

Assessing Quality and FT-MIR spectroscopic chemometrics of virgin olive oils extracted from three olive cultivars in Morocco: Haouzia, Menara and Picholine

S. Benlamaalam¹, Z. Mourhat², M. Chigr¹, M. Gouskir², A. Ait Ider², N. Belkhouya², M. Benali², M. Oubenali², and M. Mbarki²

¹Laboratory of Organic and Analytical Chemistry,
University Sultan Moulay Slimane,
Béni Mellal, Morocco

²Transdisciplinary Team of Analytical Science for Sustainable Development,
University Sultan Moulay Slimane,
Béni Mellal, Morocco

Copyright © 2016 ISSR Journals. This is an open access article distributed under the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT: Thanks to its nutritional value, its cosmetic and therapeutic properties, virgin olive oil (VOO) is becoming as a food in the «Mediterranean diet» gaining more and more interest among the people in the world. Finding a difficulty to detect the slight difference between virgin olive oils from three varieties established in Morocco «Haouzia», «Menara» and «Picholine», this work has the objective of approaching fast and less expensive tool for classification of virgin olive oil depending on its variety. This will contribute to the establishment of databases and cost-effective and efficient analytical methods in the olive industry. 11 samples of VOO from the «Haouzia» olive variety, 12 from the «Menara» variety and 16 from the «Picholine» have been collected from three plots, so that each plot is dedicated to one of these three varieties. The analysis of parameters like free acidity, peroxide content and UV spectroscopy absorbance at 232 and 270 nm have shown that a majority of the samples meet the standards of the International Olive Oil Council (IOOC). Both varieties «Menara» and «Picholine» tend to perform better in terms of the parameters analyzed. However, the results of these parameters and those in the FT-MIR spectroscopy showed a similarity confirming the same varietal origin of the three varieties. A chemometric treatment coupling the hierarchical cluster method (CHA) and that of the principal component analysis (PCA) on the one hand and spectroscopic FT-MIR in the other hand, has enabled us to approach a classification tool for such similar VOO. Such coupling of methods is an important tool to gain time and analysis cost for the purpose of quality control of the VOO.

KEYWORDS: Virgin olive oil, quality, olive varieties, *Haouzia*, *Menara*, *Picholine*, FT-MIR, classification, CHA, ACP.

1 INTRODUCTION

Virgin olive oil (VOO) is gaining more and more the interest of both communities of scientists, nutritionists, as aestheticians. The content of this herbal food lipids, omega-3 fatty acids [1,2], protein, vitamins and trace component such as phenolic compounds and minerals provide this food a nutritious and therapeutic wealth. Various previous studies have confirmed the antioxidant effects of VOO [3,4] as a "Mediterranean diet" food. Furthermore, the effectiveness of this food has already been demonstrated in favoring the increase of good cholesterol [5-8] and reducing the risk of fatal cardiovascular diseases such as ischemic heart disease [2]. Recently, results of our team work have confirmed the antioxidant properties of these phenolic compounds [9-11]. However, the problematic is that the varietal profile of the olive plots in Morocco is not yet well studied. Despite previous work, one needs work dealing with this problematic in Morocco. Moreover, the two varieties "Haouzia" and "Menara", being similar to the "Picholine" could present performance in adaptation to rainfed conditions [12]. Thus the distinction between these three varieties of olives contributes to the enhancement through scientific preparation of legal appellations of controlled origin (ACO) for olives in the olive-growing areas in Morocco, like the

"Beni Mellal-Khenifra " area, in that we carried out the present work. In fact, this work meets the strategy of the "Green Morocco" plan as a major project in which this area is highly involved. In this context we have been interested in the characterization of the parameters that play a decisive role in the quality of olive oil and the classification of its varieties. These parameters are: chemical indicators such as the free acidity, peroxide content, K270 and K290 absorbances at UV spectroscopy. However, it has proved very difficult to detect the slight difference between these three varieties. In fact, in Morocco, there is need to establish databases and cost-effective and efficient analytical methods in the olive industry. Thus, we have used the chemometric methods on mid-infrared spectroscopy data as physical and chemical analysis technique of a routine in the laboratory. This has allowed us to offer a simple, less expensive, repetitive and robust for the traceability of the Moroccan olive oil according to its varietal origin. This traceability plays a crucial and decisive role to check the label of quality in the process of controlling the quality of virgin olive oil (abbreviated hereafter to HOV) once placed in the international market.

