Chemical and Process Engineering 2022, 43 (4), 461–469
DOI: 10.24425/cpe.2022.142286

Impedance spectroscopy method used for the unpasteurized beer microbiological contamination degree assessment

Łukasz Macioszek[1*], Sylwia Andrzejczak-Grządko[2], Olga Konkol[2], Ryszard Rybski[1]

[1]University of Zielona Góra, Institute of Metrology, Electronics and Computer Science, ul. prof. Z. Szafrana 2, 65-246 Zielona Góra, Poland

[2]University of Zielona Góra, Institute of Biological Sciences, ul. prof. Z. Szafrana 1, 65-516 Zielona Góra, Poland

The main aim of the below presented work was to investigate the possibility of using impedance spectroscopy in the unpasteurized beer microbial contamination degree assessment. Advantages of the impedance spectroscopy method, a negligible number of similar published results as well as their practical aspect make the research important. Four different types of beers were investigated which were unfit for consumption due to improper storage and were heavily microbiologically contaminated. Their impedance was measured in the frequency range from 0.1 Hz to 1 kHz before and after centrifugation. Based on the measured values, an innovative electrical equivalent circuit was proposed and the parameters of the circuit elements were fitted. The obtained results show significant differences (23 up to 35%) in the values of resistance modelling the diffusion phenomenon. Such large changes, resulting from the removal of biomass from the samples, prove the validity of impedance spectroscopy in the study of the properties of unpasteurized beer. According to the authors, it would be possible to use the proposed methodology during the production of beer. With some limitations, it should aid in the early detection of microbial contamination.

Keywords: impedance spectroscopy, equivalent circuit, beer contamination, microorganisms

1. INTRODUCTION

Beer is a low-alcohol drink, produced as a result of metabolic transformations of yeast cells in a hopped malt wort or in a hopped wort obtained from malt and unmalted raw materials. For hundreds of years, it has been valued for its durability and biological stability, which results from being an unfavourable environment for the development of many microorganisms. It is mainly due to the presence of ethyl alcohol (up to 10% by weight) and a high concentration of carbon dioxide, which are products of yeast fermentation as well as the content of iso-α-acids derived from hops, low pH (3.8–4.7) and low oxygen level. Biological stability is also ensured by appropriate filtration and pasteurization (Campbell, 2003; Sakamoto and Konings, 2003).

* Corresponding author, e-mail: l.macioszek@imei.uz.zgora.pl

Presented at 3rd Seminar on Practical Aspects of Chemical Engineering PAIC 2022, 7–8 June 2022, Zaniemyśl, Poland.

The development of undesirable microorganisms in beer may result in the appearance of numerous product defects, such as discoloration of the beer, viscosity, turbidity, significant deterioration of taste and aroma, changes in appearance, disturbances in the fermentation process, loss of colloidal stability or excessive attenuation. The risk of infection increases with artisanal beers, due to errors stemming from technical or process defects in the brewery, as well as general staff and plant hygiene. The arrangement of the bottling line and the temperature of the final product after bottling also have a significant influence. The shelf life of unpasteurized beers is three months, but only if they are properly stored, that is in refrigerated conditions (Campbell, 2003; Hutzler et al., 2013; Esmaeili et al., 2015).

Microorganisms play a key role at every stage of beer production, from barley cultivation to the aging of the final product. Most of the reactions resulting from their activity are desirable because beer is a product of the metabolic activity of microorganisms. On the other hand, some microorganisms pose a risk to the quality of the end product and must be actively controlled. Depending on the production stage, beer may be contaminated by different strains of bacteria or fungi, due to the variety of environmental conditions and added components (Bokulich and Bamforth, 2013).

2. DETECTION OF ADVERSE MICROORGANISMS

In the brewing industry, beer spoilage microorganisms are defined as those that have not been intentionally introduced but are nonetheless able to survive and multiply in beer wort, fermenting wort, filtered beer or packaged beer. It has been shown that many bacterial strains remain viable in beer for longer periods, and the oxygen content is the main factor controlling their growth. However, as long as they are not able to grow in the product, they are not considered to be harmful and spoilage organisms (Campbell, 2003; Jespersen and Jakobsen, 1996).

