Effect of High Domestic Microwave Radiations at Sub-Lethal Temperature on the Bacterial Content of Raw Milk

Ahmed M. Hammad

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt

ABSTRACT:

In light of the paucity of knowledge about microwave effects on bacteria, the aim of the present study was planned to investigate the effects of high domestic microwave radiation on the safety of raw milk. Forty samples of raw milk were collected from retail shops in El Menofia governorates, Egypt. Milk samples were subjected to conventional heating in water bath to reach temperature of 40°C and 1100 Watt in domestic microwave for 3 minutes in cooled water jacket to reach the same temperature. Counts of different groups of bacteria in microwave treated samples were compared with those treated by conventional heating and non-treated raw milk samples. Additionally, to investigate the minor effects of such type of microwave treatment on the cell permeability to antibiotics, 10 of our laboratory bacterial strains with known antibiotic resistant patterns were treated by microwave and conventional heating. The zones of inhibitions for tested antibiotics were measured after each treatment. Of note, the average total aerobic, Enterobacteriaceae, Pseudomonas spp. and staphylococci counts in milk samples were 6.34, 4.20, 3.42 and 3.72 log cfu/ml, respectively. The bacterial counts were nearly similar in samples treated by conventional heating and microwave. Statistical analysis revealed non-significant effect of microwave treatment at sub-lethal temperature as P values were more than 0.05. The diameter of zone of inhibition of tested antibiotics did not change in milk treated by both methods and non-treated samples. In conclusion, this study provides evidence that high domestic microwave irradiation at sub-lethal temperature have no significant effect on the hygienic quality of milk and not provide any degree of safety to the consumer. Additionally, it confirms the hypothesis that microwave radiation under sub-lethal temperature resulting in a reversible microwave-induced poration of the cell membrane that prevents penetration of antibiotics after few minutes.

*Corresponding Author : Ahmed M. Hammad; hammad@vet.usc.edu.eg

1. INTRODUCTION

One of the modern exploitation in the food industry is the use of microwave oven irradiation. Microwave oven is electromagnetic irradiation with wavelengths ranging from one meter to one millimeter; with frequencies between 300 MHz and 300 GHz. Their traditional application in heating foods is already prevalent, especially in developed countries. Domestic microwave devices operate generally at a frequency of 2.45 GHz, while microwave systems used in factories operate at frequencies of 915 MHz and 2.45 GHz (Chandrasekaran, 2013). Microwave heating has germicidal effect on many microorganisms, such as Streptococcus faecalis, Escherichia coli, Clostridium perfringens, Salmonella, Staphylococcus aureus, and Listeria spp. (Dreyfuss and Chipley, 1980; Bookwalter et al., 1982; Farber et al., 1998; Shamis et al., 2012; Chandrasekaran, 2013). Additionally, the use of microwave radiation has become popular in the food industry for thawing, drying, and baking foods (Shamis et al., 2012).

Microwave heat intervention of food results in both thermal and non-thermal effects. Its thermal effect is characterized by emission of thermal energy inside the material being treated (Géczi et al., 2013). In fact, there are several features of microwave heating compared to conventional heating methods. The benefits of microwave heating are reduced damage to nutrient content, saving energy, lower costs, and rapid processing; all of which make the use of microwave irradiation attractive in both large and small-scale applications. Importantly, microwave technology may provide a useful alternative processing method for delivery of aseptic milk products that retain a long shelf life.
with lower sensory and biochemical changes than UHT treated milk (Clare et al., 2005). Of note, Tremonte et al. (2014) found that the exposure of milk sample to 900 W for 75 s resulted in effective microbiological sanitation, in comparison with boiling, as proofed by absence of microorganisms after the treatment.

