



The Effect of Microwaves on the Survivability of *Brucella* Microorganisms in Infected Cattle Milk



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Abstract

This study was carried out to investigate the effect of microwave radiation on the viability of *Brucella melitensis* in contaminated cattle milk through establishing a correlation between milk volumes, microwave exposure time, measured Celsius temperature and viable counts of *B. melitensis* in inoculated milk. Microwave appliances were used in houses and many food stores in Egypt and worldwide, for warming milk that processed for human consumption. Microwave heating was performed using a household electronic microwave oven (Kenwood MW 579, a frequency of 2,450 MHz, and power of 900W). *Brucella*-free milk is inoculated with *B. melitensis* in a dose of (1.3×10^8 CFU / ml) and samples are divided into 100 ml and 200 ml volumes and maintained at room temperature ($22 \text{ }^\circ\text{C} \pm 3^\circ\text{C}$) or refrigeration temperature ($5 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$) prior to microwave exposure. Microwave radiations with full power ($P = 100$) eliminate *B. melitensis* in 100 ml milk samples kept at room temperature for 80 seconds with measured temperature reached ($82 \text{ }^\circ\text{C}$) and eliminated *Brucellae* in 200 ml milk samples in 100 seconds with measured temperature of ($78 \text{ }^\circ\text{C}$). While in 100 ml refrigerated milk kept at ($5 \text{ }^\circ\text{C} \pm 2$) that exposed to 30 % of the microwave's power ($P = 30$) *Brucella* organisms still viable till 5 minutes and the organisms inactivated at 6 minutes with the measured temperature reached ($75 \text{ }^\circ\text{C}$). It was found that the warming program of the microwave either in 120 ml inoculated milk for 1 minute or 240 ml milk samples for 2 minutes, *Brucella* organisms are still viable at counts of (7.1×10^4 and 8.3×10^3 CFU/ml), respectively and considered as a potential source of human infection. In conclusion, the warming microwave program is proved to be unfit to eliminate *B. melitensis* from contaminated cattle milk.

Keywords: *Brucella melitensis*; Viability; Microwaves; Milk

Introduction

Brucellosis is a classical zoonosis of worldwide public health and economic importance. Infected animals are the main sources of human infection. The disease frequently transmitted to man by direct or indirect contact with infected animals or their products. Brucellosis infects people of all age groups and of both sexes [1]. Although there have been great efforts exerted in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and human populations. There are only a few countries in the world that are officially declared free of the disease although they still have human brucellosis due to returning citizens from endemic countries [1].

Milk is a palatable highly nutritious fluid and many people have the custom of drinking milk or add it to their beverages or food. The global per capita dairy consumption of cattle milk was recently estimated in 2015 to be 111.3 kg per year. It was also estimated that the consumption of milk in developing countries is expected to increase in the coming years [2]. Milk produced from

infected cattle, sheep, goats and camels, is frequently contaminated with *Brucella* pathogens and is considered a potential hazard as it contains large numbers of organisms. Tourists or business travelers to endemic areas may acquire brucellosis, usually by consumption of un-heat-treated milk, when visiting countries with known status of animal Brucellosis. Many heat treatment procedures including pasteurization and sterilization were employed for inactivation of pathogenic bacteria and rendering milk fit for human consumption. However, these thermal procedures have got some limitations by way of partial destruction of heat labile nutrients that affects the quality of milk, and sensory attributes [3].

Microwaves are described as they are electromagnetic radio waves that are within a frequency band ranged between 300 MHz to 300 GHz. Microwave heating refers to dielectric heating due to polarization effects at a selected frequency band in a nonconductor. Microwave heating in food beverages occurs due to coupling of

electrical energy from an electromagnetic field in a microwave cavity with the food and its subsequent dissipation within food product. This results in a sharp increase in temperature within the product. Microwave heating of milk has advantages over other conventional heat treatments due to its rapid increase in temperature, short time duration and it overcome the limitation imposed by the slow thermal diffusion process of conventional heating [4].

