Journal of Food Protection, Vol. 66, No. 9, 2003, Pages 1712–1715

Research Note

Inactivation of *Saccharomyces cerevisiae* with Radio Frequency Electric Fields†

DAVID J. GEVEKE[1]* AND CHRISTOPHER BRUNKHORST[2]

[1]*U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038; and* [2]*Princeton Plasma Physics Laboratory, Princeton University, Princeton, New Jersey, USA*

MS 02-399: Received 5 November 2002/Accepted 14 March 2003

ABSTRACT

The application of radio frequency (RF) electric fields as a nonthermal alternative to thermal inactivation of microorganisms in liquids was investigated. A novel RF system producing frequencies in the range of 20 to 60 kHz was developed. Electric field strengths of 20 and 30 kV/cm were applied to suspensions of *Saccharomyces cerevisiae* in water over a temperature range of 35 to 55°C. The flow rate was 1.2 liters/min. The *S. cerevisiae* population was reduced by 2.1 ± 0.1 log units following exposure to a 30-kV/cm field at 40°C. The results of the present study provide the first evidence that strong RF electric fields inactivate microorganisms at moderately low temperatures. Increasing the field strength, the number of treatments, and the temperature enhanced inactivation. Frequency had no effect on inactivation over the range of frequencies studied.

Consumers are drinking more freshly squeezed fruit and vegetable juices than ever before *(2)* and are demanding that these beverages contain no pathogens. In addition, they demand that these juices have freshlike qualities and nutrient contents. Although conventional thermal pasteurization assures the safety of these products, it can also affect sensorial attributes. Therefore, alternate pasteurization processes are actively being sought. Examples of such processes include the use of high-pressure and electromagnetic energy. The use of radio frequency (RF) electric fields as a pasteurization method has been studied for over 50 years *(3).* Although reports often claimed that pasteurization had been achieved under nonthermal conditions and with weak electric fields, these assertions could never be confirmed. In order to test for the existence of weak-electric-field nonthermal RF inactivation, Brunkhorst et al. *(1)* assembled an RF system that accurately and precisely controlled temperature. When an electric field strength of 0.5 kV/cm at a frequency of 18 MHz was applied to apple cider, beer, deionized water, and tomato juice, thermal effects on *Escherichia coli* K-12, *Listeria innocua,* and yeast were observed, but nonthermal effects were not detected *(5).*

Sale and Hamilton *(7)* applied square wave pulses to suspensions of vegetative bacteria and yeast cells at room temperature and concluded that a minimum electric field strength of 5 kV/cm is necessary to achieve inactivation. These authors also observed that an increase in electric field strength produced an increase in inactivation and that yeasts were more sensitive to such treatment than bacteria were. The dielectric rupture theory has been proposed to explain this inactivation *(9).* When an external electric field is applied to a cell in a suspension, an induced voltage is formed across the membrane owing to the membrane's capacitance. As the voltage is increased, the opposite charges on either side of the membrane are attracted to each other with greater force, and the membrane becomes thinner. At a sufficiently high voltage, pores are formed in the membrane and the cell ruptures *(8).* There is a lag between the applied voltage and the induced voltage across the membrane owing to the membrane's capacitance. Kotnik et al. *(6)* reported on the time course of transmembrane voltage induced by RF fields. Although these authors stated that it is generally very difficult to predict the peak value of the induced transmembrane voltage, it is clear from their analysis that the voltage is significantly reduced as the frequency is increased from 100 kHz to 1 MHz.

Geveke et al. *(4, 5)* hypothesized that nonthermal inactivation might be achieved if RF energy with a field strength of ≥5 kV/cm and/or a frequency of <18 MHz was applied. The objective of this work was to test this hypothesis by investigating the effect of frequency and field strength on the inactivation of *Saccharomyces cerevisiae* at moderate temperatures. A novel RF system that was capable of applying a field strength of 30 kV/cm over a frequency range of 20 kHz to 60 kHz was assembled. *S. cerevisiae* was selected as the initial microorganism for testing on the basis of its known sensitivity to pulsed electric fields *(7).*

MATERIALS AND METHODS

Microorganism. *S. cerevisiae* ATCC 4126 was purchased from the American Type Culture Collection (Manassas, Va.). The

* Author for correspondence. Tel: 215-233-6507; Fax: 215-233-6406; E-mail: dgeveke@errc.ars.usda.gov.

† Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of similar nature not mentioned.

