

Supercritical-Fluid Extraction of Fungal Lipids: Effect of Cosolvent on Mass-Transfer Rates and Process Design and Economics[†]

Miriam Cygnarowicz-Provost,[‡] Dennis J. O'Brien,* R. Thomas Boswell, and Michael J. Kurantz

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

Received March 14, 1994; accepted in revised form September 1, 1994

The extraction of fungal lipids by supercritical CO₂ and CO₂ with 10 wt % ethanol was studied. The lipid solubility was measured from 40–60 °C and 200–700 bar, and was found to increase with increasing solvent pressure and temperature, although the crossover effect was observed. Mass transfer coefficients were fitted to experimental data and were found to increase with increasing solvent Reynolds number. No difference in mass-transfer coefficients was observed with the addition of the cosolvent. Using the experimental data, a mathematical model for a commercial extraction process was developed, and optimal values of the extractor pressure, solvent flow rate, and extraction time were computed. Since the lipid is more soluble in the CO₂ mixture, the optimal extractor pressure and extraction time were lower than those computed for a process with a pure CO₂ solvent. Capital and operating costs for the process were estimated and the addition of the cosolvent was found to lower the costs by over 40%. A comparison of the costs for the SFE process and the costs for a liquid-extraction process show that the SFE process is not competitive for this application, although the economics would be improved if wet fungal mycelia were contacted continuously with the supercritical solvent.

Keywords: eicosapentaenoic acid, modeling, simulation, economics, liquid–liquid extraction, *Saprolegnia parasitica*

INTRODUCTION

Ingestion of polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is purported to have beneficial physiological activity, including reduced susceptibility to arthritis and cardiovascular disease. Typical sources of these fatty acids are marine oils, but the composition and content of the polyunsaturated fatty acids in these oils are dependent upon the species of fish and the season and location of the catch. It is unclear whether the supply of polyunsaturated fatty acids from marine sources will be adequate to satisfy the projected demand.¹ This fact motivates the search for alternative means of producing polyunsaturated fatty acids. Among the non-conventional sources for EPA and DHA are algae² and fungi.³ We have studied the fermentation of the filamentous fungi, *Saprolegnia parasitica*, and have

shown that it contains EPA and other polyunsaturated fatty acids in its lipid matter. We have demonstrated that supercritical fluids (CO₂ and CO₂ with 10 wt % ethanol) can be used to extract these lipids, and that the CO₂–ethanol mixture can extract both the neutral and the polar lipids.⁴ We showed that the solubility of the lipid is higher in the solvent mixture, and the fraction of EPA, the desired product, is also increased.

In this paper, we expand on our previous work and measure solubilities and mass-transfer rates in a range of temperatures and pressures. We also show the effect of the cosolvent on mass-transfer rates between a supercritical fluid and this biological matrix. Using the experimental solubility and mass-transfer data, we have developed a flow-sheet model for the extraction and recovery processes and compared the economics of supercritical-fluid extraction (SFE) and conventional liquid solvent extraction.

EXPERIMENTAL

Fungal Fermentation. *S. parasitica* was grown in a 14-L New Brunswick fermentor in which the

[†] Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[‡] Current address: U.S. Food and Drug Administration, 9200 Corporate Blvd., Rockville, MD 20850.

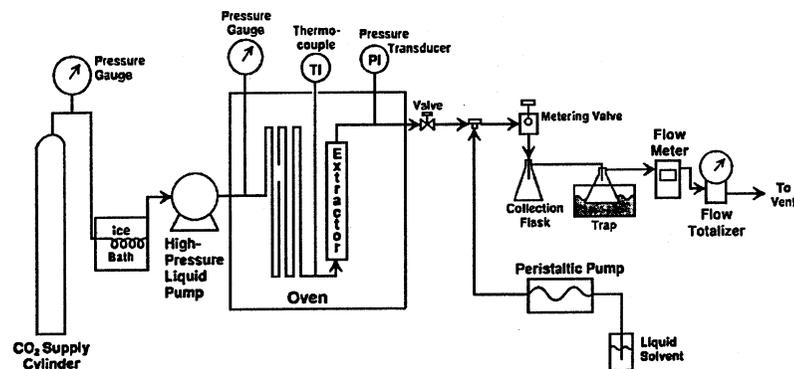


Figure 1. Experimental apparatus used for supercritical-fluid extraction studies.

agitator and baffles were removed. Mixing was provided solely by aeration. Ten liters of growth medium⁵ were used in which glucose was the carbon source. The air flow rate was 6 std. L min⁻¹ and the pH was maintained between 6.6 and 7.0 by addition of KOH. The mycelial mass was harvested after 3.5 days by filtering through a Buchner funnel using Whatman No. 2 filter paper. Four batches grown under these conditions were used for this study. Prior to extraction, the fungal mycelia was lyophilized in a Labconco Model 5 dryer and crushed until it passed through a 0.25-mm screen. The dried fungal mycelia was stored in a freezer (-5 °C) to minimize lipid degradation.

