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IMPACT OF MICROWAVE ON SURVIVAL AND RHEOLOGICAL PROPERTIES OF *Staphylococcus aureus* IN NUTRIENT BROTH AND BUFFALO'S MILK

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ABSTRACT

Staphylococcus aureus ATCC 25923 suspended in nutrient broth (168×10^4 CFU/ml.) and buffalo's milk (85×10^4 cfu/ml) were placed in glass tubes. Nutrient broth tubes were exposed to microwave of low (90w), medium (450w) and high (900w) power for 0, 2, 4, 6, 8 and 10 seconds. Milk tubes were subjected to microwave of high power for the same exposure periods. The final temperature of the media and rate of microorganism destruction and the effect of this treatment on the changes of morphological profile and rheological properties of *S. aureus* were determined.

Microwave irradiation completely destroyed the microorganism when exposed for 8 seconds and the 900 w power at a final temperature of 75°C in nutrient broth and 86°C in buffalo's milk. The athermic effect of the microwave on the microorganism was supported by the 80% destruction obtained at a final temperature of 40°C. Microwave heating decreased cell diameter, width, length, perimeter, surface area, thickness and sphericity and increased cell compactness. These changes increased by increasing microwave power and/or exposure period.

INTRODUCTION

Microwave heating of food, based on oscillation that creates considerable intermolecular friction that results in the generation of heat. Thus, the food is heated from the inside outwards and at a faster rate than the conventional heating methods (Goldblith, 1966 and Curnutte, 1980). It is thought that microwave destroys microorganisms by two effects, i.e. thermic and athermic. The athermic effect, though controversial, would cause the destruction by microwave interaction with microorganism structure (Wayland, *et al.*, 1977).

Microwave ovens became increasingly popular at homes and industry. Nowadays, the application of microwave in dairy processing is limited to certain practices, such as tempering, thawing (Young and Jolly, 1990), metling of cheese and chocolate blocks or coating (Mohr and Hanne, 1981), plastification of curds in the production of certain types of cheese (Bottazzi and Battistotti, 1976). However, research continues to study the use of microwave in various aspects of dairy industry. El-Shibiny, et al. (1982) studied the effect of microwaves on buffaloe's milk chemical and microbiological properties. Thompson and Thompson (1990) reported that aerobic plate counts in raw goat's milk were reduced after microwave treatment by up to 6 log cycles without impairing organoleptic quality. Sieber *et al.* (1996) found that microwave did not affect milk vitamins. Jaynes (1975)

studied milk pasteurization as tested by phosphatase and total bacterial count (TC). However, much of microwave researches was devoted to microorganisms destruction and the results were not in close agreement with Young and Jolly (1990); Fung and Cunningham (1980). Knuston *et al.* (1988) failed to inactivate all added cells of *Sallmonella typhi, E. Coli* and *Ps. fluorescens* in milk by microwave pasteurization. Choi *et al.* (1993) inactivated *Campylobacter* jejuni in whole milk by microwave haeting at 71.1°C for 3 minute and *Yersinia enterocolitica* for 8 min. The destruction of *Salmonella* species, *S. aureus* and *Listeria monocytogenes* in complex foods including milk heated by microwave energy were tested by Heddlson, *et al.* (1996). Heating milk to 65°C reduced *S. aureus* population by 2.4 log CFU/ml., with no obvious effect for medium composition.

One of the critical points of microwave destruction of microorganisms in foods is the fact that the results are influenced by interinsic characteristics of foods (i.e. pH, moisture level, O-R potential, composition, shape, size ...) and extrinsic characteristics (i.e. temperature, humidity, frequency, intensity of radiation, and length of time of exposure ...), (Fung and Cunningham, 1980). Therefore, much research is still needed to standardize microwave treatment for rendering product as milk safe. Actually, research is urgently needed to settle the point of the feasibility of using microwave ovens in milk pasteurization.

