PRELIMINARY RESEARCHES REGARDING THE MICROWAVES INFLUENCE ON THE MILK MICROFLORA

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Abstract. The purpose of this study was to investigate the influence of the microwaves action against the microflora existing normally in the raw milk. A number of 6 milk samples were exposed to the microwaves treatment for different periods of time (15 s, 30 s, 45 s, 60 s and 120 s) and analyzed using the Horizontal method for the enumeration of microorganisms – Colony count technique at 30°C in order to determine their T.N.G (total number of germs). The results showed a significant reduction of the colonies number as the microwave time exposure increased.

Keywords: microflora, T.N.G., microwaves, milk.

INTRODUCTION

Although the microwave oven is widely used as a means of food preparation, insufficient information is available on the consequences of microwave heating on the composition and the quality of the food. Food heating by microwaves results from the conversion of microwave energy into heat by friction of dipole molecules (water) vibrating due to rapid fluctuation in the electromagnetic filed [15]. Factors that influence microwave heating include dielectric properties, volume and shape of the material, as well as design and geometric parameters of the microwave unit [23].

The use of microwaves to reduce the population of microorganisms in many different foods such as turkeys, meats, milk, corn on the cob, chickens, frozen foods and potatoes have indicated that radiation with microwaves help preserve food, reducing the presence of active microbiological cells [1, 3, 5, 6, 8, 13, 20].

International and European Union Norms stipulate that raw milk should have a number of 100000 germs/ml [16]. For dairymen, raw milk bacteria counts represent not only an important factor that influences the processing stages but also an economic concern since in many cases the quantity of bacteria allowed in raw milk is directly related to bonus payments [17]. The potential advantages of the microwave treatment have been reported for milk pasteurization, without adverse effects on flavor during cold storage, for preparation of samples for atomic absorption analysis and for acceleration of the rate-limiting step of amino acid analysis [10].

Taking into account that there are relatively few studies that describe the effects of microwaves on the milk microflora, the aim of this study was to investigate if the exposure of the milk samples to the microwaves action for reduced time intervals, during which the samples do not reach the boiling point, can generate the decreasing of the microorganisms' population. If this microwave treatment is effective against microorganisms and considering that the global composition of microwaved milk is similar to that of conventionally heated milk as the literature shows [4], we can suggest that microwave treatment of milk can be an alternative for the classic pasteurization or sterilization processes.

MATERIALS AND METHODS

In order to determine the antimicrobial action of the microwaves against the microorganisms existing in raw milk, 6 samples of 70 ml/sample of milk were exposed to the microwaves action of a Hansa AMM 21 E80GH microwave oven at electrical power of 800 W during different periods of time: *t 0* – milk sample not treated with microwaves, *t 15* – milk sample treated for 15 seconds with microwaves, *t 30* – milk sample treated for 30 seconds with microwaves, *t 45* – milk sample treated for 45 seconds with microwaves, *t 60* – milk sample treated for 60 seconds with microwaves and *t 120* – milk sample treated for 120 seconds. The temperatures achieved by the samples after the microwave exposure are presented in Table 1.

Table 1. Samples temperature variation during the microwave exposure periods

Time (seconds)	0	15	30	45	60	120
Temperature (°C)	21	40,2	64,7	83,2	85,6	90

The antimicrobial action was investigated using the Horizontal method for the enumeration of microorganisms – Colony count technique at 30°C, according to the ISO 4833: 2003 Standard. From each sample treated at microwaves there were made 4 successive decimal dilutions: 10^{-1}, 10^{-2}, $10^{-3}$and 10^{-4}. 1 ml of inoculum of each dilution was poured in Petri dishes, mixed with 15 ml plate count agar medium and allowed to solidify. The Petri dishes were inverted and incubated at 30°C for 72 hours.

