requirement.

The results shown for SC-CO_2-extracted wet-milled oil should be considered preliminary because subsequent research has shown that 50 C and 8,000 psi are not optimum conditions for the best extraction of triglycerides. Furthermore, the amount of excess of lye used was not necessarily optimum for all wet-milled oils. However, the results are interesting from the standpoint of the flavor of the deodorized oils. Commercial refined, bleached, deodorized wet-milled corn oil is difficult to obtain in a bland state because of burnt flavors that carry over from the steeping of the corn germ with SO_2 prior to wet-milling. As a result, wet-milled corn oil received lower flavor scores than soy or cottonseed oils. SC-CO_2-extracted, wet-milled crude oil yielded a refined, bleached, deodorized oil that was substantially free of the burnt flavors prevalent in conventionally extracted oil.

The low solubility of phospholipids in SC-CO_2 yields more neutral oil and is an advantage from a processing standpoint. However, phosphatides protect the oil from autoxidation (14). Thus, if SC-CO_2-extracted oil is to be stored for any length of time, it should be handled carefully, and perhaps should even be stored in nitrogen-blanketed tanks. The mechanism by which SC-CO_2-extracted oils undergo oxidative deterioration is under investigation and will be reported later.

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Effect of Moisture and Particle Size on the Extractability of Oils from Seeds with Supercritical CO_2[1]

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ABSTRACT

Moisture level and particle size of soybeans, peanuts and cottonseed were correlated with the extraction rate and yield of oil when extracted with supercritical carbon dioxide (SC-CO_2) at a constant temperature (50 C) and pressure (8000 psig). The rate of extraction and ultimate oil yields were quite low with cracked soybeans. However, good extraction rates and nearly theoretical oil yields were obtained from ground or thinly flaked (<0.010") seeds. Moisture levels between 3% and 12% had little effect on extractability. Oil composition was not influenced by either parameter. Scanning electron microscopy was used to study seed structure before and after extraction with SC-CO_2. Micrographs of SC-CO_2-extracted seeds were similar to hexane-extracted seeds.

INTRODUCTION

German scientists have investigated the extraction of natural products using supercritical fluids since the early 1960's and have demonstrated that carbon dioxide above its critical temperature and pressure is a suitable solvent for the extraction of oil (1-4). Subsequently, Friedrich and co-workers have reported high soybean oil recovery with supercritical carbon dioxide (SC-CO_2). Phosphorus and iron contents of the SC-CO_2-extracted oil were lower and the oil color was lighter than characteristic of hexane-extracted crude oil. Flavor scores of the refined soybean oil extracted by SC-CO_2 were not different from refined hexane-extracted oils (5). Stahl et al. showed that oil yield was dependent on particle size and structure of the oilseed (6). The present study shows the effect of particle size and moisture content of the seed on the extractability of oil with SC-CO_2. The scanning electron microscope (SEM) was used to determine how the native structures of the oilseeds change during extraction and to ascertain the best configuation of the seed for efficient oil extraction with SC-CO_2.

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EXPERIMENTAL

Whole soybeans were dried or tempered to 3.5%, 6% and 12% moisture levels which were determined gravimetrically using a Brabender oven; the soybeans were then cracked and dehulled. One-third of the cracked beans were flaked to a thickness of 0.25 mm, another third were ground to a fine flour (over 94% of the flour passed through a 100 mesh screen, which corresponds to 150 microns) by an Alpine Mill, and a final third were used without flaking or grinding. The cracked, ground or flaked soybeans (950 g) were

placed into a 2-l extractor as described previously (5). SC-CO_2 extractions were conducted at 8000 psig and 50 C. Extracted oil was removed from the receiver every hour and weighed. The extraction was terminated when the oil recovered per hr dropped to less than 1 g.

In a second study, cracked, dehulled soybeans were flaked to average thicknesses of 0.10, 0.25, 0.38 and 0.81 mm and were exhaustively extracted until less than 0.25 g of oil was obtained during the last one hr of extraction. An additional experiment was conducted to determine whether soy flakes or soy flour was extracted more readily. In this case, 46 grams each of flour (> 150 μ) and flakes (0.10 mm) were separately extracted in a 150-ml vessel under identical conditions of 8000 psig and 50 C. Extracted oil was weighed after each of 5 successive 50 standard liter aliquots of CO_2 had passed through the column.

