ON THE QUANTITATIVE SEPARATION OF MALTOSE AND LACTOSE.

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THE writer, in the course of a study on the composition of sundry infant and invalid foods now on the market, undertaken to fulfil a thesis requirement for graduation from the agricultural department of the University of Vermont, found it necessary quantitatively to separate maltose and lactose. He was unable to find, either in the literature or through correspondence with several chemists well informed in food analysis, any satisfactory method to this end. Such a separation may be accomplished, however, by the use of a certain variety of yeast, *Saccharomyces anomolus*, which removes maltose completely without acting on the lactose.

It is well understood that the two bisaccharides, maltose and lactose, need to be hydrolyzed before they may be fermented, and that the former yields two molecules of glucose and the latter, one of glucose and one of galactose as a result of hydrolysis. Glucose is then easily fermented. Yeasts usually contain hydrolytic enzymes, which are capable of changing maltose to a greater or less extent, but which are inactive with lactose.

In the course of the work carried out by the writer nine different species of yeasts were used. Eight of the yeasts were obtained from Kral, and one was a pure culture from ordinary Fleischmann's bread yeast. The yeasts from Kral's laboratory were: *Saccharomyces anomolus* Hansen, *S. cerevisiae I* Hansen, *S. cerevisiae Carlsberg unterhefe I* Hansen, *S. ellipsoideus I* Hansen, *S. farinosus* Lindner, *S. Kephir* Beijerinck, *S. Marxianus* Hansen, *S. Pastorianus I* Hansen. Pure cultures of each were grown in agar, from which they were transferred to Pasteur's fluid (Strassburger and Hillhouse's formula). Samples of commercial maltose (66.36 per cent. maltose, remainder mostly dextrin) and lactose of known composition were used separately, as well as in mixtures of definite strengths.

From 25 to 100 cc. of a solution of these sugars, usually 0.50 per cent. strong and containing 1 per cent. of Pasteur's mixture (Strassburger and Hillhouse), were heated at 100° C. in a steam sterilizer for thirty minutes on three successive days. The ster-

ilized fluid was then inoculated with the particular yeast under trial and incubated at 30° C. for from two to thirty days; then again sterilized at 100° C., cooled, made up to volume, filtered, and the sugar present determined by Allihn's method. The usual precautions to prevent contamination with organisms other than the yeast under trial were observed throughout the operation from inoculation to filtration. The temperature of 30° C. was chosen, inasmuch as bread yeast appeared to act most vigorously at this temperature. The percentages of maltose removed and of lactose remaining were readily calculated from the copper reduced prior to, and that reduced subsequent to, the removal of maltose. Obviously, when working with known quantities, the completeness or the incompleteness of the removal of the maltose may be readily measured in this manner.

From about 10 per cent. to nearly all of the maltose remained unacted upon by eight of the yeasts. One of these yeasts, however, after a few preliminary trials completely and uniformly hydrolyzed and fermented the maltose.

S. anomolus was much the most active yeast used. It produced a heavy white growth in tube cultures in a few days. After two days' inoculation at least three-fourths of the maltose had disappeared and the proportion of maltose modified uniformly decreased up to sixteen days, after which there was no apparent change. It was found necessary to filter through a bacterial filter in order to remove the yeast cells and to prevent a precipitation of the flocculent matter by the Allihn solution, which was equivalent to a weight of from 1 to 2 per cent. of copper. The following table shows the result of some of the work with this yeast. The figures clearly show the relation of filtration, of the time element and of the use of the Pasteur's mixture to complete success.

Maltose taken. Gram.	Pasteur's mixture. Grams.	Volume of solution.	Solution. inoculated. cc.	Days acted on.	CuO from 25 cc. solution.	Maltose remaining. Per cent.
1	2	200	50	2	0.0138	7.47[1]
1	2	200	50	4	0.0044	2.38[1]
1	2	200	50	13	0.0030	1.63[1]
1	2	200	50	16	0.0025	1.35[1]
1	2	200	50	16	0.0000	none[2]
1	none	200	50	11	0.0198	10.68[1]
1	none	200	50	34	0.0089	4.82[2]
1	0.5	200	50	31	0.0040	2.16[2]
1	1	200	50	14	0.0063	3.42[2]
1	1	200	50	38	0.0000	none[2]

[1] Not filtered with bacterial filter. [2] Filtered with bacterial filter.

It may be remarked that when this yeast was grown for from four to twenty-four days in a solution of lactose, from 98 to 100.96 per cent. lactose was recovered, showing that this sugar was unaltered by the yeast. It may also be remarked that the fermentation method, using *S. anomolus*, has been tried upon several of the proprietary foods upon the market; that through the courtesy of the chemist of one of the companies manufacturing this class of goods the writer has been allowed to compare analytical results with manufacturer's formulas as regards percentages of maltose and lactose; and that a reasonably close agreement has been found.

It is interesting to note that in those solutions in which the other yeasts failed—as they always did—completely to transform all the maltose, reinoculation with *S. anomolus* readily hydrolyzed and removed the remaining maltose, while in no case did it appear in any way to affect their lactose content.

Vigorous growth is needed to hydrolyze the last traces of maltose. To produce this vigorous growth it is necessary to add some of the mineral elements found in the ash of yeast. Pasteur's mixture (Strassburger and Hillhouse) furnishes the necessary elements.

The method in its present form may be briefly outlined as follows: The solution containing maltose and lactose having been made approximately 0.5 per cent., and containing 1 per cent. of Pasteur's mixture, 50 cc. are heated for three successive days in a steam sterilizer, under the usual precautions, to 100° C. for thirty minutes. The fluid thus sterilized is inoculated liberally with a pure culture of *S. anomolus* and incubated at about 30° for from two to three weeks, the culture having been grown in agar and transferred to Pasteur's fluid prior to its use. After the incubation, the fluid is filtered through a bacterial filter and the copper determined in the usual manner by Allihn's method. The difference between the copper thus determined and that present prior to inoculation may be calculated as maltose, the remainder as lactose.

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