Analecta Technica Szegedinensia
Vol. 17, No. 3 ISSN 2064-7964 2023

MONITORING THE PROCESS OF YOGURT SPOILAGE BY DIELECTRIC MEASUREMENTS AND SPREAD PLATE METHOD

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Received: 10th May; Accepted: 11th July

ABSTRACT

In my research work, I primarily focused on the investigation of yogurt (made from home-made raw milk) spoilage by microbiological and dielectric measurements. During the experiment, I continuously monitored the changes in aerobic and anaerobic *Lactobacillus* cell counts of the product, as a possible spoilage process would cause the deteriorative microbes to displace the lactic acid bacteria, and I also monitored the changes in the dielectric properties of the sample material at 400 MHz frequency. The research results verified that there is a strong correlation between the variation in live cell counts and dielectric parameters in both aerobically and anaerobically cultured lactic acid bacteria. The main conclusions of the results are that the change in the bacterial count, and thus the deterioration process leading to it, can be indirectly monitored in the dairy product under study by low-frequency determination of both the dielectric constant and the loss factor.

Keywords: yogurt spoilage, dielectric measurements, microbiological properties

1. INTRODUCTION

Yogurt is a popular fermented dairy product enjoyed by many individuals around the world due to its nutritional value and taste. However, yogurt spoilage is a common problem that affects its quality, safety, and shelf life. Spoilage of yogurt can occur due to a variety of factors such as contamination with undesirable microorganisms, pH changes, and temperature abuse during storage and distribution. Yogurt contains lactic acid bacteria, which play a critical role in the fermentation process, which results in the characteristic texture, flavor, and aroma of yogurt. However, the microbiological stability of yogurt is vulnerable to various factors that can lead to spoilage. Microbial spoilage of yogurt is a significant concern for the dairy industry, as it can result in changes in product quality, sensory attributes, and safety. Understanding the microbiological aspects of yogurt spoilage is crucial for developing effective strategies to prevent or control spoilage and ensure the shelf-life of the product [1].

The electric field strength (E, unit: Vm^{-1}) is a vector of fields characterising the electric or electromagnetic field at each point in the field and showing the extent to which the electric/electromagnetic field exerts a force on the material that is in contact with it. In a time-invariant (static) electromagnetic field with a point charge of Q, the electric field strength can be written as:

$$E(r) = \frac{1}{4\pi \cdot \varepsilon} \cdot \frac{Q}{|r|^3} \cdot r \qquad (1)$$

In the equation, r is the vector from the point charge to the point of measurement and ε is the absolute dielectric permittivity of the material under test at that point. If there are n number of point charges in the field, the resultant field strength can be calculated from the superposition of the field generated by the point charges. For time-varying, i.e. dynamic electromagnetic fields, the field strength can be given by Maxwell's equations. When a material interacts with an electric or electromagnetic field E, it can cause a charge movement or dipole rearrangement in the material, depending on its composition and structure. The extent of this is characterised by the so-called electric (dielectric) shift (D), which is a vector quantity, i.e. it has

DOI: https://doi.org/10.14232/analecta.2023.3.41-47

magnitude and direction. The relationship between the electric field strength and the electric shift is given by the absolute permittivity (ε) of the materials:

$$\varepsilon = \frac{D}{E};\ \left[\frac{As}{Vm}\right] \tag{2}$$

Absolute permittivity is a constant that depends on the physical, chemical, and biological quality of the material, and is in fact a measure of the extent to which the material "responds" to the electromagnetic field strength. Since the response of real materials to the electric field E is time shifted, the dielectric shift D is also delayed, which can be understood as a kind of phase shift (δ). The magnitude of the phase and the phase shift itself can only be expressed in exact terms by complex numbers ($i = \sqrt{-1}$), so it is useful to express the permittivity of a given medium or material as a complex function of the frequency of the applied electromagnetic field, where ε^* is the complex permittivity:

$$\varepsilon^*(f) = \left|\frac{D}{E}\right| (cos\delta + i\ sin\delta) \tag{3}$$

