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.

ACKNOWLEDGMENTS

J. Johnson helped with the extractions and chemical analyses and F.L. Baker the scanning electron micrographs.

REFERENCES

1. Zosel, K., German Auslegeschr. 1 493 190 (1964).
2. Vitzthum, O.G., and P. Hubert, German Offen. 2 127 596 (1972).
3. Stahl, E., and W. Schilz, Chem. -ing. Tech. 48:773 (1976).
4. Brunner, G., and S. Peter, Ibid 53:529 (1982).
5. Friedrich, J.P.; G.R. List and A.J. Heakin, JAOCS 59:288 (1982).
6. Stahl, E.; E. Schütz and H.K. Mangold, J. Agric. Food Chem. 28:1153 (1980).
7. American Oil Chemists' Society, "Official and Tentative Methods," 3rd ed., Link, W.E. (ed.), Champaign, IL, 1975.
8. Wolf, W.J., and F.L. Baker, Cereal Sci. Today 17:125 (1972).
9. Othmer, D.F., and J.C. Agarwal, Chem Eng. Prog. 51:372 (1955).
10. Christianson, D.D.; J.P. Friedrich, G.R. List, K. Warner, E.B. Bagley, A.C. Stringfellow and G.E. Inglett, J. Food Sci. 49:229 (1984).
11. Stahl, E.; K.W. Quirin and H.K. Mangold, Fette Seif. Anstrich. 83:472 (1981).

[Received May 24, 1984]

♣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

OSAMU SUZUKI, TOSHIHIRO YOKOCHI and **SATOSHI NAKASATO,** National Chemical Laboratory for Industry, 1-1, Higashi, Yatabe-cho, Tsukuba-gun, Ibaraki 305 Japan, and **CECILE Q. CARO,** National Institute of Science and Technology/NSTA, Pedro Gil Street, Ermita, Manila 2801 Philippines

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*

var. *solani* IFO 5879, *Pellicularia filamentosa* var. *sasakii* IFO 8985 and *Pellicularia praticola* IFO 6253 obtained from the culture collection of the Institute of Fermetation, Osaka, Japan, were used throughout the study. The fungi were maintained on yeast-extract, malt-extract agar (YMA) medium. The liquid culture (in g/l contained various amounts of ammonium nitrate [6.0; C/N ratio (ratio of carbon atom weight in added carbon source to nitrogen atom weight in added nitrogen source) of 5.7, 3.0; C/N ratio of 11.4, 0.3; C/N ratio of 114] or 4.5 of urea, 6.2 of ammonium sulfate, 8.0 of ammonium chloride, 12.7 of sodium nitrate and 15.2 of potassium nitrate at C/N ratio of 5.7, respectively, as the nitrogen source; KH_2PO_4 3.0; $MgSO_4 \cdot 7H_2O$ 0.3; NaCl 0.1; $FeSO_4 \cdot 7H_2O$ 0.01; $CaCl_2 \cdot 2H_2O$ 0.01; $ZnSO_4 \cdot 7H_2O$ 0.001; $MnSO_4 \cdot 4H_2O$ 0.001; $CuSO_4 \cdot 5H_2O$ 0.0002; Thiamine HCl 0.0002; D - Biotin 0.00002 in addition to 30 g of powdery cellulose [Avicel (Tradename)] manufactured by Merck Ltd. or sugar cane bagasse. Initial pH was adjusted to 4.6. Cells were precultured aerobically for 2-3 days at 30 C in 500 ml erlenmeyer flasks containing 200 ml of the growth medium. Then 10 ml portions of the culture samples of the broth were transferred to 1000 ml flasks containing 400 ml of the culture medium. The main cultivation was performed on a rotary shaker (180 rpm) at 20 and 30 C. The series of tests concerning the effects of aging on cells and lipid formation and cellulose consumption were run at 30 C. Cultures were maintained at a uniform stir rate for 2, 3, 4, 5, 7, 8, 9, 10 and 16 days in each of the tests respectively. The 10-day cells grown in a stationary phase were harvested and separated from the medium by filtration. The fungal cells were then solvent extracted and analyzed for lipid composition.

