Assessment of poultry eggs freshness using FTIR spectroscopy combined with HCA and PCA methods

Ewelina Michalczyk[a], Rafał Kurczab[a,*]

[a] *State Higher Vocational School in Tarnów, Mickiewicza 8, 33-100 Tarnów, Poland*

Article history:
Received 6 June 2018
Received in revised form
21 June 2018
Accepted 23 June 2018
Available online 27 June 2018

Abstract

The main aim of this study was to investigate the use of Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR FTIR) and selected chemometric methods to classify eggs in terms of the laying hen farming method, as well as to identify changes in the individual egg compositions during storage. In total, 50 eggs were used for the study; 10 eggs per classes: 0, 1, 2, 3 and rural. Eggs were stored by 29 days period, which was divided on the 10 measuring days in which one egg from each class was tested by recording two FTIR spectra for the shell, albumen and egg yolk. The chemometric analysis, including Hierarchical Cluster Analysis (HCA) and the Principal Component Analysis (PCA), was performed based on the recorded FTIR spectra. Changes in chemical composition during the experiment in individual egg elements were analyzed. Furthermore, by analyzing the graphs (HCA and PCA) obtained by the chemometric analysis, it was noted that the largest changes in the chemical composition of eggs occurred in the shell and yolk, while in the albumen it was less insignificant. The chemometric analysis of the recorded spectra also showed that combination of chemometric methods and FTIR spectroscopy can potentially be used to develop a non-destructive method for classifying eggs in terms of the hen culture method and to monitor of their freshness.

Key words: egg freshness, FTIR spectroscopy, chemometrics

Introduction

Freshness and quality of eggs are very important for the health of consumers [1]. Changes occurring in eggshell, egg yolk and egg white (albumen) during storage are very complex and have a significant impact on the functional properties of eggs [2]. They are caused by gas exchange occurring through the pores of the shell and by the osmotic exchange taking place through the yolk membrane between the protein and yolk [3].

The decreasing quality of eggs is evidenced by weakening of the shell [1], weight loss, increase in the height of the air cell, increase of the pH of the protein and its liquefaction, etc. [3]. The egg freshness can be assessed by destructive and/or non-destructive methods [4]. Among destructive methods the sensory evaluation (involving determine the quality of eggs based on the senses of sight, taste, touch and smell) [2], the Haugh Units method (which combine the weight of an egg with the height of protein after it breaks down on a flat surface) [4], and by measuring the pH (for freshly laid eggs is in the range of 7.6–8.5 and increases over time) can be distinguished. An indicator of egg freshness, independent of the weight of eggs, the relative humidity of storage and the age of the hen, is present in the furosine protein, produced during the acid hydrolysis of Amadori compounds. The fact that this parameter shows high repeatability, low natural variability in fresh eggs and independence from the above-mentioned factors allows for an objective assessment of the quality and freshness of eggs [2].

Non-destructive methods are very important for the industry, which allow for unambiguous and quick assessment of the freshness and quality of eggs, without damaging them. The quality of eggs can be examined by the use of infrared spectroscopy. The advantage of using this method is that it enables non-destructive and multi-parameter measurement in a short time [2]. An important method allowing to test egg quality and freshness is Fourier transform infrared spectroscopy (FTIR - Fourier Transform Infrared Spectroscopy) [1]. It allows getting information about the structure of proteins, the interaction between them and other compounds. Another type of spectroscopy which can be used to assess the quality of eggs is fluorescence spectroscopy, which has a higher sensitivity compared to other spectroscopic methods. The fluorescent properties of aromatic amino acids that form proteins can be used to determine the structure of proteins and the interactions that occur between them. Infrared spectroscopy and fluorescence spectroscopy have the greatest potential to assess the freshness of eggs. Based on the data obtained through the application of these methods, it is possible to create a mathematical predictive model of freshness prediction of an unknown batch of eggs [2].

*Corresponding author: r_kurczab@pwsztar.edu.pl

The aim of this paper was to evaluate the possibility of using FTIR ATR spectroscopy and selected chemometric methods (i.e. HCA and PCA) for the problem of egg classification in terms of chicken farming methods and to determine changes in eggs during storage.

