TABLE II.

Williams.	Williams' + wheat emb. extract.	Williams' + alfalfa extract.	Medium F.	Medium F + alfalfa extract.	Medium F + wheat emb. extract.
130	188	286	288	284	281
140	176	253	260	256	253

288, or the same as that in Williams' medium plus the optimum concentration of alfalfa extract. Since we have constructed a medium of known constituents which gives as good results as Williams' medium plus alfalfa extract, it cannot be concluded that the stimulating effect of the extract is due to Water Soluble B or to any other unknown substances. Furthermore, when the optimum concentration of alfalfa extract was added to Medium F a count of 284 was obtained, when the optimum concentration of the wheat embryo extract was added to Medium F the count was 281.

From the above data it is evident that we have developed a medium, namely Medium F, composed of known constituents, which is not improved by the additions of vitamine-containing extracts. Therefore, vitamines are not essential as constituents of media for the growth of yeast.

Summary.

The relative potencies of 2 materials as yeast growth stimulants cannot be arrived at on an equal weight basis.

Treatment with alkali does not impair extracts of wheat embryo or alfalfa as yeast growth stimulants. Evidently the stimulant is not Water Soluble B.

Extracts of alfalfa and wheat embryo contain sufficient nitrogenous and inorganic material for the growth of yeast.

A medium of known constituents is developed which promotes the growth of yeast without the addition of vitamines. The addition of Water Soluble B does not improve the above medium.

AMES, IOWA.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE.]

THE NUTRITIONAL REQUIREMENTS OF YEAST. II. THE EFFECT OF THE COMPOSITION OF THE MEDIUM ON THE GROWTH OF YEAST.[1]

BY ELLIS I. FULMER, VICTOR E. NELSON AND F. F. SHERWOOD.

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In the preceding paper data are presented on the rôle of vitamines in the growth of yeast. In the following paper a study has been made on the influence of the nature and concentration of known components of the medium on the growth of yeast.

Experimental.

Method.—The method was identical with that outlined in the pre-

[1] Read before the meeting of the American Chemical Society at St. Louis, April, 1920.

ceding paper (p. 187) up to making the count after the addition of 5% phenol solution to stop the growth of the yeast.

Medium A was that used by Amand[1] except that disodium phosphate has been omitted. The growth in this medium is slow, the logarithmic rate of growth showing a period of reproduction of 3 hours. The yeast was transferred every other day for 3 weeks to a fresh medium, thus geometrically diluting to a negligible quantity the constituents of the wort which might have been carried over.

With Medium A as basal, the effect of the variation of one constituent at a time was studied with a view to determine the best concentration of each for the optimum growth of yeast.

In every case the concentration of the varying constituent of the medium was plotted as abscissa and C as ordinate. All concentrations of the variable are expressed as grams of the substance per 100 cc. of the medium and designated as per cent.

The composition of the various media is found in Table I. Each curve is given a number and a letter by which it may be identified on the figures designated. The letter refers to the medium used. In order to be sure that the results can be duplicated under various circumstances, the initial count and time are varied, and only in the cases indicated can time and count be directly compared.

TABLE I.
Composition of Media Used.

Constituent.	A.	B.	C.	D.	E.
K_2HPO_4	0.100	0.100	0.100	0.100	0.100
NH_4Cl	0.100	0.188	0.188	0.188	0.188
$CaCl_2$	0	0.100	0	0.100	0.100
$MgSO_4$	0.100	0.025	0	0	0
$CaCO_3$	0.020	0	0	0	0.040
Saccharose	10	10	10	10	10

The numbers represent the g. of constituent per 100 cc. of medium.

1. The Effect of the Variation of the Concentration of Ammonium Chloride upon the Growth of Yeast.—Yeast from Medium A was inoculated into Medium A with various concentrations of ammonium chloride. It was found that there is a denfiite optimum concentration of ammonium chloride (0.188% of ammonium chloride or 0.060% of ammonia), variations from which very materially decrease the yeast count. In order to save space only 2 of the many typical curves obtained are given in Fig. 1, I-A; II-A. Per cent. of ammonia signifies the concentration of the salt, calculated as NH_3, per 100 cc. of medium and is plotted on the abscissa. The count is plotted on the ordinate. The Roman numeral indi-

[1] Amand, *La Cellule*, **20**, 225 (1902).

cates the number of the experiment and the letter indicates the medium used as shown in Table I.