Determination of Dry Cell Weight

The dry cell weight of the fungal cells formed using cellulose as the carbon source was not determined quantitatively. Difficulty was encountered because of powdery cellulose on the cells. Hence, the method for the determination of the dry cell weight in the mixture of the cells and cellulose was modified as described in Figure 1. The remarkable difference in the hydrolysis rate between cellulose and the fungal cells in 0.5N methanolic NaOH was applied for this method (Fig. 2).

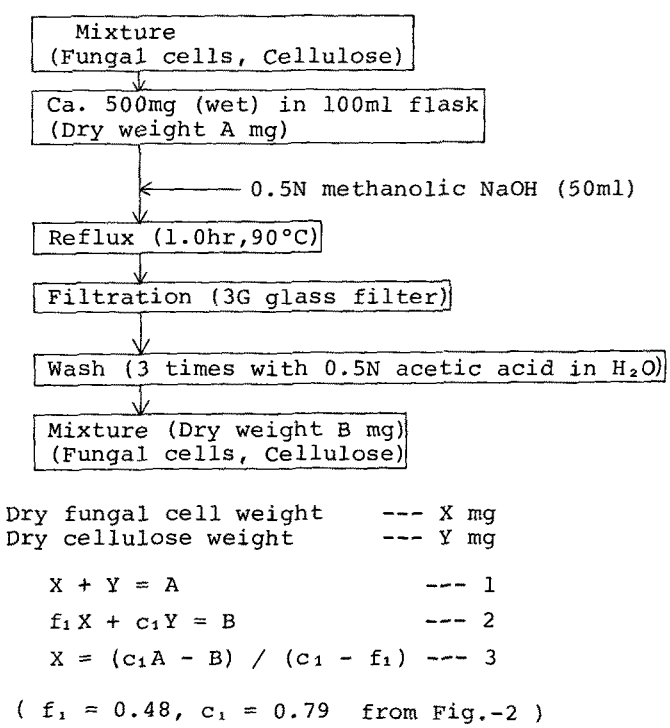

FIG. 1. Method for determination of dry weight of fungal cells formed from cellulose in mixture of cells and cellulose remained.

Extraction of Lipids

The extraction of lipids was done using a homogenizer in $CHCl_3$/MeOH (2:1, v/v) extraction solvent, a method very suitable for the complete extraction of the neutral and polar components of the fungal lipids. Glass beads were added for ease of homogenation. Five series of extractions were done on a sample to assure complete lipid separation (5). The total lipid content was measured gravimetrically. The extracted lipids were stored at 4 C on a $CHCl_3$ solution. The total lipid was fractionated to its neutral and polar lipid content by elution on a silicic acid column, initially with $CHCl_3$ and then with MeOH (6). After evaporation of eluants, the amount of each lipid was determined gravimetrically. Essentially, the entire lipid sample applied on the column was recovered into two fractions, that is, the neutral lipid eluted by $CHCl_3$ and the polar lipid eluted by MeOH.

Analytical Procedures

Neutral lipids were separated by thin layer chromatography (TLC) according to the method of Freeman and West (7) with a slight modification (8). Quantitative analysis of neutral lipid was performed by a densitometric method (using a Shimadzu densitometer, Model CS-910, with a zigzag scanning mode) (9). Polar lipids were separated by high performance liquid chromatography (HPLC), using a Shimadzu high performance liquid chromatograph Model LC-3A. The chromatography was performed on a system combined with two columns, Zorbax CN and ODS for good resolution of the polar lipid components using acetonitrile water-phosphoric acid (100:2:0.1 by volume) as the moving solvent. A complete separation and quantitation of polar lipids was achieved by this method using ergosterol as an internal standard (10). Fatty acid compositions of the total, neutral and polar lipids were analyzed as fatty acid methyl esters prepared by the BF_3/Methanol method after saponified with 0.5N methanolic KOH (11). A Shimadzu model GC-4CPF gas chromatograph equipped with a flame ioniza-

