

Characterization of dried mixture of olive cake with olive mill wastewater: elemental, spectroscopic analyses (FTIR and ^{13}C -NMR) and polyphenols dynamics

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Abstract

Morocco is a country which produces olive oil extensively and this industry within the country is currently under huge expansion. The olive mill wastewater OMW which is being dumped in the environment results in serious environmental pollution while the olive cake does have a proper disposal. In the present work, a new integrated process is described to eliminate the OMW produced by a tri-phase system using the technique of pressing. This process consists of mixing olive cake with the OMW produced and then to dry the mixture in a greenhouse to get a final moisture content of 10% (w/w). The dried mixture was characterized by elemental analysis and spectroscopic methods (Fourier transform Infra-Red: FTIR and Nuclear Magnetic Resonance: ^{13}C -NMR), in order to compare it with the crude olive cake and predict its future biotechnological valorization in the way existing as composting, lombricomposting and edible mushroom culture. The extractable polyphenols dynamics were determined by HPLC and compared to those present in OMW. The stability of the dried mixture was checked after one year of storage under the standard conditions. The results obtained will be discussed in detail.

Keywords: OMW, olive cake, solar drying, greenhouse, NMR, FTIR, Elemental analysis, polyphenols, HPLC, dynamics.

1. Introduction

Mediterranean basin concentrates 97% of the world olive oil production (Ismaili-Alaoui, 2006). The three-phase which generates two wastes (olive mill wastewater and olive cake) remains the dominating technology in the south of the Mediterranean basin. With a production of 50,000 tonnes of olive oil per year, Morocco is presently located at the sixth rank of the world olive oil producers. Fifty percent of this production still comes from traditional mills commonly called "Maasra" which use the classic "three-phase system" with the technique of pressing (Serghini et al., 2007).

Due to its residual oil content (5-10 % w/w) which depends on the extraction technology and also to its other components, olive pit (42-54% w/w), skin (10-11% w/w) and pulp (21-33% w/w) (Lopez-Villata, 1998), OC has been the subject of many valorization studies (Masghouni and Hassairi, 2000; Haddadin et al., 2002). In a country such as Morocco, its main use is still limited to the recovery of oil by extraction with hexane for the production of soap (Serghini et al., 2007). The de-oiled OC which has excellent calorific properties is usually reused directly as combustible by the OC oil extraction units to dry OC to 6-10% (w/w) humidity before the extraction step. As a consequence, OC should not be considered as waste and it is actually rarely dumped into the environment.

Contrary to OC, OMW which contains high concentrations of toxic phenolic compounds (Di Serio et al., 2008) and organic matter (100-200 g COD/l) is extremely hazardous for the environment especially the water bodies. OMW drying is actually a slow process since the olive oil and some mucilage cover the surface and make it impermeable to water (Jimenez and Lao, 2004). As so, OMW drying could be improved by increasing the surface of exchange with the atmosphere through the addition of a high surface absorbing solid matrix. The OC readily available from the same olive mill could be a good candidate for this use. Thus, the amount of wastes, generated by olive mill, could be controlled, based on the assumption that the resulting product could be valorised in the same way as crude OC.

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The objectives of the present study were to determine under which conditions all the OMW generated by a mill could be absorbed by the OC produced by the same mill and if the resulting OC-OMW mixture could be dried to 10% (w/w) humidity in order to obtain a solid matrix which could be valorized in the same way as crude OC. To achieve these objectives we (1) performed a mass balance of the different products generated by a traditional maasra mill (2) determined the OMW retention capacity by the mill OC, (3) performed several drying experiments in open sun and in a greenhouse under natural convection and (4) determined the final quality of the dried material and compared it to that of dried crude OC.

2. Materials and methods

2.1. Origin of samples

OC and OMW were obtained from an olive oil mill plant located in Beni-Mellal in the centre of Morocco. This mill used a three phase system with press and operated mostly with the "picholine marocaine" which is the main olive variety cultivated in Morocco for production of both olive oil and table olives. As so this mill can be considered as representative of the ones used in the country.

