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A Research Note

Melting Agar by Microwave Energy

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ABSTRACT

The microwave oven is very convenient for melting agar for viable cell counts. Composite data of four microwave ovens indicated that melting time for 50 ml of agar per bottle was about 1 min for one bottle, 1.5 min for two bottles, and 2.5 min for four bottles heated simultaneously. Melting time for 100 ml of agar per bottle was about 1.5 min for one bottle, 2.5 min for two bottles, and 4 min for four bottles. Melting times of agar in square or flat bottles were similar. Agar melted by microwave treatment performed in viable cell counts equally as well as agar melted by the conventional boiling method. Even after prolonged (50% longer than melting time) microwave treatment, performance of the agar remained unchanged. Agar melted by microwave treatment can remain in liquid form (48°C) in situ for about 30 min (50 ml) and 1 h (100 ml). When removed from the microwave oven immediately after melting, the agar remained in liquid form (48°C) at room temperature for about 25 min (50 ml) and 40 min (100 ml). The microwave oven is highly efficient in melting agar without detrimental effects on the performance of agar.

Microwave ovens have become a household item in the U.S. in recent years. They also have found their way into many food-related research laboratories where they are used as a general purpose heating device. We found that the microwave oven is very convenient for melting agar for the purpose of viable cell counts because the conventional method of melting agar by boiling is time-consuming and cumbersome.

Melting agar by microwaves was evaluated by Copson (*1*) and Van Zante (*4*) who studied the heating patterns of early models of microwave ovens. Our observations of microwave melting agar indicated that the number of bottles and the amount of agar in each bottle made a difference in the time needed to melt agar satisfactorily for viable cell counts.

The effect of microwave irradiation on microorganisms was reviewed by Fung and Cunningham (*2*); however, no information is available concerning the effect of microwaves on the performance of agar in relation to viable cell counts. The purpose of this investigation was to define more accurately the time and conditions needed to use microwave irradiation for melting agar for microbiological analysis. This information should be useful for laboratories planning to use a microwave oven to melt agar on a routine basis.

MATERIALS AND METHODS

Microwave ovens

Most experiments were done using a Sharp microwave oven (Paramus, NJ). For comparison, the Radarange (Amana, IA), the Radarange Plus (Amana, IA) and a Kenmore microwave oven (Sears, Roebuck and Company, Chicago, IL) were also used.

Bacteria and agar preparation

A culture of *Escherichia coli* (ATCC 11775) was grown in nutrient broth for 12 h at 37°C before use as inoculum to test the performance of standard plate count agar (Difco). The agar (50 ml or 100 ml per bottle) was placed in square bottles (250 ml; Kimble, Toledo, OH) or flat prescription bottles (6 oz; VMR Scientific Inc., Chicago, IL). The bottles with agar were first sterilized by autoclaving then held at room temperature for the agar to solidify before testing.

Melting agar by conventional method

Bottles of agar were put into a large kettle with boiling water until the agar was melted, which usually takes about 30 min. The bottles then were transferred to a 48 ± 2°C water bath to keep the agar in liquid form until needed for viable cell counts.

Melting agar by microwave

One bottle of agar (with cap loosened by 1/4 turn) was placed in the center of the Sharp microwave oven and irradiated until the agar was melted as evidenced by bubbling of the agar in the bottle. The time to reach bubbling of the agar was considered as the melting time. To melt agar in two or four bottles, the bottles were spaced equidistance from the center of the oven to facilitate even heating. They were irradiated until agar in all bottles started to bubble. All experiments were done in duplicate. All bottles with melted agar were placed in a 48 ± 2°C water bath and tempered before making viable cell counts. After the melting times of various batches of bottles were determined in the Sharp microwave oven, the entire experiment was repeated in three different microwave ovens to ascertain the melting time needed in other microwave ovens for a similar number of bottles. In this experiment, only single determinations were made of each variable and no viable cell counts were performed on these irradiated agars.

Performance of agar

Performance of agar melted by the conventional method and in the Sharp microwave oven was evaluated by making viable cell counts of

a culture of *E. coli* by standard methods (*3*). The plates were incubated at 32°C for 48 h before the numbers of *E. coli* colonies were counted. Duplicate viable cell counts were made for each batch of melted agar.

Prolonged microwave treatment of agar

After the time for melting the agar had been established, the same number of bottles then were irradiated 50% longer in the Sharp microwave oven. Viable cell counts as well as spillage of agar were evaluated to test the effect of prolonged microwave treatment on the performance of agar.

