Supercritical Carbon Dioxide Extraction of Lipids from *Pythium irregulare*

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ABSTRACT: Lipids that contain polyunsaturated fatty acids (PUFA) have therapeutic value. PUFA, however, degrade in high-temperature, oxygen-rich conditions typical of conventional hot solvent-extraction and distillation methods. Supercritical CO_2 extraction was chosen as an alternative method to recover these valuable compounds from the lower fungus, *Pythium irregulare*. Freeze-dried biomass was subjected to an aqueous phase and placed into a flow-through extraction apparatus. Extraction of oil from this biomass showed some success for moisture contents as high as 30% (wet basis). The addition of a novel CO_2-philic surfactant to the wet biomass with moisture contents as high as 95% (wet basis) increased the extraction rate of fungal oil by more than an order of magnitude. For tests with extraction times of 5 to 6 h, data for the diffusion-controlled region were modeled with an analytical solution to Fick's second law. Equilibrium data were also obtained for the fungal oil at two isotherms (40 and 60°C) over a pressure range of 13.7 to 27.5 MPa.

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KEY WORDS: Extraction kinetics, fungi, phase equilibria, polyunsaturated fatty acids, *Pythium irregulare*, supercritical fluid extraction, supercritical carbon dioxide, surfactant.

Beneficial health effects from consumption of certain fish oils have been attributed to the presence of the essential polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docasapentaenoic acid (DHA). These n-3 fatty acids have been linked to a reduced risk of coronary heart disease, arthritis, inflammation, hypertension, psoriasis, other autoimmune disorders, and cancer (1). PUFA are currently marketed as dietary supplements at health food stores in the form of concentrated fish oils. Supplementation into baby foods has lately received greater interest. PUFA are also prescribed medications for humans and pets.

Declining marine resources and an increasing demand for PUFA have prompted the search for alternative sources of EPA and DHA. Filamentous fungi have the potential to produce large amounts of EPA within the mycelial walls when grown at optimal conditions (2,3). The filamentous fungus *Pythium irregulare* converts sweet whey permeate to significant amounts of EPA (24.9 mg EPA/g dry biomass), which could potentially add value to this food-processing by-product (4).

Although the filamentous fungi are capable of producing valuable compounds such as enzymes and PUFA, the separation and purification steps impose significant restraints since many of these compounds are thermally labile or chemically unstable (5). Supercritical carbon dioxide is a potential alternative solvent for the extraction of sensitive lipids because the critical point is at 31.1°C and 73.8 bar, which is ideal for extracting thermally labile substances. Supercritical CO_2 also has the advantages of low cost, nontoxicity, high diffusivities, and low viscosity. Therefore, supercritical CO_2 is currently the leading choice for replacement of toxic organic solvents that are used in food and biological separation processes. Disadvantages of supercritical extraction systems include the high capital cost for pressurized industrial batch systems and the lack of necessary technology for successful continuous systems (6).

The extraction of lipids from fungi and algae using supercritical fluids has been accomplished for freeze-dried materials (4,7–10). In most cases, increasing pressure enhances solubility of lipids in CO_2. Lipid solubilities in freeze-dried algae reach a maximum at 24 MPa (10). Temperature generally had little effect; however, decreased solubilities were noted at 20 MPa with increasing temperature (7).

Water is only slightly soluble in CO_2 and causes significant resistance to the mass transfer of nonpolar compounds (11). Techniques for producing aqueous dispersions in supercritical CO_2 have been sought for over a decade, and only recently (12–17) have a few successful surfactants been found that result in reverse micelles in supercritical CO_2 with small quantities of water in their cores. A hybrid fluorocarbon–hydrocarbon surfactant was designed (18) with the capability of incorporating up to 10 times the amount of water normally soluble in CO_2, thus giving a water-to-surfactant ratio of 32 near room temperature. Most other surfactants give ratios near zero. The structural aggregates of quaternary mixtures of oil, perfluoropolyether (PFPE) surfactant, isopropyl alcohol, and water were verified with light-scattering measurements (19). PFPE surfactant forms reverse micelles that are soluble in supercritical CO_2 (17). These microemulsions were shown to incorporate bovine serum albumin (17). This find-

