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A STUDY OF THE AMOUNT OF LIPIDS IN THE R.
AND S. FORMS OF SEVERAL YEASTS
THESIS FOR THE DEGREE OF M. S.

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AND S FORMS OF SEVERAL YEASTS**

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Thesis

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of Michigan State College
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of Science**

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Table of Contents

Title

Acknowledgment

I. Introduction

II. Historical

III. Experimental

A. Cultural Medium

B. Apparatus

C. Growing smooth yeast

D. Harvesting and drying

E. Growing and harvesting rough yeast

F. Lipoidal extraction

G. Vitamin D assays

IV. Discussion

V. Conclusions

VI. References

I. Introduction

In recent years a great deal of emphasis has been placed on yeasts and their value as a part of the diet. As a result, extensive studies have been made on the lipids contained therein, and also of the vitamin content. It has long been known that yeast was a very good source of vitamin B and G. In the past few years it has been discovered that yeast is also a very good source of ergosterol, which when irradiated with ultra-violet light forms vitamin D. Extracts of the yeasts containing the vitamins as well as other nutrients are added to foods and medicines. By the irradiation of dried yeast a material of high antiricketic potency has been obtained. Milk of high vitamin D potency is now produced by feeding cows irradiated yeast.

Various studies have been made on yeast in regard to the lipoidal content. One of the first investigations concerned the quantitative determination of ergosterol. In later experiments the structure of the various lipids present was determined. In all of these studies the factor of variation in forms was not taken into consideration. Recent work by Fabian and

McCullough (1) has proven that yeasts dissociate as bacteria do. It was therefore felt that a study of the lipoidal content of the dissociants of several yeasts might be of interest.

II Historical

The fact that ergosterol is converted to vitamin D by irradiation with ultra-violet light brought about extensive studies of ergosterol. Bills, Massengale and Prickett (2) made a study of the factor determining the ergosterol content of yeast. Twenty-nine cultures were obtained and cultivated one per cent dextrose-nutrient agar slants. For large yields the yeasts were grown in beet molasses medium.

On repeat runs the average variation in the yield of dry yeast per liter of medium was 13 per cent. The ergosterol content of the dry yeast varied as much as 19 per cent. All yeasts of high ergosterol content grew well in culture, however, not all that gave large yields showed high ergosterol content. The yield of yeast is markedly influenced by the intensity of the aeration during cultivation.

These experiments indicate that the different yeasts may exhibit decidedly different capacities for the formation of ergosterol, capacities which may be increased or repressed by manipulation of the cultural conditions.

The above studies were made on various species of yeast. The same authors (3) made another somewhat similar study using one yeast and a series of media in which various carbohydrates were employed. They found the ergosterol content higher with di and trihexoses than with monohexoses. It was assumed that at the instant of cleavage of the more complex sugars, the resulting hexoses exist in forms more readily convertible into sterol than the familiar simple sugars. These relations, and the fact that the nitrogen content, the non-sterol lipid content, and the state of nourishment of the yeast bear no relation to its ergosterol content, indicate that ergosterol is primarily a product of carbohydrate metabolism.

Honeywell and Bills (4) reported the study of cerevisterol, a sterol accompanying ergosterol in yeast. They found it to be a stable, hexane insoluble sterol obtained from the mother liquors after the extraction of ergosterol from yeast. Irradiation does not activate it antiricketically.

Newman and Anderson (5) made a study of the chemistry of the lipids of yeasts. This work was divided into two parts, the first of which

was the composition of the acetone soluble fat.

About 6.02 per cent lipids was found to be present when calculated on a dry yeast basis. These lipids were separated into phospholipids, acetone insoluble fat, and acetone soluble fat.

In the acetone soluble fraction sterols, glycerol, and fatty acids were found to be present. The acids consisted mostly of palmitic and stearic. No appreciable amount of acid higher than stearic was found in the acetone insoluble fat.

The second part of the report was on the composition of the phospholipids. Glycerophosphoric acid, chlorine, and aminoethanol were identified among the water soluble constituents. Palmitic and stearic acids were also found to be present.