2 MATERIELS ANB METHODES

2.1 PLANT MATERIAL AND COLLECTING OLIVES SAMPLES

In 2008, we have carried out the cultivation of olive three plots in the "Beni Mellal-Khenifra" area in Morocco. The three varieties are called "Haouzia", "Menara" and "Picholine". The plots are in the same geographical zone so that the plantations of olives trees are considered being in the same environmental conditions of soil, irrigation, fertigation and weather.

2.2 COLLECTION OF OLIVE SAMPLE

In December 2012, we collected 11 olives samples from the "Haouzia" variety, 12 olives samples from "Menara" variety and 16 samples from the "Picholine". In fact, picking olives samples was performed in order to respect the representativeness of the samples within the plot. Each olive sample of 1.5 to 2 Kg was plucked manually way stage of maturity such that the sample contains green olives, reddish and blackish. So, all the 39 olive samples were stored at 4 ° C until the extraction of oil.

2.3 EXTRACTION OF THE OIL

Each of the 39 samples of olives was crushed by a mechanical crusher. The obtained pulp is placed in a centrifuge at a 2000t/min rotation of the sample to separate the solid phase from the oil phase. The resulting oil was stored at 4 ° C in the dark until the time of chemical- or spectroscopic FT-MIR analysis.

2.4 CHEMICAL CHARACTERIZATION

The determination of the free acidity and peroxide samples was performed according to the standards of the International Olive Oil Council (IOOC) [13,14]. The reagents used for the determination of the free acidity are: Ethanol, KOH, Na₂S₂O₃, KI and I₂. Free acidity of the olive oil samples, expressed as percentage of oleic acid, is determined by titration by an Ethanoic solution of potassium hydroxide. A control assay was produced in the same conditions. The peroxide value (PV), expressed in milliequivalents of active oxygen per kg of oil (meq O₂/kg oil) was determined after the dissolution of a mass of olive oil in an acetic/acid chloroform (3:2 v/v) mixture. The reaction is triggered in the dark, with a saturated solution of potassium iodide. The liberated iodine is titrated by a 0.01 N sodium thiosulfate, in the presence of starch. So, a control test (without oil sample) has been done in the same conditions.

2.5 FOURIER TRANSFORM MID-IRRED SPECTROSCOPY (FT-MIRS)

To characterize the oils in terms of organic functions of the majority chemical species, we carried out an analysis by infrared spectroscopy presenting a Fourier transform (FT-MIR). The spectra were obtained at a Bruker Vector 22 Diamond ATR spectrometer that presents a 4 cm⁻¹ resolution between 600 and 4000 cm⁻¹ with the 2.1 software integration result Thermo Nicolet. The FT-MIR spectra proved that they are very similar to be distinguished from each other. Thus we used chemometric methods in order to detect any minor differences between these spectra virgin olive oils from the three olive varieties.

2.6 CHEMOMETRICS

Classification and from oil exploration between the three varieties of olives were approached using, on the one hand, absorbances values of FT-MIR spectroscopic analysis coupled to the ascending hierarchical cluster method (CHA) and the principal component analysis (PCA) method, on the other hand.

3 RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL CHARACTERIZATION

The following Table 1 reports the results of the free acidity, peroxide content(IP) and the K232 and K270 absorbances at UV spectroscopy. This Table presents the results with values average and their standard deviation (S.D)

Table 1: Distribution of parameter: free acidity, peroxide content, and K232 and K270 absorbances at UV spectroscopy, of virgin oils extracted from three varieties "Haouzia", "Menara" and "Picholine"

		Free Acidity (% oleic acid)	IP (mill.eq. O2/Kg)	K232	K270
"Haouzia"	Min-Max	1.72-3.60	12.99-21.77	2.39-2.81	0.23-0.45
	Average	2.7236	16.6691	2.6073	0.3164
	S.D*	0.7342	2.7210	0.1124	0.0633
"Menara"	Min-Max	1.79-3.29	12.55-18.56	2.40-2.69	0.21-0.42
	Average	2.5092	16.0917	2.5275	0.3158
	S.D	0.6273	2.2108	0.1024	0.0601
"Picholine"	Min-Max	1.11-3.03	13.05-19.96	2.44-2.77	0.25-0.34
	Average	2.0775	16.8594	2.5731	0.2937
	S.D	0.6407	2.3588	0.0988	0.0299

* S.D: standard deviation

3.2 MID-INFRARED SPECTROSCOPY

The following figure 1 provides a spectrum type of virgin olive oil for each of the three varieties.

