



## Effect of microwave heating with different exposure times on physical and chemical parameters of olive oil

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### ABSTRACT

This study reflects the effect of different microwave heating times (1, 3, 5, 10 and 15 min) on physical and chemical characteristics of three Portuguese olive oils from three protected designation of origin (PDO), “Azeite de Trás-os-Montes PDO”, “Azeites da Beira Interior PDO”, and “Azeite de Moura PDO”. The parameters evaluated were free acidity, peroxide value, specific extinction coefficients ( $K_{232}$  and  $K_{270}$ ), color and chlorophylls and carotenoids content. A differential pulse voltammeter was also used to monitor the changes in  $\alpha$ -tocopherol content. The results showed that microwave heating produce losses in the quality of the different analysed olive oils. The heating time did not promote the occurrence of hydrolysis in the samples since no changes in free acidity values were found. All other parameters were affected by exposure time in a similar way: in the firsts 3 min no marked changes were observed, after that the quality of the oil decrease significantly. The microwave heating time also affects the total chlorophylls, carotenoids and  $\alpha$ -tocopherol contents which clearly decreased as long as the exposure time increases. After 15 min of heating the electrochemical signal, due to the  $\alpha$ -tocopherol, disappear completely in the voltamogram.

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### 1. Introduction

Olive oil is a premium vegetable oil that is an important component of the Mediterranean diet. This oil, contrary to the majority of the other edible oils, can be consumed in crude form, conserving all beneficial properties, like vitamins, phenols, sterols and other important natural compounds (Kiritsakis, 1998).

In the last decades, the modern life style brought procedures changes in the food and cooking processing technologies. Since its invention and development, the use of the microwave oven increased constantly, both at home and in the industry sector, due to its advantages, that includes capacity to rapidly transmit heat due to its high penetration power (Hassanein et al., 2003; Burfoot et al., 1990), convenience, ease of use (Caponio et al., 2003; Cossignani et al., 1998) and time and energy savings (Albi et al., 1997a). Another reason to the constant increase in the use of the microwave oven is the tendency of the industry to produce pre-prepared food products especially to defrost, heat or cook using this kind of equipment (Albi et al., 1997b).

Different works have been aimed to evaluate the effects of microwave heating on food and its constituents (Cossignani et al., 1998), including in lipid fraction of animal fats and vegetable oils (Yoshida et al., 1990; Yoshida et al., 1992).

The microwave heating process could accelerate oxidative reactions which promote the involvement of free radicals (Albi et al., 1997a). Several other factors could also interfere in lipid oxidation processes, such as, fatty acid composition (Gertz et al., 2000), free fatty acids, oxygen exposure (Anderson and Lingnert, 1998), heat (Fukumoto and Iibuchi, 2001; Medina et al., 2000), water (Kahl et al., 1988), physic state (Fritsch, 1994; Shimada et al., 1991), light (Chen and Ahn, 1998), trace metals (Anderson and Lingnert, 1998; Chen and Ahn, 1998; Haneda and Yoshino, 1998), antioxidants (Gertz et al., 2000), between others. Being the temperature the most important.

Tocopherols, and especially  $\alpha$ -tocopherol, are important components of olive oil and contribute to its nutritional value. Their significance is related to their vitamin action and antioxidant properties (Kiritsakis, 1998). However information regarding microwave heating and its impact in the tocopherols content is almost non-existent.

The effect of olive time storage (Pereira et al., 2002) olive fruit fly infestation (Pereira et al., 2004) olive ripening stage (Matos et al., 2007) on oil quality of Portuguese varietal olive oils was previously studied. The classification of PDO olive oils on the basis of their sterol composition by multivariate analysis was also reported (Alves et al., 2005). Nevertheless, as far as we know, there are no investigation regarding the effect of microwave heating on Portuguese PDO olive oils.

The objectives of this study were to verify the effect of different microwave heating times, 1, 3, 5, 10 and 15 min, that simulate the

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usual times used to cooking, on three Portuguese olive oils from three different Protected Designation of Origin (PDO), one from the north “Azeite de Trás-os-Montes” PDO, one from the centre “Azeites da Beira Interior” PDO, and one from the south of Portugal “Azeite de Moura” PDO. The parameters used to evaluate this effect were free acidity, peroxide value (PV), specific extinction coefficients ( $K_{232}$  and  $K_{270}$ ), color, chlorophylls and carotenoids and tocopherols content. For tocopherols determination the potentialities of the electrochemical tools were explored using the differential pulse voltammetry. This technique is very useful for the evaluation of electroactive compounds such as antioxidants. Moreover, it is a fast technique that requires a low input of chemicals (Blasco et al., 2005; Barros et al., 2008).

## 2. Materials and methods

### 2.1. Olive oil samples

Three Portuguese PDO olive oils were selected. One extra virgin olive oil from the “Azeite de Trás-os-Montes” PDO in the northeast of Portugal; one extra virgin olive oil from the “Azeites da Beira Interior” PDO in the central region of Portugal; and one virgin olive oil from the “Azeite de Moura” PDO in the south of Portugal. The samples were purchased in the local market and were stored in the refrigerator. All olive oils were filtrated in the presence of sulphate sodium anhydrous before use.