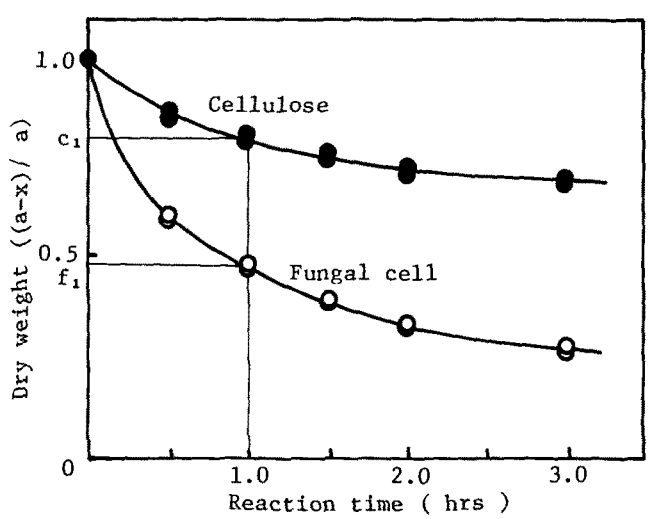

FIG. 2. Degradation curves of cellulose and fungal cell at 90 C by 0.5N methanolic NaOH.

tion detector was used in the separation of the fatty acid methyl esters. Stainless columns (200 × 0.3 cm) packed with 20% DEGS on chromosorb WAW were used in the dual system. Peaks were resolved and identified by comparing their retention times to those of authentic standards and quantified by a digital integrator.

The total amount of protein dissolved in the culture medium after filtration of the fungal cells was determined by the method of Lowrey (12). The amount of glucose formed from hydrolysis of cellulose in the culture medium also was determined, using a sugar analyzer (YSI Model-27).

RESULTS AND DISCUSSION

Effect of Age

The cell growth and cellulose consumption at 30 C with a C/N ratio of 11.4 using ammonium nitrate as a nitrogen source by *Pellicularia filamentosa* var. *solani* IFO 5879 is shown in Figure 3. A maximum dry cell weight formation of 1.5 g/400 ml of the medium was obtained after a 10-day incubation period. During culture aging, cellulose consumption was responsible for the dry cell weight increase.

Figure 4 shows the amount of total lipids and the neutral lipid (NL) and polar lipid (PL) components in the mycelium, and the protein and glucose content in the cultures determined at progressive stages of incubation. The cells proliferate on a logarithmic rate where the nitrogen source was first accumulated following an increase in the fungal lipid formation. The maximum accumulation of total lipids with a concentration of 110 mg/400 ml occurred after 4 days culture aging, when the total lipid to dry cell weight (TL/DC) content was 13%, neutral lipid to dry cell weight (NL/DC) was 7.5% and polar lipid to dry cell weight (PL/DC) was 5.5%. Results described stability of lipid accumulation from 5 to 16 days of culture aging. The protein in the liquid medium, presumed to be the enzymes activating for the hydrolysis of cellulose, was produced progressively after 2 to 5 days. After this log phase of the cell growth, the protein remained at a fairly constant concentration of ca. 160 mg/400 ml. On the other hand, the glucose concentration was at a constant level throughout the whole cultivation.

Effect of Incubation Temperature, C/N Ratio and Nitrogen Source

The effect of incubation temperature, C/N ratio of the medium and the nitrogen source on the fungal cell growth from cellulose of the *Pellicularia* genus fungi as to *P. filamentosa* var. *sasakii* IFO 8985, *P. f.* var. *solani* IFO 5879 and *Pellicularia praticola* IFO 6253 is tabulated in Table I. The strains were grown well using cellulose as the sole carbon source. A maximum dry cell weight of 3.6 g of mycelium/400 ml of the medium was obtained at 30 C with a C/N ratio of 5.7 for the strain IFO 8985. The amount of total lipids also was at its highest at this time, although the lipid content (TL/DC) was merely 5.1%. The decrease of the C/N ratio caused an alternate increase in the dry cell weight of the mycelium and the amount of the total lipids. This condition is not favorable for the IFO 5879 strain using ammonium nitrate as the nitrogen source. For the IFO 6253 strain, a 20 C culture temperature characteristically favored the cell growth at its maximum. It also was observed that, among the strains used, there was no significant difference in the total lipid content in the dry cells (TL/DC) as to the change of C/N ratio, that is reducing the NH_4SO_3 concentration from 6 g/l (C/N ratio of 5.7) to 0.3 g/l (C/N ratio of 114), at which the culture was the nitrogen limitation for the cell growth on cellulose, although a remarkable lipid accumulation in mycelium generally occurred under the nitrogen limitation (13).