The application of microwave heating to pasteurize milk has been well studied and has been a commercial practice for quite a long time. The success of microwave heating of milk is based on established conditions that provide the desired degree of safety with minimum product quality degradation. Since the first reported study on the use of a microwave system for pasteurization of milk [5], several studies on microwave heating of milk have been carried out. The majority of these microwave-based studies have been used to investigate the possibility of shelf-life enhancement of pasteurized milk, application of microwave energy to inactivate milk pathogens, assess the influence on the milk nutrients or the non-uniform temperature distribution during the microwave treatment [6]. The application of microwave energy in bovine milk proved to inactivate *Streptococcus faecalis*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Listeria monocytogenes* [7]. Up to the available literature, the effect of microwaves on the viability of *Brucella* microorganisms in milk was not yet studied. The aim of this study is to investigate the effect of the microwave radiation on the viability of *Brucella melitensis* in cow milk through establishing a correlation between milk volumes, exposure time and viable count of *Brucella* in milk, in a trial to check the efficiency of microwave to render *Brucella* infected milk safe for human consumption.

Material and Methods

Preparation of *Brucella* strain

Brucella melitensis was selected to run the current experiment on the basis that this strain is the common *Brucella* strain isolated from animals and man in Egypt and most Mediterranean countries [8]. The local strain was isolated from stomach contents of aborted ewe foetus. The organism was subjected to culture identification methods using; monospecific serum (A & M), lysis by Tiblizi and IZ *Brucella* phages and growth on thionin and basic fuchsin dyes. All biotyping methods were done parallel with and in comparison, with *Brucella melitensis* reference strain (Ether strain) obtained from NVSL, DBL, USDA. The pure *Brucella* strain was incubated at 37 °C for 72 h in tryptose soy agar (TSA) medium (Oxide). The experiment was performed in various times, fixed initial bacterial concentration and different Watts power (300 w and full power 900 w) to determine the effect of microwave radiation on viable *Brucella* organisms using viable colony counting methods.

Inoculation of Milk

Cow milk was aseptically obtained from *Brucella* free dairy herd, cooled at 4 °C and sent to the lab as soon as possible. Milk was

inoculated with fresh *Brucella* culture using a loopful of culture suspended in 9 ml of 0.85% (w/v) sterile saline solution and final density was adjusted to MacFarland 0.5. The final concentrations of the organisms were estimated through viable bacterial count of the inoculated milk.

Microwave Heating

Microwave heating was performed using household electronic microwave oven (Kenwood MW 579, a frequency of 2,450 MHz, and power of 900W). Inoculated milk divided into 100 ml and 200 ml volumes and maintained at room temperature (22 °C ± 3°C). Microwave was used at high power for heating the inoculated milk. To study the effect of warming temperature on the viability of *Brucella* in milk, a warming program were applied, using another volumes of 120 ml and 240 ml of inoculated milk were microwaved for 60 sec and 120 sec, respectively; according to the warming program listed in the instruction manual of the manufacture company. Just after microwave radiation, the temperature of the inoculated milk was taken using certified calibrated thermometer (ISO 9001). Inoculated milk was kept on bench till reach room temperature (22 °C ± 4°C). One hundred microliter of each treated milk were cultured on 3 plates of *Brucella* agar media. The experiment was carried out in triplicate and the mean of the standard error was calculated.

Determination of viable count

The milk subjected to the analysis was obtained from *Brucella*-free cattle farm. The whole amount of milk was inoculated with *Brucella* culture with the final bacterial dilution done by viable count is 1.3×10^8 CFU/ml milk [9]. Inoculated milk was divided into 100 ml, 200, ml, 120 ml and 240 ml, whereas, these aliquots were kept at room temperature 22 °C ± 2 °C. Milk samples were put in the middle of the microwave instrument in unclosed vessels and exposed to the full power of the microwave (100: P) for the following periods of time:

- a) t0 – milk samples not treated with microwaves, t10 – milk samples treated for 10 seconds,
- b) t20 – milk samples treated for 20 seconds, t30 – milk samples treated for 30 seconds,
- c) t40 – milk samples treated for 40 seconds, t50 – milk samples treated for 50 seconds,
- d) t60 – milk samples treated for 60 seconds, t70 – milk samples treated for 70 seconds,
- e) t80 – milk samples treated for 80 seconds, t90 – milk samples treated for 90 seconds,
- f) t100 – milk samples treated for 100 seconds, t110 – milk samples treated for 110 seconds, and t120 – milk samples treated for 120 seconds with microwave radiations.

