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Investigation of the shelf life of fruit yogurts as a function of the treatment of flavoring substances

Keywords: yogurt making, microwave irradiation, fruit drying, microbiological parameters, total viable count, yeast count, mold count, *Escherichia coli*, coliform

1. SUMMARY

Milk and dairy products represent one of the foundations of the human diet because of their valuable ingredients and pleasant sensory properties. The aim of our research was to investigate how different heat treatment processes (microwave irradiation, drying) affect the shelf life of dairy products (yogurt) from a microbiological point of view. In the course of our measurements, the effects of the different heat treatment parameters of the flavoring substances used in the production of the products (apples, bananas) on the microbiological properties of the products and, thus, on their shelf life were investigated. In our experiments, conventional drying (55 °C, 24 hours) and microwave irradiation technology (800 W, 55 °C, 10 min) were used as treatment forms of the additives. Comparisons were made in terms of microbiological parameters (total viable count, yeast/mold count and *E. coli*/coliform count). Based on our results, we believe that the drying process can ensure microbiological safety in food production if the air circulating in the equipment has adequate hygienic properties. The microwave irradiation technology can be used successfully to inhibit microbes in foods, in this case fruits. However, the same treatment parameters cannot be applied to different fruits.

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2. Introduction and literature review

Milk has been a mainstay in the human diet since the beginning of human history. Its useful ingredients have a beneficial effect on a person's healthy physical and mental development. The ingredients of milk are physiologically beneficial, one of the outstanding features being its high calcium content, therefore it plays a role in the bone formation of developing organisms **[1]**, and it also contains proteins that are important and easy to use for the body. Due to all these properties, dairy products can be considered as staple foods in the human diet. In the food industry, the milk of many farm animals (sheep, goats, cattle) is processed, but in Hungary cow's milk is consumed in the largest amount.

Yogurt is a dairy product consumed all over the world. Nutrition science professionals believe that this sour milk product has a high nutritional value (a significant part of its lactose content is broken down during fermentation and it has a significant concentration of Ca^{++}) and beneficial bioactive effects (prebiotic ingredients and probiotic bacteria). Natural yogurt is made by adding lactic acid bacteria that induce lactic acid fermentation in the culture medium during their basic physiological activities. Of all products manufactured from milk, yogurt is the most popular worldwide **[2]**.

In the case of fruit yogurt, when dried fruit or dried pieces are added to the yogurt, the dried products tend to absorb some of the free water in the yogurt gel, thus helping to separate the whey of the product during storage **[3]**. It is also an advantage of adding fruit that, according to some studies **[4]**, the addition of 10 v/v% of fruit significantly improves the physico-chemical properties of the product. The interior of healthy plant tissues does not contain microorganisms, so the primary microbiota of plant raw materials comes mainly from the soil, water, air and, occasionally, from insects or animals. Plant parts developing in the soil (tubers, roots) and in the vicinity of the soil are usually heavily contaminated, their microflora composition is practically identical to that of the soil. Microorganisms are present on fruit surfaces in the amount of roughly 10^3 to 10^5 CFU/g, a significant part of which are lactic acid and acetic acid bacteria. However, the largest part of the microbiota is made up of yeasts, the most common of which are *Hensaniaspora*, *Torulaspora*, *Pichia*, *Saccharomyces*, *Candida* and *Rhodotorula* species. Common spoilage microorganisms in fruits include *Alternaria*, *Aspergillus*, *Fusarium*, *Monilia* and *Mucor* species. Fruits are excellent culture media for molds, including many mycotoxin-producing ones. Contamination of the raw material and improper storage conditions often also allow the formation of toxic metabolites. For example, patulin, a toxic substance (mycotoxin) produced by *Aspergillus* and *Penicillum* fungi, can be detected in moldy fruits (mainly apples and pears) **[5]**.

During the technological processing of fruits, cutting, slicing, chopping and peeling increase the likelihood of cross-contamination from other materials, tools and equipment at different stages of production. In addition, the increased availability of sugars and other nutrients in minimally processed fruits contributes to the change in the microbiota and increases its population **[6, 7]**. The main factors in the microbiota of the raw material are the hygiene of the surface of the materials used in the production and the processing equipment, as well as the hygiene of the production environment and the food handlers, which determine the microbiological quality and safety of the final product **[8, 9, 10]**. The authors of a study on minimally processed plant-based foods detected high total aerobic microorganism counts on food contact surfaces, especially on peelers, knives and cutting boards **[10]**. The same researchers also reported high levels of facultative anaerobic bacteria of the *Enterobacteriaceae* family on cutting tables and cutting boards **[10]**. Although washing and other decontamination procedures are used in the manufacturing processes of all processing plants, it is still difficult to achieve a significant reduction in microbial contamination **[11]**. Favorable conditions for the growth of microorganisms present in fruits and vegetables can also develop during the packaging and storage periods. Lehto et al. discovered a large number of aerobic microorganisms in surface sampling of vegetable processing plants on devices and equipment in contact with already cleaned vegetables, as well as in the air space of storage, processing and packaging rooms **[10]**.

