Development of a Microwave Treatment Technique for Bacterial Decontamination of Raw Meat

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Abstract

The present study developed and verified a 'cold' microwave (MW) treatment that could lead to the inactivation of two common pathogenic species of bacteria, *Escherichia coli* and *Staphylococcus aureus*, in raw meats. A number of experimental conditions were designed and tested to maximise MW exposure without overheating the samples. The non-thermal effect was maximised by multiple exposure to attain efficient MW threshold intensities. It was shown that at sub-lethal temperatures repeated exposure using high frequency MW radiation was significantly more effective in decontaminating bacteria in raw meats compared to a single exposure. It was concluded that non thermal inactivation of pathogenic bacteria in raw meats could be achieved at defined conditions using high frequency MW radiation.

KEYWORDS: microwave effect, bacterial decontamination, microwave, raw meat, Escherichia coli, Staphylococcus aureus

1. Introduction

Raw meat is an important and arguably the major source of human foodborne infection with pathogenic bacteria (Aziz et al., 2002; Handan-Dincer & Baysal 2004; Woo et al., 2000). In spite of decades of effort, it still remains difficult to obtain raw produce free of pathogenic bacteria. The bacteria of most concern for meat and poultry include *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella serovars*, *Staphylococcus aureus* and *Yersinia enterocolitica (*Handan-Dincer & Baysal 2004*)*. High temperatures cannot be employed for the sterilisation of raw and pre-cooked meats therefore the sterilisation of these foods is referred to as cold pasteurisation (Roberts, 1998). Traditional techniques involve chemical preservation processes; however these methods are becoming replaced due to generally not being beneficial in terms of their nutritional value (Roberts, 1998, Handan-Dincer & Baysal 2004). In addition, decontamination of raw meats by chemical methods does not completely eliminate pathogenic bacteria and, because they are applied to the product before packaging, they do not eradicate possible post processing contamination (Handan-Dincer & Baysal 2004).

Currently, food irradiation using gamma rays is another preservation process of exposing raw foods to high-energy rays to improve product safety and shelf life (Roberts, 1998). The use of ionising radiation treatments has been approved for decontamination of fresh poultry carcasses in the U.S.A. and its approval to decontaminate beef, pork and lamb are under consideration (Handan-Dincer & Baysal 2004). At present, in the European Union use of gamma irradiation (GI) for meat is strongly discouraged (Smulders & Greer 1998). The safety of irradiated foods from the point of view of radioactivity or toxins, or the selection of radiation resistant bacteria in the foods is not seriously questioned (Satin 2002; Handan-Dincer & Baysal 2004). Also, application of this technology has been very limited due to the distrust of consumers of any processes based on the nuclear industry, insufficient knowledge about foodborne infections and the effectiveness of irradiation (Bruhn 1995; Lagunas-Solar 1995; Resurreccion 1995). Using gamma rays as a radiation source has further disadvantages including limited supplies available, the need for installation of personal irradiation units for each processing plant and the necessity to transport food to specialised irradiation centres. Furthermore, vitamins A, C, E, and B1 (thiamine) tend to be susceptible to irradiation (Handan-Dincer & Baysal, 2004; Roberts, 1998), and, acceptable irradiation doses do not prevent the growth and toxin production of *Clostridium botulinum* (Roberts, 1998).

There has also been a considerable amount of research into bacterial decontamination using microwave energy (e.g. Dreyfuss, & Chipley, 1980;

Geveke & Brunkhorst, 2008; Handan-Dincer & Baysal, 2004; Hyland, 1998; Heddleson & Doores, 1994; Kozempel, 2000; Shazman et al., 2007; Thuery, 1985; Welt et al., 1994; Woo et al., 2000). Microwave treatment is known to inactivate many microorganisms, such as *Escherichia coli*, *Streptococcus faecalis*, *Clostridium perfringens*, *Staphylococcus aureus*, *Salmonella*, and *Listeria* spp. (Atmaca et al., 1996; Blanco & Dawson, 1974; Bookwalter et al., 1982; Dreyfuss & Chipley, 1980; Farber et al., 1998; Fujikawa et al., 1992; Heddleson & Doores, 1994; Heddleson et al. 1994; Hyland, 1998; Pothakamury et al., 1995; Woo et al., 2000). Bacterial and mould spores have also been reported to be sensitive to microwave radiation (Welt et al., 1994). Microwave energy is a non ionizing form of radiation and it has been tested to study the effectiveness in destroying bacteria and extending the shelf life of meat products without affecting the quality, taste or reducing the weight of the product (Aziz et al., 2002; Heddleson & Doores, 1994; Kozempel, 2000).