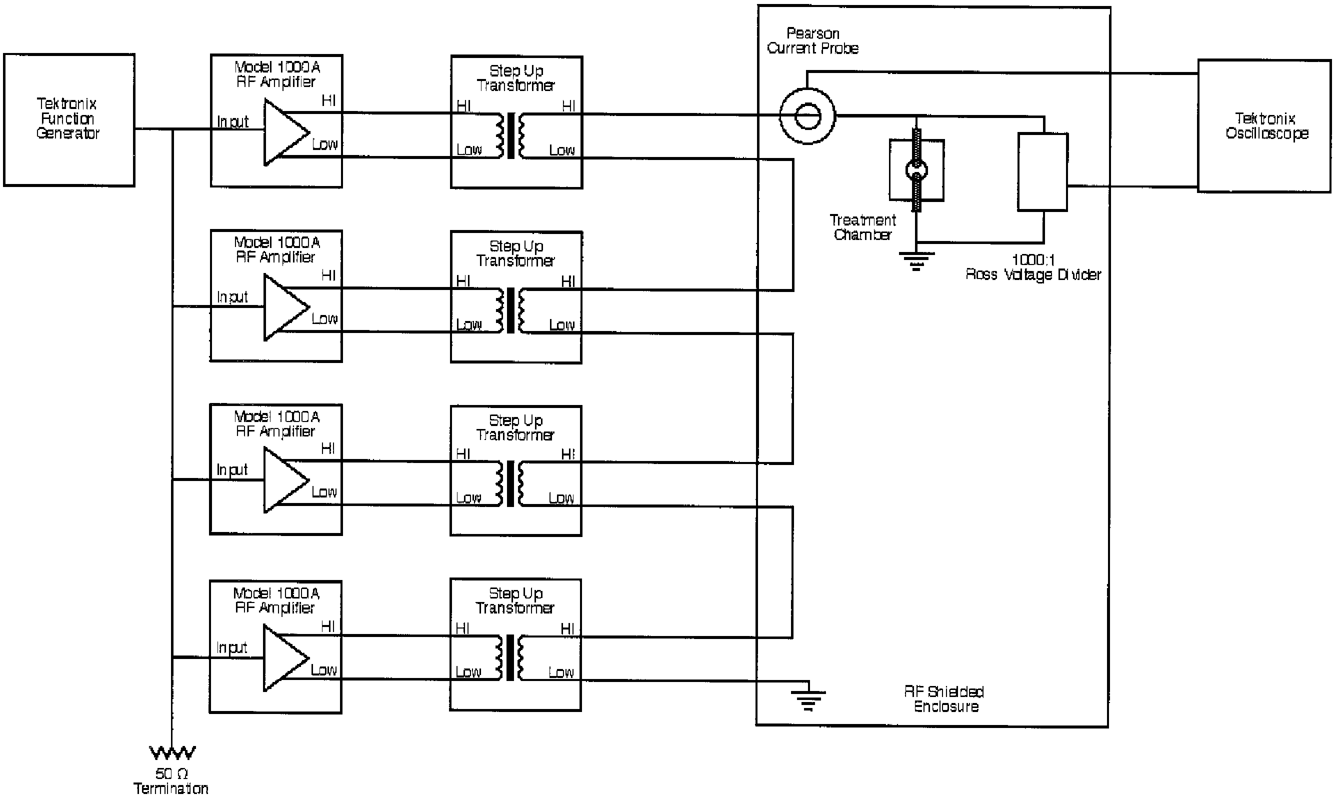

FIGURE 1. *Electrical diagram of RF system.*

yeast was cultured in yeast malt broth at 28°C for 24 h. The stationary-phase culture was diluted with sterilized deionized water to yield a population of ca. 6 log CFU/ml. The solution's pH was 7.0, and its conductivity was 60 μS/cm. This conductivity level was necessary in order to achieve the desired electric field strengths.

Equipment. An RF power supply system that produced a peak voltage of >4.0 kV over a frequency range of 20 to 60 kHz was designed, purchased, and assembled. It consisted of four 1-kW RF amplifiers (Model 1000A, Industrial Test Products, Port Washington, N.Y.) and four step-up transformers (Industrial Test Products). These components were connected in series as shown in Figure 1. A function generator (Model AFG 310, Tektronix, Beaverton, Oreg.) drove the amplifiers. The voltage input to the amplifiers and the voltage and current supplied to the RF treatment chamber were measured with an oscilloscope (Model TDS210, Tektronix), a current probe (Model 411, Pearson Electronics, Palo Alto, Calif.), and a voltage divider (Model VD15-8.3-A-KB-A, Ross Engineering, Campbell, Calif.).