Cottonseed and peanuts were cracked, dehulled and flaked before extraction; neither could be ground because of their high oil content. Extraction conditions were 8000 psig at 50 C for cottonseed and 10,000 psig at 70 C for peanuts.

Residual oil in the extracted oilseed samples was determined by AOCS Official Method Ac 3-44 (7). Extracted samples were analyzed for free fatty acids and unsaponifiable material by AOCS Official Methods Ca 5a-40 and Ca 6a-40 (7).

Methyl esters were prepared from the oil and analyzed by gas liquid chromatography (GLC) to determine fatty acid composition.

Samples for determination of surface structures of the different particle configurations were prepared for SEM examination by established methods (8).

RESULTS AND DISCUSSION

Either flaking or grinding prior to SC-CO_2 extraction, so that the surface area increased or a greater number of cell walls ruptured, was necessary for good oil recovery (Table I). These results agreed with those of Othmer and Agarwal who showed that soybeans had to be flaked or ground to permit the extraction of oil by hexane (9). After extraction with SC-CO_2, residual oil in cracked soybeans was about 20%, whereas 2% or less oil remained in the flaked and ground samples. Transfer of oil through the cell walls of the cracked beans did not occur, and only the surface oil that was exposed by the minor fracturing of the cell walls during the cracking process was removed. More cell walls were broken in the further process of flaking or grinding, resulting in a greater oil yield. Extractability of oil from flaked and ground beans was improved considerably over that obtained from the cracked beans. Oil solubility reached the apparent equilibrium solubility of 2.5% for our extraction conditions as indicated by the constant rate of extraction (straight portion of the extraction curves in Fig. 1). We found that moisture levels within the range of 3-12% had little effect on the extractability of the 3 different soybean structures. Moisture content also did not affect the extractability of the oil from dry-milled corn (10) or lupine seed (11). Oil is more soluble than water in SC-CO_2; therefore oil was removed first. Moisture content of the extracted meal was greater than full fat meal by an amount somewhat less than that due to oil removal alone. After extraction, the meal was examined. The moisture content increased steadily through the extractor in the direction of SC-CO_2. Extracted water is minimal and is observed only during the

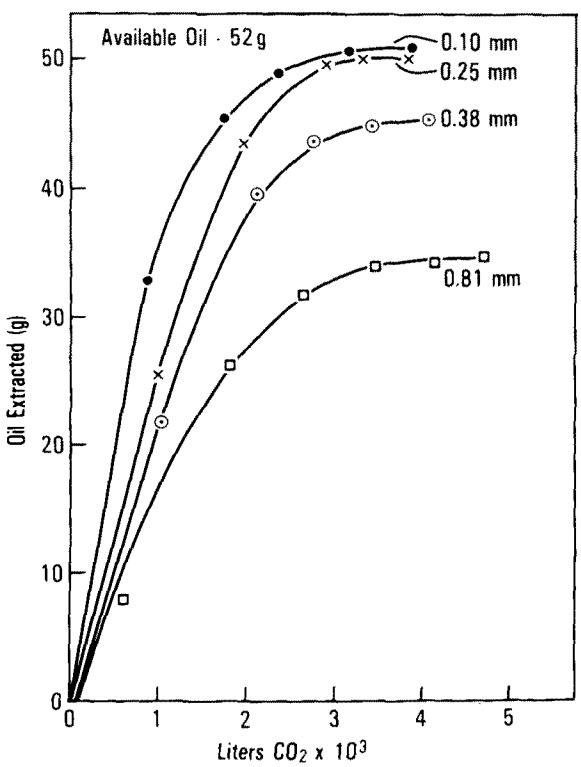

FIG. 1. Effect of soy flake thickness on oil extraction by SC-CO_2. Conditions: 8000 psig; 50 C. Available oil, 52 g.

TABLE I

Effect of Moisture and Particle Configuration on SC-CO_2 Extraction of Soybeans (Conditions: 8000 psig; 50 C)

Particle configuration	Moisture (%)	Res. oil[a] (%)	Oil solubility[b] (%)	Unsap.[a] (%)	FFA[a] (%)
Cracked	3.5	20.8	0.46	0.94	0.44
	6.0	18.4	0.39	0.81	0.42
	12.0	20.3	0.43	1.08	0.44
Flaked	3.5	2.1	2.26	0.63	0.35
(0.25 mm thick)	6.0	0.9	2.76	0.70	0.34
	12.0	1.1	2.54	0.65	0.44
Ground	3.5	0.9	2.30	0.83	0.28
(94% <100 mesh)	6.0	0.7	2.40	0.74	0.27
	12.0	1.8	2.38	0.74	0.36

[a]Determined by AOCS Official Methods.