Solidification time

After microwave treatment of various batches of bottles, one set was left in the microwave in situ and another set taken out. Temperature declines of the agar were recorded by a digital thermometer to ascertain the time for agar to reach 48°C as well as to solidify completely.

RESULTS AND DISCUSSION

The melting times for one, two and four bottles of agar in square and flat bottles containing either 50 or 100 ml of agar in four different microwave ovens, indicated that 50 ml of agar melted faster than 100 ml of agar, and the time needed to melt agar in all bottles increased as the number of bottles increased. The Sharp microwave had the slowest melting time, whereas the Radarange Plus oven had the fastest melting time. The times obtained from the Kenmore microwave oven were similar to those obtained from the Radarange oven. The time difference between microwave ovens tended to decrease when more bottles were irradiated simultaneously. The average melting times for one bottle were 59 s (50 ml) and 95 s (100 ml), two bottles were 83 s (50 ml) and 145 s (100 ml), and four bottles were 136 s (50 ml) and 248 s (100 ml). There were practically no differences between the times needed to melt agar in square bottles vs. flat bottles.

The problem encountered in melting agar in multiple bottles simultaneously was uneven melting of agar in different bottles. Agar in some bottles may start to boil while agar in other bottles remains semi-solid. Each oven seems to have certain "hot spots" and "cold spots"; therefore, it is difficult to pin-point the exact time for melting agar in multiple bottles in the microwave oven. For practical purposes, it is not necessary to find the exact time, as long as the agar in all bottles is melted before use.

The performance of agar melted by minimum time as well as prolonged time in the Sharp microwave oven is shown in Table 1. There was no difference in viable cell counts of *E. coli* on any microwave-melted agar compared with the conventionally melted agar. The slight variation of counts can be attributed to the inherent error of the viable cell count procedure (*3*). Irradiating agar in the microwave oven 50% longer than melting time had no effect on the performance of the agar. Except for tests using four bottles of 50 ml agar each, all other tests resulted in spillage of agar into the oven due to vigorous boiling.

It is of interest to an analyst to know the time for melted agar to reach 48°C (the proper agar temperature for pouring plates) and to solidify completely after melting by microwave heating. Our data (not shown) indicate that agar in bottles left in situ remained in the liquid form (48°C) for 30 min (50 ml) and about 1 h (100 ml). When the bottles were held at room temperature, agar remained in liquid form (48°C) for about 25 min (50 ml) and 40 min (100 ml). Complete solidification of the agar took more than twice the time needed for the temperature to decline to 48°C.

TABLE 1. *Performance of agar after minimal melting time and prolonged melting time in a Sharp microwave oven.*

			Minimal time treatment		Prolonged time treatment	
	Variable	No. of bottles	Time (s)	Log_{10} CFU/ml	Time (s)	Log_{10} CFU/ml
Square bottles	50 ml agar	1	65	8.23	95[a]	8.30
		2	90	8.30	135[a]	8.26
		4	145	8.26	220	8.26
	100 ml agar	1	105	8.26	150[a]	8.28
		2	170	8.26	240[a]	8.28
		4	250	8.26	375[a]	8.28
	Conventional boiling[b]		1980	8.23	1980	8.28
Flat bottles	50 ml agar	1	65	8.26	95[a]	8.26
		2	100	8.26	150[a]	8.30
		4	140	8.26	210	8.32
	100 ml agar	1	110	8.26	150[a]	8.30
		2	160	8.23	240[a]	8.26
		4	260	8.26	390[a]	8.30
	Conventional boiling		1980	8.23	1980	8.28

[a]Spillage of agar occurred after irradiation time.

[b]Conventional boiling was done by placing bottles in boiling water until agar was melted. Square bottles with 100 ml of agar each were used. The viable cell counts obtained were used as controls.

In conclusion, we defined the approximate time for the microwave oven to melt agar in two shapes of bottles, two volumes of agar, and three combinations of numbers of bottles. For practical purposes, we recommend a melting time for 50 ml of agar per bottle as 1 min for one bottle, 1.5 min for two bottles and 2.5 min for four bottles heated simultaneously; melting time for 100 ml of agar per bottles as 1.5 min for one bottle, 2.5 min for two bottles, and 4 min for four bottles. Different laboratories should experiment with their own microwave oven for more precise times using our recommended times as guidelines. We also found that microwave heating has no detrimental effects on agar, even after prolonged heating.

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