11. Moulton, K.J., G.C. Mustakas and E.C. Baker, JAOCS 58:1041 (1981).
12. Kruse, N.F., U.S. Patent 2,585,793 (1952).
13. Kruse, N.F., U.S. Patent 2,710,258 (1955).
14. Sipos, E., and N.H. White, JOACS 38:11 (1961).
15. Milligan, E.D., JAOCS 53:286 (1976).
16. Baker, E.C., G.C. Mustakas and K.A. Warner, J. Agric. Food Chem. 27:969 (1979).
17. Schwab, A.W., and H.J. Dutton, JAOCS 25:57 (1948).
18. Official and Tentative Methods of the American Oil Chemists' Society, Vol. 1, 2nd edn., AOCS, Champaign, IL, 1964 (revised to 1969), Methods AC 2-41, AC 3-44, Ba 5-49, Ba 6-61, Ac 4-41, Ba 9-58, Ba 11-65, Ca 12-55, Cd 8-53, Ca 15-75, Ca 2e-55, Ca 9F-57.
19. Hamerstrand, G.E., L.T. Black and J.D. Glover, Cereal Chem. 58:42 (1981).
20. Dupuy, H.P., E.T. Rayner and S.P. Fore, JAOCS 52:354 (1975).
21. Smith, G.N., Arch. Biochem. 19:133 (1948).
22. Black, L.T., G.G. Spyres and O.L. Brekke, AACC 44:152 (1967).
23. Smith, A.K., V.L. Johnsen and R.E. Derges, Cereal Chem. 28: 325 (1951).

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An Emulsion Method for the Sensory Evaluation of Edible Oils[1]

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ABSTRACT

The flavor intensity of soybean oils was evaluated in emulsions stabilized with gum acacia. A 10-point scale was used with a blank to establish the bland end of the scale and a standard diacetyl solution to establish a point near midscale. Tasting oils in emulsion gave significantly different scores than tasting oil directly. Evaluation in emulsion decreased panel error for poor quality oils but not for very bland oils. At least six samples could be tasted in emulsion without casusing panel fatigue or reducing accuracy. The concentration of oil in the emulsion could be adjusted to increase sensitivity to weak flavors or improve the evaluation of intensely flavored oils. Soybean oils containing various amounts of linolenic acid were evaluated by the emulsion method, and those with lesser amounts of linolenic acid were shown to be more stable. A gas chromatographic total volatile method was shown to correlate fairly well with sensory evaluation of oils tasted in emulsions under conditions where both flavors scores and total volatiles changed significantly with time.

INTRODUCTION

The traditional method for the evaluation of oil flavor grew out of research at the Northern Regional Research Laboratory of the United States Department of Agriculture (1-4), A 10-point scale was developed on which a completely bland oil scored 10. To avoid taste carry-over and fatigue, it was recommended that only two samples at a time be tasted and that the samples not be swallowed. Samples were to be smelled first and tasted in order of increasing odor.

The search for objective tests that might correlate with sensory evaluation of fats and oils has been pursued with considerably more diligence than the improvement of sensory evaluation, but satisfactory objective tests have been elusive (5-8). Recently, gas chromatographic measurement of volatiles from fats and oils has been explored, and good correlations between total volatiles and flavor evaluations have been claimed (9-22).

This paper presents a method for the evaluation of the flavor of fats and oils in emulsions stabilized by gum acacia. Correlations between the flavors of oils tasted in this way and a total volatile test are given.

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METHODS

Water for the emulsions was obtained by adding 100 mg $CaCO_3$, 35 mg $MgCO_3$, 10 mg NaCl, and 10 mg Na_2SO_4 per liter of deionized distilled water and gently carbonating with stirring to dissolve the carbonates as bicarbonates. The diacetyl standard emulsion was made by blending 2 g gum acacia powder, 300 mL of water, and 3 mL of diacetyl solution (10 μL diacetyl in 25 mL mineral oil prepared fresh daily) for 1 min. The blank was made by blending 2 g of gum acacia in 400 mL of water for 20 sec. Sample emulsions were made by blending 2 g of gum acacia, 6 mL of sample oil, and 400 mL of water for 1 min. All blending was at the highest speed of the blender (Osterizer pulsematic 10, Milwaukee, WI) in a glass blender jar.

