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Monitoring of *Brettanomyces bruxellensis* development in wine with RAMAN spectroscopy, 3D-FFFS, Flash-GC and chemometric tools

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Keywords : *B. bruxellensis*, ICA, signals, Raman, fluorescence, Flash-GC, yeast, detection.

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1. Abstract

Brettanomyces bruxellensis spoilage causes many losses in winemaking industry by volatile phenols production. The use of different spectroscopic tools such as Raman spectroscopy and 3D-Front-Face-Fluorescence Spectroscopy (3D-FFFS), in combination with chemometric tools allow for detecting this yeast growth in a ultra-fast way. Different *B. bruxellensis* concentration have inoculated in *Saccharomyces cerevisiae* fermentations of grape juice. Signals detected by Independent Component Analysis (ICA) using these spectroscopic tools after the analysis of wine samples, in combination with flavor detection by Flash-Gas Chromatography (Flash-GC), confirm the detection of wine contamination with *B. bruxellensis* at different stages.

Keywords: *B. bruxellensis,* ICA, signals, Raman, fluorescence, Flash-GC, yeast, detection.

1.1. Resumen

La contaminación *por Brettanomyces bruxellensis* causa muchas pérdidas en la industria vitivinícola por la producción de fenoles volátiles. El uso de diferentes herramientas de espectroscopía como la espectroscopía Raman y la espectroscopía frontal de fluorescencia (3D-FFFS), en combinación con herramientas quimiométricas, permite detectar el crecimiento de esta levadura de manera ultra rápida. Diferentes concentraciones de *B. bruxellensis* fueron inoculadas en fermentaciones de mosto de *S. cerevisiae*. Las señales detectadas con Independent Component Analysis en estas espectroscopías después del análisis de estos vinos, en combinación con la detección de aromas con la Flash-Gas Chromatography (Flash-GC), confirma la detección de una contaminación en vino por *B. bruxellensis* a diferentes tiempos.

Palabras clave: *B. bruxellensis,* ICA, señales, Raman, fluorescencia, Flash-GC, levadura, detección.

2. Introduction

Wine is one of the most popular drinks in the world. Wine is composed by water (highest quantity), ethanol (10-14%), glycerol, sugar, organic acids (tartaric acid, gallic acid, e. g.), polyphenols and anthocyanins (in rosé and red wines). This fermented beverage is produced by different strains of yeasts, mostly *Saccharomyces cerevisiae* or *S. uvarum.* During alcoholic fermentation of grape juice, yeasts degrade present sugars into the grape juice, producing ethanol and different aromatic compounds which change the aromatic profile of wines (Belda, Navascués, Alonso, Marquina, & Santos, 2014; Dubourdieu, 2003; Geffroy et al., 2015).

However, in grape and wine microbiota, presence of microbial contaminants are very common and unavoidable. Microorganisms such as the yeast *Brettanomyces bruxellensis* could modify the flavor of wines and tasty characteristics, producing losses in winery industry (Agatonovic-Kustrin, Morton, & Pauzi Md Yusof, 2013; Navascues & López-cordón, 2017). They appear on the grape surface, with other microorganisms such as *Saccharomyces* or LAB (Barata & Loureiro, 2012) and their development is spontaneous and depending on several factors, like residual sugars or nitrogen amount (Belda et al., 2014). Their growth is mainly related to the final stage of alcoholic fermentation, when *Saccharomyces* has finished consuming all sugars and has produced lots of ethanol as competence factor to inhibit the yeast growth of other genres. *B. bruxellensis*, on the other hand, can grow in this condition, and profit residual sugars and others molecules to increase *B. bruxellensis* population without competence by the presence of ethanol. (Navascues & López-cordón, 2017). *B. bruxellensis* receives the name by the production of small amount of molecules which causes defects in wine, decreasing quality and price of product (*B. bruxellensis* produces ethyl-phenols from hydroxycinnamic acids, which smell like horse sweat or stable) (Navascues & López-cordón, 2017).

Furthermore, quality control in winery industry cannot prevent and detect efficiently the presence of this contaminant. There are different available methods to detect wine spoilage by *B. bruxellensis* species. *B. bruxellensis* also resists to different

conditions such as high quantity of SO_2, ascorbic acid or other substances used to sterilize foods most often in liquid state (Navascues & López-cordón, 2017).

B. bruxellensis growth is very slow in comparison with *S. cerevisiae,* in winery conditions. The *S. cerevisiae's* alcoholic fermentation competence reduces growth rate of *B. bruxellensis* population(Navascues & López-cordón, 2017). On the other hand, when alcoholic fermentation has finished, sugars content is low and ethanol content is a limiting factor for *Saccharomyces* facilitating a very slow growth of *B. bruxellensis*, producing off-flavors in wine. These off-flavors are composed by volatils chemical components (fermentation secondary products) derived from ethyl phenols of wine, whose degradation by a secondary metabolism of *B. bruxellensis* leads to losses of wine quality (Carbó, Ginovart, & Vias, 2008; Martin et al., 2015; Navascues & López-cordón, 2017). The slow growth of *B. bruxellensis* causes some difficulties to detect this microorganism, because microbiological methods are very slow and unsuited. Microbiological cultures in optimal conditions to *B. bruxellensis* provide true results in 72 h or more (Carbó et al., 2008; Navascues & López-cordón, 2017), so it's relevant to detect in time and prevent. Further studies report decreases of SO_2 content in wine with the presence of *B. bruxellensis* (Navascues & López-cordón, 2017). Indeed, SO_2 can be a secondary metabolite in *B. bruxellensis* metabolism. But, on the other hand, this decrease could be visible in the stationary phase, when the secondary metabolites are being produced.

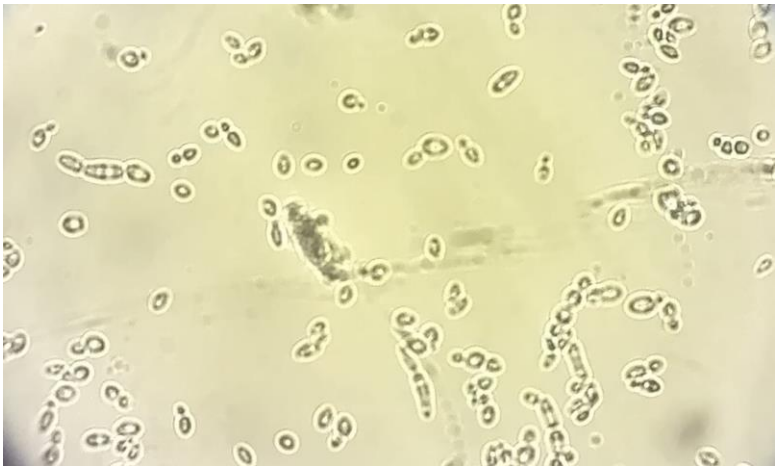

Image 1 : *Brettanomyces bruxellensis* culture observed with optic microscope (White light, x100) (Olympus Life Science, Rungis, France).

Spectroscopic methods are the most widely used analytical techniques. Non-invasive methods such as Near Infra-Red (NIR) spectroscopy, fluorescence spectrometry or Raman spectroscopy use different physical properties of molecules/particles to perform the measurement. One of these measures is Raman Spectrometry. The fundament of Raman Spectroscopy consists in the dispersion of different and specific wavelength, product of vibration and rotational properties of the particles, after a light pulse of a specific wavelength (Dent, 2005; Martin et al., 2015; Rodriguez, Thornton, & Thornton, 2013; Schulz & Baranska, 2007; Wang, Li, Ma, & Liang, 2014). The incident photon of laser interacts with the molecules producing an excitation state of them and scattering the light in a different wavelength (Stokes effect).

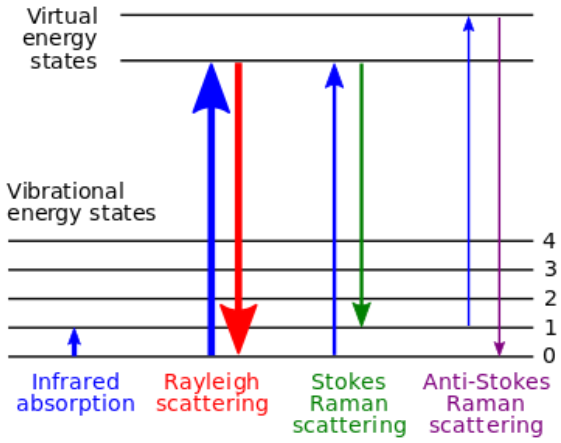

Image 2: Theory of Raman spectroscopy.

The use of this technology give us a good means to determine changes in all type of materials, being an useful tool in analytical chemistry for recording fingerprints of foods and for predicting food spoilage. The speed of this technique and the non-invasive capability allow us for detecting changes quickly and by a cheaper way. (Dent, 2005).

The use of 3D-Front Face Fluorescence Spectroscopy is a recent methodology to detect variations of molecules concentration in wine. Following the excitation-emission principle of the molecules to emit fluorescence, this spectroscopy is especially fast and sensible to detect variations in liquid and solid samples (Airado-Rodríguez, Durán-

Merás, Galeano-Díaz, & Wold, 2011; Saad, Bouveresse, Locquet, & Rutledge, 2016). This technology can perceive rapidly some markers to detect the development of the fermentation and the optimization of the process.

Other technology for monitoring the development of the winery process is the Gas Chromatography, where the sample's headspace is analysed to find some compounds produced by yeast and to detect an increase of volatile acidity (traduced by a rise of acetic acid production), for example. Gas Chromatography reach to separate the volatile compounds contained in a sample from the headspace (volatile phase) analysis. When the headspace is analyzed, the sample desorb in a trap, which condense all molecules, and after that, the sample pass in a polarized chromatographic column, impulsed by an inert gas. The retention of each molecule depending on the polarity of them in the chromatographic column induces that each molecule comes out at different times. At the end of the column, the presence of a detector permits to identify the presence of these molecules. The development of new techniques based in this theory permits to develop the e-noses, like Flash-GC, who consist in an ultra-rapid analysis of headspace to increase the velocity of the analysis by an increase of pressure in the chromatographic column, increasing rapidly the separation of molecules. The results permit to elaborate fingerprints of samples, to identify anomalies in there volatile phase, like yeast who produces specific odors, rot or organoleptic defects related to a difference in volatile phase in comparison with standard pattern. Consequently, the production of some odorant compounds by *B. bruxellensis* can be detected by Gas Chromatography (Fariña, Boido, Carrau, & Dellacassa, 2007). The analysis of the volatile compounds can detect this microorganism, because the 4-ethylphenol, the specific molecule produced by *B. bruxellensis,* is a volatile molecule.

Chemometric tools are useful for spoilage food detection. The development of spectroscopy-based instruments has provided new methods to analyse and distinguish samples. Multivariate tools such as Principal Component Analysis (PCA) issued from Hoteling's work (1933), himself inspired by Pearson's correlation calculus (1901) (Cordella, 2015) or Independent Component Analysis (ICA) issued from Common's developments (1991) (faut retrouver la reference) help to make differences between

samples, with the extraction of components which explain observed differences between them. ICA is a blind source separation method (BSS) that extracts statistical independent non-Gaussian sources from mixed signals (Rutledge & Bouveresse, 2013). This technique is very useful if we want to detect components (Signal sources) in spectra such as 3D-Front Face Fluorescence Spectroscopy, NIR, GC-MS or Raman Spectroscopy, for example (Rutledge & Bouveresse, 2013). The preferable use of this method in comparison with PCA is motivated to the separation of samples with other higher statistical orders, different to variance and covariance matrix obtained in PCA for the loadings, to calculate the vectors with the greatest independence (Rutledge & Bouveresse, 2013).

The principal aim of this study is the detection of spoilage in wine with Raman Spectroscopy and chemometric tools. With the determination of a fingerprint in different stages of fermentation, the objective is the characterization of the growth-time from which *B. bruxellensis* becomes detectable and allows for preventing possible losses in winery industry. The combination of other techniques, such as 3D-FFFS and Flash-GC will give us new perspectives to compare the techniques and develop a new sensor.

Furthermore, with the help of ICA and other chemometric tools, it could show other way to analyze data sets such as Raman spectra (Rutledge & Bouveresse, 2013). The use of chemometric tools can help to develop new technological devices and reduce the exposure and analysis time of the samples, increasing the efficiency and productivity in industrial chains. It also can help to develop new ways to prevent spoilages, frauds and adulterations in wine or other food inclusive (Rutledge & Bouveresse, 2013).

3. Materials and methods

3.1. Grape juice and yeasts

Grape juice has been collected in September 2017 in S. A. T. Vinos Coloman, a cooperative cellar placed in Pedro Muñoz, nearly to the centre of Castilla-La Mancha, located in Spain. The variety of the grapes is Airén, a white variety normally used for vinification, with a later ripening time and reduced presence of sugars. To avoid natural fermentation in grapejuice and preserve the sample, 8 g/hL grapejuice of potassium metabisulphite (SO_2) in powder was added and the must was stored at -20 °C. One of the samples has been treated by an ionic exchanger, which reduces pH by exchanging K^+-H^+ in order to limit the formation of tartrate crystals and reduces the possibility of fermentation thanks to a pH reduction.

3.2. Experiment design, inoculation and vinification

The grape juice was defrozen at 5 °C to avoid possible fermentation and, after that, 18 recipients were used to distribute 200 ml of must in each one. After that, all the recipients were sterilized with the grapejuice (121 °C 15 minutes (Jiménez, 2007)) to limit possible microbial contaminations and to ensure that the desired yeast grew in all cultures.

The inoculated yeasts used for the experiment were: *B. bruxellensis* 1009 from the *Colección Española de Cultivos Tipo* (CECT), provided by the Universitat Rovira I Virgili, and the commercial yeast QA23® (Lallemand Inc.). The yeasts were previously isolated to ensure the purity of the culture and precultured in 250 ml of Yeast Peptone Dextrose (YPD) broth in order to have a big viable population.

The distribution of the experiment has been: 9 recipients with *B. bruxellensis* at different concentration (3 with 10^6, 3 with 10^5, and 3 with 10^4 UFC/ml) + *S. cerevisiae* (10^6 UFC/ml), 3 recipients with *S. cerevisiae* (10^6 UFC/ml) (Positive control), 3 recipients with *B. bruxellensis* (10^6 UFC/ml) inoculum and 3 recipients without yeast. Before

inoculation, liquid cultures have been checked with a microscope to show the presence of yeast (Example in Image 1).

The incubation period was performed at room temperature during 21 days, taking samples per recipient once per week to ensure the homogeneity of the sample. In total, each recipient had 4 time points of monitoring (t_0, t_1, t_2, t_3). The samples were preserved at -20 °C to avoid the development of yeasts that can change the conditions between the time of taking samples and the subsequent analysis. To show the presence of *S. cerevisiae* in alcoholic fermentation, all bottles were checked by CO_2 production (Foam appears at second day after inoculation, and they disappear after 7-8 days). Foam production was checked by bottle steering. Bottle steering also permit to aerate the wine (increasing O_2 into the grape juice to stimulate the yeast growth and perform a better fermentation) (Jiménez, 2007)).

All samples were analyzed by Raman and 3D-FFF Spectroscopy for monitoring the development of the yeast, and the Flash-GC was made at first and final time points to show the difference in the volatile phase between the grape juice before and after the fermentation, with and without inoculation of *B. bruxellensis*.

3.3. Count of *B. bruxellensis* population.

All population of samples have been monitored by serial dilutions of the samples in a selective medium (Wallerstein Laboratory Nutrient (WLN) Agar) with a cycloheximide (antibiotic) concentration of 100 mg/L to ensure the exclusive growth of *B. bruxellensis* yeast whereas *S. cerevisiae* is sensitive to the used antibiotic. The incubation time was 14 days.