III Experimental

A. Culture Medium

A medium containing sugar beet molasses was selected for these studies because it is used commercially on a large scale and is inexpensive. It had the following composition:

Beet molasses----- 50 parts

Urea----- 1 part

Mono-basic ammonium phosphate----- 1 part

These ingredients were mixed and diluted with cold tap water to a reading of 5 degrees Brix. Previous to this time the pH of this media was adjusted at this point, but a great deal of trouble was encountered with a precipitate that formed upon autoclaving.

It was found that by autoclaving the medium in a mixing bucket for a period of two hours at 16 lbs. pressure, the precipitate could be removed by filtration. The medium was then placed in flasks and again autoclaved for a period of 45 minutes at the above pressure. At this point no more precipitate appeared.

The medium which after cooling had a pH of 7.3 was adjusted aseptically to a pH of 5.0 with concentrated sulphuric acid.

B. Apparatus

To obtain a large yield of yeast it was necessary to aerate the culture medium. (See drawing Fig. I) For this purpose a six liter erlenmeyer flask was used, into which were inserted four capillary tubes connected to a common inlet. An exhaust vent was also arranged for the flask. The reason for using the six liter flask instead of a large mouthed bottle was because the flask could be sterilized without any danger of its breaking.

Connected to the outlet of the capillary tubes was an air filter to render the compressed air sterile. This filter was made by packing cotton into a pyrex tube one inch in diameter by eighteen inches long. This was sterilized in dry heat at 170°C. for two hours.

After the flasks were inoculated from a starter flask, three or four drops of sterile corn oil were added to the media to prevent foaming. The entire setup was placed in a constant temperature incubator at 30° C. The air was allowed to flow gently through the medium.

C. Growing Smooth Yeast

The starters were made by inoculation of ten cc. portions of the medium in tubes from agar slants of the desired yeast. After growth had proceeded for forty-eight hours in the tube, the contents were poured into flasks containing three hundred cc. of the molasses medium. The yeast was incubated at 30°C. until the growth was heavy which usually required from two to three days.

The six liter flasks, each of which contained three liters of medium, were the inoculated with about 150 cc. of the starter. The corn oil was then added, the capillary put into place, and the air turned on. All runs were made over a five day period.

D. Harvesting and Drying

Before the yeasts were harvested agar plates were made from the large flasks to make sure that the yeast was in the desired form. Some runs had to be discarded as they were found to be the intermediate form of the yeast rather than the rough or the smooth.

At the end of the five day period the air was turned off and the capillary tubes removed. The flasks were then plugged with cotton to prevent contamination. These were allowed to stand for twenty-four hours to allow the yeast to settle. The clear liquid was drawn off by siphoning and the yeasts removed from the remainder of the liquid by centrifuging.

After all cells were removed they were washed once with physiological salt solution and once with distilled water. This washing was done in a 250 cc. centrifuge bottle. The yeast was placed in an evaporating dish and dried in an oven at 60°C. The dry yeast was then ground in a hand mill and finally in a ball mill which reduced it to a very fine powder.

E. Growing and Harvesting Rough Yeasts

The only difference in the handling of the rough yeast from that of the smooth, was that the former was not aerated.

The "R" yeast was grown in one liter Florence flasks containing 400 cc. of media. These flasks were shaken occasionally. At the end of five days were removed from the incubator and allowed to settle. The rest of the procedure was identical with that used in the case of the "S" yeast.

F. Lipoid Extraction

To determine the lipoidal content of the yeast it was dried to a constant weight in a sixty degree vacuum oven. From this one gram samples were placed in a Soxhlet extractor and extracted with anhydrous ethyl ether for 18 to 24 hours. At the end of this period the ether solution was placed in beakers of constant weight and the ether evaporated off on a steam bath. The beakers were again brought to constant weight from which the amount of extract present was determined by difference.

G. Vitamin D Assays

In this investigation a comparison of the lipoidal content of "R" and "S" forms of Saccharomyces cerevisiae, Hansen, Saaz and Saccharomyces acris-sacchari was made. The study also included Saccharomyces ellipsoidus "S", a commercial dried yeast received from the Fleischman Company.

Besides determining the amount of lipids, an index of the ergosterol content was obtained indirectly by irradiating both the yeast and the extracted lipids and comparing their anti-ricketic activity by the standard biological method.

This method involves the production of rickets in young healthy rats and then feeding the substance to be studied as a supplement to the diet to determine the degree of calcification induced in the bones.

