J Am Oil Chem Soc (2013) 90:571–578
DOI 10.1007/s11746-012-2197-5

ORIGINAL PAPER

Comparison of Lipid Extraction from Microalgae and Soybeans with Aqueous Isopropanol

Linxing Yao · Show-Ling Lee · Tong Wang · Jose A. Gerde

Received: 2 July 2012 / Revised: 30 November 2012 / Accepted: 18 December 2012 / Published online: 11 January 2013

Abstract The extraction efficiency of microalgae lipids with aqueous isopropanol (IPA) was investigated and compared with the extraction of oil from full-fat soy flour. The effects of the type of microalgae (*Scenedesmus* sp. and *Schizochytrium limacinum*), cell rupture, and IPA concentration on the yield of oil and non-lipid biomass were determined. The oil yield from intact cells of *Scenedesmus* was 86–93 % with 70, 88, or 95 % (by wt) IPA. Ultrasonic cell rupture prior to oil extraction decreased the oil yield of *Scenedesmus* to 74 % when extracting with 70 % IPA. The oil yield from intact cells of *S. limacinum* was <23 % regardless of the IPA concentration, but ruptured cells gave a 94–96 % oil yield with 88 or 95 % IPA. The different response of the two microalgae to extraction with IPA is possibly caused by differences in the cell wall structure and type and amount of polar lipids. The oil yield from soy flour with 88 and 95 % IPA was 93–95 %, which was significantly greater than yields with 50 and 70 % IPA. Cell rupture had no effect on soy flour extraction. In general, the oil yield from the ruptured cells of both microalgae and soy flour increased with increasing IPA concentration.

Keywords Biofuel · Isopropanol · Microalgae · Oil extraction · Sonication · Soy flour

L. Yao · S.-L. Lee · T. Wang (✉) · J. A. Gerde
Department of Food Science and Human Nutrition, Center for Crops Utilization Research, Iowa State University, 2312 Food Sciences Building, Ames, IA 50011, USA
e-mail: tongwang@iastate.edu

Introduction

Aqueous isopropanol (IPA) has long been regarded as an attractive solvent for seed oil extraction [1, 2] because it is less volatile and flammable than hexane, the conventional oil extraction solvent. Although IPA is currently derived from petroleum, it can be produced by fermentation [3]. A recent study in our laboratory demonstrated that IPA also can effectively extract oil from wet microalgae [4]. The removal of water is a critical challenge in algae fuel utilization [5]. The presence of water significantly lowers the extraction efficiency by hexane, but when IPA is used, dewatering and drying of algae prior to extraction is not necessary, and processing costs are reduced. Ethanol also can be used to extract oil from microalgae, but a much larger volume of solvent is needed [4, 6, 7].

Microalgae, particularly, the autotrophic species, contain polar glycolipids and phospholipids [8, 9], which constitute 17–90 % of the total lipids and may be valuable in nutraceuticals. IPA is able to extract both polar and nonpolar lipids efficiently. More than 92 % of the oil can be extracted from the marine green alga *Nannochloropsis* using a 70 % IPA solution [4], but for oil extraction from oilseeds 85 % was the lowest percentage of IPA in water that gave a satisfactory yield [1].

Cell rupture is used as a pretreatment to enhance oil extraction from microalgae, presumably by facilitating solvent penetration of the cell biomass [10, 11]. Although various means of mechanical rupture have been explored, few have been proved to be suitable for algae such as *Nannochloropsis* [5]. Oil yield from *Nannochloropsis* with IPA extraction was actually not enhanced by cell rupture as we have shown. To gain additional insight into the effects of lipid type and cell wall structure on lipid extraction, the lipid extraction efficiency of two additional types of

microalgae, *Schizochytrium limacinum* and *Scenedesmus* sp., with 50, 70, 88, and 95 % (by wt) aqueous IPA were determined, and these results were compared with the ability of aqueous IPA to extract oil from soy flour.

Materials and Methods

Our general experimental design was to extract oil from intact or ruptured cells of two algae species with four concentrations of aqueous IPA. Each extraction was done in duplicate.

Materials

The microalga *S. limacinum*, a seawater heterotrophic species, was provided by Dr. Z. Wen of Iowa State University (Ames, IA). *S. limacinum* was grown in a bioreactor using glycerol as the carbon source and with growth conditions and medium as described previously [12]. The harvested *S. limacinum* was washed with distilled water and lyophilized, which yielded dry flakes with 97 % solids. *Nannochloropsis* sp. was purchased from Seambiotic Ltd. (Tel Aviv, Israel) as a frozen paste containing 15.7–17.5 % solids. *Scenedesmus* sp. native to Louisiana were grown in raceway open ponds in Roanoke, LA using agricultural fertilizers for nutrients. The pH of the cultures was controlled between 8.5 and 9.5 with bubbling carbon dioxide. The algae culture (0.11 g solids/L) was centrifuged using an Alfa Laval BTPX-205TGD-14/34 CDP-60 centrifuge at 10,000 rpm and 23 °C. The feeding rate was 15 L/min, and the discharge interval was 30 min. The concentrated material (1.7 % solids) was further dewatered using a laboratory centrifuge (Sorvall RC 3B Plus, Thermo Fisher Scientific Inc., MC) at 5,000 rpm and 10 °C, which gave a thick paste with 12 % solids. Full fat soy flour with 91 % solids was obtained from Natural Products, Inc. (Grinnell, IA). Prior to IPA extraction, the *S. limacinum* and soy flour were each dispersed in distilled water, which yielded a slurry with about 12 % solids that was homogenized with an Ultra-Turrax T25 tissue homogenizer (Ika Works, Wilmington, NC) at 9,000 rpm for 2 min. All reagents (IPA, chloroform, and methanol) were obtained from Fisher Scientific (Pittsburgh, PA).

