APPLICATION OF DIELECTRIC MEASUREMENT FOR MONITORING THE EFFICIENCY OF ENZYMATIC PROCESSES

Sándor Beszédes[1], Réka Dobozi[2], Balázs Lemmer[2], Zoltán Jákói[1]

[1]*Department of Biosystems Engineering, University of Szeged, H-6725 Szeged, Moszkvai krt. 9, Hungary*
[2]*Department of Food Engineering, University of Szeged, Faculty of Engineering, H-6724 Szeged, Mars ter 7, Hungary*
e-mail: beszedes@mk.u-szeged.hu

Abstract
The dielectric measurements have proved their applicability in many fields of science and industry practice, as well. However, there is just limited experiences in the case of enzymatic processes. Therefore, the main aim of our research was to investigate the applicability of dielectric measurements for monitoring of enzymatic hydrolysis of lactose and cellulosic biomass. During our measurements, the dielectric constant and dielectric loss factor in the frequency range of 200-2400 MHz was determined using an open-ended coaxial dielectric probe. Based on our results, it can be concluded that the decomposition of lactose can be monitored by measuring both the dielectric constant and the dielectric loss factor at frequencies of 1000 MHz and 1800 MHz, while the enzymatic degradation of cellulose can be detected by the ratio of dielectric constant values measured at 400 and 1800 MHz frequencies. Therefore, it can be concluded that determining dielectric parameters at appropriate measurement frequencies are suitable for non-destructive and rapid monitoring of the efficiency and progress of enzymatic processes.

Key words: dielectric parameters, dielectric monitoring, enzymatic processes

Introduction
The dielectric characteristics of the materials, commonly quantified by its dielectric constant (ε') and dielectric loss factor (ε''), serve as indicators of its interactions with electromagnetic field [1]. The dielectric behaviour has significant effect on the temperature ramp, thermal efficiency, temperature homogeneity of materials subjected to microwave (MW) irradiation, for instance. In multicomponent, and heterogeneous systems, especially in solids, the different dielectric properties of the components alter the propagation of electromagnetic waves in materials under test (MUT) that presents difficulties for achieving uniform alternating electric fields. High power electromagnetic field can generate local overheating ('hot-spots') that makes difficult to maintain uniform temperature. Measurement of dielectric parameters enable to control the power absorption of MW irradiation, estimate the penetration depth of the electromagnetic waves and the conversion efficiency of electric field to heat (heat generation efficiency of MW irradiation). The dielectric constant quantifies the ability of polar components for orientational polarization (rotating) in alternating electromagnetic fields, the loss factor is related to energy absorption of electromagnetic field into the materials, which can generate heat (energy dissipation). The complex permittivity can be calculated by the Cole-Cole relaxation model equation (using static permittivity, frequency, relaxation time, conductance as variables). In some cases, the Cole-Cole dielectric relaxation model can be reduced the Debye model [2].
The dielectric measurement methods include waveguide or resonant cavity methods, open ended probes, impedance measurements and free space methods, as well. Open ended coaxial probes are widely used for the investigation of the dielectric behavior. Beside the numerous

advantages, their high price and the low penetration depth into the MUT can be considered as one of the main disadvantages, which limit the wider-scale applications. In the field of life sciences, the sensors operated at radiofrequency and microwave frequency ranges can be non-invasive alternative methods of conventional analytical techniques for the measurement of glucose concentration in biosystems (in vitro blood glucose measurement with lab-on-chip methodology, for instance) [3]. The change of dielectric loss factor measured at gigahertz frequency range is capable to detect the change of glucose concentration in aqueous solutions, but the presence of salts can reduce the alteration of dielectric behavior, therefore the sensitivity of the dielectric measurement methods decreases [4]. The problems related to electrode polarization effects of salts in aqueous system have also verified for dielectric measurements conducted at megahertz frequency ranges, the change of relative permittivity as a function of glucose concentration decreased if the concentration of ionic compounds (salts) increased [5]. The Maillard-type reactions between reducing sugars (such as glucose) and amino acids can be detected by dielectric measurements as well. In heated media, the intensity of ionic migration is enhanced that affected by the lower viscosity of the solvents, as well. Beside this, the higher temperature led to higher reaction rate between amino acids and sugars. These phenomena resulted in the increment of dielectric loss factor. However, it can be noticed, that during Maillard reaction the number of polarizable molecules decreased, therefore the contribution of dielectric polarization mechanisms to the change of dielectric behavior are decreased [6].
The analysis of the dielectric behaviour of blood plasma shows that in the frequency range of 1-8 GHz dielectric constant decreases, and dielectric loss factor (imaginary part of the complex permittivity) increases as the glucose concentration increases. However, in aqueous glucose solutions opposite trends can be observed for the relationship of glucose concentration with the dielectric parameters. At a given glucose concentration, the dielectric constant decreases while loss factor increases with increasing frequency. The lower temperature results in lower ε' and higher ε" at high concentration ranges, because the lower temperature causes increased viscosity, and in higher viscosity medium the rotation of molecules is impeded, and, furthermore, the higher viscosity is manifested in higher friction of molecules in alternating electromagnetic field [7]. During the enzymatic hydrolysis of lignocellulosic materials, change of the capacitance and the concentration of monomeric carbohydrates release. Therefore, the efficiency of the enzymatic cellulose hydrolysis can be detected by dielectric measurement [8]. In milk, the lactose interacts with water molecules forming water clustered structures. The change of physicochemical structure due the presence and concentration of lactose can have effect on the interaction with electromagnetic field. Alteration of polarisation in the electromagnetic field has effect on the dielectric behaviour of the system. However, the milk cannot be considered as homogenous system, therefore the higher penetration depth of electromagnetic field at microwave frequencies can be applied as a 'volumetric" non-destructive measurement methods compared to methods applying infrared radiation. In lactic-acid bacteria induced coagulation, the hydrogen ions form lactic acid reduces the negative charge of casein. Therefore, the calcium phosphate from casein micelles liberate which led to collapse of micelles resulted in coagulation. During the coagulation the ratio of free to bound water is changed. The relaxation of free and bound water is different in electromagnetic field (in milk: 10-20 GHz vs. 1 GHz, respectively). Because the polarization is depended on the composition and physicochemical structure of milk, analysis of the change in dielectric behaviour can be suitable to monitor the coagulation processes. For example, dielectric measurements in the frequency range of 100 MHz-20 GHz verified, that till 500 MHz the ionic conduction has the main effect on the change of dielectric parameters, and the fat content has not significant effect on the tendency of the change of dielectric loss factor vs frequency at the range of 20-4500 MHz, but there can be found differences in the absolute value of ε". The

