

Inactivation of Microorganisms with Microwaves at Reduced Temperatures[†]

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ABSTRACT

We developed a pilot-plant nonthermal flow process using microwave energy to inactivate microorganisms. The process consists of multiple passes through the microwave generator. Each passed material goes to a receiving tank for subsequent passes. The flow rate was 0.96 to 1.26 kg/min and the dwell time per pass was 1.1 to 1.5 min. Five passes were used. The microwave energy is instantaneously and simultaneously applied to the system, and thermal energy is removed by a cooling tube within the process line in the microwave generator. The cooling tube maintains the temperature below 40°C. There was significant reduction in microorganisms in water, 10% glucose solution, and apple juice, and in yeast in beer. There was a slight decrease in microorganisms in tomato juice, pineapple juice, apple cider, and beer; and no effect in skim milk.

Food processors and consumers are concerned with the reduction or elimination of microorganism populations in heat-sensitive foods. We are developing a low-temperature process which is lethal to microorganisms with little or no effect on the food being processed. In a previous paper (3), we reported ≤ 4 -log-cycle reductions of microorganisms in fruit juice by microwave energy with simultaneous cooling so that temperatures remained below 40°C.

The process was a batch full-recycle process which by its very design resulted in recontamination of the test liquid food. Even a fully successful batch process would not become a commercial process.

The objective of this research was to do an engineering process study to develop a pilot-plant semicontinuous process to kill microorganisms which minimized back-mixing and recontamination. To accomplish this objective we modified the batch full-recycle process into a process using discrete passes through the microwave generator.

MATERIALS AND METHODS

The experimental system was a modification of the batch process previously reported (3). The concept combines instantaneous energy input to the food system by microwaves with rapid removal of thermal energy. In the batch process, we used a double-tube heat exchanger inside a continuous microwave dryer. The outer tube was polypropylene, which is transparent to microwaves, whereas the inner tube was stainless steel through which tap water flowed for cooling. Process fluid circulated in the annulus countercurrently to the cooling water. The microwave energy was absorbed by the process fluid in the annulus but was reflected by the inner tube. The cooling water flowing in the inner tube removed the

thermal energy from the process fluid to prevent significant temperature rise.

Figure 1 shows the revised experimental process. There were two 190-liter stainless-steel feed tanks which alternated as receivers. A sanitary lobe pump (TriClover rotary pump, Kenosha, WI, model #PRED3-1M-UC6-ST-S) pumped the process fluid through a 7-kW continuous microwave dryer (Cober Electronics Inc., Stamford, CT) at a controlled flow rate of 0.96 to 1.26 kg/min. The double pipe heat exchanger was inside the microwave dryer. The outer pipe of the heat exchanger, which contained the process fluid, was polypropylene Sanitech-T 1-1/2" sanitary pipe, 33.8 mm i.d. The inner pipe was stainless steel, 25.4 mm o.d.

After leaving the microwave, the process fluid went through a plate and frame heat exchanger (DeLaval, Sweden, model P5VER) with tap water at 3 to 6 kg/min used for cooling. Following the heat exchanger, the process fluid went to a second feed tank which alternated as a receiver and the tap water went to the microwave cooling tube.

We initially sanitized the system before introducing the test fluid and microorganisms. The system was sanitized by circulating water through the system with microwave energy applied and no cooling. In each experiment, after the process equipment was sanitized, 40 liters of feed were charged to the feed tank and inoculated with *Pediococcus* sp. NRRL B-2354 (formerly *Micrococcus freudenreichii*). We chose this microorganism because it has a thermal resistance similar to that of *E. coli* and is used to test for pasteurization in milk. This culture was supplied by L. K. Nakamura (U.S. Department of Agriculture, Peoria, IL) and was maintained and routinely transferred on tryptose agar (TA; Difco Laboratories, Detroit, MI). Two growth media were used, tryptone glucose yeast extract (TGY) broth (tryptone, 5 g; yeast extract, 5 g; glucose, 1 g; potassium phosphate dibasic, 1 g; double distilled and deionized water 1 liter; pH 7.00) and tryptic soy broth (TSB) (Difco) plus 0.25% glucose. TGY was formulated in the lab and TSB was purchased. Either a TGY or TSB culture grown at 28°C for 8 to 9 h was used to inoculate 500 ml of broth which was allowed to grow to the post-exponential-growth phase. The feed tank containing the experimental menstuum was inoculated from the broth to give approximately log 6.5 CFU/ml.