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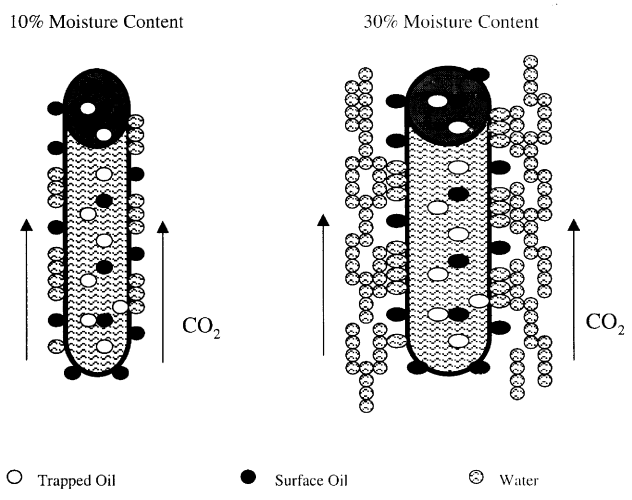
ing is very significant and possibly opens up many avenues for extraction of polar compounds in supercritical CO_2. Drawbacks of these surfactants include: the water-to-surfactant ratio attained to date has been too small for most applications involving large biomolecules, separation of surfactant from the final product must be addressed, and the known surfactants (e.g., PFPE) are very costly.

In this study, phase equilibria experiments were conducted to determine the solubility isotherms of fungal oil in supercritical CO_2. Fungi containing different levels of moisture were extracted to determine the effect of CO_2 flow rate and moisture content on the mass transfer of the system, which could then be modeled with Fick's second law of diffusion. Finally, a wet fungal suspension was mixed with a novel CO_2-soluble surfactant and extracted with supercritical CO_2 as an attempt to overcome the aqueous extraction barrier.

THEORY AND MODEL DEVELOPMENT

The economic and technical feasibility of a supercritical extraction process requires knowledge of the thermodynamic and mass transfer properties of the system (20). Therefore, the extraction kinetics and phase equilibria studies are important for design of supercritical extraction systems and economic analyses required to justify implementation for bioprocessing applications. Process models using the equilibrium and mass transfer properties are necessary to obtain design parameters concerning equipment size, operating conditions, extraction yields, and solvent flow rates.

The extraction of lipids, fatty acids, or derivatized forms of fatty acids from a microbial mass requires knowledge of the various barriers to mass transfer. Because the physical distribution of oils in processed biomass is not accurately known, understanding the controlling mass-transfer step in extraction is challenging. Observation of equilibrium and kinetic behavior of the fungal-oil system in supercritical CO_2 gives valuable insight to the overall extraction picture. This complex picture is represented by five main constituents. These constituents include the inert solid phase containing the fungal cell wall (consisting primarily of polysaccharides), the moisture adsorbed to the fungi, free moisture surrounding the fungi, the condensed oil droplets containing mainly neutral triacylglycerols, and the supercritical CO_2 solvent phase. Scheme 1 shows a heterogeneous mixture of these constituents in contact with supercritical CO_2. The right-hand fragment contains approximately three times the moisture as the left-hand fragment. This figure indicates that the barrier of surface moisture, which has highly repulsive interactions with oil, must be removed for subsequent extraction of the oil phase.



SCHEME 1

The dehydrated and shrunken fungal debris consists of both crushed and whole mycelia. A higher degree of cell fragmentation would make the oil more easily available for intimate contact with the CO_2 phase. This may be accomplished on a small scale by ultrasonic disintegration, shearing with glass beads, mastication, crushing with a ball mill (21), or freeze-drying combined with crushing (8). Once the readily accessible surface oil has been extracted, oil trapped within the tough cell walls would diffuse slowly through this barrier to the solvent phase.

A combined thermodynamic/mass transfer model is reported in the literature that applied a mathematical relationship based on the unsteady-state extraction of monocrotaline and lipids from *Crotalaria spectabilis* with supercritical CO_2 and an ethanol entrainer in a fixed-bed extractor (22). Another similar model that described the extraction of oil from soybean flakes (23) was also applied to dried algae (7) and fungi (8). These models are empirical fits to the overall mass-transfer performance of a specific extraction system and offer little in the way of fundamental understanding or transferable knowledge.

The extraction of lipids from partially crushed algae demonstrated a shift from a regime controlled by mass-transfer in the fluid phase to a regime controlled by diffusion in the condensed phase after 25% of the total lipids were extracted (24). This shift occurred near 55% of the total lipids extracted for the fully crushed algae, showing that initially more lipids were easily accessible during the extraction when the algae were fully crushed. The initial extraction indicates that the resistance to mass transfer may occur at the surface of the submerged material. This implies that the mass-transfer rate is dependent on solvent velocity and the mass-transfer coefficient may be derived from forced-convection relations (25).