Food processors and consumers are concerned with non-thermal inventions for food that reduce or eliminate the microorganisms in heat sensitive food. The existence of so-called specific microwave effects that are non-thermal in nature appears promising for the food industry. The non-thermal effects of microwave are reactions and processes, during which the physical, chemical or biological conditions of the product change without an elevation in its temperature (Géczi et al., 2013). However, much effort has been devoted to studies that have attempted to demonstrate the existence of non-thermal effects of microwave irradiation by containing end-point temperatures below thermal death points of microorganisms under investigation. Kozempel et al. (1998) reported significant decrease in microorganisms in water, 10% glucose solution, and apple juice treated with microwave at temperature below 40°C. Dreyfuss and Chipley (1980) reported some effects of microwave irradiation on different metabolic enzymes of S. aureus when exposed to sub-lethal doses of irradiation. Woo et al. (2000) examined the effect of microwave irradiation on E. coli and reported leakage of protein and DNA, severe injury on the cell walls and appearance of dark spots in bacterial cells after microwave treatment. Hong et al. (2004) reported that microwave irradiation at lower temperatures have germicidal effect on fecal coliforms in biosolids. Recently, Shamis et al. (2011) and Rougier et al. (2014) studied the non-thermal effects of microwave and reported a rearrangement in the structure of the cell membrane, resulting in the formation of pores and a significant increase in cellular permeability to ions and molecules. Importantly, the weakening of the bacterial membrane by microwaves is confirmed by the acquisition of sensitivity to some antibiotics (Nasri et al., 2013).

Based on our review of the literature, there is a relatively few reports that describe the effects of high microwave threshold at sub-lethal temperature on the microbiological quality of milk. In fact, we found only one article that discussed inactivation of microorganisms with microwaves at low temperatures in skim milk (Kozempel et al., 1998). However, in this previous study they tested only a heat-resistant nonpathogenic bacterium, *Pediococcus* spp. NRRL B-2354. In light of the paucity of knowledge surrounding the existence of specific microwave effects on bacteria, the aim of the present study was to investigate the effects of high microwave radiation from a domestic microwave under sub-lethal temperature on the safety of raw milk.

2. MATERIAL AND METHODS

2.1. Collection of samples

Forty raw milk samples (1 liter) were aseptically collected from retail shops in El Menofia governorates. At the time of sampling, the temperature of the milk was measured. All the samples were placed on ice for transportation to the laboratory. At the laboratory, the milk sample was distributed into five sterile jars each contain 200 ml of milk. Samples were analyzed within 2 h after sampling.

2.2. Conventional thermal treatment

To simulate a conventional thermal treatment at low temperature, 200 mL of raw milk was poured into a small, previously sterilized 500-mL Pyrex beaker and heated with stirring in water bath until the temperature reached 40°C. The milk temperature was monitored using a digital thermometer. The treatment was immediately stopped, and analyses were carried out within 15 min.

2.3. Microwave treatment

The microwave oven used in this study was Kenwood model (MWL425). To simulate domestic microwave treatment, 200 mL of cold raw milk (4°C) was poured into a sterilized Pyrex beaker (300 mL). The beaker was placed in 2 liters Pyrex beaker containing 750 ml of cold water (10 °C) and the treatment was performed by using a domestic microwave oven with 1100-W. The heating time was milk-dependent and was determined by prior testing with an average of 3 minutes to reach 40 °C. Immediately after treatments, the milk sample was mixed by sterile stirrer and temperature was monitored as described above.

2.4. Microbiological analysis

For microbial counts, raw milk, microwave treated milk and milk treated with conventional heating were serially diluted using 0.1% peptone water. The different microbial groups were detected on specific media and incubation temperatures
according to Wehr and Frank, (2004). In detail, total aerobic count was performed on standard plate count agar (Oxoid Ltd., Basingstoke, UK) after incubation at 30°C for 72h; Enterobacteriaceae were detected on violet red agar (Oxoid Ltd.) after incubation at 37°C for 24 h; Pseudomonas spp. count was done on Pseudomonas agar base (Oxoid Ltd.) supplemented with cephalothin-sodium fusidate-cetrimide (CFC) Pseudomonas selective supplement (Oxoid Ltd.) after incubation at 25 °C for 48 h; and staphylococci were determined on mannitol salt agar (Oxoid Ltd.) after incubation at 30 °C for 48 h.