Oil and Solid Content Determination of the Starting Materials

The oil contents of the *S. limacinum*, *Scenedesmus*, and soy flour were determined by extracting with chloroform and methanol as described previously [4]. Solids were determined by weight after a convection oven drying at 110 °C for 5 h. Each determination was duplicated.

Cell Rupture by Ultrasound

A laboratory ultrasonicator XL (Misonix, Newtown, CT, USA) was used with an energy output of 550 W at 20 kHz. The algae and soy flour pastes were sonicated for 4 min with 2 min of relaxation following each 30 s pulse. The degree of cell rupture was examined under a microscope at 100× magnification, and no intact cells were observed after the sonication.

IPA Extraction Procedure

The oil and non-lipid biomass in the algae and soy flour were separated using the aqueous IPA sequential extraction reported previously [4]. The algae and soy flour samples, both sonicated and non-sonicated, were mixed with IPA to produce a final IPA concentrations of 50, 70, 88, and 95 % (by wt) and refluxed at 80 °C while stirring at 280 rpm for 30 min, and then immediately centrifuged at 3,000*g* for 10 min (IEC Centra CL3 centrifuge, Thermo Fisher Scientific Inc., MC). The supernatant, which was referred to as the IPA_1 fraction, was decanted. The residual solids were re-dispersed in additional IPA to produce a final IPA concentration of 88 % (by wt) and refluxed for 30 min. After another centrifugation and decanting, a second supernatant was obtained, which was referred to as the IPA_2 fraction. The residual solids were referred to as the cake fraction. Each extraction was duplicated and there was no subsampling for quantification.

Determination of Oil and Non-lipid Biomass in IPA Fractions

The solvent, IPA and water, in each of the IPA_1 and IPA_2 samples was removed by rotary evaporation at 50 °C. The residual crude oil was subjected to a water wash. Briefly, the crude oil was dissolved in 12 mL of chloroform: methanol (2:1 by vol), and mixed vigorously with 3 mL of water for 30 s. After phase separation at 23 °C for 20 min, the mixtures were centrifuged at 1,800*g* for 2.5 min (IEC Centra CL3 centrifuge, Thermo Fisher Scientific Inc., MA). The upper water–methanol layer was collected and dried on a hot plate at 80 °C to determine the amount of non-lipid biomass. The chloroform in the lower phase was removed by rotary evaporation at 45 °C. Then 10 mL of IPA was added to the extracted oil (from chloroform phase) to remove the residual water by rotary evaporation at 45 °C. The resulting oil was re-dissolved in 10 mL of chloroform–methanol (3:1 by vol) and filtered through a PTFE membrane filter with a 0.45 μm pore size. The solvent in the final oil was evaporated under a stream of nitrogen at 40 °C followed by vacuum oven drying at 23 °C overnight.

To determine the oil content in the cake fraction, the wet solids from the IPA extraction were left overnight in a fume-hood in an open container to evaporate the residual IPA. The dried solids were then ground with a mortar and pestle, and the resulting fine powder was mixed with 50 mL of chloroform–methanol (2:1 by vol) with stirring continuously at 300 rpm for 2 h. Next the powder was filtered through #1 Whatman filter paper, and the solids were extracted again under the same conditions. The two filtrates were combined and the solvent was removed by rotary evaporation at 45 °C. The crude oil in the cake fraction was then subjected to the same water washing as the chloroform–methanol extract described previously. The non-lipid biomass in the cake fraction was determined from the sum of the solids obtained from the filtration plus the solids obtained from the aqueous phase of chloroform–methanol extraction and washing procedure. The quantities of oil and non-lipid biomass were determined once for each of the two replicates.

The oil mass balance, or relative oil yield, was calculated as the ratio of oil in one fraction to the total oil in the three fractions, i.e., IPA_1, IPA_2, and cake fractions. The absolute oil yield was also calculated as the ratio of actual oil in one fraction to the theoretical total oil determined by the chloroform–methanol method. The same rule was applied to the calculation and presentation of the yield of non-lipid biomass. Only the values of the oil mass balance (or the relative oil yield) were used in the discussion because the absolute yield had the same trend as the relative oil yield, but had larger errors due to variations in the solid content of replicated treatments.