Materials and Methods

Samples

Tests were carried out on L-large grade A shell eggs purchased at the local grocery store (TESCO), representing 4 different rearing methods (Table 1), and one purchased at the local small-scale family farm. For each rearing method, measurements were conducted on 10 samples of eggs, each after storage at constant conditions (i.e. at a temperature about 20°C, and at constant humidity and light exposition) for 1, 3, 8, 10, 12, 15, 17, 22, 24 and 29 days.

Table 1. Characterization of the tested egg classes. The origin of egg classes was determined based on the codes presented on the shell of eggs purchased.

Rearing method	Class	Number	Code	Origin	Expiration date[b]
0 (organic)	A	10	0-PL 26121311	świętokrzyskie, district staszowski	11.03.2017
1 (free range)	A	10	1-PL 30211341	wielkopolskie, district poznański	14.03.2017
2 (barn)[a]	A	10	2-PL 30291366	wielkopolskie, district wolsztyński	17.03.2017
3 (cage)	A	10	3-PL 30251340	wielkopolskie, district średzki	24.03.2017
small-scale family farm	N.A.	10	N.A.	małopolskie, district dąbrowski	N.A.

[a] i.e. the deep litter indoor housing,

[b] the experiment started at 04.03.2017 (the first measuring day).

Data collection

A Thermo Scientific Nicolet iS5 FTIR Spectrometer was used. The equipment included an accessory with a ZnSe single-reflexion ATR crystal (provided an angle of incidence of 45°). As a reference, the background spectrum of air was collected before the acquisition of the sample spectrum. After each sample, the crystal was rinsed first with a 98% ethyl alcohol solution, next with distilled water (to avoid denaturing egg samples), and then dried with a soft tissue. Spectra were recorded with a resolution of 2 cm^{-1}, and 32 scans were averaged for each spectrum (scan 4000–650 cm^{-1}).

The measurement consisted of recording FTIR spectra of the outer part of the egg shell, albumen and egg yolk representing each of the tested classes of eggs. For each egg, the spectra were collected in duplicates, taking a new sample of each part of the egg to minimize the inaccuracy related to sample heterogeneity.

Each egg was broken and placed in a beaker. To obtain an albumen spectrum a small amount of sample was taken with a needle and syringe and placed on the ATR plate. For the egg yolk sample, a new syringe was used to avoid mutual contamination of albumen and yolk samples. The spectrum of the egg shell was made by breaking off a small fragment of it and placing on the ATR plate in a way that the outer side of the shell adheres to the surface of the crystal. In total, 30 spectra were obtained during one measurement day, while 300 spectra were recorded during the whole experiment. A SpectraGryph program was used to visualize and transform the recorded spectra [5].

Chemometric analysis

The data of FTIR spectra is a set of multiple variables (wavenumbers and absorbance), which contains overlapping information. Multivariate data analysis makes possible to extract useful information from original spectral data, eliminate much overlapping information, and reduce the dimension of data. In order to perform chemometric analysis, recorded spectra have been encoded according to the following algorithm: the first two symbols denoted the class of egg (i.e. k0 – class 0, k1 – class 1, k2 – class 2, k3 – class 3, kw – from a local farm), the next digit in the label means the measurement day, the next symbol indicated the type of material of egg being tested (i.e. s – egg shell, b – albumen, and z – yolk), and the last symbol denoted the measurement number of the tested material (1 – the first measurement, 2 – the second measurement). The chemometric

analysis was performed using the RStudio [6] environment, by means of the ChemoSpec library [7]. As a result of the analysis, the Hierarchical Clustering Analysis (HCA) and Principal Component Analysis (PCA) plots were obtained. The HCA analysis was performed using the complete clustering method and Euclidean distance measure.

Results and Discussion

Spectra analysis

The aligned FTIR spectra of egg shell, albumen and yolk for all studied egg classes on day 1 were shown in Figure 1. The egg shell spectra (Figure 1A) are characterized by the presence of a wide and intense band in the 3700–3000 cm^{-1} range originating from the O-H stretching and N-H stretching vibrations of the peptide bond (amide A band). The two bands at 1650 and 1550 cm^{-1} wavenumbers are connected with vibrations of the amide I protein groups (combination of C=O stretching, N-H deformation, and C-N deformation vibrations) and amide II (C-N stretching, N-H deformation), respectively. The band appearing at approximately 1400 cm^{-1} is associated with the presence of carbonate groups in the egg shell (in calcium carbonate). The band in the 1170–990 cm^{-1} region may come from the polysaccharides contained in the cuticle covering the egg shell. During the experiment, the largest changes in FTIR spectra of egg shells of all classes occurred in the areas of (Figure 2): O-H stretching group (mainly from water), an amide A, amide I, amide II, and bands coming from vibrations of carbonate and polysaccharides groups. Changes in the intensity of the peak originating from the O-H stretching vibrations in water indicated that over time the water content in the egg shell decreases which is related to gas exchange processes and storage conditions. Changes in the intensity of the remaining bands may result from the degradation or formation of the compounds forming the shell, as well as from the inhomogeneity of the material tested.

