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IMPACT ASSESSMENT OF MICROWAVE TREATMENT OF RAW COW'S MILK ON ITS MICROBIOLOGICAL PROPERTIES

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ABSTRACT

The aim of our research was to examine the impact of microwave radiation on the microbiological parameters of raw cow's milk. In the measurements, our samples (raw cow's milk) were treated at different power levels [100, 200, 300, 400, 496 (~500), and 600 W], and the effects of microwave irradiation were assessed regarding total plate count and yeast cell count. Treatment temperature was maximized in all cases (40 °C) in order to eliminate the thermal effect generated by microwaves, and hence, to justify the possible microbial inhibitor or destruction impact of the non-thermal effect of radiation. Based on the results, microwave treatment had an impact on both the total plate count and the yeast cell count as well. Treatments were performed to justify the non-thermal effect of the treatments, and significant results were obtained ($p \leq 0.05$).

Keywords: cow's milk, microwave radiation, microbial destruction impact

1. INTRODUCTION

Microwave equipment are widely used today in households, in health care and in the agri-food industry as well where they are used for drying, baking, sterilizing, pasteurizing, defrosting and tempering purposes as well. For this reason, other possible uses of microwaves have been widely investigated, the importance of which is based on the fact that in traditional heat treatment procedures, food is heated up from the outside towards the inside whereas the process takes place in the exact opposite way in case of microwave treatment. The food heating property of microwaves as well as the changes that this implies is well known, however much less information is available on their actual impact on content quality and microbiological properties of food, not being associated with the temperature increase.

Microwaves are electromagnetic waves with a wavelength (λ) ranging from 1 m to 1 mm, hence, they are similar to light waves that are visible to the naked eye or to X-rays emitted by X-ray equipment, and even if they are only different with regards to their wavelength, frequency and energy, there are still huge deviations between each range [1]. The frequency of microwaves varies between 0.3 and 300 GHz, and they are classified under the so-called non-ionizing radiations which means that they do not have sufficient energy to create ions. These waves are used in various sectors, and radar technology can be probably considered the most important of them because it is thanks to this field that the heating property of microwaves has been revealed as well. They also play an important role in telecommunications, given that they penetrate much more efficiently through the atmosphere of the Earth as compared to other wavelengths. In telecommunication devices, microwaves are also used by Bluetooth and WLAN, and the 2.45 GHz frequency is used in general in household appliances for heating food [2].

Researches on the application and possible effects of microwaves have been carried out in several fields already, such as in sample preparation, wastewater treatment, increasing enzymatic activity, impact on fermentation and milk fat determination methods. It has been shown for example that using microwaves, certain processes may be shortened such as the derivatization of fatty acids in milk samples for HPLC-based analysis for example [3]. In the research conducted on the removal of ammonia nitrogen from biological wastewaters it has been shown that as compared to conventional heat transmission, thermal effects were complemented by non-thermal effects in the samples heated with microwave (700 W, 2450 MHz) to achieve a higher removal efficiency [4]. Another research conducted with microwave

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treatment of agri-food industry wastewaters has shown that the Fenton-reaction combined with microwave can reduce the biological and chemical oxygen demand [5]. Beszédes et al. [6] investigated the degradability of dairy by-products by microwave treatment. Lakatos et al. [7] worked out a method in their experiments that allowed to determine the fat content of various milk samples with sufficient accuracy using microwave treatment. In subsequent researches the influence of microwave radiation on lipase and xanthine oxidase enzyme activity was analyzed in consumer milks due to which an increase of enzymatic activity was found in the milk samples [8]. Certain researches show that a higher increase in glucose content was achieved for microwave treated vine-branch samples treated with cellulose enzyme, hence justifying the increase of enzymatic activity [9].

Besides all the above, the analysis of its impact on microorganisms also constitutes the subject of several researches. In case of analyzing the impact on microbes the phenomenon may be approached from several perspectives. It may be examined based on the frequency applied, on the power applied, on the final temperature of the irradiated sample or from the perspective of the irradiation time. When these types of experiments are conducted, it is important to differentiate thermal effects from non-thermal ones.