Staphylococcus aureus is a pathogenic organism that causes mastitis for dairy cattle and food poisoning and shock syndrome to humans (Banerjee, 1985). The absence of veterinary care for milking animals in small farms that prevails in Egypt would make *S. aureus* a like threat for the Egyptian consumers. Studies on the effect of microwave heating on *S. aureus* in milk are scant and need deep investigation.

The objective of the present study was to determine the effect of microwave heating power level and heating time on the destruction of *Staphylococcus aureus* in nutrient broth and buffalo's milk. Another objective was to evaluate the change in rheological properties of bacterial cell deformation after microwave treatment using image analysis technique.

MATERIALS AND METHODS

Staphylococcus aureus ATCC 25923 was obtained from the Microbiological Resource Center (MERCEN) at Aim-Shams University, Egypt. Stock culture was maintained on slant of nutrient agar (Difco, 1985), then stored at 4°C and sub-cultured every two weeks.

Nutrient broth and buffalo's milk were heated in a microwave oven (Model-NN-9853, National Co., Tokyo, Japan) rated at 900 watts and operated at a frequency of 2.450 MHz.

Fresh buffalo's milk was obtained from the Department of Dairy Science, Faculty of Agriculture at Fayoum, Cairo University, Egypt. Milk was sterilized at 121°C/20 min. and used to study the survival of *Staphylococcus aureus* during microwave heating.

A mercury thermometer was used to measure the final temperature of nutrient broth and milk before and after microwave heating at the end of various exposure periods. The pH values were also measured by a pH meter.

Aliquots of 5 mil of nutrient broth (pH 7.0) or sterilized milk containing initial population of 168 x 10^4 cfu/ml and 85 x 10^4 cfu/ml. *Staphylococcus aureus* ATCC 25923, respectively were placed in glass tubes. Nutrient broth tubes were subjected to the microwave at power levels of 90 w (low), 495 w (medium) and 900 w (high) for 0, 2, 4, 6, 8 and 10 sec. Milk tubes were subjected to microwave of high power for the same exposing periods. Decimal dilutions were made in 0.1% peptone water (Difco, 1985). Pour plate of salt manitol agar (Difco, 1985) were used to count viable *Staphylococcus aureus* cells. Plates were incubated at 37°C for 48 hr. Experiments were performed in triplicate. Destruction rate was calculated from the following equation:

$$\text{Destruction rate} = \frac{(\text{initial population} - \text{final population})}{(\text{initial population})} \times 100$$

Simple stain and Breed methods (Difco, 1985) were used for preparing slides of *Staphylococcus aureus* in nutrient broth and milk, respectively. The morphological profiles (shapes) of the bacterial cells were transferred to the computer by an Olympus Bx 40 microscope equipped by a Panasonic CCTV vedio color camera (Model No. WV-CP220/G). Rheological deformation of bacterial cells resulted from microwave heating were analyzed by Clemex Vision Computer Program (Clemex Technologies Inc., Longueil, Qc, J4G IT5, Canada). The images were digitally processed to produce binary images, which were measured through the system to obtain several rheological parameters. These include; cell wall thickness, width, length, sphericity, compactness, perimeter, surface area, and spherical and circular diameters.

One-way analysis of variance (ANOVA), 2 factor factorial, mean separation and correlation were analyzed by MSTAT microcomputer statistical program (ver. 2.1C, 1989).

RESULTS AND DISCUSSION

Table (1) shows the effect of microwave power and heating periods on temperature and pH of nutrient broth inoculated with *S. aureus*. Final temperature of the broth increased by increasing the above two parameters. As the power increased from 90 w into 900 w, the broth temperature increased and at a faster rate than by duration period. Final temperature of 83.5°C was reached by the 900 w power after 10 sec. heating period.

