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Development of continuous flow microwave and hot water bath system for destruction of spoilage microorganisms in food

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ABSTRACT

A continuous pasteurization system was designed based on a domestic microwave oven. Broth was pumped through helical coils of glass tubing placed in the center of the oven cavity. Inactivation of two selected spoilage microorganisms, *Bacillus cereus* and *Saccharomyces cerevisiae* in broth were evaluated under continuous flow microwave heating conditions and compared with conventional batch heating in a well stirred hot water bath. Inoculated broth was heated in a microwave oven (700 W, 2450 MHz) under continuous flow conditions to selected exit temperatures of 90°C for *B. cereus* and 60°C for *S. cerevisiae* at five power levels (210, 280, 350, 420, 490 W) and five time intervals (1, 2, 3, 4, 5 minute). Broth treated in hot water bath at 90˚C for *B. cereus* and 60˚C for *S. cerevisiae* was taken as control. There was a decrease in *B. cereus* and *S. cerevisiae* count after microwave and hot water bath treatment (control) with increasing treatment time. Higher microbial inactivation was observed at lower power levels. For all the microwave power levels, higher inactivation of *B. cereus* and *S. cerevisiae* was observed in comparison to control, this may be due to some non thermal effects associated with microwave. Heating rate and flow rate also increased with the increasing power level with decrease residence time to kill the contaminants. In future, this system may be useful for effective pasteurization of liquid foods e.g. sugarcane juice and soymilk without affecting the taste of processed juices.

Key words: *Bacillus cereus,* Broth, Hot water bath, Microwave heating, Pasteurization, *Saccharomyces cerevisiae*

Innovative approaches are required to inhibit and control food pathogens (Bevilacqua *et al*. 2008). Special attributes such as faster heating rate and greater penetration depth have made microwaves a unique tool for many industrial applications such as tempering, thawing, blanching, cooking, dehydration, sterilization and pasteurization (Knutson *et al*. 1987, Rosenberg and Bogl 1987). Even before the microwave oven was built, attempts to use microwaves to destroy microorganisms had begun (Fleming 1944). One of the earliest studies applied microwave energy to extend the shelf-life of bread (Olsen 1965). Numerous studies address the effect of microwave heating on pathogenic microorganisms in foods. Microwave (MW) lamps have been reported for the disinfection of bacteria with promising results (Barkhudarov *et al*. 2007). Food spoilage bacteria reported to be inactivated by microwave heating include *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens*, pathogenic *Escherichia coli, Enterococcus, Listeria monocytogenes, Staphylococcus aureus*, and *Salmonella* (Heddleson *et al*. 1996, Rosenberg and Bogl 1987, Knutson *et al*. 1987, Chipley 1980). A lot of research has been carried out on microwaves and their effect on several bacterial species (Vaid and Bishop1998). The actual mechanism of bacterial killing is still controversial. The authors suggested that MW radiation nonthermally induced different biological effects by changing the protein structures by differentially partitioning the ions and altering the rates and/or directions of biochemical reactions (Samarketu *et al*. 1996). It was also suggested that the turbidity of the cell suspension, protein, carbohydrate, chlorophyll a, carotenoids, and phycocyanin of microwave-exposed samples were inversely correlated with higher modulation frequencies (Samarketu *et al*. 1996). It is very difficult to precisely compare the effectiveness of microwave heating to conventional heating based on the literature, because of the different techniques employed or the lack of detail in the methods or materials used, especially in relation to temperature monitoring (Heddleson and Doores 1996). (Yeo *et al*. 1999) reported that the inactivation of *E. coli* was solely due to thermal effect, while other observed that heat generated during microwave exposure alone is inadequate

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to fully account for the nature of lethal effect (Barnes and Ho 1977, Salvatorelli *et al*. 1996). Thus combined action of heat generated by microwave may be involved in killing of bacteria. Researchers used copper cooling coil to maintain temperature. But this treatment was not effective and copper ions were toxic to the bacterial cells and cause inactivation (Cha *et al*. 1993). Other workers also controlled the temperature of the irradiated specimen through various timing, pulsing or cooling techniques (Welt *et al*. 1993). However, no available research has demonstrated a solid understanding of how MW radiation (at a defined range of frequencies) causes certain effects on microorganisms and why they occur.

The objective of this study was to experimentally evaluate and compare microbial destruction in a continuous flow microwave system operating at different power levels and hot water bath system, both set to operate at similar temperatures. Two target microbes (*B. cereus* and *S. cerevisiae*) were used for the comparison of microbial destruction. The model liquid systems used for these target microbes were their respective growth media, nutrient broth and malt extract glucose yeast extract-peptone (MGYP) broth, respectively.