To cover the effect of environmental conditions for keeping milk in the routine daily life, and to investigate the role of

microwave effect on *Brucella* organisms in milk regardless of the effect of generated heat, another 100 ml of milk samples kept in refrigerator at $5\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ were exposed directly to microwave radiations. These refrigerated milk samples were exposed to 30 % of the microwave full power (P: 30) and for longer periods of time, namely, 0-minute, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, and 8 minutes, respectively.

Statistical Analysis

Average results of the culture bacterial count (CFU) and Log_{10} of the bacterial count were calculated using Microsoft Excel (2013). While significance of the obtained results was processed using simple one-way ANOVA. All analyses were done using SPSS 20.

Discussion

In Egypt, brucellosis is still an important public health hazard due to the consumption of unheat-treated milk or dairy products produced from unpasteurized milk that could be harboring viable *Brucella* organisms. Although, there are many methods to eliminate *Brucellae* in infected milk based mostly on heat treatment, namely; pasteurization, ultra-heat treatment, and boiling, nevertheless; all these methods are of high cost and time consuming, besides; the

thermal impact on damaging milk constituents. Lowering the pH of the milk to an acidic level is also used to preserve milk. However, it was found that *B. abortus* at a concentration of 10^5 CFU/ml resists acidity of refrigerated milk at a pH of 4.0 and survive for up to 10 days of storage at 4°C [10].

Microwaves radiations are electromagnetic, non-ionizing waves with frequencies between 0.3 and 300 GHz [11]. Microwave radiation is applied on different food products through different studies to inhibit some pathogenic bacteria including, *E. coli*, *B. subtilis* [12], salmonella [13], milk flora [14], inactivate *Streptococcus faecalis*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Listeria monocytogenes* [7]. It is evident, from the obtained data that the increase in the exposure time of inoculated milk samples to microwave radiations is directly proportional with a measured temperature (Tables 1& 2) and (Figures 1 & 2). The same observation can be applied on exposed milk to microwave radiations regardless of the milk volume (either 100 ml or 200 ml) or to initial milk temperature (milk kept in room temperature or refrigeration environment). It is also clear that the measured temperature of low milk volume (100 ml) exposed to microwave radiation is higher than the measured temperature of the (200 ml) volume.

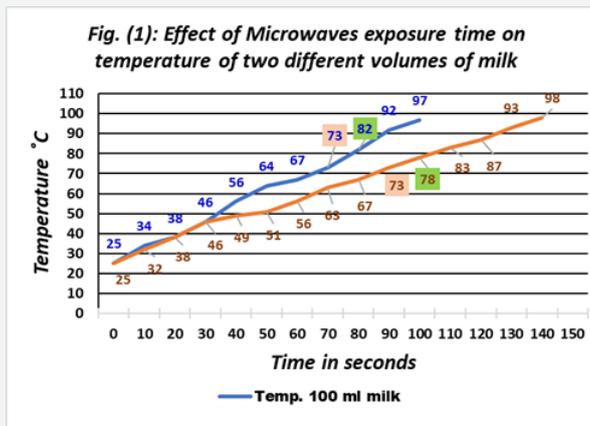


Figure 1: Effect of Microwaves Exposure Time on Temperature of Two Different Volumes of Milk.

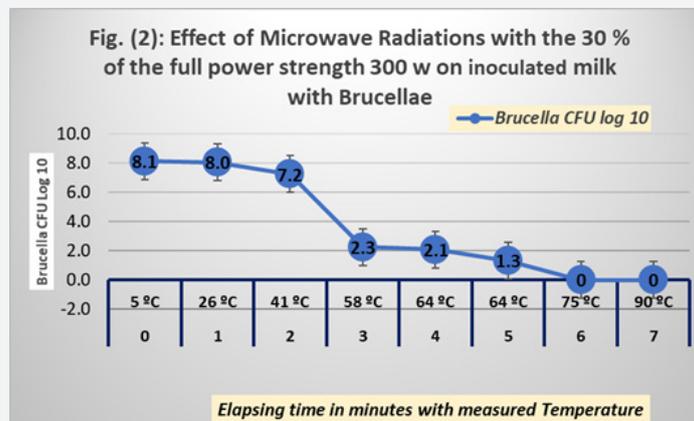


Figure 2: Effect of Microwave Radiations with the 30 % of the full Power Strength (300 w) on Brucella inoculated milk.