### 2.2. Heating procedure

To simulate conventional times used in home cooking, different times for microwave heating were selected namely, 1, 3, 5, 10 and 15 min. For each olive oil and time, three sub-samples of 50 mL were individually placed in a Petri dish (20 mm high and 110 mm of diameter) and subjected to heating in a microwave oven (SOLAC) at maximum potency (1000 Watt). Unheated olive oil was used as control (corresponding to 0 min). Afterwards, the samples were kept in Falcon tubes and refrigerated until analysis.

### 2.3. Physical and chemical parameters determination

The free acidity, the peroxide value and the coefficients of specific extinction at 232 and 270 nm ( $K_{232}$  and  $K_{270}$ ) were determined according to European Union standard methods (Annexes II and IX in European Community Regulation EEC/2568/91 from 11th July).

#### 2.3.1. Free acidity

Expressed as percent of oleic acid, was determined by titration of an oil solution dissolved in ethanol/ether (1:1, v/v) with 0.1 M potassium hydroxide ethanolic solution.

#### 2.3.2. Peroxide value

Peroxide value, given in milliequivalents of active oxygen per kilogram of oil (mequiv/kg), was determined as follows: a mixture of oil and chloroform/acetic acid 3:2 (v/v) was left to react in darkness with saturated potassium iodine solution; the free iodine was then titrated with a sodium thiosulfate solution.

#### 2.3.3. $K_{232}$ and $K_{270}$ extinction coefficients

$K_{232}$  and  $K_{270}$  extinction coefficients (absorption of 1% solution (m/v) in iso-octane at 232 and 270 nm, respectively, with 1 cm of path length) were measured using a UV spectrophotometer (Genesys 10UV).

#### 2.3.4. Color

Color was evaluated following the NP-937 (1987) method. It consists in the determination of transmittance values at 445, 495, 560, 595 and 625 nm of the sample solution, in a spectrophotometer (Genesys 10UV).

#### 2.3.5. Chlorophylls and carotenoids content

Following the procedures described by Mosquera et al. (1991) a sample of olive oil (7.5 g) was placed in a Falcon tube and filled until 25 mL with cycle-hexane. The chlorophyll fraction was measured in a UV spectrophotometer (Genesys 10UV) at 670 nm and the carotenoid fraction at 470 nm. The concentration of pigments was expressed using the following equations:

$$[\text{chlorophylls}] = \frac{\text{Abs}_{670} \times 10^6}{613 \times 100 \times \text{density}} \text{mg/Kg}$$

$$[\text{carotenoids}] = \frac{\text{Abs}_{470} \times 10^6}{2000 \times 100 \times \text{density}} \text{mg/Kg}$$

### 2.4. $\alpha$ -Tocopherol content

#### 2.4.1. Instrumentation

Differential pulse voltammetry (DPV) measurements were performed on an Autolab PGSTAT 302 potentiostat/galvanostat using a closed standard three electrode cell. A glassy carbon (BAS,  $\phi = 0.314 \text{ cm}^2$ ) was used as the working electrode and a Pt foil as the counter electrode. All potentials are refer to an Ag/AgCl 3 M KCl reference electrode (Methrom). Prior to use, the working electrode was polished in an aqueous suspension of 0.3  $\mu\text{m}$  alumina (Beuhler) on a Master-Tex (Beuhler) polishing pad, then rinsed with water. Subsequently, in a chemical treatment, the electrode was sonicated in 6 M HCl for 1 min, and then in methanol. This cleaning procedure was applied always before any electrochemical measurements.

#### 2.4.2. Procedure

The electrochemical cell was filled with a solution prepared with 0.6 g of olive oil, 0.2 mL of  $\text{H}_2\text{SO}_4$  0.1 M, 1.2 mL of tetrabutylammonium perchlorate (TBAP) solution 0.25 M, 4 mL of hexane and completed with ethanol to a final volume of 10 mL. Before the voltammetric assays the system was checked with a supporting electrolyte solution (0.5 mL of  $\text{H}_2\text{SO}_4$  0.1 M, 3 mL of TBAP 0.25 M, 10 mL of hexane and completed with ethanol to a final volume of 25 mL). All solvents used were of HPLC grade.

The differential pulse voltammograms were recorded between 0 and 1 V with a scan rate of  $0.03 \text{ Vs}^{-1}$  and pulse amplitude of 0.06 V.

For the calibration standard ( $\alpha$ -tocopherol), the concentration was set between 2.5 and 15  $\mu\text{M}$ .

All solutions were analysed after preparation and the electrochemical responses recorded immediately after the glassy carbon electrode immersion, to minimize adsorption of species onto the electrode surface prior to the run.