Rats weighing about 50 grams and from 23 to 28 days old were selected for the assay. The rats were fed a basal ricketic ration having the following composition:

Wheat gluten -----	20.0
Corn meal -----	38.0
Oat meal -----	37.5
Calcium carbonate -----	3.0
Salt (NaCl) -----	1.0
Yeast (Unirradiated) -----	<u>0.5</u>
	100.0

This ration was fed over a period of twenty one days at which time the rats were very stiff and had great difficulty in walking. They were put into individual cages and given the supplement.

In preparing the yeast for feeding 0.2 gm. was weighed out and spread as evenly as possible on a sheet of paper and irradiated for two minutes under an 110 volt ultra-violet ray lamp. The lamp was so adjusted that it was just eleven inches above the material to be irradiated. 0.1 gm. of this irradiated yeast was then used and a 1 to 100 dilution was made with commercial Northwestern yeast. Various amounts of this dilution were fed to give the desired levels.

The preparation of the extract was made by taking the extract from one gram of the yeast and redissolving it in 25 cc. of ether. This

was then irradiated in the ether solution at the same distance from the lamp and for the same length of time as the yeast itself. A small amount of corn oil was then added to take up the extract before the ether was evaporated off again on the steam bath. More corn oil was then added so that a 1 to 1000 dilution was obtained. In this dilution, 1 gm. contained the extract from 1 mg. of the dried yeast.

The supplement was fed over a ten day period and at the end of this time the rats were weighed and killed. The radii and ulnae were removed for staining.

The line test was run on these bones to determine the degree of calcification. This was accomplished by splitting the bones, immersing them in silver nitrate for two or three minutes and then placing them in distilled water. Upon exposure to light the calcified area turned dark and could be distinguished very easily from the uncalcified portion of the bone.

IV. Discussion

The results of the lipid extraction of the yeast show that the total extractable content varies considerably. This variation is not consistent for the different forms. For example in the case of Saccharomyces aceris-sacchari, the "R" form contained more extractable lipid than the "S" while in the case of Saccharomyces cerevisiae Hansen, Saaz, the opposite was true. In comparison with Saccharomyces cerevisiae, Fleischman's brewers yeast, both the Saccharomyces aceris-sacchari and Saccharomyces cerevisiae, Hansen, Saaz contained more extractable lipid than did the commercial product.

The results of the vitamin D determinations were rather irregular and none too satisfactory. It was expected that the extract when treated with ultra violet light would show a greater vitamin D activity than an equivalent amount of the dry irradiated yeast because a solution of the lipids is permeable to ultra violet light and practically all of the ergosterol should therefore be activated. On the other hand the activation would not be complete in the case of the yeast because not all of the ergosterol

would be exposed to the ultra violet light. The results did not consistently bear out the above expectation.

As the results show (Table I) Saccharomyces aceris-sacchari "R" and Saccharomyces cerevisiae Hansen, saaz "S" the irradiated extract showed more vitamin D than an equivalent amount of the irradiated yeast, as was expected.

However, in the case of Saccharomyces aceris-sacchari S, Saccharomyces cerevisiae, Hansen, saaz R and Fleischman's Saccharomyces cerevisiae the opposite was true, for the irradiated yeast showed more vitamin D than the irradiated extract from an equivalent amount of yeast. This discrepancy may be due either to the incomplete extraction of ergosterol or to the incomplete activation of the ergosterol in ether solution. Time did not permit checking these factors as possible causes for the inconsistencies.

Yeast	Form	Amount of extract per gm of dry yeast	Units of vitamin D in extract from 1/gm of dry yeast	Units of vitamin D in 1/gm of dry yeast
<u>Saccharomyces</u>	S	0.0555	800	1333
<u>accharis-sacchari</u>	R	0.0683	2000	1000
<u>Saccharomyces</u>	S	0.1602	2000	567
<u>cerevisiae,</u>	R	0.0602	- (less than 333)	2000
<u>Hansen, saaz</u>				
<u>Saccharomyces</u>	S	0.0223	500	1000
<u>cerevisiae</u>				

V. Conclusions

1. The results show that the R form of Saccharomyces aceris-sacchari has a higher lipoidal content than the S form.

2. In contrast to the above, the results show that the R form of Saccharomyces cerevisiae Hansen, saaz has a lower lipoidal content than the S form.