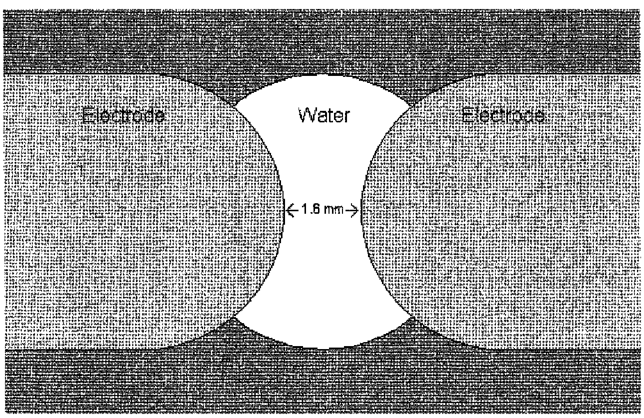

FIGURE 2. *Cross section of RF treatment chamber.*

In addition to the unique RF power supply system, a novel treatment chamber was designed and built. The chamber was made of Teflon. Liquid flowed through a bore with a diameter of 0.64 cm. Two stainless-steel electrodes were inserted into the Teflon perpendicular to and in contact with the liquid flow. The electrodes were cylindrical with a diameter of 0.64 cm, and their ends were rounded and polished. At their closest proximity the electrodes were 0.16 cm apart (Fig. 2). The output of the RF power supply was connected to the electrodes. An example of the voltage and current supplied to the RF treatment chamber is presented in Figure 3.

The experimental system included a stainless steel feed tank and a peristaltic pump (Driver Model 7523-40, Head Model 77200-62, Cole-Parmer, Vernon Hills, Ill.) that supplied the feed to the RF system at flow rates of 1.2 liters/min through Norprene pump tubing (Model 06402-15, Cole-Parmer) as shown in Figure 4. Turbulent flow (Reynolds number = 4,000) minimized local temperature gradients. On the basis of the dimensions of the electrodes and the treatment chamber, the fluid was exposed to strong electric fields for approximately 10 ms. At a frequency of 20 kHz, 200 cycles would occur in 10 ms. The inlet temperature to the RF treatment chamber was controlled with a stainless steel heat exchanger (Model SC0004, Madden Manufacturing, Elkhart, Ind.) and a temperature controller (Model CALL 9400, Cole-Parmer).

The temperatures of the process fluid immediately before and after the RF treatment chamber were measured with fiber-optic sensors (Model 790, Luxtron, Santa Clara, Calif.). The temperatures were continuously logged on a data acquisition system (Dasylab Version 5.0, Dasytec USA, Amherst, N.H.).

The process fluid was quickly cooled to <25°C with a stain-

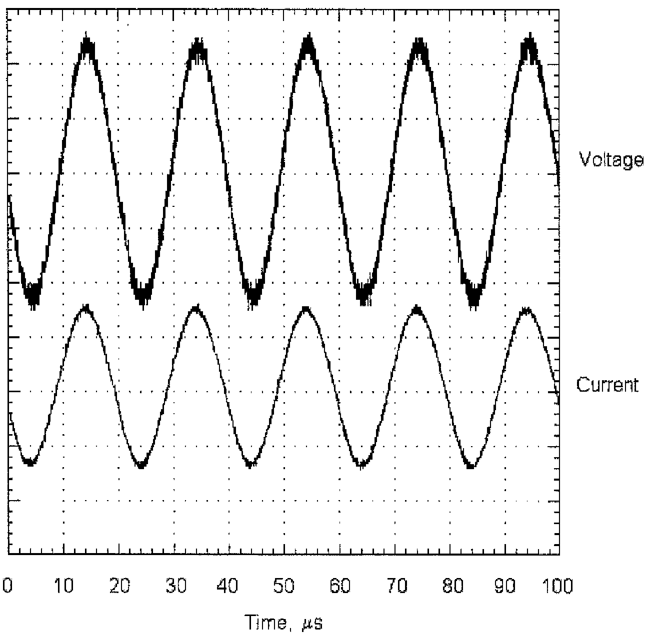

FIGURE 3. *RF treatment chamber voltage and current. Vertical scales are 2 kV and 0.2 A for voltage and current, respectively.*