In the food industry, heat treatment processes are the most important determinants of food safety. Heat treatment of milk is necessary to guarantee its microbiological safety by killing pathogenic microorganisms in milk. Several heat treatment methods are used in the food industry. The efficiency of the heat treatment is ensured by strictly defined temperatures and holding times. In addition to the raw materials of the products, it is also important to ensure the appropriate microbiological properties of the additives. „Heat treatment is an operation related to the warming or heating of milk, cream, etc., the objective of which is to reduce the number of or destroy microorganisms" **[12]**. Heat treatment during milk processing is a general technological step aimed at improving the shelf life of milk by inactivating microorganisms and enzymes. The use of a raw material with a favorable microbiological condition can also improve the texture quality of certain dairy products, such as yogurt **[13]**.

Microwave technology as a heat treatment process is primarily used in households. In the food industry, it can currently only be used reliably in certain areas. The reason for this is that heat transfer is uneven in microwave equipment, and underheated or overheated places develop in the product. In the case of liquids flowing in a pipe, e.g., when treating milk with microwave energy, this can be avoided **[5]**. where this technique can be used, it is an advantage, as the time of treatments applied to foods can be reduced, thus making the technique economical. In addition to cost-effectiveness, an additional advantage is that the directions of heat and material transport are the same, so that a dry crust that prevents flow is not formed **[14]**. Areas of application include drying, thawing of frozen meat, tempering, pasteurization, sterilization and prevention of food discoloration **[15, 16]**.

Sterilizing and antimicrobial effects are also attributed to microwave radiation. In Pozar's experiments **[17]**, this effect could be achieved using a frequency of 2,450 MHz, and in some cases even using a frequency of 915 MHz. Radiation increases the shelf life of foods by killing the microbes in the food and/or inhibiting their growth.

The effect of microwave radiation on microbes has been investigated in a wide variety of foods and food raw materials, especially in meats. The spreading of microwave pasteurization **[18]** has been facilitated by the fact that its use in foods does not cause significant damage, as opposed to traditional heat transfer methods. The reason for this is the short heat treatment and irradiation times **[19, 20]**.

Compared to the microwave treatment technology, drying is a more traditional method, the essence of which is the extraction of most of the water content from the fruit, less often, from the vegetable, by gentle heat transfer, which leaves behind an intensely flavored concentrate of significantly lower weight and size that the starting material. Thus, microscopic organisms that remain on the dried fruit lose their viability and ability to reproduce due to a lack of available water. Fresh fruits contain 90 to 95% water, which drops below 15% after drying. In this way, spoilage caused by bacteria and molds can be prevented while retaining certain nutrients, roughage and minerals, such as iron. Compared to fresh fruits, dried fruits contain a lot of carbohydrates, fiber and antioxidants, flavonoids, phenolic acids, carotenoids and vitamins in a concentrated form **[21, 22]**.

The food industry produces yogurt products of various compositions, but the technological processes used in their production are almost the same until the inoculation of milk. Milk is usually inoculated with a 2 to 3% starter culture and incubated at 40 to 45 °C. In this temperature range, the desired final acidity is reached in 3 to 4 hours. If a lower temperature (30-37 °C) is used, the operation takes longer (7-8 hours), but in this case excessive acidification of the product can be prevented **[23]**.

The bacterium *Streptococcus thermophilus* is primarily responsible for the taste, aroma and texture of the yogurt, and is in fact able to ferment pasteurized milk on its own into yogurt, however, in addition to *Streptococcus*, *Lactobacillus bulgaricus* is also used for the fermentation to produce the acids that from in the product. *S. thermophilus* and *L. bulgaricus* are usually used simultaneously, in a 1:1 ratio, when inoculating the milk (pH 6.6). Their proportion changes as the fermentation progresses **[24]**.

3. Materials and methods

3.1. Manufacture of the products

For the manufacture of the products, raw, untreated milk and 2.8% fat UHT drinking milk (Mizo) were used, which were mixed in a 2:1 ratio before making the yogurts. Raw milk was pasteurized on a hot plate at 75 °C for 15 minutes to achieve adequate initial microbiological safety, and then the drinking milk was added. After heat treatment, the temperature of the milk was allowed to drop to 30 °C, it was inoculated with the amount of thermophilic yogurt culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, YF-L812, Chr. Hansen, France) according to the instructions for use, and then it was stirred for 15 minutes to ensure proper homogeneity. The inoculated milk was dispensed into 2 dl plastic yogurt cups and the cups were placed in a 43 °C thermostat (Binder, Germany), where they were allowed to curdle for 7 hours (pH 4.6). After acid curdling, the fruits treated with the following procedures were mixed into the yogurts.

In our experiment, with the exception of the control sample, two types of heat treatment procedures were used for the fruits, microwave irradiation and a conventional heat treatment method (drying).