3.4. Raman Spectroscopy

The Raman spectra was recorded by a Raman Spectrometer (Ventana Ocean Optics, Largo, Florida, USA). All samples were analyzed in triplicate with a quartz cuvette, to enhance the homogeneity of the spectra and test the repeatability. 5

spectra acquisitions of 3 samples taken each time (Day 1, day 7, day 14, day 21) were performed for 3 weeks for a total spectra of 360. The samples were centrifugated at 3000 xg (Q-sep 3000 centrifuge, Restek Corporation, USA) during 20 min.

To monitoring the growth-stage of the yeast, each sample yeast (*S. cerevisiae* and *B. bruxellensis*) were analyzed in pure culture at different growth stages at different times to determine the differences in Raman spectra of the stages and the detection of them. To obtain the spectra, the yeast were inoculated in triplicate for each stage.

3.5. 3D-Front Face Fluorescence Spectroscopy

Fluorescence spectra were recorded using a FluoroMax-4 spectrofluorimeter (Spex-Jobin Yvon, Longjumeau, France), equipped with a variable angle front-surface accessory. The incidence angle of the excitation radiation was set at 56 ° to ensure that reflected light, scattered radiation and depolarization phenomena were minimized. As Airado Rodriguez et al.(Airado-Rodríguez et al., 2011) described in their protocol with a little modification in excitation wavelengths. Spectra were recorded from 245 to 400 nm in excitation (increment 4 nm; slits: 1 nm, both at excitation and emission), and from 300 to 500 nm in emission. Quartz cuvette of 3 ml were used for analysis in triplicate. The samples were centrifugated at 3000 xg (Q-sep 3000 centrifuge, Restek Corporation, USA) during 20 min.

3.6. Flash-GC

Gas Chromatography was perform using an FGC E-nose Heracles II (AlphaMos, Toulouse, France). The Heracles II was equipped with two columns working in parallel mode: a non-polar column (Melucci et al., 2016) (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 µm diameter) and a slightly polar column (MXT1701: 14% cyanopropylphenyl, 86% methylpolysiloxane, 10 m length and 180 µm diameter). A single comprehensive chromatogram was created by joining the chromatograms obtained with the two columns; such an approach may help in preventing/reducing incorrect identifications due to overlapping of chromatograms

obtained with two different columns, and represents a useful tool for improved identification.

To analyze volatile fractions, we used 2 ml of wine placed in vials of 20 ml. To extract the headspace, samples were incubated in 50 °C during 1 h. The chromatography parameters were: in oven at 65 °C during 15 s, with a ramp of temperature from 65 to 240 °C, staying at 240 °C during 30s, temperature of the syringe to create the headspace at 240 °C. Starting and ending temperatures of the trap were respectively 30 °C and 280 °C, and the used chromatographic columns were MTX5 and MTX12 with two Flame Ion Detectors (FIDs) placed at the end of each column.

3.7. Data treatment

For the data analysis, different analytical techniques for detecting the presence of *B. bruxellensis* were used.

3.7.1. Raman Spectroscopy

The data treatment has been done by fitting the spectra with a polynomial or gaussian curve (Gaussian degree 2, at different times to fit it (0 days, 7 days, 14 days, 21 days). This type of baseline correction was used because the Raman Spectroscopy have a fluorescence effect which can interfere in spectra increasing fluorescence signal mixed with the Raman scattering. This fluorescence signal creates a shield against the Raman signal of interest and must be removed, as we can see in the Figure 1. This curve can fitted by a Gaussian curve who follow the same trend of spectra. When the Gaussian curve is obtained, the values of the fitting curve vector can be subtracted to the Raman spectra. The output is the corrected spectra, as less fluorescence signal as possible.

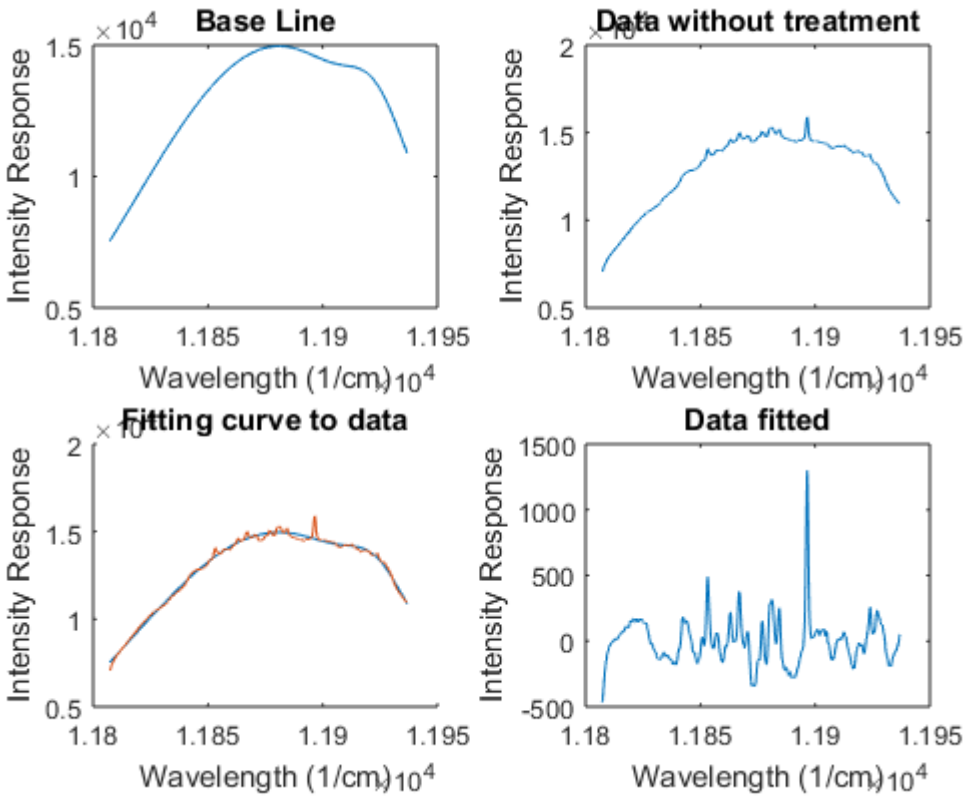

Figure 1: Model of fitting curve in Raman spectra.

The first chemometric analysis was a Principal Component Analysis. This analysis consists of a classification of samples reducing the dimensionality of the samples in principal components, orthogonally disposed in the space to catch the dispersion of the data variance. Furthermore, the Principal Component Analysis was used for the differentiation of stages in yeast growth.

3.7.2. Flash-GC

To process the data chromatography, the procedure of data treatment starts by aligning the chromatograms. This procedure is called "warping" and has for main goal to avoid mis-affectation of variables (retention times) in the data matrix leading to a bad interpretation in PCA and ICA algorithms. The COW algorithm (Correlation Optimized Warping) was used to align the peaks on all chromatograms. This algorithm used small windows to move on chromatograms for calculating correlations between i^{th} chromatogram and a chromatogram taken as reference. (Tomasi, Van Den Berg, &

Andersson, 2004). The highest the correlation, the best warping is. This new chromatogram is the product of the warping procedure, reducing the "electric effect" or "derivative effect" in the subsequent data analysis.

3.7.3. Data treatment

Principal Component Analysis is a technique aiming to reduce the data dimensions by creating different PCs (Principal Components) ; each component have the most part of the variability of the data. The representation in pairs of factorial plans of the PCs show the relative location in the component space of each sample, and each PC carries out a certain % of variance from the original dataset.

To extract the source signal on each spectrum, Independent Component Analysis (ICA) was applied on the X original dataset after pretreatment. ICA is a Blind Source Signal (BSS) method. It has been applied on Fluorescence and Flash-GC data, respectively on the unfolded data cube and on the chromatograms' matrix. ICA detects the fluorescence signals which mostly move away from a Gaussian distribution and which potentially carries interesting chemical information with respect to the phenomenon under study. In other words, this analysis can extract N unknown signals in unknown proportions, into a P mix of signals. ICA can maximize the non-Gaussianity of signals with the objective to build new combinations of variables (ICs), whose are linear combinations of the origin variables. The ICs do not have orthogonality constraints, as is the case with the components of the PCA; the consequence is a greater ability to discriminate groups when they exist between the samples. The major advantage of ICA over the PCA is that the ICs construction engine is not driven by the variance, unlike PCA because one of the first steps of ICA is precisely to apply a PCA and subtract a number of main components to obtain an isovariant dataset. The general model of ICA is a linear model such as:

$$X = A.S$$

Where X is the matrix of recorded signals, S the matrix of "pure" source signals and A is the mixing matrix corresponding to the equivalent of the PCA scores but which is called "proportions" in the case of ICA. These are the proportions of the source signals in each mixture. Unlike PCA, the ICA-generated loadings (matrix S) are much more chemically interpretable, which is an additional advantage of the technique. In recent years, the number of applications of ICA has strongly growth in the field of analytical chemistry and particularly in the signal processing in agrofood industry.

One-way Analysis of Variance (ANOVA) was performed to determine whether data from several groups of a factor have a common mean. That is, one-way ANOVA enables you to find out whether different groups of an independent variable have different effects on the response variable y. To verify the statistical significance of the variations detected in the signals, we performed a one-way ANOVA on the proportions (scores) of ICA to compare the mean of "Control" group to the mean of "*B. bruxellensis*" group and the other groups with *Saccharomyces cerevisiae*. If p-Value is less than 0,05 between the two groups, they are significantly different, that is their means are statistically different. However, it compares the general averages between all the groups, and the p-Value only represents the significance between different groups, but it is not definitively different between each group. To obtain remarkable and definitive results, the analysis should do for each two groups, to detect the difference between each group. On the other hand, this comparison is not necessary to this experiment, because the principal objective is the detection of *B. bruxellensis* in wine, without the presence of different number of cells (ANOVA results could be interesting at the second phase, to develop the sensor, with other performance of the experiment).

4. Results

4.1. Cell count

The cell count from a serial dilution of the samples at different times provided some results which can explain the ecological relationship between *S. cerevisiae* and *B. bruxellensis*, but not significantly. The presence of *B. bruxellensis* has been checked using a white light-microscope to contrast the cell presence.

Subject	T_{07}	T_{14}	T_{21}
Control-01	N/A	N/A	N/A
Control-02	N/A	N/A	N/A
Control-03	N/A	N/A	N/A
Brettan-01	N/A	N/A	N/A
Brettan-02	N/A	2,7E4	N/A
Brettan-03	N/A	1,9E4	7,4E5
Sacchar-01	N/A	N/A	N/A
Sacchar-02	N/A	N/A	N/A
Sacchar-03	N/A	N/A	N/A
CeSujet-01	N/A	N/A	N/A
CeSujet-02	N/A	1,4E3	N/A
CeSujet-03	N/A	1E3	5E3
DeSujet-01	N/A	N/A	3E3
DeSujet-02	N/A	4,3E3	N/A
DeSujet-03	N/A	3E3	N/A
EeSujet-01	N/A	N/A	3E3
EeSujet-02	N/A	3E3	N/A
EeSujet-03	N/A	4E3	N/A

Table 1: Subjects of the experiment in front of the cell count in CFU (cell/ml): Control = Without any yeast; Brettan = With *B. bruxellensis* inoculation of 10^6 cell/ml at start; Sacchar = With *S. cerevisiae* inoculation of 10^6 cell/ml at start; CeSujet = With *S. cerevisiae* and *B. bruxellensis* inoculation of 10^6 cell/ml at start each one; DeSujet = With *S. cerevisiae* inoculation of 10^6 cell/ml at start and *B. bruxellensis* inoculation of 10^5 cell/ml; EeSujet = With *S. cerevisiae* inoculation of 10^6 cell/ml at start and *B. bruxellensis* inoculation of 10^4 cell/ml;

4.2. Raman spectroscopy

The Independent Component Analysis at different times have produced some source signals that show a difference between Control samples - alcoholic fermentation of *S. cerevisiae*, and contamination of *B. bruxellensis*.

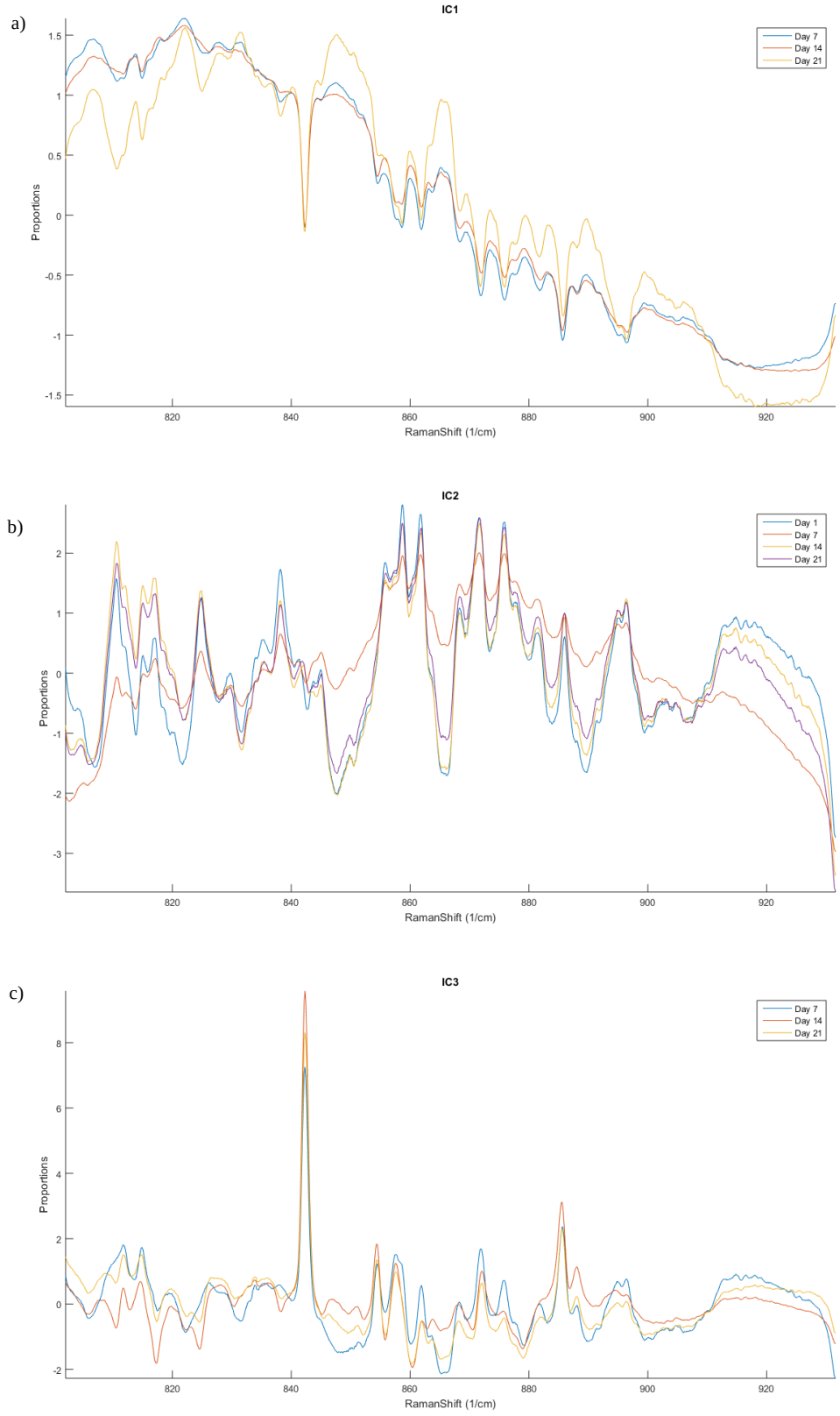

Figure 2 a), b), c): Common ICs at different times: a) IC1 common in day 7, 14, and 21; b) IC2 common in day 1, day 7, 14, and 21; c) IC3 common in day 7, 14, and 21.