Ultrastructure of Cells of Algae and Soy Flour

Transmission electron microscopic (TEM) images of the intact and sonicated cells of *S. limacinum*, *Scenedesmus*, *Nannochloropsis*, and soy flour were obtained by the Microscopy Facility (Iowa State University, Ames, IA) using a JEOL 2100 scanning/transmission electron microscope (Japan Electron Optic Laboratories, Peabody, MA). The sample preparation for TEM imaging was previously described [13]. This examination was not replicated.

Experimental Design and Statistical Analysis

All three materials, *S. limacinum*, *Scenedesmus*, and soy flour, ruptured or intact, were subjected to the extraction with 50, 70, 88 and 95 % IPA. Each extraction was repeated twice. The ranges of the two replicates are given as error bars in the figures. Mass balances of oil and non-lipid biomass of each extraction replicate were determined. The effect of IPA concentration on the extraction efficiency was analyzed using ANOVA with SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA) at a significance level of 0.05. The oil yield from intact and ruptured cells that were extracted with the same IPA concentration was compared using Student's t test at a significance level of 0.05.

Results and Discussion

Effect of IPA Concentration and Cell Rupture on Oil Extraction from Full-Fat Soy Flour

Soy flour used in this study contained 23.0 % oil on a dry weight basis (dwb). The aqueous IPA extracted over 93 % of the total extractable oil from soy flour when the IPA concentration was 88 % or higher (Fig. 1a, b). Baker and Sullivan [1], using 88 % IPA to extract soy flakes in a continuous countercurrent extractor, obtained a defatted meal with 1.1 % residual oil. In this study, the defatted soy cake contained 0.5–0.7 % residual oil. When the 50 or 70 % IPA was used, the oil extraction efficiency significantly decreased and only 9–47 % of the total oil was extracted into the first extract (IPA_1 fraction). Sonication pretreatment did not affect the extraction efficiency when the 88 or 95 % IPA was used. This suggests that solvents with high IPA concentrations can effectively penetrate cell walls of soy flour. When subjected to 70 % IPA, ruptured cells of soy flour gave an oil yield that was 11 % lower than that from the intact cells ($p = 0.0252$). However, 50 % IPA extracted 8 % more oil from ruptured cells than from intact cells ($p = 0.0111$). Probably soy oil is not completely soluble in 50 or 70 % IPA at 80 °C, leading to the low oil yield. The second extraction (by 88 % IPA) in the sequential extraction recovered a significant amount of oil from soy flour.

Effect of IPA Concentration and Cell Rupture on Oil Extraction from *S. limacinum*

Schizochytrium limacinum contained 56.7 % oil (dwb). The amount of oil extracted from intact cells varied from 7 to 23 % (Fig. 1c) with the 88 % IPA giving the highest percentage. Cell rupture significantly improved the oil yield with 88 and 95 % IPA treatments, and their IPA_1 fractions contained about 96 % of total oil (Fig. 1d), which is in agreement with the report for ruptured cells of *S. limacinum* extracted with 95 % ethanol [7]. Seemingly, the cell wall of *S. limacinum* behaved as a solvent barrier, so cell rupture was necessary for oil release. IPA levels of 50 and 70 % extracted 11 and 15 % more oil from ruptured cells than from intact cells.