## 3. Results and discussion

### 3.1. Changes in free acidity

In the present work high quality olive oils were chosen from three different PDO origins. Prior heating, “Azeite de Trás-os-Montes PDO” and “Azeite da Beira Interior PDO” were classified as extra virgin olive oil, with free acidity value of 0.49% and 0.60%, respectively; whereas “Azeite de Moura PDO” olive oil was denominated as virgin and its free acidity value was 1.05%. For the analyzed samples the free acidity values were similar at all the studied times (Fig. 1) and no statistically changes were observed when its values were correlated with heating time (Table 1). In certain way, the obtained results were expected and are in accordance with the results obtained by Albi et al. (1997a). Free acidity values results from the occurrence of hydrolysis in fatty acids. In those reactions hydrolytic enzymes are involved. Normally, these enzymes are presents in the olive fruit or surrounding microorganisms. Once in the present work we only use high quality olive oils that are filtered and dehydrated, the probability of enzymes occurrence is low or inexistent.

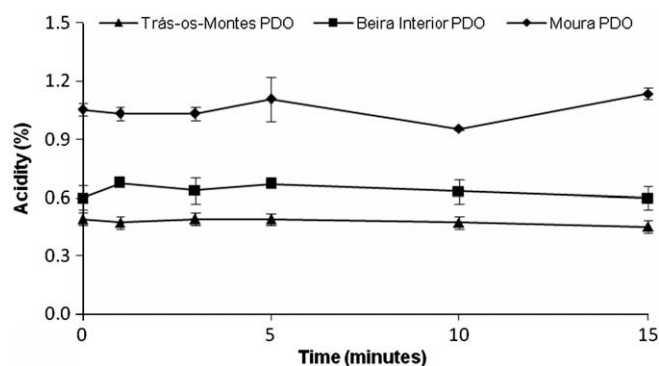


Fig. 1. Changes occurred in free acidity values (% of free fatty acids) of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times.

**Table 1**

Correlation between the evaluated parameters and microwave heating exposure times for the “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO”.

	Azeite de Trás-os-Montes PDO		Azeite da Beira Interior PDO		Azeite de Moura PDO	
	$r^2$	$p$	$r^2$	$p$	$r^2$	$p$
Free acidity	0.682	*	0.164	n.s.	0.090	n.s.
PV	0.280	n.s.	0.381	n.s.	0.874	**
$K_{232}$	0.852	**	0.743	**	0.751	*
$K_{270}$	0.961	***	0.909	**	0.853	**
$\Delta K$	0.919	**	0.925	**	0.903	**
Chlorophylls	0.962	***	0.699	*	0.880	**
Carotenoids	0.956	***	0.886	**	0.926	**
$\alpha$ -Tocopherol	0.894	**	0.900	**	0.835	**
Color parameters						
X	0.683	**	0.220	n.s.	0.798	**
Y	0.825	**	0.537	n.s.	0.883	**
Z	0.895	**	0.921	**	0.923	**
x	0.920	**	0.919	**	0.919	**
y	0.915	**	0.922	**	0.928	**
$\lambda_d$ (nm)	0.884	**	0.926	**	0.912	**
Transparency (Y%)	0.919	**	0.921	**	0.923	**

n.s., not significant correlation.

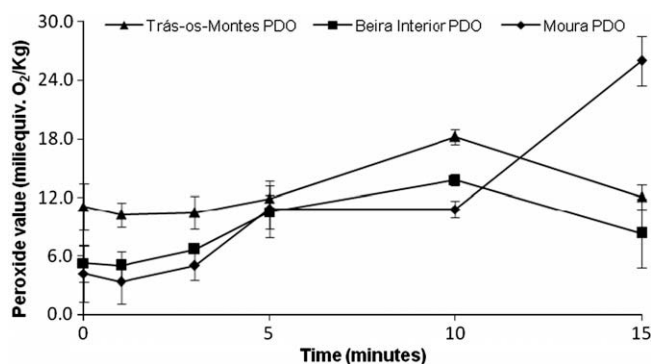
\*  $p \leq 0.05$  (significant correlation).

\*\*  $p \leq 0.01$  (very significant correlation).

\*\*\*  $p \leq 0.001$  (extremely significant correlation).

### 3.2. Changes in peroxide value

The peroxide values prior analysis (time zero) were 11.1, 5.3 and 4.2 meq.  $O_2$ /kg of olive oil for “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO”, respectively. Before treatment all olive oils presented peroxide values below the maximum permitted for their classification in each category (20 meq.  $O_2$ /kg of olive oil). In the case of “Azeite de Trás-os-Montes PDO” the limit value is even more restrictive (15 meq.  $O_2$ /kg of olive oil) than the European regulation for virgin olive oil. With the exception of “Azeite de Moura PDO”, peroxide value increased until 10 min and decreased afterwards. In “Azeite de Moura PDO”, PV increases until 15 min, overcoming the maximum permitted limit, and consequently losing the classification of virgin olive oil category (Fig. 2). A significantly positive correlation was established between heating time and peroxide values for the “Azeite de Moura PDO” but no correlation was observed for the other two oils (Table 1). The peroxide value behaviour of extra olive oils (“Azeite de Trás-os-Montes PDO” and “Azeite da Beira Interior PDO”) could be explained by changes during oxidation process, reaching this value a maximum due to hydroperoxides formation, and then decreasing due to the appearance of secondary products