If diffusion occurs primarily within the mycelium structure of the biomass and the fungi are assumed to approximate a spherical geometry after mastication, an analytical solution to Fick's second law of diffusion may be obtained (26). Because diffusion appears in the condensed phase to limit the rate of mass transfer later in the extraction after approximately 30 to 50% of the lipids are removed, this model would possibly describe data in this region of interest. Nonlinear regression of the analytical solution to Fick's second law of diffusion yields the diffusion coefficient and pseudo-r^2 value (27).

MATERIALS AND METHODS

Materials. *Pythium irregulare* (ATCC 10951; American Type Culture Collection, Rockville, MD), which is a member of the mastigomycetes (or oomycetes) class, was maintained on Corn Meal Agar (Difco Laboratories, Detroit, MI) and transferred every 3 mon to new agar slants. Cultivation of *P. irregulare* was achieved in a 2% glucose medium supplemented with 0.5% yeast extract (Difco Laboratories) and 0.1% K_2PO_4 with pH adjusted to 6.5. The cultivation was accomplished aerobically using 2.0 or 3.0 L shaker flasks in either a New Brunswick (New Brunswick, NJ) orbital shaker (NBS) at 200 rpm or a bioreactor flask placed in a constant temperature water bath operated in airlift mode. The fungi were cultivated at two temperatures, 19 and 24°C.

Harvesting the cell mass was accomplished by vacuum filtration using either Whatman #1 (Clifton, NJ) filter paper or an A/G Technology (Needham, MA) hollow-fiber microfiltration filter. This membrane cartridge would typically concentrate the 10 L biomass suspension by more than 10× over a 90-min filtration period with a pump rate of 10 L/min (28).

The cell mass was then concentrated to 10% moisture content wet basis (wb) by lyophilization and crushed with a mortar and pestle. Particles were then mixed for 20 s in an analytical mill for further cell mastication. Water activity, measured with a AquaLab (Decagon, Inc., Pullman, WA) water activity meter, was 0.48 at 25°C for the fungi with a 10% moisture content. Two fractions with different particle size, having average particle radii of 0.25 and 0.15 mm, were used for the experiments. A typical particle photomicrographed (100×) using a phase-contrast microscope (Olympus Inc., Melville, NY) with attached video processing is shown in Figure 1. This typical particle shows the clumped, circular shape of the fragmented and dehydrated fungi. The dry biomass was stored under nitrogen at −20°C.

Distilled water was added to two samples using a method (29) that would obtain moisture contents of 20 and 30%, respectively. The biomass was prepared a day before the experiment and allowed to equilibriate overnight in a closed glass container at 4°C. Moisture contents of the biomass were verified by drying in a vacuum oven under reduced pressure at 80°C overnight or until no change in weight occurred (30). The water activities for the fungi with 20 and 30% moisture contents were 0.76 and 0.87 at 25°C, respectively.

The CO_2-soluble surfactant used in this study was Krytox 157FSL (DuPont Chemicals, Wilmington, DE), $CF_3O[CF_2$-$CF(CF_3)O]_3CF_2$-CO_2-NH_4, which is the ammonium salt of a PFPE carboxylic acid (average molecular weight: 2500). A spatula was used to vigorously mix this surfactant with a high-moisture (95% moisture content, wb) preparation of fungi to make a surfactant suspension of 10% w/w. The tubing and extraction column were later cleaned with Vertrel MCA (DuPont Chemicals) solvent.

Extraction. The apparatus shown in Figure 2 was a single-pass flow system that was modified from another system (31–33). The main advantage of the single-pass flow apparatus for determining the phase equilibria is the low residence time, especially at elevated temperatures (34). This is particularly important for biomaterials that are chemically unstable or susceptible to thermal degradation such as PUFA.

The height of the extraction column was 15.2 cm and the inside diameter was 1.3 cm. The flow rate through the column was controlled with a high-pressure metering valve (Autoclave Engineering, Inc., Erie, PA). The water bath was maintained at constant temperature (±0.1°C). Pressure was monitored by a Heise (Newtown, CT) digital pressure indicator and maintained within a precision of ±0.035 MPa.

The extraction cell, shown in Scheme 2, was loaded with approximately 1.5 g fungal biomass (dry weight) mixed with 5-mm (approximately 25 beads) and 1-mm glass beads (ap-

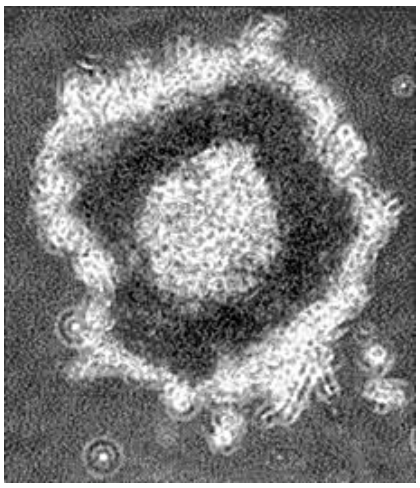


FIG. 1. Photomicrograph (100×) of lyophilized fungal particle.