Table (1): Effect of microwave power and heating periods on temperature and pH of nutrient broth inoculated with *Staphylococcus aureus*

Heating time (sec.)	Microwave power stetting					
	Low (90W)		Medium (495W)		High (900W)	
	Temp. °C	pH	Temp. °C	pH	Temp. °C	pH
0	27.0	6.73	27.0	6.73	27.0	6.74
2	28.5	6.91	30.0	7.02	31.5	6.96
4	32.0	7.04	38.0	7.11	40.0	7.09
6	36.0	7.06	47.0	7.17	51.5	7.16
8	43.0	7.09	70.3	7.25	75.6	7.21
10	51.0	7.17	80.6	7.31	83.5	7.24

Table (2) presents the destruction rate of *S. aureus* in nutrient broth when exposed to microwave irradiation of different powers and at different exposing periods. Microorganism destruction increased by the increasing the two parameters, the microwave power and the treatment period. Complete destruction was obtained at 900 w power and 8 sec. duration. The final temperature was 75.6°C (table 1). The rate of destruction was faster by the increase in the oven power than the increase in exposing period. At 6 sec. exposure, the destruction was substantial (94.5%) at the high power. This destruction was not reached after 10 sec. exposure at the low power.

Table (2): Effect of microwave power and heating period on *S. aureus survival.**

Microwave exposure periods (Sec.)	Microwave power setting					
	Low		Medium		High	
	Viable count ($x10^5$)	Reduction (%)	Viable count ($x10^5$)	Reduction (%)	Viable count ($x10^6$)	Reduction (%)
0	1.680	0	1.62	0	1.65	0
2	1.420	15.7	1.02	37.0	0.73	55.7
4	1.290	23.2	0.62	61.7	0.33	80.0
6	1.170	30.7	0.15	90.7	0.09	94.5
8	0.270	83.9	0.05	96.9	0.00	100
10	0.130	92.3	0.03	98.4	0.00	100

* Staphylococcus aureus ATCC 25923 in nutrient broth

LSD power level : (P> 0.05) = 1.4 and (P> 0.01) = 2.0

Heating time : (P> 0.05) = 2.1 and (P> 0.01) = 2.8

Actually, results in this table pointed out 3 important points. First, that the final temperature was the most important for microorganism destruction by microwave irradiation. This point was supported by the findings of other workers (Culkin & Fung, 1975 and Haddleson, *et al.*, 1994). Second, the rate of reaching this final temperature was important in determining rate of destruction. The faster the rate of reaching the temperature, the faster the rate of destruction. At the high power rate of 900 w, *S. aureus* was completely destroyed after 8 sec. and a final temperature of 75.6°C as compared to 98.3% destruction at 80.6°C after 10 sec. of exposure to the medium power. The third point, was the substantial destruction of 80% at sub lethal temperature of 40°C, obtained at 4 sec. exposure to the high power oven. This point probably support the "athermic" effect of microwave irradiation involved in microorganisms killing.

Table (3): Destruction rate of *S. aureus* in buffaloe's milk by microwave irradiation

Microwave* exposure period (sec.)	Viable count ($x10^4$)	Reduction (%)	Temp. (°C)
0	85.0	0	27
2	40.1	52.82	30
4	19.2	77.41	45
6	5.9	93.06	58
8	0	100	86
10	0	100	90

* High microwave power setting was used

Buffalo's milk was used to study the effect of medium on the above results and to study the feasibility of using microwave for milk pasteurization. Buffalo's milk was inoculated with *S. aureus* suspension (85 x 10^4 cfu/ml.) and was exposed at 900 w for different periods. Table (3) shows final temperature and microorganism destruction rates.

Complete destruction was reached after 8 sec. of exposure, and at a temperature of 86°C. A destruction of 93.06% was reached after 6 sec. exposure and at a 58°C. Of course, exposing milk for only 8 seconds to get milk free of *S. aureus* is of a great advantage for preserving milk quality and make milk safe.

Fig. (1) and (2) show the effect of microwave irradiation on the morophological profile of *S. aureus* in broth and buffalo's milk respectively. *Staphylococcus aureus* exhibited almost a perfect spherical shape arranged in staphylococci grape like groups in unheated nutrient broth (Fig. 1A). After microwave heating, the shape and arrangement of bacterial cells were altered and disrupted as shown in Fig.(1B, C, D, E, F. and Fig. 2A, B, C, D). This deformation greatly increased by extending the exposure period from 2 sec. to 10 sec.