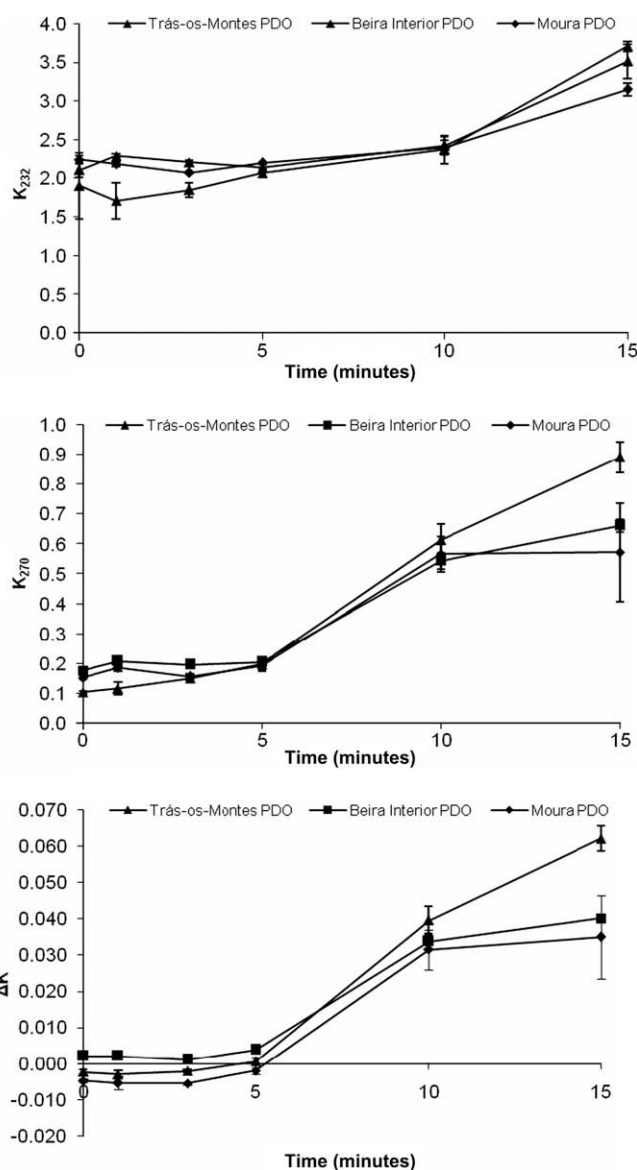
**Fig. 2.** Changes occurred in peroxide value (Milliequiv.  $O_2$ /kg) of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times.

of oxidation. The results obtained are similar to those of Caponio et al. (2003) and Albi et al. (1997a).

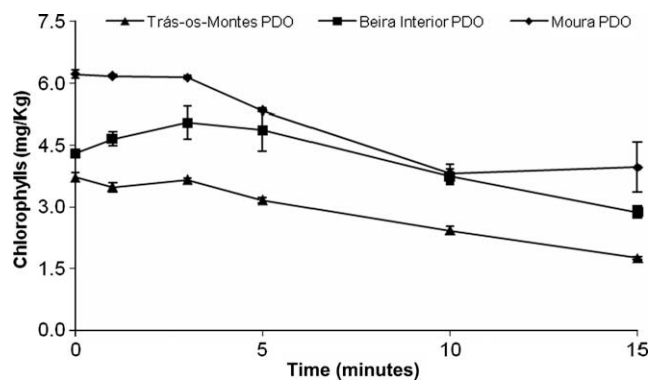
### 3.3. Changes in specific extinction coefficient at 232 nm and 270 nm

The ultraviolet spectrophotometric analysis indicates the degree of olive oil oxidation, being its values expressed as specific extinction coefficients (Annexes II and IX in European Community Regulation EEC/2568/91). The,  $K_{232}$  and  $K_{270}$ , are mainly indicative, respectively, of the conjugation of trienes and the presence of carbonyl compounds. The maximum values permitted for  $K_{232}$  and  $K_{270}$  are respectively 2.50 and 0.20 for extra virgin olive oils and 2.60 and 0.25 for virgin olive oil, respectively. For  $\Delta K$  the maximum permitted value is 0.01.

