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Effect of time and wattage power levels of microwave treatment on the microbial quality and safety of bovine raw milk

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ABSTRACT

Raw milk is sterile when secreted by healthy cows, however it is contaminated with different microorganisms and *Streptococci* commonly present in the milk ducts as well as teats. The bacterial count in raw milk ranges from a few hundred to several thousand per mL of milk, however it is harmful to human health under unpasteurized condition. Milk sanitization methods rely on the principle of preventing the growth and development of microbes and thus maintaining their nutritional quality. This study was carried out to investigate the effect of microwave radiation on the microbial quality and safety of bovine raw milk. To do so, the effect of microwaves at a frequency of 35 KH and powers of 180, 300 and 850 W for 0, 30, 60 and 90 s on the destruction of pathogens and reduction of microbial load of milk (total bacterial count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, molds and yeasts) was investigated. The results showed that microwave treatment had a significant effect on reducing the number of microorganisms in milk samples ($p<0.05$) with the power of 850 W being the most effective power for reducing the number of *S. aureus*, coliforms, molds, yeasts as well as total bacterial count. Faster heating with higher energy efficiency is the main advantage of the microwave process for foods. In fact, the microwave method can be a potential and effective method for decreasing the microbial load of raw milk.

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1. Introduction

Milk is a highly valuable food recognized as a nutritious drink for all people of all ages meeting some nutritional needs. It plays a major role in improving Intelligence Quotient and body development and retarding fatigue and aging. Thus, it is very important to pay attention to the quality properties of milk (1).

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Milk is a natural and perfect food that plays major role in human nutrition at different ages and it is crucial to control and monitor its health standards during different steps until consumption (2). Per capita consumption of milk as a basic component of other dairy products is one of the most important factors of health and nutrition development in a country. Milk production and its cost price play an important role in the per capita consumption of dairy products in addition to the general culture of people (2).

Milk is a natural and perfect food for human consumption which is extensively used worldwide regardless of its source. Raw milk is secreted when complete milking a healthy animal's udder. It is also an important source of infections with common bacteria such as *Escherichia coli* and *Staphylococcus aureus* associated with diarrhea and vomiting (3). Raw milk is still consumed in rural areas of some undeveloped countries despite its health risks (3). One of the most important indicators of healthy milk production is "reduced microbial load of milk" and so milk is pasteurized by different methods to destroy the pathogenic bacteria. Various studies on the effect of microwaves and ultrasound on reduced microbial load of some pathogens present in milk have been conducted. Due to the fact that boiling raw milk is tedious and time-consuming (3) and that microwaves (electromagnetic waves typically used at a frequency of 2450 MHz) (4), the goal of this study was to investigate its effect on reduced microbial load of raw milk (total bacterial count, *S.aureus*, *E.coli*, molds and yeasts and apply microwaves (at a frequency of ≥ 20 KHz) as a novel method for destructing the microorganisms (via acting on their cell membrane, local heating and generating free radicals) and sanitizing milk (5). Among the advantages of microwaves are less initial heating, faster heating, higher energy efficiency, less space, more precise process control, selective heating and higher nutritional quality foods. Its disadvantages include uneven heating, reduced color and flavor development, wet surface, and reduced moisture and firm texture (6). Microwaves are electromagnetic waves with a frequency ranging from 300 MHz to 300 GHz (7). By interacting with polarized (dipolar) and ionic molecules such as water, fat, amino acids and proteins in milk, microwaves cause friction-heat, thereby reducing the microbial load (8). Microwave has been applied in many food processes including drying, tempering, blanching, cooking, pasteurization, sterilization and baking because of their various advantages over commonly used heating methods especially due to energy efficiency (9). The main advantages of microwave applications are selective heating, effective process control, fast heating and portability of equipment, energy-saving, low cost and shortening of application time(10). Microwave heat is generated through the conversion of microwave energy into heat by water molecules friction based on the fluctuations of the electromagnetic field. It is characterized by volumetric heating (11,12). Microwave treatment has also been used for heating milk products to improve sensorial properties compared to the conventional heating methods (13-15). The presence of pathogenic bacteria in