2.2. Description of the greenhouse dryer

The greenhouse dryer, used in the study was located at Hassan II Agronomic and Veterinary Institute, in Rabat, Morocco. It was built on a concrete floor and oriented in the north-south direction. It had a parabolic cross-section and a size of 7 (length), 3 (width) and 2.5 m (height) giving on the ground a surface of 21 m². It was covered with polycarbonate but the facade was made of transparent extern stratum. The air ventilation was obtained by some openings at different levels of the facade. One set of iron shelves with iron trays was used to place the products to be dried. The trays were located on three different levels (0.5, 1 and 1.5 m from the ground) on the shelves. The free space between them was sufficient for loading and unloading the products. The trays were made of a wire netting to allow air circulation. A second set of identical shelves and trays were used for the outdoor drying. The structure of the greenhouse and of the shelves and trays is shown in Fig. 1.

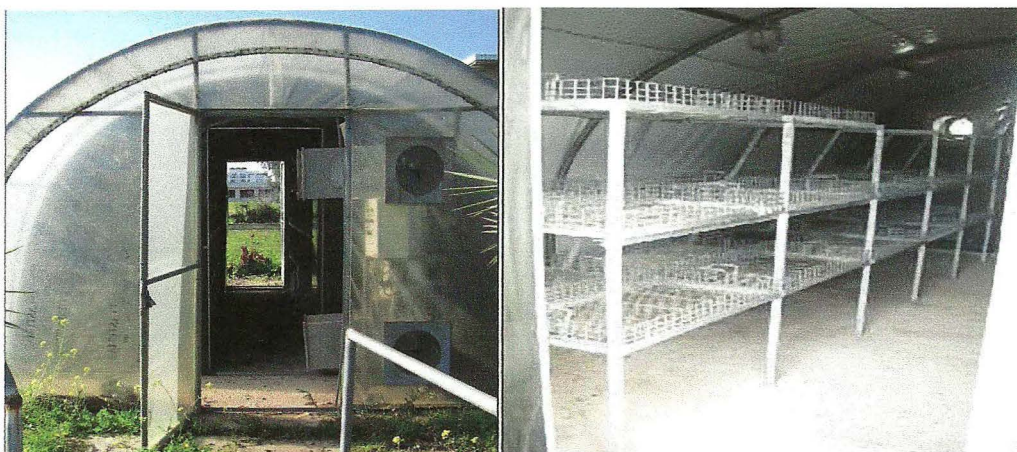


Figure 1: General view of the greenhouse solar dryer (left) and of its interior with the shelves (right). Only the lower and upper trays were used for loading the material to be dried.

2.3. Drying experiments

Outdoor and greenhouse drying were performed under identical conditions in order to be comparable. Saturated OC with OMW (no free liquid OMW present) were distributed uniformly in thin layer on the trays the bottoms of which were covered by plastic mesh. The solids on the trays were turned manually once every hour. The drying process was pursued until reaching the target moisture. For that, the whole solid present on each trays were weighted periodically at 1 h intervals using a digital balance and their moisture content was determined. Solar radiation and temperature inside and outside the greenhouse were determined periodically. In order to avoid the interference of rain on the

outdoor drying process, it was planned to cover the shelves with a plastic sheet during rainfall events. Finally this was unnecessary since no rain occurred during all the experimental period (February 2008).

2.4. Chemical characterization of dried crude olive cake and OC-OMW mixture

OC and the new product (OC-OMW mixture) both dried at 10% (w/w) humidity in the same conditions were characterized for their content in carbon, nitrogen (proteins), fats and total phenols. Carbon and nitrogen concentrations were determined by a combustion-gas chromatography technique (Flash EA 1212 Elemental Analyzer, France). The instrument was calibrated with aspartic acid standard. The amount of proteins was estimated from the nitrogen content using the general factor of 6.25 (Weinberg et al., 2008). Fats were obtained by Soxhlet extraction with hexane as solvent according to established procedures (Cordova et al., 1998). Phenol content was assessed as follows: 10 mL of 60% (v/v) ethanol-water were added to 1 g of milled solid sample. The mixture was homogenized, incubated for 1 h at 4°C and centrifuged at 4500 rpm for 10 minutes. The extraction was repeated three times. The supernatant was then filtered under vacuum. The non phenolic fraction still presents in the filtered liquid (e.g. fats) was eliminated by extraction with 10 ml of n-hexane, repeated three times. The total phenolic content of the remaining aqueous phase was quantified by the method of Folin and Ciocalteu (Bärlocher et al., 2005). The blue reactive complex formed was dosed spectrophotometrically at 760 nm. Caffeic acid being used as standard for the calibration, the phenolic content of the samples was expressed as caffeic acid equivalent.