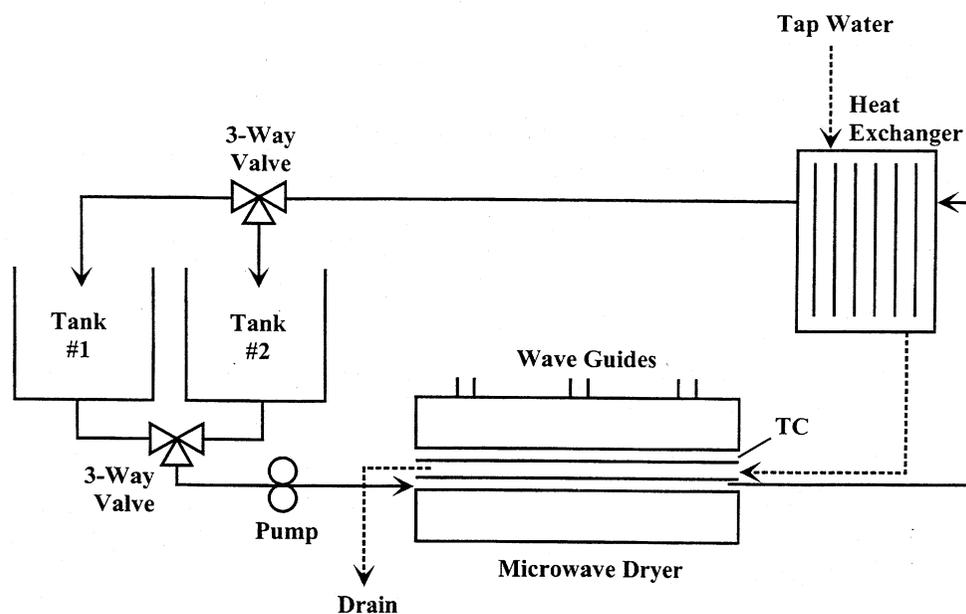


FIGURE 1. Modified experimental process flow sheet.

After inoculation, the feed was pumped through the equipment, replacing the sanitized water and achieving a nominal 16 liters of process fluid in the system and 16 liters in the feed tank. The effluent was collected in the second tank, which acted as the receiver. When the first feed tank emptied, the feed and receiver vessels were reversed by means of 3-way valves. To confirm that no extraneous cause was responsible for killing the microorganisms, we sampled the effluent, continued processing the fluid without the microwave generator on, and compared the two samples.

Microwave energy was applied at 5.0 to 5.4 kW and temperature continuously recorded at the microwave generator exit. Effluent from the plate and frame heat exchanger was sampled after each complete pass through the system.

Samples were taken in triplicate in the recycle line to the feed tank and appropriate dilutions in 0.1% peptone (Difco) were surface plated on TA plates using the spiral plating system (Spiral Systems Instruments, Inc., Bethesda, MD). The plates were then incubated at 37°C for 18 to 24 h, and the survivors were enumerated using a laser bacteria colony counter model 500A (Spiral Systems).

Except for water and glucose solution, the test fluids were purchased from local supermarkets. We made the beer from commercially available brewing kits. Apple cider, as used in this study, was the juice from freshly squeezed apples that was separated from the pomace. The cider had no further clarification.

RESULTS AND DISCUSSION

Previous results (3) showed that the exit thermocouple accurately reflected the bulk temperature of the processed fluid with only minor hot spots within the microwave. The temperature in these hot spots did not exceed 40°C. During the preprocess sanitization using the same flow rate used with the test fluids in the experiments, the sanitizing water temperature reached about 65°C with an inlet temperature of 22°C and no cooling. This would be sufficient to pasteurize the test fluids if there were no cooling. (Due to full recycle of the sanitizing water, the inlet temperature continued to rise and the exit temperature rose accordingly as we continued the sanitizing process). During the rest of the sanitizing

procedure, we adjusted flow rates to attain a sanitizing temperature throughout the entire system at or above 60°C.

After sanitizing with the microwave, the system was cooled. At time equal to zero, the process fluid was introduced and circulated through the process with the microwave generator off. When the microwave generator was started again (about 20 min), the process fluid exhibited a temperature rise through the microwave of about 25°C. However, the temperature in the microwave generator did not rise above 40°C, as indicated by the exit thermocouple. Therefore we conclude that, without cooling, the test fluids would have reached 65°C; but with cooling, they remained $\leq 40^\circ\text{C}$. There is a possibility that the temperature is not uniformly $\leq 40^\circ\text{C}$. Suppose that in the experiments there were hot spots which we did not detect, and that these hot spots reached 65°C for a time sufficient to kill the microorganisms. This scenario is not likely. Under identical process conditions, skim milk exhibited no kill. If the temperature had risen to 65°C, the microorganisms in skim milk would have shown some kill, but there was none. The skim milk experiment confirms that pasteurization conditions were not reached within the test fluids and supports the exit thermocouple measurements of $\leq 40^\circ\text{C}$.