The samples prepared in the course of the experiment:

Sample 1: fruit yogurt with the addition of raw apples (Ny-A)

Sample 2: fruit yogurt with the addition of raw bananas (Ny-B)

Sample 3: fruit yogurt with the addition of dried apples (A-A)

Sample 4: fruit yogurt with the addition of dried bananas (A-B)

Sample 5: fruit yogurt with the addition of microwave-treated apples (MH-A)

Sample 6: fruit yogurt with the addition of microwave-treated bananas (MH-B)

Raw fruits were processed in a clean, impeccable condition free of bruises and defective parts, at the optimum degree of ripeness.

For the microwave treatment, a MARS 5 (CEM Corporation, USA) microwave digestion oven was used. Diced raw apples and bananas were placed in the sample holder of the microwave apparatus and subjected to microwave irradiation. The energy transfer program on the device was set to heat the fruits to 55 °C when a power of 800 W with 100% efficiency and with a holding time of 10 minutes was applied, in a total of 15 minutes. Temperature detection and control was performed using a sensor (RTP 300) introduced into the sample holder.

The fruits used in the drying process were cut into pieces of equal size (0.5 cm), so that the drying time would be the same, and then they were placed in a drying apparatus for 24 hours at 55 °C. The dried and microwave-treated fruits were then ground. The knives and the grinder were treated with Mikrozid disinfectant before use. 5 grams each of the ground fruits were added to 150 ml of the already prepared yogurt samples. Until further analysis, the yogurt-dried fruit mixtures were stored in a refrigerator at 4 °C.

3.2. Product shelf-life analysis

The product were tested for 4 weeks in terms of shelf life. Analyses were carried out on days 0, 7, 14, 21 and 28. Microbiological properties (total viable count, yeast/mold count, *E. coli*/coliform count) were tested every week from the time of production. The experiment was carried out with 3 parallel measurements (n=3) on each sampling day, i.e., with 15 samples for each original sample (Ny-A; Ny-B, A-A; A-B; MH-A; MH-B), meaning that a total of 90 samples were processed.

A plate pouring method was used to grow the microorganisms. From food safety and technological hygiene point of view, a total viable count of $10^5/cm^3$ is the critical limit for raw milk, because normal pasteurization procedures can still be used with sufficient efficiency at this microbe count.

The determination of the total viable count was carried out on a PC (Plate Count, Biolab) medium, with an incubation time of 72 hours at 30±1 °C **[25]**. By culturing on a selective medium prescribed in standard MSZ ISO 7954:1999 at 25 °C, yeasts and molds form colonies. YGC agar (Yeast Extract Glucose Chloramphenicol Agar, Biolab) was used for their detection, as prescribed by the standard. This selective medium is suitable for isolating and counting yeasts and filamentous fungi from milk and dairy products. Plates were incubated at 25±1 °C for 48 hours, after which the colonies developed on the plates were counted **[26]**.

Co-determination of the coliform count and the *E. coli* count can be accomplished using CC agar (ChromoCULT Coliform Agar, Biolab). Differentiation between the colonies is aided by the fact that coliform colonies are salmon red, while the color of *E. coli* colonies ranges from dark blue to violet. Incubation parameters for *E. coli*/coliform were 24 hours and 35-37 °C **[27]**.

Our measurement results were plotted using Microsoft Office Excel 2016®. During the evaluation of the microbiological results, microbe counts were displayed in a logarithmic form: the slope values of the lines fitted to each point characterize the exponential growth phase of the microorganisms.

4. Results and evaluation

4.1. Test results of the yogurt samples with apples

4.1.1. Total viable count

According to ***Figure 1***, on days 0, 7 and 14 of the measurement, the total viable count showed almost the same results for the yogurt with dried apples and the yogurt with microwave-treated apples. The yogurt with raw apples already showed higher total viable count values in the second measurement (day 7) compared to the other two samples. Here we already saw a significant difference between the cell counts, which difference only increased over time (day 14). In the case of yogurts with microwave-treated apples and dried apples, the rates of increase in cell counts were approximately the same. This result is also supported by the slope values marked in ***Figure 1***.

