Fatty Acid Profiles of Some Cultured and Wild Yeasts in Brewery[1]

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ABSTRACT

A simple, rapid, microscale, gas chromatographic method was developed for the determination of fatty acids in yeast. The method includes a simplified procedure for extraction and saponification, the use of a direct pyrolytic methylation technique, and the use of a high resolution wall-coated open tubular column. A total of 19 strains of cultured, wild, and alcohol-tolerant yeasts previously grown in brewer's wort at 20°C were analyzed. Chromatographic and quantitative profiles for each strain of yeast were established. Apart from some subtle differences among the yeasts, three major distinctions were apparent: 1) Cultured yeasts contained more saturated fatty acids, among which palmitic acid was the most abundant. On the other hand, wild and alcohol-tolerant yeasts contained more unsaturated fatty acids, among which palmitoleic acid was usually the most abundant. 2) The ratio of stearic to oleic acid was greater than 1 for cultured yeasts but smaller than 1 for the other yeasts. 3) Some additional fatty acids hitherto unidentified were present in most of the wild and alcohol-tolerant yeasts. Differentiating or identifying brewery yeasts by analyzing their fatty acid composition would therefore appear possible.

Key Words: *Cultured and wild yeasts, Fatty acid profile, High resolution gas chromatography, Pyrolytic methylation, Yeast differentiation*

One of the approaches to differentiating microorganisms is to analyze their major cellular constituents or metabolic products. The feasibility of such an approach was first demonstrated by Abel et al (1) in 1963, utilizing gas-liquid chromatography (GLC). With the development of new analytical methodology and advances in instrumentation, this chemical approach has become more and more successful. For example, in clinical research, fatty acid analysis by GLC has proved to be a valuable means for rapid identification of a variety of bacteria and yeast fungi (7,17,19). In the baker's yeast industry, application of GLC was proposed to determine wild yeast contamination by lipid analysis (2).

Providing that sufficient inherent biological differences are present, the keys to the success of yeast classification are techniques for extraction and derivatization of some specific cellular constituents and subsequent chemical analysis. Martinez et al (16) used a two-phase silylation (with trimethylsilylimidazole) technique to generate chromatographic profiles for identification of yeast genera. The technique appears to be rapid and simple, but the resultant products are largely undefined and unselective because the silylation reagent reacts with all hydroxy compounds, such as carbohydrates, steroids, alcohols, phenols, organic acids, and nucleotides.

The most attractive method for derivatization of saponifiable fatty acids in small biological samples is a process called pyrolytic methylation (11,15). The process includes two sequential steps: 1) the formation of a quaternary *N*-methylammonium salt by deprotonation of the fatty acid with a strongly basic quaternary *N*-methylammonium hydroxide, and 2) thermal degradation of the salt in the heated injector of a chromatograph, to yield the volatile methyl derivative of the acid and a tertiary amine. Using stearic acid and (*m*-trifluoromethylphenyl) trimethylammonium hydroxide as an example, the mechanism of the process is illustrated in Fig. 1. The outstanding feature of the procedure is a great reduction in sample manipulation (washing, drying, evaporating, transferring, etc.), compared to that in the conventional methods for preparation of methyl esters; substantial saving in time and better accuracy can therefore be achieved.

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Recent availability of flexible glass capillary columns represents a major breakthrough in GLC column technology. The advantages of the wall-coated open tubular column over the conventional packed column are great separation efficiency, high precision, and significant reduction in analysis time. Application of a capillary column for analysis of fatty acids in biological specimens and a variety of foodstuffs has been highly successful (9,14,18).

The purposes of the present study were twofold: 1) to develop a microanalytical method for determination of the total saponifiable fatty acids of yeast by using the direct pyrolytic methylation technique and high resolution gas chromatography with capillary column, and 2) to provide some quantitative as well as chromatographic profiles of yeast fatty acids, so that the detection and differentiation of brewery-cultured and wild yeasts might be facilitated.

MATERIALS AND METHODS

Yeast Cultivation

Yeast cultures were obtained from the yeast culture collection, Molson Breweries of Canada Ltd., and from W. M. Ingledew, University of Saskatchewan, Saskatoon, Canada. The identities and characteristics of some of the yeasts are listed in Table 1. All yeast cultures were cultivated in sterile hopped wort (Molson "Export," adjusted to 12° P) at 20° C. For anaerobic growth, wort contained in a 3-L distillation flask was first purged with nitrogen, and, after inoculation, the flask was placed in a GasPak 150 system (Becton, Dickinson, and Co., Cockeysville, MD). The anaerobic chamber, in turn, was placed in a Controlled Environment Incubator-Shaker (New Brunswick Scientific Co., Inc., Edison, NJ) set at 20° C with slow-speed shaking. Yeast cells were harvested by centrifugation and washed twice with sterile distilled water. The cleaned yeast was freeze-dried immediately. The lyophilized yeast was ground in a mortar and then kept in a freezer until analysis.

Fatty Acid Extraction

About 100 mg of freeze-dried yeast powder was weighed exactly in a 50-ml centrifuge tube (29 × 123 mm, screw-cap, lined with Tuf-Bond Teflon/silicone disks, 22-mm diameter, obtained from

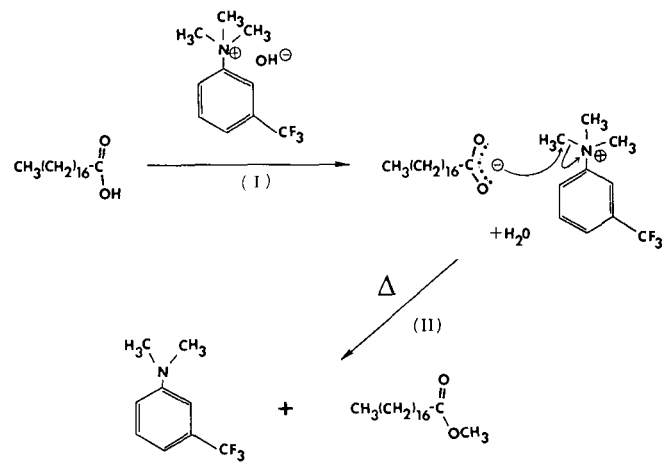

Fig. 1. The mechanism of pyrolytic methylation of stearic acid with (*m*-trifluoromethylphenyl) trimethylammonium hydroxide.

