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SPECTRAL DISTANCE NUMERICAL APPROACH FOR THERMAL BEHAVIOUR CHARACTERIZATION OF ALBUMEN-BASED PAINTING COMPONENTS

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ABSTRACT. In this work, Attenuated Total Reflectance - Fourier Transform InfraRed (ATR - FTIR) spectroscopic technique has been employed in order to investigate the temperature behavior of albumen and of its mixture with trehalose/D_2O, the interest being associated with the wide employment of albumen in painting works both as a protective varnish and as a binder. Spectra were collected, in the 4000 cm^{-1} ÷ 400 cm^{-1} range, as a function temperature from 20°C to 80°C. The spectra profiles, in the whole spectral range and for all the temperature values, have been analysed by means of the Spectral Distance (SD) and of Cross Correlation Wavelet (XWT) approaches. In order to extract quantitative information from the comparison of the albumen and albumen/trehalose/ D_2O thermal behaviours, a logistic function has been adopted. The result of this approach shows that the albumen in the presence of trehalose/D_2O solution, in respect to albumen, is characterized by an increased stabilization temperature, furnished by the inflection point curve, together with a higher thermal restraint value, connected with the total SD and XWT variation with temperature.

1. Introduction

Egg white (i.e. albumen) consists mainly of water and functionally important proteins, such as ovalbumin, ovotransferrin, ovomucoid, ovomucin, conalbumin and lysozyme and also, in less quantity, carbohydrates, ash, and trace amounts of lipids. It is often found in paintings and is commonly used as a protective varnish and in egg tempera as a binder. Tempera is one of the oldest painting techniques: in the Middle Ages and up to the fifteenth century it was the most used technique in Europe to paint on wooden planks (Lechevalier *et al.* 2007; Cluff *et al.* 2016). Among the various types of tempera, the egg tempering is the one that offers the best requirements for a good resistance over the time and for a good visual result. It is different from modern tempers because the brushstroke is never completely opaque and then it is possible to work by overlapping colours or hatching lines. It is one of the most difficult techniques but it also has the advantage that, when finished, it is more pictorial than traditional tempera which are often more “graphic” and “cold” (Painter and Koenig 1976; Tuma 2005). Moreover, the albumen is very adhesive, impermeable and, after drying, hard and resistant and it is used for varnishing while for paintings the albumen is usually diluted with an equal amount of water. Due to the absence of fats, the pictorial film with only albumen is difficult to work due to its fragility and tends easily to chap. For these reasons, it is used for small surfaces, like the miniatures of the manuscripts, together with honey, glycerine and sugar (Andreotti *et al.* 2006; Karoui *et al.* 2007). In this framework the use of trehalose in paintings could play a precious role both in terms of long time protection and in terms of temperature restraint. Trehalose (α-Dglucopyranosyl-α-D-glucopyranoside) is a nonreducing disaccharide contained in several organisms which are able to survive in environmental stress conditions (Kandror *et al.* 2002; Magazù *et al.* 2013a). Although it has the same chemical formula of the other homologous disaccharides, e.g. sucrose and maltose, it shows different bioprotective effectiveness (Barreca *et al.* 2013). In particular, due to its unique water-structuring properties trehalose produces an environment around proteins that allows to improve their thermal stability (Abate *et al.* 2003; Minutoli *et al.* 2007) Furthermore, trehalose is considered as a water-structure maker, in fact the interaction between trehalose and water results much stronger than between water and water interaction (Ballone *et al.* 2000; Lokotosh *et al.* 2000). This property gives rise to its bioprotective action (Faraone *et al.* 1999). On this purpose, it is useful to remember the tardigrades, small water bears that are able to live in different extreme conditions, such as insufficient of oxygen, low and high values of temperature, excessive salinity and in the absence of liquid water in the environment. In all these cases the trehalose is the responsible of the behaviour of the tardigrades, because thanks to the synthetization of such a disaccharide, such organisms manage to live in conditions where other organisms fail (Magazù 1996). Neutron scattering experiments reveal that the structural and the dynamical properties of water are significantly affected by trehalose (Jannelli *et al.* 1999; Minutoli *et al.* 2008; Hennet *et al.* 2011; Marchese *et al.* 2017).Those trends confirm the special role of water in may structural processes, as well as the fundamental role of hydration water in in many biological functions, including proteins, (model and real) biomembranes (Kiselev and Lombardo 2016; Lombardo *et al.* 2016b, 2018). As far as the technique employed in the present study is concerned, Attenuated Total Reflectance - Fourier Transform InfraRed (ATR-FTIR) is a non-destructive technique widely used for the characterization of Cultural Heritage pigments (Bueno and K. Lednev 2013). FTIR is based on the interaction between electromagnetic radiation and matter; it allows to measure, in a definite wavelength range, the vibrational and rotational motions. From a general point of view, the range of a FTIR spectrum spans from 14000 $cm^{-1} \div 10$ cm^{-1} that can be splitted in three different sub-region, i.e. Near-IR (14000 $cm^{-1} \div 4000$ cm^{-1}), Mid-IR (4000 $cm^{-1} \div 400$ cm^{-1}) and Far-IR (400 $cm^{-1} \div 10$ cm^{-1}) (Migliardo *et al.* 2013b). It is also useful to remember that Infrared spectroscopy is complementary to other techniques, such as Raman scattering and neutron scattering (Migliardo *et al.* 2013a, 2014). In order to study the thermal behaviour of the investigated systems we have applied the Spectral Distance approach which allows to evaluate the deviation of each registered spectrum fromthe spectrum registered at the lowest temperature.