[b]Apparent solubility determined by oil wt./CO_2 wt.

final stages of extraction.

Analysis of the extracted oil indicated no significant difference in free fatty acid content in any of the samples. Unsaponfiable matter was slightly higher in the oil from cracked beans. Moisture had no effect either on the FFA or unsaponifiable content in the samples.

Thickness of the flakes limited the oil yield and rate of extraction (Fig. 1). Rate of extraction increased and total oil yield improved as the flake thickness decreased. Oil yields of 97.4% for 0.10-mm flakes and 96.8% for the 0.25-mm flakes were not significantly different. Yield decreased rapidly as the flake thickness increased; yield was 87% oil from the 0.38-mm flakes and 66% from the 0.81-mm flakes.

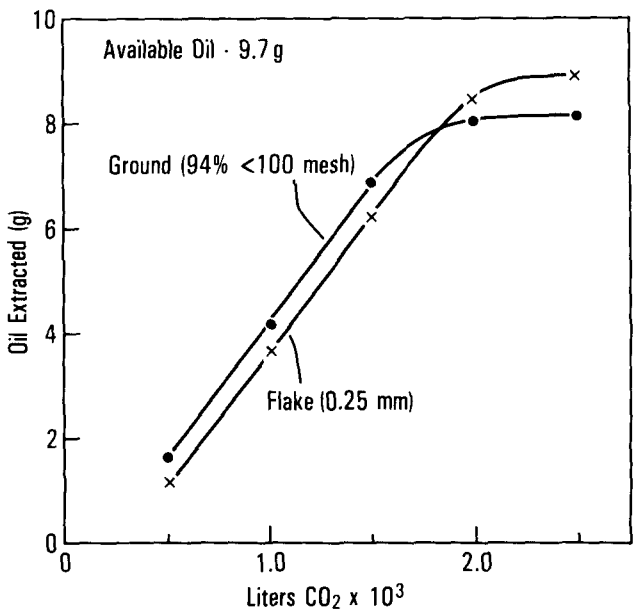

FIG. 2. Extraction of equal weights of soy flour and soy flakes with SC-CO_2. Conditions: 8000 psig; 50 C. Available oil, 9.7 g.

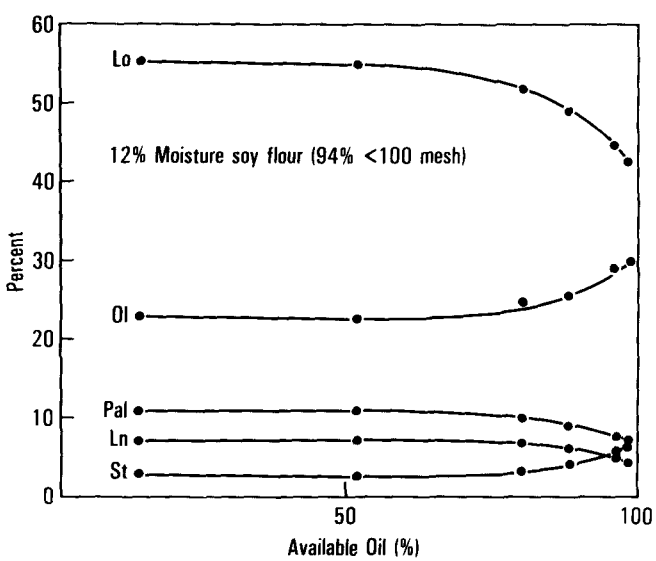

FIG. 3. Change in fatty acid composition during SC-CO_2 extraction of soybeans. Conditions: 8000 psig; 50 C. Soy flour 12% moisture. Ln=linolenate; Lo=linoleate; Ol=oleate; St=stearate; Pal=palmitate.

