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Lipid and fatty acids extraction from the cyanobacterium *Spirulina*

Ratana Chaiklahan[a,*], Nattayaporn Chirasuwan[a], Veara Loha[b], Boosya Bunnag[c]

[a] Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok 10150, Thailand

[b] Department of Chemical Engineering, King Mongkut's University of Technology Thonburi, Thung-kru, Bangkok 10140, Thailand

[c] School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok 10150, Thailand

* Corresponding author, e-mail: ratana@pdti.kmutt.ac.th, chaiklahan@yahoo.com

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ABSTRACT: Lipid extraction from *Spirulina* using a single stage extraction at 30 °C showed that a sample-solvent ratio of 1:10 and an extraction time of 120 min gave the highest total fatty acid (TFA) yield, whereas a ratio of 1:5 was suitable for a multistage extraction. An increase in extraction temperature resulted in higher lipid and TFA yields. Increasing the extraction temperature from 30 °C to 60 °C decreased the extraction time and increased the yield of lipid and TFA in the solvent by approximately 69% and 55%, respectively. In order to increase the extraction yield of the lipid and TFA, multistage cross-current extraction was implemented. Seven-stage extraction at 30 °C resulted in 85% TFA recovery, whereas only a three-stage extraction was needed to obtain the same level of TFA at 60 °C. Lipid extracted from *Spirulina* contained approximately 21% linoleic acid and 18% γ-linolenic acid.

KEYWORDS: *Spirulina*, lipid, total fatty acid, solvent extraction

INTRODUCTION

The cyanobacterium *Spirulina* is used worldwide as a health food and is also used in animal feed. *Spirulina* can produce a large number of valuable compounds such as phycocyanin and carotenoids used as antioxidants[1,2], and polyunsaturated fatty acids such as linoleic acid and γ-linolenic acid (GLA)[3]. Polyunsaturated fatty acids have an important role in human metabolic pathways, particularly as precursors of a particular type of prostaglandin E1[4]. GLA has been used in several medical applications such as in the treatment of dermatitis, diabetes, and pre-menstrual syndrome[5,6]. The use of GLA in medical and dietary applications has led to the need to search for better methods of extraction and purification of fatty acids from natural sources[7]. There have been several studies dealing with the extraction of lipids containing GLA from many sources including fungi (*Mucor* spp. and *Mortierella* spp.)[8], evening primrose seeds[9], borage seed[10] and *Spirulina*[4,11].

The lipid extraction yield depends on the nature of the solvent, the lipid or oil particle size, the sample-solvent ratio, temperature, and time of extraction. Solvents such as hexane, ethanol, methanol, acetone, petroleum ether, and a mixture of chloroform and methanol ($CHCl_3$-MeOH) are used in the extraction of lipid from vegetable, flower, and oil seeds[12]. In a previous study on finding a suitable solvent for lipid extraction, we found that the yield of lipids extracted with ethanol did not show a significant difference from that obtained from the extraction using a $CHCl_3$-MeOH mixture[13]. The aim of this work was to investigate the effect of process parameters such as the sample-solvent ratio, extraction temperature, time of extraction, number of extraction stages (multistage extraction) with ethanol as a selected solvent for maximum lipid and total fatty acids (TFA) extraction, and to optimize the extraction process.

MATERIALS AND METHODS

Dried *Spirulina* biomass provided by Siam Algae Co., Ltd., Samutprakarn, Thailand was subjected to lipid extraction. The process was performed under continuous mixing using a stirrer (EYELA, NZ-1000 series equipped with a stirring shaft and a stirring propeller type flat blade turbine) at 120 rpm. Extraction of lipids from *Spirulina* biomass was performed in a single-stage extraction at room temperature for 30 min. Nine variations of the sample-solvent ratio (1:3, 1:4, 1:5, 1:6, 1:8, 1:10, 1:12, 1:15, and 1:50 w/v) were tested in a 150 ml beaker. Dry *Spirulina* biomass samples weighing 10 g, 7.5 g, 6 g, and 5 g were placed in 30 ml of ethanol in order to prepare mixtures with sample-solvent ratios of 1:3, 1:4, 1:5, and 1:6 w/v,

respectively. Similarly, samples weighing 7.5 g, 6 g, 5 g, and 4 g were placed in 60 ml of ethanol to achieve sample-solvent ratios of 1:8, 1:10, 1:12, and 1:15 w/v, respectively. Finally, a ratio of 1:50 was obtained from 2 g of sample in 100 ml of ethanol. To determine the effect of temperature on the yield of lipid and TFA, the process was carried out at 25 °C, 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C.