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raw milk is well documented. Several studies conducted on raw milk samples collected from the market could detect the presence of different pathogens such as *Salmonella* spp., *E.coli O157:H7*, *Campylobacter* spp. and *L. monocytogenes* (15-17); Recently in the United States and in Ireland, some cases of listeriosis caused by the consumption of raw milk have been reported (18,19); In Canada, an outbreak due of *E.coli* in dairy products made from raw milk was observed (20). Stephen found that the effect of microwaves on the pathogens (*Listeria monocytogenes* and *Escherichia coli*) in raw milk resulted in their 5 log cycles reduction (3). Tremonted et al. (2014) studied the microbial quality of raw milk in Italy and found that the applied microwaves reduced the number of *Pseudomonas*, *Enterobacteriaceae* as well as coliforms (21). The use of ultrasound at 100 W and 30 KH for homogenizing Sheep milk (22) and 5.3 log reduction in the growth of *Listeria innocua* by ultrasound at 63°C for 30 min are among the applications of ultrasound to reduce the microbial load (23).

The aim of this study was to investigate the effect of microwave radiation on the microbial viability of the cow milk by establishing a correlation between exposure time and the viable count of the microbial load of milk, to check the efficiency of microwave to render microbial infected milk.

2. Materials & Methods

2.1. Sample preparation

First, the raw milk samples were collected from a farm and transferred to the laboratory at 4°C. Then three samples were selected and the pre-and post-microwave treatment parameters including total bacterial count, *S.aureus*, coliforms, *E.coli*, molds and yeasts were examined.

The effect of microwaves (Model SAMSUNG) at a frequency of 35 KH and power of 180, 300 and 850 W For the times of 0, 30, 60 and 90 s on removing the pathogens and reducing the microbial load of raw milk (total bacterial count, coliforms, *E.coli*, *S.aureus*, molds and yeasts.

2.2. Total bacterial count

Initially, a dilution was made from the milk sample at 10^{-7} concentration. To do so, 9 mL of physiological serum were poured into 7 sterile test tubes and the tubes were numbered from 1 to 7. One mL of the initial milk sample was transferred to tube 1 under sterilized conditions (next to the flame) and mixed well to obtain a uniform dilution at 10^{-1} concentration from which 1 mL was transferred to the next tube (tube 2) and so on. Finally, a dilution at a concentration of 10^{-7} was made. Next 0.1 mL of each dilution was cultured on a sterile plate containing nutrient agar with sterile ring forceps, then it was incubated at 32°C for 48 h and the colonies were counted by a colony counter. This procedure was repeated for microwave-treated milk samples.

2.3. Coliform culture and count

One mL of the respective dilutions was poured into the sterile plate to which 10-15 mL of violet red bile agar (VRBA) were added at 45°C, vortexed and cooled. Another layer of VRBA (3-4 mL) was added to the newly formed solid medium and the plate was turned upside down at 35°C for 24 h. For confirmatory tests, coliforms from the colonies formed on VRBA were transferred to a tube containing brilliant green broth (BGB) with Durham tube and the tubes were placed at

35°C for 24-48 h. Then they were observed for gas production.

2.4. *E.coli* culture

The contents of BGB positive culture tubes were cultured on MacConkey agar medium and they were placed at 44.5°C for 24 h.

2.5. Molds and yeasts culture and count

One ml of the dilutions was poured into a sterile empty plate to which melted Sabraud dextrose agar (SDA) was added at 45°C, cultured by pour plate method and shood in an 8 shape direction. The culture medium was closed and incubated at 22-25°C for 3-5 days.

2.6. *S.aureus* culture and count

Baird-Parker agar (BPA) is used for culturing and counting *S.aureus*. To do this, 0.1 and 0.01 dilutions were cultured on BPA and incubated at 37°C for 24 h. In the positive cases, black colonies with a colorless halo are produced.

2.7. Statistical analysis

Data were analyzed by SPSS software version 21 as well as Kruskal-Wallis nonparametric test. Mann-Whitney test was used to compare the treatments and determine their significant difference at a confidence level of $p<0.05$.

