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Preliminary study on the application of an electric field as a method of preservation for virgin olive oil

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ABSTRACT. The objective of this study was to analyze the effect of an electric field treatment (voltage: 3 kV cm^{-1}, frequency: 60 Hz and time: of 5 and 25 min) on the stability of unsaturated fatty acids in virgin olive oil. Unsaturated fatty acid oxidation in the virgin olive oil was analyzed by Fourier transform infrared spectroscopy in the mid infrared region, and by quality parameters (acidity, peroxide and iodine). The electric field is a suitable method to preserve this oil composition with minimal modifications without the synthetic antioxidant addition.

Keywords: electric field, fatty acids, Fourier transform infrared spectroscopy, virgin olive oil.

Estudo preliminar de uma aplicação de campo elétrico como um método de conservação de azeite virgem

RESUMO. O objetivo deste estudo foi analisar o efeito de um tratamento de campo elétrico (tensão: 3 kV cm^{-1}, frequência: 60 Hz e tempo: 5 a 25 min) sobre a estabilidade de ácidos graxos insaturados em azeite de oliva virgem. A oxidação de ácidos graxos insaturados em azeite foi analisada por meio de espectroscopia de infravermelho com Transformada de Fourier na região média do infravermelho, e pelos parâmetros de qualidade (acidez, peróxidos e iodo). O campo elétrico é um método adequado para preservar a composição do óleo, com modificações mínimas, sem a adição de antioxidantes sintéticos.

Palavras-chave: ácidos graxos, campo elétrico, espectroscopia do infravermelho com transformada de Fourier, azeite de oliva virgem.

Introduction

The olives and olive oil are the primary sources of dietary lipid Mediterranean; these foods are rich in oleic acid, a monounsaturated fatty acid (Alarcón-de-la-Lastra, Barranco, Motilva, & Herrerías, 2001). The fatty acid has a positive effect on the heart and against cancer (Alonso, Ruiz-Gutierrez, & Martínez-González, 2005). However, one of the problems in the use of olive products is the rapid oxidation of the unsaturated fatty acids that containing. This kind of spoilage affects the shelf life of olive products and their nutritional quality; moreover, it may be a risk for the health of consumers (Alarcón-de-la-Lastra et al., 2001). The techniques collectively known as electrotechnologies may have a solution to this problem by providing non-thermal preservation treatment. As a result of the interaction between vegetable tissues in a liquid environment with electric field, deleterious enzymes and microorganisms in food products are inactivated. There are reported that the products has a fresher taste and no loss of nutrients (Espachs-Barroso, Barbosa-Cánovas, & Martín-Belloso, 2003).

Electrotechnologies can be classified as electroosmosis- and electroplasmolysis- related methods. Electroosmosis is based on the electrokinetic effects of an electric field on porous systems as a result of the formation of a double electrical layer at a solid-fluid interface. Electroplasmolysis is based on the transformation or rupture of cells by an external electric field (breakdown), which results in an increase in the electrical conductivity and permeability of the cell material (Zimmermann, Pilwat, & Riemann, 1974).

In the field of food technologies, electrotreatment can be used for non-thermal pasteurization of food products, to increase yield in juice and sugar production, and in winemaking (Barbosa-Cánovas, Góngora-Nieto, Pothakamury, & Swanson, 1999). However, there is a lack of data in the literature about the impact of this emerging technology on oils and its effect on the unsaturated fatty acids. This information would be relevant since unsaturated fatty acids are very susceptible to oxidation and react readily with oxidants or under oxidizing conditions, and therefore, their behavior could vary after of an electric field treatment. Hence, the aim of this study was to analyze the effect of an electric field (voltage: 3 kV cm^{-1}, frequency: 60 Hz and with a time of application of 5 and 25 min) on the unsaturated fatty acids in virgin olive oil.

Material and methods

Sample

Virgin olive oil, manufactured and imported from Spain, was purchased on the local market in Puebla, Mexico. Oil was obtained by cold-pressing with a maximum acid rate of 2.0% of oleic acid. Nutritional information indicated 5 g of total lipid content, 3.6 g of monounsaturated fatty acids, 0.7 g of polyunsaturated fatty acids, 0.7 g of saturated fatty acids and 0 g of *trans* fatty acids.

Electric field treatment

Electric field was applied on the samples in a scale unit of electric field designed by the Research Center for Applied Biotechnology of the National Polytechnic Institute located in the Municipality of Tepetitla, belonging to the State of Tlaxcala, México. The voltage (3 kV cm^{-1}), frequency (60 Hz) and time of treatment of 5 and 25 min, were similar to those used by Castorena (2008) to inactivate polyphenol oxidase enzyme. The scale unit of electric field consisted of a generator (where high-voltage is produced). The generator is connected to a unit (model 9412A, Quantum Composers, Inc., Bozeman, MT) where the required waveform could be selected (a square form was selected for this work). The unit is connected to a chamber with two stainless steel connectors (acting as electrodes). Both electrodes are screwed to the final section of the chamber. Samples were collected after these treatments and were stored in a closed container at 25°C. Measurements of the chemical parameters (acidity, peroxide and iodine) were done. All treatments were performed in triplicate.