2.5. NMR analysis of dried crude olive cake and OC-OMW mixture

Dried crude OC and the mixture OC-OMW were used directly in NMR experiments without special preparation. Owing to their size, the samples were gently ground (IKA Labortechnik, Germany) in order to fill the rotor. Solid-state ¹³C CPMAS NMR spectra, of dried crude OC and dried mixture OC-OMW, were obtained on a Bruker Avance-400 MHz spectrometer (Bruker, Bremen, Germany) operating at a ¹³C resonance frequency of 100.7 MHz. Samples were placed in a 7-mm zirconium rotor and spun at the magic-angle at 6 kHz. All measurements were made at room temperature. The ¹³C chemical shifts were referenced to tetramethylsilane and calibrated with the glycine carbonyl signal, set at 172.5 ppm.

Deconvolution of the NMR spectra was performed using the DmFit software (Massiot et al., 2002). This software adjusts the spectra to obtain the linewidth and the peak positions (in ppm) and to integrate each peak so as to obtain the percentage of each contribution. The aromaticity of dried mixture OC-OMW and crude OC was calculated by expressing aromatic C (110–165 ppm) as a percentage of aliphatic C (0–110 ppm) + aromatic C (Wikberg and Liisa Maunu, 2004). The lignin content was calculated according to the model of Gilardi et al. (1995), which takes into account the total lignin carbon. These authors calibrated lignin content from aromatic carbons plus aliphatic carbons of lignin. The cellulose crystallinity (X_c) was expressed by the ratio between the signal at 84 ppm, assigned to the C-4 of amorphous cellulose, and the signal at 89 ppm, assigned to the C-4 of crystalline cellulose (Newman and Hemmingson, 1990).

2.6. Statistical treatment of results

Data of drying, chemical analysis and NMR spectra were subjected to analysis of variance (ANOVA). The error square was obtained from the analysis of variance and used to calculate the least significant difference (LSD, $P < 0.05$). Variations between data are significant when the difference between is more than the LSD.

3. Results and discussion

3.1. Drying of the mixture of OMW-Olive cake

After a set of trials, it was found that the best way to perform the two step absorption-drying was the one shown in Table 1.

Table 1: Optimized procedure for the two-step drying process of OMW absorbed on OC.

Substrate	Olive cake (OC)		OMW		Mixture before 1 st drying		Mixture after 1 st drying		OMW		Mixture before 2 st drying		Mixture after 2 st drying			
Process time (Hours)					8								35			
Drying cycle	Quantité (Q.) Kg	Humidité (H. %)* Kg	Q. Kg	H. % Kg	Q. Kg	H. % Kg	Q. Kg	H. % Kg	Q. Kg	H. % Kg	Q. Kg	M. % Kg	Q. Kg	M. % Kg		
1 st cycle	100	35	62	90	162	56	122	40								
2 nd cycle							122	40	25	90	147	50	82	10		
Input(kg)	100		62						25							
Output (kg)													82	10		

The first step consisted to saturate OC with OMW (resulting product with 56% moisture, w/w) and then to limit the drying of the mixture up to a final moisture of 40% (w/w). This level of humidity was found as a good compromise to achieve three objectives. It allowed the evaporation of enough water (42 L from 166 kg of OC-OMW mixture) to give to the partial dried OC-OMW mixture the capacity to absorb all the OMW remaining to absorb (25 L). It permeated to avoid the problems generated by a more intensive drying which could have affected the absorption capacity, because OC became more water repellent upon drying due to the shrink of the water absorbing solid material that it contains resulting in a loss of porosity of this material, porosity which is the driving motor of absorption (Liu and Lee, 2006). It also allowed a fast drying for this first step since the drying from a humidity of 56% to 40% (w/w) concerned mostly to free water which is easy to be removed (Ekechukwu, 1999). Quantitatively, the total mass of by-products (OC and OMW) generated by a maasra mill (78 kg / 100 kg of olives processes) can be reduced by 56.15% (final amount of 35-36 kg / 100 kg of olives processes) thanks to such drying.