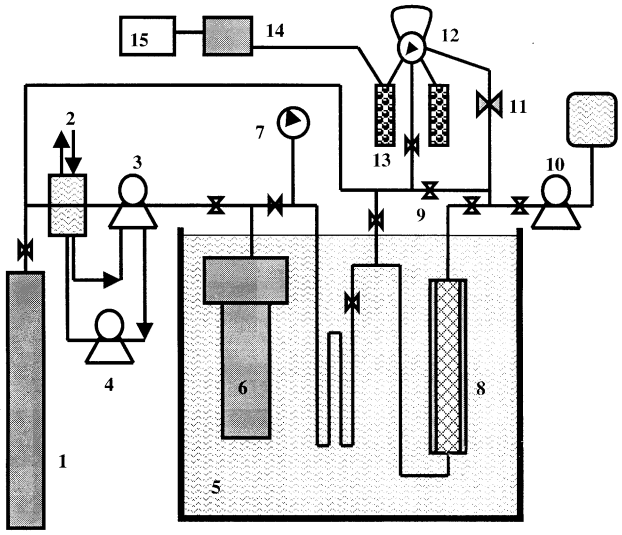
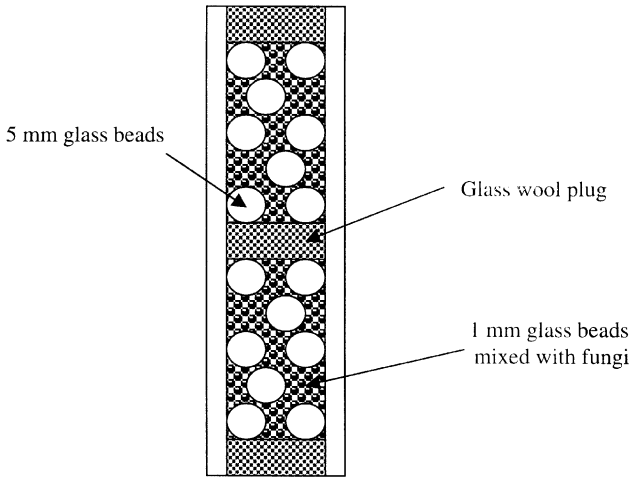


FIG. 2. Schematic of continuous-flow supercritical CO_2 system: 1. CO_2 tank, 2. refrigerated circulator, 3. high pressure liquid pump, 4. cooling pump, 5. heated water bath, 6. CO_2 pulse dampener, 7. pressure display, 8. extraction cell, 9. CO_2 purge valves, 10. liquid solvent pump, 11. metering valve, 12. 6-way sampling valve, 13. cold traps, 14. mass flowmeter, 15. flow totalizer and display.



SCHEME 2

proximately 5 g) and packed between glass wool. This packing helped to ensure good solvent mixing and prevent channeling and entrainment in the extraction cell. The extraction cell was then assembled, placed in the constant temperature water bath, flushed with CO_2, and pressurized. Samples were taken by depressurization and rinsing the valve system with 20 mL hexane (Fisher Scientific, Pittsburgh, PA) collected into a cold hexane trap. An additional trap at room temperature served as a backup and a visual aid for setting the flow rate.

The expanded CO_2 escaped to the flow meter and totalizer (Hastings-Teledyne, Hampton, VA) to determine the total volume of CO_2 that passed through the column. The sample volume and cumulative volume were recorded for each time interval after each sample was taken. The total volume of CO_2 was recorded at atmospheric pressure and converted to moles using ideal gas relations.

Procedures. Initial kinetic tests were conducted to observe the effect of flow rate on extraction rates. A six-way sample valve was periodically switched to isolate the sample loop and a sample was withdrawn. Typically about 90% of total oil sample would collect on the tubing walls immediately following the sample loop where CO_2 depressurization took place. The residual hexane was then purged with CO_2 and the valve was then switched back to repressurize the sample loop.

The same procedure was followed for the remaining kinetic tests where the flow rate was kept constant at 100 standard cm^3/min (mL/min) except the system did not contain the six-way valve. Therefore the flow was temporarily blocked (approximately 5 min) at the valve immediately following the extraction column before taking each sample. The loop was slowly depressurized with the micrometering valve, rinsed with hexane, repressurized, and a constant flow rate resumed before taking the next sample. Upon completion of all kinetic tests, the biomass was removed from the extraction column and separated from the glass beads. The biomass was tested for moisture content and residual oil for all kinetic tests to complete the mass balance.

