THE CHEMISTRY OF THE LIPIDS OF YEAST

I. THE COMPOSITION OF THE ACETONE-SOLUBLE FAT*

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Although a large number of publications are recorded on the chemical composition of yeast, very few reports are found dealing with the lipids. Early work reported by Naegeli and Loew (1), Gerard and Darexy (2), Hinsberg and Roos (3), Neville (4), and MacLean and Thomas (5) would indicate that yeast contains both saturated and unsaturated fatty acids. The presence of palmitic, stearic, and arachidic acids has been established as well as that of oleic, linoleic, and an unsaturated acid in the C18 series. Other fatty acids such as butyric, lauric, a saturated C24 acid, and various oxygenated acids have been reported but without very definite proof of their identities. Evidence has also been presented by Weichherz and Merlander (6) and by Weiss (7) for the presence of an optically active valeric acid.

In order to secure more information concerning the composition of the alcohol-ether-soluble constituents of living yeast, we have devoted some time to the study of yeast lipids. Moreover, we were interested in discovering whether the fat from a unicellular organism such as yeast contained any acids similar to tuberculosteamic or phthioic acids found in tubercle bacilli.

Through the cooperation of The Fleischmann Laboratories, New

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York, we were provided with large quantities of fresh living moist yeast. The lipids were extracted with mixtures of alcohol and ether and were separated into phospholipids and acetone-soluble fat. In this paper we report upon the general procedures employed and upon the composition of those lipids soluble in cold acetone.

The results obtained indicate that the lipids of yeast are very similar in composition to the fats found in ordinary plants and animals. The neutral fat is composed of mixed glycerides of saturated and unsaturated fatty acids; the unsaturated acids representing about 80 per cent of the total acids. No saturated liquid fatty acids similar to phthioic acid (8) are present. By judging from the acetyl value, a small amount of hydroxy acids was at hand, but we were unable to isolate any such acid. The fat obtained by our method of extraction from living yeast cells contains only a very small amount of volatile fatty acids and we did not find even a trace of the valeric acid referred to by Weichherz and Merlander (6) and by Weiss (7).

A thorough search was made for an acid of a higher molecular weight than stearic acid, such as the arachidic acid reported by Neville (4) and by MacLean and Thomas (5), but we were unable to find any such acid in any of the lipid fractions.

EXPERIMENTAL

The fresh moist yeast (*Saccharomyces cerevisiae*) was supplied in 10 or 15 pound lots as needed by The Fleischmann Laboratories. The yeast was grown for a period of 8.5 hours in a thoroughly aerated molasses medium containing ammonium phosphate. It was then collected by centrifugation, cooled, and pressed.

The extraction of the lipids as well as other operations was carried out according to the methods used in this laboratory in investigations of the lipids of acid-fast bacteria (9). The air was always excluded as much as possible by using an atmosphere of carbon dioxide or nitrogen and all solvents were freshly distilled and saturated with an inert gas before use. In every case in which filtrations or decantations were made, the insoluble material was washed with an appropriate solvent. For the sake of brevity these obviously necessary operations are not mentioned.

*Extraction of Lipids*—The yeast was first partly dehydrated by digestion with alcohol after which it was filtered and treated twice.
at room temperature for 2 days with mixtures of alcohol and ether. The extracts were combined and concentrated under reduced pressure. The lipids were extracted from the aqueous residual suspension with ether. After drying, the ethereal extracts were filtered through a Chamberland candle filter and the solvent removed. The dark oil thus obtained weighed 1063 gm. or 6.02 per cent of the dry yeast.

The extracted yeast was further treated with alcohol containing 1 per cent of hydrochloric acid for 2 days at a temperature of 30-35°. By this extraction a further 150 gm. of a dark ether-soluble oil was obtained and designated acid-extraction fat.

Additional extractions with strong acids, chloroform, or other solvents yielded only insignificant amounts of ether-soluble materials. The yeast was therefore dried in the air and stored in bottles. A total of 60 kilos of moist yeast was extracted and the air-dried residue weighed 16.4 kilos.

Separation of Lipid Fractions—The crude lipids were dissolved in 1 liter of absolute ether and 2 volumes of acetone were added. The phospholipids separated as a sticky mass and, after cooling, the supernatant liquid was decanted. This solution, after it had been concentrated until the ether was removed, was again cooled and decanted from an insoluble oily product. The final solution was concentrated to dryness and the residue, which weighed about 700 gm., was designated acetone-soluble fat.

