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ORIGINAL PAPER

Production of split table olives of the Cobrançosa cultivar: a kinetic study of the fermentation profile

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Abstract
The aim of this study was to evaluate the effect of the Cobrançosa cultivar olive ripeness on the physicochemical parameters and model their progression profile throughout the fermentation period. Green and turning color olives undertook fermentation due to fruit and environmental microbiota resulting in final brines with the required acidity values and absence of coliforms, *Escherichia coli, Salmonella* and *Listeria monocytogenes*. The Monod model was used to explain the changes of a_W, total acidity and total phenolic content in the brines, and the same kinetic with inhibition was fitted to the changes of reducing sugar concentration in the brines. The inverse power model was adjusted to salt content in brines, a_W, total acidity, reducing sugars and total phenolic content in the olives. The Naperian logarithmic function was fitted to the changes of the surface color parameter ($-a/b$) of the fruits. For both olives, the models adjusted to the experimental data were the same, showing a similar trend in the physicochemical profiles, probably due to the previously fruit splitting, which promotes nutrients diffusing into the brines and the influx of salt into the olives during fermentation. However, different model parameters were estimated, depending on the ripeness degree, namely for total acidity, reducing sugars and total phenolic content of the brines, showing lower nutrients diffusion rates from the unripe olive pulp, through the skin into the brine, due to the hardness of the cell wall structures in this maturation stage.

Keywords *Olea europaea* · Fermentation profile · Ripeness degree · Cobrançosa cultivar · Kinetics · Modeling

Introduction

The Mediterranean Diet (MD) is widely considered a healthy dietary option and a greater adherence to it has been associated with significant improvements in health and nutritional status. Is has also been recognized as a sustainable diet because of its lower environmental impact [1].

The fruits of *Olea europaea* are processed to obtain table olives or olive oil, which are core ingredients of the Mediterranean cuisine and can be consumed with vegetables in salads or in cooked foods. In southern Mediterranean countries, diets were mainly vegetarian, as only a small proportion of calories were of animal origin. Before the dissemination of western diets, cereals were the basic ingredient and pulses the main protein source. These elements of high dietary fibre, low glycemic index, and antioxidant compounds with anti-inflammatory effects are the base of the so proclaimed healthy status. That is why MD is associated with lower occurrences of cardiovascular diseases, type2 diabetes, certain types of cancer and neuro degenerative diseases and, therefore, lower mortality [2, 3].

Table olives are a source of beneficial compounds for health, such as monounsaturated fatty acids, tocopherols, phenolic compounds and triterpenic acids, namely oleanolic and maslinic acids [4–10].

The different olive cultivars are one of the most important factors that explain the variability of table olive composition and sensory characteristics. However, other factors may affect their quality, such as sanitary and ripening stages of olive drupes, environmental conditions, cultural practices, preparation and fermentation methods used [11–20].

In the Algarve region, located in the south of Portugal, olive trees cover an area of 236 ha, with 173 ton of table olive production per year [21]. They are one of the most

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cultivated crops of this region, namely the Maçanilha Algarvia and Galega cultivars. As a result, production has the potential to increase in the future, which is beneficial for the Algarve agricultural sector. Recently, in addition to these olive cultivars used in the production of table olives, special attention has been given to Cobrançosa, which, although not cultivated in Algarve, but in a neighboring region, the Alentejo, is processed in the Algarve and very much appreciated by consumers, especially tourists.

Olives from Cobrançosa cultivar are traditionally processed by natural fermentation in brines (~ 8–10% salt), and in southern Portugal they are previously prepared by splitting. At the end of fermentation, when they partly loose their natural bitterness, olives are washed and immersed in new brines with lower salt concentration, seasoned with aromatic herbs and packed for commercialization. This product, which has an increasing demand, is part of the traditional daily diet of Algarvian people and also highly valued in markets all over the country.

