liquor was removed and sterilized at 15 pounds steam pressure (121° C.) for storage.

Before use the liquor was filtered through a layer of diatomaceous earth and resterilized in desired quantities. This liquor contained an average of 0.820% sugars, of which 0.251% was reducing sugars, and 0.0053% available nitrogen. The pH varied from 4.4 to 4.5. Waste liquor from protein precipitated with sulfur dioxide, however, did not support yeast growth unless the sulfur dioxide was removed.

The organism used for propagation was *Torulopsis utilis* from culture No. Y-957 of the Northern Regional Research Laboratory, U.S.D.A. The yeast used for inoculum was grown in sweet potato media containing 1% sugar, and the quantity of cells present was estimated from the centrifuged cell volume in 15 ml. The amount of a 16-hour culture that contained 10.5 ml. of wet cells was used to seed six liters of media.

Propagations were carried out aseptically using 6-liter quantities in 9-liter pyrex bottles, 7½ x 15 inches in size, fitted with two 1-inch Aloxite ball aerators and a siphon for sampling (see Figure 1). Reducing

FIG. 1. Propagators used for experimental production of yeast from peanut protein waste liquors.

sugars, pH, and the volume of cells in 15 ml. (observed in graduated centrifuge tubes) were determined every hour. One-fourth of a cubic foot of air per minute was supplied to each propagator, and the temperature was kept at 33-34° C.—conditions found to be desirable in previous work with sweet potato waste water (7).

Sugars were determined on centrifuged culture suspensions by the method of Shaffer and Hartmann (10) before and after inversion with hydrochloric acid. Ammonia- and amino-nitrogen was estimated in the waste water according to the Sorenson formol titration method (1). Yields of yeast were determined by measuring the dry weight of yeast in 15 ml. at the end of a 24-hour propagation period, subtracting the weight of yeast added as inoculum, and comparing the increase in yeast weight to the amount of sugar originally available in the liquor.

Nutritive Value of Peanut Waste Liquor for Yeast

A deficiency of nitrogen for yeast propagation was apparent when the quantity of yeast grown in plain peanut water was compared to that grown in peanut water to which ammonium salts were added. Sugar was more rapidly and efficiently utilized with a corresponding increase in cells when ammonium chloride, di-ammonium phosphate, or ammonium sulfate were added to the media as illustrated in Figure 2, which

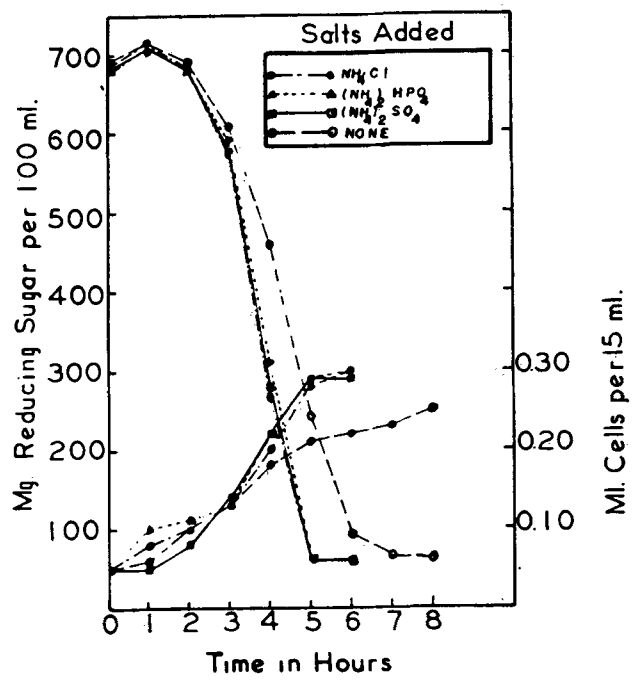

FIG. 2. Growth of the yeast, *T. utilis*, on peanut protein waste liquor supplemented with salts.

presents data from a propagation experiment. It was evident that there was sufficient sulfur and phosphorous in the peanut water since additions of these elements resulted in no increase in growth.

Maximum yields of yeast were obtained when nitrogen was provided in a ratio of one part of nitrogen to 16 parts of carbon, as shown in Table I. As much as 50.0 grams of yeast for every 100 grams of available sugar has been produced in single experiments. It was found that the complete nutrient could be provided either at the beginning, or part of the nitrogen could be added gradually by keeping the pH adjusted to 4.0-4.5 with ammonium hydroxide, with no difference in the final yields.

The sugar was utilized in five hours at 34° C. except for the residual 50 to 60 mg. per 100 ml. which always remained, but maximum yields of yeast were not reached until eight to ten hours of propagation. One hour after the practical disappearance of sugar only 75% of the maximum yield was produced.

TABLE I

Yields of Yeast on Nitrogen-Supplemented Peanut Water

	Carbon/Nitrogen Ratio	Grams of yeast per 100 g. of sugars*
Unsupplemented	63/1	41.3
$(NH_4)_2$ SO_4 added	16/1	48.5
$(NH_4)_2$ SO_4 added	8/1	47.8

*Average values from several experiments.