Despite many studies on microbial destruction by microwave radiation, the mechanisms are not fully understood. Traditionally it has been assumed that the destruction of microorganisms is mainly due to a thermal effect of microwave exposure (Banik et al., 2003; Carta et al., 2002; Heddleson & Doores, 1994; Kozempel, 2000; Shazman et al., 2007; Welt et al. 1994). However, recent reports have either shown or suggested that there are non thermal microwave effects (at frequencies above the standard 2.45GHz) in terms of the energy required to produce various types of molecular transformations and alterations (Banik et al., 2003; Hyland, 1998; Dreyfuss & Chipley, 1980; Heddleson & Doores, 1994; Pakhomov et al., 2001; Samarketu et al., 1996; Thuery, 1985). These studies have provided evidence that microwaves can cause different biological effects depending upon field strength, frequency and time of exposure. Such findings are underpinned by the destruction of microorganisms using microwaves at temperatures lower than their thermal destruction point (Hyland, 1998; Pakhomov et al. 2001; Samarketu et al. 1996; Thuery, 1985; Woo et al., 2000).

In the context of these findings, the aim of the present study was to examine a range of conditions that could lead to the inactivation of bacteria in raw meats. Two common pathogenic species of bacteria, *Escherichia coli and Staphylococcus aureus, were used as test microorganisms.* A specialised high frequency MW (set at the maximal frequency of 18 GHz) was employed to design and optimise high frequency microwave settings for non thermal decontamination of bacteria.

2. Materials and Methods

2.1. Bacterial strains and cultivation procedures

Escherichia coli ATCC 15034 and *Staphylococcus aureus* CIP 103594[T] were used as test strains in all experiments. Bacterial strains were obtained from the American Type Culture Collection (USA) and the Culture Collection of the Pasteur Institute (France). Pure cultures were stored at -80°C in nutrient broth (NB) (Oxoid) supplemented with 20% (v/v) of glycerol. Both strains were routinely cultivated on nutrient agar (NA), (Oxoid). Prior to the experiments, the test strains were cultured overnight at 37°C yielding the stationary phase cells.

2.2. Preparation of bacterial suspensions

Bacterial suspensions were freshly prepared for each independent experiment from cultures of *E. coli* and *S. aureus* grown overnight. The cell density for both strains was first adjusted to OD_{600} (1) to yield 0.8×10^8 bacterial colony forming units (cfu) per ml in phosphate buffered saline (PBS), 10 mM, pH 7.4, using a spectrophotometer (Amersham Biosciences – Gene Quant Pro). Each initial suspension was subjected to 5 serial dilution steps (dilution factor 10^5) in order to obtain the recovery of 300 cfu from 50 µl of bacterial suspension spread per plate. These suspensions optimised by the plate technique were further used in all experiments as controls.

2.3. Experiment deliberating the heating effect

A control experiment was set up to assess the thermal effect on bacteria. For each sample, 100 µl of working suspension from the final dilutions that was used as the control in all experiments was placed in duplicates into separate microcentrifuge tubes and subjected to a water bath with temperatures of 50, 60 or 70°C for one minute. After heat treatment recovery of viable bacterial cells on NA plates was compared to control plates in order to determine the lethality of the temperature at each setting.

2.4. Microwave Treatment

2.4.1. Sample preparation for microwave treatment

Raw pork loin fillets used as the meat sample were purchased at a retail supermarket. It was assumed that a sample size of $1.0 \times 1.0 \times 0.5$ cm^3 cut from a fillet would be suitable for the experiments. At 12 hours preceding microwave

treatment the samples were submerged in 70% ethanol solution to eliminate contaminants. One hour prior to treatment the samples were washed with PBS solution to cleanse out the ethanol. The samples were kept in this state until further use. For the inoculation procedure, it was determined that 30 µl of the optimised bacterial suspension would be adequate to coat the test sample. Each meat sample was placed onto a micro Petri dish (35 mm, Griener) and inoculated with one of the bacterial test strains.