TABLE I
Yeasts Used for Fatty Acid Analysis

Yeast Species/Strain	Sample Code	Source and Remarks
Brewing		
Saccharomyces cerevisiae	1	MBCL,[a] A-47, ale
S. uvarum	2	MBCL, L-5, lager
Wild		
S. diastaticus	3	MBCL, Y-41
S. ellipsoideus	4	MBCL, Y-42
S. pastorianus	5	MBCL, Y-40
S. willianus	6	MBCL, Y-39
Hansenula anomala	7	MBCL, Y-26
Kloeckera apiculata NCYC 328	8	UOS[b]
Candida utilis	9	MBCL, Y-54
Torulopsis colliculosa	10	MBCL, Y-23
Pichia membranaefaciens	11	MBCL, Y-38
Brettanomyces anomalus NCYC 449	12	UOS
B. bruxellensis NCYC 362	13	UOS
Alcohol-Tolerant		
S. cerevisiae ATCC 42858	14	UOS, tolerates 11% ethanol
S. cerevisiae ATCC 26603	15	UOS, ferments to 12.2% ethanol
S. cerevisiae var. *bobadella*	16	MBCL, Y-14, wine yeast
S. sake IFO 2347	17	UOS, produces up to 23% ethanol in proteolipid-supplemented medium
S. uvarum IFO 0205	18	UOS, produces up to 20% ethanol in proteolipid-supplemented medium
S. uvarum ATCC 26602	19	UOS, tolerates 13.3% ethanol

[a] Molson Breweries of Canada Ltd.
[b] University of Saskatchewan.

Chromatographic Specialties Ltd., Brockville, Ontario). Two hundred microliters of pentadecanoic acid solution (0.2 mg/ml of ethanol) was added as internal standard. Also added was 25 ml of an equal-volume mixture of KOH (1*N*) and methanol. A micromagnetic stirring bar (Teflon-coated, 3 × 10 mm) was placed in the tube, which was then capped and fitted into the well of an aluminum heating module (Pierce Chemical Co., Rockford, IL). The extraction and saponification were carried out at 100°C for 30 min with occasional stirring. (Because the tightly capped tube is pressurized at an elevated temperature, heating and stirring should be done with care and preferably in a fume hood.) After cooling the tube in an ice bucket, the contents were acidified with 2.5 ml of HCl (6*N*). The liberated acids were then extracted with 2 × 1.5-ml portions of hexane by shaking mechanically (reciprocating shaker, Eberbach Corp., Ann Arbor, MI) for 10 min. Hexane extract, appearing on the top layer after centrifugation (to break the emulsion) was transferred quantitatively with a long tip Pasteur pipet to a 5-ml conical graduated centrifuge tube (13 × 116 mm with glass stopper). To the pooled hexane extract was added 12 μl of Meth-Prep I—a 0.2*N* aqueous solution of (*m*-trifluoromethylphenyl) trimethylammonium hydroxide, purchased from Applied Science Laboratories, Inc., State College, PA. The tube was stoppered and shaken on a Vortex mixer for 2–3 min. The quaternary ammonium salt solution fatty acids settled down to the pointed bottom tip of the centrifuge tube on centrifugation. With a microsyringe, the salt solution was transferred quantitatively to a gas chromatograph sampler vial (100-μl capacity suitable for Hewlett-Packard automatic sampler 7671A, Chromatographic Specialties Ltd., Brockville, Ontario). The vial was crimp-sealed. Immediately before gas chromatographic analysis, 48 μl of methyl propionate/methanol (1:2) mixture was injected into the vial and the contents thoroughly mixed.

Gas Chromatography

A Hewlett-Packard 5840A gas chromatograph equipped with a glass capillary inlet system (model 18835B), a flexible fused-silica column (Carbowax 20M, 0.2 mm i.d., 25 m long, Hewlett-Packard Canada Ltd., Kirkland, Quebec), and a flame ionization detector was used. The sample injector was operated on a split mode at a ratio of 50 to 1. Carrier flow of helium was maintained at 1 ml/min through the column. Injector and detector were both set at 265°C. During a chromatographic run, the oven temperature was controlled at 120°C for 2 min and then programmed to 220°C at 10°C/min. A maximum sample size of 2 μl was introduced either manually or mechanically with the automatic sampler. The glass splitter inserted in the injector block was half filled with 10% OV-1 on Chromosorb W AW HP, 80/100 mesh.

Fatty Acid Standards and Other Chemicals

Fatty acid standards used for identification and quantitative calibration were obtained from Supelco, Inc., Bellefonte, PA. A synthetic product of 9-decenoic acid ($C_{10:1}$) was provided by C.-T. Ho, Rutgers University, New Brunswick, NJ. All chemicals were of reagent grade or better. Methanol was redistilled. *n*-Hexane was Instra-analyzed grade (J. T. Baker).

RESULTS AND DISCUSSION

Figure 2 shows a gas chromatogram of a standard mixture of fatty acid methyl esters run through the capillary column under the specified conditions. The sharp base-line separation and the short retention time of all the compounds demonstrate the superiority of the wall-coated open tubular column. For the present application, a sample inlet split ratio of 50 to 1 was chosen, because, at such a ratio, optimal sample linearity, ie, the least discrimination against sample components having extreme volatility during vaporization, can be obtained.