As any other complex function, complex permittivity can also be separated to its real and imaginary part:

$$\varepsilon^*(f) = \varepsilon'(f) - i\varepsilon''(f) \tag{4}$$

The real part of the function is called the dielectric constant (ε'), while the complex part is called the dielectric loss factor (ε''). As can be seen from the equation above, these two quantities are frequency dependent, i.e. for a given material their values vary as a function of frequency. From an electrodynamic point of view, the dielectric constant indicates how much of the energy conveyed by the electromagnetic field can be absorbed and stored by the material interacting with it, while the loss factor expresses how much of the stored electrical energy is converted into other types of energy (such as heat or kinetic energy) [2].

The application of dielectric behaviour analysis has been broadly investigated in different disciplines, such as environmental science, biotechnology and food technology. It was shown that measuring certain dielectric parameters can be used to monitor microwave sludge disintegration and water purification [3], the efficiency of microwave-oxidation process for meat industry wastewater treatment [4], as well as to examine the process of anaerobic digestion of pre-treated sludge [5], and also in enzymatic and fermentation processes regarding lignocellulose degradation [6].

The spoilage processes of different food products are the result of microbial metabolic activities, meaning that the deterioration of these products involves a whole set of different, complex biochemical and physicochemical changes. It means that the determination of dielectric properties and their changes can be a suitable alternative for monitoring these processes. While the literature has not yet addressed in depth the monitoring of dairy product spoilage processes based on the dielectric measurement principle, research has been conducted decades ago to investigate the spoilage of other types of food products and the cell number changes of different microorganisms in terms of dielectric behaviour. In 1995, Asami and Yonezawava looked for a connection between yeast growth dynamics and dielectric properties, measuring dielectric parameters in the frequency range 0.1 to 100 MHz. The results showed that the so-called dielectric dispersion (which is actually the frequency dependence of permittivity) increased exponentially at 1 MHz in the log phase of cell number change and then stabilized in the stationary phase. This relationship clearly shows that the yeast cell count can be monitored by dielectric measurement methods [7]. In an earlier study, it was concluded that the dielectric properties of different microbial suspensions measured at radiofrequency are a direct and monotonic function of the volume fraction of the suspended phase formed by the microbial cells, i.e. the method is suitable for direct estimation of the microbial biomass size [8]. In a 1983 study, the deterioration process of real frozen food raw materials was monitored by low-frequency dielectric measurements, and the experimental raw materials were various frozen fish meat. The results of this research

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led to the conclusion that the dielectric constant at 1.5 kHz was correlated with the state of spoilage of different frozen fish, while the conductivity of the test materials varied independently of spoilage [9]. In one of our earlier studies we have shown that dielectric analysis can also be used during the coagulation of raw milk: strong correlation was found between the apparent viscosity and the dielectric constant of the milk samples during the acid- and enzyme-induced coagulation of the samples [10].

The results of these earlier studies have also shown that, although the suitability of dielectric measurement as an indirect but rapid method for indicating changes in the deterioration phenomena of foodstuffs or food raw materials (e.g. microbial growth, degradation of macromolecules, etc.) has been investigated for decades, detailed microbial-specific results for a wide range of foodstuffs or raw materials are not yet available.

