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Research Article

Optimum Incubation Temperature for the Plate Count of Milk and Water

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Abstract

Three different incubation temperatures 25, 32 and 37°C were tested to determine the optimum incubation temperature for the aerobic plate count of raw and pasteurized milk for both running and stagnant water. The optimum incubation temperature for the plate count of raw milk was found to be 32°C, which revealed the highest total viable count ranging from 5.49×10^4 to 5.25×10^5 cfu/mL, the optimum incubation temperature for the plate count of [supposedly] commercial pasteurized milk was found to be 37°C which gave the highest total viable count ranging from 7.30×10^6 to 1.52×10^6 cfu/mL. The optimum incubation temperature for the running water and stagnant water was 25C which gave the highest viable count ranging from 3.00×10^2 to 3.70×10^2 cfu/mL (running water) and from 2.65×10^3 to 2.97×10^3 cfu/mL (stagnant water). The values of count given above were the highest and were obtained at 72 h of incubation but although the count at 48 h of incubation was slightly lower, this incubation time is recommended for both milk and water for reasons of economy in time and cost.

Keywords: Incubation temperature, pasteurized milk, plate count, raw milk, total viable count.

Introduction

Humans live in a world filled with microbes from birth until death (Tortora *et al.*, 1998), thereby the environment around is extensively polluted, including food especially milk and water. For many years the Sudanese people did not pay much attention to water pollution problems, but today the population is more aware of the importance of good water quality and its relation to diseases. The current interest in the formulation of standards for the quality control of food-stuffs in this country has prompted this work on the microbiological standards of milk. One of the most useful indices of the hygienic quality of milk and one on which milk grading is usually based on the count of live microorganisms in milk. This is done by the plate count method (American Public Health Association, 1971).

Total viable counts of food products do not only reflect the handling history, state of decomposition, or degree of freshness, but they may in some instances, reflect on the sanitary quality of foods. Total viable counts most effectively evaluate the sanitary quality of foods that do not support microbial growth (Jay, 1986). The objective of this work was to study the effect of incubation temperature on the plate count of milk and water and to find the optimum incubation temperature for the plate count of milk and water.

Materials and methods

Sterilization: Glassware wrapped in aluminum foil, were Sterilized in the hot air oven at 160°C for two hours (Barrow and Gelthan, 1993).

Autoclaving: It was used for sterilization of media, solutions and materials which could not withstand the dry heat. The exposure time was 15 min at 121°C under 15 pounds pressure (Barrow and Gelthan, 1993).

Preparation of media: Plate count Agar is non-selective medium for general viable counts of bacteria in food (Harrigan, 1998). It was obtained in dehydrated form (Biomark Laboratories, India). The medium composed of yeast extract, tryptone, D-glucose and granulated agar. It was prepared according to the manufacturer's instructions by using 17.5 g in 1 L distilled water and then sterilized by autoclaving at 121°C for 15 min.

Preparation of Butterfield's phosphate buffer: A stock solution of 68 g/L of monopotassium phosphate (KH_2PO_4) was prepared and the pH was adjusted to 7.2 with NaOH (1N). The volume was brought to 1 L with distilled water. The solution was sterilized for 15 min at 121°C and stored in refrigerator (FAO, 1992).

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For dilution blanks an amount of 1.25 mL of above stock solution was taken and the volume brought to 1 L with distilled water and dispensed into bottles to 90 mL and sterilized for 15 min at 121°C (FAO, 1992).

Collection of samples: Using sterile containers, a total of 8 samples of raw cow milk were obtained from the University of Khartoum's farm, plate counts were carried out within 2 h after milking. A total of 8 commercial pasteurized milk samples were brought from the market and plate counts carried out 24 h after pasteurization.

Preparation of dilutions of Samples: Ten mL from the milk sample was taken by sterile pipette and transferred to the first bottle containing 90 mL of dilution to give a 10^{-1} dilution, then, with a sterile pipette, a volume of 1 mL from the first dilution bottle was transferred to a second bottle of 9 mL sterile diluents to give a 10^{-2} dilution, then further dilutions were made similarly. In the case of water samples, the dilutions were prepared in same steps used for the milk samples.

Viable count of bacteria for milk samples (raw and pasteurized): Plate count agar (PCA) was used for non-selective cultivation and enumeration of bacteria, using the pour-plate technique as described by Harrigan and McCance (1976). In brief, 10 mL of homogeneous milk were added to 90 mL of phosphate buffer to give 10^{-1} dilution and then further dilutions were made by transferring 1mL to the next tube of 9 mL buffer. One mL from each suitable dilution was transferred aseptically into sterile Petri dishes and the plate count agar media was added. The inoculums were mixed with the medium and allowed to solidity. The plates were made in duplicates for each dilution and incubated at 25, 32 or 37°C for 72 h. The result was reported as the viable bacterial count per 1 mL of sample. Counting of the colonies was done every 24 h using colony counter (Scientific and Electronics Ltd.).

Viable count of bacteria for water (running and stagnant): Bacterial growth and enumeration for water samples was conducted using the same methods described above for the milk samples.