The number of colonies/ml, usually referred to as T.N.G., developed after the incubation time was determined using the colony-counting equipment and by applying the formula:

$$N = \frac{C}{v(n_1 + 0{,}1n_2)d}$$

Where:
C – the sum of the colonies counted in every Petri dish;
n_1 – number of Petri dishes taken into account for the first dilution;
n_2 – number of Petri dishes taken into account for the second dilution;
d – the dilution considered for the first counting;
v – the inoculum's volume poured in each Petri dish, ml.

RESULTS

The values obtained for the number of colonies/ml, N, after the colonies counting are presented in Table 2.

Table 2. The T.N.G. values obtained for the samples exposed to the microwaves action for different periods of time.

Microwaves time exposure of the sample, s	0	15	30	45	60	120
T.N.G. values, number of colonies/ml	$43x10^6$	$41x10^6$	$25x10^4$	$42x10^2$	52,7x10	16,36x10

The images of the Petri dishes containing the samples after the incubation time are presented in the Figures 1-6.

As one can observe in Figure 1, corresponding to the sample that was not treated with the microwaves, the number of the microorganisms units forming colonies is very high. In the image there can be seen the variety of the milk microflora, but the high total number of germs developing colonies is determined using the colony-counting equipment, which allows us to count also the pinpoint colonies. This device consists of an illuminated base with a dark background, fitted with magnifying lens and mechanical counter. The microorganisms developed in the milk samples are represented by bacteria, yeasts and moulds.

The bacteria developed in milk can be of technological use (lactic bacteria and propionic bacteria), unpathogenic contaminating bacteria (coliform bacteria) or pathogenic bacteria (*Mycobacterium tuberculosis, Brucella, Staphylococcus aureus*). They are prokaryote monocellular microorganisms and they present very diverse cellular forms: spherical, cylindrical, helicoidally or phylamentous [2].

The yeasts of the milk microflora are eukaryotic monocellular microorganisms and have also a great variety of shapes: oval, spherical, appiculate or cylindrical. They are saprophytes and they action against the milk proteins, for example the gens: *Torulla* and *Saccharomyces* [2].

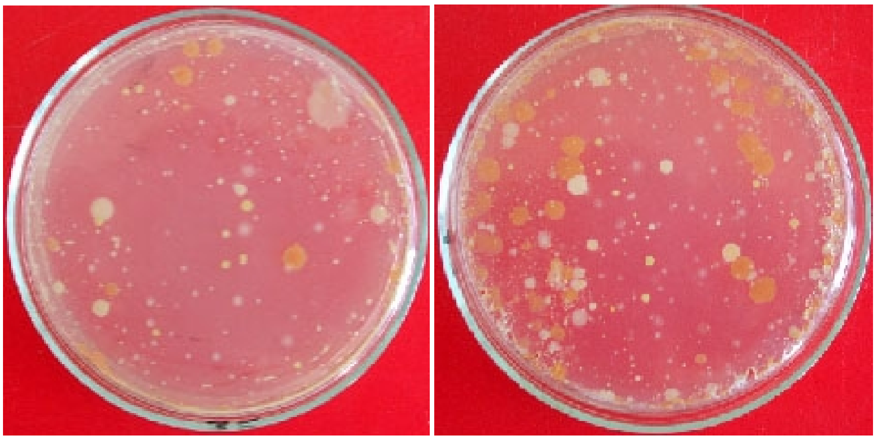
A.  B.

Figure 1. The T.N.G. of the sample 1 (0 s), dilution 10^{-3} (**A**); dilution 10^{-4} (**B**).