Day	PCs	DFs	F	Probability (<0,05)
1	IC1	89	12,97	2,35568E-09*
	IC2	89	2,76	0,0235*
	IC3	89	13,85	7,28837E-10*
7	IC1	89	37,87	3,77138E-20*
	IC2	89	11,9	1,01651E-08*
	IC3	89	23,98	6,61531E-15*
14	IC1	89	44,41	3,49241E-22*
	IC2	89	34,76	4,22715E-19*
	IC3	89	22,24	3,9323E-14*
	IC4	89	8,13	2,71689E-06*
21	IC1	89	79,28	2,51416E-30*
	IC2	89	109,21	3,06137E-35*
	IC3	89	7,96	3,51958E-06*

Table 2: ANOVA Results of each IC scores at different times (Probability <0,05 of significance).

***Probabilistic significance on interval <0,05 (DF = Degrees of Freedom, F = F-Fisher test result).**

4.3. Flash-GC

Gas Chromatography analyses provided another point of view of the analyzed samples. Chromatograms and their statistical treatment by Independent Component Analysis (ICA) provided different interesting source signals allowing us for detecting the changes during the yeast growth and wine maturation at 21 days.

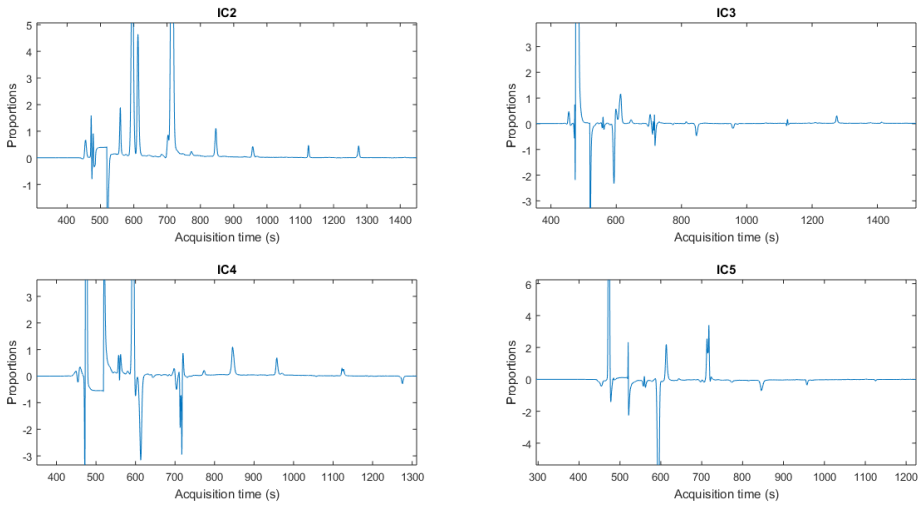

Figure 3: ICs loadings obtained in Gas Chromatography after data treatment and Independent Component Analysis.

Day	ICs	DFs	F	Probability (<0,05)
21	IC2	53	330,7	5,90896E-36*
	IC3	53	5,62	0,0004*
	IC4	53	3,45	0,0096*
	IC5	53	205,86	3,34768E-31*

Table 3: Results of ANOVA one-way analysis between the different groups.

***Significant independent with a probability of <0,05 interval (DF = Degrees of Freedom, F = F-Fisher test result).**

The list of molecules identified by Kovats Indices upon ICs signals shows the molecules, which change in proportion at the end of the experience. The confirmation of the picks and the correspondence of the identified molecules must be completed by a GC-MS and the standard analysis (for example, TRITIVIN (Chambre d'Agriculture de Gironde, Blanquefort, France), with a previous annotation of interesting picks and identification by comparison with another internet databases.

Kovats Indices (KI)	Molecule	Bibliography
459	Ethanol	(Cais-Sokolińska D., 2011)
536	Propanol	(Rychlik, Schieberle, & Grosch, 1998)
600	Acetic Acid	(Jirovetz, Buchbauer, Ngassoum, & Geissler, 2002; Rychlik et al., 1998)
627	Ethyl acetate	(Baek, Cadwallader, Marroquin, & Silva, 2006)
641	Methylbutanal	(Peterson & Reineccius, 2003)
685	Methyl methyl-propanoate	(Rychlik et al., 1998)
755	2-Methyl-1-butanol	(Jordán, Margaría, Shaw, & Goodner, 2003)
846	Ethyl Methyl butyrate	(Rychlik et al., 1998)
944	Mercaptomethylpentanone (3-mercapto-4-methyl-2-pentanone)	(Rychlik et al., 1998)
1178	4-Ethylphenol	(El-Sayed, Heppelthwaite, Manning, Gibb, & Suckling, 2005)
1293	1,3-p-menthadien-7-al	(Grosch, 1993)

Table 4: List of molecules identified upon the ICs Signals in Flash-GC with Kovats Indices.

4.4. 3D-Front Face Fluorescence Spectroscopy

The fluorescence analysis highlighted different fluorophores with ICA, after signal extraction and ANOVA analysis.

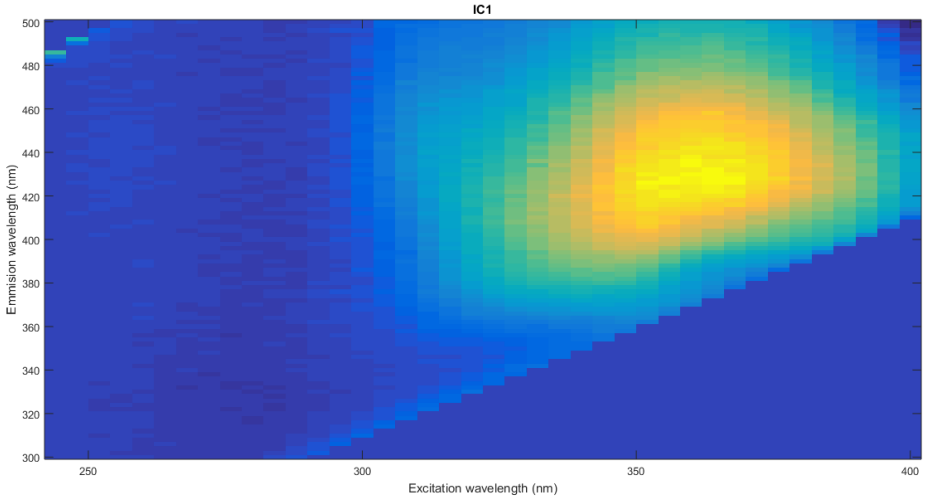

Figure 5: Signal extraction of ICA (IC1) at day 1 in 3D-FFFS (Excitation wavelength (245-400 nm)-Emission wavelength (300-500 nm)).

Table 5: ANOVA of ICs proportions at different times. (p <0,05)

*Probabilistically significance at p<0,05 (df = Degrees of Freedom, F = Fisher test result).

Day	PCs	df	F	Probability (<0,05)
1	IC1	53	2,72	0,0307*
	IC2	53	2,8	0,0207*
7	IC1	52	7,35	3,70574E-05*
	IC2	52	9,77	1,9027E-06*
	IC5	52	29,19	2,39874E-13*
14	IC5	53	14,36	1,35087E-08*
21	IC1	53	10,82	5,26394E-07*
	IC3	53	6,54	0,0001*
	IC4	53	7,85	1,82926E-05*
	IC8	53	46,08	3,46116E-17*

5. Discussion

5.1. Analytical Results

5.1.1. Cell Count

Culture cell in Petri dishes results have shown that *B. bruxellensis* is present in the fermented must. However, in comparison with the inoculated *B. bruxellensis* population, the counts were very minor, close to zero in most of the cases. This fact may reflect the loss of cultivability of *B. bruxellensis* after the inoculation in the must and probably a pre-adaptation to the must conditions should have been done. However, the existence of non-viable cells of the species *B. bruxellensis* is well known, the viable but not culturable state (VBNC) in which the cells enter during adverse conditions (Refs) and this could explain the lack of cultivability on the WLN medium that would recover just the viable cells. This effect, in combination with the low growth and the small population at the end of the alcoholic fermentation causes a non-discriminant result (Willenburg & Divol, 2012). The confirmation of the number of cells could be performed either using the quantitative Polymerase Chain Reaction (qPCR), which counts the number of cells by determining the number of DNA replicates of part of the yeast genome or by flow cytometry (Serpaggi, Remize, Grand, & Alexandre, 2010).

5.1.2. Raman Spectroscopy

In Raman Spectroscopy, PCA analysis shows a difference between different stages of the growth. In Fig. 2 a), b) and c), the PC scores show the presence of *B. bruxelllensis* in wine in comparison with *S. cerevisiae*, whose cultures do not have receive *B. bruxellensis* inoculum.

At different times (Figure 2 a), b), c)), the ICA results show the signals of different molecules which can change in each sample. IC2 shows the variation in some molecules as illustrated in Annex 1: Figure 2 a), b), c) and d). At day 1, the variations are not significantly different (Annex 1: Figure 2 a)), but during the next days (7, 14 and 21), the differences are significant. IC2 is common in all days, but in different proportions. It is a matrix signal (Grape juice composition), because the progressive changes over all days correspond to the same pattern, as illustrated by the ANOVA results of ICs scores

in Table 2 and Annex 1: Figure 2 a), b), c) and d). The variation of Raman Spectra in certain regions (>840 cm^{-1} – 900nm) show a progressive evolution caused by the yeast growth (sugars degradation (fructose) and hydroxycinnamic acids) and wine aging (Martin et al., 2015).

The same response appears in IC3, but similarly to the case of IC1 the signal appears from day 7 (After *S. cerevisiae* growth) to the end of the experiment. The comparison between IC3 and IC1 shows the difference between *S. cerevisiae* and *B. bruxellensis* spectra, finding out each yeast spectra (Figure 2 a) and c)). When IC1 is detected at day 7, proportions of each sample depends on the alcoholic fermentation of *S. cerevisiae*. The signal proportions are very different, but the trend of the data analysis starts to difference the presence of *B. bruxellensis* in comparison with Control samples and *S. cerevisiae* fermentation (Annex 1: Figure 2 b), c) and d)). The fingerprint also show a difference between the wine aging and *B. bruxellensis* growth in IC3 (Figure 2 a)), where the signals are higher at the end of the experiment than in Day 7. It appears some regions corresponding to hydroxycinnamic acids (>900 cm^{-1}) and tyrosine (~840 cm^{-1}) ((Conterno, Fondazione, & Henick-Kling, 2010; Martin et al., 2015; Schuster, Urlaub, & Gapes, 2000). IC3 corresponds to sugar degradation at wavelength <840 cm^{-1} that seems correspond to a higher fructose proportion from the alcoholic fermentation produced by *S. cerevisiae* (Martin et al., 2015). The latter consumes glucose in major proportion than fructose because *S. cerevisiae* is not capable to consume fructose (Annex 1 Figure 5: b), c) and d)). The variation of this peak also shows a fructose consumption caused by *B. bruxellensis,* which has a fructose-degradation capacity (Conterno et al., 2010).

5.1.3. Flash-Gas Chromatography

After treatment of Flash-GC data, the IC analysis extracted 4 independent signals which correspond to different flavor production. IC2 could show a fingerprint associated to the presence of *B. bruxellensis* (Figure 3, Table 3 and Annex 2: Figure 1). The presence of peak variations in the chromatogram corresponds to molecules such as acetic acid (Figure 3, Table 4, KI 600, (Jirovetz et al., 2002; Rychlik et al., 1998)), ethyl acetate (Figure 3, Table 4, KI 627, (Baek et al., 2006)) or 4-ethylphenol (Figure 3, Table

4, KI 1178 (El-Sayed et al., 2005)). These key molecules are involved in secondary flavor production in wine (Derived by yeast activity). Although the presence of other molecules in chromatogram, the fingerprint associated to these molecules shows the presence of other yeast with other aromatic profile, *B. bruxellensis*. Acetic acid production in wine is likewise associated with *B. bruxellensis*, traditionally as marker of its presence (Conterno et al., 2010; Navascues & López-cordón, 2017). Variation of this molecule between recipes with and without *B. bruxellensis* is clear, as illustrated in Annex 2: Figure 1, where the presence of *B. bruxellensis* in co-fermentation produce a high number of these molecules, compared with *S. cerevisiae alone.* The cause of the non-acetic acid production in the fermentation in presence of *B. bruxellensis* alone is due to the low growth of this yeast and the effect of glucose on the yeast. Therefore, glucose repression causes a low growth in *B. bruxellensis* (Carbó et al., 2008; Castro Martinez, 2006; Navascues & López-cordón, 2017). Glucose consumption by *S. cerevisiae* produces a glucose reduction in the wine, which leads to an increase of acetic acid issued from *B. bruxellensis* metabolism. The ester production such as ethyl acetate sustained by *B. bruxellensis* in comparison with *S. cerevisiae alone* fermentation, increases is supported by esterase activity of *B. bruxellensis* (Spaepen & Verachtert, 2018). Identification of 4-ethyl phenol in IC2 (Figure 3, Table 4, KI 1178 (El-Sayed et al., 2005)) also produces a discrimination between *B. bruxellensis* and the other samples due to the degradation of hydroxycinnamic acids. This flavor is characteristic in wine spoiled by *B. bruxellensis,* which produce these molecules in absence of nutrients at the end of the alcoholic fermentation (Carbó et al., 2008; Conterno et al., 2010; Navascues & López-cordón, 2017). IC5 is related to all these molecules in presence of *B. bruxellensis*, but in this signal the 4-ethylphenol is not detected (Figure 3, Table 4). So, in IC2, the presence of 4-Ethylphenol is more important and more discriminant than in IC5, which detects bigger variation in other key-molecules associated to the *B. bruxellensis* detection, such as acetic acid and ethanol (Figure 3, Table 4, KI 459 (Cais-Sokolińska D., 2011). An increase in ethanol concentration is another characteristic effect of *B. bruxellensis* spoilage in wine by residual glucose and fructose consumption (Aguilar-Uscanga et al., 2011).

IC3 and IC4 detect primary aromas such as 1,3-p-mentadien-7-al (Figure 3, Table 4, KI 1293 (Grosch, 1993)), or mercaptomethylpentanone (Figure 3, Table 4, KI 944 (Rychlik et al., 1998)), whose molecules are present in grape juices, and are derived from the fruit (Csóka, Amtmann, Sárdy, Kállay, & Korány, 2013). The decrease of these compounds in wine in comparison with *B. bruxellensis* and *S. cerevisiae* (Annex 2: Figure 1) is due to higher alcohols production by yeasts in their presence, because of the aldehydes and ketones reduction (Swiegers, Bartowsky, Henschke, & Pretorius, 2008). This modulation produces a reduction of these flavors leading to higher alcohols. The difference observed between scores of IC3 and IC4 is related to these molecules, whose proportions in each signal produce a discrimination of these compounds. Therefore, IC3 (Figure 3) represents the influence of 1,3-p-mentadien-7-al and IC4 is more representative of mercaptomethylpentanone.

5.1.4. 3D-Front-Face-Fluorescence-Spectroscopy

Fluorescence results show different fluorophores whose significance is a marker of *B. bruxellensis* presence.