Oil samples were presented in plastic 30-mL cups, emulsions and blank in 90-mL paper cups. All samples were coded with 3-digit random numbers. Red lighting was used to mask color differences in samples. Judging was done in individual booths which provided a quiet, comfortable atmosphere.

A 10-member panel was trained by presenting them with soybean oils and emulsions that had a range of qualities and flavor intensities. After initially scoring the samples, the panel discussed and retasted them to allow the judges to train themselves to rank samples according to the expected degree of oxidation. Efforts were made to keep the panel motivated and interested by providing treats, discussing experimental results, and soliciting suggestions.

Panel members were given the following intructions: "You will evaluate oil-in-water emulsions for flavor intensity using a 1-10 scale. The emulsion marked standard is the same intensity every day and should represent a fixed point on the scale for you. The blank has a score of 10. Score each sample using the flavor intensity of the standard as a reference and the information on the scale describing its end points. Smell the samples before tasting, taste the most oxidized samples last and do not swallow anything". Response was made by marking numbers on a 10-number scale. The number 10 was indicated as bland and tasteless and the number 1 as extremely intense. Rinsing the mouth

between samples was recommended.

Crude commercial solvent-extracted soybean oil was degummed, alkali refined, and deodorized on a laboratory scale. Results from the flavor evaluation of oil deodorized under two conditions are reported. In experiment I, deodorization was at 195 C for 1.5 hr. In experiment II, 235 C for 1.3 hr. No antioxidant was added. The two strains of soybeans having a low linolenic acid content were obtained from the breeding program of W.R. Fehr and E.G. Hammond at Iowa State University. These, along with a cultivar grown under the same conditions, were cracked and extracted in the laboratory by soaking in hexane. The hexane was evaporated and the oils were refined as before. Deodorization was at 195 C for 1.5 hr.

Oils were oxidized at 4, 30 and 55 C and by exposure to light at room temperature (25 C). Light-treated samples were kept ca. 50 cm from two 20-W white fluorescent tubes. The 30 and 55 C-sample were protected from light. Samples at 4 C were protected from light and kept tightly stoppered under nitrogen.

The fatty acid composition of the oils was determined by converting them to methyl esters with sodium methoxide and analyzing on a 2 m x 3.3 mm column packed with 15% EGSSX on Chromosorb W 100/120 mesh at 185 C with a Beckman GC-5 (Beckman, Fullerton, CA) fitted with a flame detector. Peak areas were determined by electronic integration and corrected with a standard methyl ester mixture.

Oil samples were analyzed for total volatiles by the method of Jackson and Giacherio (22). The method was modified by reducing the sample size to 0.2 mL and by using an oil bath for heating the sample tube.

RESULTS AND DISCUSSION

The emulsions required a bland emulsifier and a suitable water supply. Gum acacia was chosen as an emulsifier because it contributed very little flavor and produced stable emulsions. The available tap water contained a considerable amount of iron which might act as a prooxidant, and distilled water gave emulsions a characteristic astringent flavor, so we used distilled water with added minerals and carbonation. Triangle tests were used to determine if the flavor of the emulsions deteriorated within a period of 1 hr. No significant change was detected, allowing ample time for sample preparation and evaluation.

A diacetyl solution was used to make a standard that fixed a point near the middle of the scale for the average panel member. Diacetyl was chosen because its flavor was not objectionable to our panel members at the concentrations used, and it was readily available in pure form. Diacetyl is found in many food products and is characteristic of certain occasionally encountered stages in soybean oil oxidation (23, 24).

Oil storage experiments were conduced in which the scores for emulsions were compared to those of oil tasted directly (Fig. 1). In the first experiment, the flavor as oil and in emulsion were comparable after 1 day's storage at 55 C, but by day 5 the flavor as oil was more intense than in emulsion. In the second experiment, after two day's storage the flavor as oil was already more intense than in emulsion. The difference in scores for oil and emulsions was significant ($P<0.1\%$). Probably oil in emulsion tasted blander than when tasted directly as oil because considerably less oil was actually being tasted in the emulsion form. Since oil frequently is consumed in emulsion (e.g., mayonnaise) it may be that evaluation of oil in emulsion form would give results that reflect consumer experience more accurately.