Detecting beer spoilage microbes is difficult as they are often biochemically and physiologically very similar to brewer's yeast, as is the case with, inter alia, wild yeast of the genus *Saccharomyces*. In order to minimize the risk of microbial contamination, it is necessary to use appropriate methods of beer production monitoring, allowing for precise control and assessment of potential contamination. These methods can be divided into qualitative and quantitative. The former allow the detection and identification of specific strains and their metabolites, which leads to the confirmation or exclusion of their presence in the product. Quantitative determination of microorganisms shows the scale of contamination and allows to state whether it is at an acceptable level in relation to the applicable standards (Wawerla et al., 1999; Hill, 2015).

3. IMPEDANCE SPECTROSCOPY OF BEER

Microorganisms break down macromolecular, weakly ionized compounds, such as carbohydrates, proteins, lipids, into small metabolites, such as amino acids or organic acids, which are more electrically charged. This results in an increase in electrical conductivity, i.e. a decrease in resistance. Therefore, measuring resistance (and more often impedance) can be used to monitor the metabolism of microbes. This method is mainly used for the quantitative analysis of specific microorganisms, for example from the *Enterobacteriaceae* family, determining the shelf life of food products and estimating the condition of the product in terms of the amount of microorganisms (Wawerla et al., 1999; Hill, 2015).

On the other hand, living cells have completely different electrochemical properties than their inanimate surroundings. Hence, their mere presence causes a number of changes in the electrical properties of the test sample. For example, a different amount of yeast is associated with changes in the electrical capacity

of the sample, and bacteria cause changes in the measured electrical permeability (Asami, 2014; Brunauer et al., 2021). The changes in the relative electric permeability as a function of the bacterial membrane potential were also shown (Bot and Prodan, 2009). Hence, the variety of electrochemical phenomena that can take place in unpasteurized beer, which contains both yeast and various types of bacteria, makes the measurements of its electrical properties very interesting. On the other hand, they are also challenging, thereby the negligible amount of published papers containing experimental data similar to those shown in this article.

Impedance spectroscopy is one of the methods of analysing electrical properties of the tested material and is based on measuring the impedance of a sample at different frequencies. In a potentiostatic approach, a voltage of a certain amplitude is applied to the electrodes and the value of the resulting current flowing between them is measured. On the other hand, galvanostatic measurements force a current to flow and the value of the resulting voltage generated at the electrodes is recorded. The values of the disturbances introduced into the system are sufficiently small (typically up to 20 mV for voltage) in order not to cause additional reactions or phenomena in the sample. Measurements are repeated for a number of defined frequencies, usually starting with the highest value and ending with the lowest.

The recorded results of the sample impedance values can then be used to determine the relative dielectric permittivity or to fit the electrical equivalent circuit parameters, modelling the phenomena and reactions. These circuits may consist of commonly known basic electronic components such as a resistor, a capacitor or a coil. Over the recent years, a number of additional elements have also been proposed that reflect the observed phenomena much more accurately (Barsoukov and Macdonald, 2018). The main advantages of the impedance spectroscopy method include an uncomplicated measurement concept, a relatively short measurement time, the possibility of implementing it in a portable device and performing online measurements.

4. MATERIALS AND METHODS

The research material consisted of samples of four unpasteurized and unfiltered beers, produced by a local craft brewery. All samples were unfit for consumption due to improper storage at room temperature for eight weeks from the time of bottling. The bottles were opened on the day of the first examination. Measurements were made before and after centrifugation of the samples. The basic features and ingredients (per 1000 litres of wort) of the tested beers are listed in Table 1.

The measuring system (Fig. 1) consisted of a PC with PowerSINE software, controlling the potentiostat/galvanostat 263A and the Lock-In Amplifier 5210 phase-sensitive detector from EG&G/Princeton Applied Research. The impedance of the samples was measured in the two-electrode configuration.

A pair of the same electrodes (Fig. 2) was made of a laminate covered with a layer of copper and gold to protect against corrosion.

The electrodes with an area of 5.6 cm^2 each were spaced 2 mm apart and were immersed in the samples during the measurement. The impedance was measured at a test voltage of 20 mV RMS, for 25 frequency settings from 0.1 Hz to 1 kHz, at an ambient temperature of 24 °C. Unfiltered beer showed high electrical heterogeneity even after centrifugation, hence during all measurements the samples were mixed uniformly with a magnetic stirrer.