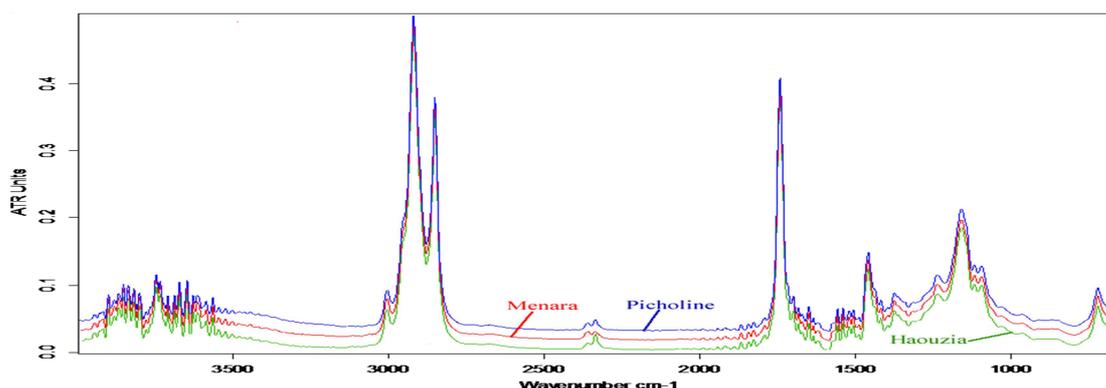


Figure 1: FT-MIR spectrum type for each of the three virgin olive oil varieties: spectrum of the "Picholine" variety (up), the spectrum of "Haouzia" (down) and between them: the spectrum of "Menara"

3.3 CHEMOMETRIC TREATMENT OF FT-MIR SPECTROSCOPY DATA

The following figures 2 and 3 illustrate the results obtained by the CHA method and the PCA one, respectively. Figure 4 shows the representation of the explained variance for the calibration and validation for the PCA. Of the total 39 samples, at this chemometric analysis we omitted 5 samples that are out IOOC standards, they are all from the Haouzia variety. Thus, the

classification shaft of the 34 remaining samples (Figure 2) has been based on the calculation of the median and the correlation between the different variables (oil sampes).