3. Results

3.1. Effect of microwaves power on microbial load of milk

Variations in the number of different microorganisms at different powers and times of microwave treatments in three milk samples are presented in Tables 1, 2 and 3.

The results showed that the use of microwaves at different powers and times reduced the microbial load of microorganisms including *S.aureus*, coliforms, molds and fungi as well as the total bacterial count in milk as in some samples increasing microwave power destroyed all microorganisms, e.g. 850 W for 90 s eliminated *S.aureus* and coliform completely in samples 1 and 2. In sample 3, microwaves powers of 300 and 850 W at all times caused *S.aureus*, total bacterial count, coliform, molds and fungi to reach zero.

Microwave power of 30 W for 60 s caused the complete elimination of *E.coli* indicating the significant effect of increased power on reduced microbial load.

Fig. 1 shows that increasing the microwave power from 180 to 850 W reduced the microbial load of *S.aureus*, total bacterial count, coliforms, models, fungi and *E.coli* as their lowest microbial load was observed at 850 W.

In this study, the effect of microwave power was examined by using Kruskal-Wallis. Given the results presented in Table 4, the microwave power had a significant ($p<0.05$) effect on reducing microorganisms in milk as the highest power (850 W) resulted in the lowest number of microorganisms so it could be concluded that increasing the microwave power to 850 W reduced the microbial load of all microorganisms.

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Table 1. Variations in number of different microorganisms at different powers and times of microwaves treatments in milk sample 1

	Time(S)	Power(W)	*Staph. C* (cfu/mL)	T.C (cfu/mL)	C.C (cfu/mL)	M&Y.C (cfu/mL)	*E.coli* (cfu/mL)
		180	6.7×10^3	8.1×10^7	7×10^4	5×10^5	1
	30	300	5×10^3	2.8×10^6	5.2×10^4	4×10^5	1
		850	2.2×10^3	2.2×10^6	3×10^4	1.4×10^5	1
		180	1.1×10^4	6.5×10^6	3.7×10^5	4×10^5	1
	60	300	3.5×10^3	3.6×10^6	1.5×10^5	1.6×10^4	0
		850	3×10^3	3×10^6	1×10^4	3×10^2	0
		180	1.2×10^4	6×10^6	2.4×10^5	1.7×10^4	1
	90	300	1.2×10^3	4.7×10^6	1×10^3	2.6×10^3	0
		850	0	3.1×10^6	0	3×10^2	0
Control Sample	0	0	7×10^3	9.2×10^7	7×10^5	6×10^5	1

Table 2. Variations in number of different microorganisms at different powers and times of microwaves treatments in milk sample 2

	Time(S)	Power(W)	*Staph .C* (cfu/mL)	T.C (cfu/mL)	C.C (cfu/mL)	M&Y.C (cfu/mL)	*E.coli* (cfu/mL)
		180	2.2×10^3	5×10^7	2.3×10^5	8.2×10^5	1
	30	300	1×10^3	2.1×10^7	2×10^5	7.5×10^5	1
		850	1×10^2	1×10^6	9×10^4	4.6×10^5	1
		180	3×10^3	4.6×10^7	1.3×10^5	9.7×10^5	1
	60	300	7×10^2	1.2×10^7	1.1×10^5	5×10^5	0
		850	1×10^2	1×10^6	1×10^2	7.5×10^4	0
		180	1.3×10^3	1.7×10^7	1.1×10^5	6.6×10^5	1
	90	300	2×10^2	1×10^6	0	5.4×10^5	0
		850	0	5×10^5	0	6×10^3	0
Control Sample	0	0	5.6×10^3	6×10^7	3×10^5	1.2×10^7	1

Table 3. Variations in number of different microorganisms at different powers and times of microwaves treatments in milk sample 3