At day 1, the more important fluorophore to discriminate the presence of yeast has been extracted on IC1 (Annex 3: Figure 1 a)). This fluorescence signal (360 nm excitation wavelength - 440 nm emission wavelength) could correspond to NADH molecules, molecule having a cell activity (Leblanc & Dufour, 2002; Yoshimura et al., 2014). NADH can discriminate between different strains, as we can see in ANOVA results of ICs at day 1, which shows the same equivalence in all wines with *S. cerevisiae* inoculation, in comparison with the control wine and the wine inoculated with *B. bruxellensis* alone (different of Control) (Table 5, Annex 3: Figure 2a)). The other source signal (>400 nm excitation wavelength-500 nm emission wavelength in Annex 3: Figure 1 a)) can correspond to Vitamin B2, a riboflavin involved in energy production (Airado-Rodríguez et al., 2011). The difference in vitamin quantity can also contribute to the identification of different yeast, as we can see in the Annex 3: Figure 2a).

At day 7, IC1 and IC2 also correspond to the same signals detected at day 1 (IC1: Riboflavin: Vitamin B2 (>400 nm excitation wavelength-500 nm emission wavelength);

IC2: NADH (360 nm excitation wavelength - 440 nm emission wavelength)). These signals can also help to the yeast identification (Annex 3: Figure 1b)). The ANOVA results show the difference in cell activity who could correspond to S. cerevisiae, which makes differences in several conditions (Table 5, Annex 3: Figure 2b)). IC5 (265-300 nm excitation wavelengths – 340-440 nm emission wavelength) can correspond to phenolic acid - cinnamic-like and benzoic-like (Table 5, Annex 3: Figure 1b) 3b)) (Airado-Rodríguez et al., 2011).

At day 14, the only source signal that it can show the difference between the different subjects is the representative marker of phenolic acids - cinnamic-like and benzoic-like (Annex 3: Figure 1c) and 3c)) (265-300 nm excitation wavelength – 340-440 nm emission wavelength) (Airado-Rodríguez et al., 2011).

At the end of the experiment (day 21), it detects five source signal that correspond to different molecules. IC3 and IC4 correspond to Vitamin B2 (>400 nm excitation wavelength-500 nm emission wavelength) and NADH (360 nm excitation wavelength - 440 nm emission wavelength) (Leblanc & Dufour, 2002; Yoshimura et al., 2014), respectively (Annex 3: Figure 1d)). IC8 could correspond to phenolic acids – cinnamic-like and benzoic-like (265-300 nm excitation wavelength – 340-440 nm emission wavelength) (Airado-Rodríguez et al., 2011). IC1 correspond to flavan-3-ol signal (Airado-Rodríguez et al., 2011) (Table 5, Annex 3: Figure 1d) 3d)), which also correspond to astringency of the wine, aging and also to preserve the color in red wines. The variation of these compounds in white wines is related to its aging, condensation of them and stabilization of these compounds. In the other hand, these molecules can show a difference in yeast activity with the use of vinylphenol, pyruvic acid and acetaldehyde in formation of malvidine-3-monoglucoside, precursor of vitisin A, vitisin B, vinylphenol - malvidine aduct and flavanol – malvidine aduct in aging wine process. Although the extract of grape juice is not in contact with some parts of the grape which can increase the quantity of flavan-3-ols, the presence of this phenolic compounds is also remarkable and could also help to detect the presence of *B. bruxellensis* (Table 5, Annex 3: Figure 2d)) (in comparison with red wines, whose process use seeds and skin grape to extract the prodelphinidins and procianydines).

5.2. Biological interpretation, Ecological relationships and flavor production

With these results, the modelling of these phenomena describes the spoilage phases and *B. bruxellensis* growth. The process describe alcoholic fermentation in the first step, to degrade the sugars producing ethanol to eliminate the most part of yeast as evolutionary strategy. In this step, *B. bruxellensis* cannot growth because of the high population of *S. cerevisiae*, which consume the most part of nutrients, so *B. bruxellensis* population decreases to small levels (Belda et al., 2014; Navascues & López-cordón, 2017). Competence relationship of *S. cerevisiae* is also remarkable with CO_2 and ethanol production by sugars consumption by Crabtree effect (Repression of oxidative pathway in high presence of sugars and aerobic conditions, activating fermentative pathway and ethanol production), which avoids the development of other strains. The presence of yeast can be highlighted in the first day day using 3D-FFFS and Raman spectroscopy, because of different scores in ICA, which show the difference between Control and all other samples in all ICs (Annex 1: Figure 2) particularly in Raman Spectroscopy. IC3 is also able to distinguish between *B. bruxellensis* and *S. cerevisiae* (Annex 1: Figure 2). Markers of hydroxycinnamic acids appear at day 7, when *B. bruxellensis* starts its growth during the senescence phase of *S. cerevisiae*, at the end of the alcoholic fermentation (Annex 3: Figure 1b)). During the latter phase, *S. cerevisiae* is still present, and the variation in NADH shows a biological difference between yeast strains by different cell activity and metabolism (Annex 3: Figure 1b), 3b)) (Blacker et al., 2014; Yoshimura et al., 2014). The variation in hydroxycinnamic acids and phenolic compounds is another point to detect the growth of *B. bruxellensis* in 3D-FFFS (Annex 3: Figure 1b)), a consequence of degradation of phenolic compounds to obtain energy, producing 4-ethylphenol (Carbó et al., 2008; Navascues & López-cordón, 2017). The same effects occur with Vitamin B2 fluorophore (Annex 3: Figure 1b)) (Dmytruk, Yatsyshyn, Voronovsky, Fedorovych, & Sibirny, 2009), which also detect a spectral variation at day 7, corresponding to *S. cerevisiae* growth during alcoholic fermentation (Residual Vitamin B2 of died yeast). Development of *B. bruxellensis* is appreciable by Raman Spectroscopy which detects sugar consumption at day 7, hydroxycinnamic acids and tyrosine proportions are constant on IC1 and IC3 during all the experiment (Figure

2a) and c)). IC2 explains the observed differences by nutrient degradation and aging perceived in all experiments from day 7. At day 14, aging wine appears in Raman spectroscopy by tannins polimerization (Figure 2b)), but also the *B. bruxellensis* growth in IC1 and IC3 (Figure 2 a) and c)). However, the only signal detected in 3D-FFFS shows a difference in cinammic-like acids, corresponding to these degradation due to *B. bruxellensis* growth(Annex3: Figure 2c)). The reason of fluorophore absence correspond to the slow growth of *B. bruxellensis* at this stage, when the growth yeast can be in latency phase (Castro Martinez, 2006; Navascues & López-cordón, 2017). At day 21, in contrast, the presence of other fluorophores show the evolutionary phase and the start of exponential phase of *B. bruxellensis*, detecting 4 fluorophores corresponding to NADH (IC4), Vitamin B2 (IC3), flavan-3-ol variation (IC1) and cinnamic-like-acids fluorophore (IC8) in Annex 3: Figure 1d). Flavan-3-ol correspond to aging wine, by combination of different flavanols such as tannins, which come from the plant and seeds. The apparition of the other fluorophores correspond to *B. bruxellensis* growth, maybe at the end of the exponential phase, when the yeast activity is more present and yeast starts more efficiently to consume another energy source and produce secondary metabolites like 4-ethyl phenol (Carbó et al., 2008; Conterno et al., 2010; Curtin, Varela, & Borneman, 2015). The variation is also appreciable in Raman spectroscopy, where the difference in IC1 and IC3 shows the *B. bruxellensis* growth with sugars consumption, reduction of hydroxycinnamic acids to produce the 4-ethylphenol (Figure 2a) and c)). Aging wine is detected by Raman spectroscopy in IC2 by difference in matrix complex of the wine (Figure 2b)). The GC-Flash shows the presence and growth of *B. bruxellensis*, by the difference in acetic acid production (One of the principal characteristics of this yeast (Carbó et al., 2008; Conterno et al., 2010)) and 4-ethylphenol production in IC2 and IC5 (Figure 3).

6. Conclusions and perspectives

Raman spectroscopy and fluorescence spectroscopy have successfully signaled the presence of *B. bruxellensis* throught its off-flavor and effects on wine. Furthermore, the chromatographic and microbiological results confirm the presence of *B. bruxellensis* into the wine and allow for highlighting the production of 4-ethylphenols from the degradation of hydroxycinnamic acids. Independent Component Analysis allows for efficient source signal extraction that can change by the presence of *B. bruxellensis*. The identification of these signals such as cofactors or hydroxycinnamic acids permit to develop a systematic monitoring of the wine fermentation and global quantification of these signals and peaks for an ultra-fast method to monitor the wine fermentation, prevent and detect spoilage in wine.

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"A veces, quedarse callado equivale a mentir. […] Venceréis porque tenéis sobrada fuerza bruta. Pero no convenceréis, porque convencer significa persuadir. Y para persuadir necesitaríais algo que os falta en esta lucha: Razón y derecho." Miguel de Unamuno, en una confrontación con el general fascista Millán-Astray en la Universidad de Salamanca. 12 de Octubre de 2018.

"At times to be silent is to lie.[…] *You will win because you have enough brute force. But you will not convince. For to convince you need to persuade. And in order to persuade you would need what you lack: Reason and Right."* Unamuno in a confrontation with fascist General Millan-Astray at the University of Salamanca on October 12, 1936.

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Annex 1: Raman Spectroscopy analysis

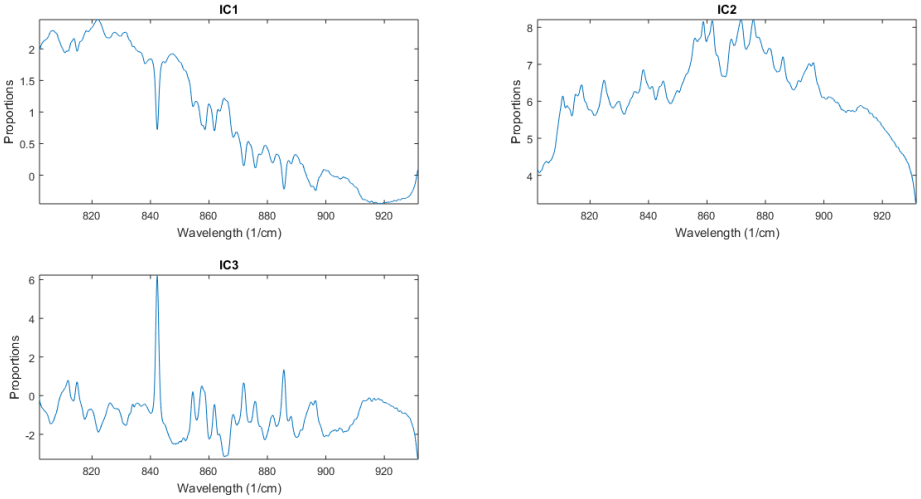
Annex 1: Tables

Day	PCs	DFs	F	Probability (<0,05)
1	IC1	89	12,97	2,35568E-09*
	IC2	89	2,76	0,0235*
	IC3	89	13,85	7,28837E-10*
7	IC1	89	37,87	3,77138E-20*
	IC2	89	11,9	1,01651E-08*
	IC3	89	23,98	6,61531E-15*
14	IC1	89	44,41	3,49241E-22*
	IC2	89	34,76	4,22715E-19*
	IC3	89	22,24	3,9323E-14*
	IC4	89	8,13	2,71689E-06*
21	IC1	89	79,28	2,51416E-30*
	IC2	89	109,21	3,06137E-35*
	IC3	89	7,96	3,51958E-06*

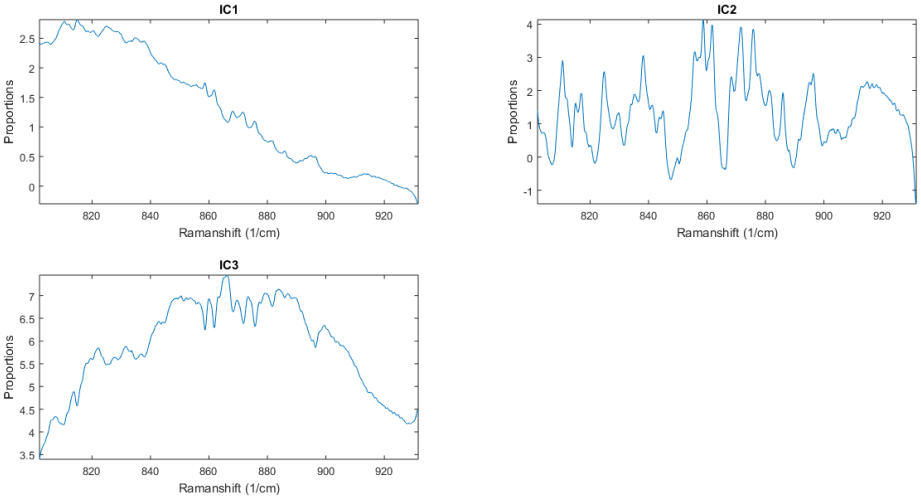
Table 2: ANOVA Results of each IC scores at different times (Probability <0,05 of significance).

Annex 1: Figures

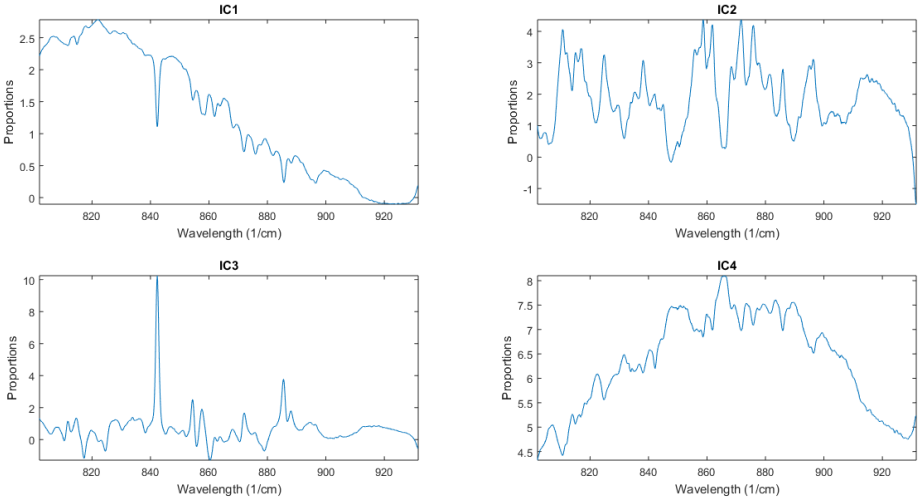
a)



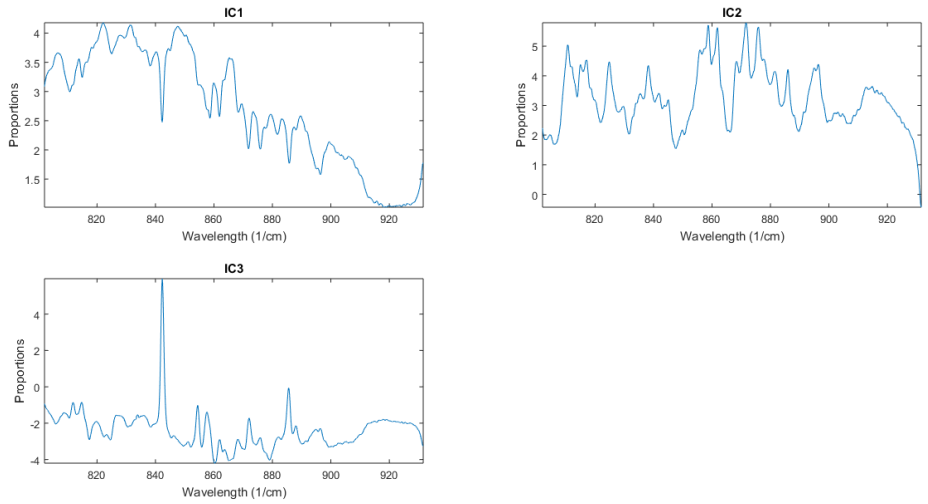
b)



c)



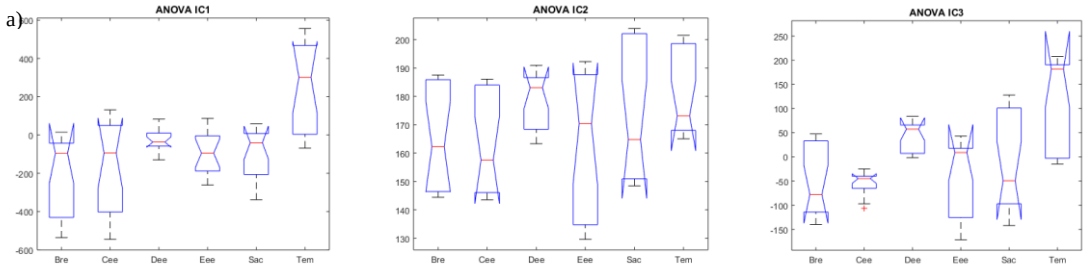
d)



Annex 1 Figure 1 a), b), c), d): ICs signals of each stage in Raman Spectroscopy at a) 1 day, b) 7 days, c) 14 days, d) 21 days.