MATERIALS AND METHODS

A continuous flow microwave pasteurization system was developed having the following design parameters: dimensions of heating tube, flow rate of pump, holding time, microwave power levels. The experimental pasteurization system was developed using a domestic microwave oven. Pasteurization experiments were carried out at laboratory scale with a continuous microwave pasteurization system (consists of four sub-systems: liquid pumping unit, heating unit, holding unit and display unit) developed in the Division of Food Science and Post-harvest Technology, ICAR–Indian Agricultural Research Institute, New Delhi. The liquid pumping unit consists of a peristaltic pump that pumps the liquid into the heating section. The flow rate (3-300 ml/min) of the pump was regulated by controlling the RPM (20-200) of peristaltic pump before entering the microwave oven. The heating unit consists of a microwave oven with technical features of about 230 V, 50 Hz, and 700 W with a frequency of 2 450 MHz has dimensions of 295, 458, and 370 mm and it also operates in pulsed mode. The microwave oven has the capability of operating at 10 different microwave output powers between 70 and 700 W. The adjustment of microwave power level and processing time is done with an analogue controller. The side of the oven was drilled with two holes for inlet and exit of liquid and fitted with reduction barb tees of borosilicate glass. A set of borosilicate helical glass coil having dimensions 160 cm length and 8 mm inner diameter was used for holding the juice and located centrally inside the oven cavity. Flexible silicone tubing was used to connect the reduction barb tees with helical glass coil and peristaltic pump. The heated liquid coming out from the oven was held in silicone tubing in hot water bath for the desired time and then collected in a borosilicate glass bottle with two port screw cap. Bottle was kept inside an ice water bath to cool the treated sample immediately. Sterile pressure equalization was made possible inside the glass bottle through use of pre-sterilized membrane filter (0.22 µm pore size). To know the liquid temperature at inlet and exit of microwave oven, two temperature electrodes were fitted in reduction barb tees. The temperature electrodes were further connected to a digital display unit. Before the pasteurization process could be started, silicone tubing was sealed with clips and all the components of the system (helical glass coil, reduction barb tees, silicone tubing, and glass bottle) were put inside an autoclavable bag and sterilized in an autoclave at 121°C for 15 minutes. After that they were assembled inside a pre-sanitized laminar flow chamber. All the tubing's were sanitized by circulating hot distilled water (65°C) through the system for 30 min before and after treatment as a precautionary measure. The liquid sample to be run through the microwave was kept in a controlled temperature environment for achieving the desired initial temperature. The liquid to be pasteurized was run through the system long enough to flush the remaining water in the system and to establish the steady-state condition indicated by a constant outlet temperature unless it was achieved the liquid was bypassed to another container with the help of a three-way valve. To prevent any contamination to the treated sample, the outlet or the glass bottle tubing was sealed with clip and open inside a laminar flow chamber which was pre-sanitized to be free from microbes. The pasteurization process was started for different combinations of microwave power and liquid flow rate.

The developed system was tested for its efficacy in reducing microbial load using two target microbes (*B. cereus* MTCC 1272 and *S. cerevisiae* MTCC 178). The model liquid systems used for these target microbes were Nutrient broth and MGYP broth. These two target microbes were purchased from IMTECH Chandigarh. These cultures were grown on their respective media and maintained as slant at 4˚C and subcultured at monthly interval.

In the experiment following combinations of five power levels (210, 280, 350, 420, 490 W) and five time intervals (1, 2, 3, 4, 5 min) were investigated for the inactivation of *B. cereus* at 90˚C and *S. cerevisiae* at 60˚C in continuous flow microwave system. Heat treated broth in hot water bath at 90˚C for *B. cereus* and 60˚C for *S. cerevisiae* was taken as control. Three replications were taken for all the experiments taking total number of experiments up to 156 (5×5×2×3=150 + 2×3=6). Initial microbial load of the model liquid system was 10 logCFU/ml for *B. cereus* and 8 logCFU/ml for *S. cerevisiae*.

Nutrient broth and MGYP broth were prepared by suspending 13 and 21 g readymade media supplied by Hi Media (Mumbai) in 1 000 ml distilled water and autoclaved at 121˚C for 15 minutes. Both the media broth were inoculated with 5% V/V inoculum of *B. cereus* and *S. cerevisiae* respectively and incubated for 24 hrs. at 35˚C in a incubator shaker.

Population of microorganisms was enumerated by

total plate count method using dilution plate technique. For enumeration of microorganisms, 20-25 ml of prepared medium was poured into pre-sterilized petri plates followed by spread plating of 0.1 ml of different dilutions and plates were incubated at 35ºC for 24-48 H. The results are expressed as LogCFU per ml of sample.