2. MATERIALS AND METHODS

To measure the dielectric constant, a laboratory dielectric measurement system was used. Dielectric parameters were determined using a DAK 3.5 (SPEAG GmBh) measuring sensor connected to a vector network analyser (ZVL-3 VNA, Rhode&Schwarz GmBh) with a 50-ohm coaxial power supply line. Based on my preliminary measurement results, I experienced a high level of electromagnetic interference in the upper frequency range, and therefore the tests were performed between 200 MHz and 2400 MHz. The values of the dielectric properties - dielectric constant, dielectric loss factor - as a function of frequency are obtained from the average of 3 to 3 measurements at 10 points, the arithmetic mean of which (from 30 recorded measurements) is used to determine the values of the parameters associated with a given measurement point. The spontaneous spoilage was investigated in a home-made yogurt sample, made out of raw milk. During the one-week study, the yoghurt was stored in a laboratory incubator at 20±1 °C in its original packaging in a plastic cup with a sealable snap-on lid. In addition to monitoring the storage of the yoghurt at room temperature by dielectric parameters, a microbiological test was performed. Instrumental analysis and microbiological sampling were always performed at the same time using yoghurt from the same production. For the microbiological assessment, I monitored the variation in the living Lactobacillus strain counts using MRS culture media. In the series of microbiological experiments, sampling was carried out in each measurement session by preparing 3 parallel decimal dilutions of stock solutions, from which independent decimal dilution series were formed until 106 dilution members were reached, and then inoculated onto MRS media using a spreading method as described above.

I extended the series of experiments to monitor the microbial counts of lactic acid bacteria under aerobic and anaerobic culture conditions, accordingly, half of the inoculated media were inoculated aerobically and the duplicate plates in anaerobic jars (Thermo Scientific™, Oxoid™, AnaeroJar™ 2. 5L, AG0025A) using an anaerobic modified atmosphere load (Thermo Scientific™, Oxoid™, AnaeroGen™ 2.5L Sachet, AN0025A) at 37±1 °C. After 48 h of incubation, live cell counts were determined using the formula described previously.

3. RESULTS AND DISCUSSION

During the first part of the experiment, I investigated the relationship between the changes in dielectric constant and the living cell count of *Lactobacillus* during aerobic conditions ($N_{aerobic}$; CFU/ml). Among the analysed frequencies, f=400 MHz showed the most distinct differences in the dielectric behaviour, therefore I chose this frequency value for interpreting the results. Figure 1 shows the variation of aerobic germ count and dielectric constant with time.

DOI: https://doi.org/10.14232/analecta.2023.3.41-47

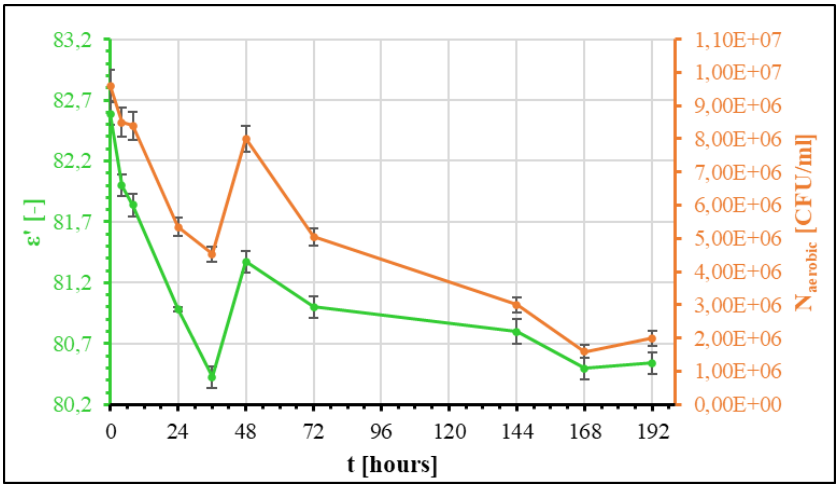

Figure 1. Changes in dielectric constant and aerobic living cell count versus time