2.4.2. Microwave apparatus

The microwave apparatus that was used in the present study had the option of a variable frequency ranging from 5 to 18GHz (Lambda Technologies Vari-Wave Model LT 1500). The LT1500 is a computer controlled variable frequency processing cavity for delivering excellent levels of control and uniformity of energy distribution into a multi-mode microwave cavity. Both the amplitude and frequency of the microwave power could be varied allowing a significant expansion of the parameter space within which a system could be optimised. A data logging option allowed processed data capture from the embedded computer system over a standard RS-232-C serial interface. A cavity characterisation option was also available which allowed evaluation of the performance of a material in the cavity to assist in determining the optimum processing conditions. Fig. 1. demonstrates the schematic diagram of the MW system used.
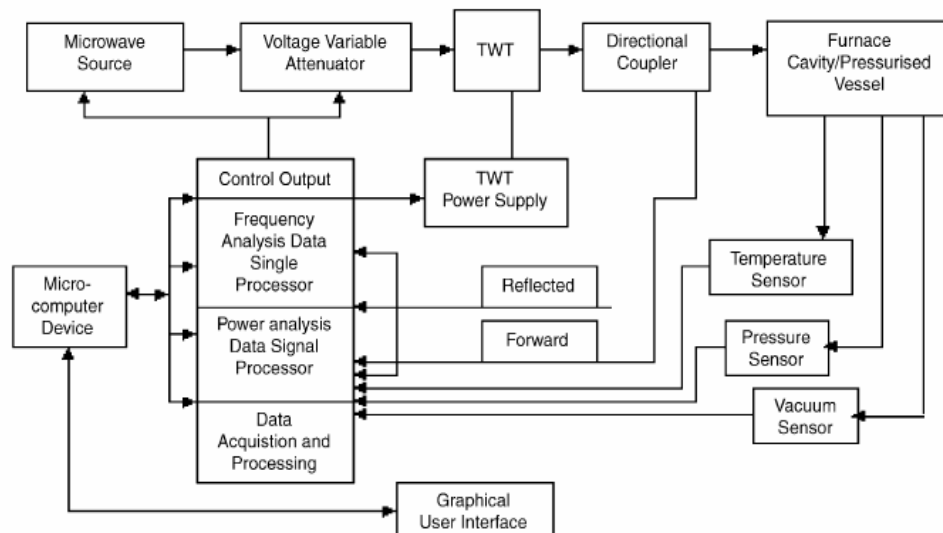

Fig. 1. Schematic diagram of the microwave system

2.4.3. Experimental set up for MW treatment

The microbial treatment using microwave radiation is a function of power, frequency range, time and temperature. To separate the thermal and non thermal effects of microwave radiation the maximal temperature reached during exposure was controlled and recorded. For the threshold temperature, 45°C was preferred. The experiments were set up using the highest available frequency of 18 GHz.

In order to achieve reproducible and reliable results the identification of the area within the MW chamber with a constant flow of radiation was critical. To obtain a uniform temperature gradient in the multimode cavity and to avoid "hot spot" and "cold spot" standing wave effects, a fixed location was determined on a Teflon platform within the microwave cavity (Fig. 2). For uniformity of exposure, each of the samples was placed in the same marked spot on the Teflon platform. In addition, to maximise the MW effect and the exposure time without having the temperature exceed 45°C, a low power was used. For the first experiment, two power settings were trialed, i.e., 6 Watt and 16 Watt incident power.

Fig. 2. MW chamber with the identified area containing a uniform flow of radiation.

Fibre optic probes attached to the sample within the microwave apparatus performed internal temperature controls (Fig. 2). Exposure times at which the sample's internal temperatures did not exceed 45°C were determined experimentally. All other environment factors were kept constant during the tests.