	Time(S)		*Staph .C* (cfu/mL)	T.C (cfu/mL)	C.C (cfu/mL)	M&Y.C (cfu/mL)	*E.coli* (cfu/mL)
		180	6.2×10^3	1.1×10^7	2.6×10^5	5.7×10^5	1
	30	300	1.1×10^3	2×10^5	2.1×10^5	1×10^5	1
		850	0	0	0	0	1
		180	2.7×10^4	4×10^7	5.5×10^4	1×10^6	1
	60	300	2×10^2	1×10^2	2×10^3	3.2×10^4	0
		850	0	0	0	0	0
		180	1×10^4	5.5×10^6	5.7×10^4	3×10^5	1
	90	300	0	0	0	0	0
		850	0	0	0	0	0
Control Sample	0	0	9.2×10^3	1.7×10^7	3.3×10^5	1.7×10^6	1

Table 4. Different variations in microorganisms at different microwave power

Parameter	power	N	Mean	SD	Test statistics	**P-Value**
Staph	180	9	8822.22	7845.18	15.385	**0.000**
	300	9	1433.33	1694.84		
	850	9	600.00	1152.17		
Total count	180	9	29222222.22	26481256.47	15.120	**0.001**
	300	9	5033344.44	7079185.98		
	850	9	1200000.000	1261942.94		
Coliform	180	9	169111.11	110559.31	12.197	**0.002**
	300	9	77555.55	90274.73		
	850	9	14455.55	30040.26		
M and Y	180	9	581888.89	321468.68	11.422	**0.003**
	300	9	260066.67	288676.88		
	850	9	75733.33	152168.22		
E.coli	180	9	1	0.00	10.400	**0.006**
	300	9	0.33	0.50		
	850	9	0.33	0.51		

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Table 5. Different variations in microorganisms at different microwave power

Parameter	Time(s)	N	Mean	SD	Test statistics	**P-Value**
Staph	30	9	2722.22	2586.88	1.506	**0.471**
	60	9	5388.89	8799.35		
	90	9	2744.44	4735.27		
Total count	30	9	18800000.00	28407217.39	0.869	**0.648**
	60	9	12455566.67	17779692.53		
	90	9	4200000.00	5355371.13		
Coliform	30	9	123888.89	100616.16	4.486	**0.106**
	60	9	91900.00	120165.78		
	90	9	45333.33	82503.03		
M and Y	30	9	415555.55	286535.82	3.211	**0.201**
	60	9	332588.89	412654.03		
	90	9	169544.44	264297.18		
E. coli	30	9	1.00	00	10.400	0.006

Table 6. Variations in microorganisms in two different groups (control and intervention)

Parameter	group	N	Mean	SD	Test statistics	P-Value
Staph	Intervention	27	3618.52	5866	14.000	0.066
	Control	3	7266.67	1814.75		
Total count	Intervention	27	11818522.22	19786201.85	6.500	0.019
	Control	3	56333333	37634204		
Coliform	Intervention	27	87040.7407	103598.63	2.000	0.007
	Control	3	443333.3	222785.4		
M and Y	Intervention	27	305896.29	331655.42	5.000	0.014
	Control	3	4766667	6288349		
E. coli	Intervention	27	0.55	0.51	22.500	0.143