We tested various fluids in the process as shown in Table 1. Initial experiments were made with water. Table 1 shows that, with a dwell time per pass of 1.1 to 1.5 min, there was a 0.3- to 1.7-log-cycle reduction in *Pediococcus* sp. after one pass. Duplicating these experiments with 10% glucose solutions (a simple model fruit juice) resulted in 0.1- and 0.7-log-cycle reductions after one pass. Unmistakably, microorganisms were killed at low temperatures with microwaves in this process. However, in skim milk there was no reduction of the microbial population.

Four runs were made with apple juice (with bacteria grown in TGY). Three of these runs are plotted in Figure 2. (All data are listed in Table 1). There was a very dramatic

TABLE 1. Effect of sublethal microwave energy on microorganisms

Fluid	Flow rate (kg/min)	Microwave exposure time/pass (min)	Log kill, one pass	Log kill, total	Temp. (°C)	Medium	Inoculum
Skim milk	1.19	1.20	0.0	0.00	39.0	TGY	<i>Ped.</i> ^a
Water	— ^b	—	1.7	3.70	35.0	TGY	<i>Ped.</i>
Water	1.26	1.10	0.3	2.30	35.0	TGY	<i>Ped.</i>
Water	1.05	1.30	0.7	3.30	35.0	TGY	<i>Ped.</i>
10% glucose	0.96	1.50	0.7	2.30	35.0	TGY	<i>Ped.</i>
10% glucose	1.06	1.30	0.1	1.50	35.0	TGY	<i>Ped.</i>
Apple juice	1.16	1.20	3.5	5.20	39.0	TGY	<i>Ped.</i>
Apple juice	1.19	1.20	4.6	5.10	40.0	TGY	<i>Ped.</i>
Apple juice	—	—	2.0	2.50	36.0	TGY	<i>Ped.</i>
Apple juice	1.21	1.20	1.4	2.10	39.0	TGY	<i>Ped.</i>
Apple juice	1.14	1.23	1.5	3.30	42.2	TGY	<i>Ped.</i>
Apple juice	1.50	0.93	0.0	0.20	38.6	TSB	<i>Ped.</i>
Apple juice	1.25	1.12	0.1	0.40	39.2	TSB	<i>Ped.</i>
Apple juice	1.31	1.07	0.2	0.70	45.5	TSB	<i>Ped.</i>
Apple juice	1.12	1.25	0.1	0.50	38.0	TSB	<i>Ped.</i>
Pineapple juice	1.08	1.30	0.1	1.10	38.0	TGY	<i>Ped.</i>
Pineapple juice	1.17	1.20	0.3	1.75	37.7	TSB	<i>Ped.</i>
Tomato juice	1.07	1.30	0.2	0.60	39.0	TGY	<i>Ped.</i>
Tomato juice	1.00	1.40	0.2	0.40	51.9	TSB	<i>Ped.</i>
Apple cider, as is	1.23	1.14	0.2	0.55	39.2	NA ^c	NA
Apple cider, as is	1.27	1.10	0.1	0.40	38.8	NA	NA
Apple cider	1.19	1.17	0.5	0.99	43.0	TGY	<i>Ped.</i>
Beer	1.21	1.16	0.2	2.01	39.0	TGY	<i>Ped.</i>
Beer	1.22	1.15	1.2	3.39	37.3	TGY	<i>Ped.</i> + yeast
Beer	1.14	1.23	0.7	2.94	35.7	NA	yeast

^a *Pediococcus* sp.

^b Flow rate was not measured.

^c NA, not applicable.

bacterial kill starting with the first pass, up to 4.6 log units in the first pass. However, in apple cider, there was little kill, about 1 log unit (Table 1). With no added bacteria and the initial count at 3 log CFU/ml there was about an 0.5-log-unit reduction in apple cider. Figure 3 shows that yeast and *Pediococcus* sp. were affected in beer. Yeast was reduced by 2.5 log CFU/ml and *Pediococcus* sp. by about 1 log unit.