2. Sample preparation

Eggs have been acquired from a local market. Trehalose and Deuterium Oxide (D_2O) were purchased from Sigma Aldrich-Chemie. The investigated samples have been prepared by adding to albumen trehalose and double-distilled water solution (95wt% albumen/5wt% (trehalose + D_2O)) ATR – FTIR spectra were registered by means of the Vertex 70V spectrometer (Bruker Optics) using Platinum diamond ATR. The spectra were measured in absorbance mode and the spectrometer was configured to register a spectrum at 4 cm^{-1} resolution. The spectra were collected between 4000 cm^{-1} ÷ 400 cm^{-1} in the 25°C ÷ 80°C temperature range. Before proceeding with the data analysis some procedures of data preprocessing have been applied, i.e. the smoothing treatment, the baseline treatment, the first derivative treatment and the normalization of spectra. By means of Mathematica and Matlab the data have been analyzed.

3. Experimental data

Figure 1 reports, as an example, the ATR-FTIR spectra of albumen in the $400<\Delta\omega<4000$ cm^{-1} range for thermal scans, from T=25°C to T=80°C, while the Figure 2 shows, as an example, ATR-FTIR spectra of albumen in presence of trehalose/D_2O in the $400<\Delta\omega<4000$ cm^{-1} spectral range from T=25°C to T=80°C.

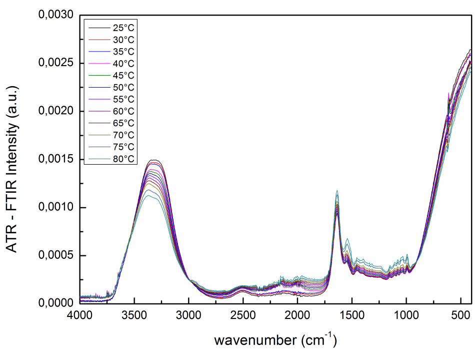

FIGURE 1. ATR-FTIR spectra of albumen in presence of trehalose and D_2O in the $400<\Delta\omega<4000$ cm^{-1} spectral range for thermal scans from T=25°C to T=80°C.

4. Results and discussion

In order to follow the thermal evolution and to extract quantitatively information, the whole spectral range has been evaluated by means of Spectral Distance, SD (Caccamo and Magazù 2017b; Caccamo *et al.* 2018b):

$$SD = \sqrt{[I(\omega,T) - I(\omega,T = 25°C)^2\Delta\omega]} \quad (1)$$

where $I(\omega,T)$ is the intensity at the frequency ω and at the temperature T and $\Delta\omega$ is the frequency resolution; SD allows to evaluate the deviation of each registered spectrum fromthe spectrum registered at the lowest temperature, that in the present study is equal

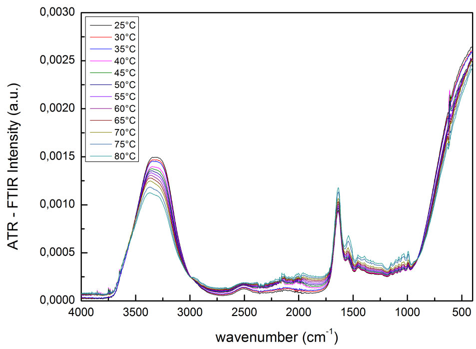

FIGURE 2. ATR-FTIR spectra of albumen in presence of trehalose and D_2O in the 400<$\Delta\omega$<4000 cm^{-1} spectral range for thermal scans from T=25°C to T=80°C.