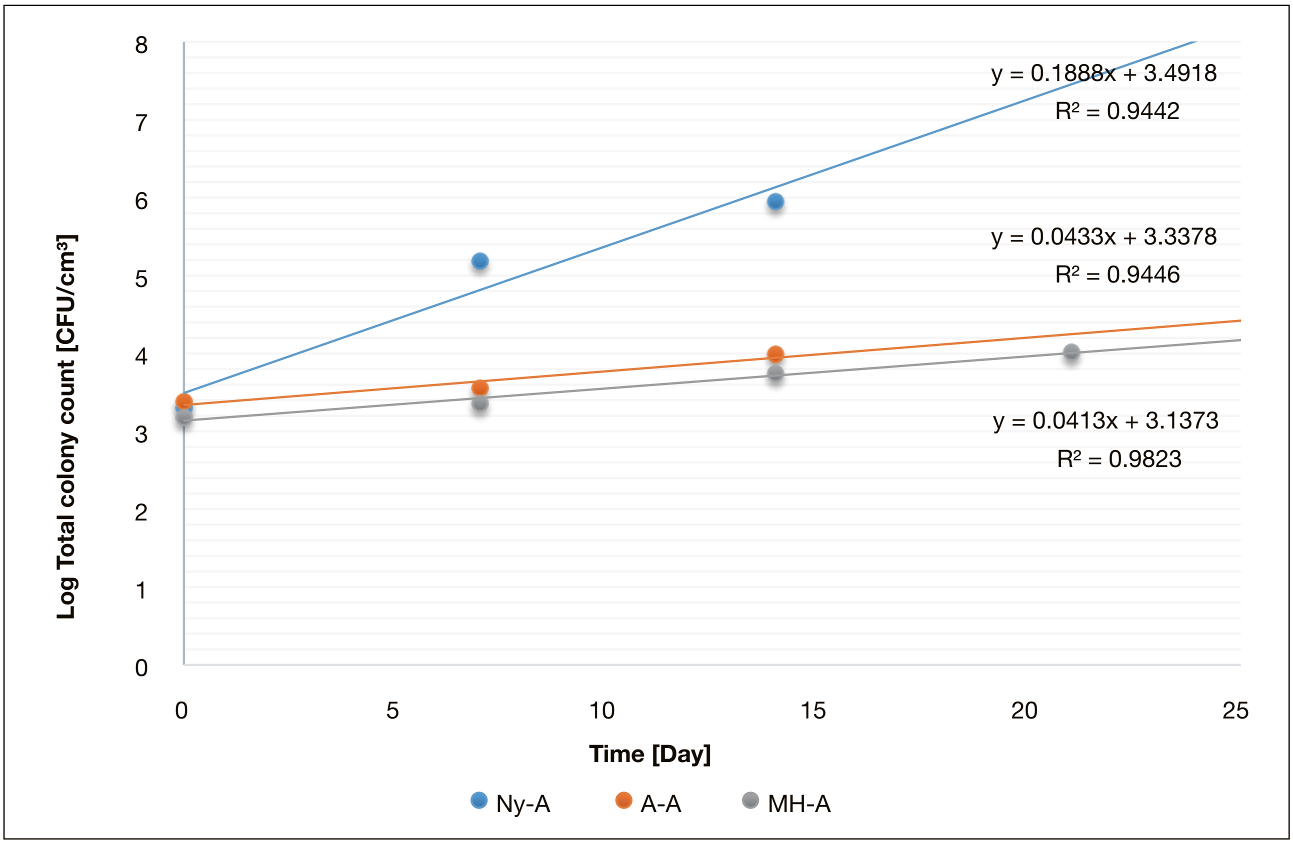

Figure 1. Results of the determination of total viable count in the case of yogurts with apples

4.1.2. Yeast/mold count

Based on ***Figure 2***, it can be concluded that from the first measurement data to the last measurement result, the yogurts with microwave-treated apples and with dried apples showed significantly lower yeast counts than the yogurt with raw apples. There was no difference of the same order of magnitude between samples MH-A and A-A, however, on day 21 of the measurement, there was a clear, significant difference in favor of sample MH-A. Based on this, microwave heat treatment proved to be more effective in inhibiting the activity of yeasts, using the treatment settings applied by us.

In the case of yogurts with apples, it is clear that the microwave technology proved to be the best treatment for both the total viable count and the yeast/mold count. The yogurt with dried fruit exhibited similar cell counts and growth tendencies. However, at the end of the storage time, larger differences between the cell counts developed here. In terms of shelf life, the worst results were obtained for the samples with raw fruit. Differences of an order of magnitude were measured compared to the other two samples, there were significant differences ($p \leq 0.05$).