The procedure for extraction and saponification of yeast fatty acids is greatly simplified by carrying out the shaking, refluxing, and centrifuging all in the same vessel. Unlike the conventional methods of extraction and methylation (5,13), the simplified process of sample preparation requires no back-washing, concentrating, or evaporating, but only two transfers. As a result of the reduction of sample manipulation, the precision of the method was increased about 100%, and the preparation time was decreased very significantly. With the aid of automatic pipets, a technician can easily prepare 15–20 samples in a working day. The procedure, therefore, lends itself to a rapid, quantitative, microdetermination of fatty acids in yeast or other biological samples.

Under similar pyrolytic conditions, the recovery of fatty acids and completeness of methylation was assessed by Gerhardt and Gehrke (6) at 88.5–100.5%. This fluctuation of recovery can be offset by the use of an internal standard (C_{15} acid). Depending on the individual fatty acid methyl ester, the relative standard deviation ranges from 1 to 15%. The low-boiling and high-boiling fatty acids tend to vary much more, presumably due to a higher degree of discrimination in the inlet vaporization system against these compounds. Overall, the coefficient of variation of the entire procedure was estimated at 6–8%.

In the original procedure of pryolytic methylation (15), the quaternary *N*-methylammonium salt of the fatty acid solution was sandwiched between two plugs of the methyl propionate-methanol mixture. The purpose of this simultaneous injection was to neutralize the initial alkalinity of the ammonium salt and thus prevent the possible isomerization of polyenoic fatty acids (11). In

the present procedure, the sample (as ammonium salt solution) was premixed with methyl propionate-methanol solution at a ratio of 1 to 4. This change permits the sample to be injected conveniently by an automatic sampler, and yet yields the same quantitative data; no migration of carbon-carbon double bond was observed.

Figure 3 shows the actual chromatograms of fatty acid methyl esters of cultured ale (MBCL A-47) and lager (MBCL L-5) yeasts. These chromatograms illustrate the similar preponderance of palmitic (C_{16}, peak 8) and stearic (C_{18}, peak 10) acids, as well as the quantitative differences of some other fatty acids. The peak labelled "N," emerging at about 3.4 min, is due to the tertiary amine that resulted from the pyrolytic methylation (Fig. 1).

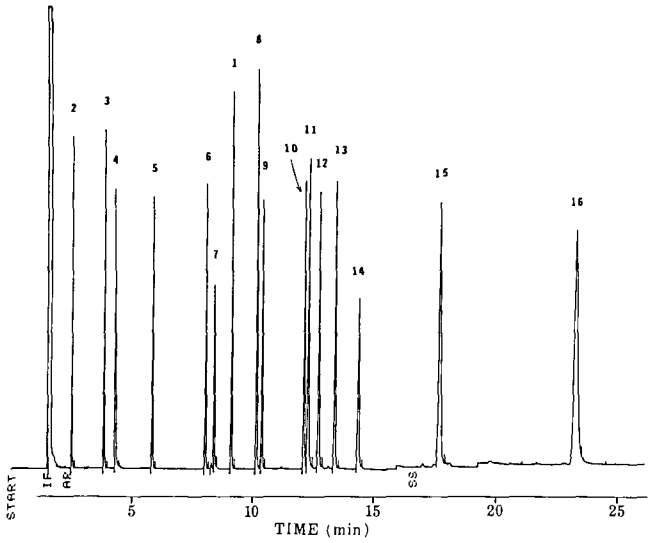

Fig. 2. Gas chromatogram of a mixture of standard fatty acid methyl esters. Peaks: 1 = C_{15} (internal standard), 2 = C_8, 3 = C_{10}, 4 = $C_{10:1}$, 5 = C_{12}, 6 = C_{14}, 7 = $C_{14:1}$, 8 = C_{16}, 9 = $C_{16:1}$, 10 = C_{18}, 11 = $C_{18:1}$, 12 = $C_{18:2}$, 13 = $C_{18:3}$, 14 = C_{20}, 15 = C_{22}, 16 = C_{24}.

Yeast is known to be a very adaptive and facultative microorganism. Several reports have indicated that yeast cellular constituents may vary substantially, depending on the environment and stage of growth (3,4,20). To assess the effect of growth temperature and oxygen content of growth medium, the same pair of ale and lager cultured yeasts were grown aerobically at 20 and 10°C and anaerobically at 20°C. Results are presented quantitatively in Table II and graphically in Fig. 4. The most striking effect on both ale and lager yeasts is the enormous increase (30–50-fold) of monounsaturated fatty acids, ie, $C_{18:1}$, $C_{16:1}$, $C_{14:1}$, and $C_{10:1}$ when growth temperature is changed from 20 to 10°C. The increase is accompanied by an appreciable reduction in C_{16} and C_{18} and the disappearance of polyunsaturated octadecanoic acids. Substantial amounts of behenic (C_{22}) and lignoceric (C_{24}) acids were found in yeast grown at 10°C. Under anaerobic conditions maintained at 20°C, C_{16} and C_{18} acids increased substantially and polyunsaturated octadecanoic acids were absent. These results clearly indicated that for comparative study of yeast cellular constitutents, such as fatty acids, the microorganisms must be cultivated under standardized specific conditions.

Seventeen strains of yeast, representing the genera *Saccharomyces*, *Hansenula*, *Kloeckera*, *Candida*, *Torulopsis*, *Pichia*, and *Brettanomyces* were analyzed for their fatty acid composition. Some of these yeasts are more likely to be found in a brewery environment and are referred to as "wild yeasts," whereas the others are wine yeasts or yeasts of laboratory strains that are capable of tolerating a higher concentration of ethanol. The reason for including the latter group of yeasts in the present study was to evaluate the relationship between the unsaturated fatty acid content in the plasma membrane of yeast and its alcohol tolerance (22).