Continuous Propagation

In a system of three bottles similar to that used for sweet potato experiments (8), two of which were provided with air (as in Figure 1), it was found entirely practicable to feed continuously peanut water of about 0.8% sugar supplemented with nitrogen to an actively growing culture of *T. utilis* and withdraw yeast liquor continuously. Feeding at a rate of 50% of the volume in the propagator per hour, the sugar was completely utilized and a yield of 40.6 grams of yeast per 100 grams of sugar was produced. At increased rates the yield dropped considerably. Continuous feed appears to be the most efficient method of using equipment and time for handling large quantities of waste water in the propagation of yeast.

Quality of Yeast Produced

Although maximum yields occur in a nutrient medium having a carbon/nitrogen ratio of 16/1, a higher-protein yeast is formed in one having a carbon/nitrogen ratio of 8/1. Estimating protein as 6.25 times the total nitrogen of dried yeast, 54.3% protein is formed at a C/N ratio of 8/1 as compared to 46.8% at 16/1 (calculated to moisture free basis), which correspond to values found by other workers for *T. utilis* (7, 9) and commercial yeast (13).

The vitamin content of dried yeast grown in peanut water is apparently similar to dried yeast from other sources (2, 11); thiamine averaged 72 gamma per gram, and riboflavin 40.9 gamma per gram in samples containing about 5% moisture.

Summary

Peanut protein waste liquor supplemented only with an ammonium salt was found to be an excellent medium for the propagation of the food yeast, *Torulopsis utilis*, in batch and continuous processes.

When nitrogen was provided to give a carbon/nitrogen ratio of 8/1, 100 grams of sugar yielded 48 grams of a high-protein yeast that was comparable in food value and vitamin content to food yeasts from other sources.

Acknowledgment

The protein analyses of dried yeast were made by V. Orr. The thiamine and riboflavin determinations were made by L. H. Charbonnet and M. Murray, using photofluorometric methods.

REFERENCES

1. Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis, 4th ed. p. 364 (1935).
2. L. E. Booher, E. R. Hartzler, and E. M. Hewston. A Compilation of the Vitamin Values of Foods in Relation to Processing and other Variants. U.S.D.A. Circular No. 638, pp. 225-8 (1942).
3. R. Braude. Institute of Brewing J., *18*, 206 (1942).
4. R. S. Burnett and T. D. Fontaine. Ind. Eng. Chem., *36*, 284 (1944).
5. T. D. Fontaine and R. S. Burnett. Ind. Eng. Chem., *36*, 164 (1944).
6. T. D. Fontaine, C. Samuels, and G. W. Irving, Jr. Ind. Eng. Chem. *36*, 625 (1944).
7. G. S. King, T. J. Klatt, and N. Porges. Manuscript in preparation.
8. T. J. Klatt, R. P. Kupperman, and N. Porges. Experiments in progress.
9. A. J. Nolte, H. W. von Loesecke, and G. W. Pulley. Ind. Eng. Chem., *34*, 670 (1942).
10. P. A. Shaffer and A. F. Hartmann. J. Biol. Chem. *45*, 349 (1920).
11. J. J. Stubbs, W. N. Noble, and J. C. Lewis. Food Industries, *16*, 694 and 751 (1944).
12. A. L. Winton and K. B. Winton. The Structure and Composition of Foods, Vol. I, pp. 504 and 511 (1939).
13. *Ibid.*, Vol. IV, p. 479.

The Fatty Acids of Menhaden Oil*

II. The Separation of the Methyl Esters of Menhaden Oil Into Saturated, Monoethylenic, and Polyethylenic Fractions by Low Temperature Crystallization. The Composition of the Saturated and Monoethylenic Fractions.

FRANK A. SMITH** and J. B. BROWN
Laboratory of Physiological Chemistry, Ohio State University
Columbus, Ohio

THE fatty acids of fish oils constitute some of the most complex mixtures of fatty acids encountered in the field of fat chemistry. The saturated acids alone embrace carbon series from C_{12} to C_{24}. The unsaturated acids occur in an equally wide range of carbon series, and in addition include unsaturation from one to six double bonds. Thus, with menhaden oil for an example, we have shown (1) that the C_{18} series of unsaturated acids includes acids of from one to four double bonds. Previous work from a number of laboratories has indicated that the C_{20} and C_{22} series of this oil and of other fish oils are even more complex in nature, the unsaturation culminating in the presence of docosapentenoic and docosahexenoic acids in the C_{22} series.

The acids of two and more double bonds in a fish oil may be isolated by the lithium soap acetone procedures, originally proposed by Tsujimoto in 1920 (2). Debromination of the ether-insoluble bromides of the esters of the oil, as carried out by Brown and Beal (3) in their study of menhaden oil fatty acids, results in a mixture of esters of three and more double bonds. In the previous report of this series (1) we

*Presented in partial fulfillment of the requirements for the degree of doctor of philosophy in the Graduate school.

**Present address, School of Medicine and Dentistry, University of Rochester, Rochester, New York.