The results in Table I showed that extraction of soy flakes and soy flour were similar. Data in Figure 2 demonstrate that flaking is more acceptable in grinding as a particle configuration for maximum extraction of oil with SC-CO_2. Initially both soybean configurations were extracted at equal rates with 2.5% solubility, but extraction of the ground material slowed after approximately 80% of the oil was extracted. The gradual decrease in the rate of extraction for the ground beans may be attributed to either a size distribution of particles or packing of the flour, the latter leading to a reduction in gas flow with potential channeling of solvent. When the extracted flour was removed from the vessel, the bottom portion of material had packed together into a solid piece. This solid portion contained a higher concentration of oil than the top section, which indicated that the CO_2 had not freely penetrated this part of the column. Determination of residual oil showed 0.5% oil in the extracted flakes and 0.9% oil remaining in the flour.

Fatty acid composition of the SC-CO_2-extracted soybean oil was typical of hexane-extracted oil with 7.5% linolenate, 55% linoleate, 23% oleate, 3.5% stearate and 11% palmitate. Fatty acid composition of individual samples remained the same throughout 85-90% of the extractions. Fractionation of the triglyceride oil may be

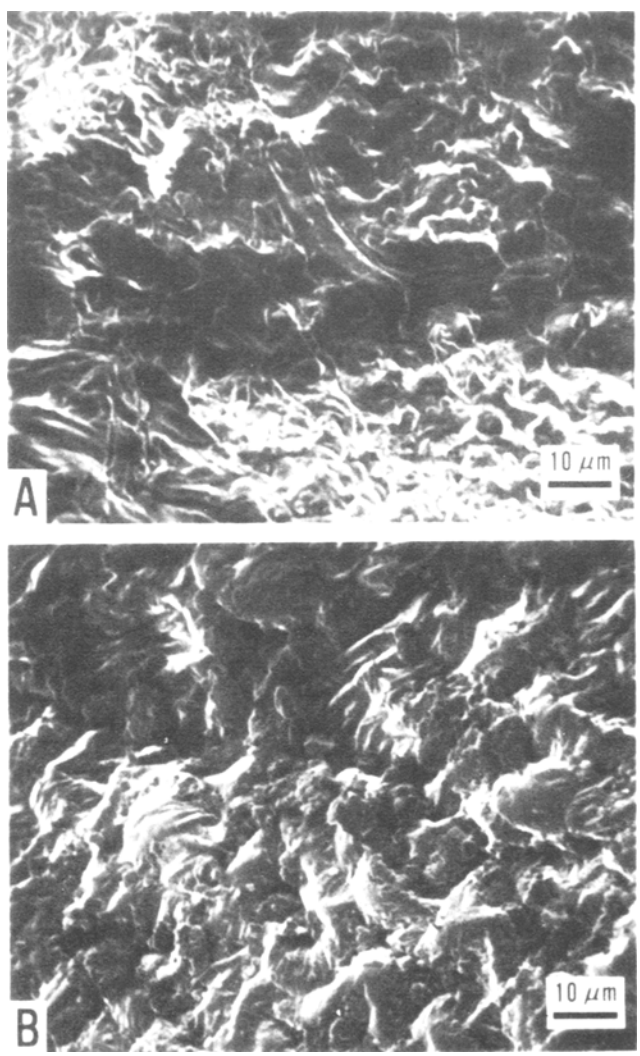

FIG. 4. Scanning electron micrograph of (a) full-fat cracked soybean, and (b) SC-CO_2-extracted cracked soybean.

