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Changes Induced by UV Radiation during Virgin Olive Oil Storage

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The effects of UV radiation on the chemical and sensory characteristics of virgin olive oils (cv. Arbequina and Picual) were assessed. Even small doses of UV radiation induced oxidation of the virgin olive oil samples. Total phenols and fatty acids contents decreased during the process as well as the intensity of the bitter and fruity sensory attributes, while the intensity of the rancid sensory attribute notably increased. Acetaldehyde, 2-butenal, 2-pentenal, octane, octanal, hexanal, nonanal, and 2-decenal were the volatile compounds most affected, showing an important increase during the irradiation process. Nonanal, hexanal, and pentanal showed high correlation with the rancid sensory attribute (90%, 86%, and 86%, respectively). 2-Decenal and nonanal concentrations allowed us to predict the alteration level of the samples by mean of multiple Ridge regression.

KEYWORDS: Virgin olive oil; UV radiation; oxidation; volatiles; off-flavors

INTRODUCTION

A typical virgin olive oil contains more than 100 volatile compounds that can be grouped in several chemical families such as acids, alcohols, esters, or carbonyls, but their concentrations depend on the olive variety, the production system, and the storage time (*1*, *2*). Virgin olive oil can be classified as extra virgin olive oil (EVOO) (high quality), ordinary quality olive oil (medium quality), and the so-called lampante olive oil (low quality) by sensory assessment. The flavor of EVOO is characterized by pleasant sensory notes, very appreciated by consumers (*3*, *4*), that correspond to a profile of volatile compounds constituted by a balanced flavor of green and fruity sensory characteristics (*5*). Several processes, however, can alter this pleasant flavor, giving rise to unpleasant sensory notes, the virgin olive oil off-flavors. Current olive oil official regulations (*6*, *7*) classify the most frequent off-flavors into four groups: fusty, mustiness−humidity, winey−vinegary, and rancid. From all of them, the rancid defect, produced by lipid oxidation, is the most studied because of its implication in the loss of quality of fat-containing foods (*8*−*10*).

Lipids are responsible for texture and flavor in food, and the oxidation of fat products leads to an undesirable taste and smell. The formed compounds usually have a low odor threshold and are responsible for the rancid off-flavor. Furthermore, besides the changes in the sensory quality, there is also a decrease in the nutritional value, aside from acceptability and safety. Indeed, when the process has reached an advanced stage of oxidation, toxic compounds may be formed which could injure the consumer's health (*11*).

The volatile profile of a rancid olive oil depends on its oxidation level since the volatile content changes along the oxidative process from a qualitative and quantitative point of view (*9*). The rancidity process mainly affects unsaturated fatty acids (FA), oleic, linoleic, and linolenic being the acids that undergo major alteration (*12*). The higher the unsaturation level, the higher the relative alteration speed (*13*). The main decomposition products formed along the oxidative process are aldehydes, most of them contributing to the typical rancid odor of the oxidized oil because of their sensory characteristics and their low odor thresholds (*8*, *9*). The oxidative rancidity is accelerated with exposure to heat, light, humidity, and the presence of trace transition metals. Several studies have reported the changes in the chemical composition of olive oils during accelerated oxidation processes using high temperatures and bubbling with air (*9*, *14*, *15*). These conditions accelerate the oxidative process, but they are not usual in bottled olive oil since the oil is not usually in contact with air and is not exposed to high temperatures.

It is well-known that light influences the rancidity process of olive oil (*10*) although scarce attempts have been done to study the contribution of only this factor to the oxidation process as well as the effect of a single radiation on olive oil (*16*). Sun energy is made up of ultraviolet, visible, and near-infrared radiation; as shorter is the wavelength of the radiation then the potential damage is greater. The aim of this work was to evaluate the effect of ultraviolet radiation, the most damaging one, on the production of off-flavors in the bottled virgin olive oil.

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EXPERIMENTAL PROCEDURES

Samples. Two EVOOs (cv. Picual (P) and Arbequina (A)) were subjected to UV radiation. The selection of these single olive oils was carried out because these varieties have different polyphenol content (*17*), which is related to the oxidative stability, different sensory characteristics (Arbequina is characterized by a very sweet, slightly pungent and green odor, while Picual is characterized by medium-high values of pungent, apple, and strength odor), and diverse chemical composition (*18*, *19*). Samples (50 g) were put inside closed glass flasks and placed in a chamber especially design for this experience. The samples were irradiated using a UV lamp (HQV L 18/73, 300−400 nm, 18 W) for 1, 2, 3, 5, 8, and 12 days. A set of 14 samples, 2 EVOO and 12 irradiated samples (6 for each variety), was analyzed. The sample codes used are related to the variety (A, Arbequina; P, Picual) and the irradiation days (1, 1 day; 2, 2 days; 3, 3 days; 4, 5 days; 5, 8 days, and 6, 12 days).