3. Variations were observed in the vitamin D content of the R and S forms of the irradiated yeasts and also of the irradiated extracts of these yeasts. None of the forms studied appear to be an exceptionally rich source of ergosterol.

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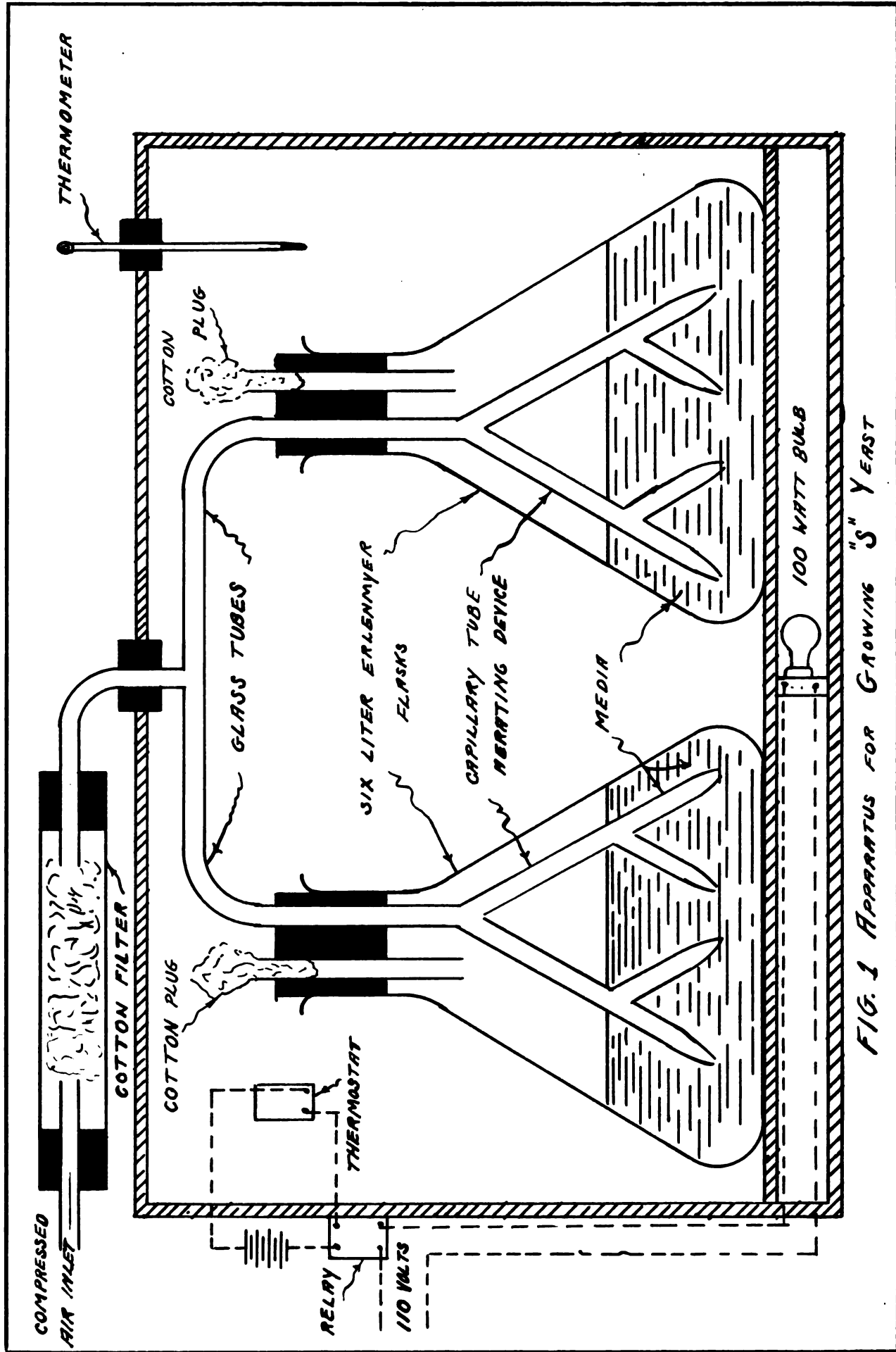


FIG. 1 APPARATUS FOR GROWING "S" YEAST

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