METHODS FOR THE CHEMICAL AND BACTERIOLOGICAL EXAMINATION OF ICE-CREAM.*

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The increased consumption of ice-cream, due to prohibition, will result in regulations, where none now exist, with reference to the fat and bacterial content of this product; therefore, the subject of the chemical and bacteriological examination of ice-cream is of sufficient importance to present the following simple methods of analysis. For routine work, the following is a very satisfactory method for the quantitative determination of fat:

QUANTITATIVE DETERMINATION OF FAT.

Accurately weigh 9 grammes of the melted and thoroughly mixed sample into a Babcock milk-bottle, add 10 Cc. glacial acetic acid, follow with 10 Cc. sulphuric acid, sp. gr. 1.84, and after mixing thoroughly by a rotary motion allow to stand for at least five minutes. Then place in the centrifuge and whirl for eight minutes, after which add hot water up to the neck of the bottle and whirl for five minutes. Then take the bottles out of the machine and place in hot water for about ten minutes, and after returning to the machine whirl for three minutes and then, by means of calipers, read the percentage of fat on the neck of the bottle; multiplying this reading by two gives the percentage of fat in the sample.

This is a simple method, is inexpensive to operate, and yields accurate results when applied to vanilla ice-cream. For chocolate, strawberry and other kinds of ice-cream the method does not work so well, and for these samples employ the Roese-Gottlieb method for determining fat in condensed and evaporated milk, and apply it to ice-cream as follows:

Accurately weigh about 4 grammes of the homogeneous sample into a Rohrig tube or similar apparatus, dilute with water to about 10.5 Cc., add 1.25 Cc. of concentrated ammonium hydroxide (2 Cc., if the sample is sour) and mix thoroughly. Add 10 Cc. of 95% alcohol and mix well. The total volume of liquid in the tube should now be somewhat less than 22 Cc. and be slightly below the stop-cock. Now add 25 Cc. of washed ethyl ether and shake vigorously for half a minute, then 25 Cc. of petroleum ether (redistilled slowly at a temperature below 60° C., preferably, and shake again for half a minute. Let stand 20 minutes, or until the upper liquid is practically clear and its lower level constant. Draw off as much as possible of the ethereal fat-solution (usually 0.5 to 0.8 Cc. will be left) into a weighed flask.

Reëxtract the liquid remaining in the tube, this time with only 15 Cc. of each ether, shaking vigorously half a minute, with each, and allow to settle. Draw off the clear solution into the same flask as before and wash the tip of spigot, the funnel, and the filter with a few cubic centimeters of a mixture of the two ethers in equal parts (previously mixed and free from deposited water). For absolutely exact results the reëxtraction must be repeated. This third reëxtraction yields usually not more than about a milligramme of fat, if the previous ether-fat solutions have been drawn off closely, an amount averaging about 0.02 percent on a 4-gramme charge. Evaporate the ether slowly over a steam pipe or electric hot plate, then dry the fat at 100° C. until loss of weight ceases. Prove the purity of the fat by dissolving in a little petroleum ether. Should a residue remain, wash the fat out completely with petroleum ether, dry the residue, weigh and deduct the weight. (This should not often be necessary.) Finally deduct the weight obtained by blank determination on the chemicals used.

This method leaves little to be desired with reference to accuracy. The tube employed by us for the extraction is on a glass foot and has the following dimensions: One inch in diameter, total length $9^1/_2$ inches, $1^{10}/_{16}$ inches from the base to the lower part of stop-cock, and holds about 22 Cc. from the base to the

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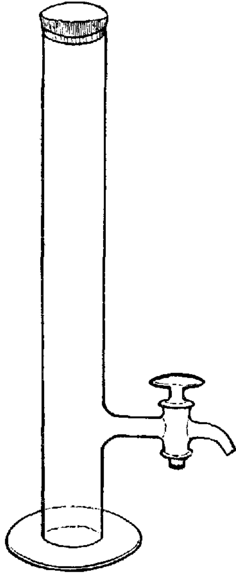
lower part of the stop-cock, thus allowing the ethers to be drawn off without disturbing the lower layer. This is much shorter and wider than the usual apparatus employed, and has the advantage that during the shaking it can conveniently be held under running water, and thus the solvents are prevented from becoming overheated. Any chemical apparatus house will make these tubes to order at a nominal cost. We employ this method and apparatus for quantitatively determining fat in emulsions, etc.



Extraction Tube for Quantitative Determination of Fat in Ice-Cream.

BACTERIOLOGICAL EXAMINATION.

The samples of ice-cream are collected in 4-ounce, wide-mouthed, glass-stoppered, sterilized bottles, and the material allowed to liquefy in the containers; after which 1 Cc. is placed, by means of a sterile pipette, in 99 Cc. of sterile water and, after shaking thoroughly, 1 Cc. of this dilution is added to another 99 Cc. of sterile water. This makes a dilution of 1 to 10,000. After shaking the bottle thoroughly add $^1/_{10}$ Cc. of this dilution to one Petri dish and 1 Cc. to another Petri dish. To each dish add about 10 Cc. liquefied agar, at a temperature of about 45° C., and after mixing by a rotary motion, allow to harden and then place in an incubator at 37° C. for 48 hours, after which count the colonies with a lens, magnifying $2^1/_2$ diameters, or what the opticians call a $3^1/_2x$ lens. The number of colonies in the dish, when 1 Cc. is employed, is multiplied by 10,000, and in the case of the 1 Cc. by a 100,000 to obtain the number of bacteria per Cc. of ice-cream.

The agar is made according to the "Standard Methods of Bacteriological Analysis of Milk," reprinted from *American Journal of Public Health.* This publication may be obtained for a few cents by writing to 126 Massachusetts Ave., Boston, Mass. Methods with reference to the sterilization of apparatus, etc., given are followed.

We incubate the plates in a Freas electric incubator, which maintains a constant temperature of 37° C. For convenience we employ an autoclave with 15 pounds' steam pressure in preparing our culture media. An Arnold sterilizer gives equally good results, but possesses the disadvantage that by its use three successive days are required to sterilize the media. Glassware, collecting apparatus, etc., are sterilized by dry heat for one hour at a temperature of about 175° C. Dilutions are made in 4-oz. French square bottles using non-absorbent cotton as plugs.

The above methods for the quantitative determination of fat and bacteria in ice-cream are simple and accurate.

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