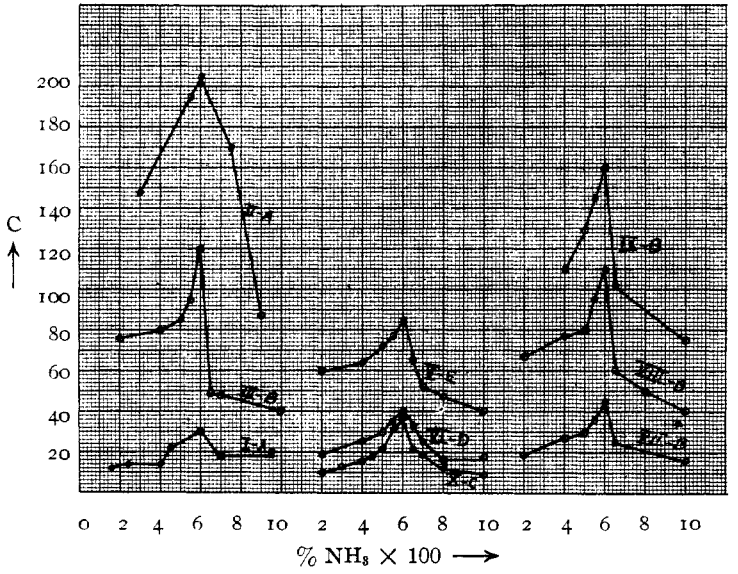

Fig. 1.—The effect of various concentrations of ammonium salts upon the growth of yeast.

2. *The Growth of Yeast in a Medium Free from Calcium and Magnesium.*—In order to free the yeast as far as possible from these salts, the yeast from Medium A was transferred to Medium C, which is free from calcium and magnesium salts. To our surprise, we found that the yeast grew in this medium. It has been growing now for a period of 3 months in this medium, having been transferred to a fresh medium every other day. At the time of publication of this paper the yeast is normal in appearance and is still growing well. Several g. of this yeast was obtained and the percentage ash determined. In the case of Fleischmann's yeast the ash amounted to 6.3 g. per 100 g. of dry material, while in the case of the yeast grown in the calcium- and magnesium-free medium the ash amounted to only 3 g. per 100 g. of dry material. The ash in the latter case was free from calcium and magnesium.

3. *The Effect of the Variation of Concentration of Clacium Chloride upon the Growth of Yeast.*—Yeast grown in Medium C (magnesium and calcium-free) was inoculated into Medium C, and also into Medium B. Containing various concentrations of calcium chloride, A few of the typical curves obtained are shown in Fig. 2, I-B; and II-C. On the abscissa is plotted

the number of grams of salt per 100 cc. of medium multipled by ten. Count is plotted on the ordinate.

It will be seen that the addition of calcium chloride up to 0.10% increases the growth of the yeast. Further additions up to 0.60% do not influence the count. The presence of magnesium sulfate does not change the optimum concentration as is shown in Curve 1-B.

From curves plotted in Fig. 1, III-B; X-C; and VI-D, it is seen that the absence or presence of magnesium sulfate or calcium chloride does not influence the optimum concentration of ammonium chloride.

4. The Effect of the Variation in Concentration of Magnesium Sulphate upon the Growth of Yeast.—Yeast from Medium C was placed in Medium C and into Medium D with various concentrations of magnesium sulfate. In order to save space only a few typical curves are given in Fig. 2, I-D; II-D; III-D; V-C. It must be remembered that in all curves the Roman numeral refers to the number of the experiment and the letter to the medium used, and whose composition is given in Table I.

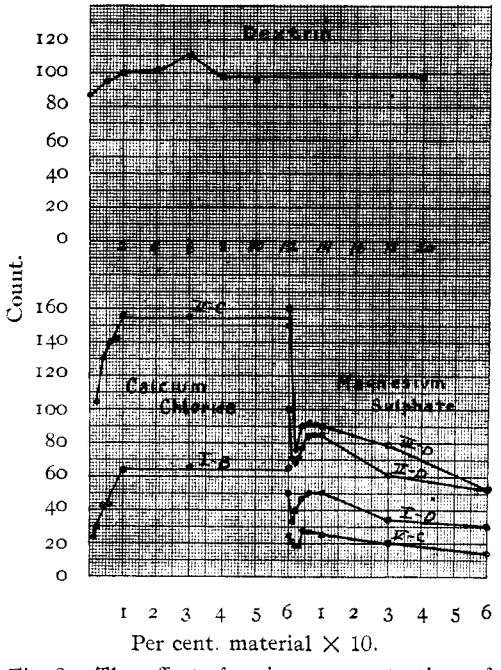

Fig. 2.—The effect of various concentrations of calcium chloride, magnesium sulfate, and dextrin upon the growth of yeast.