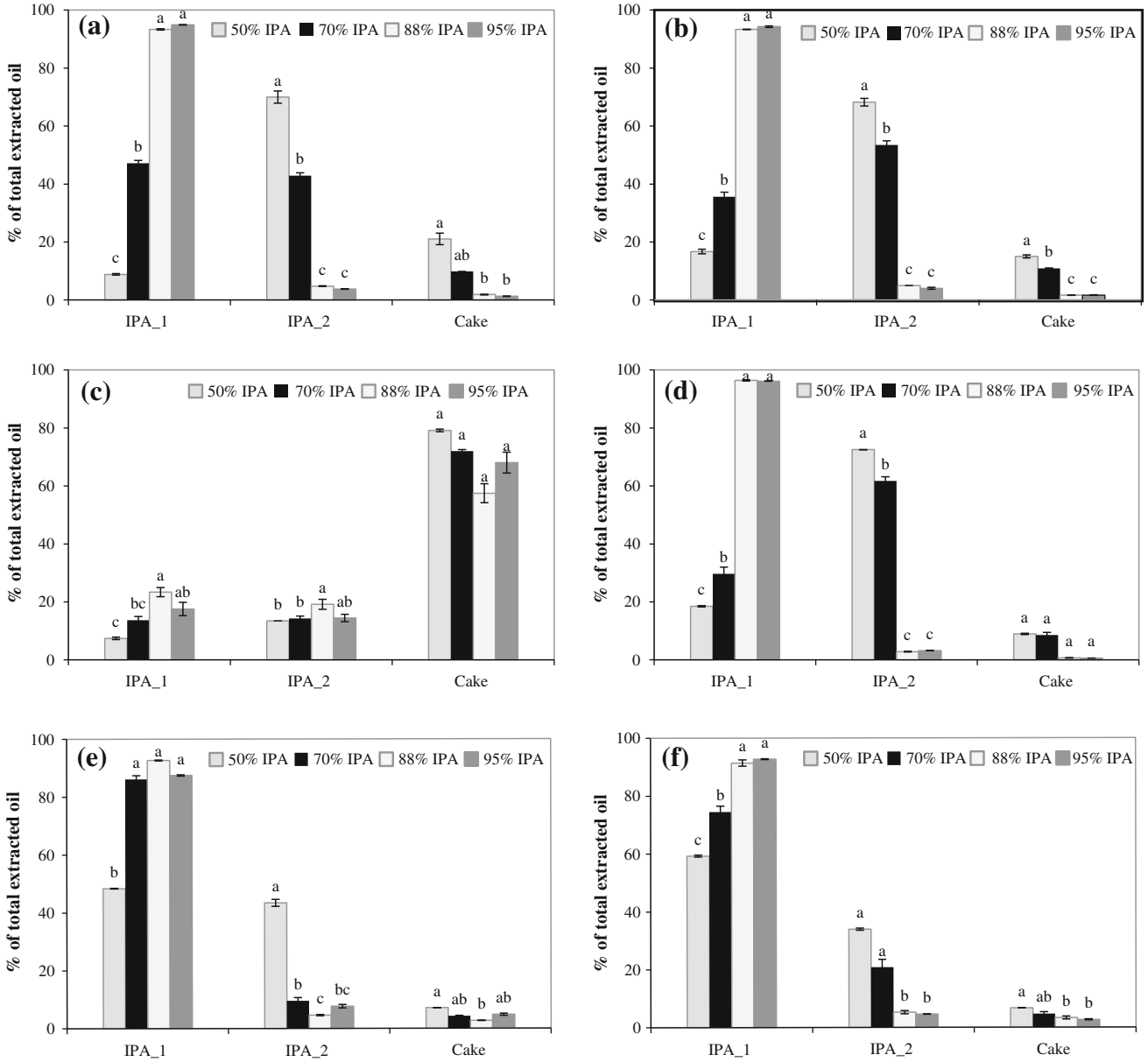

Fig. 1 Oil mass balance of fractions from IPA extractions of soy flour (**a**), sonicated soy flour (**b**), *S. limacinum* (**c**), sonicated *S. limacinum* (**d**), *Scenedesmus* (**e**), and sonicated *Scenedesmus* (**f**). The means with different letters within each fraction are significantly different with $p < 0.05$. The *error bar* represents the range value of two replicates

Effect of IPA Concentration and Cell Rupture on Oil Extraction from *Scenedesmus*

Scenedesmus contained 10.5 % oil (dwb). With intact cells of *Scenedesmus*, the oil yields of the IPA_1 fractions were 86–93 % with 70, 88, and 95 % IPA treatments. These percentages were not significantly different ($p > 0.05$), although the 88 % IPA yielded 6 to 7 % more oil than the 70 or 95 % IPA treatments. With ruptured cells, the oil yield increased with the IPA concentration. Use of 95 % IPA extracted the most oil (93 %) from ruptured cells but this yield was not significantly different from that with 88 % IPA ($p > 0.05$). A comparison of the intact and ruptured cells at the same IPA concentrations showed that sonication significantly lowered the oil yield with 70 % IPA extraction from 86 to 74 %; however, extractions with 50 and 95 % IPA were significantly increased. A similar trend was observed with another autotrophic microalga, *Nannochloropsis* [4]. Ryckebosch et al. [14] found that cell rupture lowered the total oil yield of *S. obliquus* extracted with chloroform and methanol after treatment with a bead beater and sonication. Possibly, a more intimate interaction between the oil and cell debris lowered the oil extractability.

In general, aqueous IPA with 70 % or more IPA is good for extracting oil from the intact cells of *Scenedesmus*. More than 95 % of the oil can be extracted with sequential extraction, and the defatted cake contained only 0.3–0.8 % oil.

Comparison of Isopropanol Extraction Performance on Microalgae and Soy Flour

Figure 2 shows the oil extractability from intact and ruptured cells of *S. limacinum*, *Scenedesmus*, *Nannochloropsis* and soy flour using various IPA concentrations. The extraction data of *Nannochloropsis* are from previous work and are used for comparison [4]. Regardless of cell rupture, lipid extractability with aqueous IPA depended on the type of algae and the IPA concentration. The polar lipid contents of *S. limacinum*, *Scenedesmus*, *Nannochloropsis* and soy flour were approximately 9, 23, 51 and 4 %, respectively [9, 15, 16]. The major polar lipids of *Nannochloropsis* include sulfoquinovosyl diacylglycerol, digalactosyl diacylglycerol, monogalactosyl diacylglycerol, phosphatidylglycerol and phosphatidylcholine (PC). *Scenedesmus* was of limited phospholipids [unpublished data]. *S. limacinum* contained mainly PC and phosphatidylethanolamine [16]. The dissimilarity in lipid classes of these four materials may have resulted in different solubilities of their oil in aqueous IPA, which would affect their extractability. IPA at 88 and 95 % concentration effectively extracted oil from ruptured cells of all four materials (Fig. 2b), and the oil yields ranged from 91 to 96 %. But oil extractability decreased with the decreasing IPA concentration. The extractability of oil from soy flour and *S. limacinum* were reduced more than those of the other two green algae. Soy flour and *S. limacinum* contained more neutral lipids which require a nonpolar solvent to dissolve their oils. The polar lipids in the green algae made their oil solubility better in aqueous IPA, and these polar lipids also might have acted as surfactants and assisted oil dissolution. The lipid class compositions of soy flour and *S. limacinum* were similar, so they had similar patterns of extraction in the sonicated treatments (Fig. 1b, d).

