EXPERIMENTS ON PRODUCTION OF FEED YEAST FROM CITRUS PRESS JUICE [1]

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The Citrus Products Laboratory at Winter Haven, Florida, has been interested for several years in the production of feed yeast from the press juice obtained in the manufacture of dried pulp from citrus peel. In 1942 Nolte, von Loesecke and Pulley (1) published the results of their investigations in an article entitled "Feed Yeast and Industrial Alcohol from Citrus Press Juice." In their work a batch system was used which was somewhat slow in operation, but they did demonstrate that this raw material was suitable for the production of yeast and they obtained information on the composition of the press juice and yeast.

At the Southern Regional Research Laboratory of the Bureau of Agricultural and Industrial Chemistry, New Orleans, Louisiana, the Sweetpotato Products Division had encountered a similar situation on the utilization and disposal of some liquors obtained during the manufacture of starch from sweet potatoes. They had been investigating the possibilities of making yeast from these liquors and had developed a continuous method of conducting the fermentation (3). They had also constructed a pilot plant capable of handling up to 200 gallons per hour of waste liquor for demonstrating the process (4). It appeared that this method showed excellent possibilities of adaptation to the manufacture of yeast from citrus press juice. A cooperative project was arranged with the Dr. P. Phillips Canning Company, Orlando, Florida, and the pilot plant was installed in a building adjacent to their cannery and feed mill. A similar laboratory experimental unit with propagator capacities of six gallons each was installed at the U. S. Citrus Products Station, Winter Haven, Florida.

In this discussion the term "feed yeast" is used, and some explanation of what is meant may be in order. The investigations were limited to the production of a grade of yeast suitable for feeding animals. The organism used was *Torula utilis*, one of the wild yeasts, which is fast growing and not as susceptible to contamination as the true yeasts. One might ask what particular value yeast would have in feeding. It is good for this purpose because of its high protein and vitamin contents. About half of the yeast is composed of high-quality protein, which is readily available to animals. The proteins in yeast are deficient in only one of the ten so-called essential amino acids, methionine. This deficiency can be corrected by addition to the diet of any of a number of cereals.

The yeast is high in the B vitamins, particularly B1 (thiamin) and B2 (riboflavin). Values of 1.4 to 2.7 mg. thiamin per 100 grams of yeast have been reported and a sample made in the current studies contained 2.9 mg. per 100 g. For riboflavin, values of 5.2 to 9.1 mg. per 100 g. of yeast have been reported and a sample made in the pilot plant contained 4.54 mg. per 100 g. The yeast is a good source of ergosterol which, upon irradiation, yields calciferol, one of the compounds showing vitamin D activity (D2). A sample of the yeast pro-

[1] Agricultural Chemical Research Division Contribution No. 222.
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duced in these experiments contained about one-half of one per cent of ergosterol. It is extracted commercially from yeast of particularly high content of this the compound. Significant quantities of niacin and pantothenic acid are also present.

The Medical Research Council of England in 1945 (2) published a review of some investigations on the use of yeast in the diet of humans and animals entitled "Food Yeast, A Survey of the Nutritive Value." The following is quoted from this publication:

> "The results of the above experiments with animals show that the addition of food yeast greatly improves the nutritive value of a diet whose protein is otherwise derived mainly from cereals, the biological value of the mixture of the cereal proteins with those of yeast being equal to that of a similar mixture with those of milk. The good effects of the addition of food yeast to a white flour diet have demonstrated its value as a source of B vitamins."

In Germany during World War II several plants were erected for producing yeast from wood sugar and the yeast was used in preparing "ersatz" foods.

The press juice from the citrus feed mill offers many advantages for the production of feed yeast. The juice is rich in carbohydrates which can be utilized in the growth of yeast. It is available at centralized points in sufficient quantities to make large-scale production possible, and at sufficiently uniform rates to permit continuous operation. The manufacture of yeast would not require the expensive vacuum concentration necessary for the manufacture of molasses; it is ready for use as it is. When the liquor is used for the manufacture of yeast its biological oxygen demand (B.O.D) is greatly reduced, and this simplifies disposal of the resulting effluent.

Accurate records of the amounts of press juice obtained in the citrus feed mills are generally not maintained, but an approximation of the amount and the possible yield of yeast can be made. It is estimated that a 90-pound box of citrus fruit will yield about three gallons of press juice of approximately 10 per cent soluble solids. From these three gallons, about a pound of dried yeast can be produced. Potential total production might be as much as 25,000 tons per year.