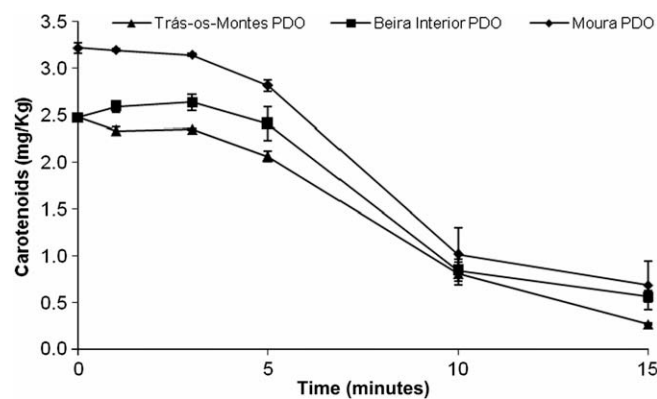
Fig. 3 shows changes in  $K_{232}$  and  $K_{270}$  specific coefficients and  $\Delta K$  under different exposure times at microwave heating. Before analysis the olive oils presented for “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO”  $K_{232}$  values of 1.91, 2.11 and 2.25, and  $K_{270}$  values of 0.10, 0.18



**Fig. 3.** Changes occurred in  $K_{232}$ ,  $K_{270}$  and  $\Delta K$  of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times.



**Fig. 4.** Changes occurred in chlorophylls (mg/Kg) of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times.



**Fig. 5.** Changes occurred in carotenoids (mg/Kg) of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times.

and 0.15, respectively. In the initial heating stages (approximately the first 5 min) the specific coefficient values showed little variations, after that a significant increase was observed for all the coefficients. At 15 min all oils presented a  $K_{232}$  higher than 3.1 that indicates an accelerated degradation process. The same situation was also observed for  $K_{270}$  and  $\Delta K$  values. Our results are in accordance to the obtained by Albi et al. (1997a) and Caponio et al. (2003). A significant positive correlation was observed between  $K_{232}$  and  $K_{270}$  specific coefficients and  $\Delta K$  with heating times (Table 1) that proves the worsening effect of microwave heating in olive oil.

### 3.4. Changes in chlorophylls content

Chlorophylls are present in olive oils and are the responsible for the greenish coloration of certain olive oils (Rahmani and Csallany, 1991; Criado et al., 2008). Those pigments are also important in olive oil stability. In the present work, before treatment, their amount varied between 3.72 mg/Kg, in the “Azeite de Trás-os-Montes PDO” oil, and 6.22 mg/Kg, in the “Azeite de Moura PDO” samples. A marked decrease was observed in its levels for all the analyzed olive oils (Fig. 4). This observation was more noticeable in the “Azeite de Trás-os-Montes PDO” olive oil. In fact, when

**Table 2**

Color characteristics, mean  $\pm$  SD, of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times (0 min -  $T_0$ ; 1 min -  $T_1$ ; 3 min -  $T_3$ ; 10 min -  $T_{10}$  and 15 min -  $T_{15}$ ).