In the experiment of Roohi and Hashemi [10] *Enterococcus faecalis, Bacillus cereus, Staphylococcus aureus* and *Shigella flexneri* hosted on carrot slices were analyzed using radiation at various power levels. Power values were selected as 200, 400 and 600 W. It has been stated that a higher power level results in a higher microbial destruction impact even without a significant temperature effect. In other researches, the analysis of microwave irradiation of *Salmonella enteritidis* in potato omelet at various power levels has shown that this microorganism is resistant to high-power microwaves in such a medium. Inactivation of the microbe population was detected even in case of radiation at 600 or 800 W only after a significant rise in temperature (60 °C) [11]. The analysis of *Escherichia coli* O157:H7 performed in salsa has shown that in case of irradiation at 915 MHz and 4.8 kW, population started to decline only due to a temperature influence and not as a result of irradiation [12]. Nevertheless, the period of treatment in the above experiments has been defined as a short time range of only several minutes, and longer exposure might lead to a different outcome.

Kapcsándi [13] proved that low-power (50 W) microwave treatment makes a positive impact on the life activity of *Saccharomyces cerevisiae* (30 °C), therefore the influence on microbes strongly depends on the power level applied. This effect has been proven by the increase of the glucose utilization capacity of yeast during fermentation processes. By the exposure of *Escherichia coli* and Bacillus *subtilis* to the same microwave radiation it has been shown that even though the cells had been inactivated, their lysis had not taken place. [14].

From a microbiological perspective, milk can be considered to be sterile, it is contaminated when milking is performed. The microbiological status of raw milk depends on various factors such as the lactation period or the cow breed [15].

2. MATERIALS AND METHODS

2.1. Applied materials and tools

In order to analyze the impact of microwaves on microorganisms raw milk has been used as raw material, procured from a raw milk vending machine (in Mosonmagyaróvár). The bottles used for transporting the milk had been sterilized by autoclave (121 °C, 20 min) to exclude contamination. Our experiments were conducted at the Department of Food Science using a MARS5 (Microwave Accelerated Reaction System, CEM Corporation, USA) microwave equipment. MARS5 is a sample preparation equipment operated with microwave, applying a frequency of 2455 MHz that corresponds to the frequency used in household microwave ovens. The power configuration settings allowed for the treatment of the samples at an output power of 400, 800 and 1600 W, with a possible modulation of the standard power levels by further increments of 1%.

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2.2. The method of microwave treatment

Before starting the measurements, the sample processing vessels provided for the microwave equipment were sterilized with hot air (103 °C) in a THELCO 70M drying oven for laboratory use. These processing vessels (HP-500 Plus) are resistant up to a pressure of nearly 2.5 MPa and a temperature of 210 °C.
During the microwave treatments, several power levels (100, 200, 300, 400, 500 and 600 W) have been defined for analyzing non-thermal efficiency. The lowest applied power level was 100 W that was increased by increments of 100 W in case of different samples. The highest power level applied for the treatment was 600 W. Due to the justification of the non-thermal effects of microwave radiation, maximum temperature of the samples was set to 40 °C, using a treatment without temperature holding. Detection of the sample temperature was performed using an optic fiber sensor (RTP-300 Plus) that can be placed in a microwave environment. At all power levels examined, 3 x 75 mL of samples were placed in the microwave treatment equipment, meaning that we were working with three repetitions and three parallel measurements.