Standards. Acetaldehyde, octane, pentanal, hexanal, 2-methyl-2-butenal, 2-pentenal, 1-penten-3-ol, heptanal, octanal, nonanal, 2-heptenal, 2-octenal, and 2-decenal were purchased from Sigma-Aldrich (St. Louis, MO).

Dynamic Headspace Gas Chromatography (DHS-GC). Volatile compounds of virgin and irradiated olive oils were analyzed by a modified dynamic headspace (DHS) technique previously reported (*20*). Samples of 0.5 g were heated at 40 °C and swept with N_2 (200 mL/min) for 15 min, and the volatiles were adsorbed onto a Tenax TA trap (Chrompack, Middleburg, The Netherlands) at room temperature. The volatiles were condensed onto a fused silica trap cooled at −110 °C with liquid nitrogen for 5 min just before injection, which was carried out by flash heating of the cold trap at 170 °C, where it was held for 5 min. The volatiles were transferred onto a fused silica DB-Wax column (60 m × 0.25 mm i.d. × 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The carrier gas was hydrogen. The oven temperature was held at 40 °C for 4 min and programmed to rise 1 °C/min to a temperature of 91 °C, and then to rise 10 °C/min to a final temperature of 201 °C, where it was held for 10 min. A Hewlett-Packard 5890 series II (Palo Alto, CA) with a flame ionization detector was employed. Volatiles were isolated and analyzed in duplicate. Quantification was carried out using isobutyl acetate as internal standard.

Identification of Volatiles. The identification of the volatile compounds was first carried out by mass spectrometry and later checked with standards if available (see Standards). The identification by GC−MS (Fisons mass detector MD800 coupled to a GC 8000 series) was carried out using identical conditions to those used for the DHS-GC analysis with the exception of the carrier gas, which was helium. The identity of the volatiles was obtained by comparison of their mass spectral data with the information from the NIST version 1.7 library. The volatile compounds were also identified using the relative retention times of the standards with respect to the internal standard (isobutyl acetate) (*5*, *20*).

HRGC Olfactometry. An HRGC-sniffing technique was applied to virgin olive oil samples (*21*) to assess the aroma notes corresponding to olive oil volatile compounds. The effluent of the GC column was split 1 to 10, to the detector and the sniffing port, respectively. The odor-active regions of the eluate were evaluated, and their aroma notes were assigned by five assessors, two with more than 10 years experience and three who were habitual consumers of virgin olive oil. The odor descriptions were noted on a form with a preprinted time scale; assessors did not see the chromatogram. Assessors basically agreed on the odors of volatile compounds, although different semantic terms were used. A consensus-building discussion was held with assessors to decide the final sensory descriptors.

Sensory Analysis. Samples were evaluated for flavor at different stages of the UV irradiation process by a panel test following the official procedure (*22*). The panel's assessors were fully trained with more than 5 years of experience in evaluating all types of olive oil (virgin, current, lampante). Assessors first evaluated the flavor descriptors of the samples, the score for each attribute being the result of the overall gustatory−olfactory− tactile perception, and finally a flavor global score was given.

Odor Thresholds. A fully refined and deodorized olive oil was the matrix for the assessment of the odor threshold values. Olive oil was refined and deodorized at a semi-industrial scale in the pilot plants of the Instituto de la Grasa, and the absence of volatile compounds in the oil was checked by the DHS-GC procedure described above. The sensory evaluation was carried out in the test room used for evaluating virgin olive oil sensory characteristics (*6*). Three samples were presented to the assessors following the triangle test whose results were statistically analyzed. A volume of 15 mL of each sample was kept in standardized glasses at 29 °C ± 2 °C for 15 min and then tested.

The odor activity values (OAVs) (ratio of the concentration to the odor threshold) (*5*, *23*) of volatile compounds were calculated to determine their sensory significance. Thus, the concentration of each volatile found in the oil samples was divided by its corresponding odor threshold value previously determined as described above.