3.2. Chemical characterization of dried crude OC and OC-OMW mixture

Chemical analysis results for the dried crude OC and the dried OC-OMW mixture are presented in table 2. Statistical analysis ($P=0.05$) showed significant differences in pH, C, N, C/N and total phenols, but no significant difference in fat content. Incorporation of OMW into OC allowed to increase by 24% of the protein content in the mixture compared to dried crude OC which is considered as a poor material for animal feed (Martin Garcia et al., 2003). However, this was counterbalanced by total phenol content which was 5 times higher than that in the dried crude OC since phenols are known for their anti-digestive properties (Grabber, 2009). On the other hand, this converted the OC-OMW mixture into a good raw material candidate for the extraction of antioxidants which are used in the food, cosmetic and pharmaceutical fields (Erbay and Icier, 2009). The fat content and dryness of the mixture indicates also that it could be used directly by the traditional oil recovery units in place of OC.

Table 2: Global chemical composition of the OC-OMW mixture and crude OC both dried at 10% moisture*

Component	OC-OMW dried mixture	Dried crude OC
pH	5.1 ± 0.1	4.9 ± 0.2
C (%)	52.49 ± 0.01	51.11 ± 0.18
N (%)	1.06 ± 0.01	0.85 ± 0.09
C/N	49.51 ± 0.64	59.77 ± 2.00
Protein (%)	6.62 ± 0.08	5.34 ± 0.56
Olive oil (%)	10.00 ± 1.02	10.00 ± 0.54
Total Phenol (%)	4.1 ± 0.81	1.20 ± 0.15

*All % in w/w, the values after ± represent standard deviation

3.3. NMR analysis of OMW-olive cake

The ^{13}C NMR spectra of dried crude OC and dried OMW-OC mixture are presented in Figure 2. The peak assignments and interpretations (Table 3) are based on previous NMR studies of organic matter (Albrecht et al., 2008; Baddi et al., 2004; Farnet et al., 2009).

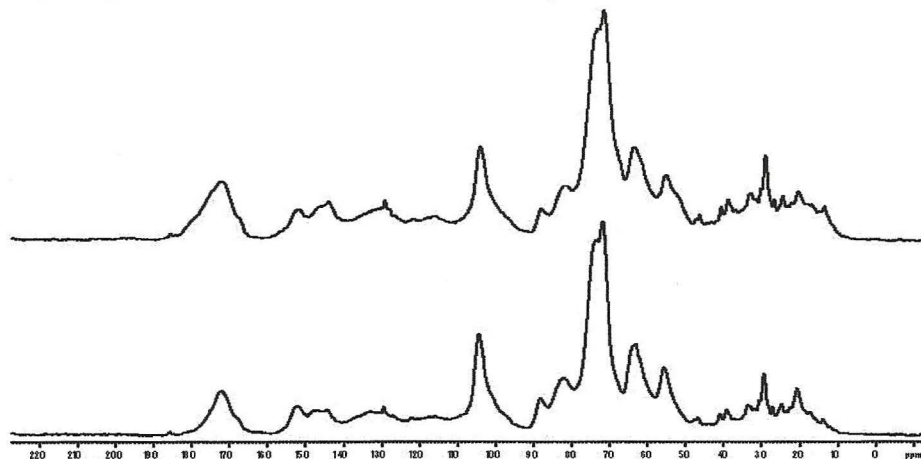


Figure 2: Solid-state ^{13}C NMR spectra of, (a) dried OC-OMW mixture and (b) dried crude OC.

Table 3: ^{13}C -NMR spectral assignment of C functional groups.

Chemical shift (ppm)	Assignment	Compounds included
0-45	Alkyl C	Lipids, Amino-acids,
45-60	N-Alkyl/methoxyl C	Amino acids
60-110	O-Alkyl C	Celluloses, hemicellulose, polysaccharides
110-145	Aryl C	Polyphenols, Lignin (aromatic C)
145-165	O-Aryl C	Phenols, lignin (phenolic C)
165-190	Carboxyl C	Organic acids, amide and ester C

Deconvolution of spectra allowed integration of 27 peaks. Figure 3 showed relative integration values for the major C-types from the dried substrates. A comparison of the spectra of dried OC-OMW mixture and crude OC showed that the major differences are in the Alkyl C (0-45 ppm), O-alkyl C (60-110 ppm) and Carboxyl C (165-190 ppm). The addition of OMW to OC led to an apparent low content of carbohydrate in the dried mixture. The high intensity of O-alkyl C in the dried mixture was consistent with total phenol content which was 5 times higher than in OC. The dried mixture showed more intense peaks at 175 ppm, because of the carboxyl group which can result from the degradation of OMW upon drying, especially, during the first stage of drying. The intensities of the peaks at 84 and 89 ppm showed a very low degree of crystallinity of cellulose both for the dried OC-OMW mixture and OC and the lignin content was not affected by the additions of OMW (Table 4).