	$T_0$	$T_1$	$T_3$	$T_5$	$T_{10}$	$T_{15}$
<b>X</b>						
Azeite de Trás-os-Montes PDO	64.772 $\pm$ 1.351	69.213 $\pm$ 5.8596	70.615 $\pm$ 2.8166	66.444 $\pm$ 0.4372	71.018 $\pm$ 1.6702	75.752 $\pm$ 1.3834
Azeites da Beira Interior PDO	69.133 $\pm$ 2.162	64.106 $\pm$ 1.5469	62.839 $\pm$ 3.9690	63.507 $\pm$ 0.3708	67.612 $\pm$ 2.7900	69.441 $\pm$ 1.7379
Azeite de Moura PDO	55.837 $\pm$ 1.858	51.935 $\pm$ 1.9762	56.895 $\pm$ 0.8732	55.361 $\pm$ 1.8632	59.512 $\pm$ 0.4307	62.665 $\pm$ 1.4449
<b>Y</b>						
Azeite de Trás-os-Montes PDO	65.384 $\pm$ 1.312	69.982 $\pm$ 6.0613	71.473 $\pm$ 2.8374	67.076 $\pm$ 0.3237	75.312 $\pm$ 2.1239	81.565 $\pm$ 1.4483
Azeites da Beira Interior PDO	70.024 $\pm$ 2.213	64.947 $\pm$ 1.5745	63.737 $\pm$ 3.9918	64.752 $\pm$ 0.3408	72.811 $\pm$ 2.8796	74.464 $\pm$ 1.9310
Azeite de Moura PDO	56.292 $\pm$ 1.889	52.318 $\pm$ 1.9890	57.373 $\pm$ 0.9233	55.807 $\pm$ 1.8281	63.478 $\pm$ 1.2464	68.222 $\pm$ 2.3090
<b>Z</b>						
Azeite de Trás-os-Montes PDO	3.805 $\pm$ 0.076	4.136 $\pm$ 0.3857	4.413 $\pm$ 0.1299	4.789 $\pm$ 0.1967	28.999 $\pm$ 4.9472	61.907 $\pm$ 2.1312
Azeites da Beira Interior PDO	3.287 $\pm$ 0.178	3.011 $\pm$ 0.0820	3.241 $\pm$ 0.2018	4.454 $\pm$ 0.4610	37.570 $\pm$ 4.0526	57.762 $\pm$ 2.3208
Azeite de Moura PDO	1.109 $\pm$ 0.068	1.054 $\pm$ 0.0139	1.245 $\pm$ 0.0488	1.784 $\pm$ 0.2031	16.827 $\pm$ 5.4812	27.115 $\pm$ 9.2832
<b>x</b>						
Azeite de Trás-os-Montes PDO	0.484 $\pm$ 0.001	0.483 $\pm$ 0.0007	0.482 $\pm$ 0.0002	0.480 $\pm$ 0.0003	0.405 $\pm$ 0.0119	0.346 $\pm$ 0.0017
Azeites da Beira Interior PDO	0.485 $\pm$ 0.001	0.485 $\pm$ 0.0003	0.484 $\pm$ 0.0004	0.479 $\pm$ 0.0019	0.380 $\pm$ 0.0117	0.344 $\pm$ 0.0018
Azeite de Moura PDO	0.493 $\pm$ 0.001	0.493 $\pm$ 0.0005	0.493 $\pm$ 0.0003	0.490 $\pm$ 0.0005	0.426 $\pm$ 0.02	0.398 $\pm$ 0.0244
<b>y</b>						
Azeite de Trás-os-Montes PDO	0.488 $\pm$ 0.001	0.488 $\pm$ 0.0004	0.488 $\pm$ 0.0002	0.485 $\pm$ 0.0010	0.430 $\pm$ 0.0095	0.372 $\pm$ 0.0020
Azeites da Beira Interior PDO	0.492 $\pm$ 0.001	0.492 $\pm$ 0.0001	0.491 $\pm$ 0.0003	0.488 $\pm$ 0.0015	0.409 $\pm$ 0.0122	0.369 $\pm$ 0.0019
Azeite de Moura PDO	0.497 $\pm$ 0.001	0.497 $\pm$ 0.0004	0.497 $\pm$ 0.0004	0.494 $\pm$ 0.0009	0.454 $\pm$ 0.0142	0.433 $\pm$ 0.0217
<b><math>\lambda_d</math> (nm)</b>						
Azeite de Trás-os-Montes PDO	577.87 $\pm$ 0.023	577.81 $\pm$ 0.0820	577.78 $\pm$ 0.0125	577.87 $\pm$ 0.0688	575.28 $\pm$ 0.5200	571.74 $\pm$ 0.1444
Azeites da Beira Interior PDO	577.75 $\pm$ 0.022	577.74 $\pm$ 0.0331	577.70 $\pm$ 0.0191	577.51 $\pm$ 0.0375	573.86 $\pm$ 0.5062	571.96 $\pm$ 0.2445
Azeite de Moura PDO	577.90 $\pm$ 0.013	577.92 $\pm$ 0.0564	577.89 $\pm$ 0.0382	577.90 $\pm$ 0.0289	575.28 $\pm$ 0.8847	573.76 $\pm$ 1.1277
<b>Purity (<math>\sigma\%</math>)</b>						
Azeite de Trás-os-Montes PDO	92.4 $\pm$ 0.06	92.3 $\pm$ 0.12	91.9 $\pm$ 0.12	90.7 $\pm$ 0.32	55.9 $\pm$ 5.74	24.4 $\pm$ 0.98
Azeites da Beira Interior PDO	93.8 $\pm$ 0.14	93.9 $\pm$ 0.05	93.3 $\pm$ 0.17	91.0 $\pm$ 0.92	43.5 $\pm$ 6.40	23.3 $\pm$ 0.97
Azeite de Moura PDO	97.4 $\pm$ 0.07	97.3 $\pm$ 0.09	97.1 $\pm$ 0.08	95.8 $\pm$ 0.33	68.1 $\pm$ 9.16	54.7 $\pm$ 12.32
<b>Transparency (Y%)</b>						
Azeite de Trás-os-Montes PDO	65.4 $\pm$ 1.312	70.0 $\pm$ 6.061	71.5 $\pm$ 2.837	67.1 $\pm$ 0.324	75.3 $\pm$ 2.124	81.6 $\pm$ 1.448
Azeites da Beira Interior PDO	70.0 $\pm$ 2.213	64.9 $\pm$ 1.574	63.7 $\pm$ 3.992	64.8 $\pm$ 0.341	72.8 $\pm$ 2.880	74.5 $\pm$ 1.931
Azeite de Moura PDO	56.3 $\pm$ 1.889	52.3 $\pm$ 1.989	57.4 $\pm$ 0.923	55.9 $\pm$ 1.822	63.5 $\pm$ 1.246	68.2 $\pm$ 2.309

correlating the exposure times to microwave heating and the chlorophylls content, an extremely significant negative correlation was established ( $r^2 = 0.962$ ;  $p < 0.001$ ). In “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” a very significant negative correlation was also obtained (Table 1).

### 3.5. Changes in carotenoids content

The behaviour of these pigments was similar to the chlorophylls. The level of carotenoids in the unheated oils (time zero) was respectively 2.47, 2.47 and 3.22 mg/Kg for “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO”, and “Azeite de Moura PDO”. Fig. 5 shows the changes occurred in carotenoid levels along the exposure times to microwave heating. The values remain practically constants until 3 min of heating and decrease drastically after that until 15 min. Similarly to chlorophylls “Azeite de Trás-os-Montes PDO” showed the most perceptible decrease, that was confirmed by the extremely significant negative correlation established between time of exposure and carotenoid content ( $r^2 = 0.956$ ;  $p < 0.001$ ). Also for “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” a very significant negative correlation was obtained (Table 1).