Supercritical-Fluid Extraction. The experimental apparatus used in these studies is shown in Figure 1. It consisted of a high-pressure liquid pump (Haskell, Inc. Burbank, CA, Model 29376-DSF-150C), a 20- or 40-mL extractor housed in an oven, a series of micrometering valves, a collection flask, a flow meter (McMilan Co.), and a flow totalizer (American Meter Co., Philadelphia, PA). The air-driven Haskell pump included an air pressure regulator which maintained a constant outlet pressure. The extractor, valves, and fittings were supplied by Autoclave Engineers, Inc. (Erie, PA).

To begin the experiments, liquid CO₂ from a supply tank was cooled in an ice bath and then compressed to the desired pressure. Once it entered the oven, the stream passed through approximately 2.5 m of 6.35-mm OD high-pressure tubing to allow it to reach thermal equilibrium. It then entered the extractor which had been packed with equal volumes of 5-mm glass beads and freeze-dried fungal mycelia (approximately 4 g were extracted in each experiment). The ends of the extractor were packed with glass wool to prevent loss of the substrate. Upon leaving the oven, the supercritical CO₂ stream, containing the dissolved lipid, passed through two metering valves which controlled the flow and maintained the back pressure. The reduction in pressure caused the solubilized lipids to separate from the CO₂ and precipitate in the collection vessel. To prevent volatilization of the solute, the collection vessel was kept in an ice bath, and an additional ice bath was placed in the line immediately following it. The depres-

surized CO₂ gas flowed through the flow meter and flow totalizer before being vented. For each experiment, the flow rate of solvent was kept constant by adjusting the micrometering valve. In these studies, we used flow rates between 1 and 6 L min⁻¹ (measured at atmospheric pressure).

The system had no provision for adding cosolvents directly to the supercritical fluid. Instead, cylinders of CO₂ mixed with 10 wt % ethanol were obtained from a supplier (Scott Specialty Gases, Plumsteadville, PA).

Extraction curves were determined by weighing the lipid that precipitated after passing a known volume of CO₂ through the extractor. Samples were collected at 15-L intervals for the first four extracts, followed by 30-L intervals for the next two, and 100-L intervals for the final two extracts. A total of 350 L (approximately 650 g) of solvent was passed through the extractor in each experiment. At the end of each sample collection, the metering valve and tubing were flushed with a liquid solvent (methylene chloride or ethanol) using a peristaltic pump to remove the small amount of lipid remaining in the transfer lines.

To determine the total lipid extracted, the collection flask and cold trap were washed with additional methylene chloride, the fluid was transferred to a preweighed flask, and the solvent was evaporated under nitrogen. If an ethanol cosolvent was used, this was evaporated as well. Prior to weighing, the flasks were stored in a desiccator overnight. The mycelia remaining in the extractor was extracted with a mixture of chloroform, methanol, and water, using the method of Folch.⁶ The solvent was evaporated under a stream of nitrogen, and the dry lipid weights determined. The percent recovery was, thus, equal to the weight of lipid extracted (including the amount precipitated in the metering valve and tubing), divided by the weight of lipid extracted plus the weight of lipid remaining in the fungal mycelia. A complete mass balance was not obtained since the starting material (i.e., the dried mycelia) contained variable amounts of lipid (between 8 and 14 wt %).

The solubility of the lipid at a given temperature and pressure was determined by computing the slope of

TABLE I
Solubilities of Fungal Lipids of *S. parasitica* in Supercritical Fluids as a Function of Temperature and Pressure

Pressure bar	100% CO ₂ g lipid/g solvent	10% ethanol g lipid/g solvent
Temperature = 40 °C		
208	0.0030	0.0089
346	0.0022	0.0076
483	0.0026	0.0067
690	0.0027	0.0070
Temperature = 50 °C		
208	0.0020	0.0075
346	0.0032	0.0082
483	0.0031	0.0066
690	0.0034	0.0082
Temperature = 60 °C		
208	0.0020	0.0071
346	0.0022	0.0072
483	0.0025	0.0080
690	0.0034	0.0092

the extraction curve during the initial period. Extractions were performed at 40, 50, and 60 °C and from 200 to 700 bar.

The fatty acid composition of the lipid extracts was determined by capillary GLC of the fatty acid methyl esters (FAMES) as outlined by Wessinger et al.⁷ Analysis of the lipid classes was performed by thin layer chromatography using the method of Kurantz et al.⁸ The EPA content of the mycelial lipid, as determined by the FAMES analysis, varied between 10 and 14 wt %.

RESULTS AND DISCUSSION

Solubility and Mass Transfer Models.

The estimated solubility of the fungal lipid as a function of temperature and pressure are shown in Table I. In agreement with our previous work,⁴ the solubility of the lipid was found to be higher in the CO₂-ethanol mixture. Generally, it increased with increasing temperature and pressure, although the crossover effect (i.e., increasing solubility at decreasing temperatures at lower pressures) was apparent. The highest solubilities were observed at 50 °C with 100% CO₂ and 60 °C with 10% ethanol. The values obtained in this work are similar to those of Sakaki et al.⁹ for the solubility of the lipid of the fungus *Mortierella ramanniana*.