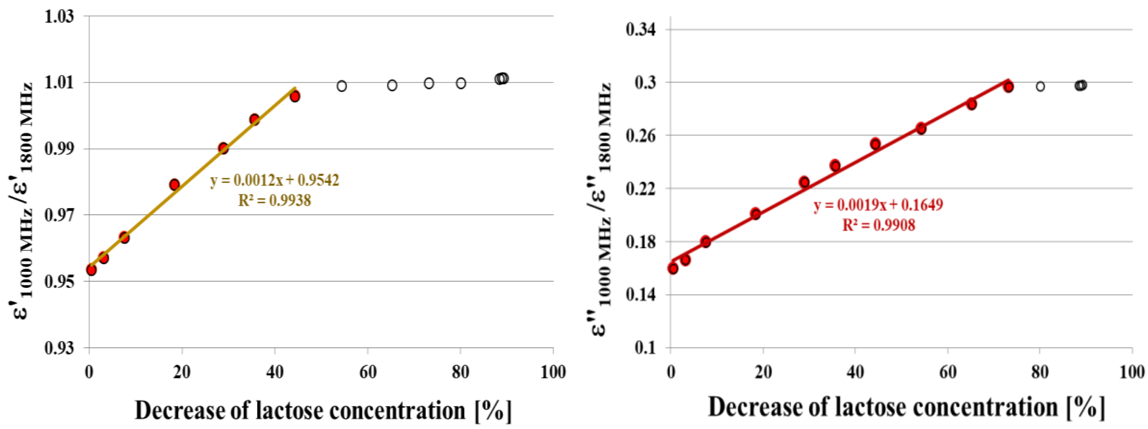
dielectric loss and dielectric constant are also negatively correlated with the pH during the milk coagulation process [9].
It is verified that dielectric behaviour of the milk and other high water contented biological systems is mainly determined by the polarization effects at frequencies below 3 GHz [10]. Mastitis is one of typical health problems on cows' health status. The change of somatic cell count (SCC) has effect on the physicochemical properties of the milk, and its techno-functional characteristics in dairy industry processing, as well. A recent study concluded that the change somatic cell count in cow milk can be detected by the analysis of the dielectric behaviour. The increment of SCC caused a higher concentration of ionic components that resulted in enhanced ionic polarization in electromagnetic field, therefore the dielectric loss factor is increase, as well. It is also observed, that at higher SCC concentration ranges, the frequency dependence of dielectric spectra reduced [11].

Experimental

The dielectric behaviour of the materials (characterized by the dielectric constant and dielectric loss factor values) in the frequency range of 200-2400 MHz was investigated by a Speag DAK 3.5 dielectric probe connected to a ZVL-3 vector network analyzer with coaxial line. The measurements were based on the determination of reflection coefficient at the interface of sample material and probe. The temperature of MUT/biosystems was controlled with water bath at 20±0.5 °C. For the calibration open, short circuit and deionized water (dielectric load) method was applied. The immersion depth of DAK probe into samples was 10 mm for all measurements.
For the cellulose enzymatic hydrolysis test corn cob residue was used as substrate which has 46±2 w%, 37±1.6 w% and 7±0.5 w% cellulose, hemicellulose and lignin content, respectively. Enzymatic hydrolysis tests were carried out in 3.5 w% suspensions at the temperature of 45°C using 0.1 M sodium acetate buffer for controlled pH of 4.8 using Cellic CTEC2 enzyme blend in a dosage of 60 FPU/g(cellulose). The reducing sugar concentration was measured by DNSA photometric assay. The samples were filtered through a 0.2 μm syringe filter (PES membrane). For the investigation of lactose hydrolysis commercially UHT milk was used with fat content of 1.5%. The incubation temperature was 20±0.5 °C (higher than applied in the dairy technologies to shorten the time demand). The dosage of β-galactosidase enzyme (Maxilact L2000, DSM) was 0.15 v/v%). After the incubation (before the analysis) the enzyme was inactivated using 2 min 90°C heat treatment. The lactose content was measured by refractometer (PAL19S, Atago), the galactose concentration was determined by galactose oxidase colorimetric detection kit (ThermoFischer, absorbance was measured at 560 nm).