2.5. Antimicrobial susceptibility tests

Antimicrobial susceptibility tests for 10 strains of fecal coliforms (5 E. coli and 5 Klebsiella pneumonia) previously isolated from milk in our lab, were performed using the agar diffusion method as previously described (Clinical and Laboratory Standards Institute, 2011). Briefly, overnight culture was prepared. The turbidity of the culture was adjusted using sterile saline to achieve a turbidity equivalent to a 0.5 McFarland standard. 200 ml each of 0.5 McFarland standard from each strain were treated by conventional heating and microwave as described above, beside untreated one used as control. Antibiotic disks were disposed manually and plates were incubated at 37 °C for 18 h. The antimicrobial agents tested (Oxoid) were as follows (µg): amoxicillin-clavulanic acid (20/10), ampicillin (10), aztreonam (30), cefepime (30), cefotaxime (30), ceftazidime (30), ceftriaxone (30), ciprofloxacin (5), gentamicin (10), nalidixic acid (30), streptomycin (10), sulfamethoxazole-trimethoprim (23/75), and tetracycline (30).

Statistical analysis

Statistical analysis of data was carried out by SPSS program version 20 using one Way ANOVA.

3. RESULTS AND DISCUSSION

Several studies showed that the bactericidal effect of microwaves was due to not only thermal but also to non-thermal mechanisms. However, there is scarce of knowledge about the non-thermal effects of microwave on the hygienic quality of food. This study provides the first detailed analysis about the effect of high microwave threshold at sub-lethal temperature on the safety of raw milk.

In 1998, Kozempel et al. reported that there is no effect of microwave treatment on microorganisms in milk at temperature below 40°C. However, in recent years the existence of non-thermal effects of microwaves on microorganisms became subject to debate as some studies showed non-thermal effects of microwaves on certain types of bacteria (Barnabas et al., 2010; Shamis et al., 2011; Nasri et al., 2013; Rougier et al., 2014). According to these studies, it may be hypothesized that high microwave irradiation at low temperature affect viable count of certain groups of bacteria and consequently provide a degree of safety to food treated by microwave. Therefore, in this study we investigated the non-thermal effect of domestic microwave using 1100 Watt of irradiations on different bacterial groups.

Aerobic plate count provides an estimate of the total number of viable aerobic bacteria in a food sample. It is used to determine milk quality and high counts might indicate that the milk was handled under unsanitary conditions (EL-Leboudy et al., 2014). The average total aerobic count in raw milk samples was 6.34 log cfu/ml. The microwave treated samples showed a slight decrease in total aerobic count (6.16 log cfu / ml) than those treated by conventional heating (6.24 log cfu / ml) Table (1) and Figure (1.a). Statistical analysis showed non-significant effect of both treatments as the P value was more than .05 as shown in table 1. From the food hygiene point of view, this decrease in number neither provide safety nor improve the hygienic quality of milk.

To test the hypothesis of the presence of some effects of microwave at sub-lethal temperature on specific groups of bacteria, we counted the Enterobacteriaceae, Pseudomonas spp, and staphylococci before and after treatment by conventional heating and microwave treatment. Enterobacteriaceae count is generally used as indicator of hygienic quality rather than of faecal contamination and therefore indicates more about general microbiological quality rather than of faecal contamination and therefore indicates more about general microbiological quality.
Pseudomonas spp count was 3.42 log cfu / ml and the P value was more than 0.05. This confirms the non-reliability of microwave treatment at low temperature in improving the hygienic quality of milk.

Table 1. Descriptive analysis of different bacteriological parameters analyzed in raw milk and milk treated by conventional heating and microwave.