The oily substance mentioned above was dissolved in ether and the solution treated with 2 volumes of acetone. A sticky mass which separated was collected and added to the original phospholipid fraction. The ether-acetone solution on evaporation to dryness left an oily residue weighing about 150 gm., which was designated acetone-insoluble fat.

Purification of Phospholipids—The phospholipids were dissolved in ether and precipitated by adding 2 volumes of acetone. The supernatant solution was decanted and the precipitated material redissolved in ether and again separated by the addition of acetone. After repeating these operations twenty times, the phospholipids were collected and dried in vacuo. The material was a brownish mass weighing 154 gm. The mother liquors were concentrated to dryness and the small residue added to the acetone-insoluble fat.
For analysis the phospholipids were dried to constant weight at 56° in vacuo over phosphorus pentoxide. The dried preparation was brittle and very hygroscopic.

\[
\begin{align*}
0.2096, 0.2231 \text{ gm. substance: } 0.0314, 0.0340 \text{ gm. } \text{Mg}_2\text{P}_2\text{O}_7; \\
0.5452, 0.6652 \text{ " " (Kjeldahl): } 6.85, 8.55 \text{ cc. } 0.1 \text{ N } \text{HCl} \\
0.4047 \text{ gm. substance gave } 0.0370 \text{ gm. ash} \\
\text{Found: } P \ 4.17, 4.24; N \ 1.75, 1.80; \text{ ash } 9.14
\end{align*}
\]

The ratio of nitrogen to phosphorus is as 1:1.07.

The composition of the phospholipids will be reported in a separate paper.

\textit{Acetone-Soluble Fat. Analytical Constants}—The following constants of the fat were determined according to the official methods (10).

\[
\begin{align*}
\text{Saponification No.} & \quad 109.6 \\
\text{Free acid No.} & \quad 28.6 \\
\text{Iodine No. (Hanus)} & \quad 61.3 \\
\text{Reichert-Meisel No.} & \quad 2.3 \\
\text{Polenske No.} & \quad 0.5 \\
\text{Acetyl No.} & \quad 20.2^* \\
\text{Unsaponifiable matter, per cent.} & \quad 46.7
\end{align*}
\]

* The acetyl number was determined after the fat had been standing in a closed flask for about 2 years. It is possible therefore that a certain amount of oxygenated acids may have been formed by oxidation of the unsaturated acids during this time.

\textit{Saponification}—A portion of the fat weighing 201.3 gm. was saponified by refluxing for 1 hour with an excess of alcoholic potassium hydroxide after which the solution was diluted with water and extracted with ether. The ether was distilled off and the residue was again refluxed with alcoholic potassium hydroxide, the unsaponifiable matter being extracted with ether as before. A light yellow oil weighing 92 gm., or 45.6 per cent of the fat, composed this fraction.

The fatty acids were extracted with ether after the soap solution had been acidified with hydrochloric acid. The acids formed a thick oily mass weighing 95.6 gm. or 47.4 per cent of the fat.

For the isolation of the water-soluble constituents, the acidified solution, after extraction of the fatty acids, was evaporated to dryness under reduced pressure and extracted in the usual manner.
with absolute alcohol. The yield of crude glycerol was 11.9 gm. or 5.9 per cent of the fat.

Unsaponifiable Matter—The unsaponifiable matter when tested by the Hanus method was found to have an iodine number of 7, thus indicating that the bulk of the material consisted of saturated compounds. A small amount of crystalline sterols which separated from the oil on standing was removed by filtration and was not further examined.

In order to determine the chemical composition of the oily portion of the unsaponifiable matter, it was necessary to remove the sterols completely. This object was successfully accomplished by the application of a modified Liebermann-Burchard reaction. A similar procedure has been used by Windaus and Resau (11) for the separation of saturated from unsaturated hydrocarbons in the cholesterol series and by Anderson and Nabenhauer (12) for the separation of saturated from unsaturated sterols.

The reaction was carried out in a separatory funnel. About 25 gm. portions of the oil were dissolved in 250 cc. of carbon tetrachloride, 50 cc. of acetic anhydride were added, and then 15 cc. of concentrated sulfuric acid were added drop by drop with shaking and cooling. The deep green reaction mixture was carefully diluted with a little water and allowed to stand until a dark colored acid layer separated on top. The nearly colorless carbon tetrachloride layer was drawn off, washed with dilute alkali and water, and dried over sodium sulfate. A light yellow oil was obtained on removing the solvent. The oil gave no sterol color reactions and did not absorb any iodine when tested with Hanus solution.