Figure 5 is an actual chromatogram (with retention time print-out suppressed) of fatty acid methyl esters of *Saccharomyces*

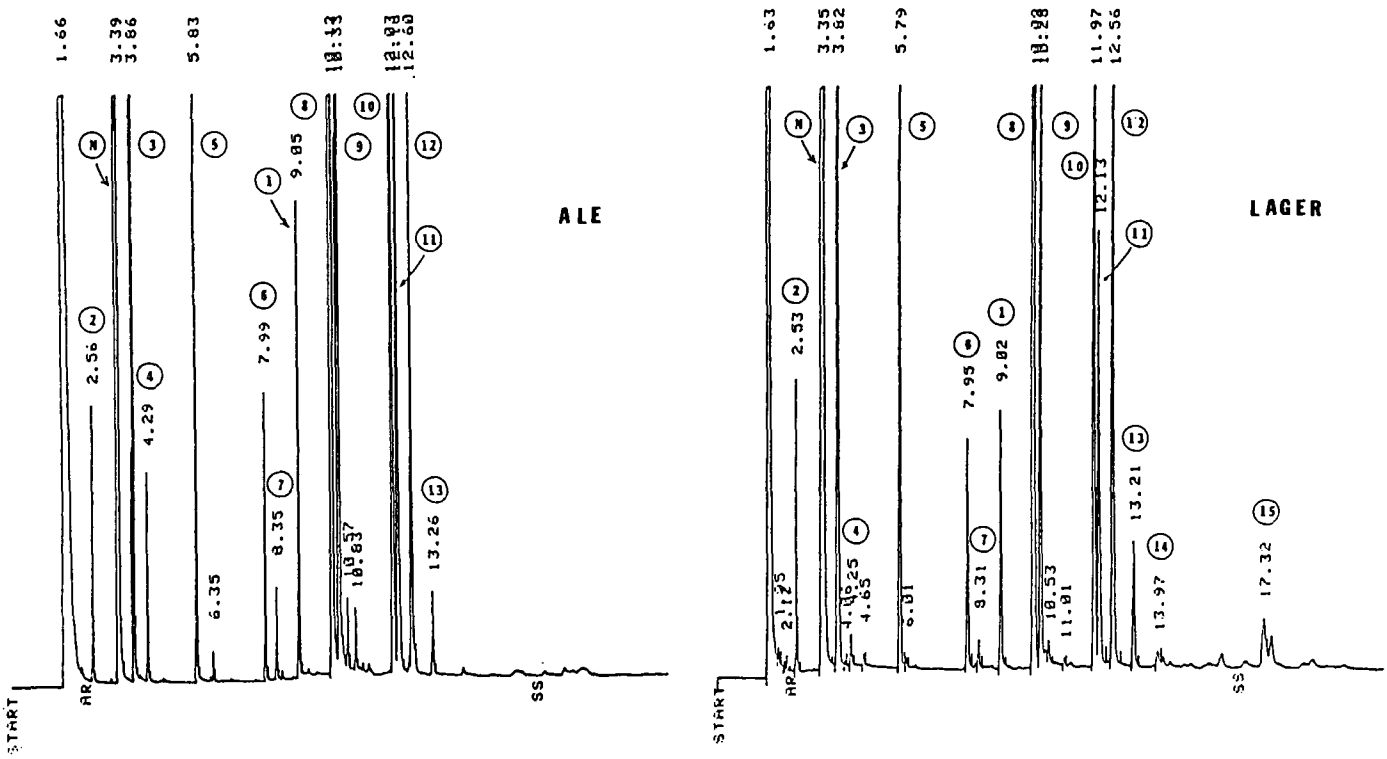

Fig. 3. Actual chromatogram of fatty acids (as methyl esters) of cultured ale and lager yeasts. N = a tertiary amine, byproduct of pyrolysis. Circled numbered peaks correspond to those in Fig. 2. Numerical print-outs are retention times in minutes.

ellipsoideus, the presence of which in beer is alleged to cause phenolic off-flavor (21). The predominance of fatty acids with 16 and 18 carbon atoms is similar to that in cultured (brewing) yeasts. However, the major characteristics are the three peaks labeled X_1, X_2, and X_3, eluted immediately after palmitoleic acid ($C_{16:1}$). The likely identities of these three peaks are polyunsaturated, hydroxy derivatives or positional isomers of palmitic acid. Peak X is probably linderic acid ($C_{12:1}$), but peak X_4 and X_5 are more difficult to speculate upon.

Chromatographic profiles of fatty acids of all the other yeasts are presented in Figs. 6 and 7. An overall view of the chromatograms verifies the distinctive differences of each strain of yeast, albeit some differences are more subtle than others. These chromatograms may serve as fatty-acid "finger prints" of the yeasts analyzed thus far.

Quantitative profiles of fatty acids of wild and alcohol-tolerant yeasts are compiled in Tables III and IV, respectively. The fatty acid contents of the 11 strains of wild yeasts vary greatly. Some wild yeasts are devoid of short-chain fatty acids but relatively rich in long-chain fatty acids. No common quantitative pattern appears among all the wild yeasts; in other words, each strain has its own characteristics. Palmitoleic is the most abundant acid in six of the 11 wild yeasts. *Saccharomyces willianus* (Yeast 6) and *Hansenula anomala* (Yeast 7) have relatively small amounts of $C_{16:1}$ but relatively large amounts of linoleic ($C_{18:2}$) acid. *Torulopsis colliculosa* (Yeast 10) and *Pichia membranaefaciens* (Yeast 11) grow poorly in brewer's wort and contain rather small amounts of fatty acids, among which oleic ($C_{18:1}$) acid is the most abundant and linolenic ($C_{18:3}$) is also quite prominent. The characteristics of *Brettanomyces anomalus* (Yeast 12) and *Brettanomyces*