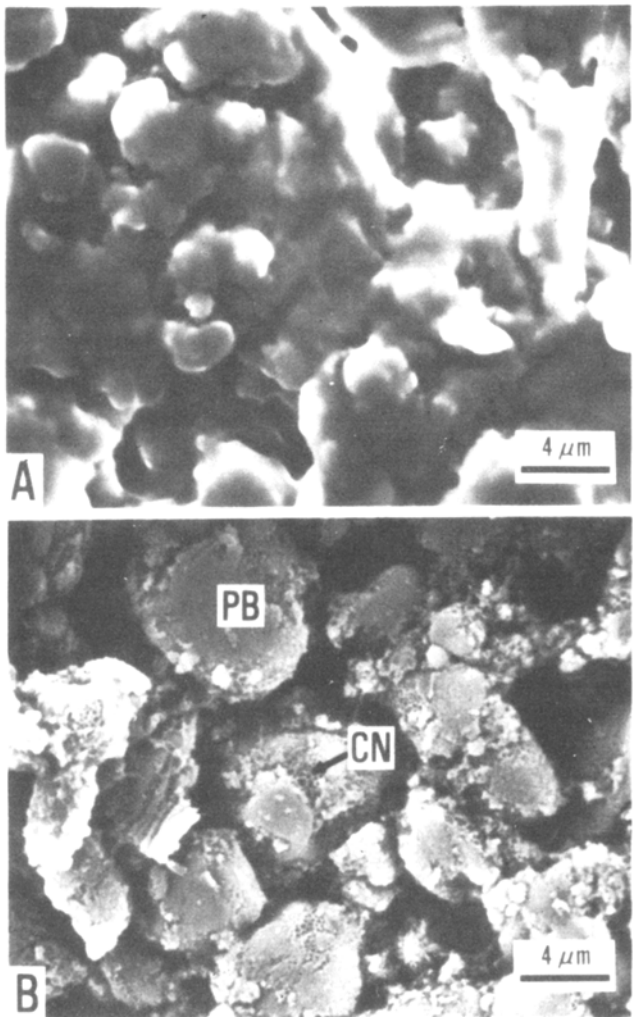

FIG. 5. Scanning electron micrographs of (a) full-fat soyflakes, and (b) SC-CO_2-extracted soy flakes. PB=protein body. CN=cytoplasmic network.

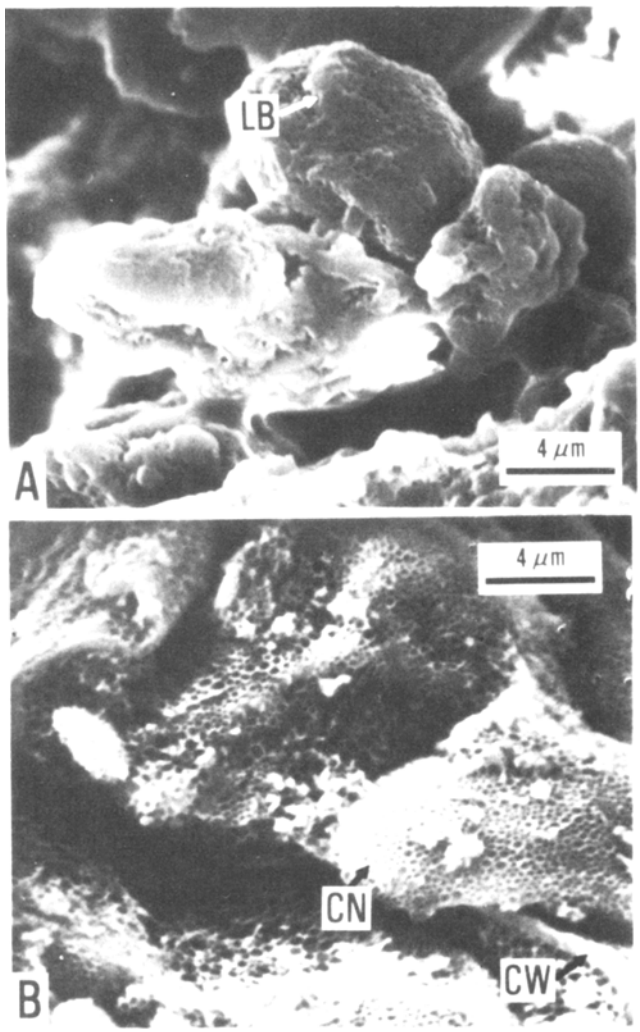

FIG. 6. Scanning electron micrographs of (a) full-fat soy flour, and (b) SC-CO_2-extracted soy flour. LB=lipid body. CN=cytoplasmic network. CW=cell wall.