Table 1: Effect of Microwaves and Exposure Time on Viability of Brucellae on 100 & 200ml of Brucella inoculated milk kept at room temperature.

Serial	Time (Sec.)	100 Milk Samples		200 Milk Samples	
		Tempe.	B. CFU/ml in Milk	Tempe.	B. CFU/ml in Milk
1	0	25°C	1.3 × 10 ⁸	25°C	1.3 × 10 ⁸
2	10	34°C	6.5 × 10 ⁷	32°C	8.6 × 10 ⁷
3	20	38°C	3.3 × 10 ⁶	38°C	5.1 × 10 ⁶
4	30	46°C	2.1 × 10 ⁵	46°C	2.7 × 10 ⁵
5	40	56°C	3.8 × 10 ⁴	49°C	8.7 × 10 ⁴
6	50	64°C	3.9 × 10 ³	51°C	1.2 × 10 ⁴
7	60	67°C	7.6 × 10 ²	56°C	1.1 × 10 ³
8	70	73°C	1.3 × 10 ²	63°C	4.5 × 10 ²
9	80	82°C	No growth	67°C	1.1 × 10 ²
10	90	92°C	No growth	73°C	0.8 × 10 ¹
11	100	97°C	No growth	78°C	No growth
120 ml*	60	P = A	<i>Brucella</i> Growth	54°C	7.1 × 10 ⁴
240 ml	120	P = A	<i>Brucella</i> Growth	56°C	8.3 × 10 ³

Table 2:

Se.	Time	Temperature	CFU of Brucella/ml
1	0 Time	4-6°C	1.3 x 10 ⁸
2	1 minutes	26°C	1.1 x 10 ⁸
3	2 min.	41°C	1.7 x 10 ⁷
4	3 min	58°C	1.8 x 10 ²
5	4 min.	64°C	1.2 x 10 ²
6	5 min.	64°C	0.3 x 10 ²
7	6 min.	75°C	0 (negative culture)
8	7 min.	90°C	0 (negative culture)
9	8 min.	-	0 (negative culture)
10	9 min.	-	0 (negative culture)

*Start Temperature 5°C ± 2°C (Refrigerated Milk) using 30 % of Microwave power (P = 30).

For milk samples kept at room temperature, *Brucella* organisms still survive in 100 ml milk samples exposed to 900 w full power of the microwave (P = 100) for up to 70 seconds and the measured temperature at that time was 73 °C. But no viable *Brucella* organisms could be isolated at 80 sec with measured temperature of 82 °C. On the other hand, *Brucellae* still present live in 200 ml milk samples for up to 90 seconds and the measured temperature at that time was 73 °C. But no viable *Brucella* organisms could be isolated at 100 sec with measured temperature of 78 °C (Table 1).

While in milk samples kept at refrigeration temperature (5 C ± 2 °C), milk contaminated with *Brucella melitensis* exposed to 30 % of the full power of the microwave (P = 30), *Brucellae* was found viable for up to 5 minutes (300 sec) with the measured temperature of 64 °C, but not viable at 6 minutes (360 sec) with a measured temperature of (75 °C). This observation may be attributed to the fact that the thermal effects of microwave treatment are related

to the heat generated following the absorption of their energy by water, organic molecules or ions [15]. Food heating by microwave is the results of the conversion of microwave energy into heat by the friction of dipole molecules vibrating due to rapid fluctuation in the electromagnetic field [16].