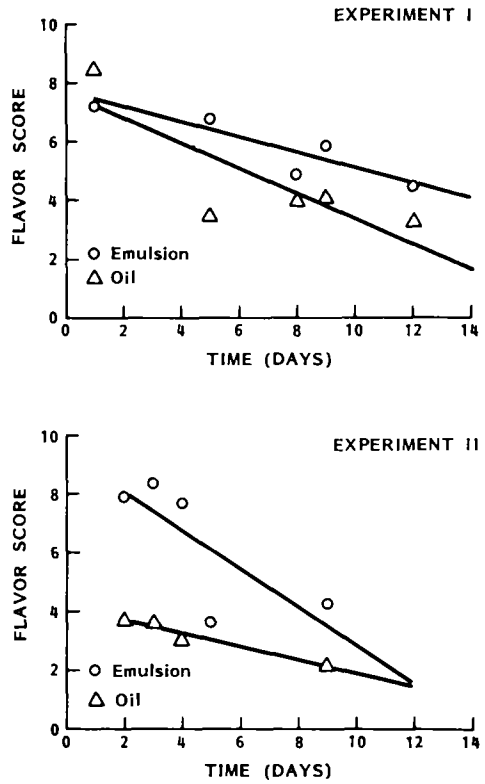

FIG. 1. Flavor scores for soybean oil oxidized at 55 C evaluated as oil and in emulsion. The oil for experiment I was deodorized at 195 C for 1.5 hr. The oil for experiment II was deodorized at 235 C for 1.3 hr.

The concentration of oil in the emulsion is important in the response, as shown in Figure 2. The regression of flavor on oil percentage was significant ($P<.01\%$). This suggestes that oils of high quality may best be tasted in an emulsion containing a higher percentage of oil, whereas scoring more oxidized oil might best be done at a lower concentration to improve the sensitivity of the method.

Oils were oxidized under several conditions to generate a variety of flavor qualities and intensities to evaluate scoring in emulsions. Figure 3 shows the relationships of emulsion flavor scores to time for different storage conditions. The flavor score of oil tasted as emulsion and stored at 4 or 30 C did not change significantly over a 2-week period, but that stored at 55 C did. The oil in experiment II started with a score of at least $\sim$ 8 as shown by the results at day's 2 and 3 for the 4, 30 and 55 C treatments. After two days the lighted sample had dropped to $\sim$ 5. The scores of the radiated sample were significantly different from any of the light-protected treatments.

The standard deviation of the panel scores can be used as a measure of panel performance. The smaller the standard deviation, the smaller the difference in flavor that can be observed. Table I gives the average standard deviation of the observations made on oils of good (score 7-8) and poor (score 4-5) quality during the course of the experiments reported in Figure 1, 2 and 3. Tasting in emulsion rather than oil did not increase panel accuracy significantly for good quality oil, but for poor quality oil, the standard deviation of the panel was significantly lower for emulsions than for oil. The standard deviation was significantly greater for poor quality oil than for good quality oil when tasted as oil but not as emulsion. Similar results were

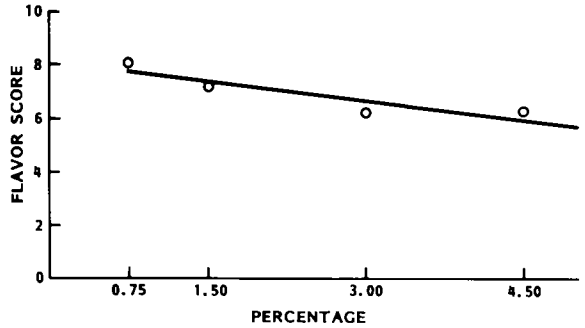

FIG. 2. Effect of oil concentration of emulsion on flavor scores. 1.5% oil was used in other experiments. The oil was that used in experiment II.