Fig. (3&4) and Table (4) illustrate the change in the rheological parameters of *S. aureus* (diameter, width, length, perimeter, thickness, surface area, sphericity and compactness) by microwave treatment. The figure shows the effect of microwave irradiation (at different powers and exposing periods) on the microorganism cells in nutrient broth. While Table (4) shows that effect in buffalo's milk exposed to high power for different periods. Generally, all parameters except compactness had a gradual decrease by increasing the power intensity from 4.314 mW/μm^2 (low power) to 43.139 mW/μm^2 (high power) and the exposure period separately or in combination. Therefore, the greatest deformation in all rheological parameters occurred by microwave heating at the highest power level and longest exposure period. This means that the killing effect of microwave irradiation was manifested through the changes in morphological and rheological properties of the cell. This of course resulted from or affecting the inside components of the cell. This was supported by the fact that morphological deformation as well as destruction increased by microwave power.

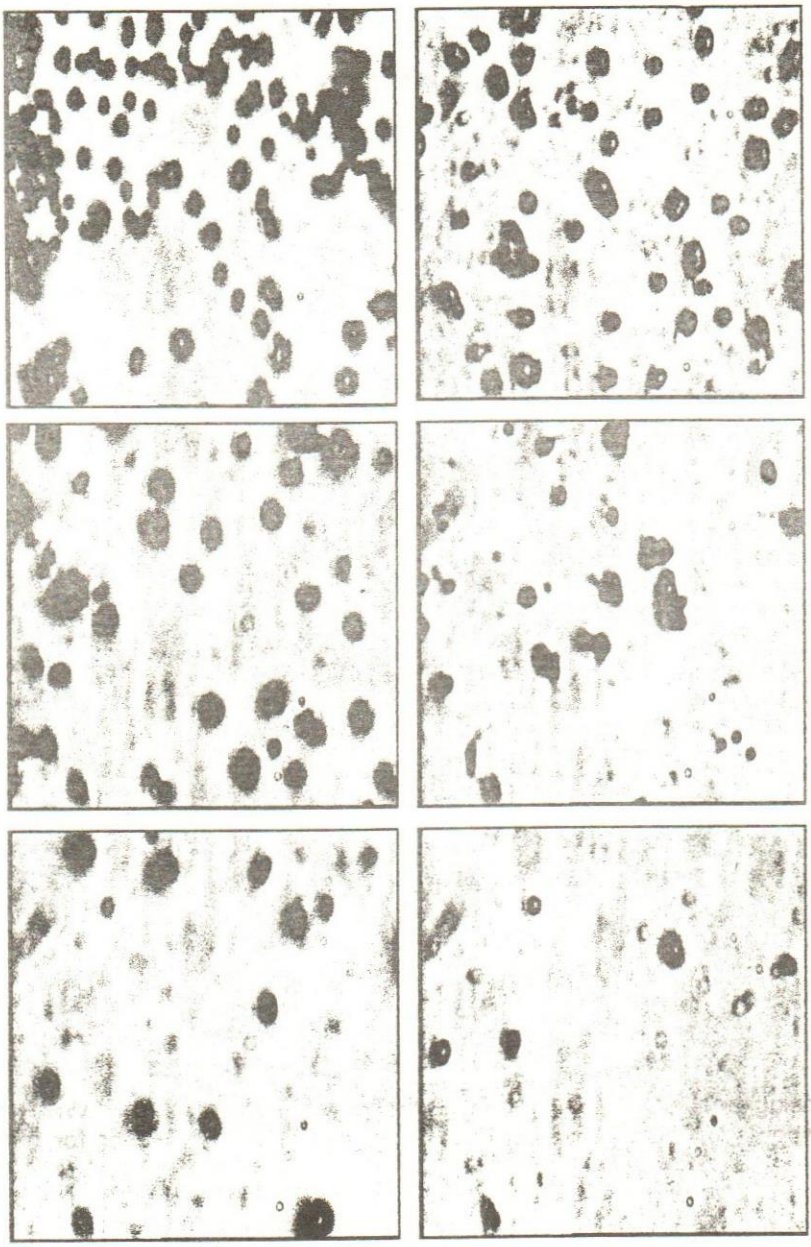


Fig (1). Morphological profile of Staph.aureus in nutrient broth heated in microwave oven at high power set for 2 sec. (B) , 4 sec (C) , 6 sec (D) , 8 sec (E) and 10 sec(F) compared with that in unheated broth (A).