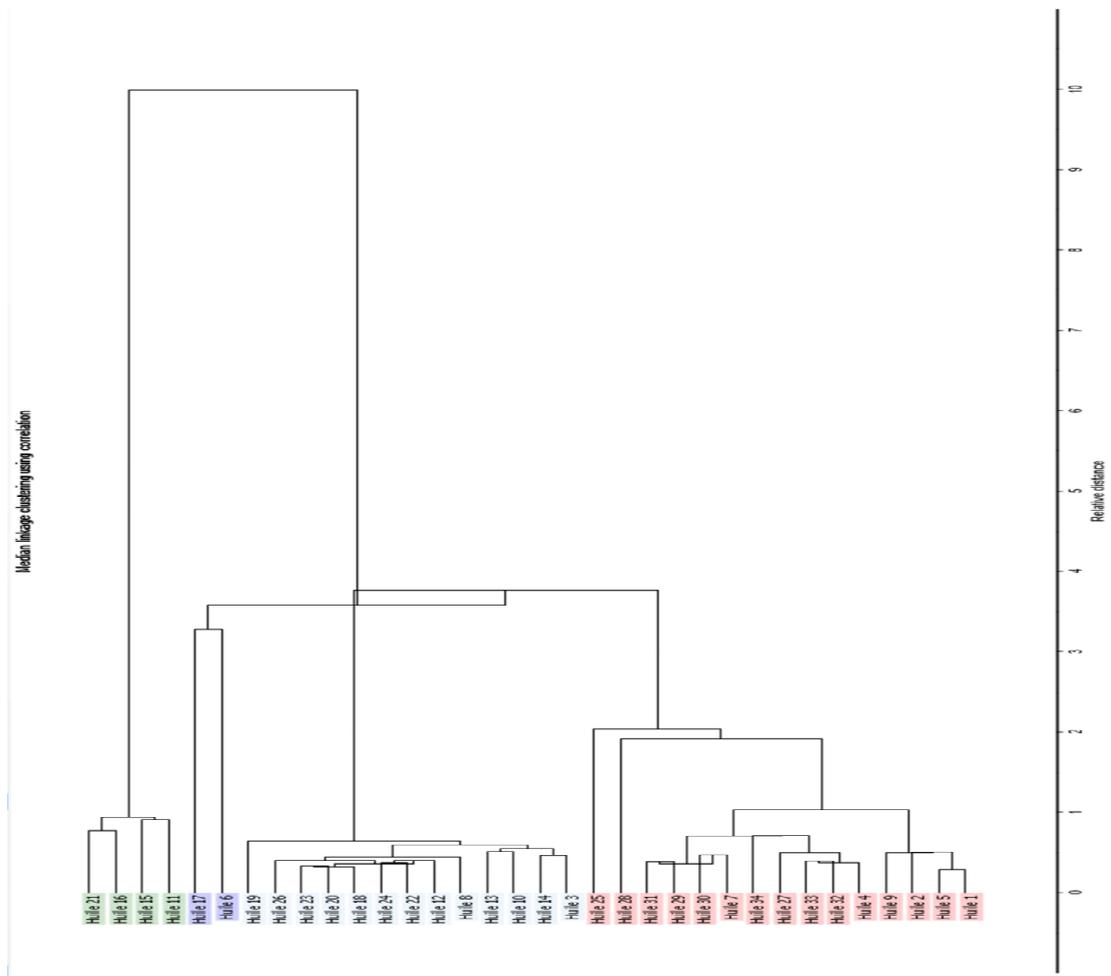


Figure 2: Classification shaft, by the CHA method, of virgin olive oil samples from the three varieties "Haouzia", "Menara" and "Picholine"

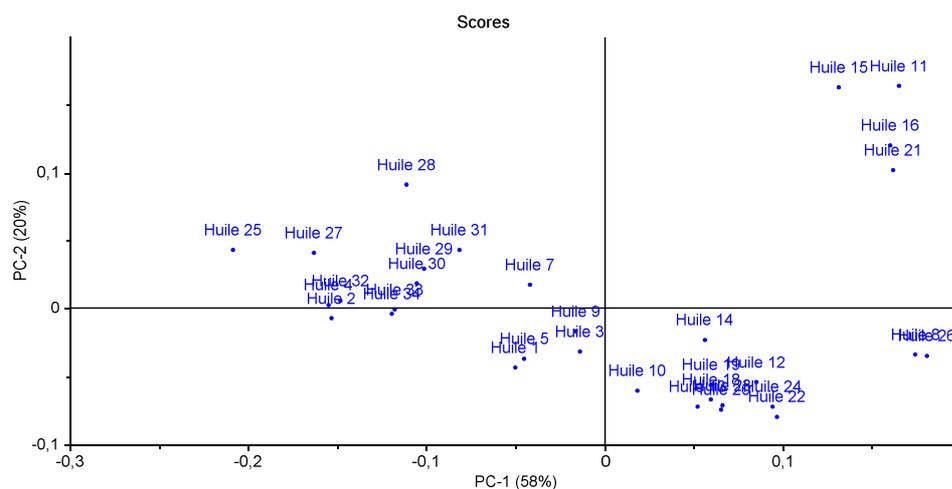


Figure 3: Classification by principal component analysis (PCA) of virgin olive oil samples from the three varieties "Haouzia", "Menara" and "Picholine"

3.4 DISCUSSION

The results in Table 1 show that a majority of VOO samples respects the IOC standards, especially in terms of free acidity and peroxide content [13, 14]. This can be explained by the fact that on the one hand the conditions of olive harvest and transportation of the olives to the place of extraction of oil and, on the other hand, the extraction and conservation of the oils have been done in good conditions.

The VOO from the "Picholine" olive variety presents the lowest free acidity, and then followed by the "Menara" variety VOO that presents the lowest peroxide content, followed by the "Haouzia" variety VOO. The standard deviations show that dispersion of free acidity values around the average is more marked in the case of the "Haouzia" variety VOO. In addition, differences between the three types of VOO in terms of UV absorbances were less marked than those in terms of free acidity and peroxide, in particular in case of K270 absorbance. Overall, these results of acidity, peroxide and UV absorbances show that the three varieties are similar to each other although the two varieties "Menara" and "Picholine" tend to exhibit more performance than the "Haouzia". Moreover, such a similarity between these three varieties confirms that the two varieties "Haouzia" and "Menara" are selected varieties derived from the "Picholine" [12].