Chlorophylls and carotenoids play an important role in the oxidative stability due to their antioxidant nature in the dark and pro-oxidant activity in the light. And are mainly responsible for the color of virgin olive oil, that varying from yellow–green to greenish gold (Criado et al. 2008). In our work the loss of color in all olive oils was well noted probably due to the loss of these pigments.

### 3.6. Changes in color

Tables 2, gives the values, determined in the “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO”, and “Azeite de Moura PDO” olive oils along the exposure time of the color parameters, chromatic coordinates ( $x$  and  $y$ ), transparency ( $Y\%$ ), and purity ( $\sigma\%$ ).  $x$  and  $y$  are the chromatic coordinates of the superficial point of the chromaticity diagram that corresponds to the light transmitted by the oil. Transparency corresponds to the values of the light transmitted after passing through the oil layer. Purity is the percentage of light transmitted by the oil with the prevailing wavelength. All analysed color parameters were strongly, with very significant and significant correlations, affected by the microwave heating, with the exception of two cases in “Azeite da Beira Interior PDO” olive oil (Table 1).

Through visual inspection, comparing the unheated and heated samples at 1, 3 and 5 min, at naked eye, no changes were detected.

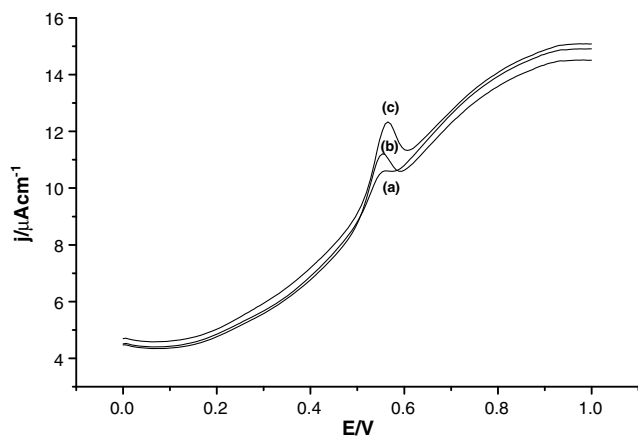


Fig. 6. Differential pulse voltamograms of  $\alpha$ -tocopherol in 0.2 mL of  $H_2SO_4$  0.1 M, 1.2 mL of TBAP solution 0.25 M (40 hexane:60 ethanol): (a) 5  $\mu$ M, (b) 10  $\mu$ M, and (c) 15  $\mu$ M.

However comparing the untreated samples with the samples heated at 10 and 15 min, the latest show some discoloration and less viscosity. This visual observation could be related also with the destruction and constant decrease in the content of chlorophylls and carotenoids pigments, as shown in Figs. 4 and 5.

### 3.7. Changes in $\alpha$ -tocopherol content

Fig. 6 shows differential pulse voltamograms at several concentrations of  $\alpha$ -tocopherol. As observed there is a peak at 0.56 V, corresponding to an irreversible oxidation of this compound at the electrode surface. The observed current density present a steady increase with concentration, expressed by a linear correlation.