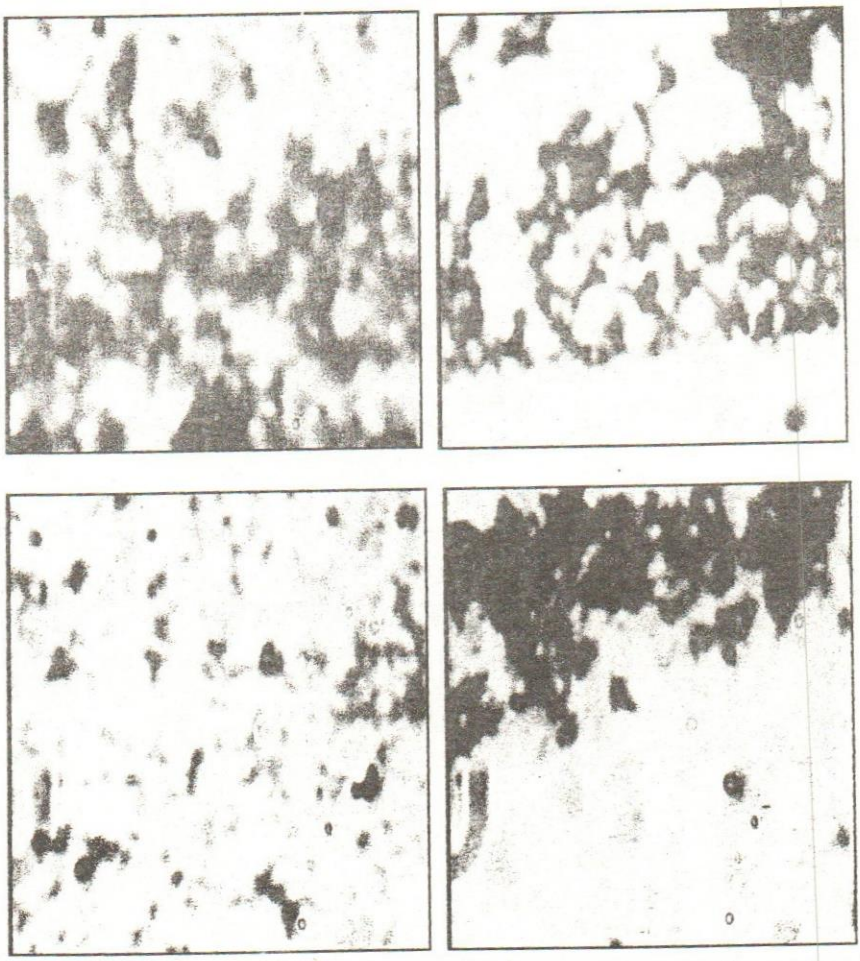
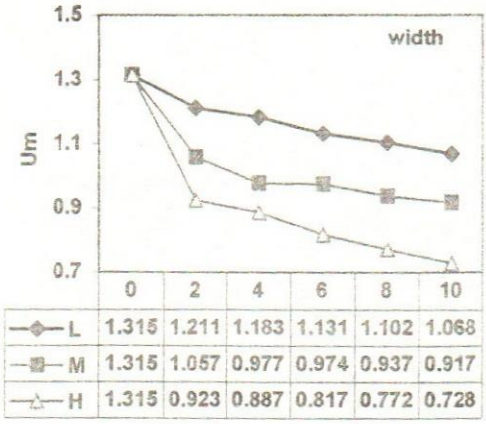
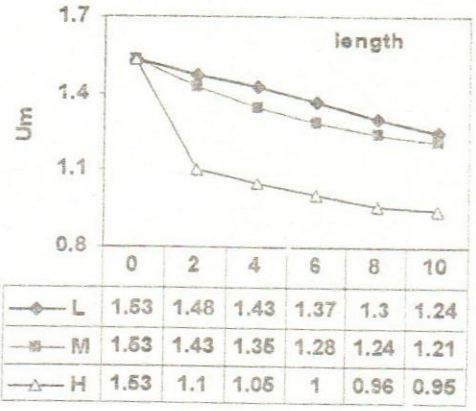