TABLE I

Average Standard Deviations[a] of Flavor Scores for Panels of 8-11 Members Tasting Oils of Good and Poor Quality Both Directly and in Emulsions

	Emulsions tasted per session		Oil tasted per session
Flavor score	2	6	2
7-8	1.72 ± .45	1.43 ± .32	1.79 ± .55[b]
4-5	2.00 ± .32	1.56 ± .49[c]	2.39 ± .41[b,c]

[a]These are panel standard deviations for individual samples averaged over several samples and days of observation. They are not standard deviations of the panel means. The number after the ± is the standard deviation of the panel standard deviations.

[b,c]Values having the same letter were significantly different at $P < .05$ by a t-test. Pooled values for all emulsions were significantly lower than pooled values for oils at $P < .05$. Pooled values for emulsions having flavors at 4-5 were significantly lower than those or the corresponding oils at $P < .05$.

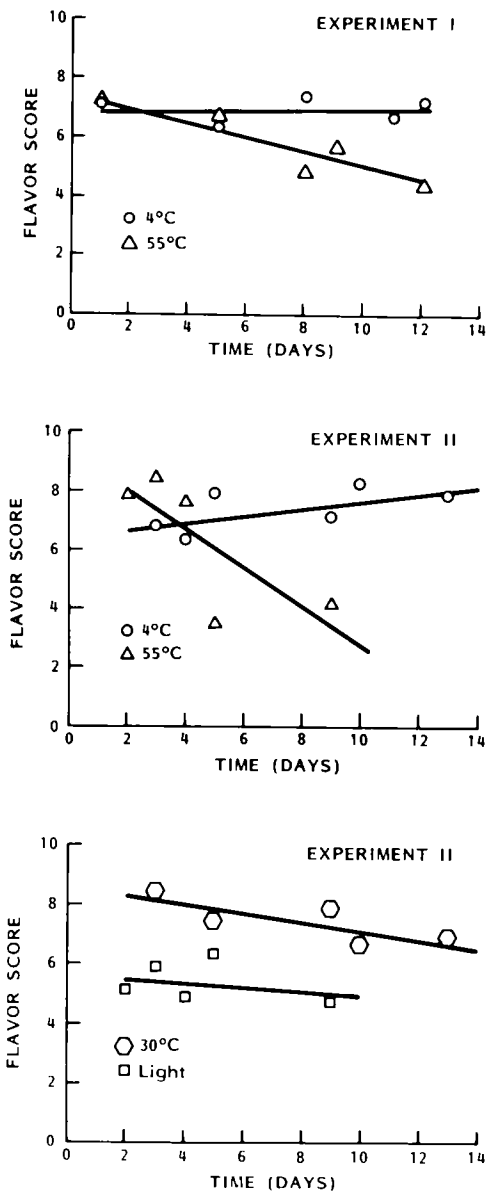

FIG. 3. Flavor scores for soybean oil oxidized under various conditions evaluated in emulsion. Experiments I and II represent two batches of oil deodorized under different conditions.

reported by Evans (4) who found average standard deviations of 0.78 when tasting oil scoring 8-10 and 1.22-1.36 when scoring oil of poorer quality. The average standard deviations of our panel were greater than those of Evans. The variance of our panel might have been reduced by more intensive selection or training to get the panel to respond more uniformly to the oxidized samples and the standard. But when such practices are used to improve panel performance, it is possible that unintended biases are induced in the panel. We had hoped that the use of the diacetyl standard would give standard deviations as small for our panel as those reported by more rigorously trained and selected panels, but this seemed not to be so. Possibly by selecting panel members who reacted uniformly to the diacetyl standard, performance might be improved without resorting to methods that may introduce biases. When the panel was asked to rate the diacetyl standard on a 10-point scale, they scored it from 3 to 7 with an average of 6.

When emulsions were tasted, the number of samples could be increased to at least six without causing an observable increase in panel standard deviation as shown in Table I. Thus, there was no indication that this many samples caused fatigue or reduced accuracy. It is assumed from reports in the literature (2, 3) that tasting more than two oil samples would have increased panel standard deviation.