During the war it was profitable to concentrate the citrus press juice into a molasses or sirup in multiple-effect vacuum evaporators. The product was used in the compounding of mixed feeds. With the return of more normal times competition with blackstrap molasses is to be expected and the additional uses for the press juice are desirable. The strength of the press juice makes it impossible to dispose of it in sewers, lakes or other bodies of water in most locations. It must be treated or processed in some manner.

In this discussion it will not be possible to describe the pilot plant in detail, but the general method of operation can be given.

The juice pressed from the ground, limed, citrus peel was pumped from the feed mill to the yeast pilot plant without any treatment. In the pilot plant it was first passed through an 80-mesh vibrating screen to remove particles of sunspended matter that might later clog pipe lines, screens, or the nozzles of the yeast centrifuge. It is not to be expected that the small amount of suspended matter remaining in the juice will be later collected with the yeast by the high-speed yeast centrifuge, but it is not objectionable in the feed.

The juice was then pumped through a heater to a large storage tank and held at 140° F. to prevent premature fermentation and destruction of the sugars. This supply tank was necessary because the feed mill was shut down for a few hours during the night while the yeast pilot plant operated continuously. From the storage tank the juice was pumped through a pasteurizer where it was heated to 200° F. to destroy microorganisms, then through a cooler to

where it was cooled to approximately room temperature, and then directly into the yeast propagators. Concentrated nutrient solution was also pumped continuously to the propagator in a quantity proportioned to the feed rate of the press juice. The nutrient solution contained the phosphate and ammonium salts necessary for yeast growth.

Three yeast propagators, each capable of holding 500 gallons of liquid, were arranged in series so that the liquid flowed successively through them. Porous aeration tubes in the bottom of each propagator provided the continuous flow of air necessary for rapid yeast growth. The propagators were fitted with cooling coils for dissipation of the heat generated by the fermentation, and temperatures were maintained at about 96° F.

In the yeast propagators the yeast multiplied and utilized the carbohydrates and other nutrient materials. Since the plant operated continuously, a suspension of yeast discharged continuously from the system. This yeast suspension was pumped to a collecting tank and thence to a continuous centrifuge. This special type of centrifuge discharged the yeast as a thick slurry suitably concentrated for drying on a steam-heated double drum drier.

The large pilot plant was placed in operation as follows: The propagators and all connecting lines were sterilized with flowing steam, and 200 gallons of pasteurized press juice and the necessary nutrients were pumped into the first propagator. About 20 gallons of an actively growing culture were added. Aeration had been started as soon as the steam was turned off. In the course of six to eight hours, 200 gallons of an active culture were built up. Approximately 200 gallons more of pasteurized press juice and nutrients were then added, and about two hours allowed for the culture to build up before continuous flow of press juice was started and maintained. No new culture was added from this point on.

During operation determinations were made of yeast counts, yeast volume, yeast weight, pH, phosphates, ammonia nitrogen, total organic matter, sugars utilized, and oxygen consumed. B.O.D. values of the effluent and observations on the amounts of liquid in each propagator and gas analyses of the air coming from the propagators were made.

The pilot plant was first placed in operation at Orlando in May and June, 1946. At the beginning trouble was encountered with foaming and it was evident that unless this difficulty were overcome, the method would not be practical. At times half the liquid and yeast were lost through the air vents in the tops of the propagators. An antifoam mixture was used which is still considered good, but even with large amounts of it, the foaming was not adequately controlled. During the latter part of this period a system of eight-inch pipes was installed at the tops of the propagators which conducted the foam from the first propagator to the second, the second to the third, and finally to the yeast collecting tank. The air vents used previously were closed. This modification was found to work very well and solved this problem. Not only was the foam kept within bounds, but it eliminated the necessity for any antifoam mixture during continuous operation. The propagators remained reasonably full of liquid. Since then, this system has proved itself over an extended period of operation.

Experimental work with the pilot plant at Orlando was resumed in March, 1947, and continued for a period of three weeks. The Dr. P. Phillips Canning Company then operated the pilot plant for a short time and produced additional quantities of yeast. Th smaller experimental unit in Winter Haven was operated during April, May and June, 1947.

The results of all the experimental operations on feed yeast will be summarized briefly:

It was found that the continuous method of propagating yeast as developed by the Sweetpotato Products Division of the

Southern Regional Research Laboratory was adaptable to citrus press juice. Continuous operation, twenty-four hours a day, has been maintained without apparent decrease in the activity of the culture for periods as long as a month. The results indicate the propagation can be continued almost indefinitely without any new culture. Experience in operation on citrus waste liquor to date indicates that three stages of propagation may not be required to obtain satisfactory yields. One stage should be sufficient and certainly not more than two stages. With a single-stage system the air required and the cost of the plant will be less. The total detention time in single-stage plant would be only about three hours.