This oil was separated by fractional distillation at very low pressures into a series of fractions ranging in boiling point from 72–200°. The various fractions were repeatedly refractionated without yielding any clear cut pure fractions. All distillates were colorless oils. The apparently purest cuts were analyzed and found to be saturated hydrocarbons corresponding to the general formulas C<sub>n</sub>H<sub>2n</sub> and C<sub>n</sub>H<sub>2n-2</sub>. The composition and molecular weight of the lowest fraction corresponded to the formula C<sub>19</sub>H<sub>38</sub>, a middle fraction to C<sub>30</sub>H<sub>60</sub>, and the highest to C<sub>34</sub>H<sub>66</sub>.

Since it appeared improbable that living cells would produce such a large amount of their ether-soluble constituents in the form of saturated cyclic and bicyclic hydrocarbons, we examined the
unsaponifiable matter prepared from a small lot of yeast grown under carefully controlled conditions in The Fleischmann Laboratories, New York. In this case we found the unsaponifiable matter to consist entirely of crystalline sterols. We conclude, therefore, that the hydrocarbons described above were not derived from the yeast fat but were present as accidental contaminations. In this connection it is interesting to note that Daubney and MacLean (13) mention a yellow saturated oil as occurring in large amount in the unsaponifiable matter of yeast fat. It is likely that this oil was introduced accidentally into their yeast in the same unknown manner in which it was introduced into the yeast used in this investigation.

Examination of Fatty Acids—The mixed fatty acids formed a semisolid mass with an iodine number of 102. They were separated by means of the lead soap-ether treatment (14) into 74.9 gm. or 78 per cent of liquid acids and 17.4 gm. or 18 per cent of solid acids.

Liquid Acids—The iodine number (Hanus) of the liquid acids was 119. After catalytic reduction (15) the acids were again separated by means of the lead soap-ether procedure (14) into 56.7 gm. of solid reduced acids and 7.4 gm. of liquid acids. The latter material still had an iodine number of 66. Several attempts were made to reduce this product after thorough purification but the final acid still had an iodine number of 52.

A larger quantity of mixed acids obtained from yeast secured from The Fleischmann Laboratories was examined in the manner described above. In this case also a small amount of liquid fatty acids, having an iodine number of 55, was obtained. These also could not be further reduced by catalytic reduction. While the nature of the unreducible fraction of the liquid acids could not be determined, it is evident that the acetone-soluble fat of yeast does not contain any liquid saturated fatty acids analogous to those obtained from acid-fast bacteria.

General Procedure for Examination of Fatty Acids—The fatty acids were converted into their methyl esters and the latter were carefully fractionated at pressures varying from 0.002 to 0.005 mm. For these fractions the melting point and index of refraction at 55° were taken as criteria of purity. The purest fractions were then saponified and the free acids recrystallized. The melting point
and mixed melting point with the corresponding authentic acid were then taken and the molecular weight determined by titration of an alcoholic solution of the acid with 0.1 \( n \) alcoholic potassium hydroxide. Finally, from a consideration of the amounts and the indices of refraction of all the ester fractions, the percentage composition of the esters, and hence the acids, was approximated.

**Solid Fatty Acids**—A small amount of a low-boiling ester was obtained. This melted at 19–20° and on saponification yielded an acid melting at 53.5–54.5°. A mixed melting point with myristic acid showed a depression of 3.5°. The molecular weight was 236 as compared with 228 for myristic acid. Hence this acid is probably a mixture of palmitic with some lower acid, possibly lauric. The amount obtained was too small to permit of complete identification.

Two pure ester fractions were obtained and were shown to be methyl palmitate and methyl stearate by the following data.

*Fraction I*—M. p. 28–29°; \( n_d^{55} = 1.4260; \) free acid m. p. 62–63°.

0.8821 gm. substance required 34.26 cc. 0.1 \( n \) KOH

\[ \text{C}_{15} \text{H}_{31} \text{O}_2 \] Calculated mol. wt., 256; found, 257

*Fraction II*—M. p. 38–39°; \( n_d^{55} = 1.4300; \) free acid m. p. 71–71.5°.

0.8156 gm. substance required 28.49 cc. 0.1 \( n \) KOH

\[ \text{C}_{17} \text{H}_{35} \text{O}_2 \] Calculated mol. wt., 284; found, 286

The indices of refraction at 55° were determined for methyl palmitate and methyl stearate and found to be 1.4260 and 1.4300 respectively.

The solid fatty acids were composed of about 75 per cent palmitic acid and 25 per cent stearic acid.

**Solid Reduced Acids**—Two pure ester fractions were obtained and were shown to be methyl palmitate and methyl stearate by the following data.

*Fraction I*—M. p. 28–29°; \( n_d^{55} = 1.4260; \) free acid m. p. 62–63°.