Fourier Transform Infrared Spectroscopy (FTIR)

Bruker spectrometer (model Vertex 70 Bruker Optics-Bruker Corporation, Billerica, Massachusetts, USA) with fast Fourier transformer in the measurement mode called Attenuated Total Reflectance (ATR) was employed. The used crystal was a ZnSe of one reflection. The infrared absorbance was measured in the mid-infrared region from 4000 to 600 cm^{-1}, with a resolution of ±4 cm^{-1} and an integration time of 60 s (1 s per scan). Data acquisition and processing were performed by OPUS 6.0 (Bruker Optics, USA). Only 20 μL of each sample was deposited on the instrument's crystal. The changes in the fatty acids in the crude oils samples were obtained by comparing using a standard of 37-components (Food Industry FAMEs Mix, Restek). The virgin olive oil that was used in this work had a concentration of 3.5 g oleic acid "C18:1", which is the main fatty acid found in this oil. Therefore, to quantify trans fatty acid was taken as parameter the elaidate fatty acid "C18:1t" (purity ≥99%, Sigma-Aldrich). The analysis was according to the methodology reported by the Association of Official Analytical Chemists [AOAC] (2005). The results were substituted into the Equation 1:

$$Trans\ as\ methyl\ elaidate(\%) = \left(\frac{g\ methyl\ eliadate\ weight\ equivalents}{test\ portion\ weight,\ g/10\ mL\ CS_2}\right) x\,100 \tag{1}$$

Characterization of the virgin olive oil

The virgin olive oil was characterized by the following chemical analysis: acidity rate, defined as the quantity in mg of KOH necessary to neutralize the free fatty acids in 1.0 g of oil or fat (Norma Mexicana, 1987a); peroxide rate, expressed as the mEq of O_2 in the form of peroxide per kg of fat or oil (Norma Mexicana, 1987b); iodine rate, determine the quantity of unsaturated fatty acids in fats and oils in cg of I_2 absorbed per gram of sample (Norma Mexicana, 1981). Each analysis was performed in triplicate.

Statistical analysis

Results were expressed as mean value ± SD. Statistical analysis was performed by analysis of variance (ANOVA) at $\alpha = 0.05$ significance. Statistical Analysis System 6.1 (SAS Institute Inc., Cary, NC, USA) was employed for analyses.

Results and discussion

Fourier Transform Infrared (FTIR) spectroscopy and quality parameters

The oil was analyzed by FTIR spectroscopy to determine the characteristic modes of vibration and the wavenumbers of absorption peaks. Several quality parameters were also determined on the samples (without treatment and with an electric field treatment). Results are shown in Table 1 and Figure 1.

Table 1. Chemical characterization of virgin olive oil.

Time treatment (min)	0	5	25
Peroxide rate (mEq O_2 kg^{-1} of oil)	3.7 ± 0.5^{a}	3.74 ± 0.7^{a}	3.76 ± 0.6^{a}
Acidity rate (% of oleic acid)	2.1 ± 0.1^{a}	2.7 ± 0.3^{a}	2.71 ± 0.6^{a}
Iodine rate with Wijs reagent (cg I_2 g^{-1})	91.6 ± 1.0^{a}	91.5 ± 1.1^{a}	91.4 ± 1.2^{a}

Average of 3 replicates ± SD. Different letters in superscripts in the same row indicate significant differences between treatments ($p < 0.05$).

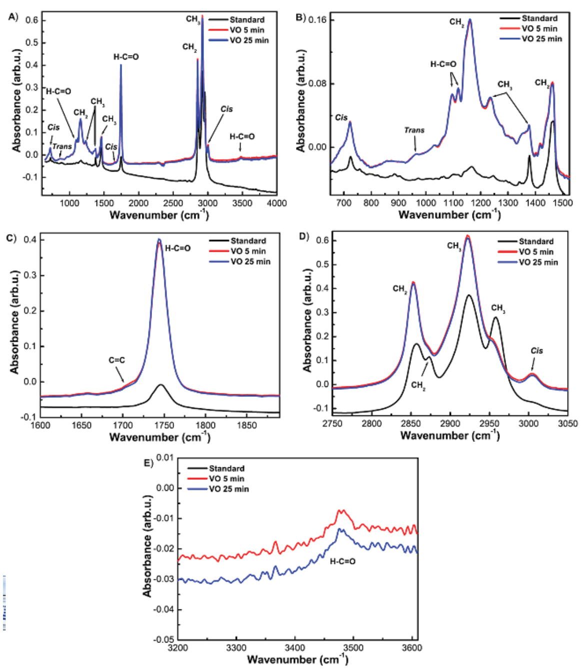

Figure 1. Fourier transform infrared spectrograms of virgin olive oil (VO) without treatment and with electric field treatment (3 kV cm^{-1}, 60 Hz, application time of 5 and 25 min) and comparison with the standard.