The fungal lipid is a complex mixture of glycerides, free fatty acids, sterols, and polar lipids. Physical properties, such as the critical temperature and pressure and the acentric factor are usually not known for these high-molecular-weight components. This precludes using a more rigorous model, such as an equation of state, to represent the solubility. Therefore, for process modeling

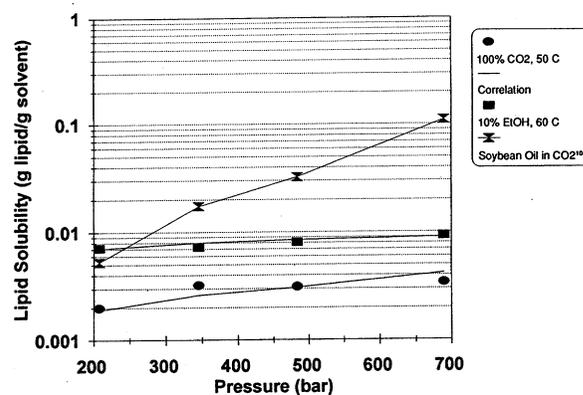


Figure 2. Experimental solubilities and values predicted by eq 1 for fungal lipids in supercritical CO₂ and CO₂ with 10 wt % ethanol as a function of pressure. For 100% CO₂, $C_1 = -23.1985$, $C_3 = 2.5359$ and for 10% ethanol, $C_1 = -11.2587$, $C_3 = 0.9444$. The solubility of soybean oil¹⁰ is shown for comparison.

purposes, we considered the lipid to be a single component and we correlated the solubilities at 50 °C (100% CO₂) and 60 °C (10% ethanol) using the empirical relationship of del Valle and Aguilera¹⁰ in which the solubility is represented as a simple function of temperature and density

$$\ln c = C_1 + \frac{C_2}{T} + C_3 \ln \rho \quad (1)$$

where C_1 , C_2 , and C_3 are constants fitted from experimental data; units of c and ρ are g L⁻¹ and temperature is in degrees Kelvin. The density of pure CO₂ was estimated using the Schmidt-Wagner equation of state¹¹ and the density of the CO₂-ethanol mixture was estimated using the modified Peng-Robinson equation of state.¹² In this work, only C_1 and C_3 were correlated since the solubility at only one temperature was modeled. Figure 2 compares the experimental solubilities and the values predicted by eq 1. The solubility of soybean oil predicted by the model of del Valle and Aguilera¹⁰ is given for comparison.

The results of the FAMES analysis for the extracts and the remainder (i.e., the lipid that remained in the mycelia) are shown in Figure 3a for extraction with CO₂ and in Figure 3b for extraction with the CO₂-ethanol mixture. The composition of the extract and the lipid remainder is nearly the same, although the lipid from the CO₂-ethanol extraction contained more of the polyunsaturated fatty acids, (C20:4 ω 6 and C20:5 ω 3) which are the valuable components of the product.

To represent the extraction curves, a model first presented by Lee et al.¹³ was used. This model was described thoroughly in our previous work.⁴ In our adaptation of this model, the mass-transfer rate was described by the following expression:

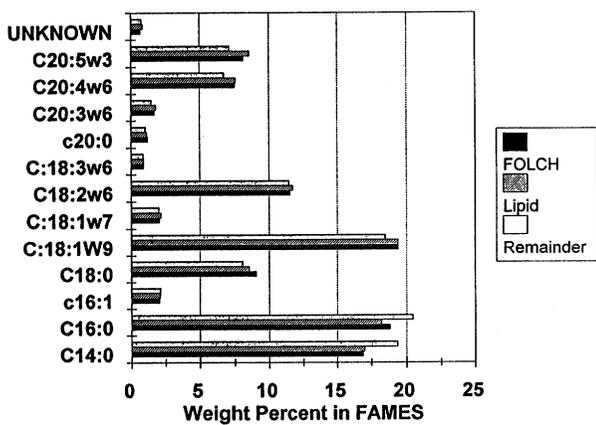
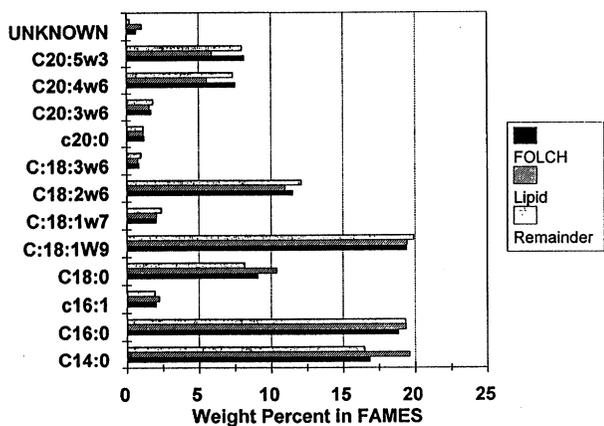