The addition of magnesium sulfate up to 0.020% decreases the growth of the yeast; further additions up to 0.040% increase the count. In concentrations from 0.040% up to 0.10% there is no change in the count but upon further additions the count is diminished. The above results are not influenced by the presence of calcium chloride. The addition of magnesium sulfate to the medium does not improve it.

5. The Effect of the Variation of Calcium Carbonate upon the Growth of Yeast.—Yeast from Medium D was inoculated into Medium D and into Medium E with various amounts of precipitated calcium carbonate. A few of the many curves obtained are shown in Fig. 3, I-D; II-E. It is seen that the addition of calcium carbonate up to 0.040% greatly improves the medium. Further additions prove decidedly detrimental.

This effect cannot be due to the addition of calcium because concentrations of calcium chloride as high as 0.60% give the same count as 0.10%. The presence of calcium carbonate does not influence the optimum concentration of ammonium chloride as is evident in Fig. 1, V-E.

6. The Effect of the Variation of the Concentration of Dipotassium Phosphate upon the Growth of Yeast.—Yeast from Medium E was placed in Media C, D and E, with various concentrations of dipotassium phosphate. Some typical curves are given in Fig. 3, I-E, II-D; III-C. Additions of the phosphate up to 0.020% greatly improve the medium, further additions up to 0.60% do not change the count.

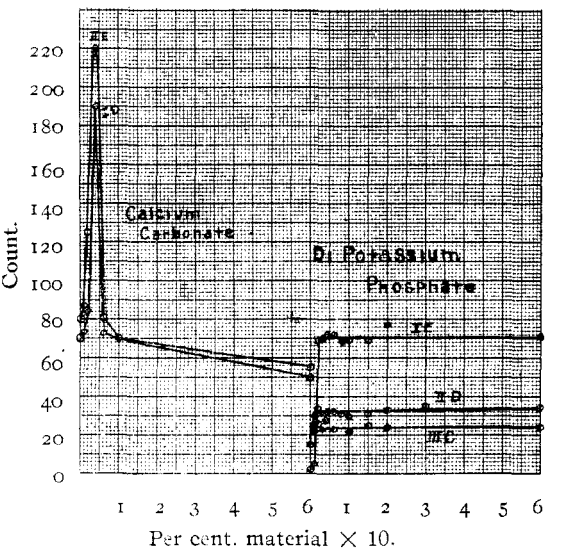

Fig. 3.—The effect of various concentrations of calcium carbonate and of dipotassium phosphate upon the growth of yeast.

7. The Effect of Various Ammonium Salts upon the Growth of Yeast.—To see whether there were optimum concentrations of other ammonium salts similar to that exhibited by ammonium chloride, yeast from Medium A was inoculated into Medium D with various concentrations of ammonium chloride, ammonium sulfate, ammonium nitrate, and ammonium tartrate. Curves are shown in Fig. 1, III-B; VII-B; VIII-B; IX-B. The order of the curves is the same as that of the salts given above. All 4 salts show the same optimum concentration, that is that concentration of the salt corresponding to a calculated value of 0.060% ammonia.

To compare the effect of the optimum concentrations of the above ammonium salts, yeast from Medium A was inoculated into Medium E, in which was contained the optimum concentration of the various salts. The results are given in Table II.

TABLE II.

Ammonium salt used.	Count after 24 hours.	Count after 40 hours.
Chloride	37	109
Sulfate	34	100
Nitrate	37	105
Tartrate	34	103

It is evident that all 4 salts are equally effective for the growth of yeast. This phenomenon of optimum concentration of ammonium salts is dependent only upon the concentration of cation.

8. The Effect of Asparagine upon the Growth of Yeast.—Yeast from Medium E was inoculated into Medium E with various concentrations of asparagine. The results are shown in Table III. The concentration of the asparagine is expressed as per cent. NH_2 to be comparable with the concentration of the ammonium salts and the concentration is also expressed as g. of the asparagine per 100 cc. of the medium.

TABLE III.