With intact cells, the oil yield was determined both by their oil solubility and the cell wall structure (Fig. 2a). The 88 and 95 % IPA worked well for soy flour, *Nannochloropsis*, and *Scenedesmus*, but not for *S. limacinum.* Oil yields of the two green algae were not significantly decreased when extracting with the 70 % IPA; however, the oil yield of soy flour with the 70 % IPA was reduced to about half the percentage obtained with 88 and 95 % IPA. Seemingly, cell rupture was not necessary for the two green algae when extracting with optimal IPA concentration. This observation is consistent with the result of Ryckebosch et al. [14] who reported that the oil extractions of several green algae were not affected by cell rupture, but our results are contrary to those of Lee et al. [10]. Possibly this discrepancy resulted from the different species of algae, and solvent extraction protocols. Oil yields from intact cells for all three types of algae with 95 % IPA were slightly lower than the yields with 88 % IPA, which may be caused by a lower cell wall penetrability of 95 % IPA compared with 88 % IPA [4].

Ultrastructure of Microalgae and Soy Flour

Figure 3 shows the characteristics of the cell walls of the microalgae and soy flour. *Nannochloropsis* and *Scenedesmus* are marine and freshwater green algae, respectively. Both had a thin outer cell wall with a trilaminar structure (TLS) [17, 18] as shown in Fig. 3a, d. Most algae with TLS have algaenans that are chemically resistant polymers that

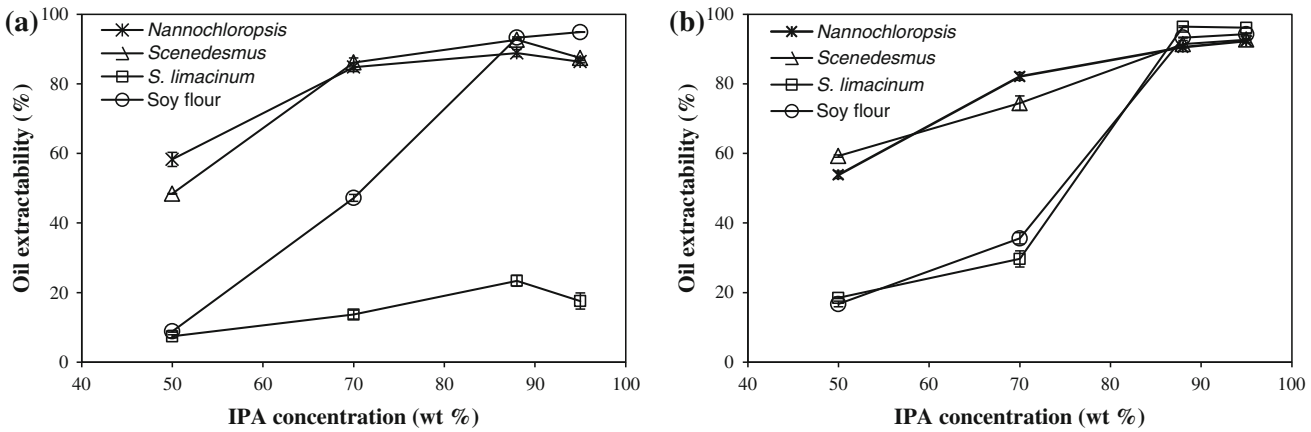

Fig. 2 The oil extractability of *Nannochloropsis*, *Scenedesmus*, *S. limacinum*, and soy flour without sonication (**a**) and with sonication (**b**). The oil extractability (%) is the relative oil yield of the IPA_1 fraction. The extraction data of *Nannochloropsis* were from Ref. [4]. The *error bar* represents the range value of two replicates