2.3. Microbiological analysis

Pour plate method was used for the development of microorganisms. From the perspective of food safety and technological hygiene, the critical limit for total plate count is at 105/cm3 in raw milk because in order to obtain sufficient efficiency, pasteurizing processes may be used in case of such microbial counts.
Sample preparation was performed based on the MSZ EN ISO 6887-1:2017 [16] standard. Milk samples had been stored in refrigerator (on 4 °C) until testing, and homogenized by shaking before the decimal dilutions were done. To prepare the decimal dilution line, 9 ml of the peptone water was put in test tubes, which were then sterilized in pressure cooker for 30 minutes at about 120 °C. Peptone water contained 8.5 g of sodium chloride (VWR International Ltd., Hungary) and 1.0 g of peptone (Merck Kft., Hungary), dissolved in distilled water, then sterilized.
Based on the MSZ EN ISO 4833-1 [17] standard, determination of the total microbial count was performed on a PC (Plate Count, Biolab) agar, with an incubation time of 72 hours, at a temperature of 30±1 °C. Determination of the yeast and mold count has been performed on the selective agar defined in the MSZ ISO 7954:1999 [18] standard, incubated at 25 °C for 48 hours. As defined in the standard, YGC agar (Yeast Extract Glucose Chloramphenicol Agar, Biolab) has been used for their indication.
Our measurement results have been plotted using Microsoft Office Excel 2016®. During the interpretation of microbiological results, the microbial count has been plotted in a logarithmized format, with the gradient values of the straight lines fitted to specific points characterizing the exponential propagation phase of microorganisms.

3. RESULTS AND DISCUSSION

3.1. Heating trends in the samples

During microwave treatments, a temperature profile has been defined for the treatment at each power level in order to (*Fig.1*) demonstrate the decrease of the treatment time occurring due to the increase of power. It can be seen that there is a linear connection between the two values, and the value of the correlation coefficient is 0.98, therefore there is a strong relationship between the applied power and the speed of heating at the power levels examined.

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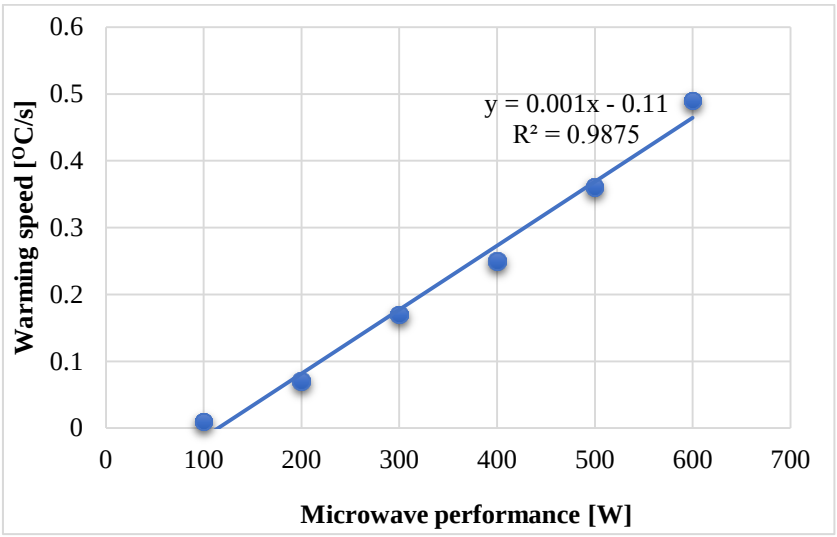

Figure 1. Heating speed of samples against power

3.2. Impact of the treatments on total plate count

Fig.2 shows that during the microwave treatment of raw milk, by increasing the microwave power, the total plate count decreases in milk. These results indicate that already in case of a treatment at 100 W, the cell count of 8.54 log CFU/cm^3 decreased to 6.24 log CFU/cm^3. This clearly represents a significant deviation ($p \leq 0.05$). Comparing the samples, it can also be seen that except for the treatments at 200 and 300 W, by comparing the results of other samples, significant differences can be found in all cases when the results of the culture are interpreted.