Ammonium sulfate and tri-sodium phosphate were found to be satisfactory as sources of nitrogen and phosphorus. Other compounds of ammonia and phosphate can doubtless be used as long as the proper ratios are maintained and the proper alkalinity furnished for pH control. It was found that the pH could be maintained in the proper range between 4 and 5 by the addition of the nutrient used.

The maximum feed rate for the large pilot plant was approximately 185 gallons per hour. This means that the liquid remained in each propagator less than three hours. Higher rates of feed resulted in the incomplete utilization of the sugars and low yields. Yields of 60% of yeast resulted when the press juice was diluted with two volumes of water. With full-strength press juice yields of 33% were obtained, based on the weight of sugar present. Perhaps with some modification the higher yields obtained by diluting the juice can be realized with full-strength press juice, but this has not been accomplished to date.

In describing the plant operation, it was mentioned that the press juice was pasteurized by heating to 200° F. This is considered advisable while a new culture is being developed in the propagator, but it was found that successful operation could be maintained without pasteurizing the press juice fed to the plant after continuous operation had been established. The organism, "*Torula utilis*," appear to outgrow the few other organisms that may be present. Generally the citrus pulp is heated before it is pressed and this greatly reduces the number of initial micro-organisms. It is fortunate that pasteurization is not necessary because the press juice rapidly deposits a scale when heated in a heat exchanger and frequent cleaning is necessary.

Yeast was produced with as little as 500 cubic feet of air per pound of yeast. Experiments are not complete on this point and it may be possible to use less air than this. In the commercial manufacture of compressed yeast by the batch system, the amount of air used per pound of yeast produced generally exceeds 1200 cubic feet.

It was found that the peel oils present in the press juice did not interfere with the propagation of yeast. These volatile oils were reduced to negligible amounts by the air blown through the propagators.

During passage through the propagators approximately 95 per cent of the sugars were utilized, about 65 per cent of the total organic matter was destroyed, the reduction in the B.O.D. was about 80 per cent and the reduction of the oxygen consumed value was about 75 per cent. This is considered efficient operation because it is not to be expected that the yeast could utilize all the different types of organic compounds present.

The centrifuge which is commonly used with compressed yeast was found to be suitable for separating the *Torula utilis* as a thick slurry. The drum drier operated in a satisfactory manner in producing dried yeast. The product was fluffy, light in color and could easily be ground to a fine powder. It had a characteristic flavor and was slightly bitter, due to the small amount of the residual citrus press juice dried with the yeast.

Most of the information needed to design a commercial plant has been obtained. Ex-

perimental work on certain phases will be continued.

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BACTERIOLOGICAL SURVEY OF SOME CITRUS CANNERIES IN FLORIDA WITH SPECIAL ATTENTION TO *ESCHERICHIA COLI* [1]

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Escherichia coli is an organism commonly associated with a certain type of bacterial contamination. The presence of *E. coli* should be given serious attention even though it is generally considered harmless because it is commonly found in the intestinal tract of warm-blooded animals. When this organism is present it is considered evidence of contamination, usually fecal, and indicates a health hazard. There is the danger that other bacteria capable of producing typhoid fever, dysentery, or other intestinal diseases may be present. The presence of *E. coli* in a food product may be determined by a series of simple tests. The American Public Health Association has recommended a standard routine check to determine the presence of *E. coli* in milk and water supplies and has been of great value in safeguarding the consumer's health. They have been a valuable aid in developing suitable sanitary measures so essential to healthful living. The results of routine analyses supported by differential tests as applied to citrus cannery equipment and unpasteurized citrus fruit products are presented in this discussion.

Samplings were made at ten citrus canneries in the Winter Haven area over a period of three years. Each plant was inspected at least once and several of them three times a season. The investigation included samples of the unwashed fruit, washed fruit, and of material from washers, conveyors, sizers, juice extractors, juice troughs, and juice blending tanks.

A brief description of the steps taken while the search for *E. coli* in unpasteurized products will be given. A known dilution is made with sterile water from the thoroughly agitated liquid portion of a sample. Petri dishes of eosin-methylene blue (E.M. B.) agar are inoculated with some of the diluted sample; observations are made at the end of 18 and 24 hours of incubation at 37° C. Eosin-methylene blue agar is a selective medium. It is a mixture of lactose (milk sugar), other nutrients, and dyes. When *E. coli* grows on the surface of this medium, the dyes are incorporated with the cell growth, and a colony is formed that is distinctive in appearance for *E. coli*. The diagnosis of colonies grown on this medium requires a skill that may be devel-

[1] Agricultural Chemical Research Division Contribution No. 223.

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