As seen in Figure 4, tomato juice yielded a slight decrease, 0.2 log cycles on the first pass and 0.6 log units after five passes. Pineapple juice produced a 0.1-log-unit kill on the initial pass but a 1.1-log-unit kill after five passes.

We tested the effect of two different growth media for the bacteria in apple juice. As already shown in Figure 2, there was up to a 4.6-log-cycle kill in the first pass with TGY. As shown in Figure 5, in comparison to apple juice inoculated with TGY-grown bacteria, in TSB the bacteria were reduced only about 0.1 log cycle on the first pass and only 0.4 log cycle total. Apparently, the bacteria grown in TSB and inoculated in apple juice were more resistant than those grown in TGY.

As listed in Table 1, the bacteria grown in TSB were

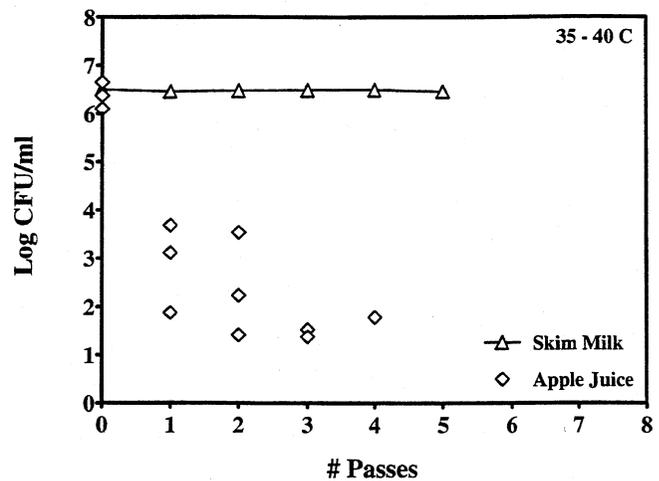


FIGURE 2. Sublethal microwave process results: Δ skim milk \diamond apple juice.

slightly more susceptible to microwave energy in pineapple juice than bacteria grown in TGY. There was little difference in tomato juice. Apparently, each food-microorganism system is independent.

For years, the literature has reported the disruption of microorganisms at sublethal temperatures with electromagnetic energy such as microwave and radio frequency (1, 7, 10, 11). Recently there has been a heightened interest in the use of pulsed electric fields to kill microorganisms in pasteurization (4, 9).

There are four predominant theories to explain a possible cold pasteurization: selective heating, electroporation, cell membrane rupture, and magnetic field coupling. There is also the dissenting opinion that there is no cold pasteurization (6): that the microorganisms are killed thermally but that the temperature distribution measurements are in error.

The selective heating theory postulates that the microorganisms selectively absorb the electromagnetic energy. The solid microorganisms get hotter than the surrounding fluid and reach temperatures required for pasteurization. Meanwhile, the surrounding fluid remains below the temperature required for pasteurization. Two of the theories are related. In the electroporation theory (2), the electrical potential across the cell causes pores to form in the weakened membrane, resulting in leakage of cellular material and cell

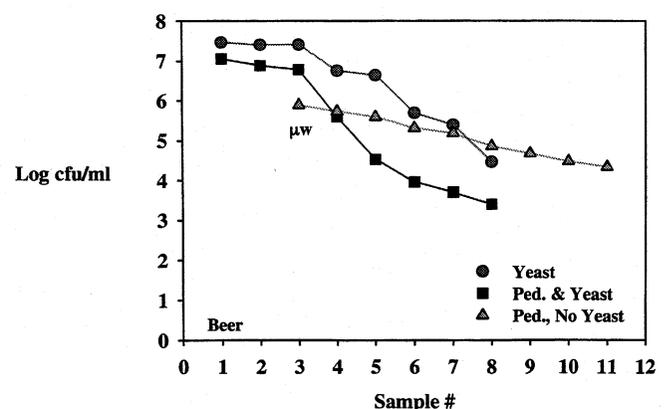


FIGURE 3. Sublethal microwave process results. beer + yeast, beer + yeast + *Pediococcus* sp., beer + *Pediococcus* sp.