to 25°C. In Figure 3 the SD behaviors referred to the spectrum at 25°C, for the albumen (green dots) and albumen in presence of trehalose and D_2O solution (purple circles) are reported. In order to extract quantitative information a logistic function has been adopted. More precisely, to obtain more information about the thermal behaviour, we have performed a fit by means of the following equation (Magazù *et al.* 2013b; Caccamo and Magazù 2016):

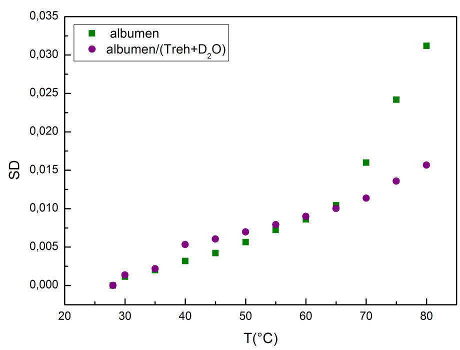

FIGURE 3. SD referred to the spectrum at 25°C, as a function of temperature, for the albumen (green dots) and albumen in presence of trehalose and D_2O solution (purple circles).

$$SD_L = \left(\frac{Ae^{-B(T-T_0)}}{1+e^{-B(T-t_0)}} \right) + C - DT \tag{2}$$

where A is the amplitude, whose inverse is connected to thermal restraint, B represents the relaxation stepness, T_0 is the curve inflection point and $C-DT$ represents a linear temperature contribution which overlaps to the registered relaxation. Figure 4 reports, on the left, the fit of the albumen spectra and, on the right, the fit of the albumen in presence of trehalose and D_2O solution spectra.

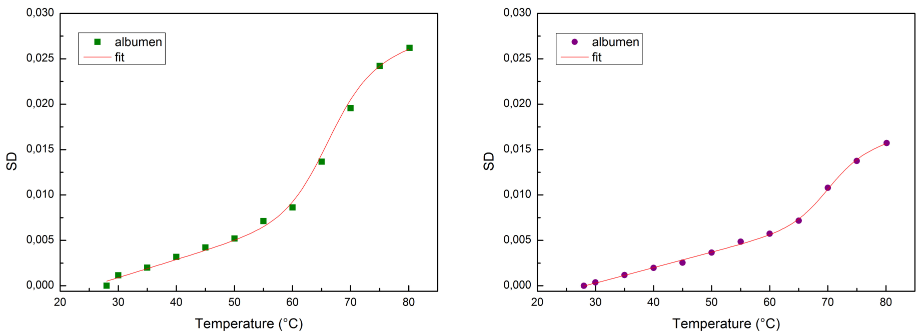

FIGURE 4. On the left: SD as a function of temperature of albumen spectra (green dots: experimental data; red continuous line: fitting curve by equation (2)). On the right: SD as a function of temperature for albumen in presence of trehalose/D_2O (purple dots: experimental data; red continuous line: fitting curve by equation (2)).

From this analysis it emerges that the value of the thermal restraint for the albumen in presence of trehalose/D_2O solution is equal to 135,13 together with a higher value of while the value of the thermal restraint for albumen is equal to 64,22 with a lower value of $T_0 = 70,1^{circ}C$. Such a result suggests that the addition of a small quantity of trehalose/D_2O to albumen gives rise to an increase of the temperature stability. To confirm the thermal behavior of these systems the wavelet cross correlation (XWT) has been applied (Grinsted *et al.* 2004; Veleda *et al.* 2012; Caccamo and Magazù 2017a,c; Caccamo *et al.* 2018a). Wavelet Transform (WT) is a powerful tool that has been applied to treatment of signals both the frequency and time domains (Caccamo and Magazù 2017d, 2018a), to engineering (Caccamo *et al.* 2017; Cannuli *et al.* 2018), to observe and to detect anomalies (Gilbert 2001) to analyse meteorological time series (Percival and Walden 2000; Caccamo *et al.* 2016; Colombo *et al.* 2018), neutron scattering data (Magazù *et al.* 2012, 2013c; Caccamo and Magazù 2018b), financial and medical data sets (Razdan 2004; Gallegati 2008). Moreover, the synergistic action of different types of soft interactions involved frequently influence the structural and dynamic properties of nanostructured material systems, and give rise to complex assembly processes that regulates the stability of a wide range of material systems (Lombardo 2014; Calandra *et al.* 2015; Lombardo *et al.* 2016a). Mathematically, WT allows to get correlation coefficients between the investigated signal and the so called wavelet mother. This latter, that can be scaled and shifted, includes a set of function (Daubechies, Coiflet, Morlet, Meyer, etc...) that can be used depending on the shape of the signal. To determine the WT, the following expression is used