The linoleic acid is the major fatty acid component of both the neutral and polar fractions of the lipid in any cultural conditions. The amount of protein produced in lipid culture is proportional to the cell growth and the lipid

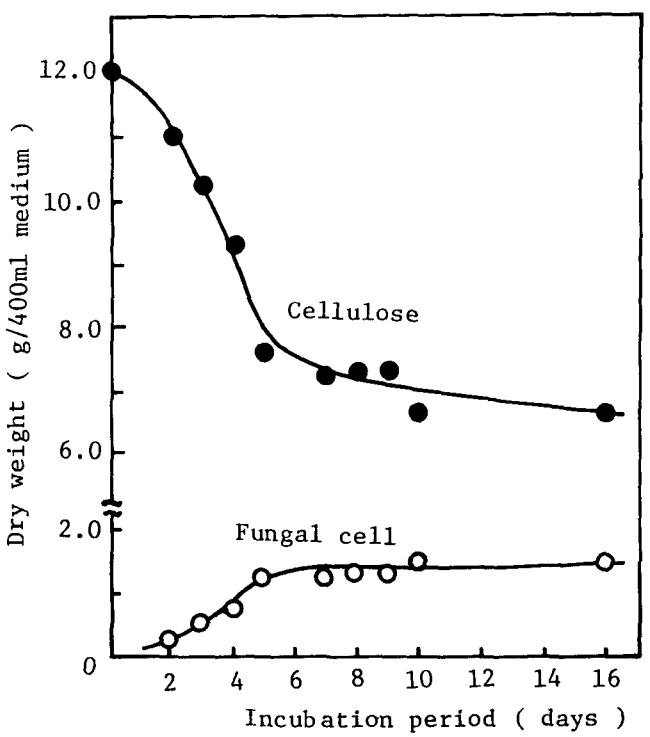

FIG. 3. The cell growth and cellulose consumption at 30 C in C/N ratio of 11.4 with NH_4NO_3 as a nitrogen source by *Pellicularia filamentosa* var. *solani* IFO 5879.

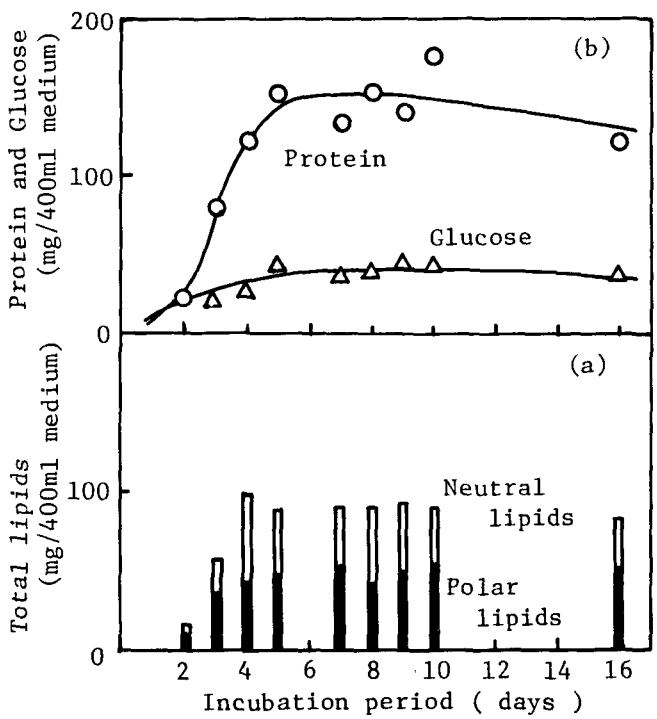

FIG. 4. Changes in the amounts of total lipids, neutral and polar lipids from *P. f.* var. *solani* IFO 5879 mycelium (a) and the protein and glucose contents in cultures (b) at progressive stages of incubation.

formation. The same is true with the glucose content of the medium.

The effect of nitrogen source on the cell and lipid formation from cellulose at 20 C with a 5.7 C/N ratio on the IFO 5879 strain is exemplified in Table I. The growth of the cell varied from 0.4 to 3.2 g using six different nitrogen sources: ammonium nitrate, ammonium sulfate, ammonium chloride, urea, sodium nitrate and potassium nitrate. Higher percentages of the dry cell weight were obtained using urea, sodium nitrate and potassium nitrate.