The aim of this work was to study the effect of the olive ripening stages (green/unripe, and turning color/semi-ripe) on the physicochemical parameters of split Cobrançosa table olives during the fermentation period. At the end of the fermentation processes a microbiological characterization of the olives was performed. To better understand the influence of the ripeness degree on the fermentation profile, the application of models, to describe the kinetics of changes of some crucial parameters, was also attempted.

Materials and methods

Sampling and preparation of split olives

Samples were collected in two maturity stages: unripe, when the drupes were green, and semi-ripe when they were turning color or purple. Only healthy olive drupes, without any kind of infection or physical damage, were selected. The olives were handpicked in the crop year 2015. After harvesting, the olive fruits were immediately transported to a local factory (Hélder Madeira - Indústria e Comércio de Azeitonas, Unipessoal Lda., Tavira, Portugal, a local, medium-size company), where they were washed, screened (to remove any damaged fruits) and split, with a machine that makes three cuts in each olive. After splitting, the fruits were transported to the Instituto Superior de Engenharia, Universidade do Algarve, Faro, Portugal, where they were separated according to their maturation degree (unripe and semi-ripe olives). Then, the two types of olives were placed in separate fermenters and left to ferment in brines of 8% NaCl. The experiments were done in duplicate at room temperature in a pilot scale (ratio olives/brine of 8.0 kg/7.4 l). Olives were fermented for 225 days, as is usually done, based on homemade/empiric knowledge, which uses traditional processing handed down from one generation to another. On an industrial scale, as in the case of the supplier factory, the olives are usually fermented for seven to eight months period of time that the manufacturer considers adequate in achieving the desired taste to be eaten. Periodical determinations of total acidity, reducing sugars, total phenolic contents and water activity (a_W) in the olives and respective brines, were done to monitor the fermentations. Furthermore, pH and Cl concentrations in the brines and the changes on olive surface color were also measured during the fermentations. Finally, the microbiological characterization of the table olives obtained at the end of the fermentation processes was also done in order to evaluate their safety and spoilage potential.

All solvents and reagents for analysis were of a chromatographic or analytical grade.

Physicochemical analyses

Twenty randomly chosen olives were manually de-pitted, chopped in a domestic food processor and then in an Ultra-Turrax homogenizer, T25, IKA-Laborthechnik, (Staufen, Germany). The olive paste pulp obtained was immediately analyzed. The physicochemical analyses were carried out in three replicates.

The pH measurements of the brines were done using a digital Crison instrument, GLP 21 pH meter (Barcelona, Spain), at 21 °C.

The total acidity of brines, expressed as g of lactic acid/100 ml brine (%, w/v), were obtained as the sum of free and combined acidities determined by the titration method of Fernández-Díez et al. [22] with some modifications [18]: 10 ml of brine added to 50 ml of distilled water was titrated with 0.1 N NaOH, up to pH 8.2, or with 0.1 HCl, down to pH 2.6, to obtain the free or combined acidities in the brines, respectively. The total acidity of olive paste pulp, expressed as g of lactic acid/100 g olive (%, w/w), was also obtained by titration, using 5 ml of olive extract added to 25 ml of distilled water. The olive extract was previously prepared with 10 g of olive paste pulp macerated in 50 ml of distilled water at 20 °C for 30 min and then filtered using a Macherey–Nagel MN 615 (Ø 70 mm) paper filter (Düren, Germany).

The concentration of reducing sugars in brines was determined according to the method of Miller [23], using a Genesys™ 10 series spectrophotometer (Waltham, MA, USA), and expressed as g of glucose equivalent/l. An adaptation of this technique, described by Saúde et al. [18], was used to obtain the concentration of reducing sugars in the olive paste pulp, expressed as g glucose equivalent/kg.

The total phenolic content (TPC) was determined with the Folin–Ciocalteu assay, according to the procedure described by Singleton et al. [24] with some modifications, using 10 g

of olive paste pulp and 25 ml of pure methanol for total phenols extraction [18]. The brines' TPC were also determined by the same procedure using 0.1 ml of brine. TPC was expressed as g gallic acid equivalent per kg fresh olive or g gallic acid equivalent per l brine.