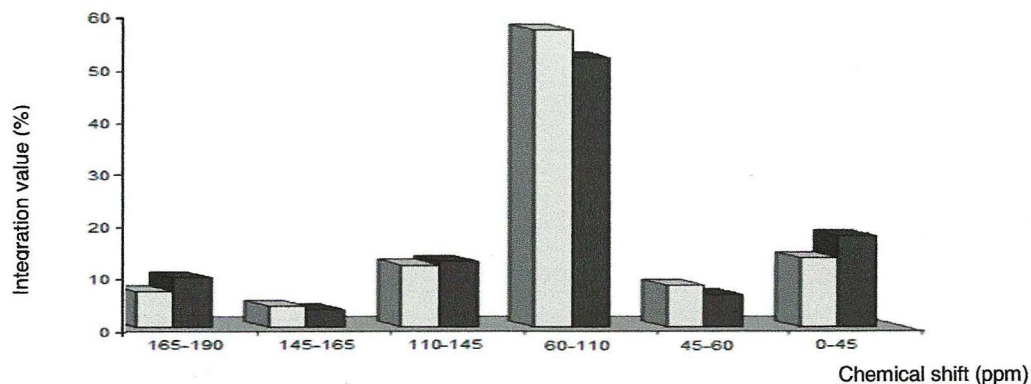


Figure 3: Integration values for the major C-types in the ^{13}C NMR spectra of dried OC-OMW mixture (■) and dried OC (▒).

Table 4: Calculated parameters from NMR data of dried mixture OC-OMW and dried crude OC

Parameters	OC-OMW dried mixture	Dried crude OC
Aromaticity %	17.42±0.26*	16.78±0.33
Lignin%	25.23±1.24	25.81±1.31
Carbohydrate%	47.11±1.02	53.00±2.32
Crystallinity (89/84ppm)	0.15±0.01	0.15±0.02

*The values after ± represent standard deviation

4. Conclusions

The absorption of all the OMW produced by an olive mill on the OC generated by the same mill was found to be possible. It was carried out in two steps with alternate drying. The experimental results showed that the drying of the OC-OMW mixture in greenhouse was very effective, and that the drying period to achieve the same level of moisture was shortened relatively to the open sun drying. The homogeneity of air in the greenhouse under natural convection eliminated any difference in drying rate at different levels of trays. The chemical and NMR analysis of the dried OC-OMW mixture showed that the new product was sufficiently similar to OC to be at least valorised in the same way with the advantage to avoid the dumping of OMW in the environment.

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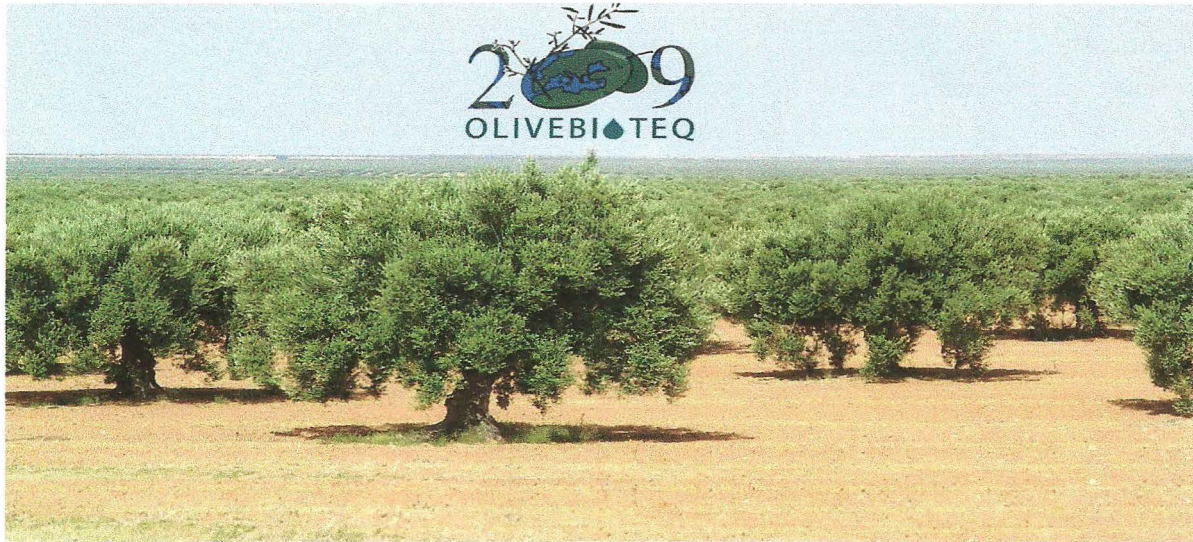
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PROCEEDINGS