There was no significant difference in the total, neutral and polar lipid content in the dry cells. Values of 5.1%-9.4% for TL/DC, 2.5-6.8% for NL/DC and 1.4-4.4% for PL/DC were observed in the cells grown on several nitrogen sources. The maximum accumulation of lipids (256 mg/400 ml) occurred when the cells were grown with potassium nitrate at which 9.4% of the total lipid content was determined.

Neutral lipid composition of the strains in fungi genus *Pellicularia* grown on cellulose by different cultivation conditions is tabulated in Table II. Triglycerides comprise 65-85% of the neutral lipids and comparable remaining proportions of diglycerides, free fatty acids and free sterol. Significant differences in triglyceride formation were dependent on nitrogen source. Cells grown using ammonium nitrate, ammonium sulfate and ammonium chloride

TABLE I

Effect of Incubation Temperature, C/N Ratio and Nitrogen Source on the Cell and Lipid Formation from Cellulose by *Pellicularia filamentosa* var. *sasakii* IFO 8985, *P. f.* var. *solani* IFO 5879, *P. praticola* IFO 6253

IFO no.	Nitrogen source	C/N ratio	Temp. (C)	DC[a] (g/400 ml)	TL[b] (mg/400 ml)	TL/DC (%)	NL[c]/DC (%)	PL[d]/DC (%)	LA content[e] NL (%)	LA content[e] PL (%)	Protein[f] (mg/400 ml)	Glucose[g] (mg/400 ml)
8985	NH_4NO_3	5.7	20	2.9	119	4.2	1.5	2.7	81.1	85.2	313	83
			30	3.6	185	5.1	2.3	2.8	80.0	82.6	229	19
		11.4	20	1.6	93	5.8	2.1	3.7	77.5	85.4	275	64
			30	2.5	116	4.7	1.9	2.8	70.7	74.4	99	5
		114	20	0.8	40	3.6	1.5	2.1	74.7	82.6	53	9
			30	0.8	47	5.7	2.3	3.4	81.2	81.4	45	3
6253	NH_4NO_3	5.7	20	2.9	196	6.7	2.0	4.7	73.9	82.7	321	21
			30	0.4	36	9.5	4.1	4.5	64.7	80.3	27	15
		11.4	20	2.6	189	7.3	2.3	5.0	77.3	84.6	263	20
			30	1.1	63	5.6	1.5	4.1	72.4	84.1	101	13
5879	NH_4NO_3	5.7	20	0.5	48	9.4	5.3	4.1	60.1	60.3	76	52
	$(NH_4)_2SO_4$	5.7	20	0.4	26	6.3	2.5	3.8	68.2	71.7	74	19
	NH_4Cl	5.7	20	0.5	25	5.2	2.6	2.6	65.4	66.3	103	19
	$(NH_2)_2CO$	5.7	20	3.2	226	7.1	2.7	4.4	71.9	78.9	428	11
	$NaNO_3$	5.7	20	3.1	161	5.1	3.7	1.4	70.0	74.5	112	23
	KNO_3	5.7	20	2.7	256	9.4	6.8	2.6	74.7	77.4	291	52

[a]Dry cell weight.
[b]Total intracellular lipids.
[c]Neutral lipid fraction of intracellular lipids.
[d]Polar lipid fraction of intracellular lipids.
[e]Linoleic acid content in each lipid fraction.
[f]Amount of protein in culture medium after separation of cells.
[g]Amount of glucose in culture medium after separation of cells.