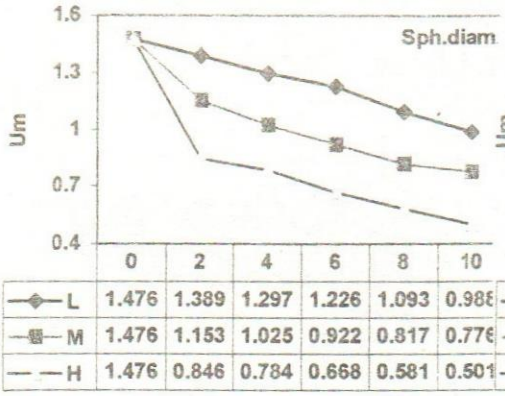
Fig (2) Morphological profile of Staph. aureus in buffalo's milk heated in Microwave oven at high power for 4 sec. (A), 6 sec. (B), 8 sec. (C) and 10 sec. (D).



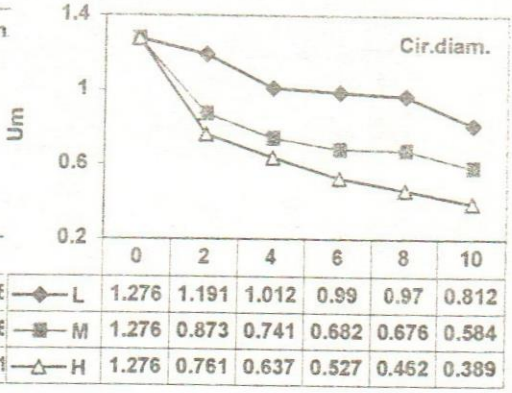
	0	2	4	6	8	10
L	1.315	1.211	1.183	1.131	1.102	1.068
M	1.315	1.057	0.977	0.974	0.937	0.917
H	1.315	0.923	0.887	0.817	0.772	0.728



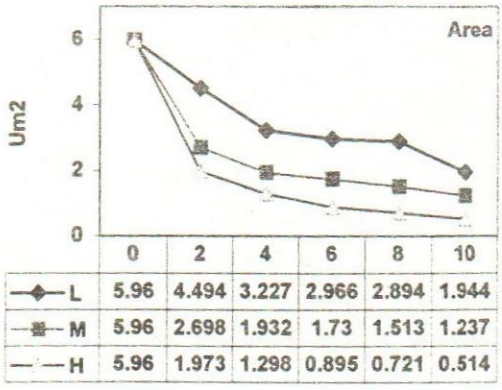
	0	2	4	6	8	10
L	1.53	1.48	1.43	1.37	1.3	1.24
M	1.53	1.43	1.35	1.28	1.24	1.21
H	1.53	1.1	1.05	1	0.96	0.95



	0	2	4	6	8	10
L	1.476	1.389	1.297	1.226	1.093	0.988
M	1.476	1.153	1.025	0.922	0.817	0.776
H	1.476	0.846	0.784	0.668	0.581	0.501

