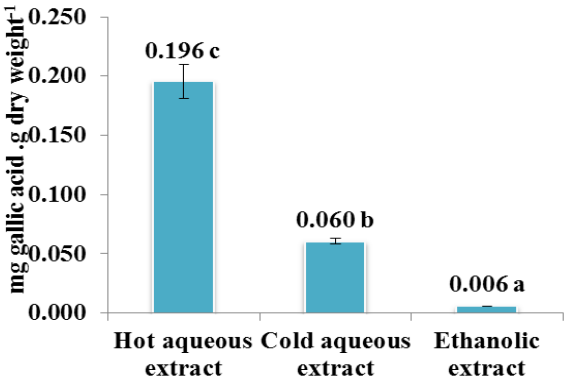

Figure 2. Ferric reducing power of extracts of microalgal residue
Note: Values with the different letters are significantly different at $p>0.05$.

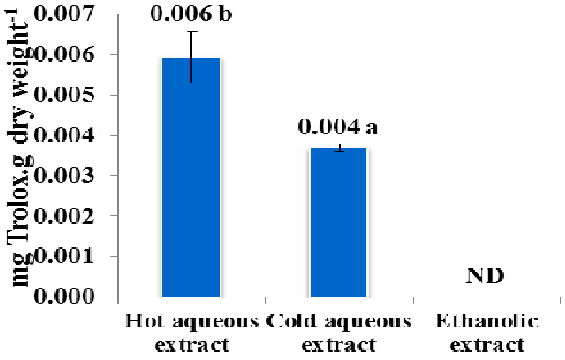

Figure 3. ABTS radical scavenging assay of extracts of microalgal residue
Note: Values with the different letters are significantly different at $p>0.05$. ND = not detected

Hot water was found to be the most suitable solvent for extraction of phenolics and antioxidants from the microalgal residue. Since the polarity of phenolic compounds varies significantly, it is difficult to optimise a method for extraction of all phenolic compounds [14]. Water is a highly polar solvent and generally not a suitable solvent for non-polar organic compounds at room temperature. However, when the temperature of water is increased, there is a steady decrease in its permittivity, viscosity and surface tension and a rise in its diffusive characteristics, which results in its capacity for dissolving a wider range of substances including some less polar compounds [15]. This may account for a slightly higher extraction yield and higher antioxidant capacities as well as higher total phenolics content of the hot aqueous extract compared to the cold extract. The results are similar to the study by Shon et al. [16], who found that hot water could extract a larger amount of total phenolic compounds than that obtained by other solvents and that the hot-water extract also exhibited good antioxidant activities. On the other hand, non-polar antioxidants and other non-polar metabolites such as chlorophyll, carotenoids, ω-3 fatty acids and γ-linolenic acid found in the

microalgae [17] were removed in the bio-oil extraction process, leading to the observed low antioxidant capacities of the ethanolic extract.

Interestingly, although the lipid-extracted microalgal biomass is a leftover material from the production process, its antioxidant activities are still comparable to that of some whole biomass algae. The total phenolics content of the hot aqueous extract (0.945 ± 0.015 mg gallic acid.g dry weight^{-1}) obtained in this study is close to that of *Turbinaria conoides* aqueous extract (1.116 ± 0.011 mg gallic acid.g dry weight^{-1}) [18] and its ABTS radical scavenging activity (0.006 ± 0.001 mg Trolox.g dry weight^{-1}) is about twice higher than that of *Padina minor* aqueous extract (0.003 ± 0.001 mg Trolox.g dry weight^{-1}) [19]. Similarly, the value for reducing power from this study (0.196 ± 0.014 mg gallic acid.g dry weight^{-1}) is higher than those derived from *Amphiroa* sp. and *Halimeda macroloba* aqueous extracts (0.022 ± 0.000 and 0.092 ± 0.000 mg gallic acid.g dry weight^{-1} respectively) [18].

However, the ratio of the microalgal population in a microalgal consortium may influence the antioxidant activities. Although the population may change with physical factors such as light intensity and water temperature, CMU03 medium was found to control the population of microalgal consortia to be in the group of green microalgae. From our previous study [7], the dominant species may be different in each consortium but they are still in the Chlorophyceae and this may not significantly affect the proximate composition, although the antioxidant activities may still change. Aboul-Enein et al. [20] reported that microalgae show different antioxidant activities, even though they belong to the same genus. It was found that *Scenedesmus dimorphus* shows higher anti-lipid peroxidation than *S. acutus* due to different antioxidant contents in each species, such as carotenoids, vitamin E and vitamin C. Thus, the microalgal population and residue at the time of harvest should be standardised.

CONCLUSIONS

A microalgal residue after lipid extraction (for biodiesel production) is found to be high in protein and may be suitable for use as a feed supplement. The hot aqueous extract of the residue is also high in total phenolics content and antioxidant activities, i.e. ferric reducing power and ABTS radical scavenging activity.

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