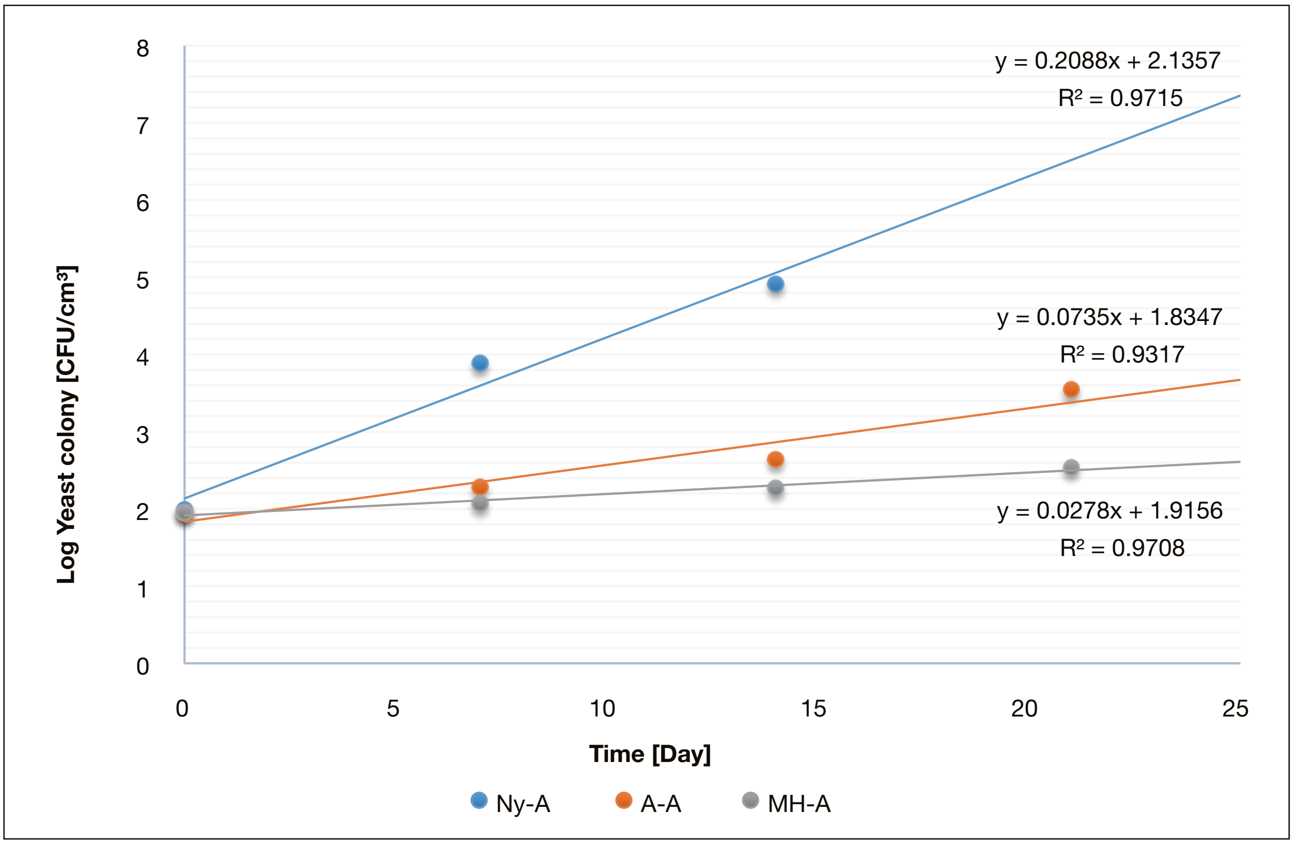

Figure 2. Results of the determination of yeast count in the case of yogurts with apples

On day 21, at the third sampling time, with the exception of yogurts with microwave-treated apples, yogurts with raw and dried apples were spoiled. After the third measurement, in addition to the high yeast count, a significant mold count was also detected in the yogurts with raw or dried apples. In contrast, in yogurts with microwave-treated apples, no mold colonies could be detected after the third measurement.

For the mold count, under the current regulation **[27]**, the level of compliance (m) is 10^2 CFU/cm^3 for fermented milk, dairy products, sour dairy products, cottage cheese and cottage cheese products, while the rejection limit value (M) is $5*10^3$ CFU/cm^3.

In terms of mold count, the presence of no molds was detected in yogurts with apples during the first two measurements, on days 0 and 7. On day 14 of the experiment, colonies of mold appeared in the samples with raw and dried apples, already with a value above the rejection limit as defined by the regulation in the case of raw apples ($3*10^4$ CFU/cm^3). However, in the case of the product with dried apples, the number of mold colonies remained at an acceptable level ($2.2*10^2$ CFU/cm^3) according to the relevant regulations **[27]**.

4.2. Test results of the yogurt samples with bananas

4.2.1. Total viable count

In the case of yogurts with bananas, it was found that the two types of treatment procedures (drying, microwave) also have an effect on the microbial count. In the case of the microwave-treated sample, the increase in the total viable count was not significant until day 21 of the experiment compared to the initial TVC ***(Figure 3)***. On the other hand, the total viable count increased from week to week for the yogurts supplemented with raw or dried fruits. In addition, it was also found that the total viable count of the sample supplemented with dried fruit had the highest total viable count, and the most intense increase in the TVC was also observed in this sample. Already on day 7 of the storage experiment, there were significant differences between the test results of the samples, with an order of magnitude difference between sample A-B and samples Ny-B and MH-B. On day 14 of the experiment, orders of magnitude differences were observed between the date of all three samples. In terms of total viable count, the increase in TVC was the lowest in the case of sample MH-B.