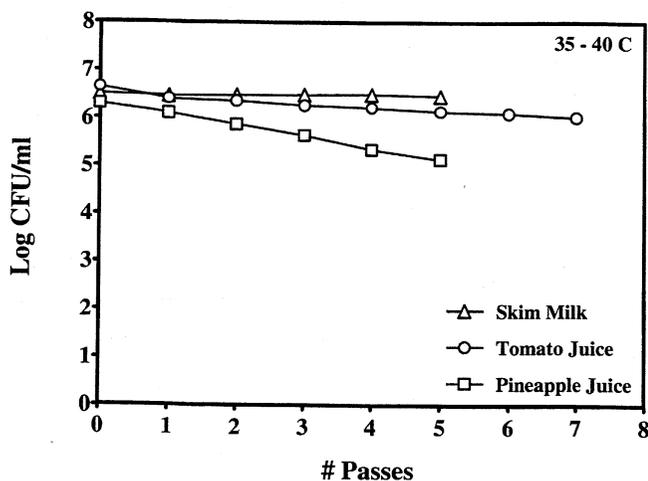


FIGURE 4. Sublethal microwave process results. Δ skim milk, \circ tomato juice, \square pineapple juice.

lysis. In the dielectric cell-membrane rupture theory (12), the voltage across the cell membrane is sufficient to burst the cell membrane, thus killing the microorganisms. In the fourth theory, cell lysis is explained by a coupling of the electromagnetic energy with critical molecules within the cells, such as protein or DNA (5, 8). Disrupting the internal components of the cells causes them to die.

Even though temperatures were normally below 40°C in the test fluids, microorganisms exhibited a reduction in some (water, glucose solution, and apple juice). There was only a slight reduction in microorganisms in apple cider, tomato juice, and pineapple juice, but there was no change in skim milk.

Bacteria are more readily destroyed in water, glucose solution, and apple juice than in apple cider or tomato or pineapple juice, and none were killed in skim milk. The obvious difference is the existence of insoluble solids in apple cider and tomato and pineapple juice where very little kill took place and essentially no insoluble solids in the clear fluids water, glucose solution, and apple juice. Yet there was no effect in milk. Water, glucose solution, and skim milk are essentially neutral whereas the juices are acidic (apple juice,

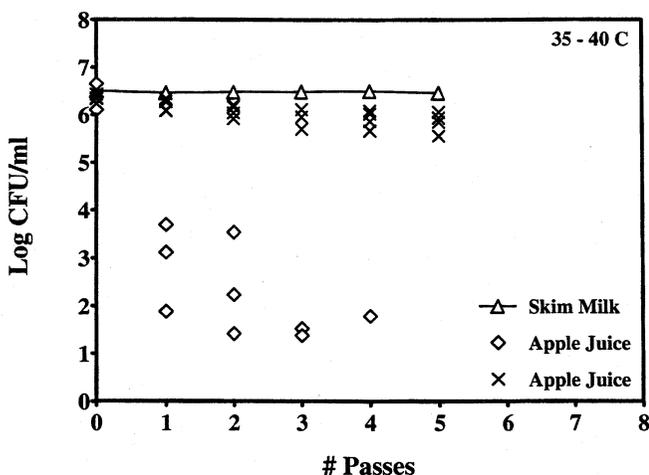


FIGURE 5. Sublethal microwave process results with bacteria cultured in different growth media: Δ skim milk, \diamond apple juice, TGY medium; \times apple juice, TSB medium.

pH ca. 4; apple cider, pH ca. 3.9; tomato juice, pH ca. 4.4; pineapple juice, pH ca. 3.7). To further confuse the issue, tomato juice (14.1 mmho), skim milk (4.7 mmho), and apple juice (2.2 mmho) are relatively conductive, whereas the other fluids are essentially nonconductive (<0.5 mmho).

There are other variables which may affect the efficacy of this process, for example, the specific microorganisms, microorganism growth media, other components of the fluid such as ethanol in beer, solids, dwell time, and power level. Dwell time and power level will be studied in the fully continuous process we are developing. Specific microorganisms, pH, other fluid components, and solids content will be studied as relevant to particular foods.

It is easy to speculate which theory fits the data. Realistically, the data neither affirm nor reject any of the theories. This process is a good beginning to studying the effects of microwave pasteurization at low temperatures. However, to elucidate the killing mechanism and develop a viable process, we must move to a new phase of the research and further modify the process to eliminate all recontamination of product with feed, effect a fully uniform temperature and dwell time distribution within the microwave generator, and maintain knowledge of the microorganism concentration throughout the process with time and location. To this end we are developing a new continuous process based on this work.

Our data show significant microorganism kills in some fluids using microwave energy at sublethal temperatures. Microorganisms become much more susceptible to stresses like acidic pH when the temperature is raised. The utility of this process depends on the microorganism-food system. The semicontinuous process forms a basis for a continuous process under development. The data do not clearly define the killing mechanism of microwave energy.

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