In Figures 1A, B, C, and D can be seen that the absorption peaks at wavenumbers 723, 1417, 1658, and 3006 cm-1 that corresponds to the cis double bonds, with move of bending and stretching respectively, according to Guillén & Cabo (1998) and Yang, Irudayaraj, & Paradkar (2005). In these wavenumbers showed not changes in the double bonds in the tested samples compared with standard. Morover, the treatment time increase did not affect the unsaturated fatty acids in the oils, these results were corroborated by iodine value, and are within the range specified (75-94 cg I_2 g^{-1}) by the international standard for virgin olive oil (Organização das Nações Unidas para a Alimentação e a Agricultura [FAO], 1999) (Table 1).

Figure 1C shows the absorption peaks wavenumbers corresponding to the stretching and overtone of the carboxyl functional group of triacylglyceride esters (Guillén & Cabo, 1998).

The virgin olive oil samples showed a minimal concentration of free fatty acids, according with the results of acidity value. On the other hand, the international standard for virgin olive oil fixes a maximum value of 3.3% of oleic acid (FAO, 1999). so post-processing values are within acceptable range as well, showing that the electrical field treatment preserved the nutritional quality of the oils (Table 1). This can be due to the decrease in lipoxygenase enzyme activity by an electric field application. This enzyme degrades to the triacylglicerols and forms free fatty acids, according to studies in soymilk, peanut oil and olive oil (Ying-Qiu, Qun, Xiu-He, & Zheng-Xing, 2008; Xin-an, Zhong, & Zhi-hong, 2010; Abenoza et al., 2013).

Figure 1D were observed increases in intensity in the functional groups of CH2 and CH3 at 2874 and 2960 cm-1 due to the chemical composition of virgin olive oil, which only contain traces of long-chain fatty acids compared with the standard (C22:1n9, C22:2, C22:6n3, C23:0, C24:0, and C24:1n9).

Figure 1E shows the stretching and overtone of the carboxyl functional group in triacylglyceride ester (Guillén & Cabo, 1998). Oil samples exhibited a weak intensity peak at 3468 cm^{-1}, and no significant changes on shift or curve intensity were observed. Therefore, the application of an electric field for long periods on virgin olive oil samples; does not significantly modify their molecular structure. Nevertheless, electric field treatment cannot prevent oil oxidation by hydrogen peroxide, and the oxidation of unsaturated fatty acids is inevitable, as was quantitified by peroxide value. Brühl (1996), exposure to oxidants, such as atmospheric oxygen and light, produces singlet oxygen species, which initiates a cascade of reactions leading to oil oxidation. It also produces configuration changes of the double bond from cis to trans form (Coolbear & Keough, 1983). Trans fatty acids were identified with a minimal intensity in all samples in the absorption peak at 968 cm-1 (see Figure 1B). Table 2 shows the results of the trans fatty acids of virgin olive oil treated with an electric field (3 kV cm-1, 60 Hz, with application times 5 and 25 min).

Table 2. Results of percentage of *trans* fatty acids in virgin olive oil treatment with an electric field.

Time (min)	C18:1t
5	0.0001^{a} ± 0.00001^{a}
25	0.0001 ± 0.00001^{a}

Means of 3 replicates ± SE. Different letters in superscripts between untreated and treated samples indicate significant difference ($p < 0.05$).

Time of application of an electric field treatment (5 and 25 min), generated a minimal value in the *trans* fatty acids concentration in virgin olive oil compared to that proposed as maximum allowed by the Food and Drug Administration < 0.5 g 100 g^{-1} of lipids (Food and Drug Administration [FDA], 2003). To prevent the oxidation of fatty acids in the treated samples, exposure to light should be minimized during oil handling, and the product should be stored in non-transparent containers (Psomiadou & Tsimidou, 2002).

Conclusion

Treatment with electric fields did not affect the total concentration of unsaturated fatty acids in virgin olive oil. Since no differences in composition or quality were observed with different treatment times, the use of minimum treatment time for oil conservation (3 kV cm-1, 60 Hz and 5 min) is suggested.

Electric field processing has good prospects for being used in the oil industry as a non-thermal preservation method. It could provide an alternative to preserve oil composition without adding a synthetic antioxidant. However, further investigation is required for more in-depth information on the parameters affecting the result of this treatment.

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