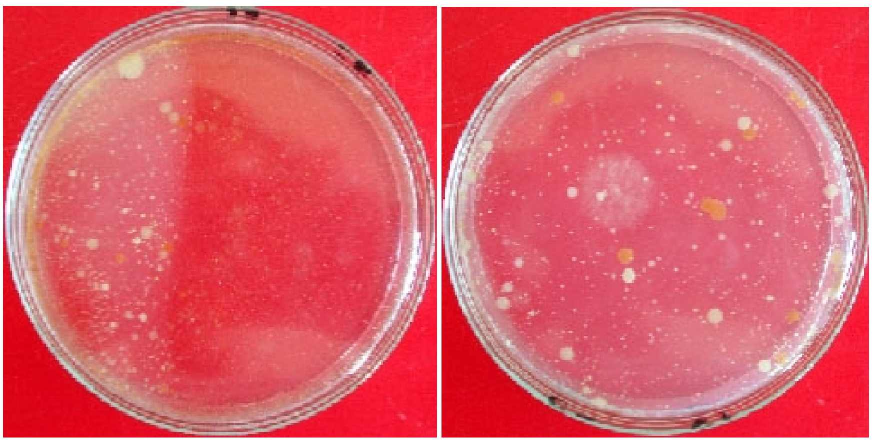
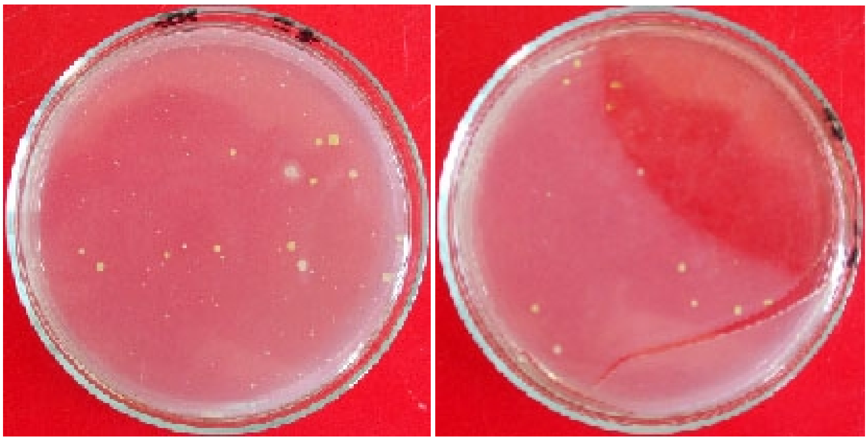
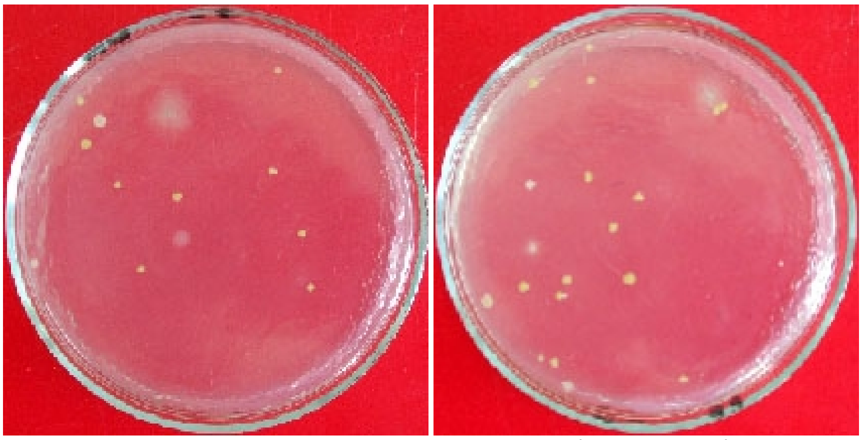
A.  B.

Figure 2. The T.N.G. of the sample 2 (15 s), dilution 10^{-2} (**A**); dilution 10^{-3}(**B**).



