

DETECTION OF TRIGLYCERIDE BASED ON MICROWAVES DIELECTRIC PROPERTIES OF TRIGLYCERIDE ENZYMATIC REACTION

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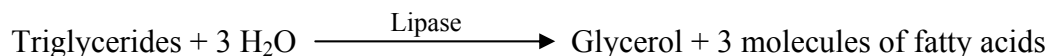
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ABSTRACT

The measurement of dielectric properties of enzymatic reaction between triglyceride (TG) and lipase was done with the product formed was fatty acids and glycerol. The product of the reaction can be seen as a cloudy upper layer liquid and a clear lower layer which can be observed after a stable condition at about 4 minutes after the reaction time. A good relation can be observed for TG determination by studying the changing in dielectric properties. This relationship is applied for the ranging of concentration of the TG from 10000 ppm to 50000 ppm. For the better sensitivity, the best frequencies of measurement are 1 GHz, 2.6 GHz, 10 GHz and 15 GHz and the preferable mixing ratio between substrate and enzyme is 1:1. Furthermore, the sensitivity observed for the above frequencies is ranging from 0.00005 ppm^{-1} to 0.00075 ppm^{-1} .

INTRODUCTION

TG can be catalyzed by enzymes as shown in figure below:



The formation of two layers can be seen when TG hydrolyses by lipase which is in upper layer contains fatty acids while at lower layer is a glycerol.

In milk fat, TG may affect the cheese flavor and causes milk rancidity. Realizing the important constituent of TG in dairy product, the measurement of dielectric changes of TG by enzymatic reaction based biosensor was successfully carried out. This measurement has been done to investigate the interaction of molecules in electromagnetic field. The measured dielectric properties are dielectric constant, ϵ' and dielectric loss, ϵ'' and dependent upon many parameters. The appropriate parameters are depend on the concentration of TG and lipase, time response during the reaction occurred, the frequency that showed the effect of dipole or ionic conductivity and ratios of substrate-enzyme reaction.

The step of hydrolysis of TG into glycerol and fatty acids in the presence of lipase which can be carried out in diverse ways [1]. Studies have been done by exploring the detection of TG based on nanostructures [2]. Estimation of triglyceride content in food material is important from the point of view of increased health awareness among

people and stringent regulatory laws in the food industry [3]. Another approach had studied about the detection of TG using amperometric biosensor [4]. A study on TG has been explored, by comparing a potentiometric and a micromechanical triglyceride biosensor [5]. An amperometric enzyme sensor for determination of TG was constructed by mounting cellulose acetate (CA) membrane-bound commercial lipase. Hence, analyzing the component in food and bio sample by investigating their dielectric properties changes is an effort to control and detect the amount of TG.

In this study, the main objective is to investigate the dielectric properties of product formed from the TG - lipase enzymatic reaction at microwave frequencies (0.2 GHz - 20 GHz) and to correlate the dielectric properties of product formed with concentration of TG at the specific frequency.

EXPERIMENTAL DETAILS

The sample used in this study is a Lipid Standard Triglyceride Mixture product from Supelco (17811) which is acted as a substrate and Lipase from *Aspergillus Niger* (62301) acted as a catalyst. The sample has been diluted in order to obtain a homogeneous TG solution and conducted at room temperature. The various concentrations of TG were done which are 10000 ppm, 20000 ppm, 30000 ppm, 40000 ppm and 50000 ppm. The mixture of chloroform and methanol with a volume ratio 1:1 respectively is used to dilute the TG samples while for lipase, water was used as a solvent to dilute the enzyme. Technique for measuring the dielectric properties of TG was using an open ended coaxial probe. Before performing any measurement, the network analyzer has to be calibrated using open air, shorting probe and water as described in the instrument's users manual and then the dielectric properties of reaction solution was measured using the network analyzer.