Table 1. Selected characteristics of the tested beers

Sample name	American Wheat (AW)	Life (L)	American Saison (AS)	Ox Bile (OB)
Alcohol (wt.%)	4.7	6.6	7.0	8.5
Malt	Pilsner 50 kg, light wheat 50 kg	pale ale 90 kg, rye 25 kg, RED 5kg, chocolate 5 kg, roasted 5 kg	Pilsen 100 kg, Munich 25 kg, wheat 5 kg	Pilsner 125 kg, pale ale 40 kg, wheat 25 kg
Hops (iso-α-acids)	Citra (13%) 1.3 kg, Columbus (13%) 0.1 kg, Azacca (12.5%) 1.3 kg	Columbus (14%) 0.3 kg, Simcoe (12.8%) 1.0 kg	Columbus (13.9%) 0.2 kg, Mosaic (12%) 1.0 kg	Centennial (8.7%) 1.5 kg, Chinook (11%) 3.2 kg
Other components	–	zinc sulphate 0.7 g	dried lemon peel 0.1 kg, coriander 0.2 kg	sugar 10 kg, zinc sulphate 0.65 g
Resting time	less than a month	7–8 weeks	over half a year	3–4 months

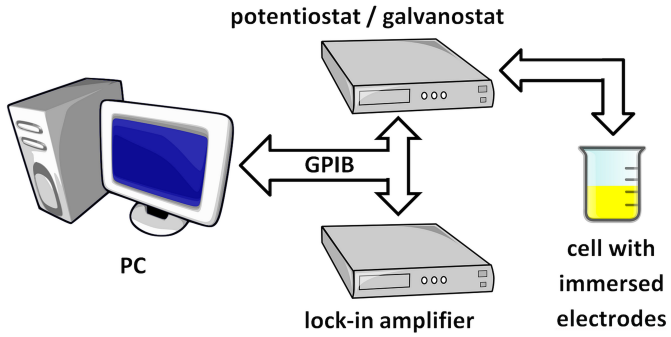

Fig. 1. The measuring system scheme

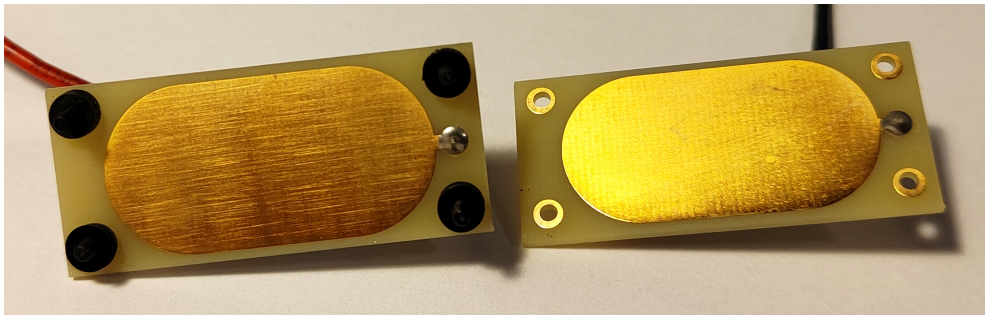

Fig. 2. Electrodes used for measurements

5. RESULTS AND DISCUSSION

The aforementioned range of measurement frequencies (0.1 Hz-1 kHz) was selected experimentally and was intended to cover only the alpha dispersion area (Brunauer et al., 2021). The limitation of the research to only one type of dispersion allowed to use a simpler electrical equivalent circuit of the cell.

After a preliminary analysis of the obtained results, the authors decided to use an innovative circuit, not found in literature dedicated to testing beer properties. The circuit (Fig. 3) had a form of a parallel connected resistor R_d with a constant phase element CPE and a resistor R_s in series with them.

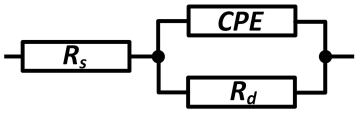

Fig. 3. Electric equivalent circuit used in the experiment

The impedance in general is the complex quantity that can be written as the sum of its real part (resistance R or Z_{re}) and the imaginary part (reactance X or Z_{im}) in the form (1):

$$Z^*(\omega) = R(\omega) - jX(\omega) \tag{1}$$

The constant phase element's impedance can be described by the formula (2):

$$Z^*_{\text{CPE}}(\omega) = \frac{1}{Q(j\omega)^n} \tag{2}$$

where: Q is the admittance value $1/|Z|$of the CPE element at $\omega = 1$ rad/s, and n is a fraction contained in the range $0 \leq n \leq 1$. At $n = 1$, the CPE element is identical to the ideal capacitor.

