Utilization of Wort Fatty Acids by Yeast During Fermentation[1]

Ernest C.-H. Chen, *Research and Development Department, Molson Breweries of Canada Ltd., Montreal, Quebec, Canada H2L 2R5*

ABSTRACT

Uniformly labeled ^{14}C-palmitic, oleic, and linoleic acids were used individually to trace wort fatty acids in ale yeast fermentations. Fatty acids extracted from the resulting beers were analyzed by gas-liquid chromatography with concurrent radioactivity monitoring. Lipid components extracted from the harvested yeast were separated by thin-layer chromatography, and the fatty acyl residues of these components were subjected to the same radio-gas chromatographic analysis. Results indicate that wort fatty acids are incorporated into the yeast cell as structure lipids, mainly in the forms of free fatty acid and phospholipid. The relationship between wort and beer fatty acid is indirect. This means that wort fatty acids contribute to the growth and maintenance of yeast, the activity of which, in turn, determines the formation and release of shorter chain fatty acids into beer.

Key words: *Beer fatty acids, Radio-gas chromatography, Utilization, Wort fatty acids, Yeast lipids*

Most lipid materials present in brewer's wort are derived from barley malt. Depending on the conditions of processing, eg, mashing and boiling, about 0.1–3.0% of total extractable malt lipids are recovered in hopped wort (6). These wort lipids include free fatty acids, glycerides, free and esterified sterols, and phospholipids (1,7). Among these lipid constituents, the free fatty acids are considered important both quantitatively and functionally.

Fatty acid composition changes greatly when wort becomes beer. A survey on fatty acid contents in three brands of a brewery's beers and their corresponding worts was conducted recently in our laboratory, using the method described in this article. Results of the survey, shown in Table I, indicate that palmitic (C_{16}), linoleic ($C_{18:2}$), stearic (C_{18}), and oleic ($C_{18:1}$) acids account for 85–90% of total fatty acids in wort, whereas caprylic (C_8), caproic (C_6), and capric (C_{10}) acids make up 75–80% of total beer fatty acids. The change of fatty acids from long-chain to medium-chain as a result of fermentation was also reported elsewhere (7,8,15)..

Speculation about a direct correlation of wort fatty acids with the resulting beer fatty acids has been raised from time to time. Taylor and Kirsop (14) investigated the possibility of a biochemical route by which long-chain fatty acids are degraded to shorter chain beer fatty acids. Because they were not able to detect any nonanoic (C_9) acid in beer prepared from wort supplemented with substantial amounts of heptadecanoic (C_{17}) acid, they concluded that such a route was unimportant. However, the addition of heptadecanoic acid to wort in amounts much greater than the total wort fatty acid content (130–670 mg/L, in this case) may not be appropriate. The large quantity of exogenous fatty acid may disturb the balance of other wort constituents. Enzyme systems that act upon the naturally occurring even-numbered carbon fatty acids may not catalyze the odd-numbered carbon fatty acids. Furthermore, heptadecanoic acid may not disperse in fermenting wort because of its limited solubility. An alternative approach to the study, therefore, is to trace the wort fatty acids by using uniformly labeled compounds in the yeast fermentation. This article describes the application of radio-gas chromatography to assess the role of wort fatty acids during fermentation. Specific attention was paid to the uptake of wort fatty acids by yeast and to the origin of beer fatty acids.

[1] Presented at the 46th Annual Meeting, Minneapolis, MN, May 1980.

0361-0470/80/04014806/$03.00/0

EXPERIMENTAL

Fermentation

Ale fermentations were conducted at 20° C in 1-L Erlenmyer flasks placed in a shaker-water bath (model 66123, New Brunswick Scientific Co., New Brunswick, NJ). All-malt hopped wort adjusted to 12° P was used throughout. Pure ale culture yeast (*Saccharomyces cerevisiae*) was pitched at a level of approximately 5×10^6 cells per milliliter. Quantity and radioactivity of each labled compound used in individual 500-ml batch fermentations were as follows: ^{14}C(U)-palmitic acid, 10 μg in 0.2 ml of hexane, 25 μCi; ^{14}C(U)-linoleic acid, 7 μg in 1 ml of hexane, 25 μCi; ^{14}C(U)-oleic acid, 7 μg in 1 ml of hexane, 25 μCi; 1,2-^{14}C-acetic acid (sodium salt), 100 μg in 1 ml of ethanol, 100 μCi. All the radioactive compounds were purchased from New England Nuclear Canada Ltd., Lachine, Quebec. Fermentations were terminated in 5–6 days when 80% extracts were fermented. All experiments were carried out in duplicate.

Fatty Acid Analysis

Upon completion of fermentation, beer was separated from the yeast by centrifugation (10,000 rpm for 10 min at 0° C) and subjected to three successive solvent and alkaline extractions. The fatty acid extract was then concentrated and methylated. Details of the procedure as well as gas chromatographic conditions were reported earlier (3).