The ratio scaling technique called magnitude estimation was tried in an attempt to improve the panel's performance. In this technique, one sample is assigned an arbitrary numerical value and others are scored relative to it. For example, if the second sample is judged half as intense as the first, it is assigned a score half as big (25). No statistically significant improvement was found for samples scored by magitude estimation vs the 10-point scale. The panel generally preferred the 10-point scale.

Soybean lines with reduced linolenic acid content were available from a local breeding program. We wished to see if the panel could detect differences in flavor stability of oil from these strains and normal soybean oil. Figure 4 shows the regression lines for the emulsion flavor scores for these soybean oils oxidized at 55 C. The fatty acid composition of the low-linolenic acid strains and the cultivar with which they were compared are shown in Table II. The cultivar and FA 27168 were grown in 1980, FA 9656 in 1979.

In Figure 4 only two lines appear for flavor scores vs time because the scores from the two low-linolenic acid varieties were almost identical. Even so, the flavor scores for FA 27168 generally were slightly better than those for

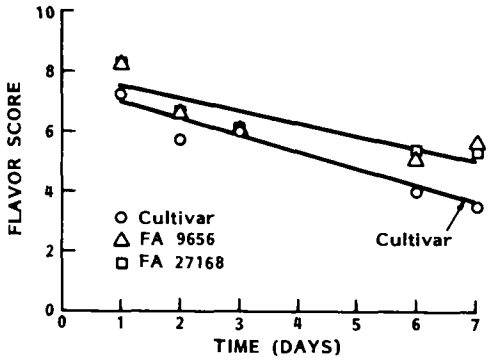

FIG. 4. Flavor scores obtained during storage at 55 C of oil from low linolenic acid soybeans and a commercial variety.

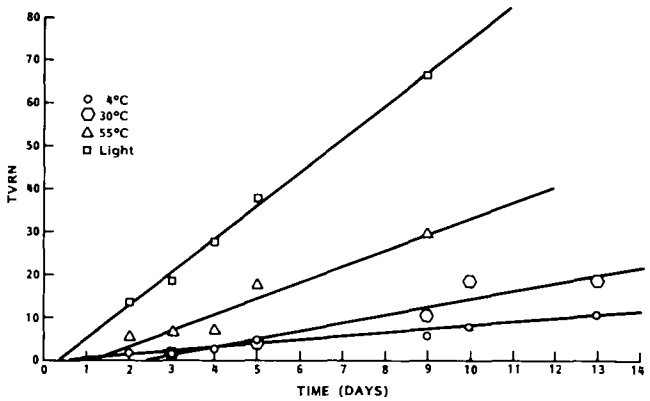

FIG. 5. Total volatiles relative to nonane for soybean oil stored under a variety of conditions.

TABLE II

Fatty Acid Composition of Soybean Oils

Variety	16:0	18:0	18:1	18:2	18:3
Cultivar	10.81	3.73	24.71	53.42	7.30
FA 9656	9.59	5.28	42.73	37.81	4.57
FA 27168	9.85	3.86	43.77	38.59	3.69
Oil used in storage tests	11.51	4.35	24.32	53.03	6.76

FA 9656. The regression for the low-linolenic acid varieties is significantly different ($P<.05$) from the line for the cultivar. An analysis of variance showed that the means of the cultivar and low-linolenic varieties also were significantly different ($P<.01$). Clearly, the flavor scores for the oils having lower than normal linolenic acid contents are higher. This supports the belief that linolenic acid is mainly responsible for the flavor instability of soybean oil (7, 26).

The relative error of the total-volatile measurements varied considerably and at times were greater than the 8% reported by Jacksion and Giacherio (22). It is not clear if this is caused by our modifications or incomplete specifications of the controls that are needed. In any event, this contribution to variance generally was small compared with that of the taste panels.

Deodorization conditions influenced the initial level of volatiles. In the course of this study, oils were deodorized at temperatures ranging from 190 to 240 C and for times varying from 1.5 to 3 hr. In agreement with Moser et al. (27), we found that these conditions gave quite bland oils. The total volatiles relative to the internal standard, nonane (TVRN), averaged 18.4 and ranged from 1.7 to 67 in the freshly deodorized oils, and the TVRN correlated poorly with initial flavor scores ($r^2=0.31$) tasted as emulsions. This may be because the kinds of volatiles left in the oil vary with deodorization conditions.