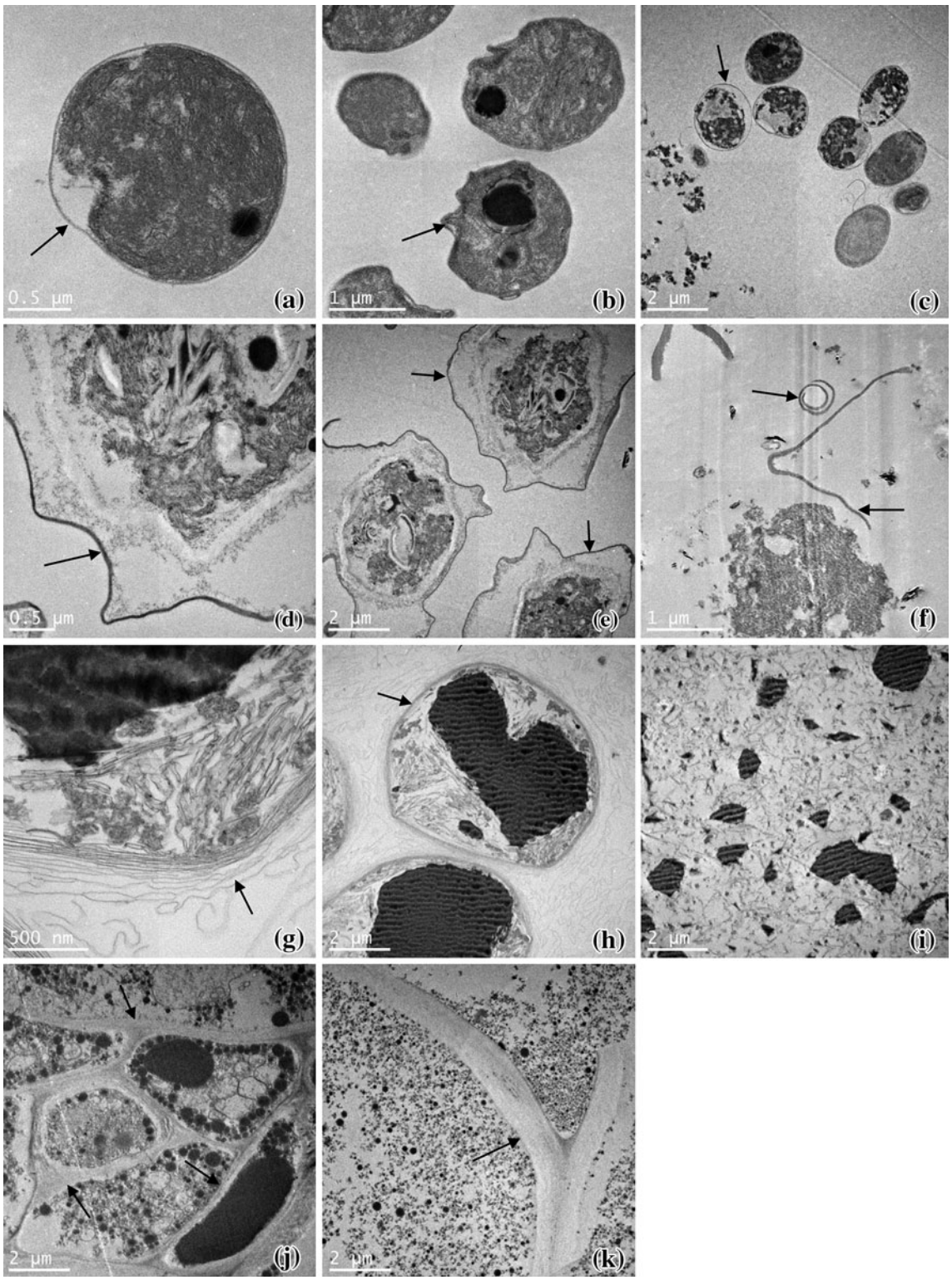

Fig. 3 Transmission electron micrographs of microalgae and soy flour. **a**, **b** *Nannochloropsis* before sonication; **c** *Nannochloropsis* after sonication; **d**, **e** *Scenedesmus* before sonication; **f** *Scenedesmus* after sonication; **g**, **h** *S. limacinum* before sonication; **i** *S. limacinum* after sonication; **j** soy flour before sonication; **k** soy flour after sonication. *Arrows* point to the cell walls

make the algae cell wall difficult to open. *Scenedesmus* seemed to have an inner microfibrillar layer protected by a TLS with a light electron density (Fig. 3d, e) [19, 20]. The multilayered cell wall (Fig. 3g) of *S. limacinum* is a characteristic of the Thraustochytriaceae [21]. This high oil-production species had large oil bodies (Fig. 3h) that

exhibited a fine structure with alternating electron light and dense regions representing the saturated and unsaturated fatty acids [22]. The cell walls of *Nannochloropsis* and *Scenedesmus* were more resistant to ultrasonic cell rupture than *S. limacinum.* Some cells of *Nannochloropsis* seemed to have intact cell walls after sonication even though part of their intracellular materials or structure was lost (Fig. 3c). Under the same sonication condition, the cells of *Scenedesmus* and *S. limacinum* ruptured into small pieces (Fig. 3f, i); however, the cell walls of *Scenedesmus* were more resistant (Fig. 3f).

Based on the results of oil extraction yield and the cell wall structure of the algae, we suggest that there may be two possible scenarios that influence oil extraction. First, the algaenans in the TLS of *Nannochloropsis* and *Scenedesmus* may help maintain the porosity of their cell walls and allow IPA solvent penetration. On the other hand, the multilayered cell wall of *S. limacinum* which lacks algaenans may undergo physical changes in the cell wall network when in contact with the hot IPA solution, so the pores in the cell wall are closed and solvent penetration is inhibited [23]. Thus, oil can be easily dissolved in IPA ?tul?> and transported outside the cells of *Nannochloropsis* and *Scenedesmus*, but not with *S. limacinum.* Secondly, the multilayered cell wall of *S. limacinum* may form a condensed network without pores that makes a formidable barrier to solvent access.