Results and discussion

Our results shown, that during the enzymatic decomposition of lactose components in milk the dielectric constant (ε') show increasing tendency. The change of dielectric constant and loss factor due to the decreasing of lactose (and increasing of galactose and glucose monomers) was different in the varying frequency ranges (data not shown). The difference between dielectric properties vs. lactose concentration was the highest at the frequency of 1000 MHz, and has been slightly in the frequency range of 1800-2400 MHz. Therefore, the ratio of dielectric constant and dielectric loss factor (ε") measured at 1000 MHz to that of determined at 1800 MHz was used as dielectric control parameter of the enzymatic process. Our results show, that the dielectric constant is suitable to monitor the lactose hydrolysis process till approximately 45% lactose decomposition degree (Fig. 1.a), while the dielectric loss factor ratio has good linear correlation with the change of lactose concentration in the range of 0-70% (Figure 1.b), respectively. The hydrolysis of lactose to its monomers increase the number of easier polarizable molecules (with lower molecular weight) in the system that can be manifested in the change of dielectric parameters, for example the increasing tendency of dielectric loss factor [6,9].



a) *b)*

Figure 1. Relationship between the lactose hydrolysis (decrease of lactose concentration) and dielectric constant (a) and dielectric loss factor (b) ratio
(at 1000 MHz and 1800 MHz, t=20°C)

Analysis of the dielectric behaviour during enzymatic hydrolysis of lignocellulosic biomass show, that in the frequency range of 200-2400 MHz the dielectric constant has better correlation with the reducing sugars (RS) concentration compared the sensitivity of dielectric loss factor to the change of RS yield (data not shown). Similar to the experiences related to the dielectric behaviour of milk during lactose hydrolysis, there can be found 'characteristic' measurement frequencies (for minimum and maximum value of loss factor) for the monitoring of cellulose hydrolysis. Ratio of the dielectric constant at the frequency of 400 MHz to 1800 MHz has good linear correlation with reducing sugars yield (mg RS./g dry matters). Progress of cellulose degradation caused decreasing of dielectric constant ratio (Figure 2).

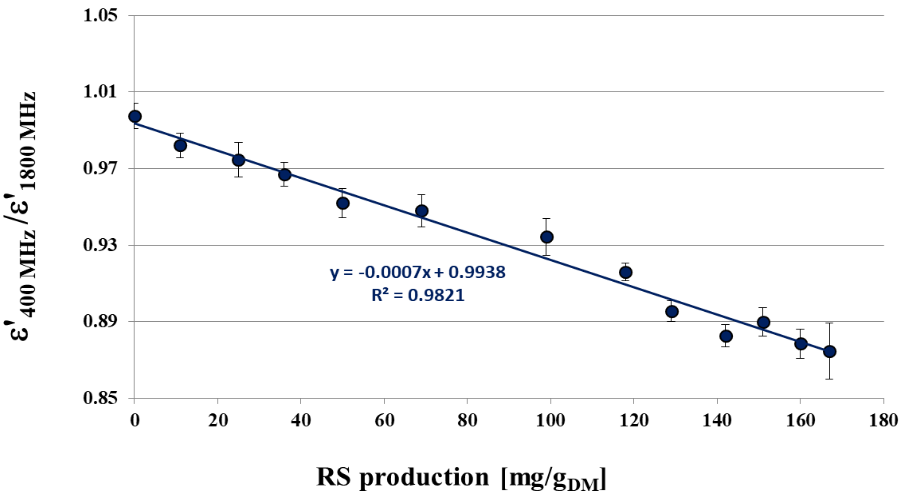


Figure 2. Correlation between RS yield and dielectric constant ratio (measured at 400 MHz and 1800 MHz)

Other studies have also verified, that the presence of sugar monomers (such as glucose) has effect on the relaxation time in electromagnetic field, and increasing of glucose concentration resulted in lower permittivity and dielectric constant in aqueous systems [7, 8].

Conclusion

Our research has verified that both the enzymatic lactose decomposition process used in dairy production technologies and the enzymatic hydrolysis of lignocellulosic biomass can be detected based on the measurement of the dielectric properties. However, to apply a dielectric monitoring method with adequate accuracy for the specific raw material and enzymatic process, the determination of the most sensitive dielectric parameters and appropriate measurement frequencies for the substrate and hydrolysis products can be considered as crucial. However, due to their non-destructive characteristics, the rapid dielectric measurements can be suitable for application in industrial manufacturing technologies, as well.

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