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BOOK OF ABSTRACTS

P-121

MODELING AND PREDICTING SECOND and THIRD-ORDER FLUORESCENCE SPECTROSCOPY DATA AS A NOVEL QUALITY CONTROL STRATEGY ON MAYONNAISE

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Fluorescence spectroscopy has found in food analysis a large use due to that it is fast, gives direct measurement, is not destructive and noninvasive [1].

In this work, the potential of excitation emission fluorescence matrix (EEM) data recorded with a front face system along with chemometric methods was investigated for the determination of spoilage on mayonnaise and for the rapid prediction of changes of microbial flora (counts of bacteria).

Made in home mayonnaise and commercial mayonnaises samples were maintained at 25°C during four days, and stored at 37°C for three days. The microbial load on mayonnaise samples was determined per day. Total viable count (TVC), aerobic mesophilic bacterias, Staphylococcus aureus, Escherichia coli and Salmonella were determined on mayonnaise at each step using culture methods.

In parallel, mayonnaise samples were analyzed by 3D front-face fluorescence spectroscopy, without prior preparation, at 8 and 6 different times for the samples stored 25 °C, and 37°C, respectively. The data matrices were recorded varying the excitation wavelength between 230 and 400 nm each 10 nm, and registering the emission spectra from 300 to 600.5 nm each 0.5 nm. Thus, the EEMs were of size 18×602. PARAFAC analysis allowed capturing the changes occurring in the fluorescence spectral data. The best PARAFAC models showed 3 components for data recorded both temperatures. Profiles of main compounds were extracted with this algorithm describing quality evolution on the time. In order to meet those compounds that their concentration decreases and those that are produced (specifically amino acids) a chromatographic analysis was performed [2]. The chromatographic analysis confirms the decrease of tyrosine and the production of tryptophan in the time.

Partial least squared discriminant analysis (PLSDA) was applied to data set formed by concatenating of all the fluorescence spectra at same temperature for testing the allocation of the spectra of the individual samples within the five and four groups corresponding to the five and four investigated storage times. The results showed that 100% of good classifications were obtained using 3 PLS factors. These results allowed the classification of samples as a function of stored time. In addition, this information can be related to the microbial counts.

In order to evaluate other quality parameters, a study with optical fibers is being performed. EEMs were obtained through the degradation kinetics of mayonnaise produced by irradiation the samples each two minutes for a total of 20 minutes. Third order data analysis will be directed to the fat content evaluation as well as at the content of different dressing of mayonnaise for obtaining information about the quality mayonnaises.

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P-144

COMPARATIVE STUDY OF DIFFERENT THIRD-ORDER DATA GENERATION APPROACHES

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Over the last years, it has been demonstrated that the increase of multiway data dimensions has a positive impact on analytical figures of merit, e.g. higher sensitivity, lower limits of detection and quantitation, better selectivity, among others. First- and second-order data analyses have become excellent tools for the resolution of complex samples which would result experimentally challenging from the univariate calibration standpoint. On the other hand, even though no additional analytical advantages have been yet proved, third-order data analysis for analytical applications constitutes a field worth to be explored [1].
Although multidimensional instrumental signals are easy to be obtained with the available modern instrumentation, and several chemometric algorithms have been successfully developed to solve multi-way data problems, the way in which the multi-way data are generated may have a significant effect on the final results. In this work, a comparative study of different third-order data generation approaches was carried out. Three methods based on identical liquid chromatographic conditions but coupled to different emission and excitation fluorescence detection systems were developed for the quantitative analysis of antibiotics in aqueous matrices.
The first approach included the collection of several fractions at the end of the chromatographic procedure by means of a custom-built device that allows to collect fractions in a 96-wells ELISA plate. Then, emission and excitation spectra were registered for every fraction by using a spectrofluorometer equipped with a plate reader accessory coupled to an optical fiber and a gated photomultiplier. In this way, 25 emission-excitation matrices (EEM, size: 17×25) were obtained for each chromatographic run. [2] A second strategy was developed by using a 10 µL flow-cell connected at the end of the chromatographic instrument and placed in a fast-scanning spectrofluorometer. Here, it was possible to register sequential EEMs for a unique chromatographic run. Since the fast-scannig spectrofluorometer takes only few seconds to register each EEM, it was necessary neither to stop the chromatographic flow nor to collect fractions after the chromatographic procedure, and 25 sequential EEMs (size: 7×45) were obtained for each chromatographic run. Finally, a multi-chromatographic run method involving a liquid chromatographic instrument coupled to a fast-scanning fluorescence detector which allowed to register time-emission fluorescence data matrices in a specific spectral range at a fixed excitation wavelength was developed. In order to build excitation-emission data matrices, eight chromatographic runs at different excitation wavelengths (time-emission matrix size: 45×150) were required for the same sample.
The three methodologies aforementioned were evaluated using different algorithms, such as PARAFAC, *A*PARAFAC, PLS-RTL and MCR-ALS, and selectivity, sensitivity, robustness, and time processing were evaluated. Since the data generation was different, each methodology required a particular data pre-processing including smoothing, peak alignment, and baseline correction, among others. Furthermore, due to differences in sensitivity provided by the implementation of a variety of detection mode it was necessary to assess several sample preparation methods in order to reach good analytical figures of merit.