The cell walls of soybean are mainly polysaccharides. After sonication, large piece of broken cell walls were observed (Fig. 3k), and the lipid bodies were broken into much smaller oil droplets (Fig. 3j, k). Since oil extraction from soy flour was unaffected by sonication, it suggests that its wall structure also is permeable to IPA.

Effect of IPA Concentration and Sonication on Non-Lipid Biomass Recovery from *S. limacinum* and *Scenedesmus*

Figure 4 shows that 64–86 % of the non-lipid biomass was recovered in the cake fractions of the two algae regardless of cell rupture. The non-lipid biomass yield generally increased with the IPA concentration except for the treatments on the intact cells of *S. limacinum.* The major loss of non-lipid biomass, about 20–35 % for *S. limacinum* and 14–21 % for *Scenedesmus*, were found in the IPA_1 fraction. Regardless of cell rupture, *Scenedesmus* had about 10 % higher non-lipid biomass yield in its cake fraction than *S. limacinum.* Sonication increased the biomass loss of *S. limacinum*, but had no effect on *Scenedesmus.* The yields of non-lipid biomass were similar to those of *Nannochloropsis* [4].

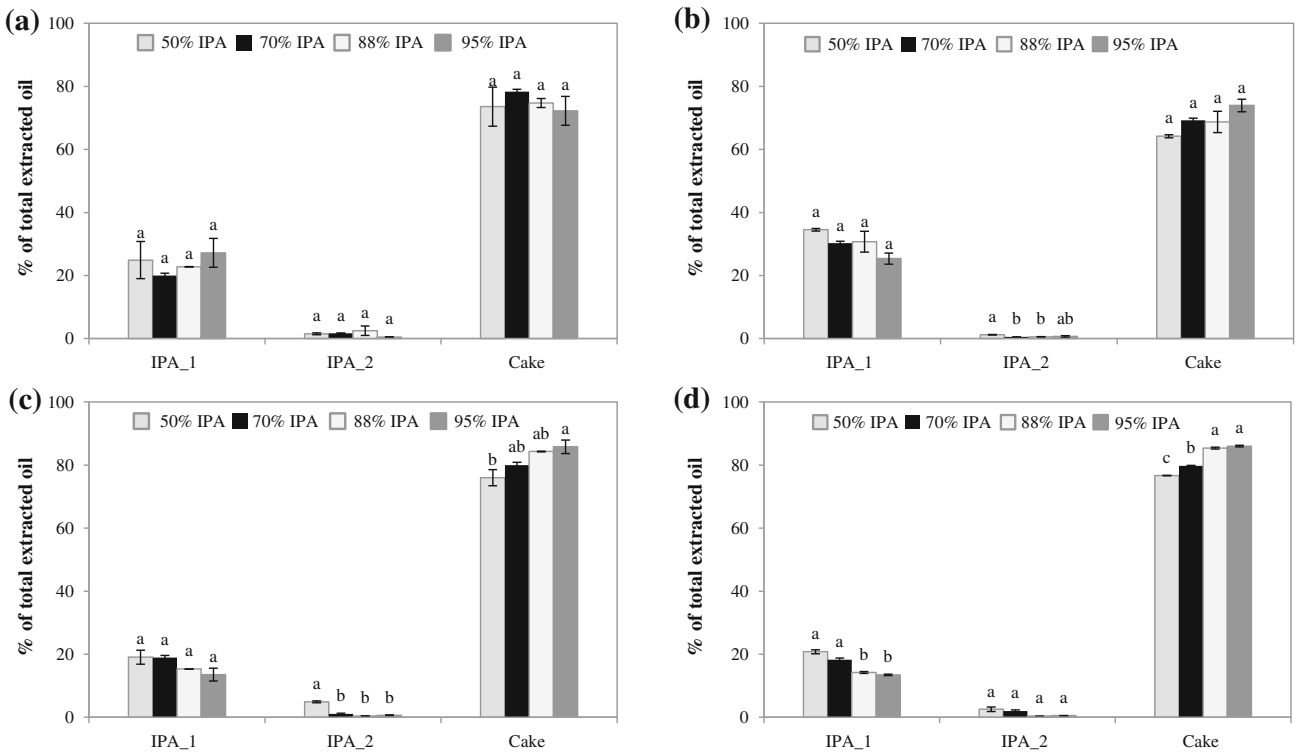

Fig. 4 Non-lipid biomass mass balance of fractions obtained from IPA extractions of *S. limacinum* (**a**), sonicated *S. limacinum* (**b**), *Scenedesmus* (**c**), and sonicated *Scenedesmus* (**d**). The means with different letters within each fraction are significantly different with $p < 0.05$. The error bar represents the range value of two replicates

Conclusion

Aqueous IPA has proven itself to be an effective solvent for extracting oil and precipitating non-lipid biomass from both autotrophic and heterotrophic microalgae. The optimal concentration of IPA varies with the species of microalgae. A higher IPA concentration is needed for the alga with a high percentage of non-polar lipids. Cell rupture is only needed for the alga whose cell walls resist solvent penetration. The lipid composition and cell wall structure are the factors that determine the optimal IPA concentration for oil extraction. This study provides further evidence that aqueous IPA extraction of lipids is particularly suitable for green microalgae.