The FTIR spectra of albumen contain three bands at the wavenumbers identical to water spectra [8]. In the range 1550–1500 cm^{-1} of the FTIR spectra of albumen (Figure 1), a narrow band with low intensity, and partially covered by a water band is visible. Due to the fact that in an egg albumin, apart from a water, contains proteins, therefore it can be assumed that this band comes from C-N stretching vibrations and bending of N-H peptide bond (amide II band) [9]. Moreover, it can be concluded that during the storage the water content in the albumen decreases, which can be caused by gas exchange through the porous shell, as well as the diffusion of water into the yolk through the vitelline membrane.

The FTIR spectra of egg yolk (Figure 1) also show many similarities between the studied egg classes. The wide band in the 3700–3000 cm^{-1} range comes from the O-H stretching vibration of water molecules, and also from the N-H stretching vibrations of peptide bonds (amide A band) [9]. In the yolk spectrum, bands appeared at the 2900 and 2850 cm^{-1} wavenumbers can be associated to the stretching vibrations of the C-H group in lipid molecules. The band at the region of 1750 cm^{-1} can be connected with stretching vibrations of the C=O group of saturated aliphatic esters. In the areas of 1650 and 1550 cm^{-1}, there are two amide bands (respectively: amide I and II) associated with vibrations of peptide bonds of proteins. During the experiment, the largest changes in spectra are visible in the area of the stretching vibrations of O-H group and amide A band. Changes in the intensity of the amide I and II bands may indicate a breakdown of peptide bonds, and thus an increase of free amino acids in egg yolks.

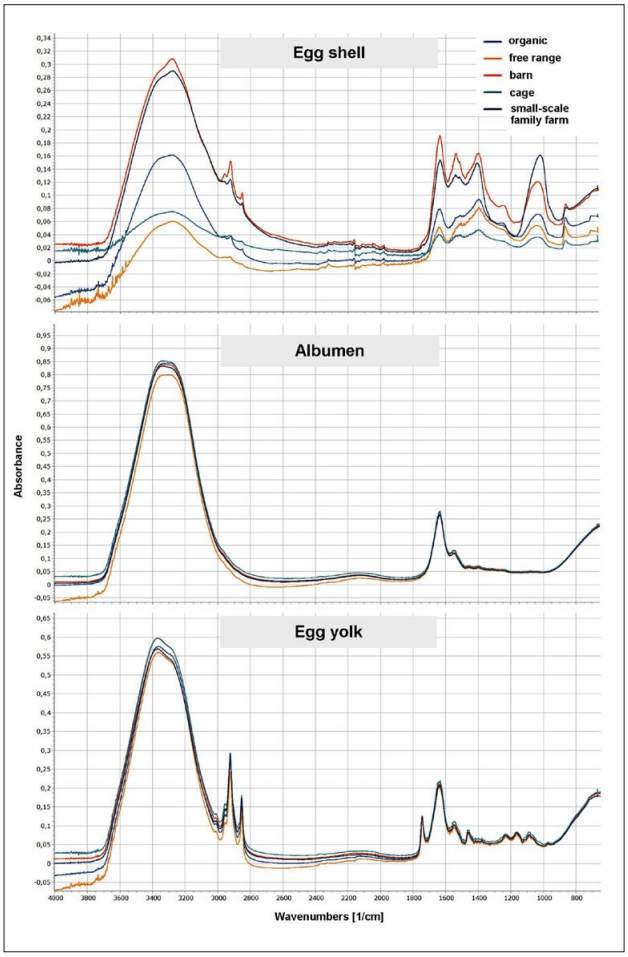

Figure 1. Alignment of the FTIR ATR spectra of all egg classes measured at the beginning of the experiment (day 1) for egg shells, albumen, and yolk, respectively

Evaluation of egg classification possibilities based on spectroscopic measurements

The chemometric analysis was performed using the full FTIR range (4000–650 cm^{-1}). At first, the analysis was focused on discrimination properties for egg classes using FTIR spectra of an individual part of the eggs (i.e. shell, albumen, and yolk) at the beginning of the experiment (i.e. day 1). The second analysis was performed using FTIR spectra recorded for all measurement days and was aimed to illustrate the dynamics of changes of individual egg samples during aging.