Results and discussion

Effect of incubation temperature on the plate count of raw milk: There were three temperatures tested (25, 32 and 37°C). Temperature 25°C gave the least growth and incubation in 32°C gave the highest viable count (Table 1). With respect to incubation time, it can be seen that counts at 32, 37 and 25°C reach the maximum at 48 h or 72 h of incubation. This result disagrees with Dirar (1976) who found that incubation at 37°C gave the highest viable count and both counts 37°C and 25°C reached the maximum at 48 h of incubation.

It might be during the 30 years, new strains of microorganisms have appeared or new practices followed. In United States of America, for instance, the incubation for plate count of milk is 23±1°C for 48±3 h (Haussler, 1972). These specifications were originally set up by a research committee of bacteriologists (Babel *et al*., 1955). Other work shows that incubation temperatures of 10, 20, 27 and 30°C gave higher counts than 33°C or 37°C and the selected organisms from plates incubated at the different temperatures grew best at 20°C and 27°C. The author recommended the use of 27°C as incubation temperature, instead of the present 32°C for the plate count of raw milk. Smith *et al*. (1973) obtained highest counts when plates were incubated at 29.9°C for 48 h. Our results show clearly that, the incubation temperature of 32°C is by far more superior to the lower temperature of 25°C for the plate count of raw milk. The viable count of chilled farm raw less than 10^5 per mL and the total viable count under aseptic conditions was less than 10^3 per mL (Harrigan and Mc Cane, 1976). It should be noted that, milk samples under test differ because the atmospheric variation between cold countries and tropical countries like Sudan. This fact shows that, it is unwise copying of standards of one country to another without testing.

Effect of incubation temperature on the plate count of pasteurized milk: The comparison of the three tested temperatures in Table 2 revealed that the lowest count was given at 25°C while 37°C gave the highest count. Plates incubated at 32°C and 37°C attained the maximum count only after 3 d of incubation. This result also disagrees with Dirar (1976) in Sudan and committee's finding in America (Babel, *et al.,* 1955). It might be that the differences are due to using different sanitations materials for cleaning the flours and utensils.

Effect of incubation temperature on the plate count of water: Results presented in Table 3 and 4 show that, the optimum incubation temperatures are not the same as in the case of milk. It can be seen that 25°C gave the maximum count (running water and stagnant water). In this study, the result disagrees with Dirar (1976) who found that incubation at 37°C gave the highest viable counts and 25°C gave the least growth, in U.S.A on incubation.

Conclusion

In conclusion, we recommend the incubation temperature of 23°C for raw milk and temperature of 37°C for pasteurized milk. For running water, the recommended temperature is 25°C. In all cases, we recommend the incubation time of 48 h for more time and cost although incubation for 72 h gave slightly higher counts.

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Table 1. Effect of incubation temperature on the plate count of raw milk.

Incubation Temperature (°C)	Incubation Time (h)	Viable Count (cfu/mL)	Log of Viable Count
25	24	1.19×10^5	5.07
	48	1.31×10^5	5.11
	72	1.34×10^5	5.12
32	24	5.26×10^5	5.72
	48	5.49×10^5	5.74
	72	5.49×10^5	5.74
37	24	4.47×10^5	5.65
	48	4.57×10^5	5.66
	72	4.61×10^5	5.66

Table 2. Effect of incubation temperature on the plate count of pasteurized milk.

Incubation Temperature (°C)	Incubation Time (h)	Viable Count (cfu/mL)	Log of Viable Count
25	24	5.70×10^5	5.75
	48	8.10×10^5	5.90
	72	8.30×10^5	5.91
32	24	1.12×10^6	6.05
	48	1.18×10^6	6.07
	72	1.27×10^6	6.10
37	24	1.30×10^5	6.11
	48	1.45×10^6	6.16
	72	1.52×10^6	6.18

Table 3. Effect of incubation temperature on the plate count of running water.

Incubation Temperature (°C)	Incubation Time (h)	Viable Count (cfu/mL)	Log of Viable Count
25	24	3.70×10^2	2.56
	48	3.00×10^3	3.47
	72	3.00×10^3	3.47
32	24	9.80×10^2	2.99
	48	1.45×10^3	3.16
	72	1.52×10^3	3.18
37	24	1.32×10^3	3.12
	48	1.55×10^3	3.19
	72	1.59×10^3	3.20

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Table 4. Effect of incubation temperature on the plate count of stagnant water.

Incubation Temperature (°C)	Incubation Time (h)	Viable Count (cfu/mL)	Log of Viable Count
25	24	2.65×10^3	3.42
	48	2.90×10^3	3.46
	72	2.97×10^3	3.47
32	24	2.20×10^3	3.34
	48	2.25×10^3	3.35
	72	3.26×10^3	3.35
37	24	9.80×10^2	2.99
	48	1.87×10^3	3.27
	72	1.93×10^3	3.28

More studies and researches should be done because it is not correct to designate an incubation temperature for the plate count of milk and water on one or two researchers' results. Team work is needed to obtain the correct results to contribute to finding standards and specifications special for our country Sudan.

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