RESULTS AND DISCUSSION

Dielectric Properties of Sample after Reaction

The dielectric constant, ϵ' and dielectric loss, ϵ'' for upper layer is presented in Figure 1. Figure 1 shows the dielectric properties of the product after reaction at various TG concentrations. The pattern is almost similar to previous work studied by [6] which is reported that as the TG content is reduced and the water content increased the dielectric properties of the mixture is no longer the dielectric properties of TG itself. For this reason, it is believe that the presence of TG will dilutes the water dielectric properties behavior. Furthermore, the dielectric properties of aqueous solutions or mixtures is decreased by two mechanisms which is the replacement of water by a substance with a lower dielectric constant, ϵ' and dielectric loss, ϵ'' and the binding of water molecules [7]. This also may indicate that the dielectric properties are influenced by hydrophilic interactions of TG component. This effect is similar to the study on dielectric properties with frequencies ranging from 1 and 20 GHz [6].

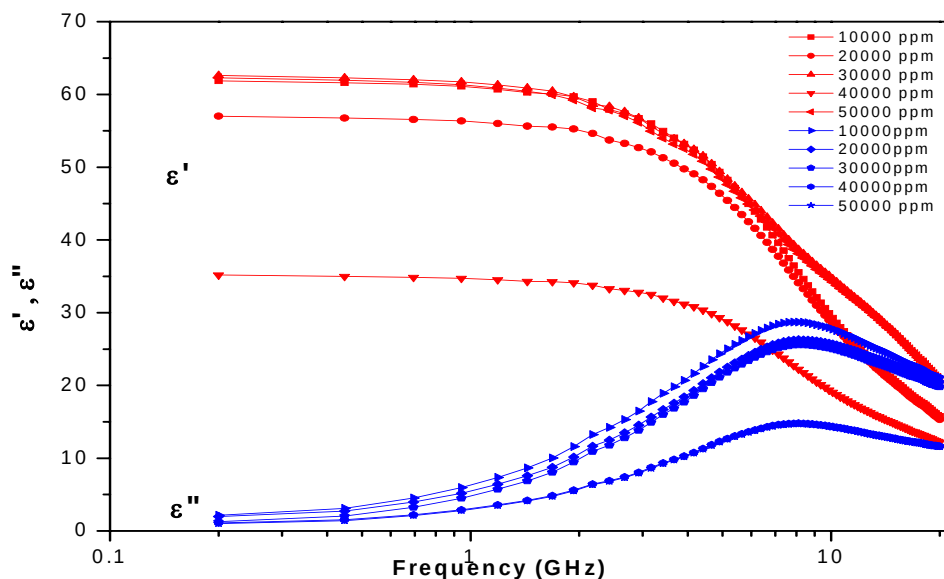
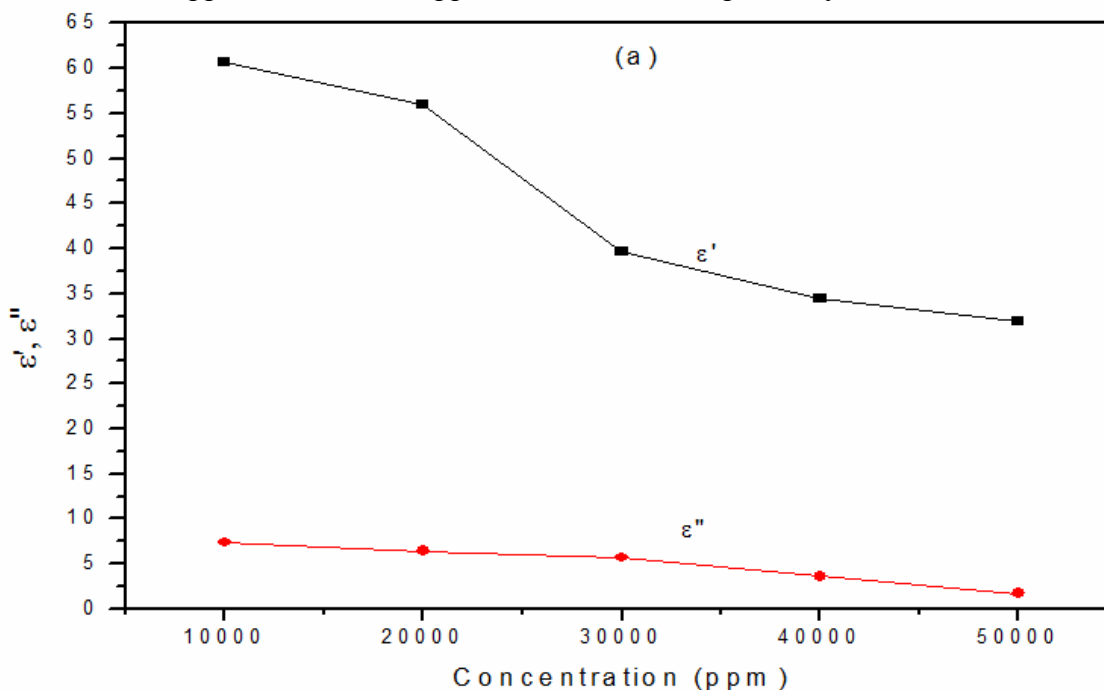