TABLE II

Neutral Lipid Compositions in *Pellicularia* Genus Fungi, 3 Strains, Incubated at Different Cultural Conditions Using Cellulose as a Carbon Source

IFO no.	Nitrogen source	C/N ratio	Temp. (%)	NL/DC (%)	Neutral lipid (% of total) SE	TG	1,2-DG	1,3-DG	FA	FS	MG
8985	NH_4NO_3	5.7	20	1.5	3.7	65.6	2.8	13.1	5.5	9.0	0.0
			30	2.3	8.4	65.2	4.9	12.8	1.5	4.6	2.0
		114	20	1.5	4.2	42.6	7.5	19.0	10.1	12.2	3.8
			30	2.3	7.0	61.4	5.9	13.5	3.5	5.3	3.1
6253	NH_4NO_3	5.7	20	2.0	2.1	62.3	5.5	12.3	6.9	10.0	0.3
5879	NH_4NO_3	5.7	20	5.4	0.8	19.7	9.9	25.0	28.5	10.1	5.3
	$(NH_4)_2SO_4$	5.7	20	2.5	0.7	26.2	4.5	22.3	20.4	6.1	18.9
	NH_4Cl	5.7	20	2.6	1.0	31.0	8.7	28.3	19.0	11.5	0.0
	$(NH_2)_2CO$	5.7	20	2.7	3.3	56.8	3.5	19.0	2.5	13.4	0.0
	$NaNO_3$	5.7	20	3.7	5.1	62.7	2.9	14.6	5.4	8.9	0.0
	KNO_3	5.7	20	6.8	2.2	54.2	4.5	18.0	4.0	15.4	0.0

SE = Sterol ester; TG = Triglyceride; DG = Diglyceride; FA = Free fatty acid; FS = Free sterol; MG = Monoglyceride.

caused a decrease of triglyceride content, that is 19-31%, and an increase in the ratio of other components. Cells grown utilizing urea, sodium nitrate and potassium nitrate as nitrogen source caused an increase in the ratio of triglycerides and a decrease in the ratio of other components. Triglycerides in the neutral lipid varied considerably depending on the cells and the lipid formation.

Analyses of the polar lipid composition in fungi genus *Pellicularia* showed no quantitative change among the cells in different strains and among those grown under different culture conditions. Table III illustrates the polar lipid composition of the strains incubated at different culture conditions using cellulose as the carbon source. Phospholipids are the major substance of the polar lipids. Of the total polar lipids (TPL), phosphatidylcholine (PC) comprises 27-39%, phosphatidylethanolamine (PE) 12-22%, phosphatidylserine (PS) 3-7% and phosphatidylinositol (PI) 8-13%. As for the total phospholipids, PC comprises 43-59%, PE 21-29%, PS 4-9% and PI 12-18%. The HPLC analysis also detected minor phospholipids such as lysophosphatidylcholine (LPC) 0.4% of TPL, lysophosphatidylethanolamine (LPE) 0.2% of TPL, and glycolipids containing phosphorous (p-GL) 3-14% of TPL and glycolipid (GL) 3-11% of TPL. TLC analysis spotted trace amounts of phosphatidylglycerol (PG) and the diphosphatidylglycerol (DPG).

Polar lipid composition was unaffected by the C/N ratio of the liquid medium, but an increase of growth temperature from 20 to 30 C led to a substantial decrease in phospholipids, an increase in the PC content and a decrease in the PE and the PS.

Cells and Lipid Formation from Sugar Cane Bagasse

The cell growth and lipid formation utilizing the sugar cane bagasse as a substrate on *Pellicularia* genus fungi was investigated for practical use of the strains. The physical and chemical properties of the sugar cane bagasse are listed in Table IV.

The results obtained by growing *P. f.* var. *sasakii* IFO 8985 from sugar cane bagasse at different cultivation conditions are summarized in Table V. The strain was observed to grow well using sugar cane bagasse as a substrate and using urea as the nitrogen source where the maximum dry cell weight of 2.0 g/400 ml was obtained. Extractable total lipids (ETL) from the mycelium of the fungus and from the remaining unused bagasse substrate in the medium

TABLE IV

Physical Properties and Chemical Composition of Sugar Cane Bagasse used as a Substrate

Grain size	120> mesh
Yield from bagasse	19.7%
Moisture content	15.7%
Ash	5.5%
Alcohol:Benzene extractable matter	2.9%
Pentosan	23.4%
Lignin	20.0%
Holo cellulose	73.3%
Crystallization index (X-ray method)	69.8%

TABLE V

Effect of Incubation Temperature and Nitrogen Source on Cell and Lipid Formation from Sugar Cane Bagasse by *Pellicularia filamentosa* var. *sasakii* IFO 8985