The HCA plot (dendrogram) obtained for egg shells (Figure 2) indicated that egg class 1 and 2 were the most similar from the remaining ones because they originate from the same branch of the dendrogram. In addition, it can be seen that the egg shells of class 0 and 3 are not chemically homogeneous, because the first and second measurement of their spectrum have been classified into two different branches, which indicates differences in the spectra between the first and the second measurement, as well as the similarity of the egg shells belonging to these classes. The shells of eggs from the local farm showed the highest similarity to shells of class 0 and 3 tested during the second measurement. Similar conclusions can be drawn from the analysis of the PCA plot for egg shells (Figure 2), where the five main clusters can be indicated. The identity of the spectra obtained for egg shells of class 1 caused there overlap, therefore, the cluster representing this class was illustrated as one point.

For albumen, the dendrogram (Figure 2) showed that rural egg had the lowest similarity to the remaining egg classes because they form a separate branch in the graph. In addition, it can be seen that class 0 had a small heterogeneity because their spectra for measurement 1 and 2 have been classified into different branches. The fact that the branch representing egg class 1 and 2 eggs is divided into two groups at a distance slightly higher than 0 shows that the egg albumens of these classes are very similar to each other. Identical conclusions can be drawn on the basis of PCA plot analysis that albumen of particular egg classes do not show significant differences in chemical composition, however, by using chemometric analysis it is possible to recognize the individual classes.

When it comes to the HCA plot generated on the FTIR spectra of egg yolks, it can be concluded that class 0 is the least similar in chemical terms to the remaining classes (forms a separate branch). Interestingly, egg yolks of rural and class 3, despite the fact that they originate from one branch, were also classified into separate groups, which indicates the differences in the chemical composition of egg yolks of these classes. The fact that