TABLE I
Fatty Acid Contents in Worts and Corresponding Beers

	Fatty Acid Concentration, mg/L[a]												
Brand	C_6	C_8	C_{10}	Iso-C_{11}	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	C_{20}	C_{22}
I													
Wort	0.15	T	T	···	T	0.18	2.14	0.11	0.65	0.19	1.19	0.22	0.06
Beer	0.58	3.13	0.54	0.10	T	0.06	0.48	0.08	0.27	0.27	0.06	0.06	T
II													
Wort	0.07	T	T	···	T	0.15	3.26	0.14	0.29	0.30	2.47	0.29	0.08
Beer	0.78	3.45	0.57	0.16	T	0.06	0.52	0.09	0.21	0.17	0.05	T	T
III													
Wort	T	T	0.07	···	0.07	0.20	3.37	0.18	0.67	0.32	1.86	0.28	0.09
Beer	0.56	3.64	0.30	0.11	0.05	0.07	0.48	0.16	0.17	0.35	T	T	T

[a] T = trace (less than 0.05 mg/L).

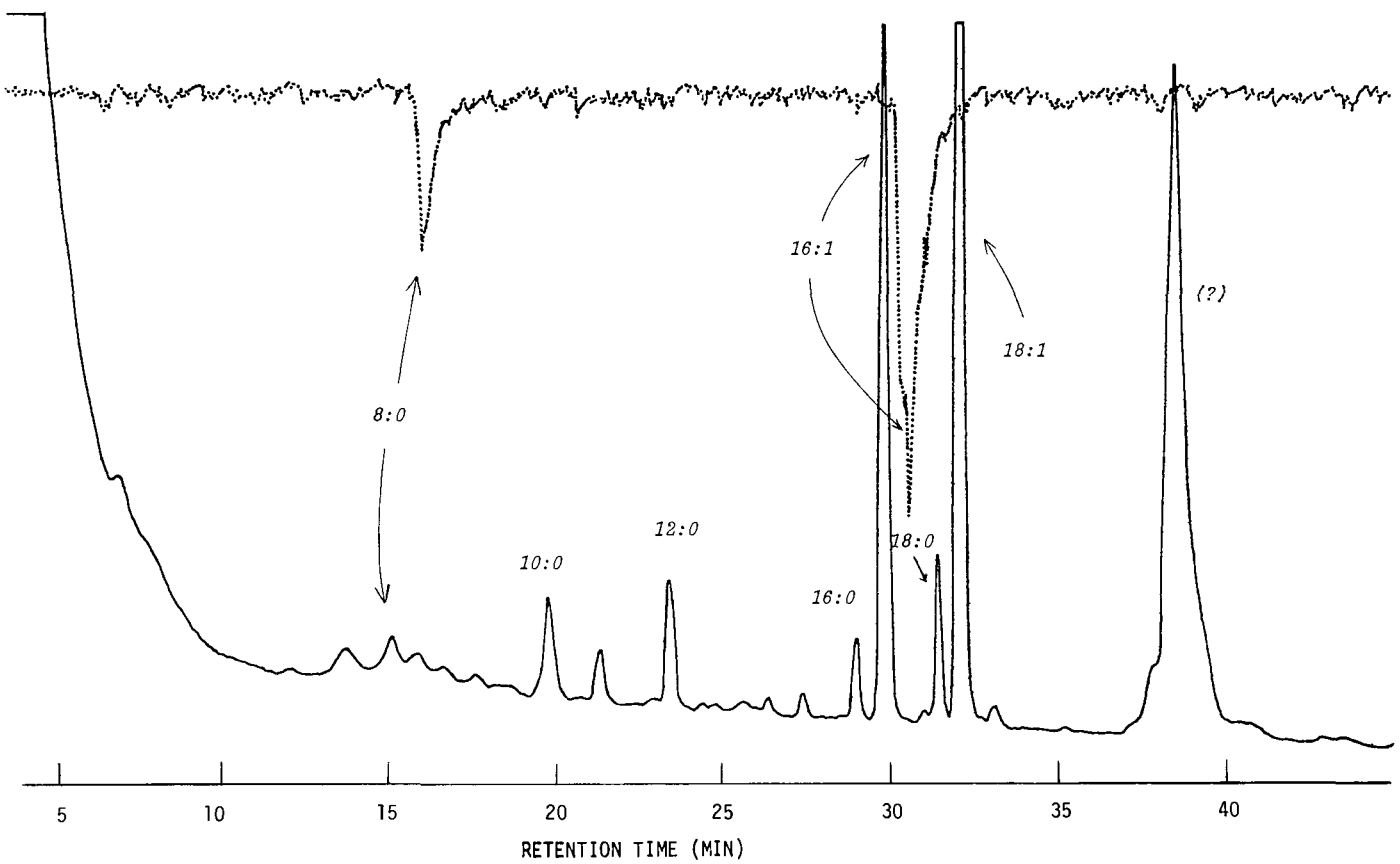

Fig. 1. Radio-gas chromatogram of fatty acyl moieties (as methyl esters) of sterol esters fraction from yeast grown in wort containing ^{14}C(U)-palmitic acid. Dotted line represents ^{14}C tracing, 1K.