Asparagine. %.	NH_2. %.	Expt. I. Count.	Expt. II Count.
0	0	58	65
0.070	0.017	62	67
0.130	0.031	64	68
0.200	0.048	58	69
0.270	0.065	62	63
0.330	0.079	55	61
0.400	0.096	60	58

The addition of asparagine does not improve the medium.

9. The Effect of Temperature upon the Optimum Concentration of ammonium Chloride.—Yeast from Medium E was inoculated into 4 series of flasks containing Medium E with various concentrations of ammonium chloride. One series each was incubated at the following temperatures: 21°, 30°, 35°, 40° for 48 hours. The curves are shown in Fig. 4. It is evident that the optimum concentration varies with the temperature, the higher the temperature the greater the concentration of the salt required to give the maximum count. It follows that there must be a different concentration of ammonium salt for each temperature for best results. This may also be true for the other constituents of the medium. Extensive experiments are being undertaken in this laboratory on the relation of temperature to the optimum concentrations of the various constituents for yeast growth.

10. A Possible Explanation of the Optimum Concentration of Ammonium Chloride and of the Effect of the Temperature upon the Same.—The effect upon the growth of yeast of various concentrations of ammonium chloride is a discontinuous function of the concentration of the salt. Since such discontinuous functions are characteristic of certain colloidal phenomena, and are exhibited strikingly in the case of the effect of various reagents upon the swelling of proteins, it seemed possible that there might be some relation between the effect of the salt upon the growth of yeast and upon the swelling of protein.

Wheat gluten was used in the work here described. The method used was that of Upson and Calvin[1] and Gortner and Doherty.[2] The results

[1] Upson and Calvin, THIS JOURNAL, **37**, 1295 (1915).

[2] Doherty, *J. Agr. Research*, **13**, 389 (1918).

are plotted in Fig. 4. On the ordinate is plotted the grams gain in weight per 100 g. of gluten. The experiments at 30° were conducted with gluten obtained from "White Swan, Perfect Patent" flour, while the experiment at 21° is a typical curve obtained with "Gold Medal" flour. On each curve is designated the number of hours during which the gluten remained in contact with the given solutions. The number of hours up to 4 does not influence the minimum point.

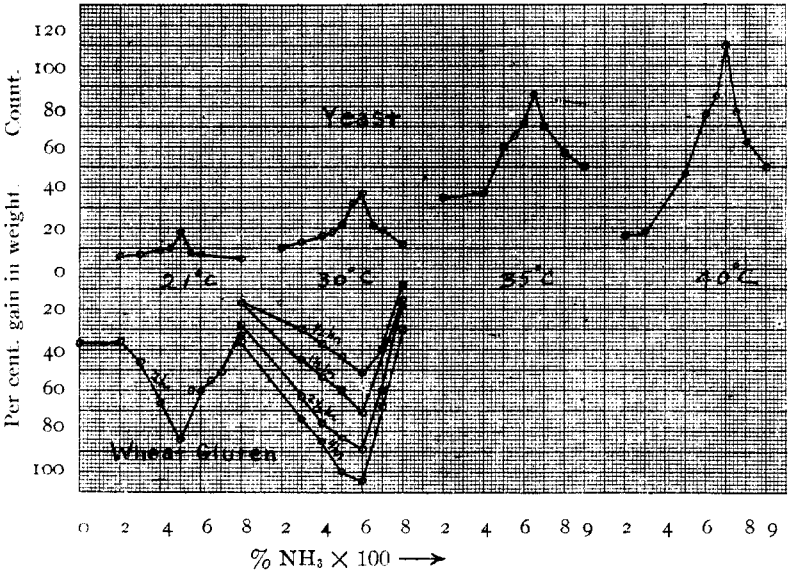

Fig. 4.—The effect of temperature upon the optimum concentration of ammonium chloride for the growth of yeast and upon the swelling of wheat gluten in the presence of ammonium chloride.

It is evident that at the 2 temperatures tested (21 and 30°) the optimum concentration of ammonium chloride for the growth of yeast is identical with the concentration of the salt producing the least swelling of wheat gluten, thus giving a possible explanation not only of the fact that there is an optimum concentration of ammonium chloride for the growth of yeast but also of the effect of temperature upon that optimum. We are studying in detail similar relations with other constituents of the medium with several proteins at different temperatures.

