ELECTROMETRIC pH DETERMINATIONS OF DIFFERENT AGAR MEDIA AT 25 AND 45 C

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ABSTRACT

In all tests performed using antibiotic test medium No. 1, APHA standard reference, BBL standard methods, violet red bile, and desoxycholate lactose agars, the overall mean values of pH readings were significantly higher ($p<0.01$) when determined at 25 C than at 45 C. This difference varied between 0.14 and 0.38 pH unit. Whether this difference has practical significance in routine laboratory use of these media was not determined.

In determining the pH of Standard Methods Agar, the 11th edition of *Standard Methods for the Examination of Dairy Products* (SMEDP) stipulates: "Make determinations at 45 C without diluting medium or, providing laboratory can demonstrate that results are equivalent, at lower temperatures (20 C to 35 C) if medium is diluted 1:1 with freshly distilled water or 1:2 with freshly boiled distilled water". The 12th edition of SMEDP specifies: "Determine hydrogen-ion concentration of culture media at 25 C either electrometrically or colorimetrically and record reaction in terms of pH".

No published reports have been found indicating the advantages of one temperature over the other for making pH determinations. However, correspondence from representatives of Difco and Bioquest (BBL) indicates that they adjust their media at 25 C and that when the pH of Standard Methods Agar was determined at 45 C low and erratic values 0.1 to 0.3 of a pH unit below the pH specified on the bottles of the media were obtained.

The present study was conducted to obtain specific information regarding pH values of Standard Methods Agar as well as of several other media at 25 C and 45 C.

MATERIALS AND METHODS

pH meter

A Beckman (Zeromatic II, Model 96 A) was employed.

Media

The following media were employed: APHA Reference Standard as used in the Productivity Test for Standard Methods Agar; Standard Methods Agar, BBL Lot. No. 610639; Violet Red Bile Agar BBL Lot No. 903607; Desoxycholate Lactose Agar BBL Lot No. 708642; and Antibiotic Medium No. 1, Difco Lot No. 485630.

TABLE 1. COMPARISON OF pH DETERMINATIONS ON APHA STANDARD REFERENCE AGAR MEDIUM[1, 2]

Trial	Before autoclaving		After autoclaving	
	25 C	45 C	25 C	45 C
1	7.02	6.79	7.05	6.73
	7.00	6.78	7.00	6.75
	7.01	6.73	7.07	6.78
	7.02	6.70	7.03	6.70
2	7.09	6.85	7.09	6.83
	7.08	6.85	7.08	6.83
	7.09	6.87	7.10	6.83
	7.09	6.86	7.09	6.82
3	7.00	6.87	7.02	6.82
	6.98	6.89	7.01	6.82
	7.00	6.90	6.99	6.82
	6.99	6.89	7.01	6.82
4	7.05	6.79	7.08	6.79
	7.05	6.80	7.03	6.80
	7.00	6.75	7.00	6.78
	7.01	6.83	7.04	6.79

[1]At 25 C: overall mean 7.04 with standard deviation of 0.04
[2]At 45 C: overall mean 6.80 with standard deviation of 0.05

Water baths

A National Appliance Company (NAPCO) Model 8725, water bath was maintained at 45 C ± 2 C and another NAPCO bath Model 8735-4 was maintained at 25 C ± 1 C.

Procedure

Six hundred milliliters of each medium were prepared. The Standard Methods agars and the antibiotic medium were each divided into two 300 ml samples. One portion was then autoclaved. Neither coliform medium was autoclaved. All samples were prepared and divided randomly by one technician into four 75 ml portions contained in 250 ml beakers. The other technician determined the pH values of the media, not knowing the identity of any sample.

The water bath contained the numbered beakers of media, distilled water, certified Coleman buffer (pH 7.00 ± 0.01 at 30 C), and certified Coleman buffer (pH 9.02 ± 0.01 at 30 C).