Thus, the selected equivalent circuit's impedance is given by Equation (3):

$$Z^*(\omega) = \frac{R_d}{1 + QR_d(j\omega)^n} + R_s \tag{3}$$

The elements R_d represent the resistance related to the diffusion phenomenon also described by the CPE element. Resistance R_s reflects the series resistance resulting from the electrodes' properties and their connection.

The measured impedance values of individual samples before and after centrifugation are shown in Figures 4–7. American Wheat beer samples are shown in the Nyquist plot in Figure 4. The impedance

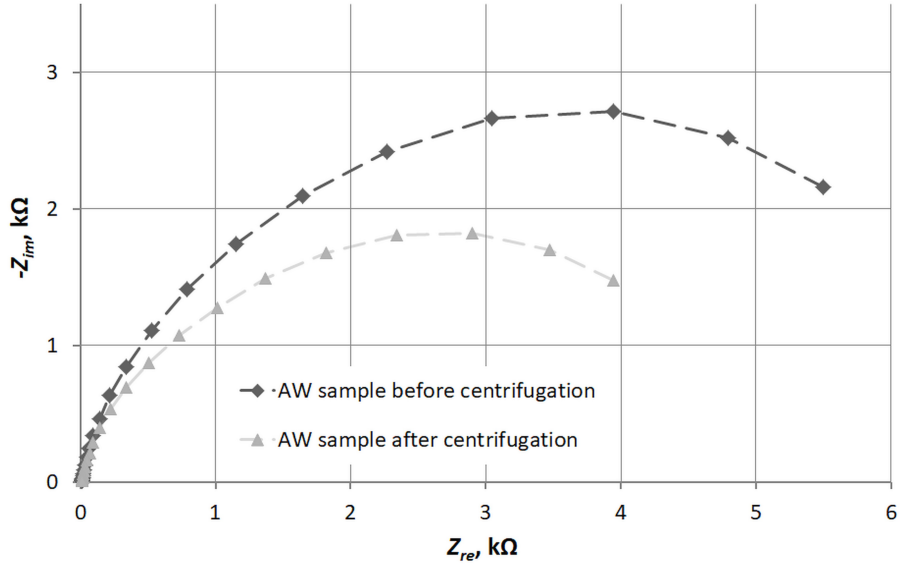

Fig. 4. The American Wheat sample Nyquist plot

values form single semi-circles with centres below the Z_{re} axis. This proves the problematic values matching the equivalent circuit elements without the use of the CPE element. It is worth noting that as the measuring frequency is lowered, the points begin to appear on the right side of the semicircles, i.e. they present higher real impedance part values. The sample, after centrifugation, is characterized by much lower impedance values, which in general suggests increased electrical conductivity.

The same observations arise after analysing the remaining beer samples. The Life sample shown in Figure 5 has generally slightly higher impedance values than the AW specimen.