Yeast Lipid Extract and Thin-Layer Chromatographic Separation

After the removal of beer, the yeast was washed twice with distilled water and resuspended in the aqueous medium to a concentration of about 10%. The suspension was placed in a jacketed, conically shaped glass vessel (cooled at −10° C with a circulating methanol bath) and subjected to ultrasonification (Ultrasonifier, model J-17A, Branson Sonic Power Co., Danbury, CT) for about 15 min. The disintegrated cell suspension was then extracted with an equal volume of chloroform/methanol (3:1) mixture. The organic phase was retained and concentrated to about 1 ml by using a rotary vacuum evaporator.

The concentrated lipid extract was streaked on a precoated thin-layer chromatography glass plate (Silica gel G, 0.5-mm thick, Alltech Associates Inc., Neward, DE). To locate the lipid fractions, a mixture of lipid standards was also spotted on the side of the plate. The chromatogram was developed in a chamber saturated with a solvent system composed of *n*-hexane/diethyl ether/acetic acid (80:20:1.5), according to Kupke and Zeugner (9). A 0.2% ethanolic solution of Rhodamine 6G was used as visualizing agent. The lipids were separated, in order of decreasing migration distance, into sterol esters, triglycerides, free fatty acids, sterols, diglycerides, monoglycerides, and phospholipids. With the

TABLE II
Relative Distribution of Radioactivity in Lipid Components of Yeasts Grown in Worts Containing ^{14}C(U)-Fatty Acids

	Labeled Fatty Acid					
	C_{16}		$C_{18:1}$		$C_{18:2}$	
Lipid Components	cpm[a] ($\times 10^{-3}$)	Percent of Total	cpm ($\times 10^{-3}$)	Percent of Total	cpm ($\times 10^{-3}$)	Percent of Total
Sterol esters	189.4	12.6	148.5	10.0	185.0	10.7
Triglycerides	171.6	11.4	108.1	7.4	99.6	5.8
Free fatty acids	712.0	47.5	529.4	40.0	672.9	39.0
Diglycerides and monoglycerides	174.4	11.6	161.4	11.0	119.2	6.9
Phospholipids	251.4	16.8	523.2	35.6	648.0	37.6

[a]cpm = Counts per minute.