Fatty Acid and Total Phenols Analysis. Olive oils were subjected to methylation according to the European Communities procedure (*24*) and injected in a Hewlett-Packard 5890-II gas chromatograph with a flame ionization detector. The oven temperature was held at 160 °C for 15 min and raised 1.5 °C/min to a final temperature of 190 °C, where it was held for 20 min. A fused silica column SP-2380 (60 m × 0.25 mm id × 0.2 μm) was employed. The carrier gas was H_2.

Phenolic compounds were isolated by extraction of a solution of oil (10 g in samples with phenol content higher than 100 mg/kg and 30 g in those with phenol content lower than 100 mg/kg) in hexane (50 mL) with 20 mL of a water−methanol mixture (60:40) three times. Folin−Cicalteu reagent and sodium molybdate 5% in ethanol 50% reagent (both Merck), respectively, were added to suitable aliquots of the combined extracts. The absorption of the solution was measured at 725 nm on a spectrophotometer (Hewlett-Packard 8450 A UV−vis). The results are given as mg/kg of caffeic acid.

Mathematical Analysis. The whole set of volatile compounds data was imported to Excel from the HPChemstation program (Hewlett-Packard, revision A.05.01 (273)) and merged to sensory and chemical data. Statistica release 6.0 (*25*) was used to perform the data processing and to implement multivariate data analyses.

Cluster analysis was applied to determine the natural conformation of groups of single olive oils gathered around the oxidation degree. Ward's method and city-block (Manhattan) were used as the linkage rule and distance measure, respectively.

Multiple regression (Ridge regression, $\alpha = 0.05$) was applied to evaluate the correlation between volatile compounds and sensory attributes.

RESULTS AND DISCUSSION

Previous studies on the oxidation process have established that total FA content decreases during oxidation; oleic, linoleic, and linolenic acids being the most altered during this process (*9*, *12*). FA analysis of irradiated samples agreed with these results since 20% of the FA were altered at the end of the irradiation period; the quantification was based on the major saturated acid (C 16:0) initially present, to show the real content of unaltered FA (*26*). Unsaturated FA analysis showed no significant reduction during the first 5 days of irradiation in any of the varieties; however, a progressive reduction of the FA content was observed from 5 to 12 days of irradiation in all the unsaturated FA, this reduction corresponding to linolenic (36%), linoleic (31%), and the w9 isomer of oleic (32%) acids. Saturated FA did not show, on the contrary, significant variation during the irradiation process.

An abrupt decrease of the total content of phenols was also observed during the irradiation time, so contributing to the progressive oxidation of the samples. The decrease was more drastic during the first 5 days and, in particular, the first day (>45%). These results agree with those of FA that began their degradation once the concentration of phenols was lower enough. **Figure 1** shows the evolution of the total content of phenols together with the (*E*)-2-hexenal content, the major

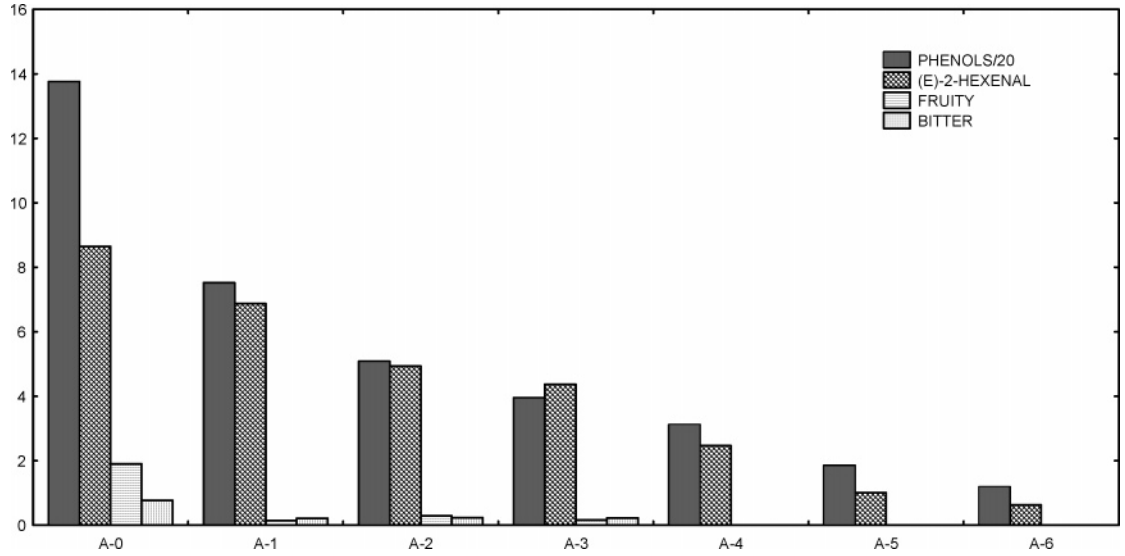

Figure 1. Evolution of green and bitter sensory attributes, the total content of phenols, and (*E*)-2-hexenal (mg/kg) during the irradiation process of an EVOO (cv. Arbequina). Note: the phenol concentration is divided by 20.