The computer modeling and simulation software (CST Microwave studio 5.1) was also used to develop and refine microwave set-up to verify the generation of a uniform electric field pattern within a cavity and a uniform flux through the sample (Fig. 3).
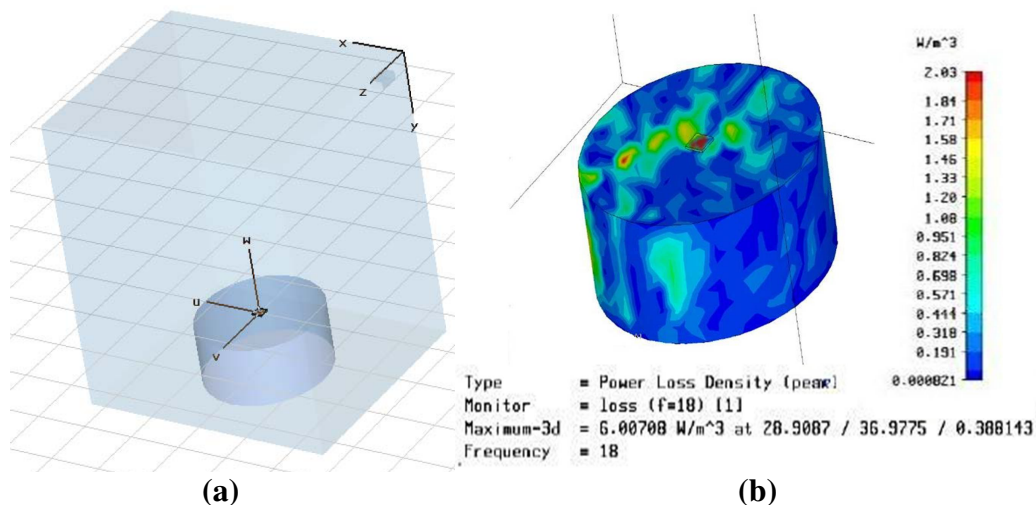

(a)   **(b)**

Fig. 3. General set-up for modelling (a): Electric field patter on Teflon and (b): sample.

2.4.4. Sample handling after microwave treatment

Immediately after MW treatment the temperature of each sample was measured using a handheld infrared temperature sensor (HLP, Model 8866) to ensure that internal temperature of the samples did not exceed 45°C. Following MW treatment, each sample was removed from the Petri dish and 'washed' in 500µl of PBS solution to remove the inoculated bacteria. For each sample, five 50µl aliquots taken from the washed solution were spread onto nutrient agar plates to determine recovery of viable bacterial cells. Controls for this experiment were set up by inoculating the meat samples with 30µl of bacterial suspension, washing it in 500µl of PBS solution and spreading ten 50µl aliquots onto nutrient agar plates.

2.4.5. Statistic analysis

Statistical data processing was performed using SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA). Three independent T-tests were performed to compare consistency of decontamination rates across conditions, experiments and bacterial test strains.

3. Results and Discussion

3.1. Heat effect on bacterial cells viability

The results of experiments evaluating the heat effect on *E. coli* and *S. aureus* cells after 1 minute of incubation under 50°C, 60°C, or 70ºC are shown in Table 1. It follows from the table that the incubation under 50°C resulted in 31.5% and 22.6% decrease of cell viability for *S. aureus* and *E. coli,* respectively, while there was no recovery of bacteria after incubation under 60°C and 70°C in water baths. These results confirmed that conditions for non thermal microwave treatment could not be conducted at temperatures of 60°C and 70°C. The treatment performed at 50°C should also be analysed with caution taking into account the 'pure' heating effect.

Table 1
Heating effect on *E. coli* and *S. aureus* cell viability

Temperature	Cell Viability			
	S. aureus		*E. coli*	
	cfu/50µl (average)	Heating effect	cfu/50µl (average)	Heating effect
25°C (Control)	89	0	102	0
50°C	61	31.5%	79	22.6%
60°C	0	100%	0	100%
70°C	0	100%	0	100%

3.2. Treatment of inoculated sample with microwave radiation

The microbial treatment using microwave radiation is a function of power, frequency range, time and temperature. Therefore in order to achieve reproducible and reliable results the identification of the area within the MW chamber with a constant flow microwave radiation was critical. As it can be seen from Fig. 2, such an area was indentified within the microwave chamber. This was achieved by attaining a constant rate in temperature rise in that area across

trials. In addition, to maximise the MW effect and the exposure time without having the temperature exceed 45°C, a low power was used.