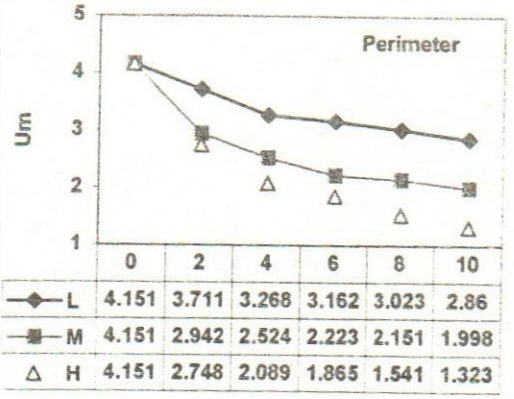


	0	2	4	6	8	10
L	1.276	1.191	1.012	0.99	0.97	0.812
M	1.276	0.873	0.741	0.682	0.676	0.584
H	1.276	0.761	0.637	0.527	0.462	0.389



	0	2	4	6	8	10
L	5.96	4.494	3.227	2.966	2.894	1.944
M	5.96	2.698	1.932	1.73	1.513	1.237
H	5.96	1.973	1.298	0.895	0.721	0.514

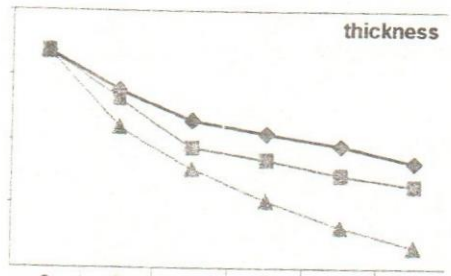
Exposure period (sec.)



	0	2	4	6	8	10
L	4.151	3.711	3.268	3.162	3.023	2.86
M	4.151	2.942	2.524	2.223	2.151	1.998
H	4.151	2.748	2.089	1.865	1.541	1.323

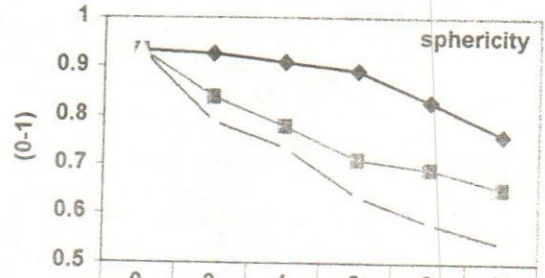
Exposure Period (sec.)

Fig. (3):. The change in rheological parameters of Staph.aureus in nutrient broth heated in microwave at low (L) , medium (M) and high (H) powers for various exposure periods.



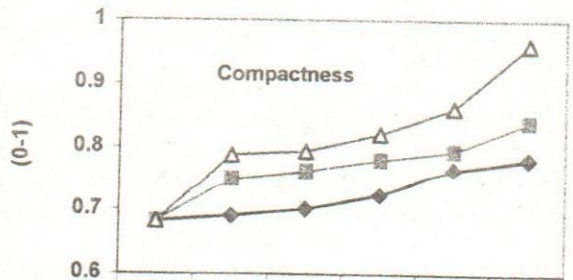
0	2	4	6	8	10
0.171	0.153	0.139	0.133	0.127	0.119
0.171	0.149	0.126	0.1204	0.113	0.108
0.171	0.136	0.116	0.101	0.089	0.08

Exposure period (sec.)



	0	2	4	6	8	10
L	0.933	0.926	0.91	0.892	0.83	0.76
M	0.933	0.84	0.78	0.71	0.69	0.65
H	0.933	0.79	0.736	0.635	0.58	0.54

Exposure period (sec.)



	0	2	4	6	8	10
L	0.682	0.691	0.702	0.724	0.766	0.783
M	0.682	0.75	0.763	0.781	0.795	0.841
H	0.682	0.788	0.794	0.822	0.863	0.962

Exposure period (sec.)

Fig. (4): The change in rheological parameters of *Staph.aureus* in nutrient broth heated in microwave at low (L) , medium (M) and high (H) powers for various exposure periods.

In conclusion, as for the pathogen *S. aureus*, microwave treatment could be a good method for rendering milk pathogen free and at a such short exposure time that does not adversely affecting the milk quality. However, these result was drown by exposing milk in a glass tube, but as mentioned earlier , any change in the extrinsic and intrinsic characteristics of exposed sample, the new effect should be determined experimentally.