Table 1. Volatile Compounds Content Showing Significant Differences during the Irradiation Process

		Arbequina (mg/kg)		Picual (mg/kg)				
compd	significant difference[a]	conc 0 days	conc 12 days	conc 0 days	conc 12 days	hydroperoxide precursor[b]	sensory characteristics	odor threshold (mg/kg)
acetaldehyde	**	0.004	0.941	0.015	0.873	LnOOH	pungent	
octane	**	0.376	25.798	1.075	25.564	10-OOOH	sweet, alcane	0.940
propanal	*	0.068	0.494	0.109	0.763	LnOOH	pungent	
pentanal	**	0.553	1.858	0.372	1.395	13-LOOH	wood, bitter, oily	0.240
2-butenal	**	0.029	0.682	0.030	0.924	15-LnOOH		
hexanal	**	0.655	7.849	0.349	4.440	12,13-LOOH	fatty, strong, oily, grass	0.320
2-methyl-2-butenal	*	0.116	0.496	0.132	0.295		paint, grassy	
2-pentenal	**	0.00	0.312	0.002	0.292	13-LnOOH	paint	0.300
1-penten-3-ol		0.069	0.181	0.008	0.130		oxidized	
heptanal	*	0.174	0.269	0.110	0.154	OOOH	oily, fatty, wood	0.500
octanal	**	0.024	0.621	0.040	0.801	11-OOOH	fatty, pungent	0.320
2-heptenal	*	0.019	0.349	0.066	0.602	12-LOOH	oxidized, tallowy, pungent	0.005
nonanal	**	0.027	0.318	0.039	0.310	9,10-OOOH	fatty, waxy, paint	0.150
2-octenal	**	0.453	0.926	0.247	0.982	LOOH	spicy, grassy	0.004
2-decenal	**	0.044	0.454	0.023	0.485	9-OOOH	paint, fish, fatty	0.010

[a] Significant differences at 95%. ** Indicates significant differences at 99%. [b] OOOH, oleic acid hydroperoxide; LOOH, linoleic acid hydroperoxide; LnOOH, linolenic acid hydroperoxide.

volatile compound in virgin olive oils. It is remarkable that the concentrations of phenols and (*E*)-2-hexenal followed a similar evolution as both are responsible for the bitterness of EVOO (*3*).

Several sensory attributes showed a similar trend in the process. **Figure 1** also displays the scores of fruity and bitter sensory attributes. The progressive decrease in the intensity of the fruity attribute was observed from the initial to the most oxidized samples. This reduction is related with the decrease in the concentration of (*E*)-2-hexenal that is characterized by fruity and bitter notes (*3*, *27*). The bitter attribute also showed a decreasing evolution in the process that is related with the falling off of the phenols content since these compounds contribute to olive oil flavor with bitter notes (*28*, *29*) and with the reduction in (*E*)-2-hexenal concentration. The decrease in the concentration of these compounds from the fifth day of irradiation explains the lack of fruity and bitter attributes in the organoleptic evaluation by the analytical panel.

Sixty-four volatile compounds were quantified in the samples. All volatile compounds did not show the same behavior since some of them increased during the process while others decreased. Most of the compounds present in the virgin sample decreased their concentrations, (*E*)-2-hexenal being the most remarkable in both varieties. The evolution of this compound in the oxidation process of Arbequina EVOO is shown in **Figure 1**; a similar evolution was found in Picual EVOO.

Student's *t*-test was applied to the whole set of 64 volatile compounds quantified to identify those that showed more variation during the oxidation process. **Table 1** shows the compounds that displayed significant variations at a $p < 0.01$ and $p < 0.05$. All of them increased their concentrations during the irradiation process, it being remarkable that no acid was found in the volatile analysis that means a low-medium oxidation degree (*8*). Acetaldehyde, 2-butenal, and 2-pentenal formed from hydroperoxides of linoleic acid and octane, octanal, nonanal, and 2-decenal from 9,10,11-hydroperoxides of oleic acid were the compounds that showed the highest variation, which agrees with other results obtained in previous studies (*12*).