At the mid-infrared spectroscopy of the samples we observe that the spectrum types, from the three varieties, are very similar (Figure 1). Effectively, this result has been expected given the fact that the three varieties do not present very different chemical characteristics in terms of free acidity, peroxide content and UV absorbance.

According to the figure 1, FT-MIR spectra would show same characteristics of the olive oil. Two intense bands around 2924 and 2853 cm^{-1} respectively are due to vibration of asymmetric and symmetric elongation of CH_2 . Another moderately intense band, that is observable around 2953 cm^{-1} , is due to the asymmetrical vibration of elongation of the CH_3 group. In the low frequency zone, only the CH_2 deformation vibration is observable. The 1458 and 1232 cm^{-1} bands are attributable to shearing deformation and vibrations out of the plane. The band, around 724 cm^{-1} , is representative of the rocking vibration and deformation. The $=\text{CH}$ vibration mode that presents to a low intensity band is observed around 3008 cm^{-1} . The $\text{C}=\text{O}$ vibration mode appears around 1750 cm^{-1} . A clearly observable band is located at 1150 cm^{-1} , this band is due to the stretching vibration of $\text{C}-\text{O}$ group of esters. The intensity of this peak would be, also, inversely proportional to the number of carbon atoms.

The dendrogram result of the CHA method, given by figure 2, shows that there is a classification between the three sample groups, each group of which comes from one of the three varieties. In fact, in 2011, Mr. De Luca et al. used the CHA coupled to FT-IR spectroscopy to classify the VOO from Morocco [15]. Recently, a work of our team on the FT-MIR data of "Picholine" variety VOO, in the Moroccan "Beni Mellal-Khenifra" area, has been able to classify VOO based on its

geographical origin[16]. Such classification is more delicate than that based on the variety of olives as it is the case of the present work. Moreover, the chemometric methods have been very useful for the classification, discrimination and authentication of vegetable oils as it has been reported in other work carried out by our team [17].

According to the figure 3, the first principal component (PC1) and the second principal component (PC2) explain respectively 58% and 20% of the total variance. In fact, PC1 ensure the maximum of information and PC2 provides the majority of the residual information. Thanks to the PCA method there is, also, a classification between 3 groups of oils from the three varieties. The CPA is actually one of the most applied methods of classification. Figure 4 confirms that both PC1 and PC2 axes are sufficient to have a majority (over 70%) of the information from the FT-MIR spectra and four axes would explain almost all of the information. Moreover, in order to detect the adulteration of olive oil extra edible oils, Rubén M. et al. have established in 2010, a new chemometric strategy based on the coupling between, on the one hand, the methods of analysis and, on the other hand, algorithms such as PCA and MPA [18]. After the present work, PCA and CHA analyses algorithms would allow automatic classification based on mid-infrared spectroscopy characteristics of Moroccan VOO.

Thus, with the coupling between the FT-IR spectroscopy and chemometric methods could classify the VOO from the varieties even they are very similar. In fact, previous studies have discussed various varietal and geographical characteristics of olive oils produced in the world [19-22] and such a coupling would be of great importance.

4 CONCLUSION

The analysis of quality parameters like free acidity and peroxide content and UV absorbances of virgin olive oil three varieties "Haouzia", "Menara" and "Picholine" have shown that a majority of samples meet the standards of the International Olive Oil Council. Both varieties' Menara " and " Picholine " tend to perform better in terms of parameters analyzed.

The coupling of the FT-MIR spectroscopic method and chemometric methods such PCA and CHA is a fast and less expensive tool for classification and discrimination between oils from these three varieties although they are similar. Such a tool would be useful to gain in terms of physical and chemical analyzes and time in the field of quality control of virgin olive oil and other products.