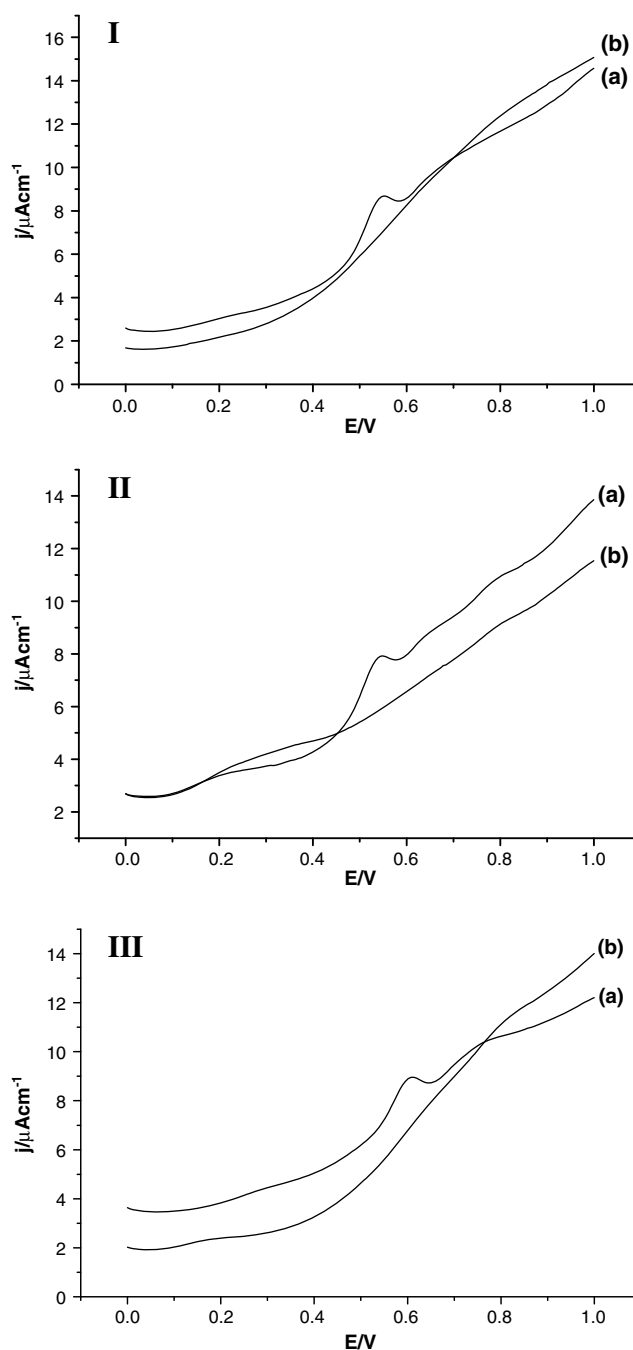
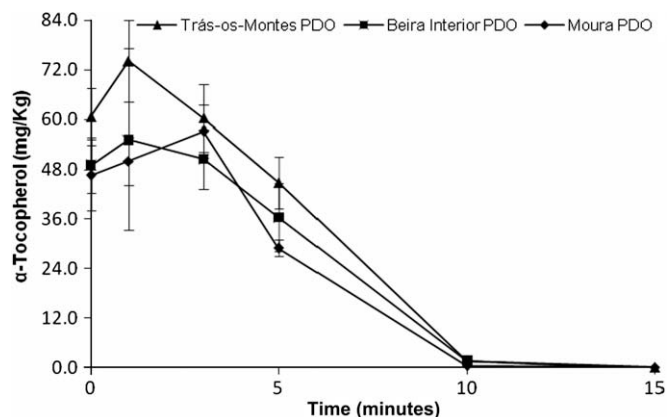


Fig. 7. Differential pulse voltamograms of  $\alpha$ -tocopherol signals in the (I) “Azeite de Trás-os-Montes PDO”, (II) “Azeite da Beira Interior PDO” and (III) “Azeite de Moura PDO” before (a) and after 15 min (b) microwave heating.



**Fig. 8.** Changes occurred in  $\alpha$ -tocopherol (mg/Kg) of “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” subjected to different microwave heating times.

This performance allows the use of this electrochemical technique to access the content of this vitamin in food matrix. The main differences in the oil samples were found when comparing unheated and heated samples with 15 min on microwave (Fig. 7), with a total disappearance of the electrochemical signal on the heated samples. As observed above for the pigments content this behavior is common for the three olive oils and reflect the degradation of the antioxidant components of the olive oil.

The profile of  $\alpha$ -tocopherol content for the different olive oils submitted to microwave heating at different exposure times can be observed in Fig. 8. The values of “Trás-os-Montes PDO”, “Azeite da Beira Interior PDO”, and “Azeite de Moura PDO” unheated olive oils are  $61 \pm 7$ ,  $49 \pm 7$  and  $47 \pm 8$  mg/kg, respectively. Is to rescue that “Trás-os-Montes” olive oil, contains a higher content of  $\alpha$ -tocopherol than the others olive oils analyzed. “Azeite de Trás-os-Montes PDO” and “Azeite da Beira Interior PDO” had similar behavior, with an increase of  $\alpha$ -tocopherol content until one minute of microwave heating, followed by a continuous decrease until 10 min, after that the content is approximately zero (Fig. 8). The virgin olive oil also reported a similar behavior as the other two oils. The difference is that the rise in the content of  $\alpha$ -tocopherol was seen until 3 min of microwave heating, instead of one minute. At 10 min, the content was not significant. The increase in the content of  $\alpha$ -tocopherol during the first minutes of heating could be explained due to the destruction of others compounds of the olive oil that are linked to  $\alpha$ -tocopherol. Therefore only when this bond is broken the electrochemical signal is observed at the right potential, increasing then its content. Very significant correlations, with  $r^2 = 0.919$ ,  $0.921$  and  $0.923$  respectively for “Azeite de Trás-os-Montes PDO”, “Azeite da Beira Interior PDO” and “Azeite de Moura PDO” and  $p < 0.01$ , were obtained between exposure time and  $\alpha$ -tocopherol content (Table 1).

The present work proves that microwave heating produces significant losses in olive oil quality and in their nutritional value. The extension of losses is higher when the time of exposure increases. With the exception of free acidity values, all parameters are significantly affect by the time of heating. In what concern to pigments such chlorophylls and carotenoids content, we can conclude that they are thermal labile, once that their quantity decrease as long as the exposure time increase. This fact is also observable in the  $\alpha$ -tocopherol that disappears completely in the voltamogram at 15 min of heating.

#### Conflict of interest statement

The authors declare that there are no conflicts of interest.

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