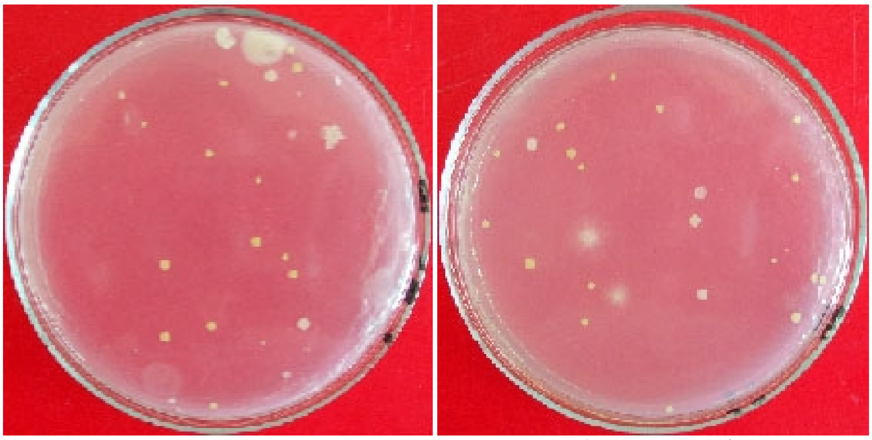
A. B.

Figure 3. The T.N.G. of the sample 3 (30 s), dilution 10^{-2} (**A**); dilution 10^{-3}(**B**).



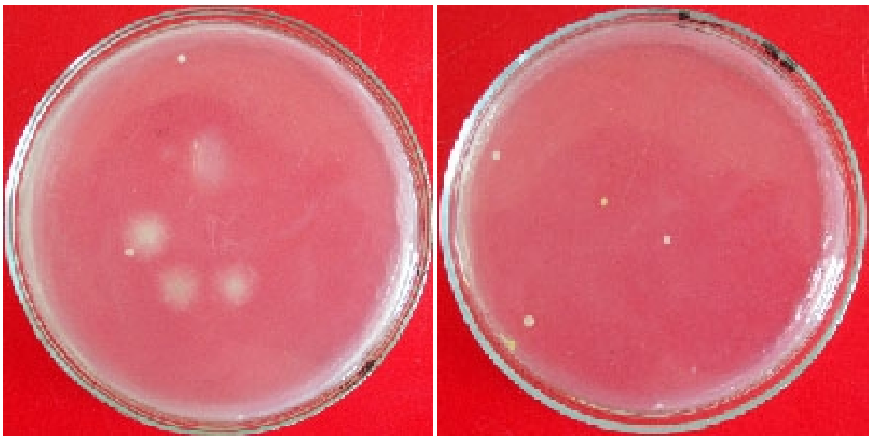
A. B.

Figure 4. The T.N.G. of the sample 4 (45 s), dilution 10^{-2} (**A**); dilution10^{-3} (**B**).



A. B.

Figure 5. The T.N.G. of the sample 5 (60 s), dilution 10^{-1} (**A**); dilution10^{-2} (**B**).



A. B.

Figure 6. The T.N.G. of the sample 6 (120 s), dilution 10^{-1} (**A**); dilution 10^{-2} (**B**).

The moulds are also eukaryotic monocellular or pluricellular microorganisms, saprophytes which action also against the milk proteins, for example the gens: *Mucor, Aspergillus, Penicillium* or *Oidium* [2].

Comparing the all 6 Figures, there can be seen the gradually decreasing of the number of colonies of the Petri dishes corresponding with the increasing of the microwave time exposure. This fact was also indicated by the number of colonies counted for every sample using the colony-counting equipment.

DISCUSSIONS

After exposing the milk samples to the microwaves action and determining the number of colonies/ml with the Horizontal method for the enumeration of microorganisms – Colony count technique at 30°C, one can observe a significant reduction of the colonies number as the microwave time exposure is increased though the samples do not reach the boiling point. The variation of the colonies number/ml depending on the time exposure is presented in Figure 7.

The absorption of microwave energy can increase the temperature of foods rapidly, deactivating microorganisms and performing pasteurization or sterilization. Studies have shown that the thermal effect is essential in destroying microorganisms [21].