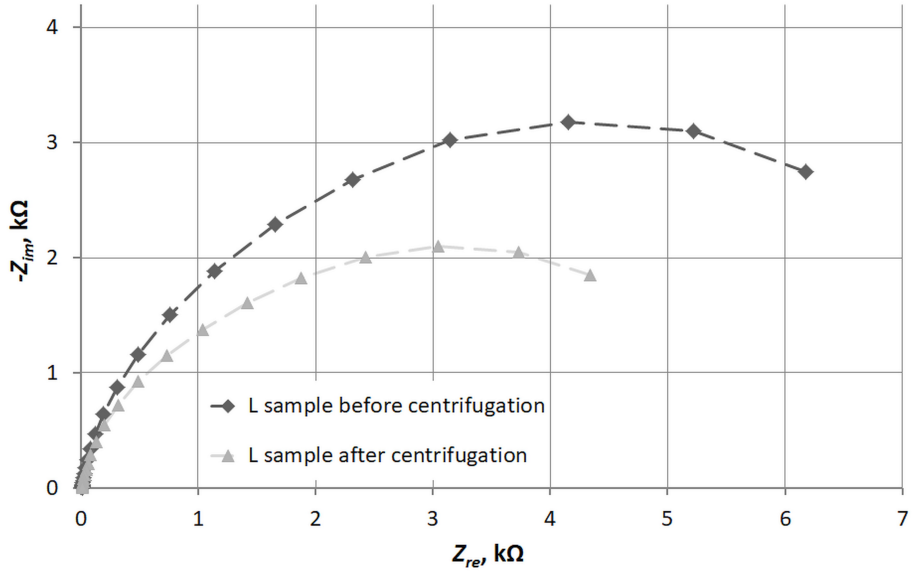

Fig. 5. The Life sample Nyquist plot

On the other hand, the American Saison sample shown in Figure 6 shows an increased conductivity (smaller semicircles) relative to the previous results.

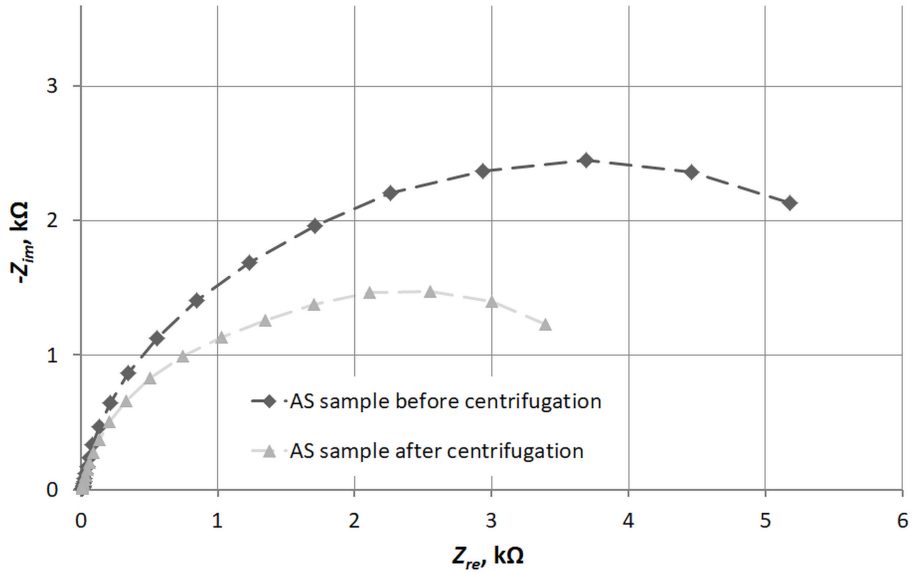

Fig. 6. The American Saison sample Nyquist plot

The last sample tested, Ox Bile (Fig. 7), has similar impedance values before centrifuging to the Life sample, but after centrifuging the impedance did not decrease so significantly.