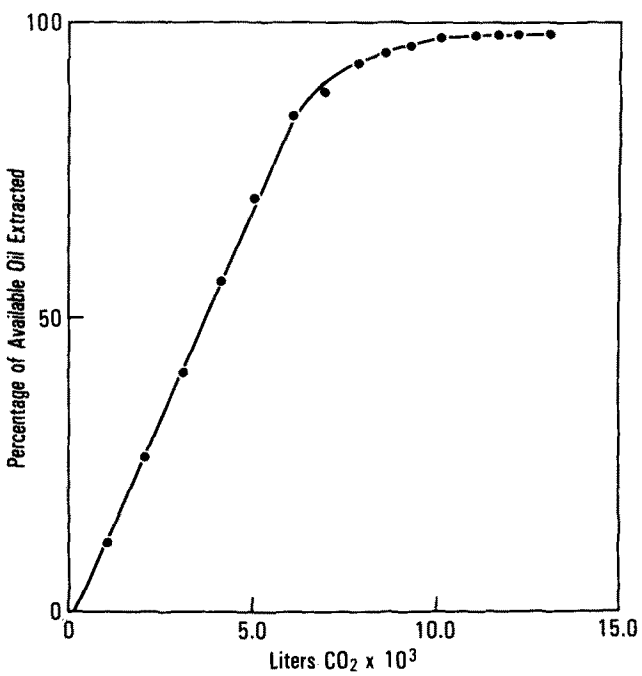

FIG. 7. SC-CO_2 extraction of cottonseed flakes. Conditions: 8000 psig; 50 C. Available oil, 340 g.

indicated by the composition change in the final 10-15% of the oil extracted, shown in Figure 3; linoleate, linolenate and palmitate decreased while oleate and stearate increased.

Surface structures of the different soybean particle sizes were examined with the scanning electron microscope (Figs. 4-6). When the soybeans were cracked mechanically, some protein bodies and other cellular contents were exposed, but most of the cell walls were intact (Fig. 4a). Little change was observed in the appearance of the extracted cracked bean (Fig 4b). The poor oil yield shown in Table I probably is derived from the small amount of surface oil exposed during the cracking process.

When the cracked beans were flaked, cell walls were disrupted, exposing more lipid bodies (Fig. 5); this exposure greatly improved the rate of extraction and oil yield. Lipid bodies in full-fat flakes (Fig. 5a) were full of oil, and definition of cell material was not clear in the micrograph. When the oil was removed from the flake by extraction with SC-CO_2 (Fig. 5b), the cytoplasmic network covering the protein bodies could be recognized. The SC-CO_2 -extracted flakes were similar to hexane-extracted flakes. Oil was not transported through the unbroken cell walls, and only surface oil was removed.

Grinding the beans to a finer flour caused cellular content to be fractured to a greater extent. Intact protein bodies were still present, and swollen lipid bodies were

numerous in the full-fat flour shown in Figure 6a. After extraction of lipid bodies, cytoplasmic network on the protein bodies was well defined and the flour had a honeycomb appearance (Fig. 6b). The micrograph of the SC-CO_2-extracted soy flour resembled that of hexane-extracted soy flour (8).

Extraction plots of cottonseed flakes (Fig 7) and peanut flakes (Fig. 8) showed that satisfactory extraction was achieved. Extraction times were longer because of the high oil content (30% oil in cottonseed and 50% in peanut). Oil solubility from the cottonseed extractions was 2.5%,

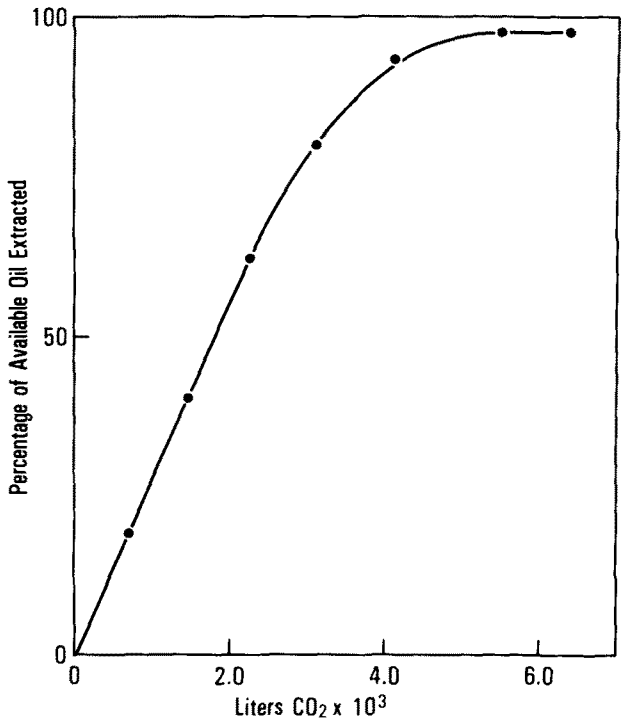

FIG. 8. SC-CO_2 extraction of peanut flakes. Conditions: 10,000 psig; 70 C. Available oil, 870 g.

