REVEALING THE INVISIBLE CHANGES IN FTIR SPECTRA UPON STORAGE OF EDIBLE COMPOSITE FILMS USING TWO DIMENSIONAL CORRELATION ANALYSIS

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Abstract–Attenuated Total Reflectance/Fourrier Transform Infra Red (ATR/FTIR) Spectroscopy is a powerful technique for the rapid extraction of information from complex matrices such as the composite films that are based on edible biopolymers. These films are used for the protection of food products against moisture uptake and are consumed with them. In order to be suitable for food packaging they need to be stable against physical and chemical changes upon storage. FTIR is a convenient tool to examine the film compounds and changes due to their interactions during ageing. However, the incorporation of a hydrophobic compound into the plasticized biopolymer matrix leads to a complex FTIR spectrum due to peak overlapping, which increases uncertainty concerning peak assignment to specific compounds. Moreover, the complexity of the spectrum prevents the identification of small changes during storage. Literature on FTIR data mostly relies on chemometric approaches. In this work, the effectiveness of the Two Dimensional Correlation Analysis (2D-Cor) to increase spectral resolution by analyzing spectrum into two dimensions and hence allows to differentiate overlapping peaks is exemplified. 2D-Cor analysis was carried out in ATR/FTIR spectra of composite oil bodies-sodium caseinate based edible films plasticized by glycerol during storage under mild conditions for 60 days. Invisible, otherwise, physical changes, due to water uptake and glycerol migration to the film surface, and chemical changes, due to lipid hydrolysis, were revealed using this mathematical treatment of spectra. Correlations and sequences of changes at various wavelengths are presented and discussed.

Keywords: *FTIR, 2Dimensional Correlation Analysis, edible films, oil bodies, storage*

1. INTRODUCTION

Edible films have gained research attention at the last three decades due to the many advantages of their potential application on the food sector. They may serve as protection covers or coatings of food products and they are consumed along with the product. Edible biodegradable biopolymeric materials, such as proteins, polysaccharides or combinations of them are used for the preparation of edible films [1]. Plasticizers, such as sugars and polyols, are also employed to improve film’s mechanical properties [2]. The fact that edible films are food products themselves, their stability during storage is essential. However, edible films are consisted of natural and usually hydrophilic materials and, thus, they may be susceptible to changes during storage, especially, due to their interaction with moisture. In order to reduce their affinity to water and improve their water barrier properties, lipids and lipophilic materials are incorporated into the biopolymeric matrix in the form of emulsified lipid/oil droplets [3].

Oil bodies are small organelles of spherical shape, in the form of which, lipids are stored in vegetable cells. A mixed membrane of phospholipids and proteins, mainly oleosins (Fig. 1) [4], surrounds the core of oil bodies, consisting of triacylglycerols.

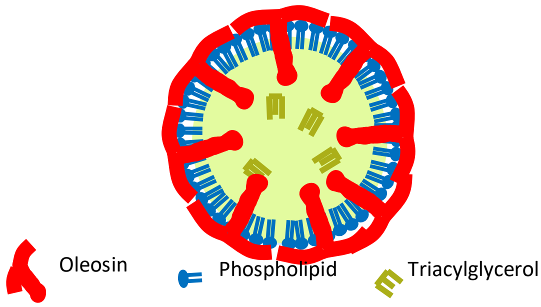

Figure 1.Oil body structure.

Oil bodies are extracted from oleaginous materials with water, without the use of organic solvents [5], achieving high yields [6] and leading to a stable natural emulsion [7], which may then be exploited for the preparation of food products. Recently, composite edible films were prepared by incorporating oil bodies, extracted from an industrial maize germ by-product, into sodium caseinate matrix [8].

The complex matrix of composite edible films may be affected by storage conditions and be vulnerable to chemical changes, such as lipid oxidation [10], lipid hydrolysis, Maillard reactions [11] or physical changes such as water uptake [12], phase separation [13], plasticizer migration [14]. It is rather challenging to identify these changes. FTIR-ATR is a powerful technique for the rapid extraction of information from biopolymeric matrices [14], [15]. It is mostly used to identify interactions between edible films' components [12], [16]. However, the incorporation of lipids into the matrix leads to a complex spectrum due to peak overlapping [10], [12]. Scientists have employed FTIR spectorscopy to study the interactions between the components of edible films. However, a small number of studies have used FTIR spectroscopy to identify changes through storage time [10], [17]. These publications mainly focus on extreme storage conditions under which chemical changes in lipids are easily visualized with new peak generation or peak shifts. Film changes, that have been often reported [3], [14] and mainly are due to physical changes, such as plasticizer migration or creaming, under mild storage conditions, have not been studied by FTIR since it is almost impossible due to spectrum complexity. However, the combination of FTIR spectroscopy and the mathematical technique of generalized 2Dimensional Correlation analysis (2D-Cor) has proved to be valuable to reveal invisible changes in many fields of research [18], [19].

According to 2D-Cor spectroscopy the sample is subjected to an environmental perturbation (time, temperature etc). The series of spectra obtained are spread into a second dimension and two maps, synchronous and asynchronous, are provided. The result of this analysis is the cross correlation of a given spectral variable with every other spectral variable in the data. The most important features of this method are the transformation of complicated spectra into simpler ones, the enhancement of spectra resolution by expanding them in two dimensions, the unambiguous assignments of peaks and the peak changes sequences [20], [21]. The goal of this work was to emphasize the advantages of applying 2D-Cor FTIR-ATR Spectroscopy for identifying small changes in composite edible films matrices during aging in mild conditions.