Exposure of milk to microwave radiation directed to a molecular level through the molecular interaction with the electromagnetic field, precisely, through molecular friction resulting from dipole movements of polar solvents and from the conductive migration of dissolved ions. Water in milk is the primary dipolar component responsible for the dielectric heating. In an alternating current electric field, the polarity of the field is varied at the rate of microwave frequency and molecules attempt to align themselves with the changing field. As a result, thermal effect is produced as a result of internal molecular friction. In addition, microwave radiations generate heating through the polarization of ions as a

result the movement of the ionic molecules [17].

It is noteworthy to mention that the warming beverage microwave program (A - 6) failed to decontaminate *Brucella* organisms in tested milk volumes of (120 ml or 240 ml), regardless to the time of the exposure (1 min or 2 min). From the obtained results, it is evident that milk contaminated with *Brucellae* is unsafe for consumers in warm exposure program of the microwave. In this study, it was found that *Brucella* microorganisms in milk exterminated when exposed to microwave radiations with a measured temperature of 82 °C of the volume of 100 ml for about 80 seconds and 78 °C of the 200 ml volume for 100 seconds (Table 1). While it was proved by many authors that *Brucella* organisms when subjected to heat, the organism disintegrated at 63 °C for 30 minutes [9].

Based on this results, it is evident that *Brucella* microorganisms eliminated in milk exposed to microwave radiations not from thermal effect alone, as; the measured temperature for this short time (80 sec and 100 sec in 100 ml and 200 ml of milk) can not alone e responsible for deactivation of *Brucellae* in milk. Our findings substantiate the conclusions of many authors that microwave radiations alone has a decontamination effect on the bacterial organisms regardless of the thermal effect. It was found that microwaves found that microwaves at 835 MHz showed a resonant- like effect on the growth of some yeasts, including *Saccharomyces cerevisiae*, which was not dependent on the amount of absorbed energy from the microwaves [18]. The growth was interchangeably enhanced or decreased at steps of 10 MHz within the frequency range studied. In another study in which *Escherichia coli* cultures were exposed to microwaves (18 GHz, absorbed power 1500 kW/m², electric field 300 V/m) at temperatures below 40°C (to avoid the thermal effects of microwaves), transient morphological changes (dehydrated appearance) and openings of pores in the cell membrane (approximately 20 nm in diameter, enabling passage of dextran molecules) were observed [19].

It seems that this effect is electro kinetic in nature (caused by increased movements of anions and cations), causing localized structural disarrangements of the cell membrane, which results in the emergence of pores. Large membrane pores cause leakage of vital intracellular molecules out of the bacterial cells, which may lead to their death. The effect was reversible because more than 87% of exposed cells remained viable. Regardless to the effect of the microwave radiations on the cell membranes, microwaves generate non-thermal effect that enhances enzymatic reactions in bacterial cells, such as non-aqueous esterification (formation of ester-type of chemical compounds without participation of water), and this effect is substrate concentration-dependent [20, 21].

Up to our knowledge, there is no available literature investigated the effect of microwave radiation on *Brucella* organisms in milk. However, it was found that wild *E. coli* and *Bacillus subtilis* organisms radiated with microwave at the power

of 600 MHz at 70 °C led to inactivation of both organisms and *B. subtilis* did not show any t lysing or destructing cell wall while *E. coli* showed destruction [20]. Exposure of food including milk to microwave radiation is a relatively new procedure in this study we investigated its impact on the viability of viable *Brucella* organisms in milk. In view of this experiment it could be concluded that microwave radiation with full power (P = 100) and frequency of 2,450 MHz, and power of 900W, eliminate *Brucellae* (1.3 X 10⁸ CFU of / ml) in 100 ml milk kept in room temperature in 80 seconds in 200 ml milk samples in 100 seconds. While in refrigerated milk (5 °C± 2) exposed to 1/3 of the microwave power (P = 30) *Brucella* organism still viable till 5 minutes and the organisms killed at 6 minutes. It is noteworthy to mention that the warming program of beverage in the microwave either in heating 120 ml milk for 1 minute or 240 ml milk samples for 2 minutes, the organism is still viable. Microwave radiations can be a tool for keeping milk safe for consumption but with the exposure time followed in this study and taking into consideration milk kept in room temperature or refrigerating temperature.

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