The optimum extraction time and number of extraction stages were also determined. Lipid extraction was carried out at two different temperatures (30 °C and 60 °C) using a sample-solvent ratio of 1:5 (100 g of dry biomass in 500 ml of ethanol). The extraction time ranged from 5 to 150 min and the extraction time selected at each temperature was used to study the effect of the number of extraction stages under each condition.

To determine the optimum number of extraction stages, multistage cross-current extraction was used. The extraction process was performed in an agitated tank. In the first stage, the solvent was added to the feed (a ratio of 1:5 w/v), mixed and settled to separate the residue. The residue was reextracted by fresh ethanol at each extraction stage (10 stages).

Whatman No. 4 filter papers were used in the filtration step for cell residue separation and the extracts were collected. Solvents were removed by vacuum rota-evaporation (Büchi Rotavapor R-200) at a pressure of 175 mbar and at a temperature of 40 °C which was maintained by the use of a cooling tower. The yield of the extracted lipids was measured as the percentage of dry weight (lipid/dry biomass).

To determine the TFA yield in the solvent (TFA/dry biomass) and the remaining amount in the residue, the samples were transesterified by using a modified version of Lapage and Roy's method[14]. Freeze-dried samples (100 mg) or lipid extracts (at least 10 mg) were direct transmethylated in 5% HCl in methanol at 85 °C for 1 h, and heptadecanoic acid (C17:0; Sigma Co.) was added as an internal standard. Fatty acid methyl esters were analysed by gas chromatography (GC 17-A; Shimadzu, Japan) using capillary columns of fused silica glass (30 cm × 0.25 mm, SP-2330, Supelco, USA) with a film thickness of 0.20 µm. The injector temperature was 205 °C and the spit ratio was 1:50. Identification was carried out by co-chromatography[15] with authentic standards (Sigma Co.).

RESULTS AND DISCUSSION

Sample-solvent ratio

Spirulina contains 6–13% lipids, half of which is TFA[16]. In these experiments, the average value of TFA in raw *Spirulina* was 4.86% of the dry weight (Table1). This value was used as the basis for the calculation of TFA recovery. In the single-stage extraction, an increase in sample-solvent ratio led to an increase in both lipid and TFA yields in the solvent. When the sample-solvent ratio increased from 1:3 to 1:4, 1:4 to 1:5, and 1:5 to 1:6 (w/v), the yield of extracted lipids increased by approximately 60%, 50%, and 11%, respectively, and the yield of TFA in the solvent also increased by 33%, 33%, and 12.5%, respectively. Increases in sample-solvent ratios from 1:6 to 1:8, 1:8 to 1:10, and 1:10 to 1:12, resulted in a slight increase in the yield of lipids and TFA in the solvent. A sample-solvent ratio of 1:10 was found to be the initial ratio necessary for a stable TFA yield in the solvent (2.2% of dry weight). A sample-solvent ratio of 1:5 resulted in a yield of lipid and TFA in the solvent of 3.6% and 1.6% of dry weight, respectively (Table 2). In spite of the fact that a sample-solvent ratio of 1:50 obtained a yield of lipids (7.9% of dry weight) and TFA yield in the solvent (2.6% of dry weight) higher than a 1:5 ratio, a 10-fold increase in the amount of solvent was needed for extraction. In the single-stage extraction method, TFA remained in the residue despite the use of large quantities of solvent. Therefore, a multistage method using cross-current extraction was applied in order to increase the yield of TFA and to decrease the amount of solvent used.

Table 1 Total fatty acid content of *Spirulina*.

Analysis no.	no.1	no.2	no.3	no.4	mean	SD[a]
TFA/dry biomass (wt %)	5.12	4.56	4.92	4.86	4.86	0.23

[a] Standard deviation

With the same volume of solvent, results showed that TFA recovery in solvent from a sample-solvent ratio of 1:5 using a two-stage extraction technique was no different to that of a single-stage technique using a ratio of 1:10. When cells were extracted using a three-stage extraction method with a 1:5 ratio, TFA recovery in the solvent was found to be higher than that using a single-stage extraction method with a ratio of 1:15 (Table 3). However, it was found that TFA recovery in solvent from a sample-solvent ratio of 1:5 using a three-stage extraction method (10 min each) was no different to that using a single-stage method and a ratio of 1:15 for 30 min (Table 4).