Figure 5 shows typical relationships of time to TVRN for oil oxidized under several conditions. Correlations of TVRN and flavor scores for all experiments are shown in Table III. Generally the volatiles increased linearly with time. Although the flavor scores were fit with linear regressions because the variance was great enough to obscure any nonlinear effects, some of the data suggest that flavor changes nonlinearly with time. In spite of this, the correlation between flavor and TVRN generally was good under

TABLE III

Correlations of TVRN with Flavor Scores for All Experiments

Experiment	Correlation
Oil vs emulsion/storage condition	
Expt. I	
4 C emulsion	0.06
4 C oil	0.26
55 C emulsion	-0.89[a]
55 C oil	-0.82
Expt. II	
4 C emulsion	0.74
4 C oil	-0.63
55 C emulsion	-0.87[a]
55 C oil	-0.81
30 C emulsion	-0.87[a]
Light-treated emulsions	-0.31
Low 18:3-55 C emulsions	
Commercial cultivar	-0.67
FA 9656	-0.34
FA 27168	-0.82

[a]Significant $P < .06$.

conditions in which there was sufficient change in flavor scores with time, that is, at 55 C. These results are similar to correlations between flavor scores and analytical data obtained previously (17, 19, 22). At 4 C the correlation was poor because the panel detected no significant score change, although the TVRN increased slightly. The rather large positive correlation between TVRN and flavor score for emulsion at 4 C in experiment II reflects these small and insignificant flavor score changes. In this instance the small changes in flavor score chanced to show a fairly steady positive trend and resulted in a large but insignificant and meaningless positive correlation. In the light-treated sample, correlation of TVRN and flavor score also was poor because the flavor scores had already dropped to a low value in the two days before tasting began and therefore the flavor score did not decrease much. The TVRN showed a contrasting steady rise over the period of observation. To obtain good correlation between TVRN and flavor scores, it is important to have both variables change significantly during the period of observation.

REFERENCES

1. Cowan, J.C., Conference on Deterioration of Fats and Oils US Quartermaster Food Institute, Washington, DC, 1945.
2. Moser, H.A., C.M. Jeger, J.C. Cowan and H.J. Dutton, JAOCS 24: 291 (1947).

3. Moser, H.A., H.J. Dutton, C.D. Evans and J.C. Cowan, Food Technol. 4:105 (1950).
4. Evans, C.D., JAOCS 32:596 (1955).
5. Fioriti, J.A., M.J. Kanuk and R.J. Sims, JAOCS 51:219 (1974).
6. Gray, J.I., JAOCS 55:539 (1978).
7. Smouse, T.H., JAOCS 56:747A (1979).
8. Jackson, H.W., JAOCS 58:227 (1981).
9. Scholz, R.G., and L.R. Ptak, JAOCS 43:596 (1966).
10. Evans, C.D., G.R. List, R.L. Hoffman and H.A. Moser, JAOCS 46:501 (1969).
11. Selk, E., H.A. Moser and W.K. Rohwedder, JAOCS 47:393 (1970).
12. Jarvi, R.K., G.D. Lee, D.R. Erickson and E.A. Butkus, JAOCS 48:121(1971).
13. Herman, K.T., L.C. Rose and R.L. Vandaveer, JAOCS 48:178 (1971).
14. Dupuy, H.P., S.P. Fore, and L.A. Goldblatt, JAOCS 48:876 (1971).
15. Dupuy, H.P., S.P. Fore and L.A. Goldblatt, JAOCS 50:340 (1973).
16. Dupuy, H.P., E.T. Rayner and J.I. Wadsworth, JAOCS 53:628 (1976).
17. Dupuy, H.P., E.T. Rayner, J.I Wadsworth and M.G. Legendre, JAOCS 54:445 (1977).
18. Williams, J.L., and J.H. Willie, JAOCS 53:634 (1976).
19. Williams, J.L., and T.H. Applewhite, JAOCS 54:461 (1977).
20. Warner, K., C.D. Evans, G.R. List, H.P. Dupuy, J.L. Wadsworth and G.E. Goheen, JAOCS 55:252 (1978).
21. Waltking, A.E., and H. Zmachinski, JAOCS 54:454 (1977).
22. Jackson, H.W., and D.J. Giacherio, JAOCS 54:458 (1977).
23. Seals, R.G., and E.G. Hammond, JAOCS 43:401 (1966).
24. Seals, R.G., and E. G. Hammond, JAOCS 47:278 (1970).
25. O'Mahony, M., J. Dairy Sci. 62:1954 (1979).
26. Dutton, H.J., C.R. Lancaster, C.D. Evans and J.C. Cowan, JAOCS 28:115 (1951).
27. Moser, H.A., P.C. Cooney, C.D. Evans and J.C. Cowan, JAOCS 43:632 (1966).