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P-145

THIRD ORDER MULTIVARIATE CALIBRATION APPLIED TO THE QUANTITATION OF AZINPHOS-METHYL IN FRUITS BY EXCITATION-EMISSION-KINETIC MEASUREMENTS

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Azinphos-methyl (AZM) is a widely used organophosphate insecticide and acaricide, with demonstrated negative impacts on the environment [1]. The weak natural fluorescence of AZM in aqueous solution can be enhanced in a variety of ways, including UV photolysis, inclusion into cyclodextrins, and base hydrolysis. Upon absorption of UV-A radiation, this molecule undergoes photolysis to the highly fluorescent compound N-methylanthranilic acid, which undergoes subsequent photolysis to photochemically stable products [2].

Four-way data generated by excitation-emission fluorescence matrices (EEFMs) measured as a function of reaction time can be modelled with appropriate higher order algorithms to achieve the second order advantage and improve both the selectivity and sensibility of the method.

In this work, we developed a fluorescent kinetic method for the determination of AZM in fruit samples. It is based on third-order data, obtained by measuring the time evolution of the EEFMs of the photolysis of AZM in alkaline medium.

The experiments were carried out with an instrument consisting in an optic fiber connected to an UV radiation source, coupled to a fluorescence spectrophotometer. The matrices were recorded in a quartz cell of 1 cm of path length. The readings were made in the excitation range of 220 to 320 nm every 5 nm, and emission from 320 to 500 nm every 5 nm at a scan rate of 24,000 nm min^{-1}. EEFMs were measured every 30 seconds for six minutes, thus having 21 × 33 × 13 data points.

Calibration and validation sets, consisting in five concentration levels of AZM each, were analyzed. The validation samples were prepared by adding fuberidazole, bitertanol and thiabendazole as uncalibrated interferences. Data modelling was performed with the algorithms MCR-ALS, PARAFAC and U-PLS/RTL. Relative error prediction and analytical figures of merit were calculated. The results obtained were similar for the three algorithms.

The method was further used for the quantitation of AZM in apple, pear, peach and plum. For this purpose, the standard addition calibration method was used in order to overcome matrix effect, which involved spiking three levels of AZM standard to the real samples. The sample preparation procedure consisted in an extraction with acetonitrile followed by dispersive liquid-liquid microextraction. Recoveries and figures of merit were also calculated for real samples.