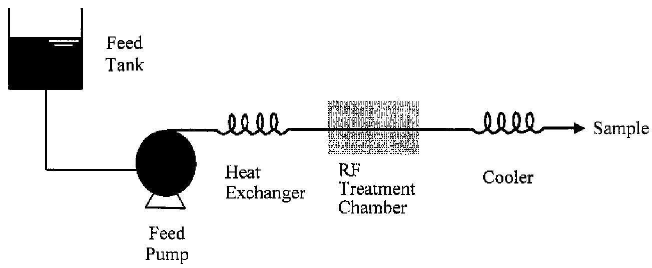

FIGURE 4. *Schematic diagram of continuous RF process.*

less steel cooling coil submerged in a water bath after it exited the treatment chamber. The time taken for the fluid to travel from the treatment chamber to the outlet of the cooler was approximately 3 s.

In some cases, the effect of exposure to multiple treatments was desired and the fluid was recycled. Product from the outlet of the cooler was collected in a carboy and was processed through the system a second or third time.

Each experiment was performed in duplicate. Results were expressed as the means of the values obtained ± standard deviations. The significance of differences in results obtained for the RF electric fields was calculated with Microsoft Excel statistical analysis algorithms.

Sampling and analysis. Duplicate samples of the feed and the products were taken. Appropriate dilutions of the samples were plated on tryptose agar with a spiral plater (Model Autoplate 4000, Spiral Biotech, Bethesda, Md.) and incubated at 28°C for 48 h. Enumerations were carried out with a colony counter (Model 500A, Spiral Biotech).

RESULTS AND DISCUSSION

RF electric fields successfully inactivated *S. cerevisiae* under nonthermal conditions. The extent of microbial inactivation was dependent on the electric field strength, the number of treatments, and the temperature.

A series of experiments were performed at a frequency of 20 kHz to determine the effect of electric field strength and temperature, and the results of these experiments are presented in Figure 5. The *S. cerevisiae* population was reduced by 3.1 ± 0.1 log units after exposure to a 30-kV/cm electric field with a treatment chamber inlet temperature of 26°C and an outlet temperature of 45°C. When the electric field was eliminated and the inlet temperature was raised to match the outlet temperature, 45°C, a reduction of only 0.3 ± 0.2 log was achieved. A field strength of 30 kV/cm with an outlet temperature of 40°C reduced *S. cerevisiae* by 2.1 ± 0.1 log units. Geveke et al. *(4, 5)* hypothesized that nonthermal inactivation might be achieved if RF energy with a field strength of ≥5 kV/cm and a frequency of <18 MHz was applied. The results of the present study confirm this hypothesis. The nonthermal inactivation is believed to be due to the dielectric rupture of the cells *(8)*.

At 55°C, *S. cerevisiae* levels were reduced by 4.7 ± 0.5 log units. Inactivation was significantly more extensive with outlet temperatures of 40 to 55°C when the field strength was 30 kV/cm rather than 20 kV/cm (critical value of Student's *t* test, $P < 0.01$). This was true even though the inlet temperatures of the treatment chamber were higher for the 20 kV/cm treatments. These results prove that electric field strength, in addition to temperature, plays an important role in inactivation.

Experiments were conducted at frequencies of 20, 40, and 60 kHz. A 20-kV/cm electric field at a temperature of 50°C was applied to *S. cerevisiae.* The reductions achieved ranged from 1.8 to 2.0 log units and were not significantly different across the limited range of frequencies ($P > 0.05$).

Inactivation increased significantly with increasing numbers of treatments ($P < 0.05$), as shown in Figure 6. A single treatment with a 30-kV/cm electric field at 35°C reduced the population of *S. cerevisiae* by 0.8 ± 0.1 log units, whereas three treatments resulted in a reduction of 3.8 ± 0.4 log units.

Additional studies are recommended. The effects of RF electric fields on microorganisms in liquid foods should be investigated. For such an investigation, an 80-kW RF power supply has been purchased and a new RF system is being

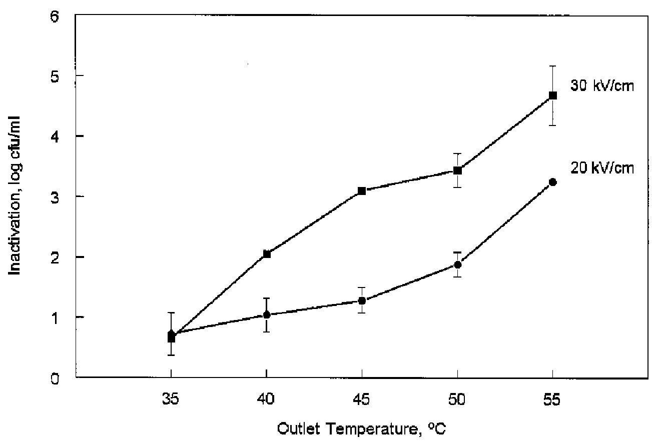

FIGURE 5. *Effect of temperature and electric field strength on the inactivation of* S. cerevisiae *at 20 kHz.* ●, *20 kV/cm;* ■, *30 kV/cm. Error bars indicate standard deviations.*