$$WT(s,b) = \frac{1}{\sqrt{s}} \int_{-\infty}^{+\infty} y(t)\psi^{*}\left(\frac{t-b}{s}\right) dt \tag{3}$$

where $y(t)$ is the signal in the time domain, ψ is the mother wavelet, * is the complex-conjugate, $s > 0$ is the scale parameter and b is the shift parameter, finally $WT(s,b)$ provides the obtained coefficients as a function of the scale and shift parameters. For the present study we used, as mother wavelet, the Morlet wavelet:

$$\psi(t) = e^{-\frac{t^2}{2}} cos(5t) \tag{4}$$

In such a way, it is possibile to have one maximum in the Fourier space for each value of the scale parameter. There is a relation that associates to each scale the frequency (fr) with which the Fourier transform of the wavelet has a peak. For the Morlet wavelet the relation is $fr = \frac{5\pi}{2s}$. Let's focus attention on the wavelet transforms of two signals: $y(t)$ and $x(t)$, namely $WT_y(s,b)$ and $WT_x(s,b)$ and define the cross-wavelet spectrum:

$$WT_{yx}(s,b) = WT_y^{*}(s,b)WT_x^{*}(s,b) \tag{5}$$

and on the Wavelet Spectrum WS, as defined:

$$WS(s,b) = \int_{-\infty}^{+\infty} |WT(s,b)|^2 dt \tag{6}$$

This method allows to estimate the degree of similarity to which two signals are correlated, by shifting or leading one relative to the other. In this work we correlate couple of registered spectra to determine the the system thermal behaviour. On this purpose, to extract quantitative information from the spectra, we calculate the wavelet cross-correlation coefficients (CXWT):

$$CXWT = \frac{\int WT_{yx}(s,b)}{\sqrt{WS_y(s)WS_x(s)}} \tag{7}$$

where $WS_y(s)$ and $WS_x(s)$ are the Wavelet Spectrum of the signal $y(t)$ and $x(t)$, respectively.

In Figure 5 the calculated CXWT for albumen (a) and albumen in presence of trehalose/D_2O (b) in the 400±4000 cm^{-1} spectral range are reported. As it can be seen, the amplitude of albumen in presence of trehalose/D_2O solution results to be lower than that of albumen suggesting an higher thermal restraint for albumen in presence of trehalose/D_2O; in fact the value of the thermal restraint for albumen is equal 7,19 while that of albumen in presence of trehalose/D_2O that is equal to 18,42. Such a result confirms the conclusion obtained by SD, i.e. that by adding a small quantity of trehalose to albumen, the system becomes thermally more stable.

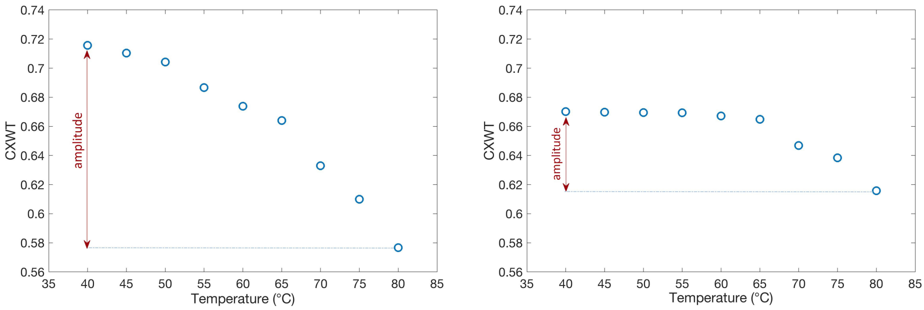

FIGURE 5. On the left: CXWT as a function of temperature of albumen spectra. On the right: CXWT as a function of temperature for albumen in presence of trehalose/D_2O.

5. Conclusions

The present work has been addressed to investigate the temperature behavior of egg white and of its mixture with trehalose/D_2O through ATR - FTIR. The collected ATR-FTIR spectra have been analyzed by means of the SD and XWT approaches. The obtained results reveal that adding a small quantity of trehalose/D_2O to albumen, the system increases its thermal stability. This finding can play a key role in painting works since albumen is widely used as a protective varnish and as a binder. However, the pictorial film with only albumen is difficult to work due to its fragility and therefore, it is used together with honey, glycerine and sugar. Therefore, the use of trehalose in paintings could play a precious role both in terms of long time protection and in terms of temperature restraint.

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