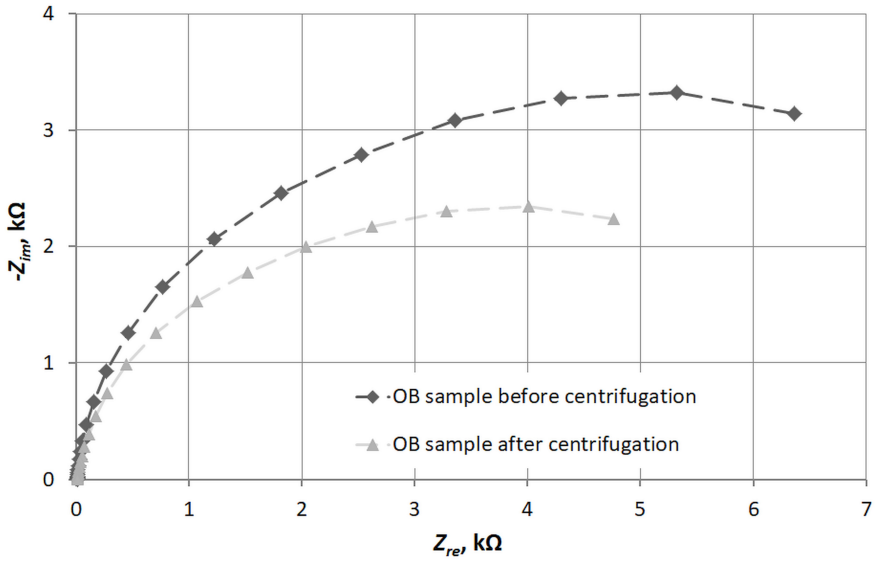

Fig. 7. The Ox Bile sample Nyquist plot

The equivalent circuit elements formulated in the proposed way were possible to fit with the modelling error χ^2 (reduced) not exceeding 0.01. The error of matching individual elements did not exceed 5.6% in the worst case, and most often oscillated around 2–3%. Such error values are perfectly acceptable. For values fitting pyZwx program was used (Kobayashi, 2021). Electrical equivalent circuit elements' fitted values and modelling errors are summarized in Table 2.

Table 2. Electrical equivalent circuit elements' fitted values and modelling errors

Sample name	American Wheat (AW)	Life (L)	American Saison (AS)	Ox Bile (OB)
R_s, Ω – before centrifugation	11.91	12.67	14.16	10.44
R_s, Ω – after centrifugation	11.77	10.91	9.63	9.80
R_d, kΩ – before centrifugation	6.54	7.26	6.09	7.55
R_d, kΩ – after centrifugation	4.70	5.26	3.94	5.82
Q, μS·s^n – before centrifugation	80.28	77.40	81.14	68.82
Q, μS·s^n – after centrifugation	104.92	101.76	110.63	96.92
n – before centrifugation	0.8810	0.8894	0.8790	0.9197
n – after centrifugation	0.8490	0.8569	0.8472	0.8681
χ^2 error – before centrifugation	0.00871	0.00215	0.00846	0.00996
χ^2 error – after centrifugation	0.00820	0.00819	0.00918	0.00616

As expected, the circuit's element that changed its value the least, regardless of the tested sample and whether it was before or after centrifugation, was the series resistance R_s. Depending on the sample, this value fluctuated in the range from 9.6 to 14 Ω and it was the case with the largest component matching

errors. The parameter n of the CPE element, which oscillated between 0.85 and 0.92, showed similarly low variability. These values are close to one, but with the use of an ideal capacitor in place of the CPE, an attempt to match the elements resulted in unacceptably large errors.

Significant changes were noted for the value of the resistance R_d, which in all tested samples decreased remarkably after the material was centrifuged. On the other hand, similarly notable changes in value relate to the Q parameter describing the CPE element. For the AS beer sample, R_d value decreased the most (by 35%) from 6.09 to 3.94 kΩ after centrifugation. For the remaining beers, the declines were also significant and amounted to 23, 27 and 28% for OB, L and AW samples, respectively. The OB beer was characterized by the largest increase in Q value from 68.82 to 96.92 μS·s^n (41%) after centrifugation. The remaining samples also showed a significant increase in this value by 30, 31 and 36% for AW, L and AS specimens, respectively.

Such large changes in the value of resistance R_d before and after centrifuging the samples are, according to the authors, related to getting rid of the vast majority of biomass that was in them. The samples after centrifugation were very clear, while before this treatment a significant turbidity of the beer was visible, especially at the bottom of the vessel. Live cells are able to increase their impedance along with decreasing the frequency of the test voltage (Polk and Postow, 1995). Thus, getting rid of them from the samples resulted in the reduced tested materials' resultant resistance. The increased Q value of CPE element in the vast majority of cases is associated with the decrease in R_d value. Therefore, both changes should be considered as a manifestation of the same phenomenon, as either Q and R_d parameters mainly describe diffusion taking place in the sample.

Unfiltered, unpasteurized beer is highly heterogeneous, and therefore, at different heights of the fermentation tank the electrical properties of the material are varied. Hence, according to the authors, simultaneous measurement of impedance at several points should allow for microbiological differentiation of beer.

Unfortunately, until the publication of this article, the authors did not find similar studies that could be used to compare the presented results. However, the subject of research is so interesting that they shall be published shortly.