The effect of microwaves on microorganisms present in foods are influenced by the intrinsic characteristics of the products being processed (pH, humidity, Ox reduction potential, antibodies present, biological structures, chemical composition, amount and geometry of the food) and extrinsic factors (temperature, humidity, ambient gases, frequency and intensity of the radiation, time of exposure, position of the foods in relation to the effective radiation field, among others). Also important are: the chemical and physical composition of the microorganisms being irradiated, their stage of development (vegetative cell, spore or development phase, wet or dry, etc.) and their initial amount. Bacteria respond differently to inactivation by microwaves [21]. There was also suggested that microwaves may catalyze certain oxidative reactions, possibly in membrane lipids, the by-products of which adversely affect the cells during sublethal heating [11].

The mechanism of destruction of microorganisms through microwaves is controversial. Some have stated that inactivation of microorganisms by microwave is entirely by heat, through the same mechanisms as other biophysical processes induced by heat, such as denaturation of proteins, nucleic acids or other vital components, as well as disruption of membranes [7, 9].

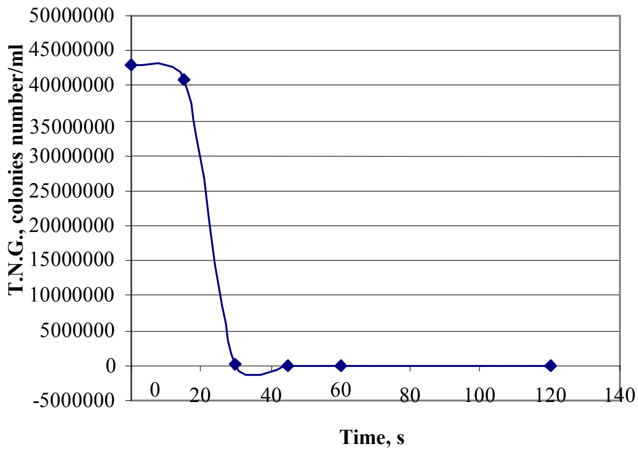


Figure 7. The T.N.G. variation during the different periods of microwaves exposure.

Others have linked destruction to non-thermal effects, as a lower final temperature may be needed to kill microorganisms. Four predominant theories have been used to explain non-thermal inactivation by microwaves or "cold pasteurization": selective heating, electroporation, cell membrane rupture, and magnetic field coupling. The selective heating theory states that solid microorganisms are heated more effectively by microwaves than the surrounding medium and are thus killed more readily. Electroporation is caused when pores form in the membrane of the microorganisms due to electrical potential across the membrane, resulting in leakage. Cell membrane rupture is related in that the voltage drop across the membrane causes it to rupture. In the fourth theory, cell lyses occurs due to coupling of electromagnetic energy with critical molecules within the cells, disrupting internal components of the cell [12].

Also, the intermediates of the Maillard reactions that occur during the microwave exposure are shown to be acting directly as mutagens of some bacteria [14]. In contrast, another experiment suggested that even if the microorganisms that experience stress at higher temperature are more likely to respond by increasing the mutation rate, there was no mutagenic effect of the absorbed high power microwave radiation of the different systems tested [19].

Although there is a controversy about the mechanisms of microwave-induced death of microorganisms, there is no doubt about the destructive effect of microwaves. Microwave destruction of many microorganisms has also been reported, including: *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, E. coli, Enterococcus, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteridis, Salmonella sofia, Proteus mirabilis* and

Pseudomonas aeruginosa, Aspergillus niger, Penicillium and *Rhizopus nigricans* [22]. No pathogen has been reported to be microwave resistant [7].

In general, food is characterized by safety quality, which implies all contaminants absence, physical, chemical and especially microbiological [18]. In conclusion, the microwave treatment of milk can be an important alternative of the pasteurization or sterilization processes due to its destructive effect on the existing microorganisms.

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