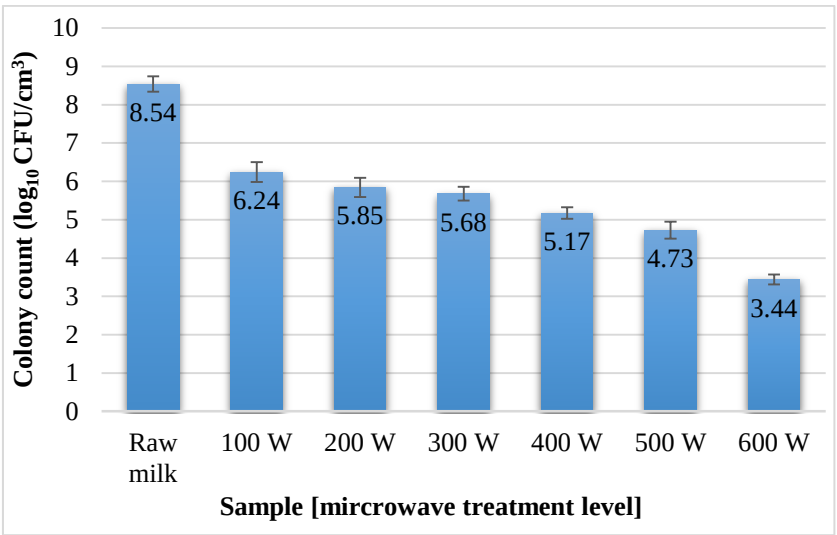

Figure 2. Total plate count results of raw milk and microwave treated

Tremonte et al. [19] conducted similar experiments. Samples were also procured from milk vending machines, then treated with microwave at 750 and 900 W and a treatment time of 75 s, using a household

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microwave oven. The results of their total plate count measurements in raw milk were similar to those of our experiments. In their experiment, a 750 W treatment resulted in a destruction of 1 magnitude regarding total plate count, whereas the 950 W treatment gave an almost sterile result. In our case, the 600 W treatment resulted already in a destruction of 5 magnitudes, with a treatment time of 47 s.
Jaynes [20] performed research on pasteurization of raw milk in a microwave system. A countercurrent heat-exchanger has been used in the equipment for heating up the inflowing milk and for cooling back the outflowing milk. During the treatment, the pasteurization temperature was tried to be kept at 72 °C. The experiments were performed with various flow rates, in case of which temperature differences were corrected by modulating the power. In addition to the microwave treatment, control samples have been treated in a 62.8 °C water bath that produced a similar result with regards to microbial destruction. The most important microbial destruction was stated in case of the treatment with a flow rate of 300 mL/min and a power level of 735 W.

3.3. The impact of the treatments on yeast count

In addition to the determination of yeast count, cultivation on a YGC agar is suitable for determining the mold count as well. However mold was not present in such volumes in our samples that would allow for drawing numerical data and conclusions, therefore in our analysis, the emphasis was put on the results obtained for yeast counts.
Fig.3 indicates that microwave radiation of various doses has an impact on the presence of yeast as well. Although, no significant difference can be shown between the samples of 200-300-400 W ($p \leq 0.05$), a clear destruction can be identified as compared to the results of raw milk, in case of which a decrease of yeast cell count of 1 magnitude can be observed from an initial cell count of 5.56 log CFU/cm^3 to cell counts of 4.28-4.23-4.15 log CFU/cm^3. In case of a power level of 400 W, the results obtained are even more striking, during which the cell count of yeast decreased to 2.06 CFU/cm^3, and, in case of even higher power levels (500 and 600W), to 0.65 and 0.18 log CFU/cm^3 as compared to the initial value. This severe destruction means a destruction rate of 89.4% in case of 500 W and 96.8% in case of 600 W.