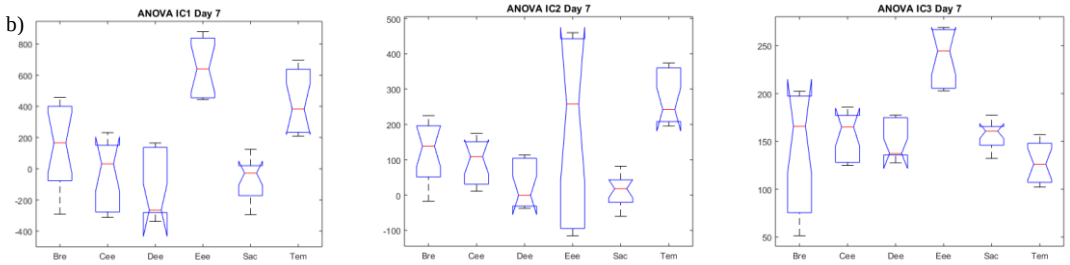
Day 1:

a)



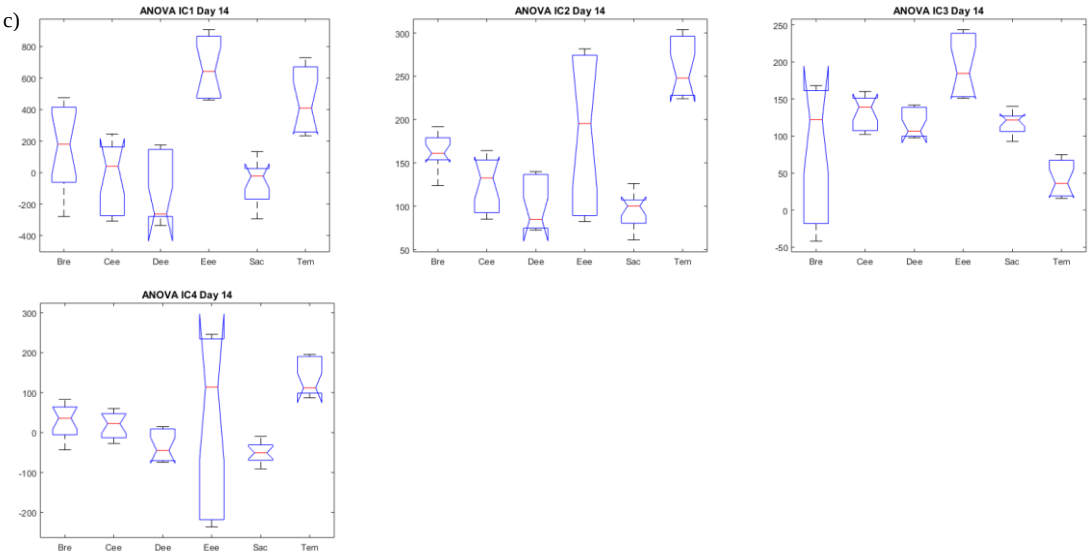
Day 7:

b)



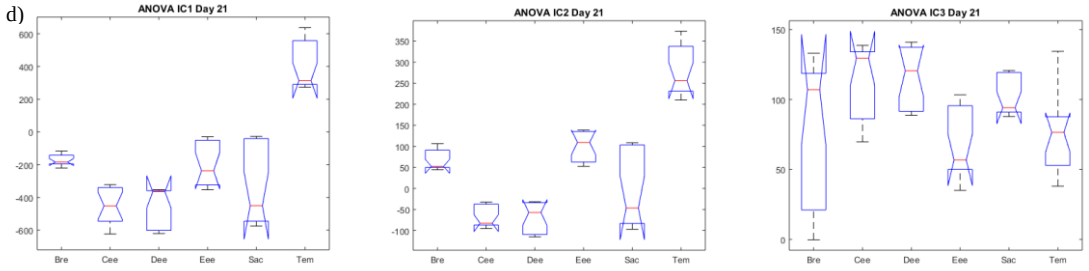
Day 14

c)



Day 21:

d)



Annex 1: Figure 2: ANOVA of IC scores obtained at different times: 4a) Day 1, 4b) Day 7, 4c) Day 14 and 4d) Day 21.

Annex 2: GC-FID.

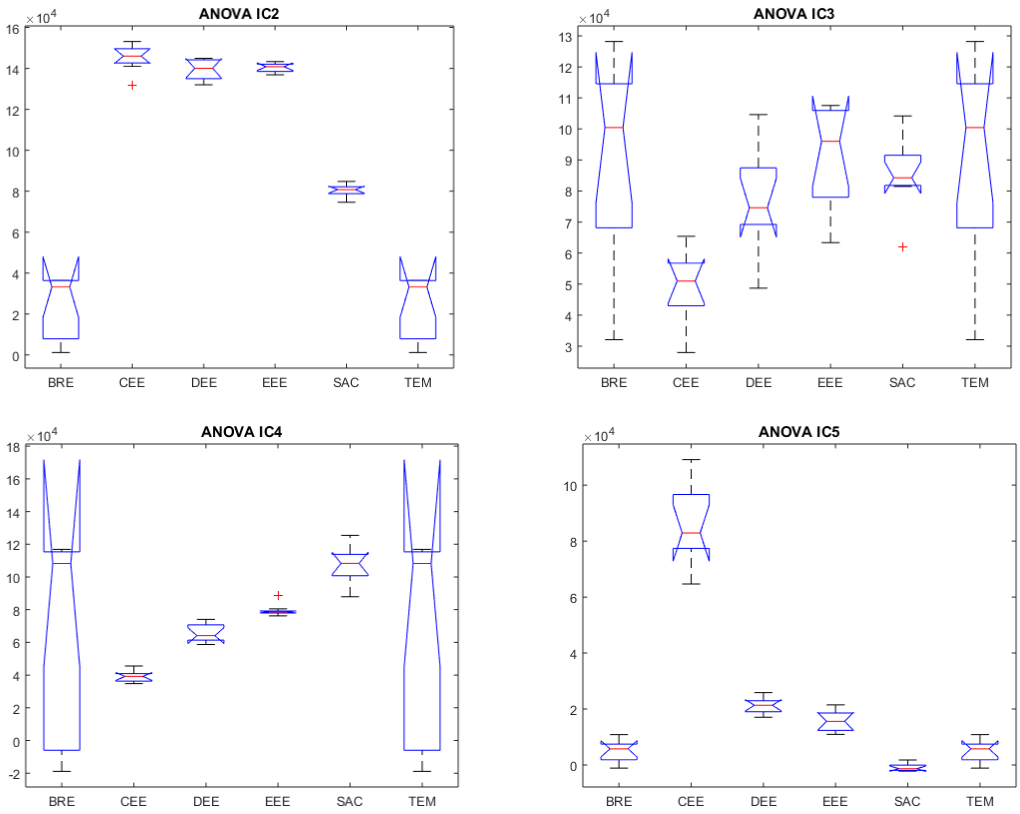
Annex 2: Tables

Day	ICs	DFs	F	Probability (<0,05)
21	IC2	53	330,7	5,90896E-36*
	IC3	53	5,62	0,0004*
	IC4	53	3,45	0,0096*
	IC5	53	205,86	3,34768E-31*

Table 3: Results of ANOVA one-way analysis between the different groups.

***Significant independent with a probability of <0,05 interval (DF = Degrees of Freedom, F = F-Fisher test result).**

Annex 2: Figures



Annex 2: Figure 1: ANOVA results of GC-Flash results at 21 days.

Annex 3: 3D-Front Face Fluorescence Spectroscopy

Annex 3: Tables

Table 5: ANOVA of ICs proportions at different times. (p <0,05)

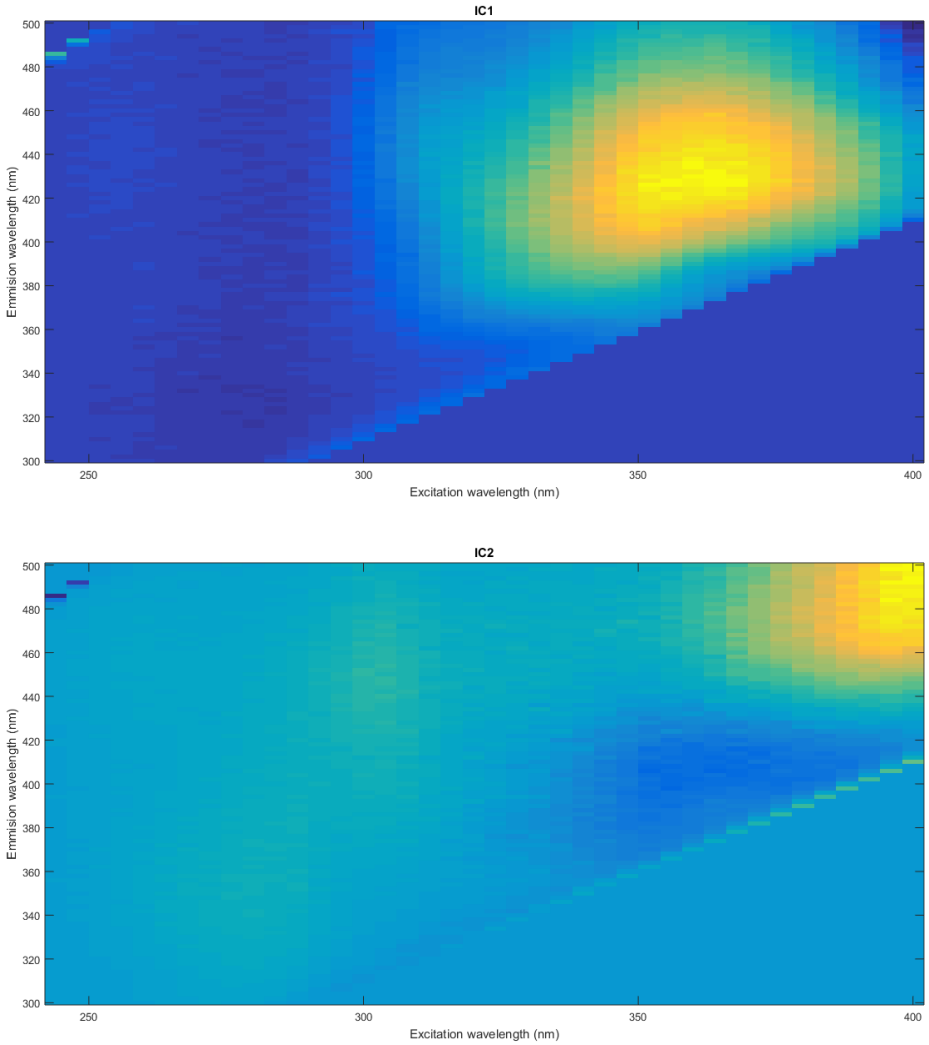
***Probabilistically significance at p<0,05 (df = Degrees of Freedom, F = Fisher test result).**

Day	PCs	df	F	Probability (<0,05)
1	IC1	53	2,72	0,0307*
	IC2	53	2,8	0,0207*
7	IC1	52	7,35	3,70574E-05*
	IC2	52	9,77	1,9027E-06*
	IC5	52	29,19	2,39874E-13*
14	IC5	53	14,36	1,35087E-08*
21	IC1	53	10,82	5,26394E-07*
	IC3	53	6,54	0,0001*
	IC4	53	7,85	1,82926E-05*
	IC8	53	46,08	3,46116E-17*

Annex 3: Figures

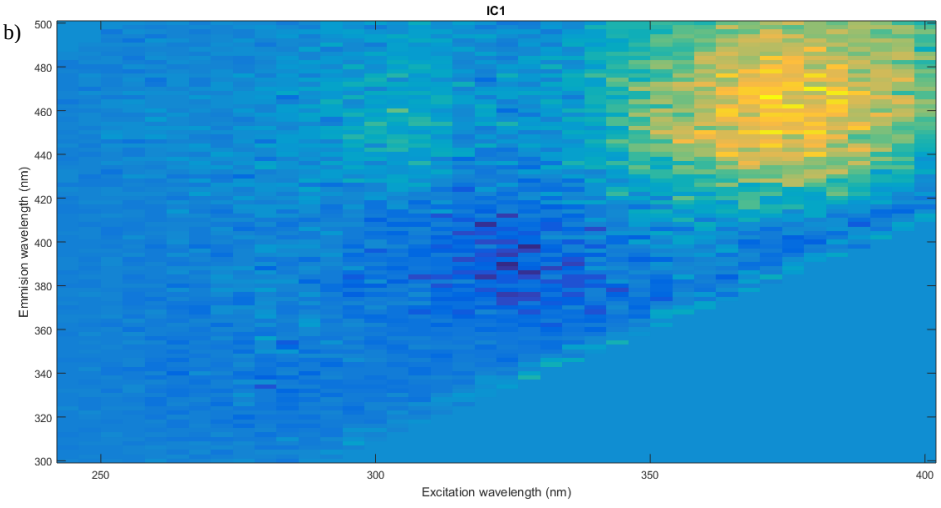
Day 1

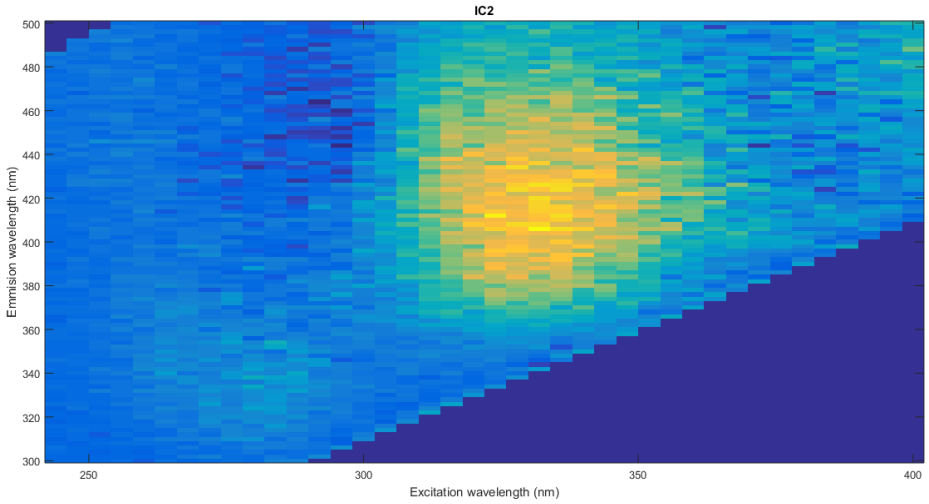
a)