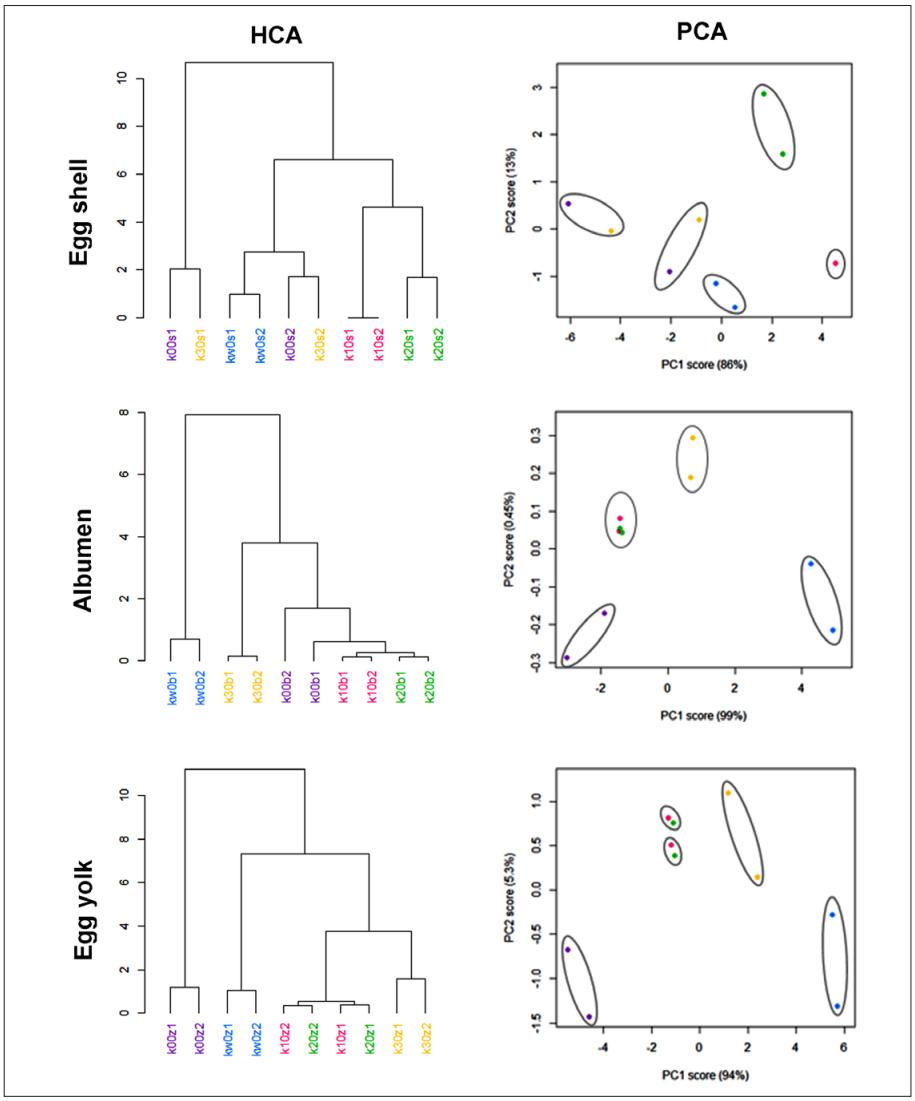

Figure 2. The HCA dendrograms (Euclidean distances are marked on the Y-axis) and PCA plots obtained for egg shell, albumen and yolk individually based on the FTIR spectra measured at the beginning of the experiment (i.e. day 1). For each egg fragment, two spectra were recorded

the branch of egg yolk of class 1 and 2 branches off at a distance slightly higher than 0, indicated that the egg yolks of these classes are the most chemically similar to each other. The spectra of egg yolk classes 1 and 2 from measurement 1 were classified to a different branch, then the spectra of egg yolk classes 1 and 2 from measurement 2, which may indicate similarity in the chemical composition of the tested samples, as well as the heterogeneity of egg yolks of these classes. The same conclusions can be drawn from the PCA plot for egg yolks, where five clusters (out of which three collected egg yolks of the same classes) were distinguished. The egg yolks of class 1 and 2 from the first measurement and the egg yolks of these classes from the second measurement have been grouped into two separate clusters located close to each other. This may indicate that the egg yolks of these classes show a high similarity in terms of chemical composition.

To determine the dynamics of changes occurred in eggs during 29 days of storage, the PCA plots for all recorded FTIR spectra were generated (Figure 3). In general, the results indicated that the major changes in the chemical composition occurred for egg shells of class 0 and 3 (they showed the greatest dispersion in variables space). At the same time, it can be stated that the smallest changes occurred in egg shell of class 1, 2 and rural eggs – the corresponding points are closely related, only a few showed significant differences, which resulted from inhomogeneity of samples. The points corresponding to the albumen of individual classes formed a dense group (with only a few exceptions) which indicated that during the experiment changes in the chemical composition of albumen were not significant. This conclusion is consistent with the spectra of proteins of particular classes during the experiment indicates that the largest changes in the chemical composition occurred in class 0 and egg yolk (purple points representing this class are the most dispersed in the space of variables, see Figure 3). Nevertheless, significant changes in the chemical composition during the experiment also occurred in egg yolk class 1–3 (points corresponding to the egg yolks of these classes also showed a clear dispersion).

In summary, during storage of eggs, the prominent change in chemical composition occurred in egg shells and yolks. The lowest area of variable spaces occupy points representing the albumen of all classes during the experiment, which indicates a small change in their chemical composition during the experiment.

Conclusions

It can be concluded that there is a possibility of classification of eggs in terms of the chicken farming method using a combination of FTIR spectroscopy and chemometric methods. Despite small differences in recorded spectra between individual elements of all egg classes, it is possible to assign eggs to individual classes, both on the basis of measurements of the egg shell spectra as well as albumen and yolk. The chemometric analysis used to monitor the dynamics of changes in chemical composition occurred in eggs during storage showed that the changes in albumen are the least visible, while the largest ones occurred in shells and yolks. It means that a non-destructive discrimination/classification model of the egg freshness assessment based on the measurement of egg shell FTIR spectra may be generated. Of course, a broader experiment (e.g. containing more egg classes, more spectra measurement, and application of machine learning algorithms) have to be carefully planned and performed.