2. EXPERIMENTAL

Aqueous extraction of oil bodies from maize germ was performed as described elsewhere [6], [22]. Oil bodies were recovered in the form of condensed "milk" after ultrafiltration of the extract [22]. Sodium caseinate-based composite edible films were prepared according to Matsakidou, Biliaderis & Kiosseoglou [8]. Films stripes were then cut in order to fit on FTIR-ATR Zn-Se cell and stored for 60 days at 25 ^{o}C and relevant humidity of 53%. FTIR spectrum was obtained periodically at wavenumber range between 4000 and 700 cm^{-1}, performing 32 scans. The spectrum of the clean and dry crystal at atmospheric air was used as background. Spectra were manipulated by performing linear baseline correction, ATR correction and 17 points smoothing. The sample was immediately placed back to the storage chamber after FTIR measurement. Normalized data were expanded in two dimensions with the aid of the 2D-Shige free software.

Synchronous 2D-Cor spectrum corresponds to the in phase changes of two separate spectral intensity variations under a given external perturbation. Correlation peaks appear at both diagonal positions, which corresponds to the autocorrelation function of spectral intensity variations (autopeaks) and at off-diagonal positions (cross peaks) which represent simultaneous or coincidental changes of spectral intensities observed at two different spectral variables (v_1, v_2). The regions of a spectrum will show strong autopeaks when great extent of intensity changes takes place under a given perturbation (time). Otherwise, the regions that remain near constant will generate little or no autopeaks. The asynchronous spectrum consists exclusively of off-diagonal positions (cross peaks). An asynchronous cross peak corresponds to accelerated or delayed change features (out of phase) (v_1,v_2). This feature is exploited in differentiating overlapped bands arising from spectral signals of different origins. Moreover, the signs of cross peaks at both maps provide information on the sequential order of events observed by the spectroscopic technique applied along with the external variable. Same signs of both synchronous and asynchronous cross peaks (v_1, v_2) lead to the following sequence of events $v_1 \rightarrow v_2$. On the contrary, if different signs correspond to synchronous and asynchronous cross peaks (v_1, v_2) then the order of events is $v_1 \leftarrow v_2$, according to Noda rules [21]. Fig. 2 shows a flow diagram of the course of actions in order to identify sequences of the changes at spectra.

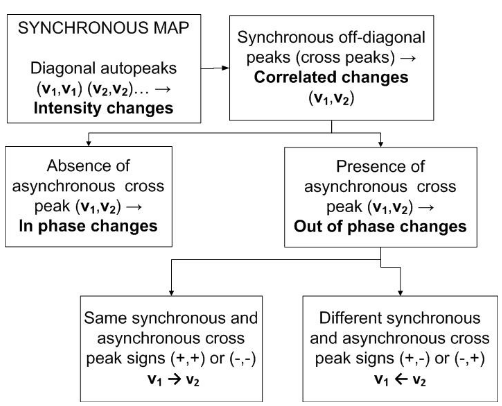

Figure 2. Flow diagram of actions for 2D-correlation analysis according to Noda rules

3. RESULTS AND DISCUSSION

In Fig. 3 FTIR-ATR spectra of composite sodium caseinate: oil bodies edible film surface before and after storage is presented. The two spectra are almost identical. The most prominent change that was observed was the broadening of the main band in the area between 3500 and 3000 cm^{-1}(intra and intermolecular H bonds) [11].

Peak intensities changes around 1710, 1589, 1415, 1398, 923 and 781 cm-1 were also observed but there were marginal in terms of significance. Therefore, the elucidation of the changes happened to the film matrix was impossible by comparing the spectra.

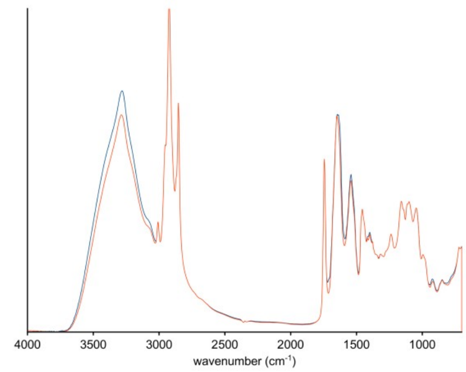

Figure 3. FTIR-ATR spectra of composite sodium caseinate:oil bodies edible film surface before (red line) and after (blue line) storage for 60 days at 25°C and relative humidity 53%.

The generalized 2D-Cor analysis revealed invisible, otherwise, physical and chemical changes. No autopeaks at the previously mentioned wavenumber were obtained. On the contrary strong autopeaks were developed at 1744, 2852 and 2921 cm^{-1}. Further analysis of the maps showed that the above mentioned changes are in phase and can be attributed with certainty to the presence of oil. So it seems that creaming has occurred during aging inside the film matrix.

The study of the signs of synchronous and asynchronous crosspeaks and the sequences of the order of events revealed that hydrolysis of oil may have occured after creaming followed by changes in water state. No apparent glycerol migration was determined.

4. CONCLUSIONS

In this work, the effectiveness of the Two Dimensional Correlation Analysis (2D-Cor) to increase spectral resolution by analyzing spectrum into two dimensions allowed the differentiation between overlapping peaks. 2D-Cor analysis was carried out in ATR/FTIR spectra of composite edible films during storage under mild conditions. Invisible, otherwise, physical changes, due to creaming and water uptake, and chemical changes, due to lipid hydrolysis, were revealed.

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