Table (4):The change in rheological parameters of *Staph. aureus* in buffalo's milk caused by microwave heating at high levelw power settings

Property	Exposure periods (sec.)						Change, (%)				
	0	2	4	6	8	10	2	4	6	8	10
Thickness (um)	0.171	0.139	0.121	0.108	0.095	0.086	-18.7	29.23	36.84	44.44	49.7
Width (um)	1.315	0.989	0.901	0.893	0.785	0.739	-24.8	31.49	32.1	40.32	43.81
Length (um	1.53	1.165	1.114	1.021	0.986	0.973	-23.9	27.2	33.28	35.57	36.42
Sphericity (0-1)	0.933	0.812	0.751	0.677	0.624	0.606	-13	19.54	27.46	33.14	35.18
Area (um2)	5.96	2.191	1.759	1.342	0.985	0.676	-63.2	70.49	77.48	83.47	88.66
Perimeter (um)	4.151	2.799	2.263	2.112	1.814	1.529	-32.6	45.48	49.12	56.3	63.17
Compactness (0-1)	0.682	0.772	0.784	0.805	0.842	0.923	13.2	14.96	18.04	23.46	35.39
Sph. diam (um)	1.476	0.976	0.847	0.813	0.623	0.568	-33.9	42.61	44.91	57.78	60.29
Cir. Diam. (um	1.276	0.835	0.705	0.618	0.581	0.426	-34.5	44.73	51.55	54.45	66.6

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تأثير الأشعة الميكروفية على الحيوية والخواص الريولوجية لبكتريا *Staphylococcus aureus* في البيئة السائلة وفي اللبن الجاموسي

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** كلية الزراعة – جامعة القاهرة – فرع الفيوم – قسم الميكروبيولوجيا.

يهدف البحث إلى دراسة تأثير الأشعة الميكروفية بمستويات مختلفة من القوة ولفترات تعرض مختلفة على حيوية ومعدل قتل الميكروب *Staphylococcus aureus* ATCC 25923 وكذلك تقييم مدى تأثيرها على التغير والتشوه الذي تحدثه في خواص الميكروب المورفولوجية والريولوجية.

وقد أجرى البحث بتلقيح الميكروب *Staphylococcus aureus* ATCC 25923 في البيئة السائلة بمعدل ١٠×١٦٨^{٤} خلية/مل، وكذلك في اللبن الجاموسي بمعدل ١٠×٨٥^{٤} خلية/مل، ثم وضعت البيئات الملقحة بالميكروب في أنابيب زجاجية، ثم عرضت أنابيب البيئة السائلة للأشعة الميكروفية بثلاث مستويات من القوى هي: منخفضة (٩٠ وات)، متوسطة (٤٥٠ وات)، عالية (٩٠٠ وات) وذلك لفترات: ٢، ٤، ٦، ٨، ١٠ ثوان. بينما عرضت أنابيب اللبن الجاموسي للأشعة الميكروفية عالية القوة (٩٠٠ وات) لنفس الفترات السابقة.

وقد أشارت النتائج إلى أن الأشعة الميكروفية نجحت في إبادة الميكروب *S. aureus* تماماً عند تعرضه للأشعة ذات المستوى العالي (٩٠٠ وات) لفترة قدرها ٨ ثوان سواء في البيئة السائلة أو اللبن الجاموسي حيث بلغت حرارة البيئة السائلة ٧٥°م وحرارة اللبن ٨٦°م. وقد اتضح التأثير غير الحراري للأشعة الميكروفية على إبادة الميكروب حيث تم إبادة ٨٠% من أعداد الميكروب الحية على حرارة ٤٠°م وهي أقل من الدرجة المميتة للميكروب. وقد ظهر بوضوح تأثير الأشعة الميكروفية أيضاً على الخواص المورفولوجية والريولوجية للميكروب فقد تغيرت جميع القيم لهذه الخواص بدرجة معنوية. وبزيادة قوة الأشعة أو إطالة مدة تعرض الخلايا الميكروبية لها أو كلاهما معاً زادت التغيرات الحادثة في الخواص الريولوجية للميكروب.