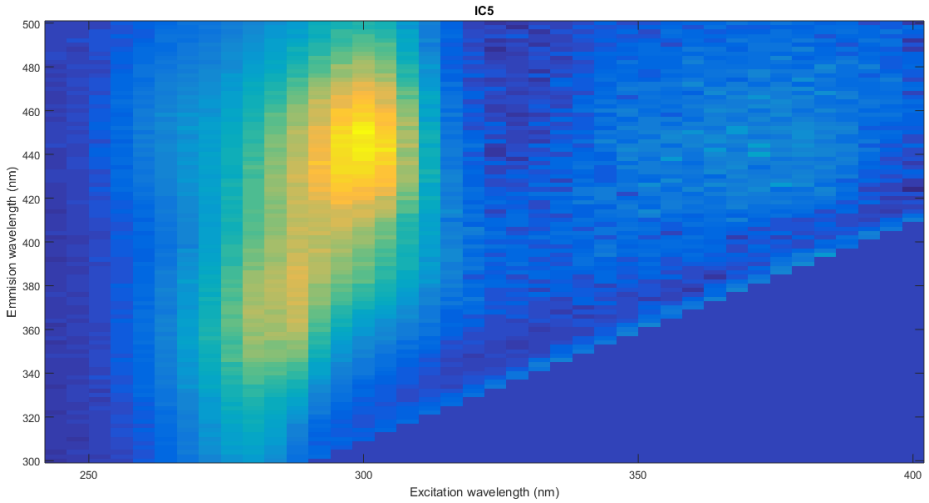


ICA: Signals (ICs) Detected at day 7

b)

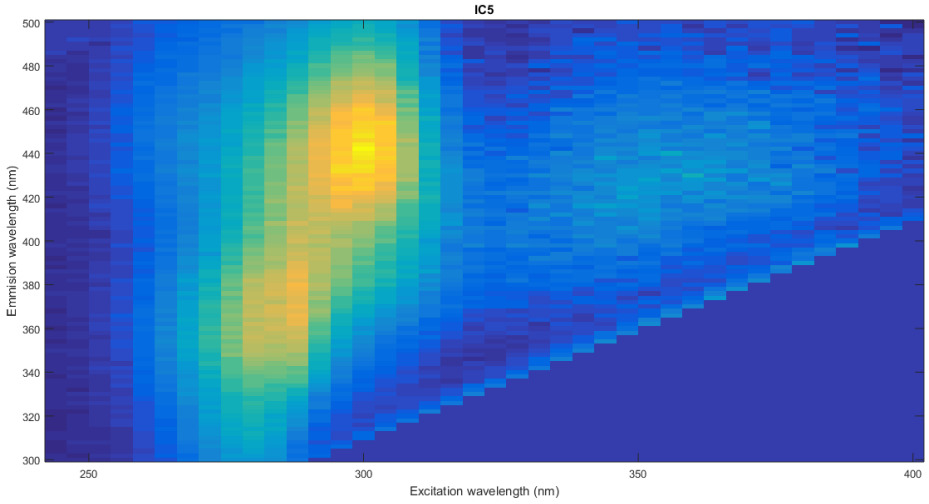






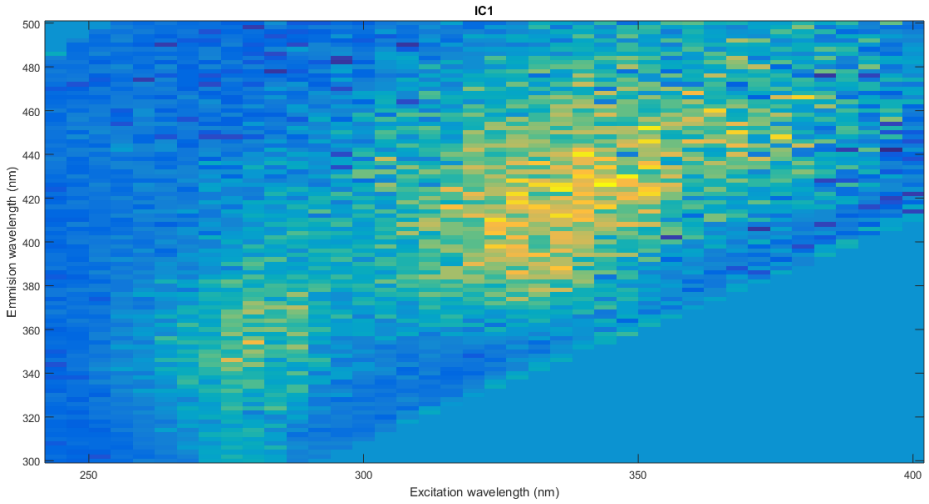
Day 14

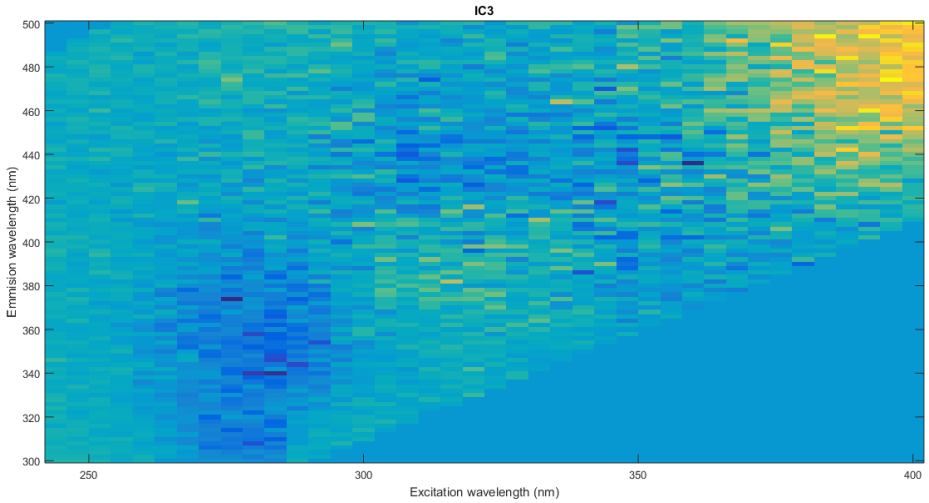
c)

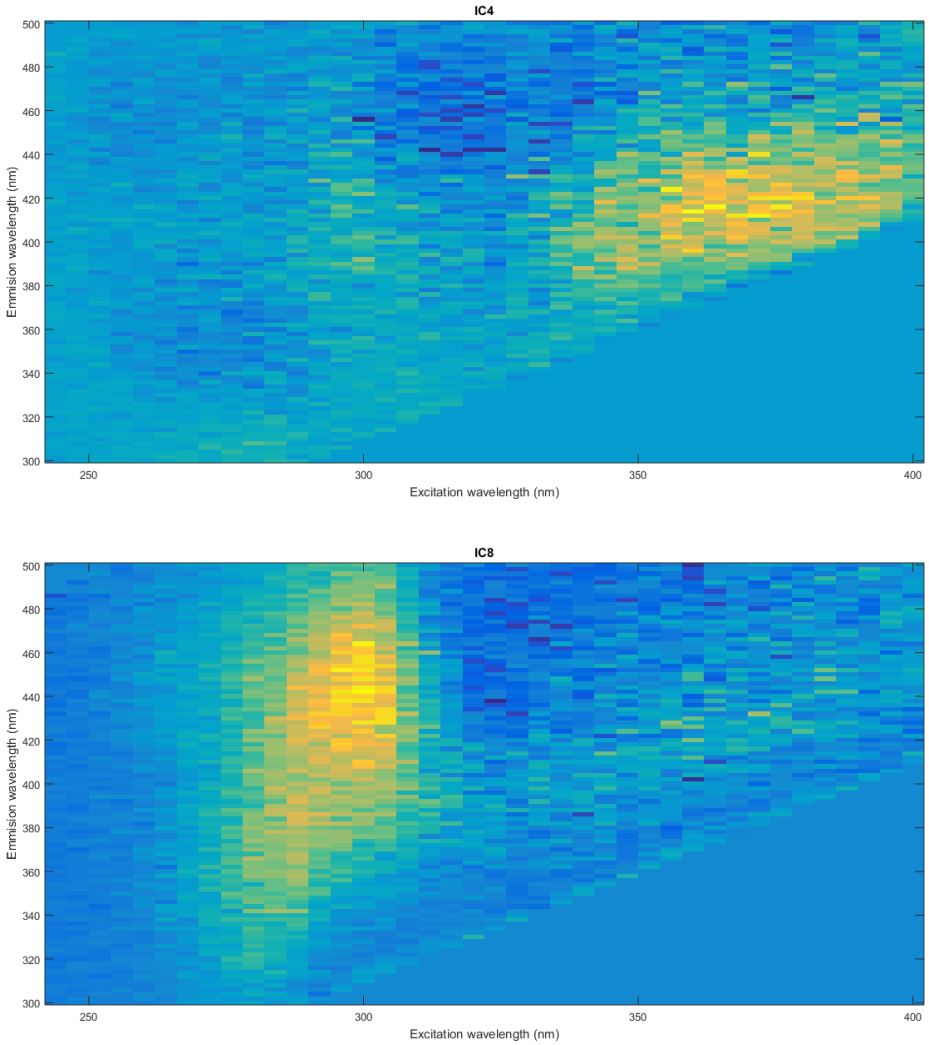


Day 21

d)

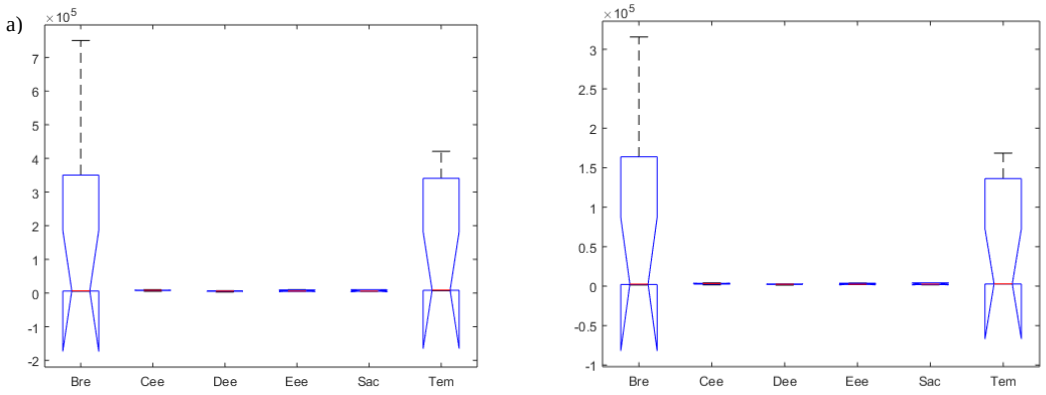

IC1
Emmision wavelength (nm)
Excitation wavelength (nm)
500
480
460
440
420
400
380
360
340
320
300
250
300
350
400


IC3
Emmision wavelength (nm)
Excitation wavelength (nm)
500
480
460
440
420
400
380
360
340
320
300
250
300
350
400



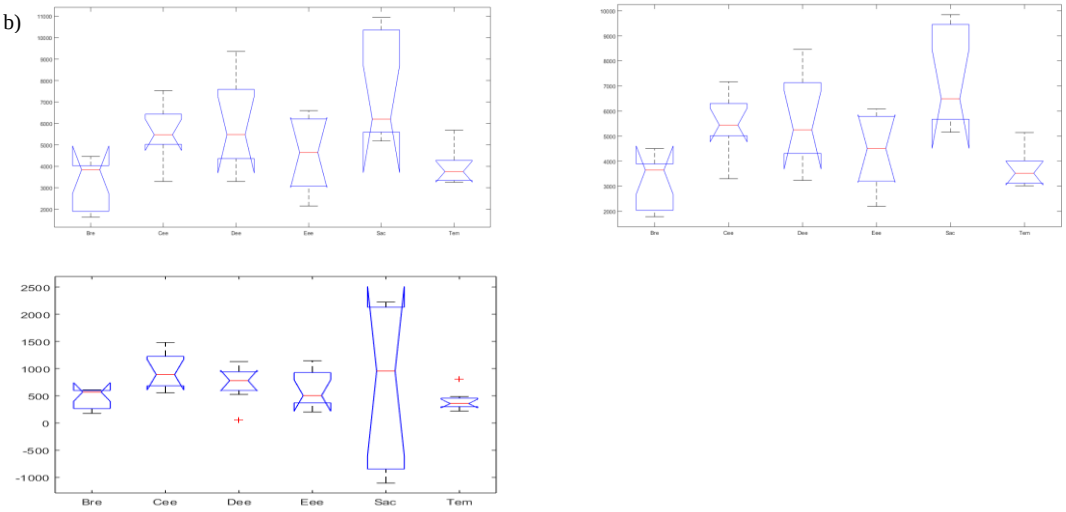
Annex 3: Figure 1: ICs obtained at different times after IC Analysis 2a) Day 1, 2b) Day 7, 2c) Day 14 and 2d) Day 21.

Day 1



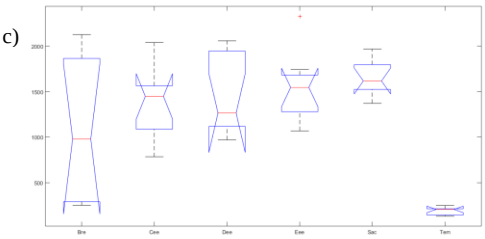
Day 7

b)



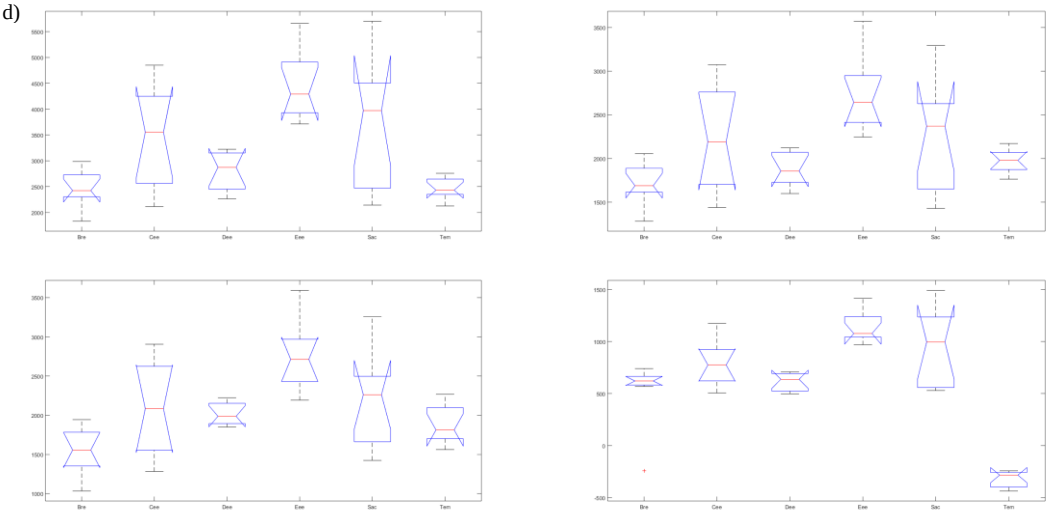
Day 14

c)



Day 21

d)



Annex 3: Figure 2: ANOVA of ICs scores at different times: 3a) Day1 (IC1, IC2), 3b) Day7 (IC1, IC2, IC5), 3c) Day 14 (IC5), 3d) Day 21 (IC1, IC3, IC4, IC8). In order, each graphic correspond to these ICs left-right, and up-down.