The phase equilibrium tests were conducted in a similar manner to the single-flow kinetic tests. Flow rates of 20 mL/min or lower provided stable equilibrium results when a small sample loop (0.25 mL) was used and at least 2 vol of CO_2 were passed through the sample loop before sampling. Higher flow rates would result in slightly lower oil concentration in the fluid phase, which indicated that mass transfer limited the oil concentration at flow rates of 50 mL/min and above. The equilibrium tests were usually stopped after 2 h because significant depletion of the fungal lipids would possibly lead to mass-transfer limitation of lipids contacting the fluid phase at the surface of the fungal particles. This limitation could occur from the transition to a regime limited by diffusion in the fungal matrix. Residual oil and moisture were not measured for the phase-equilibrium experiments, which typically ran for only 90 to 120 min.

Analysis. Either pentadecanoic acid or heptadecanoic acid methyl esters (Sigma Chemical Co., St. Louis, MO) served as the internal standard for quantification. A mixture of 37 fatty acid methyl esters (FAME) was supplied by Supelco, Inc. (Bellefonte, PA) for qualitative identification of the FAME present in the fungi. A modified rapid transmethylation procedure (35) was then implemented for a 1-mL sample volume to convert the triglycerides to FAME. All reagents used in the rapid transmethylation procedure were supplied by Fisher Scientific. The samples were then flushed with nitrogen and taken directly for gas chromatography (GC) analysis. The FAME profile containing EPA and arachidonic acid (ARA) methyl esters were determined with a Hewlett-Packard (Wilmington, DE) 6890 capillary gas chromatograph equipped with a flame-ionization detector and a 30 m × 0.25 mm ChromPac (Raritan, NJ) CPwax-52 capillary column. The injection volume was 1 μL. Temperature programming was 130°C for 2 min, 10°C/min to 180°C, 2°C/min to 215°C, 10°C/min to 230°C, and held for 2 min. The resulting areas were integrated and analyzed with HP Chemstation software. All samples were then stored at –20°C. Lipids from the initial biomass and residual lipids remaining in the extracted biomass were also determined (36).

RESULTS AND DISCUSSION

Table 1 shows the average distribution of fatty acids (converted to the respective methyl esters for GC analysis) for the fungi after lyophilization and homogenization. Saturated fatty acids account for about 38% of the total fatty acids. EPA made up approximately 11% of the total fatty acids and about 30% of the PUFA. It should be noted that a small amount (1.2%) of γ-linolenic acid, another high-value product, was also produced.

Phase equilibria. Table 2 shows the solubilities of the fungal lipids in weight percentage compared to literature values of linoleic acid (37), a ternary system of CO_2/oleic acid ($C_{18:1}$)/triolein (38) and fungal oil from *Saprolegnia parasiticus* (8), which is another filamentous mold. The solubility of the triolein in the ternary system was similar to the solubility of oil from both filamentous molds. The free fatty acids had much higher solubilities than the triglycerides, particularly at higher pressures. The evidence of retrograde condensation, where sol-

TABLE 1
Typical Fatty Acid Methyl Ester Distribution in *Pythium irregulare*

Fatty acid methyl ester (FAME)	Carbon compound	Molecular weight	% of total FAME
Myristic acid	C14:0	242.40	16.8
Myristoleic acid	C14:1	240.38	0.4
Palmitic acid	C16:0	270.45	18.6
Palmitoleic acid	C16:1	268.43	4.1
Stearic acid	C18:0	298.51	1.1
Oleic acid	C18:1n-9*cis*	296.49	17.3
Linoleic acid (LNA)	C18:2n-6*cis*	294.48	16.0
γ-Linolenic acid (GLA)	C18:3n-6	292.46	1.2
Arachidic acid	C20:0	326.56	1.6
cis-11-Eicosenoic acid	C20:1n-9	324.54	1.8
Arachidonic acid (ARA)	C20:4n-6	318.50	8.2
cis-5,8,11,14,17-Eicosapentaenoic acid (EPA)	C20:5n-3	316.48	10.5
Unknowns			

TABLE 2
Comparison of Solubilities (wt%) of *Pythium irregulare* Oil in Supercritical Carbon Dioxide with Selected Literature Values

Temperature (°C)	Pressure (MPa)	*P. irregulare* oil	Linoleic acid (38)	Oleic acid (39)	Triolein (39)	*S. parasiticus*[a] oil (8)
40	13.8	0.12	0.45			
40	20.7	0.43	2.2	2.9	0.4	
40	27.6	0.52	2.6	1	0.6	
60	13.8	0.0028	0.11			
60	20.7	0.1	1.8	0.5	0.1	0.18
60	27.6	0.23	1.9	1	0.4	0.2

[a]*Saprolegnia parasiticus.*

ubility decreases with increasing temperature, was seen for all constituents within the temperature–pressure range shown. The solubility also increases with increasing temperature at higher pressures (310 bar) for CO_2-triolein systems (39).