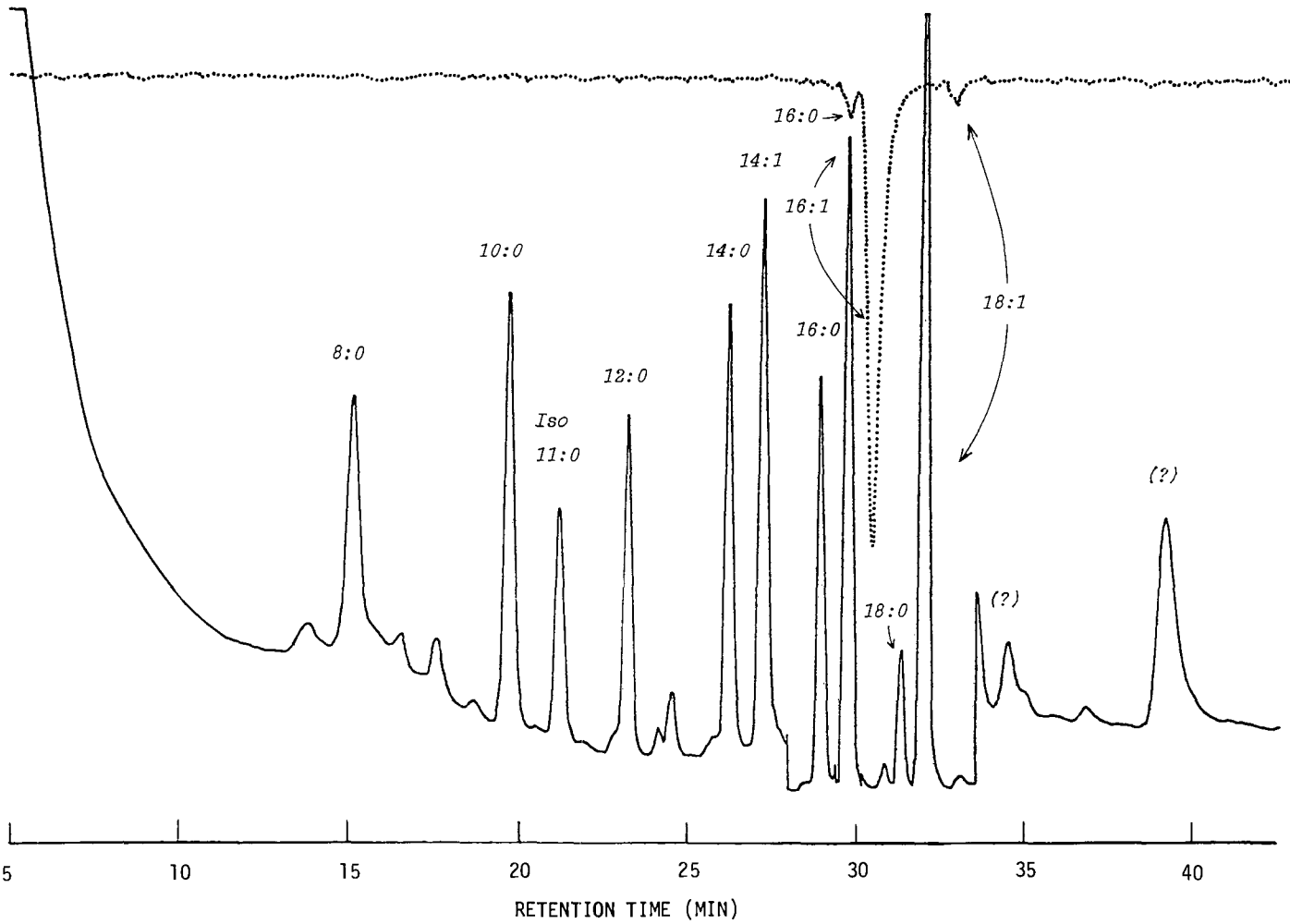

Fig. 2. Radio gas chromatogram of fatty acyl moieties (as methyl esters) of triglycerides fraction from yeast grown in wort containing ^{14}C(U)-palmitic acid. Dotted line represents ^{14}C tracing, 5K.

exception of sterols, all the fractions were scraped and collected in screw-capped vials (7.4 ml, 17 × 60 mm, cap with Teflon™ lining). Three milliliters of methanolic solution of boron trifluoride (14%, Pierce Chemical Co., Rockford, IL) and 0.5 ml of *n*-hexane were added to each vial. After the contents were mixed thoroughly on a Vortex mixer, the vial was placed on a Reacti-Therm heating module (Pierce Chemical Co.) and refluxed for 15 min at 100° C to transesterify the fatty acyl residues of the lipid fraction. The cooled methylating mixture was then shaken with 1 ml of NaCl-saturated water. On settling, the hexane layer of methyl esters floated to the top of the mixture. Five microliters of the hexane solution was injected for each radio-gas chromatographic analysis.

Radio-Gas Chromatography

A gas chromatograph (model 427, Packard Instrument Co., Inc., Downers Grove, IL) was coupled with a gas proportional counter (Packard, model 894). The responses from the flame ionization detector of the gas chromatograph and the proportional detector of the radioactivity counter were registered, with a constant time gap, on a 10-mV dual pen recorder (Packard, model 586). Helium (carrier gas) was maintained at 30 ml/min through the column and 60 ml/min through the proportional counter. A 6-ft, 4-mm id glass column packed with 10% SP-2330 on Chromosorb™ W AW, 100/120 mesh (Supelco Inc., Bellefonte, PA) was used. Detector and injector temperatures were 300 and 250° C, respectively. Oven temperature was set at 75° C for 8 min, then programmed to 220° C at a rate of 5° C/min and held for 30 min. A 10:1 stream splitter was used (the larger portion of the effluent was directed to the counter). The operating conditions of the proportional counter were as follows: furnace temperature, 700° C; sample inlet temperature, 250° C; time constant, 10 sec; sensitivity range, 1,000 or 5,000 cpm; voltage, 1,700 V; and quench gas, propane, 6 ml/min.

Scintillation Counting

A Packard Tri-Carb, model 3380, scintillation counter was used to assess the radioactivity of each yeast lipid fraction and beer. One hundred μl of hexane extract (methyl esters of fatty acids derived from a lipid fraction or beer) was mixed with 15 ml of Aquasol™ cocktail (New England Nuclear Canada Ltd). Counting efficiency was 75–80%.

Reagents and Lipid Standards

All chemicals were of reagent grade or better. Instraanalyzed grade (J. T. Baker) *n*-hexane was used to extract methyl esters of fatty acids. Chloroform and methanol were redistilled. Cholesteryl stearate, triolein, stearic acid, cholesterol, 1,2-diolein, 1-monolein, and phosphatidyl choline (obtained from Applied Science Division, State College, PA) were used as lipid standards in thin-layer chromatography.

RESULTS AND DISCUSSION

Table II shows the radioactivity, in 10^3 cpm (counts per minute), of yeast lipid fractions when each of the three labeled fatty acids was used in fermentation. One of the yeast lipid components, sterols, was not included because it was not radioactive. Because equal amounts of hexane extract prepared from each lipid fraction were used in scintillation counting, the percentage of radioactivity of each lipid component would represent its relative distribution. When ^{14}C(U)-palmitic acid (C_{16}) was used, 47.5% of the total lipid