Figure 3. Composition of fatty acid methyl esters (FAMES) in fungal lipids extracted with supercritical fluids, and in the lipid remaining in the fungal mycelia after extraction. The extractions were performed at 60 °C and 346 bar. The composition of the lipids extracted using the method of Folch⁶ are also given. (a) 100% CO₂. (b) CO₂/10% ethanol.

$$R(x, y) = A_p K_o C^{f(x)} (y^* - y)$$

$$f(x) = \left[\frac{(x_o - x)}{(x_o - x_{\text{shift}})} \right] \quad (2)$$

where y^* is the equilibrium weight fraction of lipid in the solvent, y is the weight fraction of lipid in the solvent at any time, x_o is the initial weight fraction of lipid in the fungal mycelia, x is the weight fraction of lipid in the mycelia at any time, and x_{shift} is the weight fraction of lipid in the mycelia at which the extraction shifts from a mass-transfer controlled regime to a diffusion-controlled regime. If the constant, C , is less than one, the mass-transfer rate will decrease asymptotically as the extraction proceeds. We found C equal to 0.001 to give good representation of the curves.

The overall volumetric mass-transfer coefficients ($A_p K_o$) were determined through a least squares fit of the experimental data. The successive quadratic programming

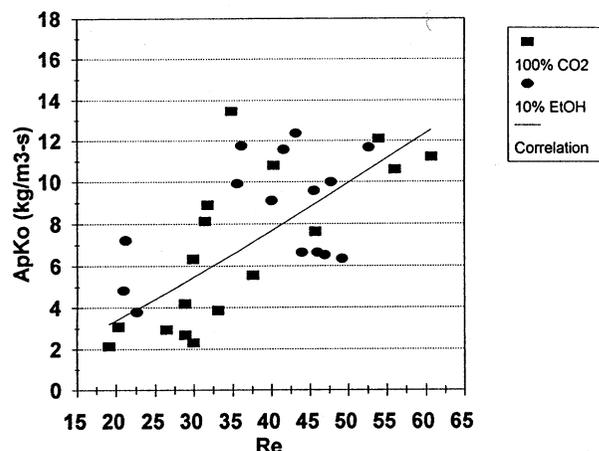


Figure 4. Volumetric mass-transfer coefficients computed for extractions with CO₂ and CO₂/10 wt % ethanol as a function of the solvent Reynolds number.

algorithm was used to minimize the sum of the squares of the residuals.¹⁴ Our previous work⁴ has shown that the model is able to represent the entire extraction curve well. Mass-transfer coefficients were determined for extractions with solvent flow rates ranging from 1–6 L min⁻¹ (measured at atmospheric pressure and room temperature). As shown in Figure 4, the mass-transfer coefficients computed for extraction with 100% CO₂ and 10% ethanol fell on roughly the same curve. The % AAD (average absolute deviation) for the fits ranged from 2 to 24%, and the values are in agreement with those computed by Lee et al.¹³ and Schaeffer et al.¹⁵ We computed x_{shift} to be 0.58 (± 0.08) for extractions with 100% CO₂ and 0.46 (± 0.07) for extractions with 10% ethanol. This is consistent with our observation that the lipids are more soluble in the mixed solvent. Since the polar lipids are not soluble in CO₂, the lipid available for extraction near the surface is quickly depleted, shifting the extraction to the slower, diffusion-controlled regime earlier in the extraction (i.e., with more lipid remaining in the mycelia).

For modeling purposes, the mass-transfer coefficients were correlated as a function of the fluid Reynolds number as follows:

$$A_p K_o = 0.0998(\text{Re})^{1.17696} \quad (3)$$

We used the experimental data published by Vukalovich and Altunin¹⁶ to determine the pure CO₂ viscosity and the correlations of Reid et al.¹⁷ to estimate the CO₂ mixture viscosity. The correlation coefficient for eq 3 was 0.53. The variability in the results may be due to either differences in the starting material (i.e., the fungal mycelia) among the experiments or changes in the composition of the CO₂–ethanol mixture.¹⁸ We studied the sensitivity of the process design results (discussed in the next section) to the mass transfer coefficient, $A_p K_o$. For a 60% de-

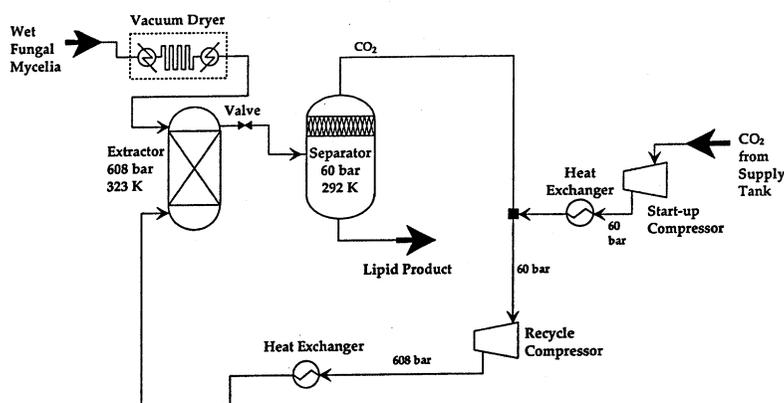


Figure 5. Schematic diagram of a commercial process to extract lipids from filamentous fungi using supercritical CO₂.