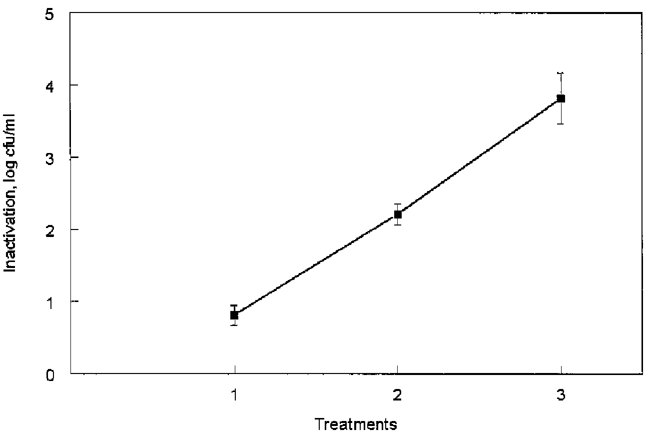

FIGURE 6. *Effect of number of treatments on the inactivation of* S. cerevisiae *at 35°C, 20 kHz, and 30 kV/cm. Error bars indicate standard deviations.*

assembled. In addition to allowing the processing of higher-conductivity liquids such as fruit and vegetable juices, the new system will provide power to several treatment chambers in series, thus eliminating the need for recycling. The system under construction may be able to provide power over a wider frequency range, thus enabling the study of frequencies of >60 kHz.

CONCLUSIONS

RF electric fields significantly reduced *S. cerevisiae* populations in water at 35°C. The results of the present study constitute the first well-documented evidence that strong RF electric fields inactivate microorganisms at low temperatures. Inactivation was dependent on electric field strength, temperature, and number of treatments; it was found to be independent of frequency in the range of 20 to 60 kHz. With an increase in power, the RF process should be applicable to higher-conductivity fluids such as vegetable and fruit juices.

ACKNOWLEDGMENTS

The authors thank A. Bigley for assistance in performing the experiments, as well as O. J. Scullen for microbiological support. The Princeton Plasma Physics Laboratory is funded by the U.S. Department of Energy and managed by Princeton University.

REFERENCES

1. Brunkhorst, C., D. Ciotti, E. Fredd, J. R. Wilson, D. J. Geveke, and M. Kozempel. 2000. Development of process equipment to separate nonthermal and thermal effects of RF energy on microorganisms. *J. Microwave Power Electromagn. Energy* 35:44–50.
2. Dahm, L. 2000. Refreshing the juice category. *Food Process.* 61:42–47.
3. Decareau, R. V. 1985. Microwaves in the food processing industry. Academic Press, Orlando, Fla.
4. Geveke, D. J., M. Kozempel, O. J. Scullen, and C. Brunkhorst. 2000. The combined effects of RF energy and thermal energy on microorganisms. Paper no. 6104. American Society of Agricultural Engineers Annual International Meeting, Milwaukee, Wis.
5. Geveke, D. J., M. Kozempel, O. J. Scullen, and C. Brunkhorst. 2002. Radio frequency energy effects on microorganisms in foods. *Innov. Food Sci. Emerg. Technol.* 3:133–138.
6. Kotnik, T., D. Miklavcic, and T. Slivnik. 1998. Time course of transmembrane voltage induced by time-varying electric fields—a method for theoretical analysis and its application. *Bioelectrochem. Bioenergetics* 45:3–16.
7. Sale, A. J. H., and W. A. Hamilton. 1967. Effects of high electric fields on microorganisms. I. Killing of bacteria and yeasts. *Biochim. Biophys. Acta* 148:781–788.
8. Zimmermann, U. 1986. Electrical breakdown, electropermeabilization and electrofusion. *Rev. Physiol. Biochem. Pharmacol.* 105:176–256.
9. Zimmermann, U., G. Pilwat, and F. Riemann. 1974. Dielectric breakdown of cell membranes. *Biophys. J.* 14:881–899.