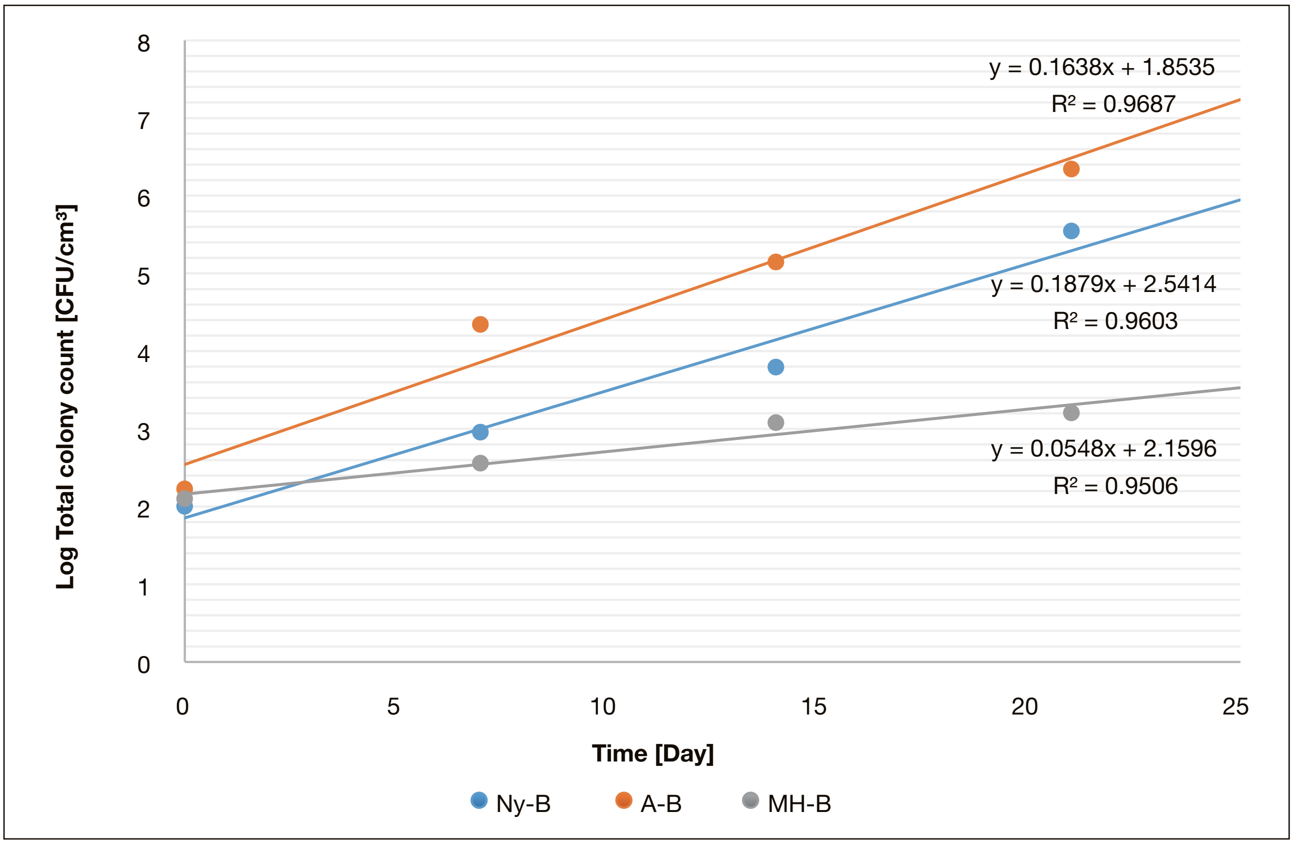

Figure 3. Results of the determination of total viable count in the case of yogurts with bananas

4.2.2. Yeast/mold count

Based on the yeast count results ***(Figure 4)***, it was found that there was no significant difference between the yogurts with raw and microwave-treated bananas in terms of the colony counts of the samples and the growth trends of the microorganisms. However, yogurts with dried apple were characterized by a rapid increase in cell number, which also affected the organoleptic properties of the product. From day 7 of the experiment, there were already significant differences between samples MH-B and Ny-B and samples A-B and Ny-B. Microwave treatment proved to be the most effective in this case as well.

The evolution of the mold count during the shelf life was examined also in the case of yogurts with bananas. The results showed that during the first two measurements, on days 0 and 7, no mold colonies developed. However, on day 14 of the experiment, molds appeared in an amount of $1.4*10^4$ CFU/cm^3 in the sample with dried bananas, a number which well exceeds the compliance limit value according to the regulation (10^2 CFU/cm^3), moreover, it falls into the rejection category ($5*10^3$ CFU/cm^3). For the other two samples (raw and microwave-treated bananas), no molds were present on day 14. On day 21 of the experiment, molds also appeared in the yogurt supplemented with raw bananas in an amount of $3.0*10^1$ CFU/cm^3, which does not yet exceed the compliance limit value. Mold was still not detectable in sample MH-B. On day 28 of the experiment, the mold count of the yogurt with raw bananas also exceeded the rejection limit value by approximately 1 order of magnitude. No mold could be detected in sample MH-B even on day 28.