Nitrogen source	Conc. N[a] (g/l)	Temp. (C)	DW[b] (g)	DC (g)	ETL[c] (mg)	LA[d] (%)
$(NH_2)_2CO$	4.5	20	6.4	2.0	236	81.5
		30	6.4	2.0	254	75.2
$(NH_2)_2SO_4$	6.2	20	9.6	0.8	203	76.4
		30	9.5	0.9	210	74.5
NH_4NO_3	6.0	20	9.4	1.0	242	75.4
		30	8.6	1.2	209	77.3
Control		30	11.7	–	151	22.5

[a]Concentration of N-source.
[b]Dry weight of sugar cane bagasse remaining in 400 ml of culture medium.
[c]Extractable total lipids in cells grown from sugar cane bagasse and in the bagasse remaining in 400 ml of culture medium.
[d]Linoleic acid content in ETL.

TABLE III

Polar Lipid Compositions in *Pellicularia* Genus Fungi, 3 Strains, Incubated at Different Cultural Conditions Using Cellullose as a Carbon Source

IFO no.	C/N ratio	Temp. (C)	Polar lipid (%) PC	PE	PS	PI	LPC	LPE	p-GL	GL	TPL (%)	TPh.L (%)
8985	5.7	20	39.2	22.1	4.6	10.4	3.0	0.6	5.1	5.7	90.7	79.9
			49.1	27.7	5.8	13.0	3.8	0.8				100
		30	32.3	13.0	3.2	10.6	0.3	0.3	5.3	5.6	70.9	59.7
			54.1	21.8	5.4	17.8	0.5	0.5				100
	114	20	37.3	19.6	7.3	13.2	Tr	1.9	9.0	8.4	96.7	79.3
			47.2	24.8	9.2	16.7	Tr	2.4				100
		30	35.8	13.8	3.6	9.2	0.7	0.2	3.3	6.4	73.0	63.3
			56.6	21.8	5.7	14.5	1.1	0.3				100
6253	5.7	20	42.9	21.8	6.5	10.4	Tr	0.6	6.4	8.5	97.1	82.2
			52.2	26.5	7.9	12.7	Tr	0.7				100
5879	5.7	20	27.6	18.6	5.8	11.4	0.2	0.7	13.7	10.9	88.9	64.3
			42.9	28.9	9.0	17.7	0.3	1.1				100

PC = Phosphatidylcholine; PE = Phosphatidylethanolamine; PS = Phosphatidylserine; PI = Phosphatidylinositol; LPC = Lysophosphatidylcholine; LPE = Lysophosphatidylethanolamine; p-GL = Glycolipid containing phosphorus; GL = Glycolipid; TPL = % of total polar lipids detected by HPLC analysis; TPh.L = % of total phospholipids detected by HPLC analysis; Tr = Trace (0.05>).

were not variable depending on the difference of the culture conditions (203-254 mg/400 ml). A higher level of linoleic acid was extracted from ETL grown on cellulose (74.5-81.5%), compared with 22.5% linoleic acid in ETL using sugar cane bagasse medium under any cultivation conditions.

ACKNOWLEDGMENT

S. Yokoyama, National Chemical Laboratory for Industry, provided advice on these experiments.

REFERENCES

1. Koyama, M., Yukagaku, 30:354 (1981).
2. Suzuki, O., T. Yokochi and T. Yamashina, Ibid., 31:494 (1982).
3. Suzuki, O., T. Yokochi and T. Yamashina, Ibid., 30:869 (1981).
4. Suzuki, O., Y. Jigami, S. Nakasato and T. Hashimoto, US Patent 4,281,064.
5. Suzuki, O., Y. Jigami and S. Nakasato, Agric. Biol. Chem. 43: 1343 (1979).
6. Vance, D.E., and C.C. Sweeley, J. Lipid Res., 8:621 (1967).
7. Freeman, C.P., and D. West, Ibid., 7:324 (1966).
8. Jigami, Y., O. Suzuki and S. Nakasato, Lipids, 14:937 (1979).
9. Jigami, Y., O. Suzuki and S. Nakasato, Yukagaku, 28:867 (1979).
10. Yokochi, T., and O. Suzuki, Ibid., 31:580 (1982).
11. Metcalf, L.D., and A.A. Schmitz, Anal. Chem., 33:363 (1961).
12. Lowrey, O.H., et al., J. Biol. Chem. 193:265 (1951).
13. Suzuki, O., T. Yokochi and T. Yamashina, Yukagaku, 31:921 (1982).