0.6503 gm. substance required 25.27 cc. 0.1 \( n \) KOH

\[ \text{C}_{15} \text{H}_{31} \text{O}_2 \] Calculated mol. wt., 256; found, 257

*Fraction II*—M. p. 38–39°; \( n_d^{55} = 1.4300; \) free acid m. p. 71–71.5°.

0.8847 gm. substance required 31.24 cc. 0.1 \( n \) KOH

\[ \text{C}_{17} \text{H}_{35} \text{O}_2 \] Calculated mol. wt., 284; found, 283
These acids were composed of about 25 per cent palmitic acid and 75 per cent stearic acid.

Examination of Acetone-Insoluble Fat and Acid-Extraction Fat—With regard to the presence of an acid of higher molecular weight than stearic acid, the acetone-insoluble fat and the fat obtained by extracting the cell residues with alcohol containing 1 per cent of hydrochloric acid were also examined.

The acetone-insoluble fat had an iodine number of 42. About 45 gm. of fat were saponified and worked up in the manner described above for the acetone-soluble fat. The unsaponifiable matter amounted to 24.7 gm. or 56 per cent, the fatty acids to 17.4 gm. or 39 per cent, and the water-soluble fraction to 2 gm. or 4.5 per cent. The fatty acids were separated by the lead soap-ether treatment (14) into 1.6 gm. of solid acids and 14.9 gm. of liquid acids having an iodine number of 105.

The acid-extraction fat had an iodine value of 54. About 65 gm. of fat were saponified as above, yielding 23.9 gm. or 37 per cent of unsaponifiable matter, 31.4 gm. or 49 per cent of fatty acids, and 9.2 gm. or 14 per cent of water-soluble constituents. The fatty acids were separated as above into 2.9 gm. of solid acids and 27.3 gm. of liquid acids having an iodine number of 101.

The solid acids from these two sources were combined, converted into methyl esters, and separated by fractional distillation under highly reduced pressure into two fractions. The lower boiling fraction weighed 2.5 gm., melted at 24° and $n_5^5 = 1.4285$. The higher boiling fraction weighed 1.36 gm., melted at 30° and $n_5^5 = 1.4307$. On saponification, this fraction yielded an acid melting at 67° after several recrystallizations. The molecular weight was 288. It is evident from the above data that no appreciable amount of an acid higher than stearic acid is present.

Identification of Glycerol—The crude glycerol mentioned before was a dark colored thick syrup. When a portion was heated with acid potassium sulfate, the characteristic odor of acrolein was noticed. The syrup did not give the Molisch test for carbohydrates and did not reduce Fehling's solution either before or after boiling with dilute acid. At a pressure of 2 to 3 mm. the syrup distilled at 144–146° giving a light yellow distillate. 1 gm. of this syrup was benzoylated according to the method of Einhorn and Hollandt (16). The reaction product was recrystallized from
absolute methyl alcohol. Beautiful prismatic needles were obtained which melted at 75–76° and showed no depression of the melting point when mixed with authentic glycerol tribenzoate. The results indicate that glycerol is the chief water-soluble constituent of the acetone-soluble fat of yeast.

In conclusion we acknowledge with pleasure our indebtedness to Dr. C. N. Frey of The Fleischmann Laboratories, New York, for supplying the yeast used in this investigation.

Anyone interested in further details of this work may consult the thesis by M. S. Newman on file in the Yale University Library.

SUMMARY

Fresh living yeast was extracted with alcohol and ether. The lipids obtained amounted to 6.02 per cent calculated on the dried yeast as a basis. Further extraction of the partly defatted cells with alcohol containing 1 per cent of hydrochloric acid yielded material amounting to 0.86 per cent calculated on dry yeast.

The alcohol-ether-soluble lipids were separated into phospholipids, acetone-insoluble fat, and acetone-soluble fat.

The acetone-soluble fat was saponified and the following substances obtained: sterols, a mixture of saturated cyclic and bicyclic hydrocarbons ranging from C_{12}H_{28} to C_{34}H_{66} present as an impurity in the yeast used, glycerol, and fatty acids. The saturated acids consisted of about 75 per cent of palmitic and 25 per cent of stearic acid, together with a trace of some acid lower than palmitic acid. The unsaturated acids on catalytic reduction gave a mixture of about 25 per cent of palmitic and 75 per cent of stearic acid.

No appreciable amount of an acid higher than stearic acid was found in the acetone-insoluble fat or in the fat obtained by extraction of the yeast cells with alcohol containing 1 per cent of hydrochloric acid.

BIBLIOGRAPHY

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