Annex 4: Scripts

3D-FFFS

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```
%% correction des spectres pqr le masque
% creqtion de la liste des spectres
listSpec = who;
listSpec = char(listSpec);
% on enleve la 1ere colonne a chaque spectre
fluo ={};
for i=1:size(listSpec,1)
aux = evalin('base',listSpec(i,:));
evalin('base',strcat('aux(:,1)=[]; fluo{',num2str(i),',1}= aux;'));
end;
mask = Sacch_001_01_02_1;
figure;surf(mask)
mask(mask>2E5)=0;
mask(mask<6000)=0;
figure;surf(mask);
mask(mask>0)=1;
figure;surf(mask)
spec = Sacch_001_01_02_1.*mask;
figure;surf(spec)
%Delete 1st column
mask (:,1) = [];
spec (:,1) = [];
fluoCorrected = {};
for i=1:size(listSpec,1)
spec = fluo{i,1}.*mask;
%spec(spec==0)=NaN;
fluoCorrected{i,1}= spec;
end;
%% creation du cube de donnees
cube = [];
for i=1:size(fluoCorrected,1)
cube = cat(3, cube, fluoCorrected{i,1});
end;
% depliement du cube en conservant la dimension des echantillons
cubemat = reshape(cube, 101*40, 215);
mydata = matrix2saisir (cubemat');
matrixday1 = selectrow (mydata, [1:9, 37:45, 73:81, 108:116, 144:152, 180:188]);
matrixday2 = selectrow (mydata, [10:18, 46:54, 82:89, 117:125, 153:161, 189:197]);
matrixday3 = selectrow (mydata, [19:27, 55:63, 90:98, 126:134, 162:170, 198:206]);
matrixday4 = selectrow (mydata, [28:36, 64:72, 99:107, 135:143, 171:179, 207:215]);
mypca001 = saisir_pca (matrixday1);
mypca007 = saisir_pca (matrixday2);
mypca014 = saisir_pca (matrixday3);
mypca021 = saisir_pca (matrixday4);
```

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```
myica001 = saisir_ica (matrixday1, 6, 'Normal');
myica007 = saisir_ica (matrixday2, 6, 'Normal');
myica014 = saisir_ica (matrixday3, 6, 'Normal');
myica021 = saisir_ica (matrixday4, 6, 'Normal');
%% 001
figure ('color', [1 1 1]); colored_map1 (mypca001.score, 1,2, 1,3);
figure ('color', [1 1 1]); colored_map1 (mypca001.score, 1,3, 1,3);
```

```
loadings_rebuiltday1 = reshape (mypca001.eigenvec.d, 101,40, 53);
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday1(:,:,1))); title ('PC1'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday1(:,:,2))); title ('PC2'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday1(:,:,3))); title ('PC3'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(myica001.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(mypca001.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(mypca001.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(matrixday1.d,10);
myica007 = saisir_ica (matrixday1, 6, 'Normal');
signals_rebuiltday1 = reshape (myica001.signals.d, 101,40,6);
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday1(:,:,1))); title ('IC1'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday1(:,:,2))); title ('IC2'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday1(:,:,3))); title ('IC3'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday1(:,:,4))); title ('IC4'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday1(:,:,5))); title ('IC5'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday1(:,:,6))); title ('IC6'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(myica001.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
```



```
myanovah = anova1(myica001.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(myica001.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(myica001.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(myica001.score.d(:,5), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday1, 1,3);
myanovah = anova1(myica001.score.d(:,6), groups.d);
set(gca, 'XTickLabel', groups.g.i);
%% 007
loadings_rebuiltday7 = reshape (mypca007.eigenvec.d, 101,40, 52);
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday7(:,:,1))); title ('PC1'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday7(:,:,2))); title ('PC2'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday7(:,:,3))); title ('PC3'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure ('color', [1 1 1]); colored_map1 (mypca007.score, 1,2, 1,3);
figure ('color', [1 1 1]); colored_map1 (mypca007.score, 1,3, 1,3);
```

```
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(mypca007.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(mypca007.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(mypca007.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(matrixday2.d,10);
myica007 = saisir_ica (matrixday2, 6, 'Normal');
signals_rebuiltday7 = reshape (myica007.signals.d, 101,40,6);
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday7(:,:,1))); title ('IC1'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday7(:,:,2))); title ('IC2'); axis xy
```



```
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday7(:,:,3))); title ('IC3'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday7(:,:,4))); title ('IC4'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday7(:,:,5))); title ('IC5'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday7(:,:,6))); title ('IC6'); axis xy
; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(myica007.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(myica007.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(myica007.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(myica007.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(myica007.score.d(:,5), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday2, 1,3);
myanovah = anova1(myica007.score.d(:,6), groups.d);
set(gca, 'XTickLabel', groups.g.i);
%% 014
figure ('color', [1 1 1]); colored_map1 (mypca014.score, 1,2, 1,3);
figure ('color', [1 1 1]); colored_map1 (mypca014.score, 1,3, 1,3);
loadings_rebuiltday14 = reshape (mypca014.eigenvec.d, 101,40, 53);
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday14(:,:,1))); title ('PC1'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday14(:,:,2))); title ('PC2'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday14(:,:,3))); title ('PC3'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(mypca014.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
```



```
myanovah = anova1(mypca014.score.d(:,2), groups.d);
```

```
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(mypca014.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(matrixday3.d,10);
myica014 = saisir_ica (matrixday3, 7, 'Normal');
signals_rebuiltday14 = reshape (myica014.signals.d, 101,40,7);
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday14(:,:,1))); title ('IC1'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday14(:,:,2))); title ('IC2'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday14(:,:,3))); title ('IC3'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday14(:,:,4))); title ('IC4'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday14(:,:,5))); title ('IC5'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(myica014.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(myica014.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(myica014.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(myica014.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(myica014.score.d(:,5), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday3, 1,3);
myanovah = anova1(myica014.score.d(:,6), groups.d);
set(gca, 'XTickLabel', groups.g.i);
%% 021
figure ('color', [1 1 1]); colored_map1 (mypca021.score, 1,2, 1,3);
figure ('color', [1 1 1]); colored_map1 (mypca021.score, 1,3, 1,3);
loadings_rebuiltday21 = reshape (mypca021.eigenvec.d, 101,40, 53);
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday21(:,:,1))); title ('PC1'); axis
```



```
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday21(:,:,2))); title ('PC2'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(loadings_rebuiltday21(:,:,3))); title ('PC3'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(mypca021.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(mypca021.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(mypca021.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(matrixday4.d,10);
myica021 = saisir_ica (matrixday4, 8, 'Normal');
signals_rebuiltday21 = reshape (myica021.signals.d, 101,40,8);
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,1))); title ('IC1'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
```

```
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,2))); title ('IC2'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,3))); title ('IC3'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,4))); title ('IC4'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,5))); title ('IC5'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,6))); title ('IC6'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,7))); title ('IC7'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
figure; imagesc(Lex, Lem, squeeze(signals_rebuiltday21(:,:,8))); title ('IC8'); axis
xy ; xlabel ('Excitation wavelength (nm)'); ylabel ('Emmision wavelength (nm)');
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i);
```

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```
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,5), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,6), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,7), groups.d);
set(gca, 'XTickLabel', groups.g.i);
groups = create_group1(matrixday4, 1,3);
myanovah = anova1(myica021.score.d(:,8), groups.d);
set(gca, 'XTickLabel', groups.g.i
```

RAMAN

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```
%% Script for processing and fitting spectroscopic Raman data.
% We load the matrix. After that, we select different columns to extract
% the curve. In this case, we will extract the curve of each time (time = 0
% days, time = 7 days, time = 14 days and time = 21 days). We will use as a
% reference the Saccharomyces spectra. The data was ordered in subject, so
% to extract the signal, we have to choose depends on the Saccharomyces
% position.
spectrum1 = data (:,121);
waveL = RamanShift;
[xData, yData] = prepareCurveData( waveL, spectrum1);
%% We use the app Fitting curve to fit the curve with a gaussian tendence
% of the curve. After that, we will obtain the model fitting to extract the
% pure signal and fit the spectra.
[modelCorrection1, gof] = fit( xData, yData, fittedmodel);
baseLine = modelCorrection1(waveL);
```

```
corrSpectra001 = bsxfun (@minus, data (:,[1:15, 61:75, 121:135, 181:195, 241:255, 301:
315]), baseLine);
names001 = names ([1:15, 61:75, 121:135, 181:195, 241:255, 301:315],:);
%% We use with all the spectras the same process.
spectrum2 = data (:,136);
[xData, yData2] = prepareCurveData (waveL, spectrum2);
[ModelCorrection, gof] = fit (xData, yData2, fittedmodel);
baseLine2 = modelCorrection2 (NewRamanShift);
corrSpectra002 = bsxfun (@minus, data (:,[16:30, 76:90, 136:150, 196:210, 256:270,
316:330]), baseLine2);
names002 = names ([16:30, 76:90, 136:150, 196:210, 256:270, 316:330],:);
spectrum3 = data (:,151);
[xData, yData3] = prepareCurveData (waveL, spectrum3);
[ModelCorrection3, gof] = fit (xData, yData3, fittedmodel3);
baseLine3 = modelCorrection3 (waveL);
corrSpectra003 = bsxfun (@minus, data (:,[31:45, 91:105, 151:165, 211:225, 271:285,
331:345]), baseLine3);
names003 = names ([31:45, 91:105, 151:165, 211:225, 271:285, 331:345],:);
spectrum4 = data (:, 166);
[xData, yData4] = prepareCurveData (waveL, spectrum4);
[ModelCorrection4, gof] = fit (xData, yData4, fittedmodel3);
baseLine4 = modelCorrection4 (waveL);
```

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```
corrSpectra004 = bsxfun (@minus, data (:,[46:60, 106:120, 166:180, 226:240, 286:300,
346:360]), baseLine4);
names004 = names ([31:45, 91:105, 151:165, 211:225, 271:285, 331:345],:);
%% Union of all the traited spectra and all the names.
namesnew = cat (1, names001, names002, names003, names004);
datanew = cat (2, corrSpectra001, corrSpectra002, corrSpectra003, corrSpectra004);
datanew = datanew'
figure; subplot (2,2,1); hold on; plot (RamanShift, datanew(301, :),'-b'); plot
(RamanShift, datanew(346,:),'-r');
legend ('Saccharomyces time 21 days', 'SacxBrettanomyces 10E4 time 21 days'); axis
tight;
subplot (2,2,2); hold on; plot (RamanShift, datanew (301,:),'-b'); plot (RamanShift,
datanew (331,:),'-r');
legend ('Saccharomyces time 21 days', 'SacxBrettanomyces 10E5 time 21 days'); axis
tight;
subplot (2,2,3); hold on; plot (RamanShift, datanew (301,:),'-b'); plot (RamanShift,
datanew(316,:),'-r');
legend ('Saccharomyces time 21 days', 'SacxBrettanomyces 10E6 time 21 days'); axis
tight;
subplot (2,2,4); hold on ;plot (RamanShift, datanew (301,:),'-b'); plot (RamanShift,
datanew (276,:),'-r');
legend ('Saccharomyces time 21 days', 'Brettanomyces time 21 days'); axis tight;
%% PCA
%001
mydata_001 = selectrow (mydata, [1:90]);
mypca001 = saisir_pca (mydata_001);
figure('color', [1 1 1]);
colored_map1(mypca001.score, 1, 2, 1, 8); title ('PC1 vs PC2 Day 1');
figure('color', [1 1 1]);
colored_map1(mypca001.score, 1, 3, 1, 8); title ('PC1 vs PC3 Day 1');
figure; subplot (2,2,1); plot (NewRamanShift, mypca001.eigenvec.d(:,1)); title
('Loadings PC1 Day 1'); xlabel ('Wavelength (1/cm)'); ylabel = ('Proportions'); axis
tight;
subplot (2,2,2); plot (NewRamanShift, mypca001.eigenvec.d(:,2)); title ('Loadings PC2
Day 1'); xlabel ('Wavelength (1/cm)'); ylabel = ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, mypca001.eigenvec.d(:,3)); title ('Loadings PC3
Day 1'); xlabel ('Wavelength (1/cm)'); ylabel = ('Proportions'); axis tight;
```

```
% ICA 001
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata_001.d,10)
myica001 = saisir_ica (mydata_001,3, 'Normal');
figure; subplot (2,2,1); plot (NewRamanShift, myica001.signals.d(:,1)); title ('IC1');
```

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```
xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,2); plot (NewRamanShift, myica001.signals.d(:,2)); title ('IC2'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, myica001.signals.d(:,3)); title ('IC3'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
figure; subplot (2,3,1); plot (x, myica001.scores.d(:,1), 'o'); title ('IC1'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,2); plot (x, myica001.scores.d(:,2), 'o'); title ('IC2'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,3); plot (x, myica001.scores.d(:,3), 'o'); title ('IC3'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,4); plot (x, myica001.scores.d(:,4), 'o'); title ('IC4'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,5); plot (x, myica001.scores.d(:,5), 'o'); title ('IC5'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,6); plot (x, myica001.scores.d(:,6), 'o'); title ('IC6'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
%subplot (2,4,7); plot (RamanShift, myica001.signals.d(:,7)); title ('IC7'); xlabel
('Wavelength (1/cm)'); ylabel = ('Proportions'); axis tight;
groups = create_group1(mydata_001, 1,3);
myanovaTete = anova1(myica001.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC1');
groups = create_group1(mydata_001, 1,3);
myanovaTete = anova1(myica001.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC2');
groups = create_group1(mydata_001, 1,3);
myanovaTete = anova1(myica001.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC3');
% ANOVA PCA
groups = create_group1(mydata_001, 1,8);
myanovaTete = anova1(mypca001.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC1');
groups = create_group1(mydata_001, 1,8);
myanovaTete = anova1(mypca001.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC2');
groups = create_group1(mydata_001, 1,8);
myanovaTete = anova1(mypca001.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC3');
%% 007
mydata_007 = selectrow (mydata, [91:180]);
mypca007 = saisir_pca (mydata_007);
```

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```
figure('color', [1 1 1]);
colored_map1(mypca007.score, 1, 2, 1, 8); title ('PC1 vs PC2 Day 7');
figure('color', [1 1 1]);
colored_map1(mypca007.score, 1, 3, 1, 8); title ('PC1 vs PC3 Day 7');
figure; subplot (2,2,1); plot (NewRamanShift, mypca007.eigenvec.d(:,1)); title
('Loadings PC1 Day 7'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis
tight;
subplot (2,2,2); plot (NewRamanShift, mypca007.eigenvec.d(:,2)); title ('Loadings PC2
Day 7'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, mypca007.eigenvec.d(:,3)); title ('Loadings PC3
Day 7'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
% ANOVA PCA
```

```
groups = create_group1(mydata_007, 1,8);
myanovaTete = anova1(mypca001.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC1');
groups = create_group1(mydata_007, 1,8);
myanovaTete = anova1(mypca001.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC2');
groups = create_group1(mydata_007, 1,8);
myanovaTete = anova1(mypca001.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC3');
% ICA 007
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata_007.d,10)
myica007 = saisir_ica (mydata_007,3, 'Normal');
figure; subplot (2,2,1); plot (NewRamanShift, myica007.signals.d(:,1)); title ('IC1');
xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,2); plot (NewRamanShift, myica007.signals.d(:,2)); title ('IC2'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, myica007.signals.d(:,3)); title ('IC3'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
groups = create_group1(mydata_007, 1,3);
myanovaTete = anova1(myica007.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC1 Day 7');
groups = create_group1(mydata_007, 1,3);
myanovaTete = anova1(myica007.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC2 Day 7');
groups = create_group1(mydata_007, 1,3);
myanovaTete = anova1(myica007.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC3 Day 7');
figure; subplot (2,3,1); plot (x, myica007.score.d(:,1), 'o'); title ('IC1'); xlabel
```