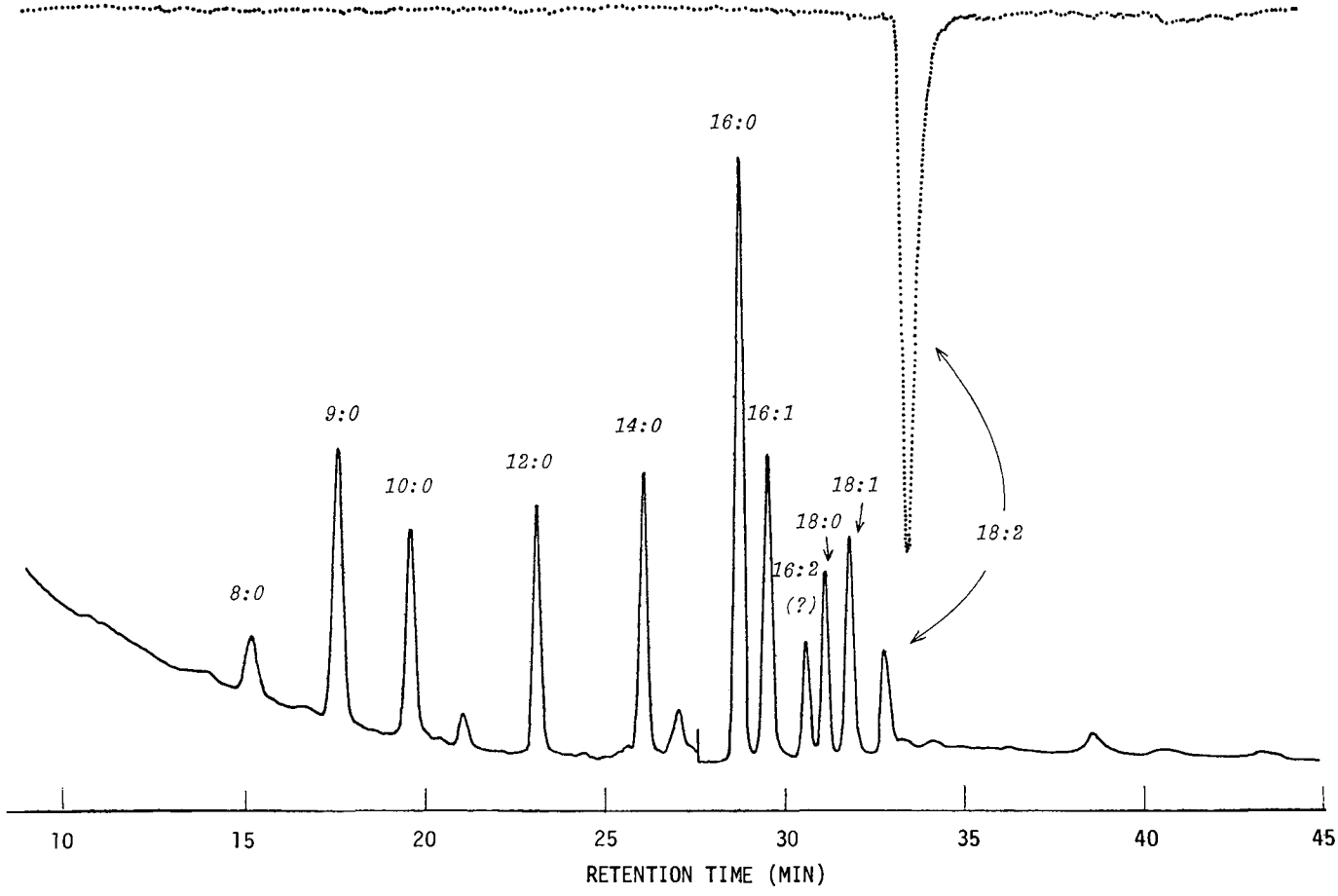

Fig. 3. Radio-gas chromatogram of free fatty acids fraction (as methyl esters) from yeast grown in wort containing ^{14}C(U)-linoleic acid. Dotted line represents ^{14}C tracing, 5K.

radioactivity was found in the free fatty acid fraction, whereas 16.8% was in phospholipids. When ^{14}C(U)-oleic acid ($C_{18:1}$) was used, 40.0 and 35.6% of the radioactivity were found in the free fatty acids and phospholipids fractions, respectively. Similarly, large proportions of radioactivity (39.0 and 37.6%, respectively) were recovered from these two lipid fractions when ^{14}C(U)-linoleic acid was used. Free fatty acids are important constituents of cytoplasm, as phospholipids are of cell membranes. Subcellular fractionation and analysis are required to determine whether the accumulation of radioactivity of the two main lipid components is restricted to these two cellular sites.

Some typical radio-gas chromatograms are reproduced and presented in Figs. 1–5. Figure 1 shows the fatty acyl moieties (as methyl esters) of the sterol esters fraction obtained from yeast grown in wort labeled with ^{14}C(U)-palmitic acid. The main fatty acyl residues of yeast sterol esters are predominantly unsaturated C_{16} and C_{18} acids. This is in agreement with the observation made earlier by Madyastha and Parks (10). Radioactivity was also found in C_8 and $C_{16:1}$ acids. Although caprylic acid was rather small quantitatively, the level of its radioactivity was appreciable. The identity of a large compound peak emerging at about 38 min is not certain, although it may be the sterol moiety of the esters. Figure 2 represents the fatty acids moieties of triglycerides separated from yeasts cultured in wort tagged with ^{14}C(U)-palmitic acid. Among all fatty acids found in this lipid fraction, palmitoleic ($C_{16:1}$) was highly radioactive, whereas palmitic (C_{16}) and oleic ($C_{18:1}$) acids were only moderately radioactive. Free fatty acids in yeast were found to range from C_8 to $C_{18:2}$, as shown in Fig. 3, and only $C_{18:2}$ was found radioactive when ^{14}C(U)-linoleic acid was used in the fermenting medium. Fatty acyl residues are smaller in quantity in the diglyceride and monoglyceride fraction of yeast lipids. Figure 4 shows that only linoleic acid was radioactive when the same fatty acid was labeled in the wort. Figure 5 illustrates the variety of fatty acyl moieties found in the phospholipid fraction of yeast lipids. It also shows that the $C_{18:1}$ moiety of phospholipid was highly radioactive when ^{14}C(U)-oleic acid was used in fermentation.