<table>
<thead>
<tr>
<th>Bacteriological parameter</th>
<th>Descriptive analysis</th>
<th>Raw milk</th>
<th>Conventional heating</th>
<th>Microwave treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacterial count</td>
<td>Mean (log cfu/ml)</td>
<td>6.3474</td>
<td>6.2479</td>
<td>6.1610</td>
</tr>
<tr>
<td></td>
<td>Std. Error</td>
<td>0.09917</td>
<td>0.10275</td>
<td>0.10259</td>
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<tr>
<td></td>
<td>P value</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae count</td>
<td>Mean (log cfu/ml)</td>
<td>4.2047</td>
<td>4.1422</td>
<td>4.0705</td>
</tr>
<tr>
<td></td>
<td>Std. Error</td>
<td>0.09115</td>
<td>0.09039</td>
<td>0.09213</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas count</td>
<td>Mean (log cfu/ml)</td>
<td>3.4287</td>
<td>3.3790</td>
<td>3.3098</td>
</tr>
<tr>
<td></td>
<td>Std. Error</td>
<td>0.06502</td>
<td>0.06568</td>
<td>0.06562</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcal count</td>
<td>Mean (log cfu/ml)</td>
<td>3.7251</td>
<td>3.6375</td>
<td>3.5606</td>
</tr>
<tr>
<td></td>
<td>Std. Error</td>
<td>0.05997</td>
<td>0.06217</td>
<td>0.06337</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.17</td>
<td></td>
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</tr>
</tbody>
</table>

-P value: is the probability of obtaining an effect at least as extreme as the one in a sample data, assuming the truth of the null hypothesis. Level of significance is 5% and result with a p value more than 0.05 is non-significant (Goodman, 2008).

Figure 1. Mean counts of different bacteriological parameters of raw milk and milk treated with conventional heating and microwave.

Fig. 1a. Total bacterial count

Fig. 1b. Enterobacteriaceae count

Fig. 1c. Pseudomonas count

Fig. 1d. Staphylococcal count
To investigate if there are specific effects of microwave treatments on Gram positive bacteria, we counted staphylococci in raw milk and treated samples. Total staphylococcal count is an important indicator used in production facilities to ensure their safety to the consumer. As shown in table 1 and figure 1(d), the average staphylococcal counts were 3.72, 3.63 and 3.56 log cfu / ml in raw milk, milk treated by conventional heating and milk treated by microwave, respectively. The P value was above 0.05. The results of this study give evidence for non-reliability of microwave treatments at low temperature for improving the microbiological quality of raw milk.

The main theories that try to explain the biological effects of microwaves are based on the possible effects on the permeability of ionic channels in the membrane (Galvanovskis and Sandblom, 1998). Modifications in sensitivity or resistance of bacteria to antibiotics after irradiation with microwaves will confirm alteration of bacterial membrane by this type of radiation (Nasri et al., 2013). Tadevosyan et al. (2008) reported that extremely high frequency electromagnetic radiation triggered sensitivity of E. coli toward different common antibiotics (chloramphenicol, ceftriaxone, kanamycin, and tetracycline). Pickering et al. (2003) have shown that applying a pulsed magnetic field (72 Hz) improved the effectiveness of gentamicin against Staphylococcus epidermidis biofilm. Recently, Nasri et al. (2013) reported appearance of sensitivity in Salmonella typhimurium to some antibiotics after exposure to microwave at sub-lethal temperature. In this study, the zone of inhibition to the tested antibiotics were nearly the same for strains treated by both treatments and non-treated samples. The lack of effect of microwave treatment on the sensitivity of the tested strains to antibiotics observed in this study confirms the results of Shamis et al. (2011) that microwave effect, is temporary, and after 10-min of treatment, the cell morphology appeared to revert to a state that was identical to that of the untreated controls. In other words microwave radiation under sub-lethal temperature resulting in a reversible microwave-induced poration of the cell membrane that prevents penetration of antibiotics.

In conclusion, this study demonstrated that microwave irradiation at sub-lethal temperature have no significant effect on the hygienic quality of milk. The results of research on non-thermal effects of microwave should be interrupted with cautions as they may give a false indication for consumers about the effects of microwave irradiation at low temperatures on the safety of foods. Finally, in lack of information regarding the sensitivity of pathogens and their toxins to different time, threshold and temperature combinations of microwave treatments, safety standards for milk should be set based solely upon the thermal effect of microwave with an internal temperature of milk not less than 95°C as recently described (Tremonte et al., 2014).

4. REFERENCES


Géczi G., Horváth M., Kaszab T., Alemany G.G. 2013. No major differences found between the effects of microwave-based and conventional heat treatment
methods on two different liquid foods. PloS one, 8:1-12.