Analysing the data, it can be seen that the number of aerobic lactic acid bacteria decreases significantly in the first 24-36 hours, almost linearly, taking into account the standard deviation, and then increases significantly between 36 and 48 hours. One possible explanation could be that the culture used for yoghurt production contained several microbial strains that initially competitively displaced lactic acid bacteria, while producing metabolic intermediates that later (36-48 hours) provided favourable conditions for their proliferation. However, further research and detailed microbiological - biochemical studies as well as further instrumental analysis are needed to explain this phenomenon precisely. A gradual decrease in the aerobic cell count was observed after 48 h, which was accompanied by organoleptic changes and the development of new microorganisms on the MRS medium used, which produced new red-pink colonies with a round morphology. It can be concluded that the deterioration of the product started on the second day of the storage experiment and that at this stage the spoilage bacteria continuously outcompeted the lactic acid bacteria.
The change in the dielectric constant, similar to previous experiments, tended to follow the cell number change, i.e. it decreased with a similar slope in the decreasing phases and increased in the increasing phase in a closely correlated manner (Figure 1). This can be explained by changes in the number of germ cells on the one hand, and by chemical and physical changes in the structure of the matrix on the other. The correlation between the two parameters was again investigated by plotting the relative cell counts and dielectric constants against the initial values (Figure 2).

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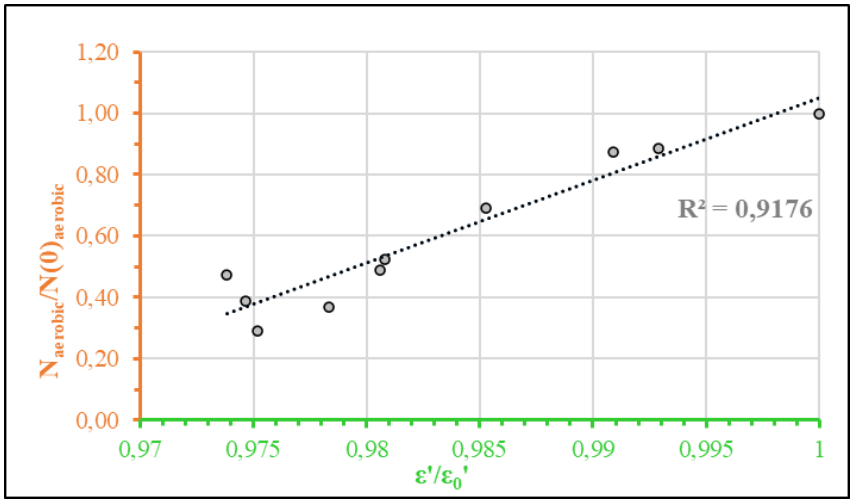

Figure 2. Correlation between the aerobic lactic acid bacteria count and the rate of change of the dielectric constant (f=400 MHz)

Figure 2 clearly shows that the correlation between cell number change and dielectric constant change is close, the coefficient of determination (R^2=0.92) indicates that the two parameters are correlated and the relationship between them is linear. In the light of the above, it can be demonstrated that the measurement of the dielectric constant can be used for this type of dairy product to provide a high level estimate of cell count change and thus indirectly to detect the spoilage process. The decreasing tendency of the dielectric constant during storage and the influence of microbial processes and the resulting pH changes during storage and spoilage (and their effects on, for example, protein structure) on dielectric behaviour were also described in a study by Szerement et al [11].

In the second phase of the research, I also investigated the connection between the dielectric constant and the anaerobic living cell counts of *Lactobacillus*. Figure 3 shows the obtained data versus time.