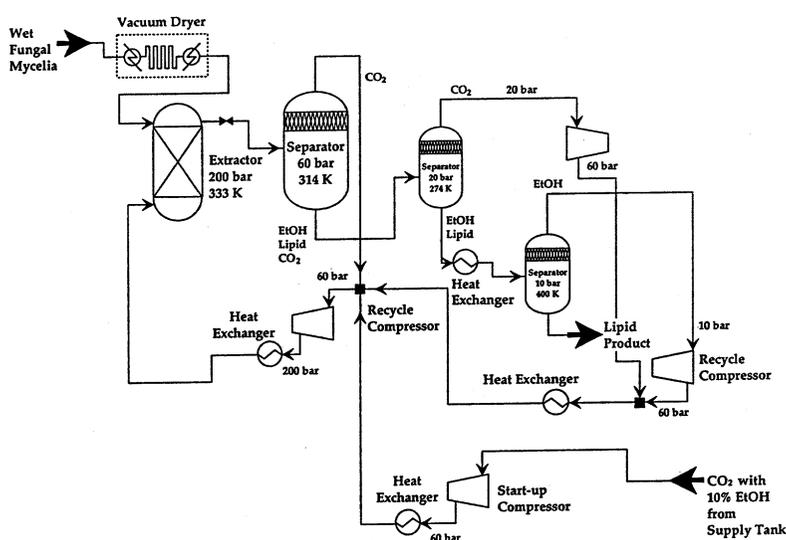


Figure 6. Schematic diagram of a commercial process to extract lipids from filamentous fungi using supercritical CO₂ with 10 wt % ethanol.

crease at a fixed solvent flow rate (the largest observed variation) the design results differed by only 6.5%.

Process Modeling, Simulation, and Optimization. Using the experimental data and the models described in the previous sections, a process flow sheet model was developed and was used to estimate the economics of a SFE process to concentrate the fungal lipids. Only the downstream section of the process was modeled in this work, therefore, the separation costs per kilogram were estimated, not the complete production cost. With a pure CO₂ solvent, the flowsheet model included a vacuum dryer, extractor, separator, compressors, and heat exchangers and is shown schematically in Figure 5. With a CO₂-ethanol solvent, a significant amount of CO₂ and ethanol remained in the liquid phase after the initial separator, and additional separators were designed to concentrate and recycle the solvent mixture. This design is shown schematically in Figure 6.

Since the process operates batch-wise, dynamic simulation techniques must be employed to rigorously solve the process models. This would require writing the equations for the separator, compressors, and heat exchangers as differential-algebraic equations. Dynamic simulation of process flow sheets is complex, however, since the integration has to be coordinated for process units which may require widely different time steps. To avoid these difficulties, the flow rates into and out of the separator, compressor, and heat exchangers were assumed constant. The amount of lipid that had been collected in the separator over the entire extraction time was computed by doing a steady-state flash of the total CO₂ extracted and the total lipid recovered. We assumed that the process operated batch-wise, with start-up and down-times of 60 min. The heating, cooling, and compression loads were estimated using the Group-Contribution Equation of State.¹⁹

TABLE II
Optimal Process Design and Costs Analysis for SFE of Fungal Lipids.
All Equipment Assumed to be Made of Stainless Steel. All Costs
Updated to 1993 in US Dollars.

	100% CO ₂	10% EtOH	10% EtOH eliminate vacuum drying step
Extractor temp (°C)	50	60	60
Extractor pressure (bar)	608	200	200
Flow rate of solvent (kg min ⁻¹)	13.0	15.5	15.5
Extraction time (min)	212	93	93
Equipment Costs			
Extractors (\$)	1,626,000	430,000	430,000
Separators (\$)	1,082,000	322,000	322,000
Compressors (\$)	1,500,000	1,572,000	1,572,000
Heat exchangers (\$)	162,000	70,000	70,000
Vacuum dryers (\$) ¹	488,000	488,000	0
Total fixed capital (\$) ²	5,724,000	3,401,000	2,824,000
Working capital (\$) ³	572,000	341,000	282,000
Total capital investment (\$)	6,296,000	3,741,000	3,107,000
Utilities and raw materials (\$)	100,000	59,000	52,000
Labor (\$) ⁴	302,000	302,000	230,000 ⁷
Maintenance and repair (\$) ³	572,000	341,000	282,000
Operating supplies and lab charges (\$)	165,000	101,000	101,000
Overhead, local taxes, and insurance (\$)	784,000	552,000	471,000
Depreciation (\$) ⁵	572,000	340,000	282,000
Administrative, distribution, selling, and R&D costs (\$)	621,000	434,000	371,000
Total operating costs (\$)	3,167,000	2,146,000	1,828,000
Return-on-Investment (%)	20	20	20
After-tax profit (\$)	687,000	408,000	339,000
Profit (\$) ⁶	1,041,000	618,000	514,000
Revenues required (\$)	4,157,000	2,764,000	2,342,000
Yield per batch (kg)	6.71	9.98	9.98
Annual production (kg)	19,390	22,533	22,533
Separation costs (\$ kg⁻¹)	214	123	104

¹ Assumed drying rate was similar to penicillin,²⁴ 0.976 kg m⁻²-h. Costs were given by Perry and Chilton.²⁵

² Includes 18% contingency and fees.