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P-170

FORENSIC SINGLE FIBER DISCRIMINATION BY COMBINING EXCITATION-EMISSION FLUORESCENCE MICROSCOPY WITH LINEAR DISCRIMINANT ANALYSIS

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The potential of total excitation-emission fluorescence microscopy combined with multi-way linear discriminat analysis (LDA) was investigated for the forensic analysis of visually indistinguishable textile fibers. Forensic fiber comparison involves one questioned fiber (i.e the fiber found in the crime scene) versus one or more fibers (called "known fibers") collected from a textile of the same color belonging to a suspect. Our approach takes fluorescence microscopy to a higher level of selectivity with the collection of excitation emission matrices (EEMs) [1, 2]. For that, four pairs of visually indistinguishable fibers from four different textiles were selected. The fibers consisted of nylon 361 dyed with acid yellow 17 and acid yellow 23, acetate satin 105B dyed with disperse blue 3 and disperse blue 14, polyester 777 dyed with disperse red 1 and disperse red 19, and acrylic 864 dyed with basic green 1 and basic green 4, and were investigated using a non-destructive approach, which is of paramount importance to preserve the physical integrity of the fibers for further court examination. Excitation emission matrices were recorded with the aid of an inverted microscope coupled via a bifurcated fiber-optic probe to a commercial spectrofluorimeter [3]. The full information content of excitation-emission matrices was processed with the aid of PARAFAC supervised by linear discriminant analysis (LDA), and discriminant unfolded partial least squares (DU-PLS). The ability of the latter algorithm to classify the four pairs of fibers, and distinguish the questioned fiber and the known fiber to exclude the possibility that both could have originated from a common source, demonstrates the advantage of using the multidimensionality of fluorescence data formats for the non-destructive analysis of forensic fiber evidence.

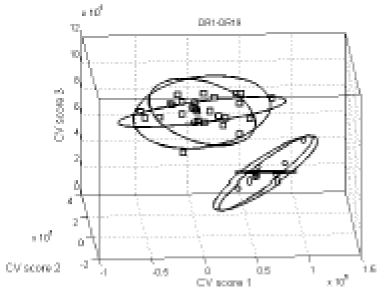

Figure 1.- LDA canonical variate (CV) scores (3 components model) for 40 samples of disperse red 1 (DR 1) (1 fiber, 10 replicates; circles) and disperse red 19 (DR19) (3 fibers, 10 replicates each; squares) polyester 777 fibers.

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P-205

CHEMOMETRIC METHODS TO ENHANCE SPECTRA QUALITY AND EVALUATE DATA OBTAINED BY A NOVEL LASER-BASED IR TRANSMISSION SETUP FOR PROTEIN ANALYSIS

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Chemometrics has been proved to be an excellent complementary tool in several research fields. In combination with advanced instrumentation technology, it could significantly improve the performance of analytical methods. In the analysis of protein secondary structure by infrared spectroscopy, the limits of the method are defined by the high absorption of the solvent (i.e. water) as well as the low emission powers of the thermal light sources commonly employed in conventional FTIR spectrometers. Recently, a novel IR transmission setup has been developed, employing an external cavity-quantum cascade laser (EC-QCL) as light source that provides spectral power densities several orders of magnitude higher than thermal sources. In spite of being commercially available, these new light sources still suffer from imperfections in the tuning mechanism and shifts in the mode-hope fine structure of the emission curve within consecutive scans. To overcome this constant and variable offset between scans, correlation optimized warping (COW) was applied. This well-known algorithm, previously employed for spectra and chromatographic peak alignment, is here used to eliminate high noise levels in absorbance spectra obtained by QCL-IR spectroscopy [1].

Furthermore, to showcase the potential and quality of the IR absorbance spectra obtained by QCL-IR spectroscopy, dynamic changes of proteins secondary structure in aqueous solution were studied at varying pH values and across a wide concentration range. To this end, extended-MCR-ALS was employed. Pure spectral and concentration profiles of the temporal transition between different protein conformational structures were obtained [2].

We successfully demonstrate the application of COW to significantly reduce the noise level in IR spectra acquired with EC-QCL, thus getting this novel type of light source ready for routine measurements. The high quality of the obtained spectra has allowed to build chemometric models for describing dynamic protein secondary structure changes at varying experimental conditions.

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