The evolution of olive oil flavor during the irradiation process was also studied by the profile of 64 volatile compounds. **Figure 2** shows the result of applying cluster analysis to the whole set of volatiles. Two main groups were found, one of them constituted by the most irradiated samples of both varieties (8 and 12 days for Arbequina and 5, 8, and 12 days for Picual)

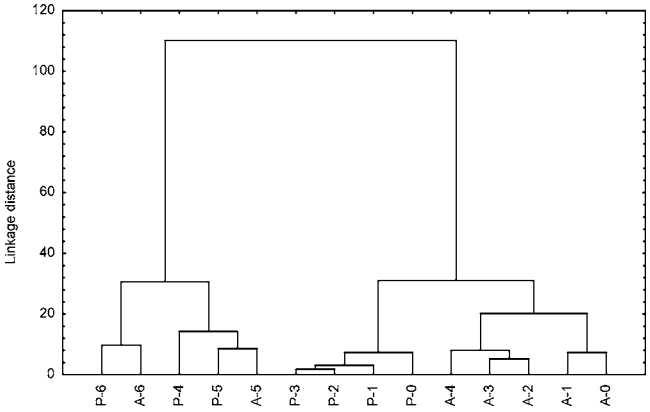

Figure 2. Cluster analysis of the irradiated samples from cv. Arbequina and Picual.

and the other by the least irradiated ones. The latter group was divided in two groups, each one of them formed by samples of the same variety which displays that the initial volatile composition of each variety was different. Arbequina samples were separated in two subgroups, one constituted by the initial and 1 day irradiated sample and the other by the samples irradiated for 2, 3, and 5 days. Concerning the Picual variety, the initial sample constituted a subgroup, while the other was constituted by the samples irradiated for 1−3 days.

This different behavior of the varieties against the irradiation process could be explained by the content of the antioxidant compounds in both varieties (*17*, *18*). These compounds include carotenoids, tocopherols, and phenolic compounds and protect the olive oil against oxidation, showing the phenolic compounds to be the major contribution to the stability of olive oil (*10*, *29*). The higher phenolic content of the Picual variety (420 mg/kg) is in part responsible of its higher stability, and it retards the oxidation process. A gradual oxidation arose in the Picual samples up to the third irradiation day, after which the samples showed an important increase in the concentration of some volatile compounds formed by oxidation, due to a noticeable decrease in the phenolic content. The oil from the Arbequina variety, on the contrary, showed remarkable variations in the chemical composition from the second day of UV radiation due to the reduction of its initial lower content of phenols (275 mg/kg).

The volatile compounds described in **Table 1** were subjected to discriminant analysis to evaluate their discriminant ability to distinguish the most altered samples (8 and 12 days of irradiation) from those not irradiated or less irradiated (0, 1, 2, 3, and 5 irradiation days). The result showed that acetaldehyde, 2-pentenal, octane, octanal, and 2-decenal allowed 100% correct classification of both groups individually. A canonical equation based on propanal, 2-methyl-2-butenal, 2-heptanal, and pentanal also allowed 100% correct classification of both groups.

Once the volatile compounds with the higher concentration in the irradiated samples were known, the next step was to identify those compounds with the major contribution to the appearance of the rancid sensory defect. Sensory analysis of the samples was carried out by an analytical panel according to International Olive Oil Council method (*6*). The intensity of positive attributes (green, fruity, bitter, and pungent) decreased along the process in both varieties, while the intensity of negatives ones (mustiness, vinegary, muddy sediment, and metallic), with a very low initial value, did not display important variations, except the rancid attribute that showed a great increase in both varieties. An inverse relationship between both attributes was found.