³ Estimated as 10% of total fixed capital investment.

⁴ Assumes 3.5 laborers per shift, 3 shifts per day, 1 supervisor.

⁵ Assumes 10 year, straight-line depreciation.

⁶ Assumes 34% income tax rate.

⁷ Reduced laborers by one.

The variables that have the greatest impact on the cost of the process are the extractor pressure and temperature, the flow rate of solvent and the extraction time. In this analysis, the extractor temperature and the separator pressure were fixed and nonlinear programming was used to determine the optimal value of the other parameters. The design objective was to minimize the annualized cost per kilogram of lipid produced. The annualized cost was the sum of the operating costs and the installed cost of the equipment, multiplied by the rate of return on investment. The installed cost of the equipment was determined using correlations of the graphical data of Ulrich²⁰ which depicts the costs of basic processing equipment as a function of

size, operating conditions, and materials of construction. Details of the equipment cost correlations were given in our previous work.²¹ The constraints included a product recovery specification (95% of the lipid extracted) and upper and lower bounds on the design variables. The nonlinear program was solved using the successive quadratic programming method.¹⁴

The results of the design optimization are shown in Table II. Since the lipid is more soluble in the CO₂-ethanol mixture, the optimal extractor pressure and extraction time were lower than those computed for extraction with pure CO₂. For the CO₂-ethanol process, the minimum annualized cost was achieved when the pressure was

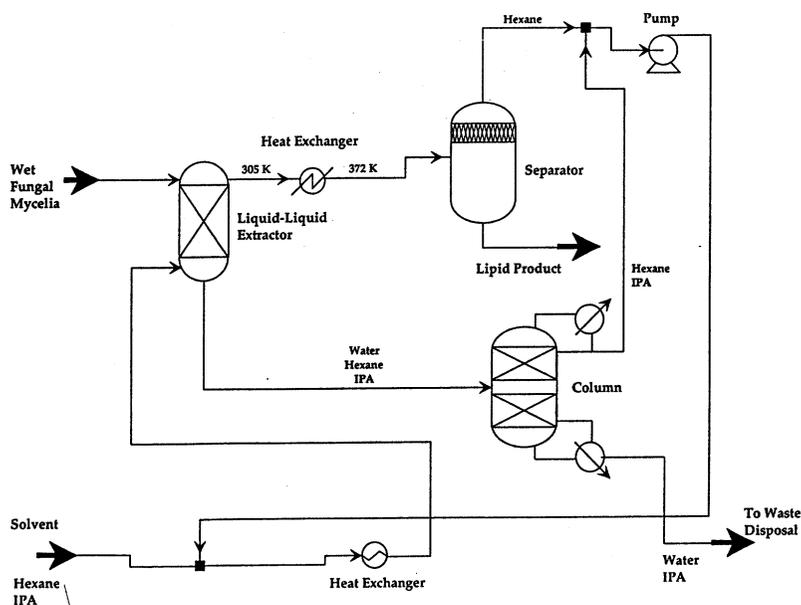


Figure 7. Schematic diagram of a commercial process to extract lipids from filamentous fungi using a mixture of hexane and isopropanol.

set to the lower bound. This indicates that the increased solubility that occurs at higher pressures cannot compensate for the higher equipment costs. The lipid extracted per batch was also higher, since the solvent mixture was able to extract both the neutral and the polar lipids. These results are reflected in the equipment costs, as shown in Table II. Both the utility and the equipment costs are higher for extraction with pure CO₂, giving a separation cost per kilogram of product of \$214 as compared to \$123 for the extraction with the CO₂-ethanol mixture.

In completing the cost analysis, it was assumed that maintenance and repair was 10% of the total fixed capital, the operating supplies were 20% of the maintenance and repair, the lab charges were 20% of the operator labor costs, and the overhead was 7% of the sum of labor and maintenance. The administrative costs were then assumed to be 25% of the overhead. Local taxes and insurance were assumed to be 2 and 1%, respectively, of the total fixed capital. Distribution, research, and development costs were assumed to be 10 and 5%, respectively, of the total expense.