Thus, the first experiment was set up using a frequency of 18 GHz and two power settings, i.e. 6 Watt and 16 Watt incident power. Exposure times were found to be inconsistent (60-340 seconds) for the 6 Watt treatment condition. From this finding, it was concluded that an incident power of 6 Watt was not sufficient enough to generate a uniform electromagnetic field as it did not allow the sample to absorb a consistent level of radiation. The decontamination rate at 6 Watt was 17.0% for *E. coli* and 14.3% for *S. aureus* (Fig. 4)*.* Due to the inconsistency in exposure times and weak decontaminating effect, the 6 Watt setting was discarded from further experiments.
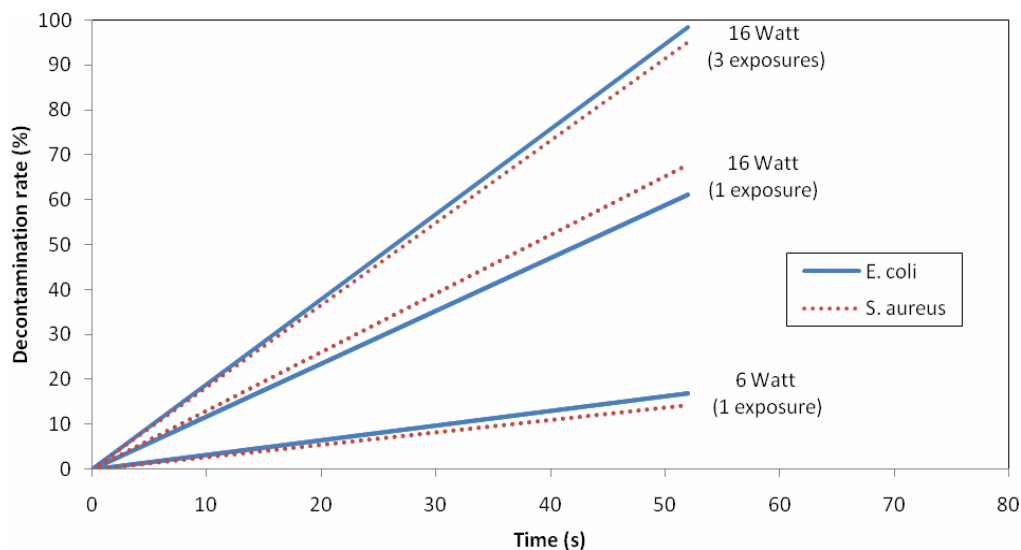

Fig. 4. Decontamination rates of *E. coli* and *S. aureus* after microwave treatment at various incident powers.

For the second setting, 16 Watt, exposure times were found to be an average of 52 seconds at which the sample's internal temperatures did not exceed 45°C. The decontamination rate at 16 Watt was 61.2% for *E. coli* and 67.8% for *S. aureus* (Fig. 4). These results suggested that the bacterial decontamination rate could be increased if a longer exposure time was used to maximise the non thermal microwave effects. Since longer exposure times would lead to sample temperatures being greater than 45°C, it was decided that repeated MW exposure with sufficient cooling in between trials would be adequate.

3.3. MW treatment using repeated exposure

The next stage of experiments was designed to maximise MW exposure without overheating the samples. This was achieved by using repeated MW exposure at 16 Watt for 52 seconds. The samples were exposed one, three or four times to the MW radiation allowing the temperature of the samples to return to room temperature (*ca.* 22°C) in between trials. The results of these experiments are shown in Table 2.

Table 2
Decontamination rates of *E. coli* and *S. aureus* after multiple exposures of raw pork loin fillets to MW radiation at 16 Watt.

	E. coli		*S. aureus*	
	cfu/30µl (average)	Decontamination rate (%)	cfu/30µl (average)	Decontamination rate (%)
Control	301±129	0	268±62	0
1 exposure				
Trial 1	103.4±10	65.6	88±18	67.2
Trial 2	101±7	66.5	76±18	71.6
Average	102.2	66.0	82	69.4
3 exposures				
Trial 1	3.2±3	98.9	14±11	94.8
Trial 2	6.4±2	97.9	12±12	95.5
Average	4.8	98.4	13	95.2
4 exposures				
Trial 1	16.6±5	94.5	18±7	93.3
Trial 2	23.2±11	92.3	20±14	92.5
Average	19.9	93.4	19	92.9

The decontamination rates after sole exposure were reconfirmed in the second experiments as being 66.0% for *E. coli* and 69.4% for *S. aureus.* The statistics analysis of the results obtained showed no significant differences between experiment one and two in the decontamination rates using single MW exposure at 16 Watt for 52 seconds for *E. coli, $t(3)$ = 1.41, $p > 0.05$,* and *S. aureus*, $t(3) = 0.18$, $p > 0.05$.