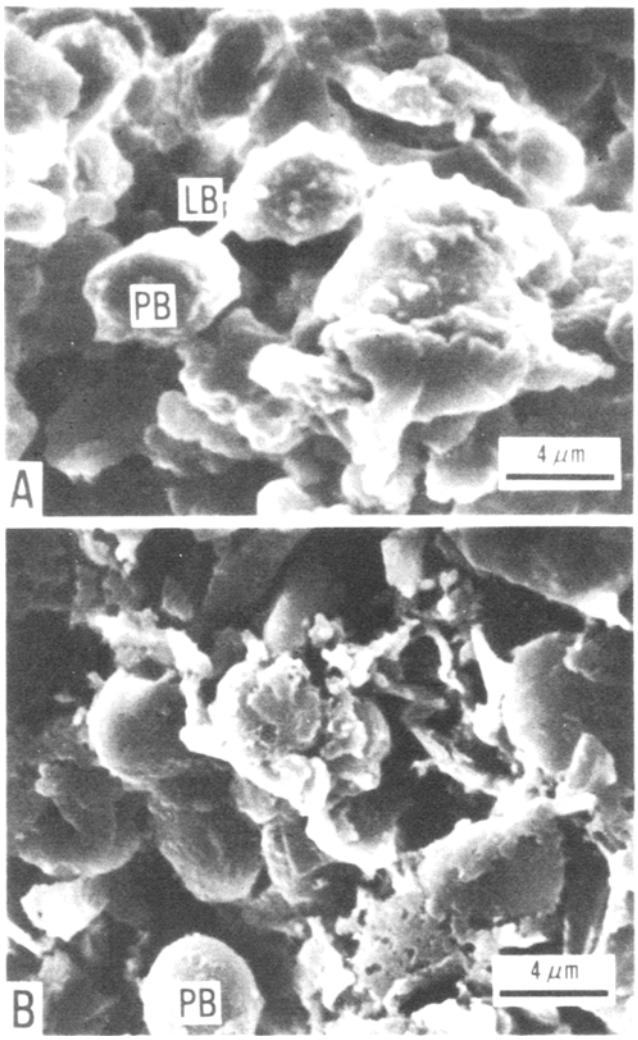

FIG. 9. Scanning electron micrographs of (a) full-fat cottonseed flakes, and (b) SC-CO_2-extracted flakes. LB=lipid body. PB=protein body.