Figure 1: Spectra of dielectric properties for product after reaction at upper layer.

The Frequencies from the Pattern of Spectrum after Reaction (1 GHz and 2.6 GHz)

Figure 2 shows the variation of dielectric properties with the concentrations of TG at 1 GHz and 2.6 GHz. From the above graphs, the dielectric properties, ϵ' and ϵ'' were increased significantly with a decrease in TG content. This trend will give the possibility to develop microwave measurement system for determination of TG concentration in the sample. Both frequencies give about the same sensitivity which are about 0.00075 ppm^{-1} and 0.00005 ppm^{-1} for ϵ' and ϵ'' respectively.



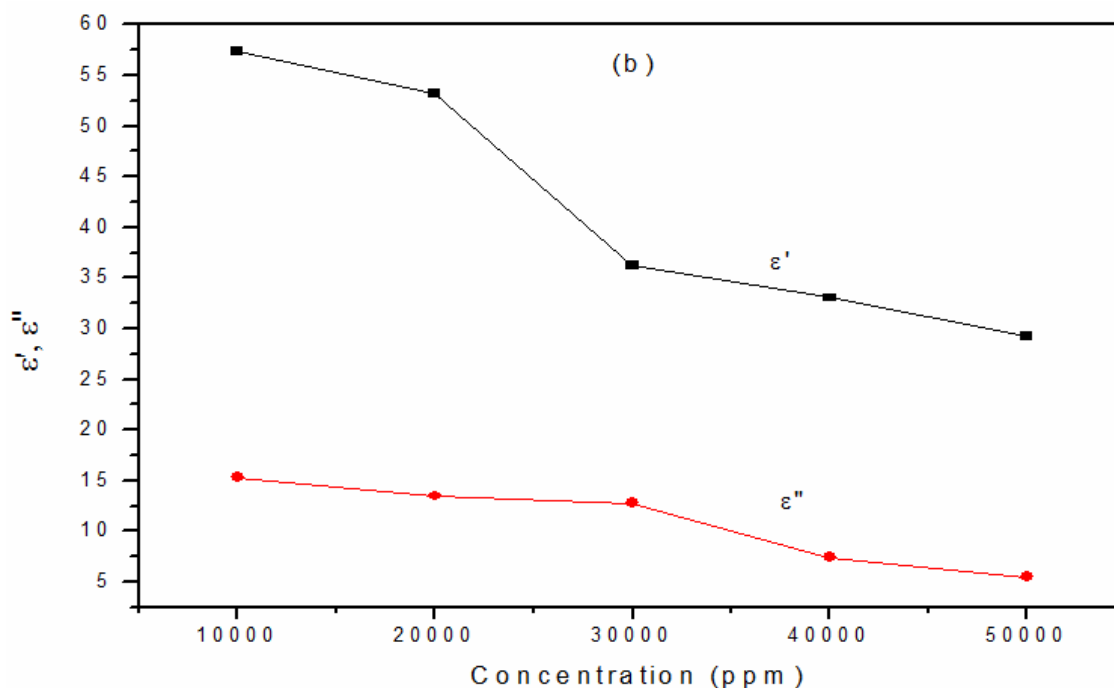


Figure 2: Dielectric properties for upper layer at (a) 1 GHz (b) 2.6 GHz

CONCLUSION

An experimental investigation of dielectric properties of TG enzymatic reaction over microwaves frequency range was evaluated using NA and OCP. The dielectric properties of TG and product after reaction are useful for detecting the dielectric changes and these findings give a potential approach in developing the microwave measurement system for TG detection in milk samples. Meanwhile, the dielectric properties of reaction give a good sensitivity, reproducibility and stability.

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