The total time taken for extraction using a sample-solvent ratio of 1:5 with a three-stage extraction of 30 min/stage was 10% less than for TFA recovery in solvent using 10 min/stage (Tables 3 and 4). Therefore, the sample-solvent ratio of 1:5 was

Table 2 Yields of extracted lipid and TFA when dried *Spirulina* was extracted using various sample-solvent ratios at room temperature.

Ratio (w/v)	Volume of solvent	Lipids/ DB[a] (wt %)	In solvent		In residue TFA/DB[a] (wt %)
			TFA/DB[a] (wt %)	TFA recovery (%)	
1:3	15	1.5 ± 0.04	0.9 ± 0.03	18 ± 0.5	3.8 ± 0.03
1:4	20	2.4 ± 0.11	1.2 ± 0.02	24 ± 0.4	3.4 ± 0.09
1:5	25	3.6 ± 0.11	1.6 ± 0.04	33 ± 0.8	2.8 ± 0.20
1:6	30	4.0 ± 0.12	1.8 ± 0.04	38 ± 0.8	2.8 ± 0.01
1:8	40	4.3 ± 0.04	2.0 ± 0.04	42 ± 0.9	2.5 ± 0.11
1:10	50	4.9 ± 0.18	2.2 ± 0.06	46 ± 1.2	2.4 ± 0.03
1:12	60	5.3 ± 0.11	2.2 ± 0.05	47 ± 1.0	2.3 ± 0.11
1:15	75	5.6 ± 0.05	2.3 ± 0.13	48 ± 2.6	2.2 ± 0.06
1:50	250	7.9 ± 0.01	2.6 ± 0.08	54 ± 1.7	2.1 ± 0.09

[a] Dry biomass

In this and subsequent Tables, values are expressed as mean ± SD of n= 2

Table 3 Yield of TFA in the solvent when cells were extracted using a sample-solvent ratio of 1:5 (two and three-stage), a single-stage of 1:10, and 1:15 ratio at room temperature.

Ratio (w/v)	Total time of extraction (min)	Total volume of solvent (ml)	In solvent	
			TFA/DB (wt %)	TFA recovery (%)
1: 5 (single-stage)	30	25	1.6 ± 0.04	33 ± 0.8
1: 5 (two-stage)	60	50	2.3 ± 0.05	47 ± 1.0
1: 5 (three-stage)	90	75	2.9 ± 0.03	60 ± 0.6
1:10 (single-stage)	30	50	2.2 ± 0.06	46 ± 1.2
1:15 (single-stage)	30	75	2.3 ± 0.04	48 ± 0.8

Table 4 Yield of TFA in the solvent when cells were extracted using a sample-solvent ratio of 1:5 (three-stage, time consumed for each stage was 10 min) and sample-solvent ratio of 1:15 (single-stage, 30 min).

Ratio (w/v)	Number of extraction stages	Time/stage (min)	In solvent	
			TFA/DB (wt %)	TFA recovery (%)
1:5	3	10	2.5 ± 0.04	50 ± 1.1
1:15	1	30	2.3 ± 0.07	48 ± 1.5

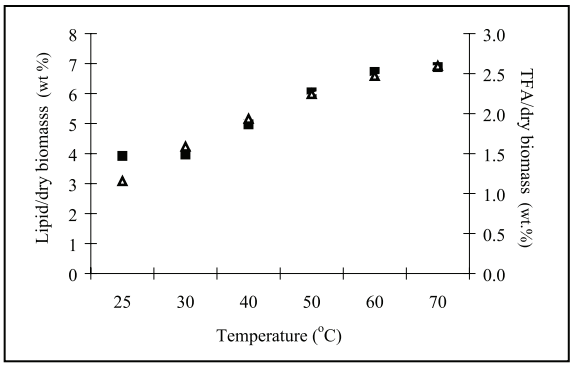

Fig. 1 Yield of extracted lipid (■) and TFA in the solvent (Δ) when dried *Spirulina* was extracted at various temperatures.

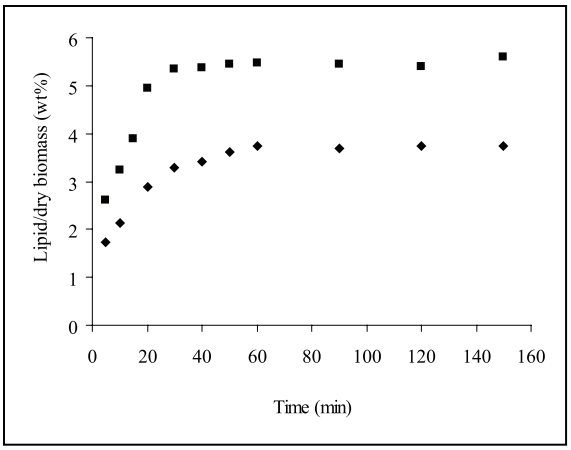

Fig. 2 Yield of extracted lipid when dried Spirulina was extracted using sample-solvent ratios of 1:5 at 30 °C (◆) and 60 °C (■).

considered suitable when using multistage extraction of lipid extraction from *Spirulina*.