By using the process simulator, ASPEN (Aspentech, Cambridge, MA), these costs can be compared to the costs of a conventional liquid extraction process. Experimental work in our laboratory has indicated that a wet milling-solvent extraction process with a 47/53 (mole basis) hexane-isopropanol mixture can extract more than 90% of the lipid in the mycelia.²² We have found that the use of this solvent system does not require drying the mycelia prior to extraction. The input to this process is therefore the fermentation broth containing wet mycelia, which is contacted continuously with the solvent. To accurately model the liquid-liquid extraction process, *K*-values for the lipid in the hexane-isopropanol

mixture were determined experimentally.²² Based on similar work on the solvent extraction of oils from yeasts,²³ a solvent-to-lipid ratio of 6 L kg⁻¹ was assumed.

A schematic diagram of the separation process is shown in Figure 7. After exiting the liquid-liquid extractor, the extract is flashed in a separator, which concentrates the lipid in the bottoms product. The raffinate is sent to a distillation column which concentrates the isopropanol in the distillate. This is mixed with the overhead from the separator (which is nearly pure hexane), cooled and recycled to the extractor. The costs for this process are shown in Table III. Both the equipment and the operating costs are significantly lower than those computed for the SFE process, giving a separation cost per kilogram of \$37. Note, however, that this process does not require the expensive drying step. If the cost of vacuum drying were eliminated from the SFE process, the cost per kilogram falls to \$104 (see Table II). Elimination of the drying step may also allow the process to be operated continuously, increasing the annual production rate and further lowering the costs. Although this analysis demonstrates that the costs for the SFE process are prohibitive, environmental and regulatory concerns about liquid solvents may prompt further consideration of SFE at a future date.

In addition to providing some insight into the commercial acceptability of this technology, the process modeling and optimization strategy also points out areas for future research. Since the cost analysis indicated a significant benefit to eliminating the drying step, we have studied the extraction of wet fungal mycelia with CO₂ and CO₂ with 10 wt % ethanol and these results will be presented in future communications.

TABLE III

Results of Process Simulation Including Design Variables and Processing Costs for a Conventional Liquid-Liquid Extraction of Fungal Lipids Using 47/53 Hexane-Isopropanol Mixture (Molar Basis). All Equipment made of Stainless Steel, Costs Updated to 1993 in US Dollars

Design Variables and Costs

Flow rate of feed stream (kg min ⁻¹)	
water	0.726
lipid	0.046
Flow rate of solvent make-up (kg min ⁻¹)	
Isopropanol	0.038
Hexane	0.02
Extractor	
Temperature (K)	305
4 stages, diameter = 1.0 m, Height = 6.1 m	
Installed cost (\$)	81,000
Distillation column	
Top stage temperature (K)	354.0 K
Bottom stage temperature (K)	356.9 K
10 trays, tray efficiency = 50%	
Diameter = 0.3 m, height = 13.1 m	
Installed Cost (\$)	173,000
Heat exchangers	
Total area (m ²)	7.3
Installed cost (\$)	90,000
Separator	
Diameter = 0.12 m, height = 0.24 m	
Installed Cost (\$)	4,000
Mixers	
Installed Cost (\$)	4,000
Total Installed Cost of Equipment (\$)	352,000
Total fixed capital ¹ (\$)	415,000
Working capital ² (\$)	42,000
Total capital investment (\$)	457,000
Utilities and raw materials (\$)	213,000
Labor ³ (\$)	230,000
Maintenance and repair (\$)	42,000
Operating supplies and lab charges (\$)	45,000
Overhead, taxes, and insurance (\$)	203,000
Depreciation ⁴ (\$)	42,000
Environmental ⁵ (\$ yr ⁻¹)	12,000
Administrative, distribution, selling and R&D costs (\$)	160,000
Total operating expenses (\$)	747,000
Return-on-investment (%)	20
Alter-tax profit (\$)	50,000
Profit ⁶ (\$)	76,000
Revenues required (\$)	823,000
Annual production (kg yr ⁻¹)	22,080
Separation cost (\$ kg⁻¹)	37

¹ Includes 18% contingency and fee.

² Assumes 10% of fixed capital investment.

³ Assumes 2.5 laborers per shift, 3 shifts per day, 1 supervisor.

⁴ Assumes 10 year, straight-line depreciation.

⁵ Assumes waste-stream clean-up cost to be 2.75% of the fixed capital investment.

⁶ Assumes 34% income tax rate.

CONCLUSIONS

Supercritical-fluid solvents were used to extract fungal lipids. The solubility of the lipids increased with increasing solvent pressure and temperature, although the crossover effect was observed. Mass-transfer coefficients fitted to experimental data increased with increasing solvent Reynolds number and were found to follow the same trends for extractions with CO₂ and CO₂ mixed with 10 wt % ethanol. The process flowsheet optimization demonstrated that the addition of the cosolvent lowers the processing costs by over 40%. A comparison of the costs for the SFE process and the costs for a liquid extraction process shows that the SFE process is not competitive for this application, although the economics would be improved if wet fungal mycelia were contacted continuously with the supercritical solvents.

ACKNOWLEDGMENTS

The authors would like to acknowledge the assistance of Raymond Kwoczek in performing the experiments. The GC-EOS program was provided by Professor Rafiqul Gani, Danmarks Techniske Højskole and the use of this program is gratefully acknowledged.