Mass transfer. Figure 3 shows the effect of flow rate on the mass transfer of lipids in fungi with a 10% moisture content (wb) and 0.15 mm particle size at 50°C. The flow rate appeared to affect the extraction rate initially; therefore, diffusion of oil within the fungal matrix did not appear to be the main form of resistance to mass transfer for lipid removal up to 40 to 50% of the total lipids. The linear portion of the curve shows the brief pseudo-steady-state conditions in the extraction. The lipids were assumed to be available at the surface of the fungal particles and the resistance to mass transfer occurred primarily in the surface film. The extraction rates decreased substantially after 40 to 50% of the lipids were removed, which indicated the transition to a diffusion-controlled regime. Therefore, only the later part of the extraction was modeled using the analytical solution to Fick's second law for the 60 and 100 mL/min runs, where unsteady-state conditions also prevailed. The effective diffusivities were nearly equal at 50°C for both flow rates and averaged 4.3×10^{-9} cm^2/s predicted with an average 0.94 pseudo-r^2 (shown in Table 3), which indicated that the model reasonably explains the phenomena for the range of data representing the later part of the extraction.

Figure 4 shows the percentage of total lipids extracted vs. the total moles of CO_2. The similarity in the slopes for the 20 and 50 mL/min flow rates confirm that equilibrium conditions prevailed initially at the 20 mL/min flow rate. The slopes decreased significantly for the higher flow rates toward the end

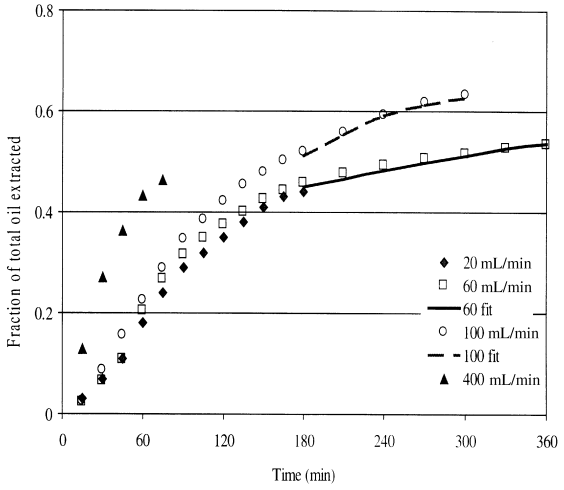


FIG. 3. Effect of flow rate on the extraction rate of fungal oil at 50°C, 20.6 MPa, 10% moisture content, and 0.15 mm particle radius. Lines indicate the fit of data using the analytical solution of Fick's second law of diffusion.

TABLE 3
Effective Diffusivities (D)[a] for *P. irregulare* at 20.6 MPa

Temperature (°C)	Flow rate (cm^3/min)	Particle size (mm)	D (cm^2/s)	Pseudo-r^{2b}
40	100	0.15	1.15×10^{-9}	0.96
40	100	0.25	2.05×10^{-9}	0.92
50	60	0.15	4.60×10^{-9}	0.95
50	100	0.15	4.01×10^{-9}	0.93

[a]According to the analytical solution to Fick's second law of diffusion (26).
[b]See Reference 27.

of the extraction, which further indicates that intraparticle diffusion resistance was important (40).

The effect of particle size and moisture content is shown in Figure 5 for the extraction of fungal oil at 40°C and 20.6 MPa. Two particle sizes (0.15 and 0.25 mm radius) for the 10% moisture fungi were compared. The effective diffusivities for both particle sizes averaged 1.6×10^{-9} cm^2/s. Table 3 shows that increasing temperature resulted in an increase in the effective diffusion coefficient of the fungal oil.