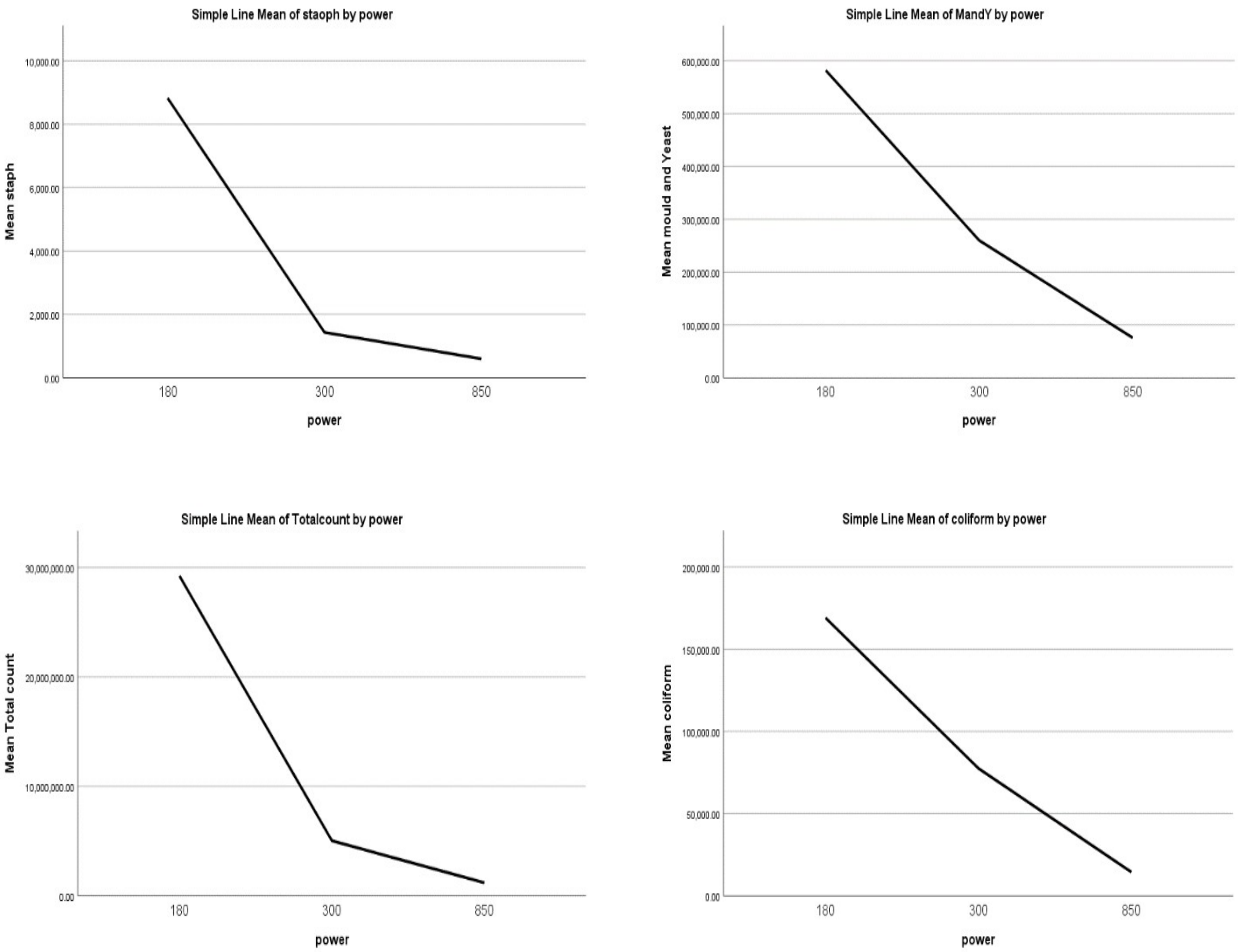

Figure 1. Variations in different microorganisms at different microwave powers

3.2. Effect of microwave time on microbial load of microorganisms in milk

The results of Kruskal-Wallis test for the effect of microwave time are displayed in Table 5.

It was shown that the microwave time had no significant ($p>0.05$) effect on *S.aureus*, total bacterial count and coliforms in milk samples. It had only a significant effect on *E.coli* that showing a standard deviation of 0.006.

3.3. Effect of microwave power and time on microbial load of microorganisms in milk samples in two groups (control and intervention)

Mann-Whitney test used for comparing the microorganisms in control and intervention groups showed a significant decrease in *S.aureus*, total bacterial count, coliforms, molds and yeasts in milk samples treated by microwaves however this decrease in *E.coli* count was not significant.

4. Discussion

The severity of microbial contamination in raw milk depends on how hygienic standards are met during its preparation, transportation and storage (24). The initial quality of raw milk is affected by factors such as animal's nutrition and health, the chemical composition of milk and microbial activity. Even in the best conditions, milk contains a number of bacteria entering the tank. The amount and type of contamination highly depend on cleanliness, environmental hygiene and the way of milking. Rapid cooling of milk to below 4°C on the farms has a great effect on the microbial quality of milk (25). A research showed a high correlation between the consumption of milk and its products and IQ, the incidence of infectious diseases and regulation of metabolic processes. Consumption of milk and its products is an indicator of the society's development. Daily intake of 1000-1500 mg calcium can reduce blood pressure preventing cardiovascular diseases. Since milk and whey contain biopeptides their consumption may prevent cancer, diabetes and osteoporosis and improve dental health. Also, the probiotic bacteria and fat contained in milk prevent many diseases including gastrointestinal disorders (30).