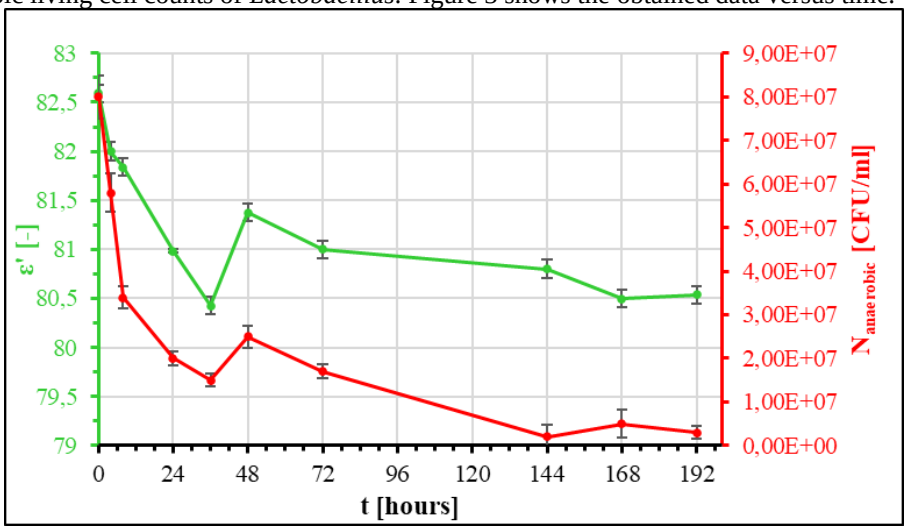

Figure 3. Changes in dielectric constant and anaerobic living cell count versus time

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Examining the anaerobic lactic acid bacteria counts in the samples, phases with similar trends to the aerobic bacteria counts could be obtained, but in this case the initial decreasing phase was more pronounced, while the increasing phase between 36-48 hours was less pronounced, i.e. the lactic acid bacteria with anaerobic metabolism were less able to proliferate in the sample during this phase (Figure 3). It should be noted, however, that the anaerobic cell count was found to be inherently higher throughout the study, suggesting that a large proportion of the mixed culture used for yoghurt production had facultative anaerobic metabolism (i.e. they were able to carry out their energy-producing processes both aerobically and anaerobically), while a significant proportion of the bacteria remained viable under anaerobic conditions only, i.e. they had obligate anaerobic metabolism. After the second day, there was also a clear decline in anaerobic bacterial counts, but of a somewhat smaller slope than aerobic counts; the microbes causing the deterioration thus gradually replaced the anaerobic lactic acid bacteria in the feedstock. The change in the dielectric constant tended to follow the cell number change in this case as well, and the strength of the correlation between the two parameters is illustrated in Figure 4.

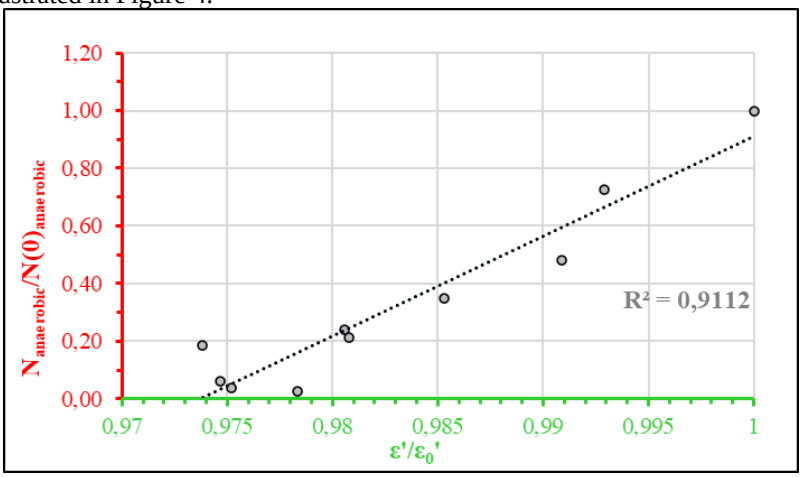

Figure 4. Correlation between the anaerobic lactic acid bacteria count and the rate of change of the dielectric constant (400 MHz)

Similar to the aerobic bacterial count change, the change in anaerobic cell count and dielectric constant values show a strong, clear correlation (R2=0.91) and the relationship is linear.

4. CONCLUSIONS

Based on the experimental data gathered, it can be confidently stated that there is a strong correlation between changes in live cell counts of both aerobically and anaerobically cultured lactic acid bacteria and dielectric parameters. The main conclusions of the results are that the change in the bacterial count, and thus the deterioration process leading to it, can be indirectly monitored in the dairy product under study by low-frequency determination of the dielectric constant.

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ACKNOWLEDGEMENTS

The author is grateful for the financial support provided by the New National Excellence Program, under project number UNKP-22-2-SZTE-199.