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Lipid from Yeast Fermentation: Effects of Cultural Conditions on Lipid Production and Its Characteristics of *Rhodotorula glutinis*

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ABSTRACT

To produce lipids from microbial origins, *Rhodotorula glutinis* (syn. *Rhodotorula gracilis*) NRRL Y-1091 was cultured in batch and continuous systems under nitrogen- and carbon-limited conditions. The lipid production patterns are shown to be different from each other depending on growing conditions. In continuous cultures under nitrogen-limited conditions, the maximum lipid accumulation was observed at the lowest dilution rate examined, giving the efficiency of substrate conversion of 16.4 g lipid per 100 g glucose consumed. As the dilution rate increased, cell biomass, lipid content, lipid productivity and lipid yield decreased. In carbon-limited continuous cultures, cell biomass decreased with increasing dilution rate, but lipid content remained almost constant. Neutral lipid portions in nitrogen-limited cultured yeast cells decreased as the dilution rate increased, and glyco- and phospholipid portions showed the reverse trend. Major components in the neutral lipid portions in yeast cells are triglyceride, free fatty acid, steryl ester and sterol. Phosphatidylserine was the predominant phospholipid in yeast cells. The dilution rate also affected the fatty acid composition of all lipid portions; polyunsaturated fatty acids increased and saturated and monounsaturated fatty acids decreased with increasing dilution rates. The degrees of unsaturation of each lipid class and total lipids were also increased by increasing the dilution rate.

INTRODUCTION

With the increasing demands for fats and oils for edible and industrial purposes, many assessments have been made to find new possible lipid sources other than the conventional plant and animal sources. As a result of such assessments, the lipid from microbial origins was found to be a possible source. As a matter of fact, the microbial lipid production has long been an attractive subject of research interests in both laboratories and industries (1–4).

Most of the organisms used for lipid production were yeast, fungi and algae (5, 6). Lipid production with *Rhodotorula glutinis* (syn. *Rhodotorula gracilis*), one of the oleaginous yeasts used widely, have been carried out for a long time and continued to recent years (7–9). The characteristics of lipids of *R. glutinis* obtained from batch cultures were well established with respect to the effects of nutrient sources, pH and temperature (10–12).

The continuous cultivation of *R. glutinis*, however, was first performed by Ratledge and Hall to study the oxygen demands under nitrogen- and carbon-limited conditions (13). In another work of Ratledge and Hall (14), they reported that the fatty acid compositions of total lipids were unchanged depending on the growth rates under nitrogen-limited conditions, but slight changes were observed under carbon-limited conditions. In contrast to the results of Ratledge and Hall (14), we found that the fatty acid compositions of total lipids were changed as the growth rate altered under nitrogen-limited conditions and, therefore, the degrees of fatty acid unsaturation were also changed (15).

The analyses of lipids from *R. glutinis* in the abovementioned studies (14, 15) were all aimed at the analyses of total lipids. The characterization of fractionated lipid classes in *R. glutinis* and fatty acid compositions of each lipid class have not been reported elsewhere to our knowledge. In this paper, we report the lipid production data obtained from batch and continuous cultures and the results of analyses of lipid classes in *R. glutinis*; we believe this is the first report to elucidate the quantitative and qualitative changes in lipid classes depending on the growth conditions.

EXPERIMENTAL PROCEDURES

Materials

Standard materials used in thin layer chromatography

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