Acknowledgments We thank Professor Zhiyou Wen for providing the microalga *S. limacinum*, Dr. Hui Wang for assisting in concentrating the algae cells in the CCUR pilot plant, and Phillips 66 for funding the research.

References

1. Baker EC, Sullivan DA (1983) Development of a pilot-plant process for the extraction of soy flakes with aqueous isopropyl alcohol. J Am Oil Chem Soc 60:1271–1277
2. Johnson LA, Lusas EW (1983) Comparison of alternative solvents for oils extraction. J Am Oil Chem Soc 60:229–242
3. Subbian E, Meinhold P, Buelter T, Hawkins AC (2008) Engineered microorganisms for producing isopropanol. U.S. patent, 20080293125
4. Yao L, Gerde JA, Wang T (2012) Oil extraction from microalga *Nannochloropsis* sp. with isopropyl alcohol. J Am Oil Chem Soc 89:2279–2287
5. Cooney M, Young G, Nagle N (2009) Extraction of bio-oils from microalgae. Sep Purif Rev 38:291–325
6. Kale A (2011) Methods of and systems for isolating nutraceutical products from algae. U.S. patent 8,084, 038
7. Wang G, Wang T (2012) Lipid and biomass distribution and recovery from two microalgae by aqueous and alcohol processing. J Am Oil Chem Soc 89:335–345
8. Harwood JL, Jones LA (1989) Lipids metabolism in algae. Adv Bot Res 16:1–53
9. Yao L, Gerde JA, Wang T, Wen Z, Lee S-L (2012) Characterization of lipids in five microalgae species. American Oil Chemists' Society, 103rd American Oil Chemists' Society Annual Meeting & Expo. Long Beach, CA, May 2012. Abstract
10. Lee J-Y, Yoo C, Jun S-Y, Ahn C-Y, Oh H-M (2010) Comparison of several methods for effective lipid extraction from microalgae. Biores Technol 101:S75–S77
11. Zheng H, Yin J, Gao Z, Huang H, Ji X, Dou C (2011) Disruption of *Chlorella vulgaris* cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, Bead Milling, Enzymatic Lysis, and Microwaves. Appl Biochem Biotechnol 164:1215–1524
12. Ethier S, Woisard K, Vaughan D, Wen Z (2011) Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. Biores Technol 102:88–93
13. Majoni S, Wang T, Johnson L (2011) Enzyme treatments to enhance oil recovery from condensed corn distillers solubles. J Am Oil Chem Soc 88:523–532
14. Ryckebosch E, Muylaert K, Foubert I (2012) Optimization of an analytical procedure for extraction of lipids from microalgae. J Am Oil Chem Soc 89:189–198
15. Wang T, Hammond EG, Fehr WR (1997) Phospholipid fatty acid composition and stereospecific distribution of soybeans with a wide range of fatty acid composition. J Am Oil Chem Soc 74:1587–1594
16. Wang G, Wang T (2012) Characterization of lipid components in two microalgae for biofuel application. J Am Oil Chem Soc 89:135–143
17. Allard B, Templier J (2000) Comparison of neutral lipid profile of various trilaminar outer cell wall (TLS)-containing microalgae with emphasis on algaenan occurrence. Photochemistry 54:369–380
18. Grossi V, Blokker P, Sinninghe Damsté JS (2001) Anaerobic biodegradation of lipids of the marine microalga *Nannochloropsis salina*. Org Gerochem 32:795–808
19. Staehelin AL, Pickett-Heaps JD (1975) The ultrastructure of *Scenedesmus* (Chlorophyceae). I. Species with the "reticulated" or "warty" type of ornamental layer. J Phycol 11:163–185
20. Atkinson AW, Gunning BES, John PCL (1972) Sporopollenin in the cell wall of *Chlorella* and other algae: ultrastructure, chemistry, and incorporation of 14C-acetate, studied in synchronous cultures. Planta 107:1–32
21. Honda D, Yokochi T, Nakahara T, Erata M, Higashihara T (1998) *Schizochytrium limacinum* sp. nov., a new thraustochytrid from a mangrove area in the west Pacific Ocean. Mycol Res 102:439–448
22. Wong MKM, Tsui CKM, Au DWT, Vrijmoed LLP (2008) Docosahexaenoic acid production and ultrastructure of the thraustochytrid *Aurantiochytrium mangrovei* MP2 under high glucose concentrations. Mycoscience 49:226–270
23. Röder T, Sixta H (2004) Thermal treatment of cellulose pulps and its influence to cellulose reactivity. Lenzinger Berichte 83:79–83