Flow rate and residence time of broth in microwave oven at different microwave power levels was measured with the help of a stop watch and measuring cylinder. Heating rate was calculated using the following formula

$$HR = (T_o - Ti)/ \Delta t$$

where, HR is heating rate (°C/s); T_o is outlet temperature (°C); Ti is inlet temperature (°C); Δt is residence time in microwave oven.

The experiment was conducted using a factorial CRD with 3 replications. Statistical analysis was done using analysis of variance (ANOVA) technique in the factorial CRD (Panse and Sukhatme 1984). The data was analyzed using SAS software SPSS (1998).

RESULTS AND DISCUSSION

Effect of continuous flow microwave pasteurization on microbial inactivation

The continuous flow microwave pasteurization was reported as an appropriate alternative to the conventional thermal processes because microwave is a volumetric and rapid heating method (Gerard *et al*. 2004, Clare *et al*. 2005, Giuliani *et al*. 2010). Inactivation of the *B. cereus* and *S. cerevisiae* in nutrient broth and MGYP broth by continuous flow microwave heating is illustrated in Fig 1. Initial microbial load of the untreated broth was 10 logCFU/ml for *B. cereus* and 8 logCFU/ml for *S. cerevisiae* on average. There was a decrease in *B. cereus* and *S. cerevisiae* count after microwave and hot water bath treatment (control) with increasing treatment time and significant differences were observed in *B. cereus* and *S. cerevisiae* count before and after heat treatment at 60° and 90°C. There were 9, 9, and 8.9 log reductions in the microbial counts of *B. cereus* when nutrient broth was treated at 90°C for 5 min at 420W, 490W, and control, respectively. Where as 7, 7, 6.9, and 6.33 log reductions in the microbial counts of *S. cerevisiae* were achieved when MGYP broth was treated at 60°C for 5 minutes at 350W, 420W, 490W, and control, respectively. Complete inactivation of *B. cereus* had taken place at 210, 280 and 350W at 3, 4 and 5 min where as *S. cerevisiae* was completely inactivated at 210 and 280W in 5 min. Higher microbial inactivation was observed at lower power levels due to increased heating time at lower power levels because of less power available per ml of broth. For all the microwave power levels, higher inactivation of *B. cereus* and *S. cerevisiae* was observed in comparison to control this may be due to some non-thermal effects associated with microwave. This finding is consistent with the study of (Nikdel *et al*. 1993), who reported that the microbial inactivation's in unpasteurized, conventionally pasteurized and microwave-pasteurized orange juice were 2×10^4, 550, and less than 10 CFU/ml, respectively. Koutchama *et al*. (2001) reported that microbial destruction occurs much faster under microwave heating than under thermal heating suggesting some enhanced effects associated with microwave heating. Similar results were also reported for *Staphylococcus aureus* in nutrient broth (Dreyfuss and Chipley 1980), *E. coli* in peptone water (Yaghmaee and Durance 2004), and *Saccharomyces cerevisiae and Lactobacillus plantarum* in apple juice (Tajchakavit *et al*. 1998) indicating that microwave treatment is more effective in reducing the microbes. Inactivation of bacteria by microwave treatment is thought to be the result of protein denaturation and aggregation in cytoplasm indicated by dark spots and the leakage of nucleic acids (Woo *et al*. 2000).

Effect of microwave power

The heating rate of broth increased with the increasing power level. Highest heating rate of 0.90°C/sec was found at 490W power level where as lowest heating rate of 0.33°C/sec was found at 210W power level when the MGYP broth

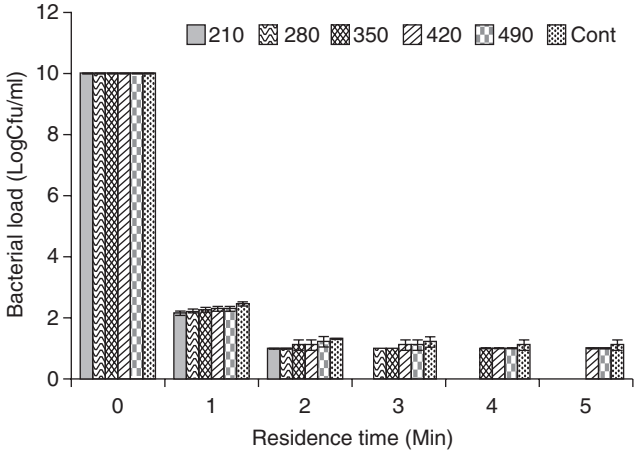

Fig 1 Effect of heating of nutrient broth at 90°C at different microwave power levels on *B. cereus* population