The initial mass transfer rates were decreased considerably for the fungi at higher moisture contents. The lower observed rates occurred for longer periods as the initial moisture content increased. Figure 5 shows an initial lag in the extraction rate that lasted about 90 min for the 30% moisture fungi and only about 60 min for the 20% moisture fungi. After the initial lag period, the mass-transfer rate increased substantially to nearly the rate of the 10% moisture fungi. Significant drying was observed during the process as nearly 30 to 50% of the initial moisture in the higher moisture samples was removed by the end of the process. The fungi with 10% moisture content typically lost approximately 20 to 30% of the total moisture by the end of the process. These results suggest that the initial water was readily available for coextraction with the fungal oil and was substantially removed, which then provides greater access of the surface oil to the surrounding fluid phase.

Figures 6 and 7 show the distribution of FAME plotted against time for the extracts from fungi with 10 and 30% moisture, respectively. The results were obtained by GC quantification for the 14:0 (myristic acid), 16:0 (palmitic acid), 18:2 (linolenic acid), ARA, and EPA fractions. The results showed differences in the affinity of supercritical CO_2 for lower-molecular weight compounds vs. higher-molecular weight compounds. The degree of unsaturation does not affect the solubility of fatty acids or FAME, except for highly unsaturated compounds such as EPA, where greater solubility is favored (41).

The higher solubility of lower-molecular weight compounds was evident for extracts from fungi at both moisture contents. The 14:0 and 16:0 fractions were extracted rapidly at the beginning of the process and were nearly depleted toward the end of the extraction. Higher-molecular weight components such as EPA appeared in greater amounts toward the end of the extraction process. The fractions, however, were obtained from neutral triglycerides where the EPA and ARA typically occupy the second (middle) position of the triacylglycerol molecule, and may share the other two positions with lower-molecular weight compounds. Approximately 83% of

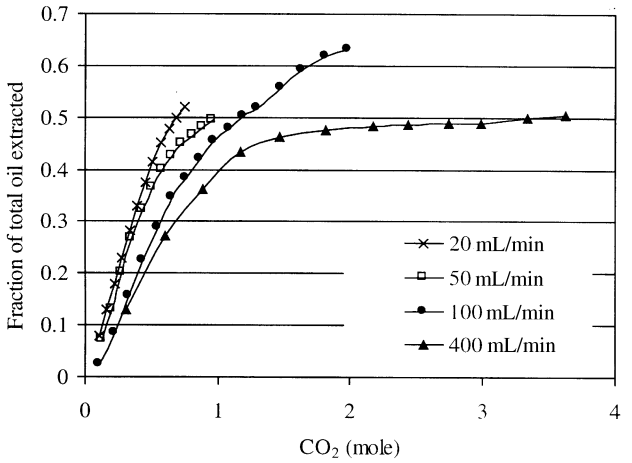


FIG. 4. Extraction of lipids at selected flow rates at 50°C and 20.6 MPa from fungi with 10% moisture content and 0.15 mm particle size vs. total moles of CO_2 consumed.

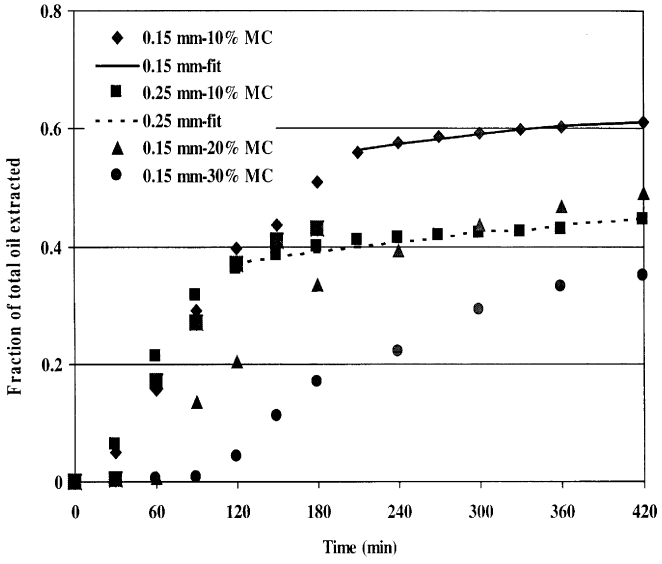


FIG. 5. Effect of moisture content (MC) and particle size (mm) on the extraction rate of fungal oil at 40°C and 20.6 MPa. Lines indicate the fit of data using the analytical solution of Fick's second law of diffusion.