The incorporation of radioactivity of labeled wort fatty acids into yeast lipids is summarized in Table III. The level of radioactivity of each ^{14}C-fatty acyl moiety was approximated by the height of the corresponding radioactive peak. One significant feature of the results is that wort C_{16} acid is desaturated into $C_{16:1}$ and incorporated as yeast lipid, whereas the unsaturated wort fatty acids $C_{18:1}$ and $C_{18:2}$ do not undergo further desaturation. Fatty acid desaturase is believed to associate with the microsomal fraction, and, along with other fatty acid synthesizing enzyme systems, is located outside the mitochondria within the cytoplasm of the yeast cell (13). Of the several different fatty acid desaturases, each is specific in stereochemical structure and in the exact location of the double bond to be introduced (2). Therefore, the desaturase that acts upon palmitic acid does not function on oleic or linoleic acids. Apparently, the nonradioactive $C_{18:2}$, as seen in Fig. 5, is not derived from the labeled $C_{18:1}$ but from another precursor, possibly through the combined mechanisms of desaturation and elongation. Because no detectable $C_{18:3}$ (radioactive or nonradioactive) is found in any lipid fraction, one may speculate that brewer's yeast does not have an effective desaturase for the acid. Deficiency of this nature has already been found in animals, including humans. Evidence shows that the human body cannot introduce a double bond at carbon 12 (designated as n-6) or carbon 15 (n-3) positions, and therefore both $C_{18:2}$ and $C_{18:3}$ are considered essential fatty acids whose supply must depend on a dietary source (4).

No radioactive fatty acid was detectable in any of the beers resulting from fermentation with the labeled long-chain fatty acids, as analyzed by radio-gas chromatography. In each case, the radioactive wort fatty acid was apparently taken up and incorporated into the yeast cell in the form of structure lipids. However, when 1,2-^{14}C-acetic acid (sodium salt) was used in fermentations under otherwise identical conditions, some of the resulting beer fatty

TABLE III
Incorporation of Radioactivity of Labeled Wort Fatty Acids into Yeast Lipids

Lipid Components	Fatty Acyl Moieties and Radioactivity[a] Acquired from Wort Labeled with		
	C_{16}	$C_{18:1}$	$C_{18:2}$
Sterol esters	C_8*, $C_{16:1}$*	C_{12}*, C_{16}*, $C_{16:1}$**, C_{18}*, $C_{18:1}$***, C_{24}*	$C_{18:2}$*
Triglycerides	C_{16}*, $C_{16:1}$***, $C_{18:2}$*	C_{18}*, $C_{18:1}$***	$C_{18:2}$**
Free fatty acids	C_{16}*, $C_{16:1}$*****	C_{18}*, $C_{18:1}$******	$C_{18:2}$****
Diglycerides and monoglycerides	$C_{16:1}$	$C_{18:1}$**, C_{22}*	$C_{18:2}$**
Phospholipids	C_{16}*, $C_{16:1}$***	$C_{18:1}$******	$C_{18:2}$***

[a] Level of radioactivity, in 1×10^3 cpm (counts per minute): * = < 1, ** = 1–2, *** = 2–3, **** = 3–4, ***** = 4–5, and ****** = > 5.

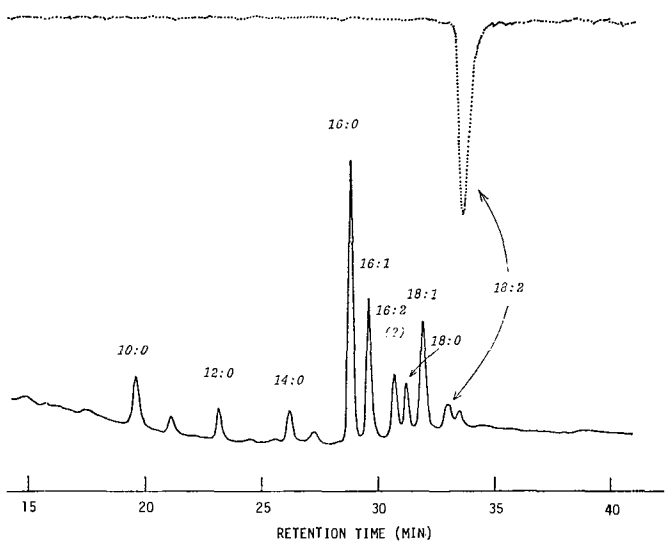

Fig. 4. Radio-gas chromatogram of fatty acyl moieties (as methyl esters) of diglyceride and monoglyceride fraction of yeast grown in wort containing ^{14}C(U)-linoleic acid. Dotted line represents ^{14}C tracing, 5K.