As can be seen from Table 2, three repeated exposures resulted in an increased decontamination rates reaching 98.4% for *E. coli* and 95.2% for *S. aureus*. Such an increase in the decontamination rates was found to be

statistically significant, $t(2) = 48.09$, $p < 0.05$ and $t(2) = 11.56$, $p < 0.05$, respectively. Notably the decontamination rates were slightly lower after four times repeated exposure (Table 2). However, the analysis indicated that there was no significant difference in the decontamination rates between three and four times exposures for *E. coli, $t(2) = 4.14$, $p > 0.05$* and *S. aureus, $t(2) = 4.23$, $p > 0.05$*.

The final analysis confirmed that there was no significant difference in decontamination rates between the two bacterial test strains, across all experimental conditions, $t(22) = 0.07$, $p > 0.05$.

4. Discussion

In retrospect, the limitation of many of the studies that examine the effects of microwave energy on microorganisms is that the treatment of the bacterial cells is conducted at lethal or close proximity to lethal temperatures (50-60°C). Such temperatures could initiate the denaturation of enzymes, proteins, nucleic acids, as well as the disruption of membranes (Heddleson & Doores, 1994), causing the basis of cell damage (non thermal or thermal) to become hard to distinguish. One recent publication reported a non thermal inactivation protocol for *E. coli* K12 in apple cider using radiofrequency electric fields (Geveke & Brunkhorst, 2008). While the study successfully demonstrated the inactivation *E. coli* using radiofrequency radiation, the method of action was completed at temperatures well above the thermal degradation thresholds of the target bacteria implicating that thermal denaturation mechanism may have contributed to the elimination factor as well.

In this study, the non thermal effectivity of high frequency MW radiation on the decontamination of two common pathogens, *E. coli* and *S. aureus,* was assessed. Results obtained showed that high frequency MW radiation could be used to eliminate bacteria from raw meats at sub lethal temperatures, i.e. via non thermal mechanisms. The present data was consistent with that of Arndt et al. (2005) who showed that microwave energy at 29.8 GHz strongly affected the viability of the bacteria *Burkholderia cepacia* while only weak effects below 5 GHz were observed. However, the efficiency of the effect of high frequency MW radiation at 29.8 GHz was not evaluated.

The results evaluating the efficiency of the 18 GHz MW radiation on bacterial elimination reported in this paper are novel. Previously, a number of studies have merely demonstrated a perturbation in bacterial cellular activity as well as cell death at MW frequencies above the conventional 2.45 GHz setting (Arndt et al., 2005; Hyland, 1998; Pakhomov et al., 2001; Samarketu et al., 1996 & Thuery, 1985). Nevertheless, the effects of high frequency MW radiation remain poorly understood, where the mechanisms of 'cold inactivation" are

highly debated. To our knowledge, there is only one report indicating that 36.2-55.9 GHz microwave irradiation of *Enterobacter aerogenes* and *E. coli* resulted to either an inhibition or stimulation of protein, DNA, RNA synthesis as well as cell growth (Pakhomov et al., 2001). It is also worth noting that Arndt et al. (2005) reported that human muscle and nerve cells were found to be strongly affected at low frequencies and weakly affected at high frequencies. These findings provide important implications when considering the decontamination of raw meats. Given that higher frequencies were found to affect bacteria and not muscle cells, it implies that multiple exposure of raw meats using MW radiation at low temperatures and high frequencies could be used to inactivate bacteria without compromising the meat quality.

In conclusion, the present study is the first in its kind to demonstrate microbial inactivation using microwave radiation at temperatures below the thermal destruction point of the bacteria. It was demonstrated that repeated exposure to high frequency MW radiation was significantly more effective in decontaminating raw meat of bacteria compared to single exposure. These findings provide important implications for the food industry when considering non-thermal preservative mechanisms of raw foods. In particular, the inactivation of common contaminants such as *Escherichia coli* and *Staphylococcus aureus* from raw meats could be targeted.

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