REFERENCES

- [1] S. K. Srivastava and K. Kaur, "Stability of Impulsive Differential Equation with any Time Delay," *International Journal of Innovation and Applied Studies*, vol. 2, no. 3, pp. 280–286, 2013.
- [2] Rahman I. U, Qureshi M. N. and Shakeel A, "Comparative Study of Fatty Acid Components in Oils of Different Olive Varieties Grown in Different Regions of Pakistan by Gas Chromatography-Mass Spectrometry", *Journal of the chinese chemical society*, Vol.59 pp.46-50,2012.
- [3] Brouwer I. A., Katan M. B., Zock P. L, "Dietary α -Linolenic Acid Is Associated with Reduced Risk of Fatal Coronary Heart Disease, but Increased Prostate Cancer Risk: A Meta-Analysis", *Journal of Nutrition*, vol.134 pp. 919-922,2004.
- [4] Kiritsakis A. K., Lenart E. B., Willet W. C., Hernandez R., "From tree to the table, Food and Nutrition Press, Trumbull, Connecticut", *Journal of Olive Oil*, USA, 1998.
- [5] Cinquanta L., Esti M., Notte E., *J. Am. Oil Chem. Soc.*, vol.74 pp1252-1264, 1997.
- [6] Keys A., Mienotti A., Karvonen M. J., Aravanis C., Blackburn H., Buzina R., Djordjevic B. S., Dontas A. S., Fidanza F., Keys M. H., Kromhout D., Nedeljcovic S., Punsar S., Seccariccia F., Toshima H., *Am. J. Epidemiol*, vol.124 pp 903-915, 1986.
- [7] Willet W. C., *Monogr. Epidemiol. Biostat.* vol. 15 pp.341-379, 1990.
- [8] WHO, Report of a WHO study group. *WHO Technical Report Series 797*, Geneva, 1990.
- [9] Kafatos A. G., *Olivae*, vol.56 pp.22-24, 1995.
- [10] Elbir, M., Moubarik, A., Rakib, E. M., Grimi, N., Amhoud, A., Miguel, G., Hanine, H., Artaud, J., Vanloot, P., Mbarki, M., *Maderas ciencia y tecnologia*, vol.14 pp.361-371, 2012.
- [11] El bir M., Amhoud A., Mbarki M., Visioli F., *Nutraceutical Research*, vol.12 (1/2) pp.9-12, 2014.
- [12] Leouifoudi I., Ziad A., Amechrouq A., Oukerrou M. A., Ait Mouse H., Mbarki M., *Food Sci. and Technol Campinas*, vol.34 (2) pp. 249-257,2014.
- [13] Essiari R., Zouhair R., *Chimi H. OLIVAE*, vol.112 pp.8-22, 2014.
- [14] Olive oile Codex Stan 33, *Codex Alimentarius*.vol.8 pp.25-39, 2001.
- [15] Conseil Oléicole International. *COI/T.15/NC (3/Rév.6)*, 2011.
- [16] M. De Luca, W. Terouzi, G. Ioele, F. Kzaiber, A. Oussama, F. Oliverio, R. Tauler, G. Ragno, *Food chemistry*, vol.124 pp.1113-1118,2014.

- [17] S. Benlamaalam, M. Chigr, A. Hirri, A. Auhmani, M. Mbarki, *International Journal of Science and Research*, vol.4(6) pp. 2443-2448, 2015.
- [18] M. El bir, Thesis, Contribution à la valorisation des produits et sous produits de l'olivier (*olea europaea* L): Application chimométrique à la détermination de l'origine géographiques des huiles d'olives et valorisation des composés phénoliques des noyaux d'olives marocaines, Maroc, 2014.
- [19] M. Rubén, C. Lorenzo, C. Emma, S. K. Teodoro, B. Alessandra, *Food Control*, vol. 21 pp. 890-89, 2010.
- [20] G. Pristouri, A. Badeka, M. G. Kontominas, *Food Control*, vol.21 pp.412-418, 2010.
- [21] D. Ollivier, J. Artaud, C. Pintel, J. P. Durbec, M. Guerer, *Food Chemistry*, vol.97 pp.382-393, 2006.
- [22] S. Veillet, V. Tomao, I. Bornard, K. Ruiz, F. Chemat, *C. R. Chimie*, vol.12 pp.895-904, 2009.
- [23] O. Galtier, N. Dupuy, Y. Le Dréau, D. Ollivier, C. Pinatel, J. Kister, J. Artaud, *Analy Chimica Acta*, vol.595 pp.136-144, 2007.