[Received May 8, 1984]

♣Sensory Characteristics and Oxidative Stability of Soybean Oil and Flour Extracted with Aqueous Isopropyl Alcohol

K. WARNER and **E.C. BAKER**, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604

ABSTRACT

Soybean flakes extracted with hexane or aqueous isopropyl alcohol (85%, 87.7% and 90.5% IPA by weight) were processed to toasted flours and the miscellas to refined soybean oils. These products were evaluated for sensory characterisitics and oxidative stability. Sensory analyses of initial oils and flours indicated good quality products. Initial flavor scores of IPA-extracted oils and flours were not significantly different from those of hexane-extracted oil and flour. Flour samples aged at 49 C for 1 mo and 37 C for 3 mo were rated slightly lower in flavor score than the initial flours. Flavor scores of oils decreased after aging but remained acceptable. Oils extracted with aqueous IPA concentrations of 85% and 90.5% received significantly lower scores than oils extracted with hexane or 87.7% IPA after 8 hr of fluorescent light exposure. Oxidative stability measured by the induction of weight increases of the oils during aging was similar. Residual solvent flavors were slightly detectable in unaged IPA flours and in those aged 3 mo at 37 C.

INTRODUCTION

In the extraction of oil from soybeans and other oilseeds, hexane is used almost exclusively as the solvent in this country. Hexane is an excellent solvent for such extractions. However, concerns about availability, flammability and toxicity have stimulated interest in alternative extraction solvents. Some of the solvent systems examined include alcohols (1), water (2), halogenated hydrocarbons (3) and supercritical carbon dioxide (4).

Extraction of soybeans with ethanol was evaluated at the Northern Regional Research Center in the mid-1940's. Low solubility of oil in 95% ethanol at ambient temperature required the extraction to be carried out under slight pressure to raise the temperature above 90 C, where the oil and ethanol are completely miscible. Isopropanol has better solvent properties than ethanol for the extraction of oilseeds. Recently, Shell Development Company (5) and NRRC scientists (6) have developed a pilot-plant process for extracting soybeans with isopropanol. The purpose of this study was to evaluate the sensory properties of the oil and meal products prepared by this process.

MATERIALS AND METHODS

Soybean flakes were extracted with hexane or aqueous isopropyl alcohol at concentrations of 85%, 87.7% and 90.5% IPA by weight, with a solvent-to-meal ratio of 2:1. The complete process of extraction, oil refining and meal desolventizing was presented in detail by Baker and Sullivan (6). Characteristics of the crude and refined oils such as free fatty acids and metal content were included in that paper, as was information on the desolventized meal such as residual alcohol, nitrogen solubility index and trypsin inhibitor content. The oils contain 0.01% citric acid added on the cooling side of deodorization.

Oils were aged for 8 days at 60 C or for 8 hr of fluorescent light exposure. For the storage tests, 8-oz narrow-mouth clear glass bottles were filled 2/3 with oil and loosely stoppered (air in the headspace) with cellophane-covered corks. The 60 C storage samples were aged in a forced draft oven in the dark for 8 days. For the fluorescent light exposure test, the bottles were placed on a revolving platform in the middle of a 17.5-in. diameter stainless steel drum, 17.5 in. high, which contained six 15-in., 14-watt daylight fluorescent bulbs mounted on the perimeter (7). The light intensity was 7,535 lux or 800 ft candles.

Soy flours were evaluated for flavor initially and after aging at 49 C for 1 mo or at 37 C for 3 mo. Flours were packaged in 4-oz wide-mouth clear glass bottles and sealed with screw-cap closures. Packaging was done with air in the headspace.

Sensory Evaluation

Oils were evaluated for flavor by a trained, experienced 15-member panel. Each tester was given 10 ml of oil maintained at 50 C in a 50-ml clear glass beaker covered with a watch glass. Overall flavor intensity of each oil was rated on a 1-10 scale, with 10 as bland and 1 as extreme intensity. Panelists also described the predominant flavors detected and rated the intensity of each description on a