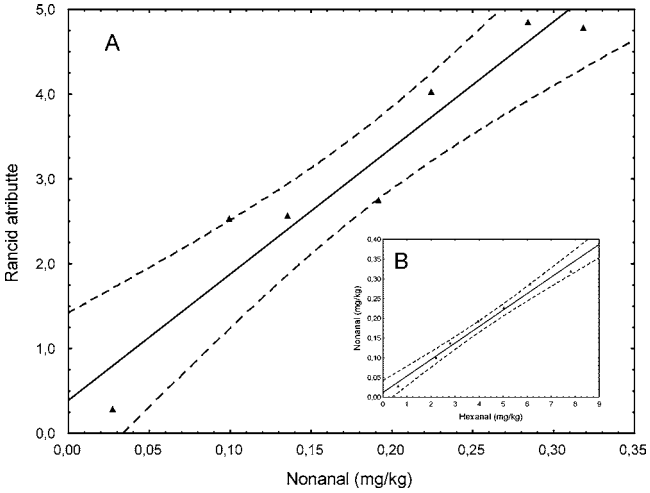

Figure 3. Regression line between rancid sensory attribute and nonanal concentration in irradiated samples of the Arbequina variety (confidence limit, 95%) (**A**). Regression line between hexanal and nonanal content (confidence limit, 95%) (**B**).

A correlation study between the rancid attribute and the 10 compounds, that showed significant differences at a confidence level of 99%, was carried out. All of them displayed R values higher than 70%; the most remarkable compounds were 2-decenal (82%), pentanal (86%), hexanal (86%), and nonanal (90%), that are characterized by "oily" or "fatty" sensory notes. Nonanal showed the highest correlation with the rancid attribute. However, its odor threshold (0.15 mg/kg) indicates that it would not have contributed to the rancid perception of the samples irradiated in the initial stages. OAVs higher than 1 were obtained after 3 and 5 days of irradiation in the Arbequina and Picual samples, respectively. Thus, the contribution of nonanal to the rancid defect was at an intermediate or advanced stage of oxidation, although its contribution to the detection of initial stages of oxidation cannot be discarded since its effect could be increased by synergistic mechanisms with other compounds. On the other hand 2-decenal (odor threshold = 0.01 mg/kg), pentanal (odor threshold = 0.24 mg/kg), and hexanal (odor threshold = 0.32 mg/kg) showed OAV higher than 1 in all the studied samples.

Regression analysis between rancid defect intensity and nonanal concentration was carried out, the following equations were obtained: $Y = 0.39 + 14.88X$ for Arbequina and $Y = 0.50 + 15.34X$ for Picual ($R = 0.96$ and $R = 0.84$, respectively). **Figure 3** shows the result of applying regression analysis to cv. Arbequina and the high correlation found between hexanal and nonanal ($R = 0.99$ and $R = 0.94$ for Arbequina and Picual, respectively), that agrees with the results found in previous studies on olive oil oxidation (*10*).

This study points out that it is possible predict the value of the rancid attribute of a sample submitted to UV radiation from its nonanal concentration. This fact would allow one to establish the alteration level of a sample from the objective measurement of only one analyte.

On the other hand, and from a practical point of view, it would be very interesting to predict the rancidity level of a sample using the concentrations of a reduced group of analytes. Ridge regression was applied to the set of volatiles having correlation with the rancid sensory defect of the samples. Concentrations of 2-decenal and nonanal allowed us to predict the alteration level of an irradiated sample by the following equation: 4.43 + 1.43[nonanal] − 2.53[2-decenal] ($R^2_{ajust} = 0.93$; F to enter = 0.80) (**Figure 4**). **Figure 4** shows that the responses of both

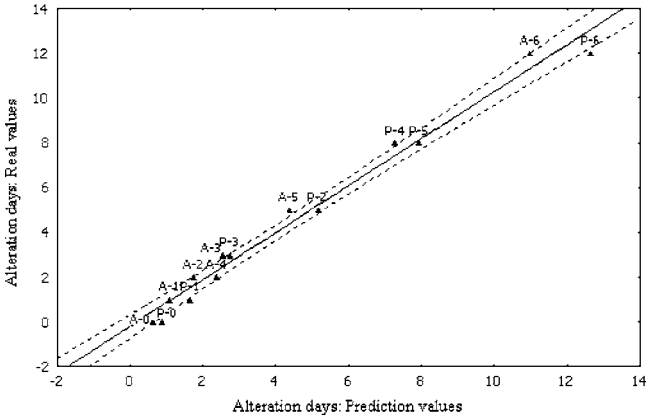

Figure 4. Result of applying the obtained regression equation to predict the rancidity of virgin olive oils of Picual and Arbequina varieties irradiated with UV radiation (confidence limit, 95%).

varieties to the rancidity induced by a UV irradiation process are similar, with a correct prediction for the nonirradiated samples and those with low and high alteration level, while the one with intermediate level shows a different behavior for each variety. This result points out that the lowest and highest alteration levels could be predicted, while the intermediate ones depend on the antioxidant content of each variety.

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