12. Effect of Colloids upon the Growth of Yeast.—It is evident that Medium E is the best medium for the growth of yeast that can be made from the constituents used. It seemed probable, however, that the medium might be further improved by protecting the yeast from certain toxic substances, particularly alcohol, formed during the fermentation. Söhngen[1] reported that certain colloidal substances accelerated fermenta-

[1] Söhngen, *J. Inst. Brewing*, **20**, 720 (1914).

tion and reproduction by yeast. He attributed the effect to the adsorption of carbon dioxide by the colloid.

Wort contains many colloidal substances, among them being dextrin. It is probable that the colloidal material in the wort protects the yeast from the poisonous substances formed. With this idea in mind a study is being made of the influence of various colloids upon the growth of yeast.

Yeast from Medium E was inoculated into Medium E with various concentrations of dextrin. The dextrin was prepared from Argo cornstarch as follows. One g. of citric acid was added to each 100 g. of starch, and enough water to make a paste. The mixture was heated for 3 hours at 9 kg. pressure. The product was dried and ground and subsequently extracted for 12 hours with 95% alcohol in a continuous extractor in order to remove the citric acid. The results are plotted in Fig. 2. It will be seen that the addition of dextrin improves the medium and the maximum effect is at a concentration of 0.60 g. of dextrin per 100 cc. of medium.

In Table IV further data are presented on the effect of the optimum concentration of dextrin and also on the effect of the addition of 0.10 g. of starch per 100 cc. of medium.

TABLE IV.

Medium E.	Medium E + 0.60 g. dextrin per 100 cc.	Medium E + 0.10 g. starch per 100 cc.
29	...	62
67	...	86
79	...	127
87	111	...
151	288	...
159	260	...

Not only does the addition of dextrin improve the medium but it is seen that starch likewise increases the growth of yeast.

12. The Composition of the Optimum Medium for the Growth of Yeast.—From the above data we can conclude that the following is the best possible medium composed of the given constituents for the growth of yeast at 30°. This medium is designated in the preceding paper as Medium F and has the following composition. 100 cc. of the medium contain 0.188 g. of ammonium chloride; 0.100 g. of calcium chloride; 0.100 g. of dipotassium phosphate; 0.040 g. of precipitated calcium carbonate; 0.60 g. of dextrin; 10 g. of cane sugar.

Summary.

1. A method is given by which is determined the optimum concentration of each of the constituents of the medium for the growth of yeast.

2. The optimum concentration of several ammonium salts was found to be identical with that for ammonium chloride. These salts are equally efficient at the optimum concentration.

3. The optimum concentration of ammonium chloride for the growth of yeast is the concentration of the salt in which a protein (wheat gluten) is least swollen.

4. The optimum concentration of ammonium chloride varies with the temperature. At the temperatures tested the concentrations of the salt causing the least swelling of a protein (wheat gluten) are identical with those most favorable to yeast growth.

5. The effect of certain colloidal materials upon the growth of yeast has been determined.

AMES, IOWA.

[CONTRIBUTION FROM OIL, FAT AND WAX LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

AN ANALYSIS OF OTOBA BUTTER.

BY WALTER F. BAUGHMAN, GEORGE S. JAMIESON AND DIRK H. BRAUNS.

Received October 26, 1920.

A sample of otoba butter from Colombia, South America, was recently submitted to this laboratory for examination by Dr. David Fairchild, of the Office of Foreign Seed and Plant Introduction of the Department of Agriculture, and it is believed that the results of the examination are of sufficient importance to warrant making a permanent record of them.

Otoba butter is the common name for the fat expressed from the fruit of *Myristica otoba*. It is also sometimes called American nutmeg butter, American mace butter, and otoba wax. It has long been used by the people of Colombia, S. A., as a medicament for skin diseases of domestic animals.

E. Uricoechea[1] made a cursory examination of this product and reported that it resembled nutmeg butter, and consisted of the glycerides of myristic and oleic acids, with a considerable amount of unsaponifiable matter. From the unsaponifiable constituents he separated a crystalline compound, to which he gave the name "otobite" and the formula $C_{24}H_{26}O_5$. He makes no mention of a volatile oil.

Experimental Part.

Physical and Chemical Characteristics of the Fat.—The freshly extracted fat has been described as being white, having the consistency of butter, and an odor resembling that of nutmegs. The sample used in this investigation had acquired a brown color and a disagreeable odor, a change which had been noted by previous observers. The chemical and physical characteristics are given in Table I. It will be noticed that the butter is a mixture of a volatile or essential oil, a fixed oil and unsaponifiable matter.

[1] E. Uricoechea, *Ann.*, **91**, 369 (1854).