Nomenclature

T	temperature
c	solubility (g L ⁻¹)
C, C_1, C_2, C_3	constants
$A_p K_o$	volumetric overall mass-transfer coefficient, (kg m ⁻³ -s)
x	weight fraction of lipid in the fungal mycelia at time t
y	weight fraction of lipid in the supercritical fluid at time t
x_o	weight fraction of lipid in the fungal mycelia at time $t = 0$
x_{shift}	weight fraction of lipid in the fungal mycelia when the extraction shifts from a mass transfer-controlled regime to a diffusion-controlled regime
y^*	equilibrium weight fraction of lipid in the supercritical fluid
Re	Reynolds number, $\frac{\rho u d}{\mu}$
u	solvent velocity, m s ⁻¹
d	particle diameter, m
μ	solvent viscosity, kg m s ⁻¹
ρ	density (g L ⁻¹ or kg m ⁻³)

REFERENCES

- (1) Yongmanitchai, W.; Ward, O. P. *Process Biochem.* **1989**, *24*, 117.
- (2) Heoksema, S. D.; Behrens, P. W.; Gladue, R.; Arnett, K. L.; Cole, M. S.; Rutten, J. M.; Kyle, D. J. In *Health Effects of Fish Oils in Foods*; Chandra, R. K., Ed.; ARTS Biomedical: Newfoundland, 1989; Chapter 21.
- (3) Gellerman, J. L.; Schlenk, H. *Biochim. Biophys. Acta* **1979**, *573*, 23.
- (4) Cygnarowicz-Provost, M.; O'Brien, D. J.; Maxwell, R. J.; Hampson, J. W. *J. Supercrit. Fluids* **1992**, *5*, 24.
- (5) Powell, Jr., J. R.; Scott, W. W.; Kreig, N. R. *Mycopath. Mycol. Appl.* **1972**, *47*, 1.

- (6) Folch, J.; Lees, M.; Sloane-Stanley, G. H. *J. Biol. Chem.* **1957**, *226*, 497.
- (7) Wessinger, E. W.; O'Brien, D. J.; Kurantz, M. J. *J. Ind. Microb.* **1990**, *6*, 191.
- (8) Kurantz, M. J.; Maxwell, R. J.; Kwoczak, R.; Taylor, F. J. *J. Chromatogr.* **1991**, *549*, 387.
- (9) Sakaki, K.; Yokochi, T.; Suzuki, O.; Hakuta, T. *J. Am. Oil Chem. Soc.* **1990**, *67*, 553.
- (10) del Valle, J. M.; Aguilera, J. M. *Ind. Eng. Chem. Res.* **1988**, *27*, 1551.
- (11) *CRC Handbook of Tables for Chemical Analysis*; Bruno, T. J.; Svoronos, P. D. N., Eds.; CRC: Boca Raton, FL, 1989.
- (12) Panagiotopoulos, A. Z.; Reid, R. C. In *Equations of State: Theories and Applications*; Robinson, R. L.; Chao, K. C., Eds.; American Chemical Society: Washington, DC, 1986; pp 571–582.
- (13) Lee, A. K. K.; Bulley, N. R.; Fattori, M.; Meisen, A. *J. Am. Oil Chem. Soc.* **1986**, *63*, 921.
- (14) Biegler, L. T.; Cuthrell, J. E. *Comput. Chem. Eng.* **1985**, *9*, 257.
- (15) Schaeffer, S. T.; Zalkow, L. H.; Teja, A. S. *J. Supercrit. Fluids* **1989**, *2*, 15.
- (16) Vukalovich, M. P.; Altunin, V. V. *Thermophysical Properties of Carbon Dioxide*; Collet's: London, 1968.
- (17) Reid, R. C.; Prausnitz, J. M.; Sherwood, T. K. *The Properties of Gases and Liquids*, 3rd ed.; McGraw-Hill: New York, 1977.
- (18) Schweighardt, F. K.; Mathias, P. M. *J. Chromatogr. Sci.* **1993**, *31*, 207.
- (19) Skjold-Jorgensen, S. *Ind. Eng. Chem. Res.* **1988**, *27*, 110.
- (20) Ulrich, G. D. *A Guide to Chemical Engineering Process Design and Economics*, 1st ed.; John Wiley and Sons: New York, 1984.
- (21) Cygnarowicz, M. L.; Seider, W. D. *Biotech. Progress* **1990**, *6*, 82.
- (22) O'Brien, D. J.; Senske, G. E. *J. Am. Oil Chem. Soc.* **1994**, *71*, 947.
- (23) Davies, R. J. In *Single Cell Oil*; Moreton, R. S., Ed.; John Wiley and Sons: New York, 1988.
- (24) *Freeze Drying of Foods*; Academy Research Council: Chicago, 1961; p 130.
- (25) Perry, R. H.; Chilton, C. H. *Chemical Engineers' Handbook*, 5th ed.; McGraw-Hill: New York, 1973; pp 20–24, 20–25.