It is also worth noting that despite the fact the tested eggs of class 0–3 had different expiration periods, and during the experiments, no significant changes in the spectra of individual egg fragments were noticed after exceeding the asset date of consumption. This outcome indicates that duration of the further experiments should be extended to even 60 or more storing days to catch any significant changes after the expiration days.

Moreover, the presented experiments show that the FTIR method in combination with the chemometric analysis allows

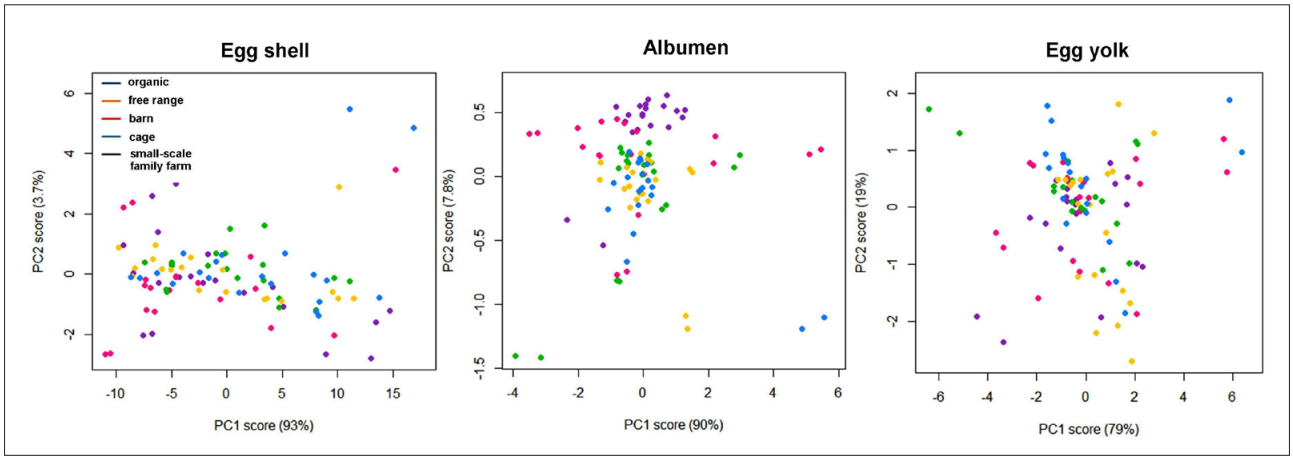

Figure 3. The PCA plots illustrating the distribution of the FTIR spectra of all five classes of eggs during the 29 days of storage under constant conditions divided into egg shell, albumen, and yolk

the classification of eggs (according to their classes) and may be used as the basis for the development of more sophisticated tool based on the machine learning (i.e. artificial intelligence).

Acknowledgments

This study was supported by the statutory funds of the State Higher Vocational School in Tarnow, Poland.

References

1. Rodríguez-Navarro AB, Dominiguez-Gasca N, Muñoz A, Ortega-Huertas M. Change in chicken eggshell cuticle with age and egg freshness, Poultry Science, 2013, 92, 3026–3035.
2. Karoui R, Kemps B, Bamelis F, de Ketelaere B, Decuypere E, de Baerdemaeker J. Methods to evaluate egg freshness in research and industry: A review, European Food Research and Technology, 2006, 222, 727–732.
3. Giunchi A, Berardinelli A, Ragni L, Fabbri A, Silaghi FA. Non-destructive freshness assessment of shell eggs using FT-NIR spectroscopy, Journal of Food Engineering, 2008, 89, 142–148.
4. Abdel-Nour N, Ngadi M, Prasher S, Karimi Y, Prediction of Egg Freshness and Albumen Quality Using Visible/Near Infrared Spectroscopy, Food and Bioprocess Technology, 2011, 4, 731–736.
5. Menges F, Spectragryph – optical spectroscopy software, Version 1.0.7, 2017, http://www.effemm2.de/spectragryph/
6. R. Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
7. Hanson BA. ChemoSpec: Exploratory Chemometrics for Spectroscopy, 2016. R package version 4.4.x.
8. https://webbook.nist.gov/cgi/cbook.cgi?ID=C7732185&Type=IR-SPEC&Index=1
9. Barth A. Infrared spectroscopy of proteins, Biochimica et Biophysica Acta – Bioenergetic, 2007, 9, 1073–1101.