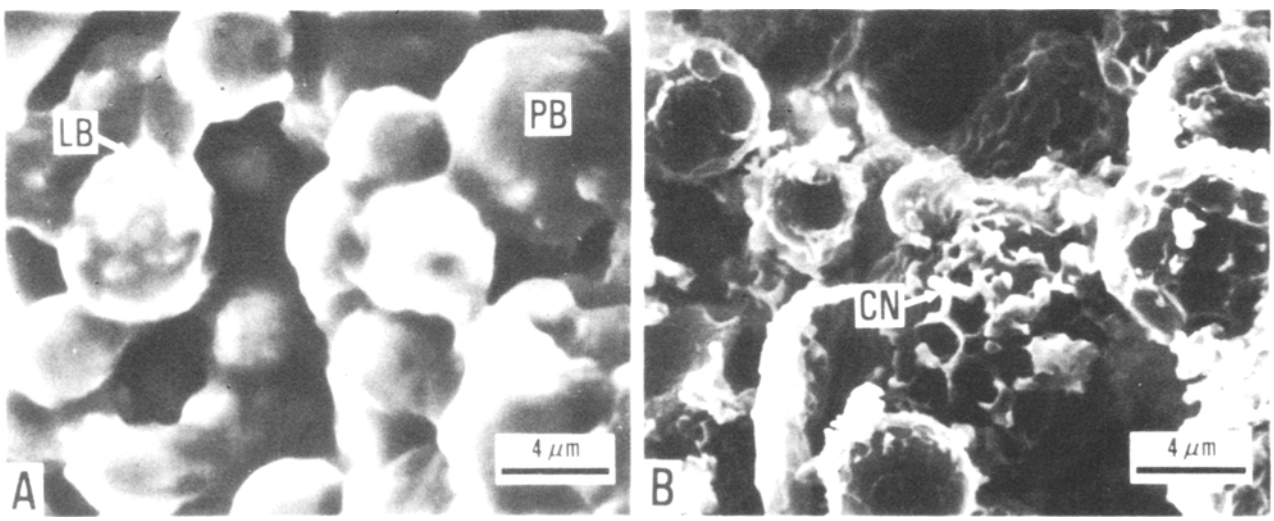

FIG. 10. Scanning electron micrograph of (a) full-fat peanut flakes, and (b) SC-CO_2-extracted peanut. LB=lipid body. PB=protein body. CN= cytoplasmic network.

comparable to soybean flakes at the same conditions of 8000 pounds pressure at 50 C. Peanut oil was extracted at 10,000 pounds pressure and 70 C to decrease the extraction time; solubility at these conditions was 5.5%.

Cottonseed flakes were examined before and after extraction with SC-CO_2 (Fig. 9). Cottonseed particulates had a wide size-distribution from 0.1 to 400 microns; numerous protein bodies were smaller than soybean protein bodies. The cytoplasmic membranes of lipid bodies are not evident after extraction (Fig. 9b) because of the small size of the lipid bodies.

The morphology of the full-fat peanut flakes was not easily recognized due to the large amount of oil present (Fig. 10a). After extraction with SC-CO_2, lipid bodies being larger in peanut than in cottonseed or soybean enabled a clearer definition of the material. Organization of the cytoplasmic network around the protein bodies was especially visible in the extracted peanut flakes because of the large size of the protein bodies (Fig. 10b). Oil yield was greater than 95%.

The results reported here indicate that the most efficient SC-CO_2 extraction is achieved with the flaked oilseed. There appears to be no significant difference in the morphology of the defatted meal when extracted by either SC-CO_2 or hexane.

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⁂Studies on the Production of Lipids in Fungi XIII. Changes of Amount and Composition of Lipids in Fungi in Species of the Genus *Pellicularia* from Cellulose by Cultural Conditions

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ABSTRACT

The influence of growth temperature, carbon to nitrogen (C/N) ratio of the medium and the nitrogen source on the cell and lipid formation from cellulose by the species *filamentosa* and *praticola* of the genus *Pellicularia* were investigated. The strains of the *Pellicularia* genus fungi can be grown well utilizing powdery cellulose and sugar cane bagasse as the carbon source. The amount of lipids accumulated in the mycelium varied considerably depending on the difference in the cell growth associated with the cultivation condition, and the difference in the strain, C/N ratio and nitrogen source. The maximum accumulation of lipids in the mycelium (256 mg/400 ml of the medium) from cellulose was observed at 20 C with a C/N ratio of 5.7 using potassium nitrate as the nitrogen source for *Pellicularia filamentosa* var. *solani* IFO 5879. Protein formation in the liquid medium is at its peak when the cell growth is at its maximum. The fatty acid compositions of the neutral and polar lipid fractions also were determined. Linoleic acid is the major fatty acid component of both fractions. The change in the total lipid content is less than 10% under any cultivation condition.

INTRODUCTION

Many studies have been reported on the production of liquid fuels and raw materials for chemical industries from biomass (1). Foremost is the action of microorganisms on indigenous raw materials. In these studies, the saccharide solution formed from the breakdown of cellulosic material was used mostly for cultivation and fermentation. However, little has been reported on the production of unicellular protein and of other cellular components, particularly lipids, by using cellulosic materials directly.

The cultivation factors affecting the lipid formation from glucose and changes in the lipid composition of the genus *Pellicularia* in Basidiomycetes have been studied previously (2). It was found that the lipids in *Pellicularia* genus fungi grown on glucose had a high linoleic acid content (3.0). The fungi of the genus *Pellicularia* are grown aerobically on cellulosic materials. In order to obtain a complete picture of what is happening to the cell growth and the lipids of *Pellicularia* genus fungi cultured by cellulose, the amount of the cells and lipids and the change in the lipid component of the mycelium grown by different culture conditions were investigated in this study. The cells and lipids obtained from sugar cane bagasse as the carbon source also were included in this investigation.

MATERIALS AND METHODS

Microorganisms and Culture Conditions

Strains of cellulose utilizing fungi, *Pellicularia filamentosa*