TABLE II
Fatty Acid Composition (ng/mg)[a] of Ale and Lager Yeasts[b] Grown in Different Environments

Yeast		Fatty Acid													
Sample Code	Growth Conditions	8	10	10:1	12	14	14:1	16	16:1	18	18:1	18:2	18:3	22:0	24:0
1	20°C	8.7	69.7	5.5	25.3	7.0	2.3	173.4	94.6	63.3	44.6	27.1	2.2	···	···
	10°C	4.4	4.3	13.2	6.1	10.8	20.4	83.6	1,210.8	33.0	609.1	···	···	8.1	8.9
	Anaerobic	5.4	68.3	8.5	15.5	2.8	3.9	204.5	122.0	78.7	63.8	2.5	···	···	···
2	20°C	8.3	86.1	1.0	45.9	11.3	1.3	250.1	65.8	68.5	30.7	87.9	9.1	···	···
	10°C	3.3	3.9	11.9	5.6	9.3	18.1	73.8	1,033.6	29.0	521.6	···	···	7.8	9.5
	Anaerobic	7.3	95.3	8.7	42.2	9.5	4.2	335.4	150.6	100.5	83.1	···	···	···	···

[a]Each figure, expressed in nanograms per milligram of dried yeast, represents the average of nine determinations.
[b]Yeasts are identified in Table I.

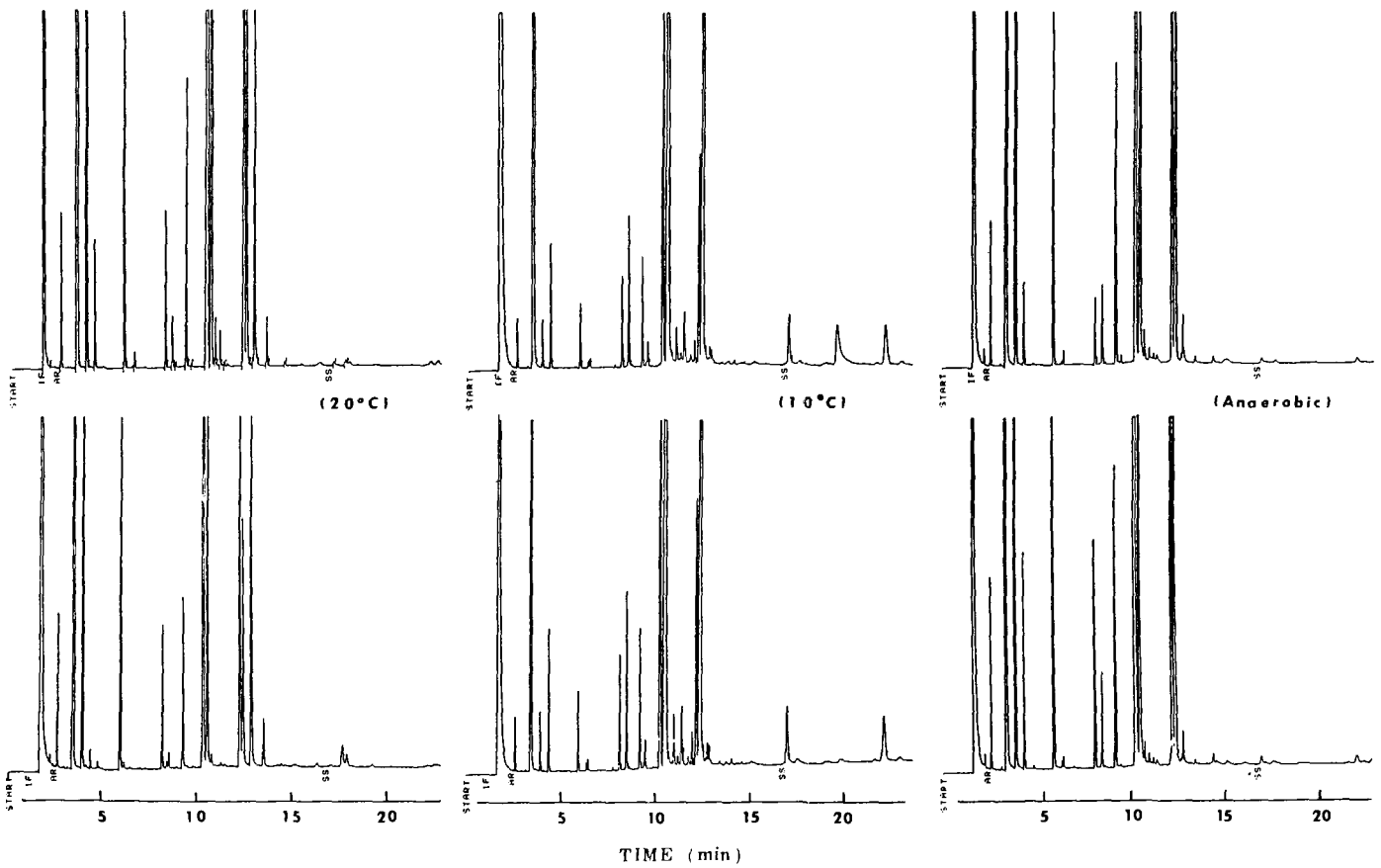

Fig. 4. Effect of growth temperature and oxygen content in growth medium on the fatty acid composition of ale **(1)** and lager **(2)** yeasts.

bruxellensis (Yeast 13) are the high levels of lauric (C_{12}) acid, the equal prominance of C_{16} and $C_{16:1}$, and the low content of $C_{18:1}$.

The fatty acid composition of the six wine or laboratory yeast strains appears to be more uniform, in the sense that they have about the same number of fatty acids identified or unidentified, and they all have $C_{16:1}$ as the most abundant acid. In terms of total saponifiable fatty acids, the alcohol-tolerant yeasts do not differ significantly from the wild yeasts as one would anticipate. This may be, again, due to the facultative nature of the yeast. The elevated level of fatty acids in plasma membrane of alcohol-tolerant yeast may simply be brought about when the yeast is maintained in a medium containing a high concentration of ethanol or other factors, such as proteolipid (8).