The pH meter electrodes were washed with distilled water and allowed to equilibrate at 45 C in the distilled water. The

TABLE 2. COMPARISON OF pH DETERMINATIONS ON BBL STANDARD METHODS AGAR AT TWO TEMPERATURES BEFORE AND AFTER AUTOCLAVING[1, 2]

Trial	Before autoclaving		After autoclaving	
	25 C	45 C	25 C	45 C
1	7.00	6.70	6.91	6.72
	7.02	6.72	7.01	6.72
	7.00	6.72	7.01	6.70
	7.02	6.69	7.02	6.69
2	7.08	6.82	7.05	6.83
	7.00	6.83	7.08	6.87
	7.09	6.83	7.09	6.83
	7.08	6.83	7.10	6.86
3	7.00	6.82	6.98	6.80
	7.00	6.82	6.98	6.80
	7.04	6.87	6.99	6.80
	7.00	6.87	6.97	6.80
4	7.02	6.73	7.04	6.77
	7.05	6.77	7.02	6.79
	7.05	6.78	7.01	6.80
	7.02	6.80	7.00	6.75

[1]At 25 C: overall mean 7.02 with standard deviation of 0.04
[2]At 45 C: overall mean 6.78 with standard deviation of 0.06

TABLE 3. COMPARISON OF pH DETERMINATIONS OF ANTIBIOTIC MEDIUM NO. 1 AT TWO TEMPERATURES BEFORE AND AFTER AUTOCLAVING[1, 2]

Trial	Before autoclaving		After autoclaving	
	25 C	45 C	25 C	45 C
1	6.47	6.22	6.48	6.22
	6.48	6.22	6.47	6.21
	6.46	6.22	6.47	6.22
	6.43	6.22	6.47	6.22
2	6.47	6.22	6.48	6.21
	6.42	6.21	6.46	6.22
	6.45	6.21	6.48	6.22
	6.48	6.19	6.45	6.22
3	6.43	6.25	6.43	6.25
	6.43	6.22	6.43	6.22
	6.43	6.23	6.43	6.23
	6.43	6.21	6.45	6.23
4	6.43	6.23	6.45	6.23
	6.45	6.22	6.43	6.23
	6.45	6.22	6.45	6.23
	6.43	6.22	6.43	6.23

[1]At 25 C: overall mean 6.45 with standard deviation of 0.02
[2]At 45 C: overall mean 6.22 with standard deviation of 0.01

temperature compensator was set at 45 C and the pH meter was then standardized at 45 C with the 7.00 buffer and checked for accuracy with the 9.02 buffer. The electrodes were washed with distilled water between measurements and carefully wiped with Kimwipes. The pH of the medium in each beaker was then determined.

All of the beakers of media and buffers were then transferred to a 25 C water bath. Fresh distilled water was used. The solidified agar was macerated with a glass rod while the pH meter was equilibrating at 25 C. The temperature compensator was set at 25 C and the pH meter was restandardized. The pH of the media at 25 C was determined following the same procedure as given for the measurement at 45 C. Electrodes were then inserted into boiling distilled water to insure removal of agar. When not in use, electrodes were placed in distilled water.

Similar pH determinations were made on split samples of the plate count agars after media had been autoclaved at 121 C for 15 min. All data were analyzed statistically by analysis of variance.

RESULTS

A comparison of pH findings at 25 C and 45 C before and after autoclaving APHA standard reference agar media is presented in Table 1. No significant difference was found between the readings obtained before and after autoclaving and therefore the data at the respective temperatures were pooled. The mean pH value of 7.04 at 25 C was significantly greater ($p<0.01$) than the mean pH value of 6.80 at 45 C. Similar results were found when BBL standard methods agar was tested (Table 2), viz. at 25 C the mean pH was 7.02 and at 45 C it was 6.78