Extraction temperature

When *Spirulina* biomass was extracted in a single stage, the extraction at 25 °C and 30 °C gave the same levels of lipid yield. An increase in the extraction temperature increased both lipid and TFA yields in the solvent. When the biomass was extracted at 30 °C, 40 °C, 50 °C, and 60 °C the yield of lipid was 3.97%, 4.97%, 6.04%, and 6.72% of dry weight and that of TFA was 1.59%, 1.93%, 2.24%, and 2.47% of dry weight, respectively (Fig. 1). The results shows that at 60 °C, the yield of lipid and TFA in the solvent were 69% and 55% higher than those extracted at 30 °C, respectively.

Elevated temperatures are sometimes used in order to keep the viscosity low and thereby minimize mass-transfer resistance. As the extraction temperature increases, the diffusivities of the solute (oil) and solvent increase, resulting in a higher oil yield[17]. At higher extraction temperatures, the oil yield might decreases due to the low solvent density in the sample. Therefore, heating solvents above their boiling points does not necessarily improve the oil yield[12,17]. In agreement with this, we found that extracting at 70 °C (which is close to the boiling point of ethanol, 78 °C), the yield of lipid (6.88% of dry weight) and TFA (2.60% of dry weight) did not show a significant difference from that obtained when extracting at 60 °C (Fig.1).

The extraction of soluble sugar in soybean[18] revealed that interactions between solvent and temperature, solvent and sample-solvent ratio, and temperature and sample-solvent ratio did play significant roles. When extracting from *Spirulina*, the higher the extraction time, temperature, and/or dielectric constant of the solvent, the higher the extraction yield[19]. Consistent with these findings, both the temperature and pH of the extraction medium were found to affect the effective diffusion coefficient and the extraction of anthocyanin[20].

Optimal extraction time at 30 °C and 60 °C

To determine the optimum time needed for lipid extraction, two temperatures were selected (30 °C and 60 °C). An increase in the yield of extracted lipid and TFA at 30 °C was seen after 3 extraction phases. The yield of lipid and TFA in the first phase (extraction time from 5 to 30 min) showed a rapid increase with only a slight increase during the second phase (30 to 60 min), and a stationary phase was noted after 60 min (Figs. 2 and 3). An extraction time of 60 min was the initial point of saturation of lipid and TFA yields in the solvent. An extraction time of 120 min

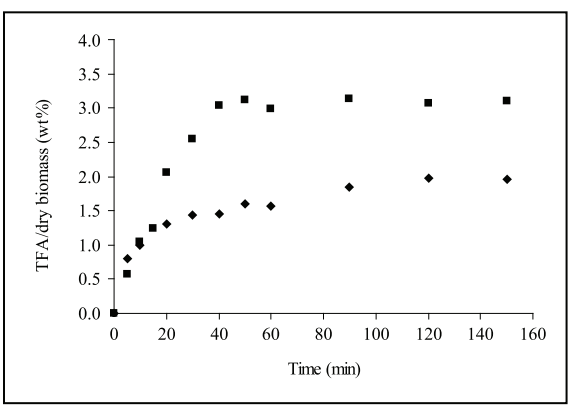

Fig. 3 Yield of extracted TFA in the solvent when dried *Spirulina* was extracted using sample-solvent ratios of 1:5 at 30 °C (◆) and 60 °C (■).

Table 5 Fatty acid composition of lipid extracted at 30 °C and 60 °C for 30 min.

Temperature (°C)	Fatty acid composition (% of total fatty acid)					
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
30	49.9 ± 0.6	6.4 ± 0.2	1.2 ± 0.2	2.7 ± 0.3	21.2 ± 0.6	18.5 ± 0.2
60	49.2 ± 0.5	5.9 ± 0.0	1.7 ± 0.2	2.9 ± 0.2	22.7 ± 0.1	17.5 ± 0.1

C16:0: Palmitic acid; C16:1: Palmitoleic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; C18:3: Linolenic acid.

resulted in the highest yields of lipid (3.75% of dry weight) and TFA in the solvent (1.97% of dry weight). However, the yields of lipid and TFA at 30 min were approximately 3.29% and 1.44% of cell dry weight which were, respectively, 12% and 27% lower than those extracted after 120 min. Considering that it requires 90 min to extract a further 12% of lipid and 27% of TFA, 30 min was considered a suitable time for a multistage extraction at 30 °C.