Two major differences are apparent between cultured brewing yeasts and wild or wine yeasts. One is the ratio of $C_{18:0}$ to $C_{18:1}$. Ale and lager yeasts have the $C_{18:0}$-$C_{18:1}$ ratio larger than unity, whereas wild or wine yeasts have the ratio much smaller than unity. The other major difference is that the wild and wine yeasts possess some additional fatty acids labeled X, X_1, X_2, and X_3 in Fig. 5 and indicated in Tables III and IV. Identification of these additional compounds will provide further ground for differentiation of yeasts.

When the analytical data were subjected to discriminant analysis by using a "STATPAK" (statistical package) program developed by the McGill University System for Interactive Computing (MUSIC) Center (10), an average of 95% associated probability was found for each strain of yeast. This means that each of the yeasts is different from others in fatty acid composition and that these data files may afford some basis for yeast typing. However, when more yeast strains and more variates are involved, a much more sophisticated computing system or chemometric methodology is needed. The pattern recognition technique advanced by Kowalski (12) would seem to be a valuable tool.

On the bases of present findings, differentiation of cultured brewery yeast from wild yeasts appears feasible by analyzing the fatty acid composition. The high resolution, sensitivity, and speed

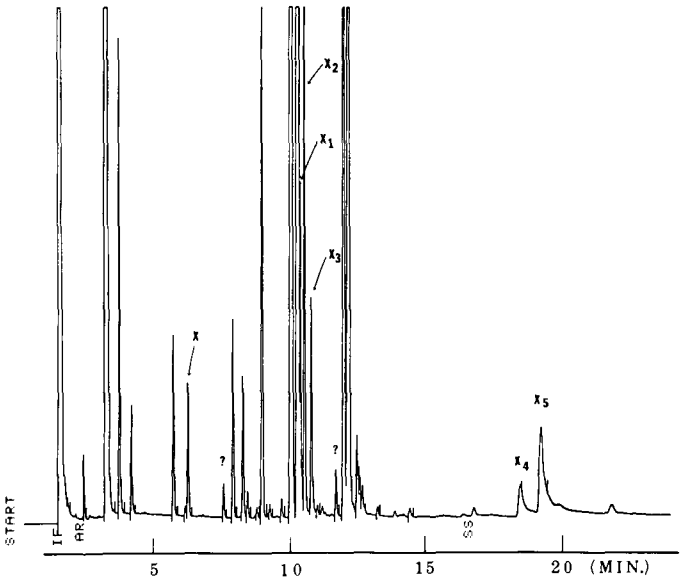

Fig. 5. Gas chromatogram of fatty acids (as methyl esters) of *Saccharomyces ellipsoideus*. Extraneous unidentified peaks are indicated (X-X^5).

TABLE III
Fatty Acid Composition (ng/mg)[a] of Some Wild Yeasts

Yeast Sample[b]	Fatty Acid													With Presence of[c]
	8	10	10:1	12	14	14:1	16	16:1	18	18:1	18:2	18:3	20	
3	2.4	22.6	3.8	5.1	5.6	2.7	267.8	470.7	130.1	346.9	9.9	···	···	X, X_1, X_2, X_3
4	2.1	15.8	5.1	5.3	6.0	5.3	131.0	290.8	42.7	123.7	2.8	···	···	X, X_1, X_2, X_3
5	3.7	40.0	6.8	12.0	9.3	4.7	206.2	273.2	82.2	172.1	4.6	···	···	X, X_1, X_2, X_3
6	0.4	···	···	3.8	13.8	2.4	118.7	55.6	12.8	147.5	165.2	46.1	···	
7	···	0.8	···	4.8	7.2	4.3	127.8	81.9	26.9	109.5	121.3	39.5	1.9	X_3
8	···	110.1	2.2	25.8	59.8	15.4	316.8	624.6	15.7	88.2	14.4	···	···	C_{24}
9	8.4	24.0	6.1	11.5	5.8	5.5	109.3	307.0	30.6	141.0	3.5	···	···	X, X_1, X_2, X_3
10	···	···	···	···	1.6	···	54.2	17.0	86.8	103.4	38.8	34.8	9.8	C_{22}, C_{24}
11	···	···	···	5.9	8.7	4.0	70.7	79.6	22.9	164.9	72.4	54.8	···	X, X_1, X_3
12	···	13.0	···	89.1	36.8	13.4	209.9	234.7	29.7	84.6	95.0	···	···	X, X_1, X_3
13	2.2	42.6	···	75.0	17.5	6.0	247.2	220.8	35.5	60.8	11.3	···	2.6	X, X_1

[a] Each figure, expressed in nanograms per milligram of dried yeast, represents the average of nine determinations.
[b] Yeasts are identified in Table I.
[c] Figure 5 shows unidentified peaks X–X_3.

TABLE IV
Fatty Acid Composition (ng/mg)[a] of Some Alcohol-Tolerant Yeasts

Yeast Sample[b]	Fatty Acid												With Presence of[c]
	8	10	10:1	12	14	14:1	16	16:1	18	18:1	18:2	18:3	
14	5.8	55.3	7.1	17.1	3.4	4.5	193.5	266.3	68.8	111.3	2.7	···	X, X_1, X_2, X_3
15	7.5	64.3	3.0	13.7	7.1	2.0	202.9	224.2	56.5	139.1	4.8	···	X, X_1, X_2, X_3
16	3.0	15.9	4.7	5.7	14.1	6.5	142.3	382.3	36.9	225.3	1.8	···	X, X_1, X_2, X_3
17	9.5	60.4	12.3	27.1	11.0	4.2	208.8	234.2	57.5	134.8	3.2	···	C_6, X, X_1, X_2, X_3
18	1.2	18.1	2.2	10.5	2.6	2.7	158.4	276.6	61.4	163.9	3.1	···	X, X_1, X_2, X_3
19	5.7	42.0	4.7	13.4	6.1	3.3	175.6	242.0	49.4	128.3	2.3	···	X, X_1, X_2, X_3

[a] Each figure, expressed in nanograms per milligram of dried yeast, represents the average of nine determinations.
[b] Yeasts are identified in Table I.
[c] Figure 5 shows unidentified peaks X–X_3.