The results obtained for dried products suggests that the 24-hour drying with a gentle heat treatment did not sufficiently improve the microbiological condition of the materials used. It can be assumed that the hygienic condition of the air flowing through the drying apparatus was also inadequate. We believe that fruits prepared for yogurt products should only be dried in a room and equipment that has impeccable air, and have exhaust and adequate air filtration systems.

4.2.3. E. coli/coliform results of the yogurts

The bacterium *Escherichia coli* is the most important microbe in the normal intestinal flora, making it a natural component of the digestive system of all warm-blooded animals and humans. It can enter foods from fruits and vegetables if they had not been cleaned thoroughly enough, but it can also be found in raw milk or dairy products made from it.

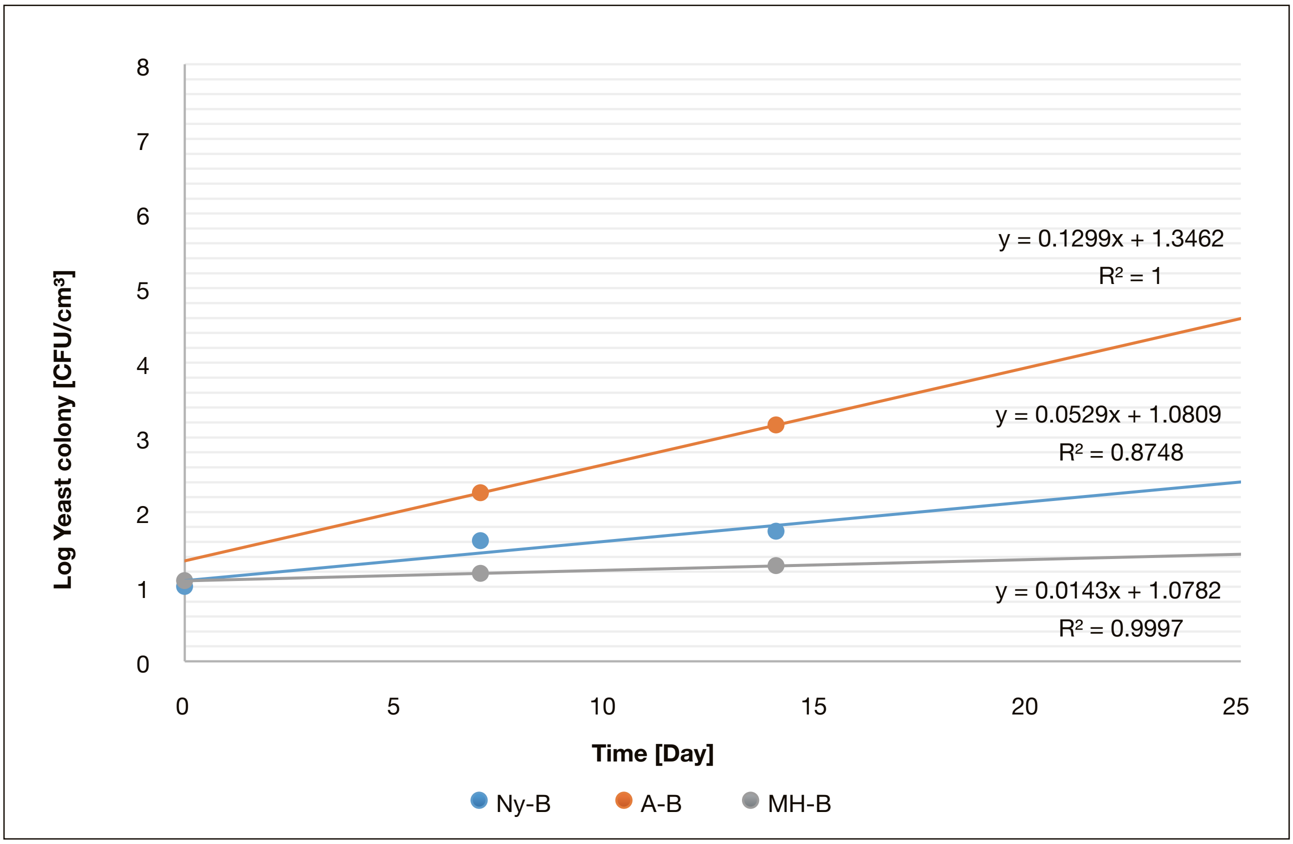

Figure 4. Results of the determination of yeast count in the case of yogurts with bananas

Under current regulation **[28]**, the compliance level is (m)<1/CFU/cm^3 for fermented milk, dairy products, sour dairy products, cottage cheese and cottage cheese products, while the rejection limit value is (M)<10/CFU/cm^3.

During the tests carried out on days 0 and 7 of the experiment, no *E. coli* bacteria were detected in any of the samples prepared by us. However, on day 21 (week 3) of the experiment, the bacterium became detectable in all yogurts except the samples with raw bananas and microwave-treated bananas. By week 4 of the experiment, *E. coli* also appeared in the yogurt with raw bananas. Thus, by the end of the study, only the yogurt supplemented with microwave-treated bananas met the legal requirements.