6. CONCLUSIONS

The conducted research confirms legitimacy of using the impedance spectroscopy method in unpasteurized beer microbial contamination degree assessment. The measurement results presented in the paper show significant differences between the samples before and after centrifugation, which were possible to record with the use of presented methodology. Beer, as a research material, is a relatively complicated mixture, which makes it difficult to analyse. Despite this, the authors are of the opinion that the impedance spectroscopy method allows to test the properties of beer. The legitimacy of its use, even as part of comparative measurements, seems to be also confirmed in this work. There are still many interesting directions for further research with its application, such as measurements at other frequency ranges, with variable voltage values or with other types of electrodes.

SYMBOLS

CPE	constant phase element described by parameters Q and n
j	imaginary unit
n	exponent in the CPE element description

Q admittance value $1/|Z|$ of the CPE element at $\omega = 1$ rad/s, $\mu S \cdot s^n$
R resistance, Ω
R_d diffusion resistance, Ω
R_s series resistance, Ω
X reactance, Ω
Z electrical impedance, Ω

Greek symbols

χ model fit error
ω angular frequency

REFERENCES

Asami K., 2014. Low-frequency dielectric dispersion of bacterial cell suspensions. *Colloids Surf.*, *B*, 119, 1–5. DOI: 10.1016/j.colsurfb.2014.04.014.

Barsoukov E., Macdonald J.R., 2018. *Impedance spectroscopy: Theory, experiment, and applications*. 3^{rd} edition, Wiley, New York, 440–441. DOI: 10.1002/9781119381860.

Bokulich N.A., Bamforth C.W., 2013. The microbiology of malting and brewing. *Microbiol. Mol. Biol. Rev.*, 77, 157–172. DOI: 10.1128/MMBR.00060-12.

Bot C., Prodan C., 2009. Probing the membrane potential of living cells by dielectric spectroscopy. *Eur. Biophys. J.*, 38, 1049–1059. DOI: 10.1007/s00249-009-0507-0.

Brunauer G.C., Meindl A., Rotter B., Gruber A., Slouka C., 2021. A case report: Electrochemical impedance spectroscopy as an Al-ternative for cell counting chambers of yeast (*Saccharomyces cerevisiae*) for brewery applications. *Arch. Food Nutr. Sci.*, 5, 027–031. DOI: 10.29328/journal.afns.1001029.

Campbell I. 2003. Wild yeasts in brewing and distilling, In: Priest F.G., Campbell I. (Eds.), *Brewing Microbiology*. 3^{th} edition, Springer, Boston, MA, 247–264. DOI: 10.1007/978-1-4419-9250-5_7.

Esmaeili S., Mogharrabi M., Safi F., Sohrabvandi S., Mortazavian A. M., Bagheripoor-Fallah N., 2015. The common spoilage microorganisms of beer: occurrence, defects and determination – a review. *Carpathian J. Food Sci. Technol.*, 7(4), 68–73.

Hill A.E., 2015. 13 – Traditional methods of detection and identification of brewery spoilage organisms, In: Hill A.E. (Ed.), *Brewing Microbiology*. Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing, 271–286. DOI: 10.1016/B978-1-78242-331-7.00013-7.

Hutzler M., Müller-Auffermann K., Koob J., Riedl R., Jacob F., 2013. Beer spoilage microorganisms – A current overview. *Brauwelt International*, 2013/I, 23–25.

Jespersen L., Jakobsen M., 1996. Specific spoilage organisms in breweries and laboratory media for their detection. *Int. J. Food Microbiol.*, 33, 139–155. DOI: 10.1016/0168-1605(96)01154-3.

Kobayashi K., Suzuki T. S., 2021. Free analysis and visualization programs for electrochemical impedance spectroscopy coded in Python. *Electrochem.*, 89, 218–222. DOI: 10.5796/electrochemistry.21-00010.

Polk C., Postow E., 1995. *Handbook of biological effects of electromagnetic fields*. 2nd edition, CRC Press.

Sakamoto K., Konings W.N., 2003. Beer spoilage bacteria and hop resistance. *Int. J. Food Microbiol.*, 89, 105–124. DOI: 10.1016/S0168-1605(03)00153-3.

Wawerla M., Stolle A., Schalch B., Eisgruber H., 1999. Impedance microbiology: Application in food hygiene. *J. Food Prot.*, 62, 1488–1496. DOI: 10.4315/0362-028X-62.12.1488 .

Received 27 June 2022
Received in revised form 25 July 2022
Accepted 29 July 2022