At 60 °C increasing the extraction time from 5 to 30 min resulted in an increase in the yields of lipid and TFA. The TFA yield reached a stable level at 40 min (3.04% of dry weight) whereas the lipid yield reached a stable level at 30 min (5.34% of dry weight). However, an extraction time of 20 min was selected for the multistage extraction at 60 °C as the lipid and TFA yields at this time were 4.94% and 2.06% of dry weight, respectively, which were approximately 25% and 66% higher than those at 15 min (3.94% and 1.24% of dry weight), respectively.

At 60 °C, the yields of lipid and TFA at saturation level were, respectively, 47% and 60% higher than those extracted at 30 °C. These findings agree with those of Mendes et al[4] who reported that an increase in pressure and temperature had a positive effect on the extraction of GLA. In addition, raising the temperature from 100 °C to 120 °C increases the yield of extracted saturated and unsaturated fatty acids, and especially that of GLA[21]. However, these results show that increased fatty acid levels are not achieved by further raising the temperature, as the fatty acid yield slightly decreased at 150 °C. Similar results were obtained in the present study. The proportion of palmitoleic acid and GLA at 60 °C were slightly less than the levels obtained when the extraction was performed at 30 °C (Table 5).

Multistage method using cross-current extraction at 30 °C and 60 °C

As some TFA remained in the residue of the

Table 6 TFA recovery in each extraction stage when *Spirulina* was extracted at 30 °C and 60 °C.

Number of stage	Total volume of solvent (ml)		Total time of extraction (min)		TFA recovery in solvent (%)	
	30 °C	60 °C	30 °C	60 °C	30 °C	60 °C
1	250	250	30	20	31 ± 0.2	43 ± 0.7
2	500	500	60	40	47 ± 0.5	74 ± 2.1
3	750	750	90	60	60 ± 0.3	85 ± 1.5
4	1000	1000	120	80	73 ± 1.1	88 ± 0.9
5	1250	1250	150	100	80 ± 0.6	91 ± 0.8
6	1500	1500	180	120	83 ± 0.4	93 ± 1.0
7	1750	1750	210	140	85 ± 0.5	94 ± 0.4
8	2000		240		86 ± 1.0	
9	2250		270		87 ± 0.7	
10	2500		300		87 ± 0.8	

Spirulina biomass when extraction was performed using a single stage, we also investigated a multistage method using cross-current extraction at 30 °C and 60 °C to determine the optimum number of extraction stages. For the extraction at 30 °C, TFA recovery was 80% and 85% when biomass was extracted using five-stage and seven-stage extraction (30 min/stage) respectively, whereas five-stage and seven-stage extraction (20 min/stage) at 60 °C, resulted in TFA recovery of 91% and 94%, respectively. Moreover, only a three-stage extraction was needed to obtain a TFA recovery of 85% when extraction was performed at 60 °C (Table 6). When seven-stage extraction was performed at 60 °C, TFA recovery was nearly 100%. However, seven-stage extraction took 80 min and 1,000 ml of solvent to extract a further 9% of TFA. Hence the optimum number of stages for extraction at 60 °C is three.

CONCLUSIONS

An increased yield of lipid and TFA in the solvent from biomass were observed when the extraction temperature was raised. The extraction time and the optimum number of extraction stages were also reduced when extraction was carried out at 60 °C. Repeated extractions increased the percentage of TFA recovery. For operations that require very few stages, cross-current operation is particularly practical and economical and offers a great deal of flexibility. A three-stage extraction (20 min/stage) using a sample-solvent ratio of 1:5 at 60 °C was the best procedure for extracting lipid from *Spirulina* and is therefore recommended for industrial scale-up.