Coliform bacteria are found in wetlands, in soil and on the vegetation, and are usually present in large numbers in the feces of warm-blooded animals.

According to the relevant regulation (EüM decree 4/1998 (XI. 11.) – EÜM: former Ministry of Health Affairs), the compliance level is (m)<10 CFU/cm^3 for fermented milk, dairy products, sour dairy products, cottage cheese and cottage cheese products, while the rejection limit value is (M)<102 CFU/cm^3.

Coliform bacteria were detected in all samples on day 0 of the experiment, however, the compliance limit value was exceeded only by the results of the yogurt samples supplemented with dried fruits. After 1 week, however, coliforms could only be detected in the yogurt with dried bananas. It is likely that the decrease in the pH value of the yogurt prevented the bacteria from growing and surviving.

At week 3 of the experiment, coliform bacteria were detected in the samples supplemented with raw fruits, they were not present in the other samples. In our case, the samples supplemented with raw fruits reached the M value, so after 21 days the products were not suitable for human consumption.

During the microbiological studies, the determination of *Salmonella* and *Staphylococcus aureus* was also performed, as required by the regulation. These tests were negative in all cases.

Schnabel et al. infected raw fruits with seven microbial strains (including the *E. coli* bacterium also tested by us) with a cell count in the 10^8 order of magnitude. The samples were then treated with microwave-assisted plasma, which reduced the cell count by 4 orders of magnitude already after 5 minutes of treatment. The treatment was performed under non-thermal conditions (at 30 °C), thus excluding the microbicidal effect of the temperature **[29]**.

This phenomenon was manifested during the measurements of our working group in the fact that *E. coli* could not be detected in the yogurt with microwave-treated bananas even on day 28 of the experiment, in contrast to the yogurts with raw or dried bananas. On the other hand, unfortunately, the presence of *E. coli* was detected by the end of the experiment in the yogurts mixed with apples.

Picouet et al. showed that microwave treatments had a similar effect on the *E. coli* O157: H7 and total viable count values, i.e., a 1.01-1.16 log CFU g^{-1} decrease was detected. The same treatment parameters greatly affected *L. innocua*, with population below the detection limit (10 CFU g^{-1}) in most cases. In apple puree samples, the total viable count remained stable during storage at 5 °C, with a slight increase on day 14 **[30]**. This trend was also observed during our own measurements. Our results confirm that the objective of our research was achieved, which was to verify the microbicidal and inhibitory effect of microwave treatment.

Our results are also supported by the fact that 5 to 25 seconds of microwave treatment (65 °C, 1200 W, 2.45 GHz) can reduce the *Salmonella* cell count in vegetables by 4 to 5 orders of magnitude, thus confirming the stronger microbicidal effect of microwave treatment compared to other ones **[31]**.

5. Conclusions and recommendations

When using fruits as flavoring agents in yogurts, two types of heat treatment were applied to increase the shelf life of the products. The effect of the microwave treatment method on shelf life was characterized by the length of shelf life after the addition of conventional and untreated fruits to yogurt.

Based on the microbiological studies, it was found that microwave treatment was the most effective of the various heat treatments. Of the methods of heat treatment of fruits, microwave irradiation resulted in a lower total viable count compared to untreated fruits and drying technology.

Based on our microbiological results, we believe that the contamination or texture of the raw fruit fundamentally influences the effectiveness of the treatment. After microwave treatment of bananas, the presence of *E. coli* could not be detected in the yogurt by the end of our experiments (day 28), as opposed to the treated apples, where its presence was already detected on day 14. This may be noteworthy because the presence of *E. coli* was not detected in either case on the day the products were prepared. It can be assumed that microwave irradiation exerted a more intense germicidal effect in the softer texture of bananas than in apples which have a harder consistency.

It was found that the drying procedure is suitable for the production of microbiologically safe food if the microbiological condition of the air circulating in the equipment is also adequate.

Based o our results, it is hypothesized that microwave irradiation technology can be applied successfully to foods, in this case fruits, to inhibit microorganisms living inside and on the surface of fruits.

While in the case of yogurts flavored with apples, the microbiological characteristics of samples with raw fruit were worse, in the case of bananas, the drying technology proved to be the most unfavorable from a microbiological point of view. The most likely reason for this may be that, compared to apples, bananas contain on average three times more carbohydrates which became more concentrated as the result of drying. This high carbohydrate fruit mixed with the yogurt may have served as a culture medium for various microorganisms.

In order to determine whether or not the use of different microwave temperatures (other than 55 °C), power and treatment times would lead to better shelf-life results, further tests are required.

Of the heat treatment procedures, microwave may be suitable both for treating milk, thus reducing the number of microbes, and for reducing the total viable count of the flavoring agents (spices, fruits, vegetables) used.

6. Acknowledgment

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