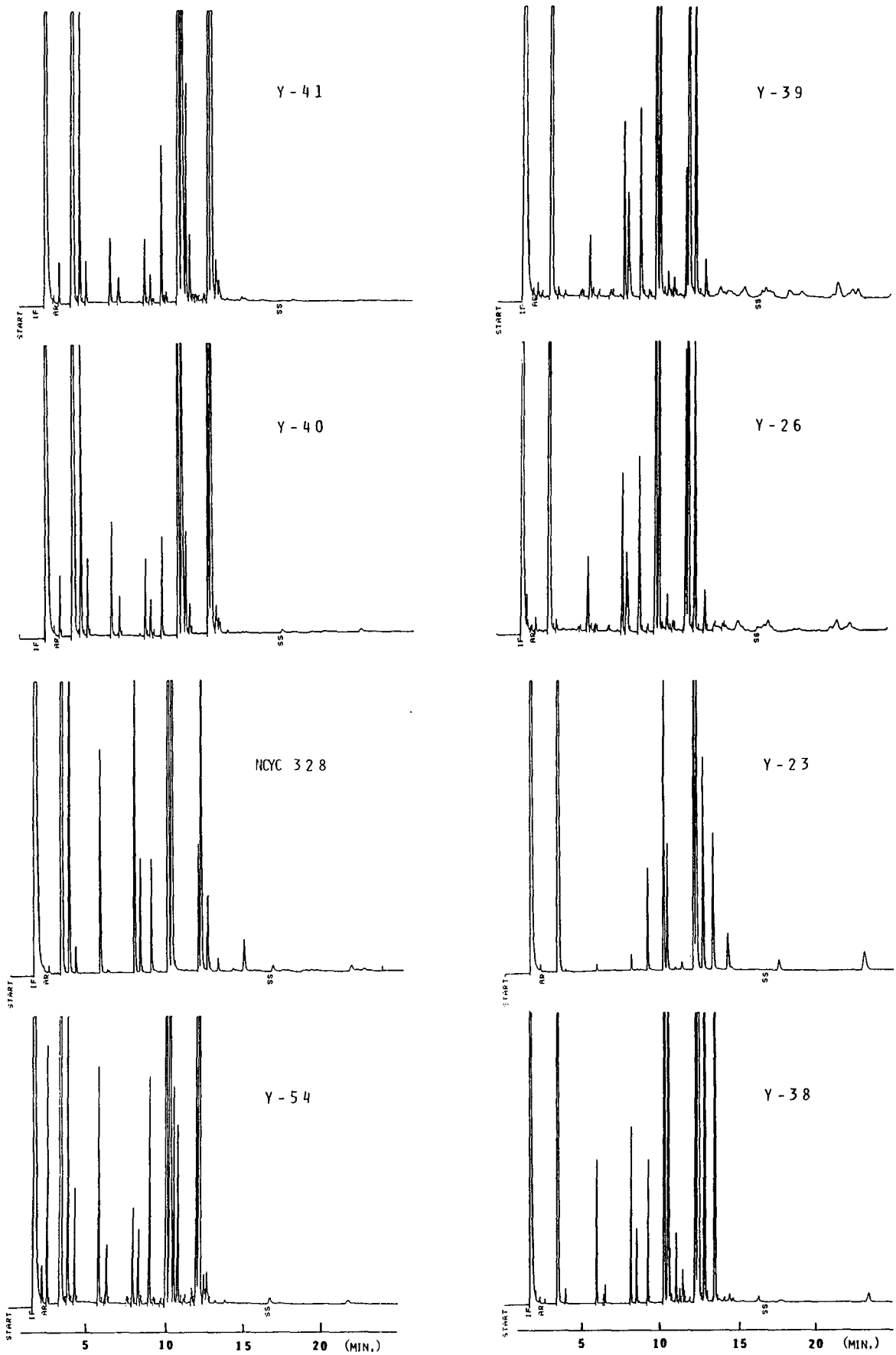

Fig. 6. Chromatographic profiles of yeasts. Yeast identifications are given in Table *1*.