TABLE 4. COMPARISON OF pH VALUES OBTAINED ON VIOLET RED BILE AGAR AND DESOXYCHOLATE LACTOSE AGAR AT TWO TEMPERATURES

Trial	Violet red bile		Desoxycholate lactose	
	25 C	45 C	25 C	45 C
1	7.30	7.00	7.19	6.87
	7.29	6.95	7.19	6.85
	7.29	6.97	7.19	6.90
	7.31	6.93	7.20	6.90
	7.32	6.98	7.19	6.90
	7.30	6.97	7.20	6.90
	7.32	6.90	7.19	6.90
	7.35	6.98	7.19	6.89
2	7.27	6.92	7.18	6.85
	7.30	6.93	7.13	6.87
	7.30	6.93	7.18	6.83
	7.30	6.92	7.18	6.85
	7.30	6.91	7.18	6.82
	7.31	6.92	7.18	6.83
	7.30	6.92	7.19	6.83
	7.30	6.91	7.19	6.82
3	7.42	7.00	7.25	6.83
	7.40	7.02	7.25	6.84
	7.38	7.01	7.23	6.86
	7.43	7.01	7.24	6.85
	7.45	7.02	7.25	6.84
	7.50	7.01	7.22	6.83
	7.39	7.02	7.24	6.84
	7.40	7.00	7.24	6.85
Overall means	7.34	6.96	7.20	6.86
Standard deviation	0.06	0.04	0.03	0.03

—a difference of 0.24 unit. Likewise, antibiotic test medium No. 1 gave significantly higher readings at 25 C—a mean pH value of 6.45 as compared to one of 6.22 at 45 C—a difference of 0.23 unit (Table 3).

Violet red bile and desoxycholate lactose agars were investigated and pH readings obtained at 25 C were significantly higher than those at 45 C (Table 4). With violet red bile this difference was 0.38 pH unit, whereas, 0.14 unit was the difference with the desoxycholate lactose agar.

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NUTRITIONAL ASPECTS OF DAIRY PRODUCTS

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provided 76% of the calcium, 44% of the riboflavin, 38% of the phosphorus, 23% of the protein, 12% of the vitamin A, 10% of the thiamine, as well as significant amounts of other nutrients, such as vitamin D, magnesium, and niacin. In no other food can we obtain the quality and quantity of nutrients per calorie as in milk, all very vital to functions of body processes.

In nutrition, a variety of foods consumed as part of a meal, more completely and efficiently meet nutrient needs than single foods taken separately, no matter how necessary each one alone may be. Recent studies also show that the body will make better use of those necessary nutrients when they are obtained along with a variety of other nutrients. In thinking of the nutrient interactions that exist in whole milk, let us first evaluate the protein in milk. Remember there are five general groups of essential nutrients.

PROTEIN IN MILK

The excellent biological value of milk has been attributed to the amount and manner in which the amino acids are arranged in its proteins. Milk is a combination of several proteins. An important one is casein which is found only in milk and comprises around 80% of the total milk proteins.

The proteins of milk are of high quality. They contain, in varying amounts, all of the amino acids commonly found in protein, essential and otherwise. Moreover, the pattern of the distribution of amino acids in milk protein is excellent. A pint of milk can provide more than a woman's or man's minimal daily requirements for all the essential amino acids except the sulfur containing amino acid, methionine.

Milk proteins are only slightly affected by the processing temperatures used in pasteurization. Even the sterilization of evaporated milk and the modern technological processes used in the manufacture of dry milk yield products of high protein quality.

FAT IN MILK

Another important nutrient to be discussed is fat. (In recent years fat has almost become a bad word.) The fat I'm going to mention is not that 50 million pounds of excess that Americans are accused of lugging around, but the butterfat of milk.

Butterfat has withstood the brunt of criticism for many years. Whole milk is believed to be fattening. We all know the fallacy of this argument. Although the diet-heart controversy has exploited the saturation of butterfat, it has stimulated a multitude of very basic studies into butterfat chemistry. At this time there is no scientific evidence to substantiate the claim that animal fats in a balanced diet can cause heart attacks. Here are a few facts about milk fat. Butterfat is 60% saturated. However, only certain saturated fatty acids are believed to raise blood cholestreal levels. These fatty acids collectively comprise only 36% of butterfat, of which milk in turn is only 4% fat; they do not enter the diet in significant amounts from dairy foods. In a recent report, butterfat has been shown superior to vegetable fats in increasing calcium absorption; particularly at low levels of calcium intake.

Both vitamin A and carotene are present in high concentration in the fat portion of milk. The carotene, which forms vitamin A in the body, gives milk its characteristac creamy color. Although the natural vitamin D content of milk is low, about 85% of all fluid whole milk in the U.S.A. is fortified with vitamin D. Traces of other vitamins also occur in the fat of milk.

CARBOHYDRATES OF MILK

Lactose, the major carbohydrate of milk, is synthesized by the mammary gland, in a manner that is not yet completely understood. It accounts for about

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