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REFERENCES

1. Romay C, Armesto J, Remirez D, González R, Ledon N, García I (1998) Antioxidant and anti-inflammatory properties of C-phycocyanin from blue-green algae. *Inflamm Res* **47**, 36–41.
2. Herrero M, Martín-Álvarez PJ, Señoráns FJ, Cifuentes A, Ibáñez E (2005) Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga. *Food Chem* **93**, 417–23.
3. Cohen Z, Vonshak A (1991) Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. *Phytochem* **30**, 205–6.
4. Mendes RL, Reis AD, Palavra AF (2006) Supercritical CO_2 extraction of γ-linolenic acid and other lipids from *Arthrospira* (*Spirulina*) *maxima*: Comparison with organic solvent extraction. *Food Chem* **99**, 57–63.
5. Biagi PL, Bordoni A, Hrelia S, Celadon M, Horrobin DF (1991) γ-linolenic acid dietary supplementation can reverse the aging influence on rat liver microsome Δ6-desaturase activity. *Biochim Biophys Acta* **1083**, 187–92.
6. Wainwright PE, Huang YS, Lévesque S, Mutsaers L, Mccutcheon D, Balcaen P, Hammond J (1996) Effects of dietary γ-linolenic acid and prenatal ethanol on mouse brain and behavior. *Pharmacol Biochem Behav* **53**, 843–52.
7. Reis A, Lobo-Fernandes H, Empis JA, Novais JM (1998) Effect of extraction and purification methods on fatty acid composition and gamma-linolenic acid yield and purity from *Arthrospira (Spirulina) maxima* biomass. In: Marine microorgannisms for industry (Le Gal Y, Muller-Feuga A, eds) pp 34–38, Ifremer, ISBN 2-905434-94-5.
8. Čertík M, Andráši P, Šajbidor J (1996) Effect of extraction methods on lipid yield and fatty acid composition on lipid classes containing γ-linolenic acid extracted from fungi. *J Amer Oil Chem Soc* **73**, 357–65.
9. Favati F, King JW, Mazzanti M (1991) Supercritical carbon dioxide extraction of evening primrose oil. *J Am Oil Chem Soc* **68**, 422–7.
10. Illes V, Szalai O, Szebenyi NI, Grosz M, Hethelyi I (1994) Oil recovery from Borage seed with carbon dioxide and propane solvent. In: Proceedings of the 3rd international symposium on supercritical fluid, pp 511–6, Institute National Polytechnique de Lorraine.
11. Mendes RL, Reis AD, Pereira AP, Cardoso MT, Palavra AF, Coelho JP (2005) Supercritical CO_2 extraction of γ-linolenic acid (GLA) from the cyanobacterium *Arthrospira* (*Spirulina*) *maxima*: experiments and modeling. *Chem Eng J* **105**, 147–52.
12. Mani S, Jaya S, Vadivambal R (2007) Optimization of solvent extraction of Moringa (*Moringa oleifera*) seed kernel oil using response surface methodology. *Food Bioprod Process* **85**, 328–35.
13. Chaiklahan R, Chirasuwan N, Loha V, Bunnag

B (2006) Optimum condition for lipid extraction from *Spirulina platensis*. Poster presentation on the 17th International Symposium on Plant Lipid, July16-21, East Lansing, Michigan USA.
14. Lapage G, and Roy CC (1984) Improved recovery of fatty acid through direct transesterification without prior extraction of purification. *J Lipid Res* **25**, 1391–6.
15. Cohen Z, Reungjitchachawali M, Siangdung W, Tanticharoen M (1993) Production and partial purification of γ-linolenic acid and some pigments from *Spirulina platensis*. *J Appl Phycol* **5**, 109–15.
16. Cohen Z (1997) The chemical of *Spirulina*. In: *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology (Vonshak A, ed) pp 175–204, Taylor and Francis Inc., Philadelphia, USA.
17. Trebal RE (1980) Mass transfer operations. McGrew Hill, New York, USA.
18. Giannoccaro E, Wang YJ, Chen P (2006) Effect of solvent, temperature, time, solvent-to-sample ratio, sample size, and defatting on the extraction of soluble sugars in soybean. *J Food Sci* **71**, C59–C64.
19. Herrero M, Ibáñez E, Señoráns J, Cifuentes A (2004) Pressurized liquid extracts from *Spirulina platensis* microalga determination of their antioxidant activity and preliminary analysis by micellar electrokinetic chromatography. *J Chromatogr A* **1047**, 195–203.
20. Türker N, Erdoğdu F (2006) Effect of pH and temperature of extraction medium on effective diffusion coefficient of anthocyanin pigments of black carrot (*Daucus carota* var. L.) *J Food Eng* **76**, 579–83.
21. Schäfer K (1998) Accelerated solvent extraction of lipids for determining the fatty acid composition of biological material. *Anal Chim Acta* **358**, 69–77.