The present study shows that increasing microwave power from 180 to 850 W reduced the microbial load of *S.aureus*, total bacterial count, coliforms, molds, fungi as well as *E.coli*. So increased time of microwave treatment resulted in a decrease in microbial load of microorganisms in milk. Tremonte et al. (2014) studied the effect of boiling, microwave treatment and cooling on the microbial quality of milk and found that increasing microwave power to 900 W for 75 s reduced the number of *Pseudomonas*, *Enterobacteriaceae* and coliforms in raw milk (21).

Kindle (1996) investigated the effect of microwaves at 2450 MHz and 600 W on *pseudomonas aeruginosa*, *E.coli*, *Enterobacter bakazaki*, *Klebsiella pneumonia*, *S.aureus*, *Candida albicans*, *Mycobacterium leprae* and polio vaccine viruses. The results revealed that the use of microwaves is a fast and convenient way of reducing the microbial load of infant milk. The results of our study showed that microwave power had a significant effect on reducing the microbial load of microorganisms which is consistent with the results of the two aforementioned studies (4). The results showed no significant effect of microwave time on reducing the microbial load.

Stephen Thompson et al. (1990) investigated the effect of microwaves on raw milk pasteurization. The results showed that the use of microwaves resulted in a 5-cycle reduction in the number of pathogenic bacteria (*L. monocytogenes* and *E.coli*) (26). Also, Hammad (2015) studied the effect of microwaves on the bacteria in raw milk. 40 raw milk samples were exposed to microwaves at 110 W for 3 min. The results revealed that the microwave treatment had a significant effect on the bacteria in milk. Our results suggested the effect of microwaves on the microbial load of microorganisms in milk which is in agreement with the mentioned results (27). Bermudez-Aguirre et al. (2009) investigated the modeling of the growth of *L.innocua* using ultrasound and found that ultrasound at 63°C for 30 min could reduce the *L.innocua* growth (23). Abbaszadeh et al. (2003) examined the effect of sodium chloride, pH and the type of acid on *L. monocytogenes* destruction by microwaves and observed that sodium chloride (0.5-3%) in liquid media had no significant effect on *Listeria*. Also, the common pH value of foods (5-7) had no significant effect on the destructive action of microwaves on Listeria. The effect of lower pH values were significant. Although at the same pH, organic acids compared to mineral ones increased the sensitivity of *L. monocytogenes* to heat, it is not true for microwave treatment (used in the present study) as the decreasing trend of bacterial count by microwaves was similar in both cases (acetic acid vs cloridric acid) (28). Ali et al. (2015) studied the effect of microwaves on the quality of raw milk and found that the application of microwaves reduced the microbial load of raw milk. Therefore this method reduced the microbial load within a shorter time preserving the milk nutrients (29). The results revealed that the power of microwaves had a significant ($p<0.05$) effect on reducing the number of microorganisms in milk with the power of 850 W being the most effective power in reducing *S.aureus*, total bacterial count, coliforms, molds, fungi and *E.coli* in milk. A comparison of microorganisms in milk in control and intervention groups showed the significant effect of microwaves on reducing *S.aureas*, molds, fungi and yeats however this reduction in *E.coli* was not significant ($p>0.005$). Therefore, due to the high efficiency and easy availability of microwaves, it could be an effective method for reducing the microbial load of microorganisms including *S.aureus*, total bacterial count, coliforms, mold, fungi as well as *E.coli* in raw milk.

5.Conclusion

This study showed that microwave processing had a significant effect on the reduction of the number of microorganisms in milk samples with the power of 850 W being the most effective power for reducing the number of *S. aureus, coliforms, molds, yeasts* as well as total bacterial count. Faster heating with higher energy efficiency is the major benefit of the microwave process for food. Indeed, the microwave method can be a potentially effective method to reduce the microbial load of raw milk.

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Conflict of interest

Authors declare no conflict of interest.

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