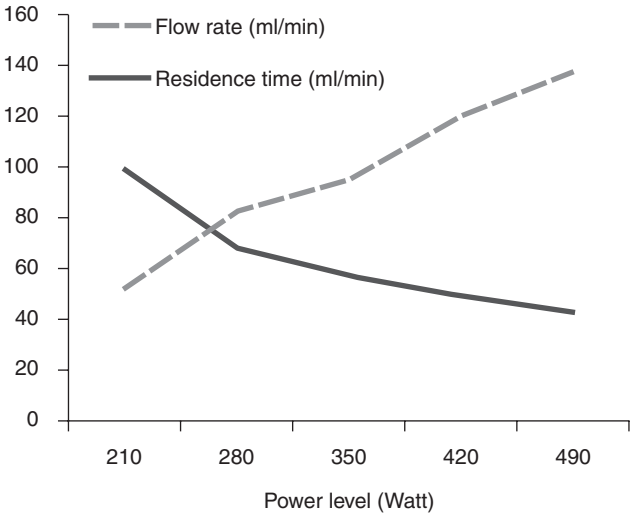

Fig 2 Effect of heating at different microwave power levels on flow rate and residence time of MGYP broth at 60°C

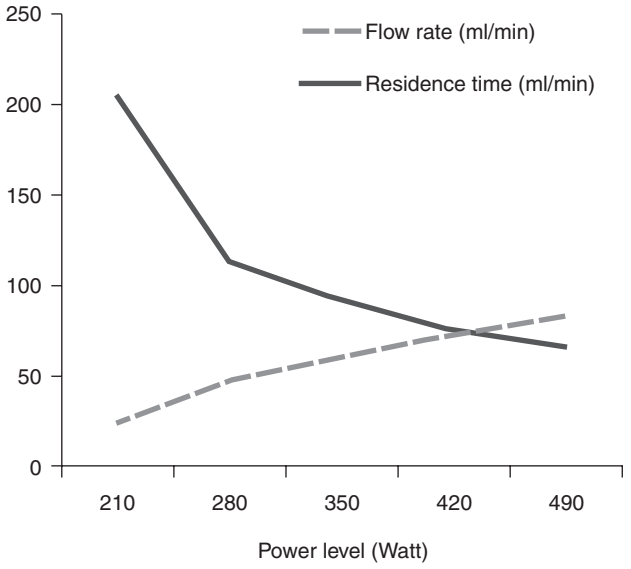

Fig 3 Effect of heating at different microwave power levels on flow rate and residence time of nutrient broth at 90°C

temperature at exit was 60°C. For nutrient broth at 90°C exit temperature highest heating rate of 1.02°C/sec was found at 490W power level where as lowest heating rate of 0.39°C/sec was found at 210W power level. This may be due to more energy available per ml of broth at higher power levels. This finding is consistent with the study of (Gentry and Robert 2005), who reported that highest heating rate was found at maximum power level of 2 000W. Flow rate also increased with the increasing power level where as residence time decreased with the increasing power level. Flow rate and residence time of MGYP broth at exit temperature of 60°C varied between 52 to 137 ml/min and 43 to 99 sec whereas flow rate and residence time of nutrient broth at exit temperature of 90°C varied between 24 to 83 ml/min and 67 to 205 sec, respectively (Fig 2 and 3). This may be attributed to the fact that at higher power levels, more energy is available per ml of broth so to maintain the temperature of the broth coming out from the oven flow rate has to be increased because the temperature of the broth at inlet, is kept constant which resulted in the decrease in residence time. Gentry and Robert (2005) reported that the time for the water to heat to a given temperature using 900W was over 2.5 times more than the time using 2 000W. Gonzalez *et al*. (2014) also observed that process heating time for guava nectar was reduced from 3.45 to 1.76 min with an increase in microwave power from 500 to 950W. Thus, operating the pasteurizer at lower power levels would result in a significant decrease in the flow rate in order to increase the residence times necessary to reach pasteurization temperature.

The application of microwave energy for destruction of spoilage microorganisms in broth was explored. Both microorganisms showed to be easily inactivated by both microwave and thermal treatments. Higher microbial inactivation was observed at lower power levels due to increased heating time and less power available per ml of broth. For all the microwave power levels, higher inactivation of *B. cereus* and *S. cerevisiae* was observed in comparison to control. This suggests the existence of some enhanced thermal effects associated with microwaves resulting in a higher rate of microbial destruction as compared to conventional hot water bath heating. Heating rate and flow rate also increased with the increasing power level with decrease residence time to kill the contaminants. In future, this system may be useful for effective pasteurization of liquid foods, e.g. sugarcane juice and soymilk without affecting the taste of processed juices.

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