```
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,2); plot (x, myica007.score.d(:,2), 'o'); title ('IC2'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,3,3); plot (x, myica007.score.d(:,3), 'o'); title ('IC3'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
%% 014
mydata_014 = selectrow (mydata, [181:270]);
mypca014 = saisir_pca (mydata_014);
figure('color', [1 1 1]);
colored_map1(mypca014.score, 1, 2, 1, 8); title ('PC1 vs PC2 Day 14');
figure('color', [1 1 1]);
colored_map1(mypca014.score, 1, 3, 1, 7); title ('PC1 vs PC3 Day 14');
figure; subplot (2,2,1); plot (NewRamanShift, mypca014.eigenvec.d(:,1)); title
('Loadings PC1 Day 14'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis
tight;
subplot (2,2,2); plot (NewRamanShift, mypca014.eigenvec.d(:,2)); title ('Loadings PC2
Day 14'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, mypca014.eigenvec.d(:,3)); title ('Loadings PC3
Day 14'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
% ANOVA PCA
groups = create_group1(mydata_014, 1,8);
myanovaTete = anova1(mypca014.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC1');
groups = create_group1(mydata_014, 1,8);
myanovaTete = anova1(mypca014.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC2');
groups = create_group1(mydata_014, 1,8);
myanovaTete = anova1(mypca014.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC3');
% ICA 014
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata_014.d,10)
```

```
myica014 = saisir_ica (mydata_014,4, 'Normal');
figure; subplot (2,2,1); plot (NewRamanShift, myica014.signals.d(:,1)); title ('IC1');
xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,2); plot (NewRamanShift, myica014.signals.d(:,2)); title ('IC2'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, myica014.signals.d(:,3)); title ('IC3'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,4); plot (NewRamanShift, myica014.signals.d(:,4)); title ('IC4'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
```



```
groups = create_group1(mydata_014, 1,3);
myanovaTete = anova1(myica014.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC1 Day 14');
groups = create_group1(mydata_014, 1,3);
myanovaTete = anova1(myica014.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC2 Day 14');
groups = create_group1(mydata_014, 1,3);
myanovaTete = anova1(myica014.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC3 Day 14');
groups = create_group1(mydata_014, 1,3);
myanovaTete = anova1(myica014.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC4 Day 14');
figure; subplot (2,3,1); plot (x, myica014.score.d(:,1), 'o'); title ('IC1'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,3,2); plot (x, myica014.score.d(:,2), 'o'); title ('IC2'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,3,3); plot (x, myica014.score.d(:,3), 'o'); title ('IC3'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,3,4); plot (x, myica014.score.d(:,4), 'o'); title ('IC4'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,3,5); plot (x, myica014.score.d(:,5), 'o'); title ('IC5'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,3,6); plot (x, myica014.score.d(:,6), 'o'); title ('IC6'); xlabel
('Wavelength (1/cm)'); axis tight;
%% 21
mydata_021 = selectrow (mydata, [271:360]);
mypca021 = saisir_pca (mydata_021);
figure('color', [1 1 1]);
colored_map1(mypca021.score, 1, 2, 1, 8); title ('PC1 vs PC2 Day 21');
figure('color', [1 1 1]);
colored_map1(mypca021.score, 1, 3, 1, 8); title ('PC1 vs PC3 Day 21');
figure; subplot (2,2,1); plot (NewRamanShift, mypca021.eigenvec.d(:,1)); title
('Loadings PC1 Day 21'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis
tight;
subplot (2,2,2); plot (NewRamanShift, mypca021.eigenvec.d(:,2)); title ('Loadings PC2
Day 21'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, mypca021.eigenvec.d(:,3)); title ('Loadings PC3
Day 21'); xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
% ANOVA PCA
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(mypca021.score.d(:,1), groups.d);
```



```
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC1');
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(mypca021.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC2');
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(mypca021.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA PC3');
```

```
% ICA 021
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata_021.d,10)
myica021 = saisir_ica (mydata_021,3, 'Normal');
figure; subplot (2,2,1); plot (NewRamanShift, myica021.signals.d(:,1)); title ('IC1');
xlabel ('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,2); plot (NewRamanShift, myica021.signals.d(:,2)); title ('IC2'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (NewRamanShift, myica021.signals.d(:,3)); title ('IC3'); xlabel
('Wavelength (1/cm)'); ylabel ('Proportions'); axis tight;
groups = create_group1(mydata_021, 1,3);
myanovaTete = anova1(myica021.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC1 Day 21');
groups = create_group1(mydata_021, 1,3);
myanovaTete = anova1(myica021.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC2 Day 21');
groups = create_group1(mydata_021, 1,3);
myanovaTete = anova1(myica021.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC3 Day 21');
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(myica021.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC4 Day 14');
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(myica021.score.d(:,5), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC5 Day 14');
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(myica021.score.d(:,6), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC6 Day 14');
groups = create_group1(mydata_021, 1,8);
myanovaTete = anova1(myica021.score.d(:,7), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC7 Day 14');
figure; subplot (2,4,1); plot (x, myica021.score.d(:,1), 'o'); title ('IC1'); xlabel
('Wavelength (1/cm)'); axis tight;
```



```
subplot (2,4,2); plot (x, myica021.score.d(:,2), 'o'); title ('IC2'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,4,3); plot (x, myica021.score.d(:,3), 'o'); title ('IC3'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,4,4); plot (x, myica021.score.d(:,4), 'o'); title ('IC4'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,4,5); plot (x, myica021.score.d(:,5), 'o'); title ('IC5'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,4,6); plot (x, myica021.score.d(:,6), 'o'); title ('IC6'); xlabel
('Wavelength (1/cm)'); axis tight;
subplot (2,4,7); plot (x, myica021.score.d(:,7), 'o'); title ('IC7'); xlabel
('Wavelength (1/cm)'); axis tight;
%% ICs Plot comparison
figure; hold on; plot (NewRamanShift, myica001.signals.d (:,1)); axis tight; xlabel
('RamanShift (1/cm)'); ylabel ('Proportions'); title ('IC1');
plot (NewRamanShift, myica007.signals.d (:,1)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
plot (NewRamanShift, myica014.signals.d (:,1)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
plot (NewRamanShift, myica021.signals.d (:,1)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
legend ('Day 1', 'Day 7', 'Day 14', 'Day 21'); hold off;
figure; hold on; plot (NewRamanShift, myica001.signals.d (:,2)); axis tight; xlabel
('RamanShift (1/cm)'); ylabel ('Proportions'); title ('IC2');
plot (NewRamanShift, myica007.signals.d (:,2)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
plot (NewRamanShift, myica014.signals.d (:,2)); axis tight; xlabel ('RamanShift
```

```
(1/cm)'); ylabel ('Proportions');
plot (NewRamanShift, myica021.signals.d (:,2)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
legend ('Day 1', 'Day 7', 'Day 14', 'Day 21'); hold off;
figure; hold on; plot (NewRamanShift, myica007.signals.d (:,3)); axis tight; xlabel
('RamanShift (1/cm)'); ylabel ('Proportions'); title ('IC3');
plot (NewRamanShift, myica014.signals.d (:,3)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
plot (NewRamanShift, myica021.signals.d (:,3)); axis tight; xlabel ('RamanShift
(1/cm)'); ylabel ('Proportions');
legend ('Day 7', 'Day 14', 'Day 21'); hold off;
xic1 = [myica007.signals.d(:,1) myica014.signals.d(:,1) myica021.signals.d(:,1)];
[n1, p1] = size (xic1);
meanXIC1 = mean(xic1);
XIC1_Centred = xic1 - ones(n1,1) * meanXIC1;
figure; hold on; title ('IC1');
plot (NewRamanShift, XIC1_Centred); axis tight; xlabel ('RamanShift (1/cm)'); ylabel
('Proportions');
```

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```
legend ('Day 7', 'Day 14', 'Day 21'); hold off;
xic2 = [myica001.signals.d(:,2) myica007.signals.d(:,2) myica014.signals.d(:,2)
myica021.signals.d(:,2)];
meanXIC2 = mean(xic2);
[n2, p2] = size (xic2);
XIC2_Centred = xic2 - ones(n2,1) * meanXIC2;
figure; hold on; title ('IC2');
plot (NewRamanShift, XIC2_Centred); axis tight; xlabel ('RamanShift (1/cm)'); ylabel
('Proportions');
legend ('Day 1','Day 7', 'Day 14', 'Day 21'); hold off;
xic3 = [myica007.signals.d(:,3) myica014.signals.d(:,3) myica021.signals.d(:,3)];
meanXIC3 = mean(xic3);
[n3, p3] = size (xic3);
XIC3_Centred = xic3 - ones(n3,1) * meanXIC3;
figure; hold on; title ('IC3');
plot (NewRamanShift, XIC3_Centred); axis tight; xlabel ('RamanShift (1/cm)'); ylabel
('Proportions');
legend ('Day 7', 'Day 14', 'Day 21'); hold off
```

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```
%% MTX5
mydata001 = DonnesvinChromatoFin';
names = char(DonnesvinChromatoFin);
mydata001 = matrix2saisir(mydata001);
mydata001.i = names;
mypca001 = saisir_pca(mydata001);
figure ('color', [1 1 1]); colored_map1 (mypca001.score, 1, 2, 1, 7);
figure ('color', [1 1 1]); colored_map1 (mypca001.score, 1, 3, 1, 7);
x = [0:0.01:110];
figure; plot (x, mypca.eigenvec.d(:,1));
figure; plot (x, mypca001.eigenvec.d(:,1)); title ('Loadings PC1 Day 1'); axis tight;
xlabel ('Time acquisition (s)'); ylabel ('Proportions');
figure; plot (x, mypca001.eigenvec.d(:,2)); title ('Loadings PC2 Day 1'); axis tight;
xlabel ('Time acquisition (s)'); ylabel ('Proportions');
figure; plot (x, mypca001.eigenvec.d(:,3)); title ('Loadings PC3 Day 1'); axis tight;
xlabel ('Time acquisition (s)'); ylabel ('Proportions');
```

```
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata001.d,10);
myica001 = saisir_ica (mydata001,7, 'Normal');
figure; plot (x, myica001.signals.d(:,1)); title ('IC1'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,2)); title ('IC2'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,3)); title ('IC3'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,4)); title ('IC4'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,5)); title ('IC5'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,6)); title ('IC6'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,7)); title ('IC7'); xlabel ('Time acquisition
(s)'); axis tight;
%% 21
data = DonnesVinChromato021';
names = char(DonnesVinChromato021);
mydata = matrix2saisir(data);
data.i = names;
mypca = saisir_pca(data);
figure ('color', [1 1 1]); colored_map1 (mypca1.score, 1, 2, 1, 3);
figure ('color', [1 1 1]); colored_map1 (mypca1.score, 1, 3, 1, 3);
x = [0:0.01:110];
figure; plot (x, mypca.eigenvec.d(:,1));
figure; plot (x, mypca.eigenvec.d(:,1)); title ('Loadings PC1 Day 1'); axis tight;
xlabel ('Time acquisition (s)'); ylabel ('Proportions');
figure; plot (x, mypca.eigenvec.d(:,2)); title ('Loadings PC2 Day 1'); axis tight;
```



```
xlabel ('Time acquisition (s)'); ylabel ('Proportions');
figure; plot (x, mypca.eigenvec.d(:,3)); title ('Loadings PC3 Day 1'); axis tight;
xlabel ('Time acquisition (s)'); ylabel ('Proportions');
figure; subplot (2,2,1); plot (x,mypca1.eigenvec.d(:,1)); title ('Loadings PC1');
xlabel ('Acquisition time (s)'); ylabel ('Proportions'); axis tight;
subplot (2,2,2); plot (x,mypca1.eigenvec.d(:,2)); title ('Loadings PC2'); xlabel
('Acquisition time (s)'); ylabel ('Proportions'); axis tight;
subplot (2,2,3); plot (x,mypca1.eigenvec.d(:,3)); title ('Loadings PC3'); xlabel
('Acquisition time (s)'); ylabel ('Proportions'); axis tight;
%subplot (2,2,4); hold on; plot (x,mypca1.eigenvec.d (:,1), 'b'); plot (x, mypca1.
eigenvec.d(:,2), 'r'); plot (x, mypca1.eigenvec.d (:,3), 'g'); hold off; axis tight;
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata001.d,10);
myica001 = saisir_ica (mydata001,7, 'Normal');
figure; plot (x, myica001.signals.d(:,1)); title ('IC1'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,2)); title ('IC2'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,3)); title ('IC3'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,4)); title ('IC4'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,5)); title ('IC5'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,6)); title ('IC6'); xlabel ('Time acquisition
(s)'); axis tight;
figure; plot (x, myica001.signals.d(:,7)); title ('IC7'); xlabel ('Time acquisition
(s)'); axis tight;
%% 021
[DW_all,SigTemp,ScoTemp,Correlations,W] = ICA_DW_Corr_W(mydata1.d,10);
myica021 = saisir_ica (totaldataprocessed, 5, 'Normal');
```

```
figure; %subplot (2,2,1); plot (RT,myica021.signals.d (:,1)); axis tight; title
('IC1'); xlabel ('Acquisition time (s)'); ylabel ('Proportions');
subplot (2,2,1); plot (RT,myica021.signals.d (:,2)); axis tight; title ('IC2'); xlabel
('Acquisition time (s)'); ylabel ('Proportions');
subplot (2,2,2); plot (RT,myica021.signals.d (:,3)); axis tight; title ('IC3'); xlabel
('Acquisition time (s)'); ylabel ('Proportions');
subplot (2,2,3); plot (RT,myica021.signals.d (:,4)); axis tight; title ('IC4'); xlabel
('Acquisition time (s)'); ylabel ('Proportions');
subplot (2,2,4); plot (RT,myica021.signals.d (:,5)); axis tight; title ('IC5'); xlabel
('Acquisition time (s)'); ylabel ('Proportions');
groups = create_group1(totaldataprocessed, 1,3);
myanovaTete = anova1(myica.score.d(:,1), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC1');
```

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```
groups = create_group1(totaldataprocessed, 1,3);
myanovaTete = anova1(myica.score.d(:,2), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC2');
groups = create_group1(totaldataprocessed, 1,3);
myanovaTete = anova1(myica.score.d(:,3), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC3');
groups = create_group1(totaldataprocessed, 1,3);
myanovaTete = anova1(myica.score.d(:,4), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC4');
groups = create_group1(totaldataprocessed, 1,3);
myanovaTete = anova1(myica.score.d(:,5), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC5');
groups = create_group1(totaldataprocessed, 1,3);
myanovaTete = anova1(myica.score.d(:,6), groups.d);
set(gca, 'XTickLabel', groups.g.i); title ('ANOVA IC6
```

ANNEX 2

FITXA DE SEGUIMENT DEL TUTOR/A del TFM

Nom i Cognoms de l'Alumne/a: Alberto Rodríguez Izquierdo

Nom i Cognoms del Tutor/a: María del Carmen Portillo Guisado

Data de la entrevista amb l'alumne: 5 de Julio de 2018

Recomanacions durant el seguiment: El alumno me entrega la memoria el 4 de Julio aún sin acabar para su revisión y seguimiento. Me comunica que falta la interpretación de datos y los anexos. Tras una hojeada a la estructura parece correcta, aunque faltan partes fundamentales como resumen y palabras claves, objetivos, discusión, conclusiones y posibles perspectivas, datos del centro donde ha realizado las prácticas. Además, le comento algunas correcciones sobre figuras y tablas (tamaño, número, explicación del pie de figura…). Después de leer el texto, también he sugerido una intensa corrección del inglés y algunas aclaraciones sobre metodología y resultados.

Observacions

Observacions Darrera revisió:

Signatura del Tutor/a

Signatura del Alumne/a

RODRIGUEZ IZQUIERDO ALBERTO - 47529979L

Firmado digitalmente por RODRIGUEZ IZQUIERDO ALBERTO - 47529979L
Fecha: 2018.08.18 19:13:42 +02'00'

Tarragona a 5 de Julio 2018