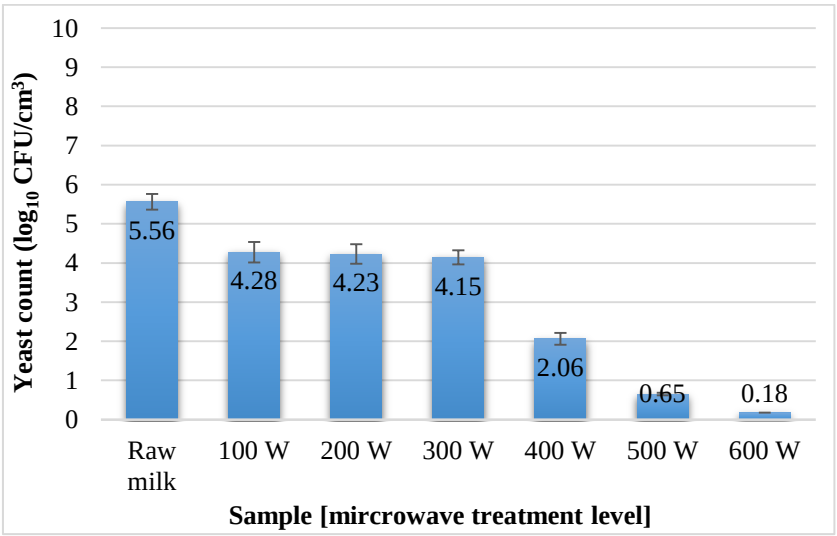

Figure 3. Yeast cell count results of raw milk and microwave treated samples

The data indicate that microwave irradiation has a smaller impact on yeast at lower power levels, however, a sharp increase can be identified when a power level of 600 W is applied, reaching a rate of 96%.

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This corresponds to the experiments of Kapcsándi [13] where *Saccharomyces cerevisiae* suspensions were examined at similar power levels and treatment times. In their case, the survival rate of yeast presented a higher value, but the extent of destruction followed the same trend. With regards to deviations in survival rates it should be noted however that in their case one type of yeast was examined, the size of the sample treated at once was more than twice as large, and the medium containing the microbes was also different, hence, the much higher destruction rates might have been caused by these factors.

Ali et al. [21] conducted experiments to examine the impact of 900 W microwave treatment on microbes, namely yeast amongst others in raw buffalo milk, using various treatment times and hence temperatures. In their case, the initial 5.23 log CFU/cm^3 value decreased to 3.6 log CFU/cm^3 using a treatment time of 30 s. However, based on our results, the initial 6.61 log CFU/cm^3 value decreased to 4.18 log CFU/cm^3 using a treatment time of 47 s with the highest power level of 600 W. In our case, the more severe microbial destruction can be justified by the fact that the liquid column of our samples was much narrower, and this way, microwaves penetrated even into the middle of the sample with less damping as compared to the above mentioned experiment. Hence, it can be concluded that microwave treatments are most efficient in narrow processing vessels because a better penetration into the material can significantly increase the destruction impact of microwaves.

Although, several papers have been published already on the non-thermal microbe destruction impact of microwaves, the microwave pasteurization processes currently used in the dairy industry are still only based on the thermal impact. Our experiments have shown that an increased power can significantly hold back microbiological life activities already at a lower temperature of 40 °C, even in case of using very short treatment times. It should be noted that in our case, samples have been treated in predefined portions with a small sample size, and in order to be able to apply this microbial inhibitor property to larger extents, the size of the treatable sample should be increased.

The simplest solution for increasing the sample size is to use a continuous flow treatment equipment in which the materials to be treated flow through a coil in the microwave treatment zone. Such an equipment would offer a large variety of control options in order to reach the most suitable result with regards to the microbe inhibitor effect. Given that in this case, non-thermal effects of microwave are desired to be used, power should be kept static, but in this case, the final temperature of the material would depend on the initial temperature, and parameters could only be controlled by modulating the flow rate or by modifying the dimensions of the treatment equipment. Nevertheless, if power control was possible, by building such an equipment, it would be possible to define more precisely the power level where the method could be applied in the most cost-efficient manner.

The results indicate that the microbial destruction impact is more present with regards to the decrease in the yeast cell count as compared to the total plate count. Taking this into account, the destruction properties of other microorganisms that can be found in milk may be subject to further analysis.

4. CONCLUSIONS

Heat treatment of raw milk is one of the key technological steps in the agri-food industry, and especially at milk processing plants. Heat treatment of the raw material, and hence, guaranteeing its microbiological safety are in the interest of both the producers and the consumers as well. Besides the conventional heat treatment process, microwave treatment has been present in various sectors of the agri-food industry. This type of process could be introduced as a new technology in the dairy industry, using which the non-thermal effect produced by the microwaves could be applied during the manufacturing of the products, enjoying the benefits of low-impact raw material handling at a lower temperature, and reducing the deterioration of content quality parameters.

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