Pour un secteur oléicole rénové, rentable et compétitif en Méditerranée

For a renovated, profitable and competitive Mediterranean olive growing sector

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Présentation

Le premier séminaire sur les Biotechnologies et qualité des produits de l'olivier dans le bassin méditerranéen (Olivebioteq-2004) a eu lieu à Errachidia au Maroc en novembre 2004 (www.olivebioteq.ird.fr). Le deuxième Olivebioteq a eu lieu à Palermo en Sicile Italie en octobre 2006 et le 3^{ème} Olivebioteq aura lieu à Sfax Tunisie. L'Institut de Recherche pour le Développement (IRD) et plus particulièrement la Délégation à l'Information et à la Communication (DIC) ont participé à la création et à l'essor de ce séminaire de formation permanente des principaux acteurs méditerranéens en oléiculture.

L'histoire de la culture de l'olivier se confond avec celle de la méditerranée au point que l'olivier est devenu le symbole de cette région dans le monde. La gestion et la mise en valeur de l'espace agricole méditerranéen sont de fait fortement liées à la culture de l'olivier et aux conséquences économiques et sociales de la production, de la transformation et de la commercialisation de l'huile d'olive. L'oléiculture traditionnelle prédomine dans la plupart des pays méditerranéens, malgré l'émergence depuis le début des années 1980 d'une oléiculture moderne conduite en irrigué, en intensif et en hyper intensif.

L'évolution des filières huile d'olive dans les différents pays est liée au progrès technique et les politiques mises en œuvre pour favoriser l'organisation de ces filières afin de tirer le meilleur parti des potentialités des marchés. Elles ont bénéficié durant les dernières décennies de plusieurs types d'innovations technologiques résultant des travaux de recherche et des politiques de promotion, de formation, d'incitation et de vulgarisation. Ces innovations se retrouvent dans les principaux maillons des filières: exploitations agricoles, huileries, usines de conditionnement et circuits de distribution.

Malgré l'adaptation de l'olivier aux conditions naturelles et à l'écologie méditerranéennes et les efforts d'innovation déployés par la plupart des pays producteurs, le devenir de ce secteur reste tributaire notamment de la dynamique du marché mondial, des nouvelles dispositions en matière de politique agricole et commerciale au niveau multilatéral (PAC, Cycle de Doha, Accords de libéralisation bilatéraux et régionaux), ainsi que de la structure et du fonctionnement des systèmes de production et d'exportation des différents pays oléicoles.

La plupart des pays méditerranéens se trouvent actuellement dans un contexte économique incertain au sein duquel la prise de conscience des enjeux d'avenir, la rénovation des systèmes de production et d'exportation et l'identification de nouveaux usages et alternatives de valorisation des produits de l'olivier (nutrition, santé, cosmétique, énergie, environnement...) sont devenues impératives afin de renforcer le rôle économique, social et environnemental et la compétitivité de l'olivier.

Pour enrichir le débat autour de cette problématique, l'Institut de l'Olivier organise la troisième édition d'Olivebioteq sur le thème "**Pour un secteur oléicole rénové, rentable et compétitif en Méditerranée**". L'objectif principal de cette édition est de faire connaître et de discuter les acquis de recherches et d'examiner les alternatives de leur valorisation pour l'innovation de la filière huile d'olive en Méditerranée.

La troisième édition d'Olivebioteq a lieu du **15 au 19 décembre 2009 à Sfax (Tunisie)**. Elle est organisée en collaboration avec des organismes nationaux (l'ONH, AVFA, DGPA, DGPCQA, OTD, UTAP, CC, APIA, API et CEPEX) et internationaux (COI, IRD, FAO, Commission Européenne, CIHEAM, AFIDOL).

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