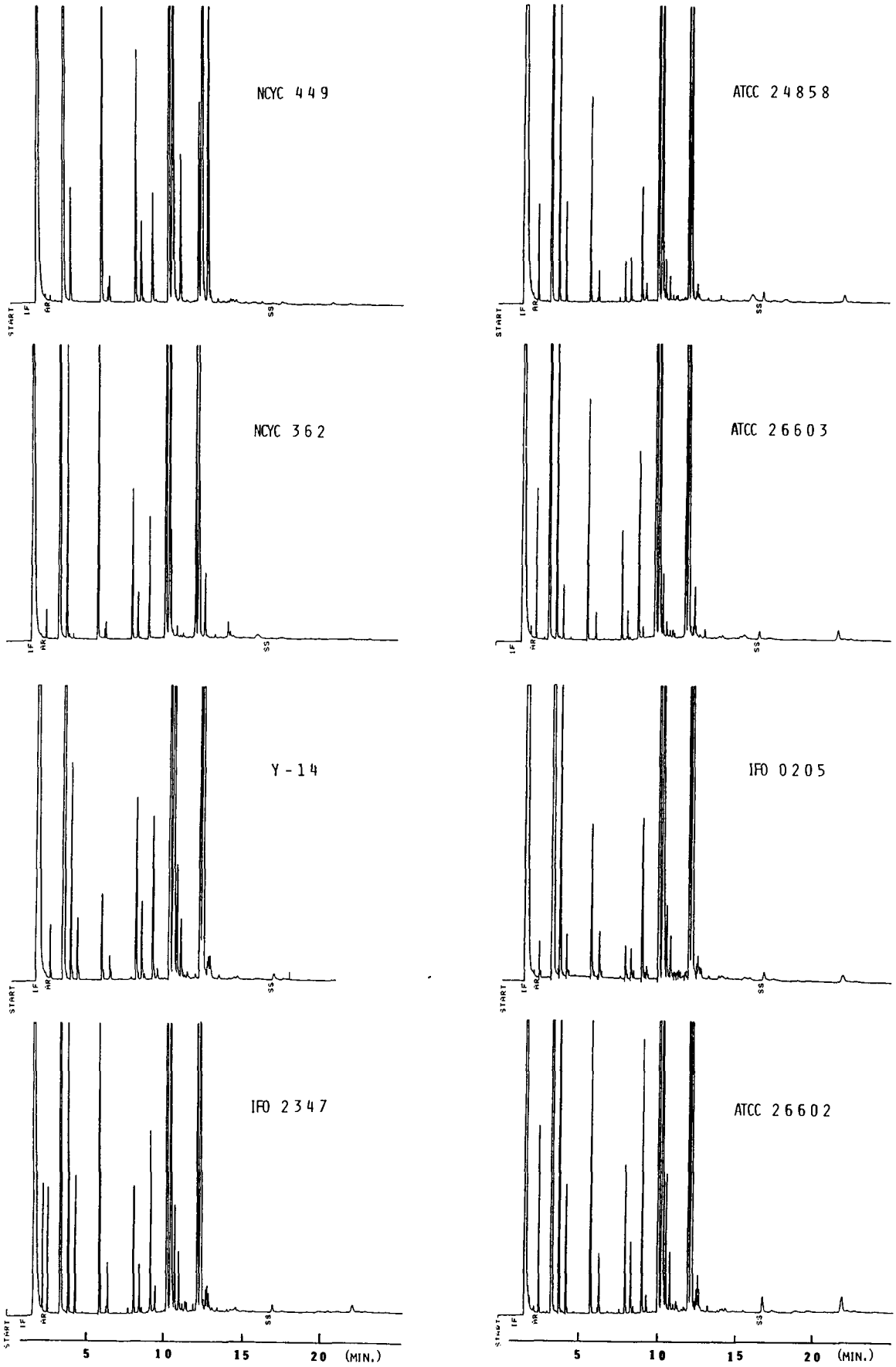

Fig. 7. Chromatographic profiles of yeasts. Yeast identifications are given in Table I.

of currently available gas chromatography techniques can certainly complement the physiological, morphological, and serological techniques conventionally used for yeast differentiation.

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LITERATURE CITED

1. Abel, K., Deschmertzing, H., and Peterson, J. I. *J. Bacteriol.* 85:1039, 1963.
2. Biacs, P. A. *Acta Alimentaria* 8(1):57, 1979.
3. Brown, C. M., and Rose, A. H. *J. Bacteriol.* 99:371, 1969.
4. Cejkova, A., and Jirku, V. *Folia Microbiol.* 23:372, 1978.
5. Chen, E. C.-H., Jamieson, A. M., and Van Gheluwe, G. *J. Am. Soc. Brew. Chem.* 38:13, 1980.
6. Gerhardt, K. O., and Gehrke, C. W. *J. Chromatogr.* 143:335, 1977.
7. Gunasekaran, M., and Hughes, W. T. *Mycologia* 72(3):505, 1980.
8. Hayashida, S., Feng, D. D., Ohta, K., Chaitiumvong, S., and Hongo, M. *Agric. Biol. Chem.* 40(1):73, 1976.
9. Jaeger, H., Klor, H. U., and Ditschuneit, H. *J. Lipid Research* 17:185, 1976.
10. Jarvis, G. T. MUSIC STATPAK Reference Manual.Computing Centre, McGill University: Montreal, Canada, 1974. p. 120.
11. Kossa, W. C., MacGee, J., Ramachandran, S., and Webber, A. J. *J. Chromatogr. Sci.* 17:177, 1979.
12. Kowalski, B. R. *Anal. Chem.* 47:1152A, 1975.
13. Kuksis, A. *J. Chromatogr.* 143:3, 1977.
14. Lanza, E., Zyren, J., and Slover, H. T. *J. Agric. Food Chem.* 28:1182, 1980.
15. MacGee, J., and Allen, K. G. *J. Chromatogr.* 100:35, 1974.
16 Martinez, M., Nurok, D., Zlatkis, A., McQuittay, D., and Evans, J. *J. High Resolu. Chromatogr. Chromatogr. Commun.* 3:528, 1980.
17. Moss, C. W., and Dees, S. B. *J. Clin. Microbiol.* 10:390, 1979.
18. Moss, C. W., Dees, S. B., and Guerrant, G. O. *J. Clin. Microbiol.* 12:127, 1980.
19. Moss, C. W., Weaver, R. E., Dees, S. B., and Cherry, W. B. *J. Clin. Microbiol.* 6:140, 1977.
20. Okuyama, H., Saito, M., Joshi, V. C., Gunsberg, S., and Wakil, S. J. *J. Biol. Chem.* 254:12281, 1979.
21. Piesley, J. G., and Lom, T. Page 183 in: H. M. Broderick, ed. The Practical Brewer. Master Brewers Assoc. Am.: Madison, WI, 1977.
22. White, F. H. *Inst. Brew. (Aust. N.Z. Sect.), Proc. 15th Conv., Christchurch, N.Z., 1978,* p. 133.

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