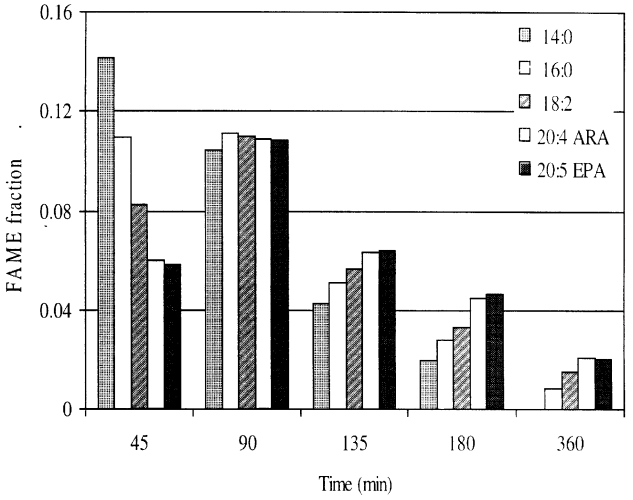


FIG. 6. Fatty acid methyl ester fraction for fungi with a 10% moisture content, wet basis extracted at 50°C and 20.6 MPa. Myristic acid is 14:0, palmitic acid is 16:0, linoleic acid is 18:2, arachidonic acid is 20:4 (ARA), and eicosapentaenoic acid is 20:5 (EPA).

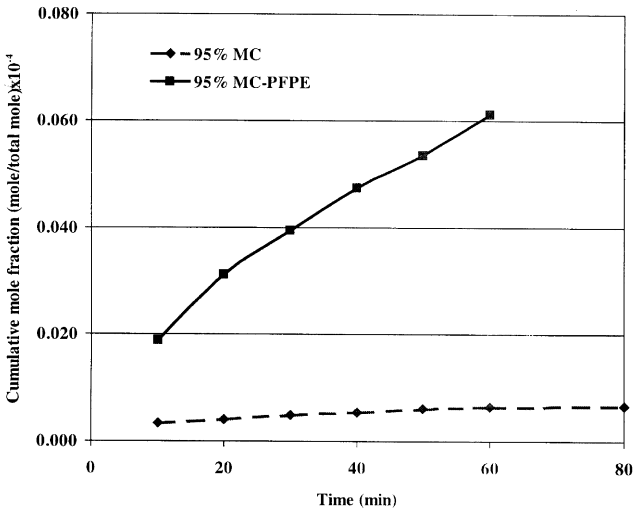


FIG. 8. Effect of surfactant (10% w/w PFPE) on extraction of fungal oil in *Pythium irregulare* with a 95% moisture content (MC). PFPE, perfluoropolyether surfactant.

the lipids in *P. irregulare* are in the neutral triglyceride fraction and about 10% of the lipids are in the polar (e.g., phospholipid) fraction (4).

Figure 8 shows that very little extraction takes place for fungi with 95% moisture content that would be typical for fermentation broth filtered with cross-flow microfiltration membranes or filter presses. However, the addition of 10% w/w PFPE surfactant resulted in an order of magnitude increase in extraction of total lipids over 60 min. The addition of the PFPE surfactant to the 95% moisture sample demonstrated a potential use for extraction of oils under extremely adverse conditions where the water-to-surfactant ratio is important for decreasing the aqueous barrier. The size of the micelles was unknown, but the system was thought to form a limited microdispersion because of the simple mixing technique used. More complete extraction of fungal oil would be expected with a greater degree of dispersion, which would create greater micellar surface area resulting in more intimate contact of the micelles with the fluid phase. The increased extraction rate could potentially lead to a significant savings in the drying costs. Further exploration of the use of surfactants to aid supercritical CO_2 extraction of biomolecules from high moisture biomass suspensions is recommended.

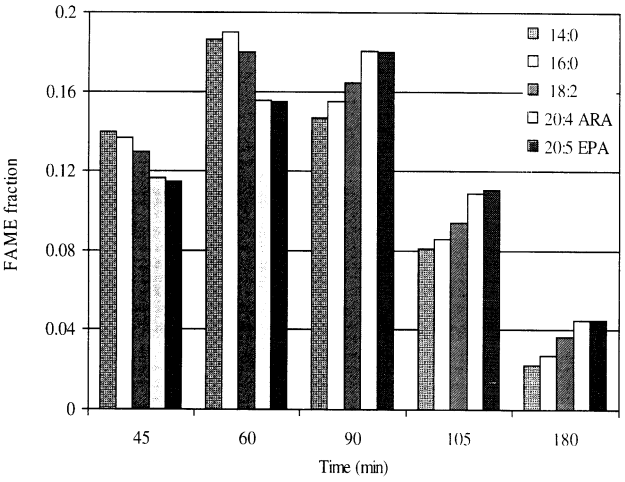


FIG. 7. Fatty acid methyl ester distribution for extract from fungi with a 30% moisture content, wet basis extracted at 50°C and 20.6 MPa. For abbreviations see Figure 6.

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