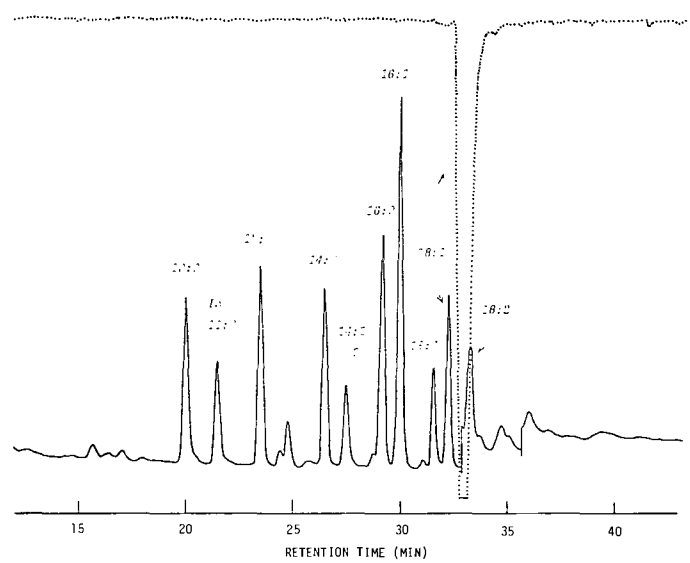

Fig. 5. Radio-gas chromatogram of fatty acyl moieties (as methyl esters) of phospholipids fraction from yeast grown in wort containing ^{14}C(U)-oleic acid. Dotted line represents ^{14}C tracing, 5K.

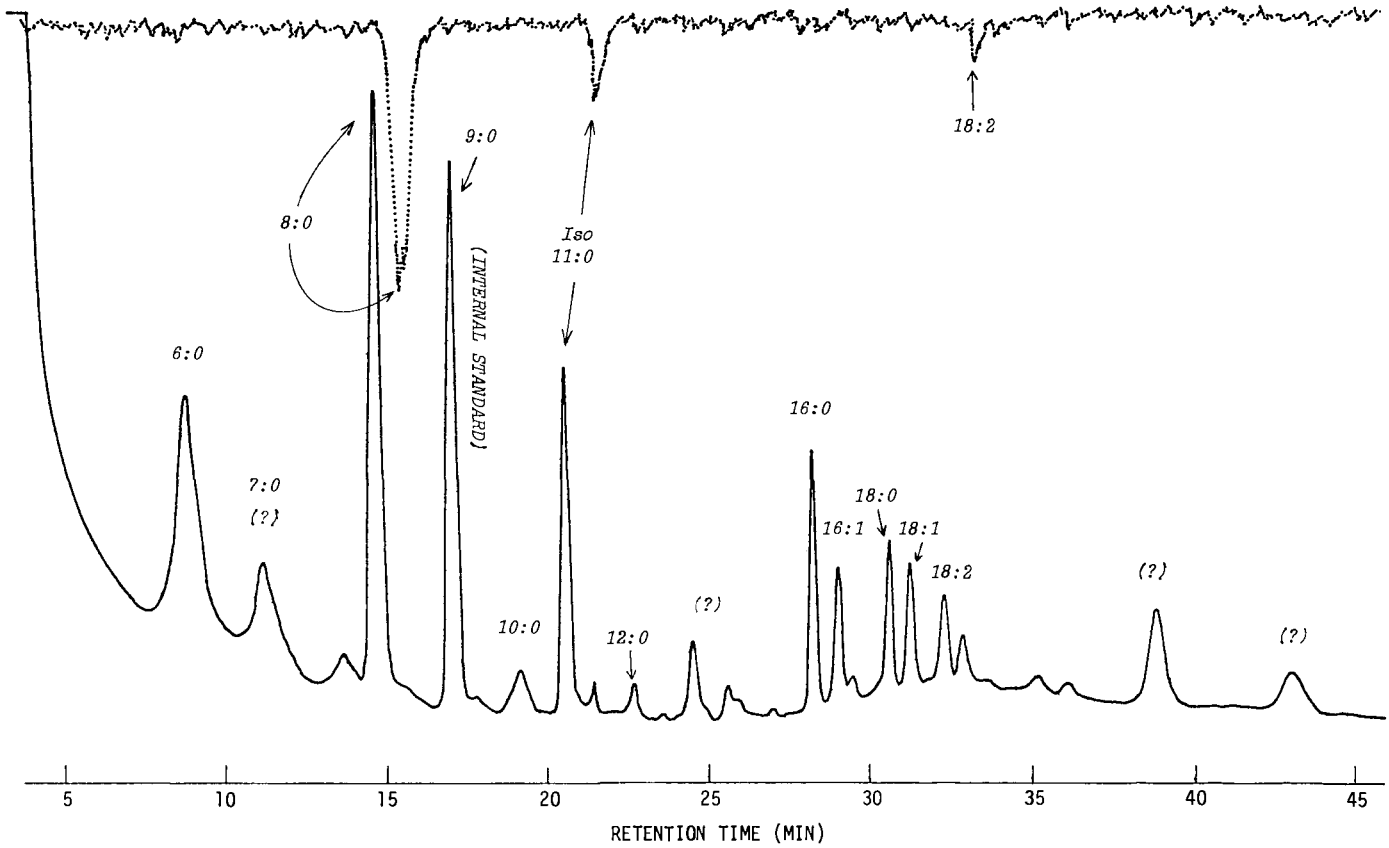

Fig. 6. Radio-gas chromatogram of beer fatty acids (as methyl esters) obtained from fermentation in which 1,2-^{14}C-acetic acid was used. Dotted line represents ^{14}C tracing, IK.

acids were found to be radioactive. Figure 6 presents a radio-gas chromatogram of beer fatty acids obtained from fermentation with 1,2-^{14}C-acetic acid. Radioactivity was found in the most prominent beer fatty acid, C_8, as well as in iso-C_{11} and $C_{18:2}$. Why not all, or almost all, of the beer fatty acids were radioactive is not readily clear. Nevertheless, results obtained thus far indicate that beer fatty acids are not degradation products of long-chain wort fatty acids. The medium-chain beer fatty acids are apparently released from the yeast cell, inside which fatty acids are synthesized through the concerted actions of acetyl CoA carboxylase, synthetase, elongation, and desaturation enzyme systems (13).

Wort fatty acids are therefore utilized primarily for the growth and maintenance of yeast, the well-being and activity of which, in turn, determine the formation of compounds such as fatty acids and a host of other flavor compounds in beer. An area of interest that has attracted a great deal of attention recently is the role of wort polyunsaturated fatty acids in the maintenance of yeast viability. Rose (12) recently recounted some evidence to suggest that polyunsaturated fatty acyl residues of cell lipids were important in conserving and enhancing yeast's tolerance to alcohol. The relationship of wort fatty acids to beer fatty acids is indirect. The effect of long-chain wort fatty acids on the formation of medium-chain beer fatty acids is similar, although different in magnitude and mechanism, to that of growth factors such as biotin and minerals (5). The combined influence of other factors, eg, fermentation environment (11), may accentuate the relationship.

ACKNOWLEDGMENTS

Sincere appreciations are due to J. E. Van Lier, Department of Nuclear Medicine and Radiobiology, University of Sherbrooke, Que., and I. M. Yousef, Department of Biochemistry, University of Montreal, Que., for helpful discussions and use of radio-gas chromatographic and scintillation counting facilities; to G. Van Gheluwe and R. L. Weaver for continuous interest and encouragement; and to M. Dionne for technical assistance. Permission by the management of Molson Breweries of Canada Limited to release this study is also gratefully acknowledged.

LITERATURE CITED

1. Äyräpää, T., Holmberg, J., and Sellmann-Persson, G. *Eur. Brew. Conv., Proc. Congr. 8th, Vienna, 1961,* p. 286.
2. Brenner, R. R. *Mol. Cell. Biochem.* 3(1):41, 1974.
3. Chen, E. C.-H., Jamieson, A. M., and Van Gheluwe, G. *J. Am. Soc. Brew. Chem.* 38:13, 1980.
4. Food and Agriculture Organization of the United Nations. Food and Nutrition Paper No. 3. Report of the Joint FAO/WHO Expert Consultation on the Role of Dietary Fats and Oils in Human Nutrition. FAO: Rome, 1977, p. 17.
5. Forch, M., Krauss, G., and Proksch, H. *Proc. Am. Soc. Brew. Chem.* 33:148, 1975.
6. Forch, M., and Runkel, U.-D. *Eur. Brew. Conv. Monograph-I (Wort Symposium), Zeist, Nov. 1974.* p. 269.
7. Jones, M. O., Cope, R., and Rainbow, C. *Eur. Brew. Conv., Proc. Congr. 15th, Nice, 1975,* p. 669.
8. Klopper, W. K., Tuning, B., and Vermeire, H. A. *Eur. Brew. Conv., Proc. Congr. 15th, Nice, 1975,* p. 659.
9. Kupke, I. R., and Zeugner, S. *J. Chromatogr.* 146:261, 1978.
10. Madyastha, P. B., and Parks, L. W. *Biochim. Biophys. Acta* 176:858, 1969.
11. Pfisterer, E., Hancock, I., and Garrison, I. *J. Am. Soc. Brew. Chem.* 35:49, 1977.
12. Rose, A. H. *Eur. Brew. Conv. Monograph V (Fermentation and Storage Symposium), Zoeterwoude, Nov. 1978.* p. 96.
13. Schweizer, E., Werkmeister, K., and Jain, M. K. *Mol. Cell. Biochem.* 21(2):95, 1978.
14. Taylor, G. T., and Kirsop, B. H. *J. Inst. Brew.* 83:241, 1977.
15. Tripp, R. C., Timm, B., Iyer, M., Richardson, T., and Amundson, C. H. *Am. Soc. Brew. Chem., Proc. 1968,* p. 65.

[Received May 13, 1980]