The water activity (a_W) of brines and olive past pulp was measured at 25 °C using a lithium chloride humidity sensor Rotronic DT Hygroskop (DMS-100H, Bassersdorf, Switzerland).

The Cl concentrations in brines were measured by titration according to Mohr´s method with 0.1 N $AgNO_3$ [25] and expressed as NaCl equivalents (%, w/v).

Surface color analysis was done on olives using a Dr. Lange Spectro-colour (Berlin, Germany) colorimeter, according to Saúde et al. [18], to describe a three-dimensional color space which is interpreted as follows: *L* indicates lightness (0 black to 100 white), *a* indicates redness (+)/greenness (−) and b indicates yellowness (+)/blueness (−) on the hue-circle. The data for each parameter are the mean average of the values from 20 randomly chosen olives. The ratio − *a*/*b* was also obtained as it reduces the variance within the samples.

Evaluation of microbiological characteristics

At the end of the fermentations, the olives were studied in relation to the microbiological safety parameters, *Salmonella* sp. and *Listeria monocytogenes*, as well as coagulase positive staphylococci. The mesophilic and psychrotrophic microorganisms, lactic acid bacteria (LAB), yeasts, filamentous fungi, coliforms, *Escherichia coli, Pseudomonas* sp., were also enumerated in the final product. *Salmonella* spp., *Listeria monocytogenes* and positive staphylococci were evaluated according to the ISO 6579:2002 (Microbiology of food and animal feeding stuffs – Horizontal methods for the detection of *Salmonella* spp.), ISO 11290 1:1996 FDAM1:2004 (Microbiology of food and animal feeding stuffs—Horizontal method for the detection and enumeration of *Listeria monocytogenes*—Part 1: Detection method.) and ISO 6888-1:1999 (Amd 1:2003) (Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species)—Part 1: Technique using Baird-Parker agar medium), respectively. The mesophilic and psychrotrophic microbial counts were performed according to the ISO 4833 (2003) (Microbiology of food and animal feeding stuffs—Horizontal methods for enumeration of microorganisms—Colony-count at 30 °C) and to the ISO 17410: 2001 (Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of psychrotrophic microorganisms), respectively. The Fungi were enumerated following the ISO 21527-1 (2008) (Microbiology—General guidance for enumeration of yeasts and molds. Colony count technique at 25 °C). The LAB were enumerated following the ISO 15214 (British Standard), 1998 (Microbiology of food and animal feeding stuffs – Horizontal methods for enumeration of mesophilic lactic acid bacteria – Colony-count at 30 °C). The total coliform and *Escherichia coli* were counted using the Chromocult Agar, (Merck, Darmstadt, Germany) and grown for 24 h at 37 °C [26]. The *Pseudomonas* sp. group was investigated using Pseudomonas agar base with CFC Supplement (Scharlau, Spain), incubated at 30 °C for 48 h.

Data analysis and models fit

Using the data obtained during the fermentations, the following kinetic models were tested:

(i) The Monod model was used to explain the changes of a_W, total acidity and TPC in the brines, *A*, (Eq. 1), where A_{max} is the maximum asymptotic value estimated by the model and *a* is the half saturation constant, i.e., the time for half the total acidity or the TPC to be obtained as a result of diffusion into the brine from the olives and, in the case of acidity, also due to the production of acids during fermentation,

$$A = A_{max}\frac{t}{a+t} \tag{1}$$

(ii) The Monod model with inhibition was used to explain the changes of reducing sugar concentrations in brines, *B*, (Eq. 2), where B_{max} is the maximum value estimated by the model, *b* is the half saturation constant for the increasing phase, due to the diffusion from the olive tissues and (*c*–*b*) is the half saturation constant for the inhibition or disappearance phase, i.e., the time needed for the reducing sugar concentrations in brines to attain half of the maximum value reached in the increasing phase as a result of the physiological activity of microorganisms.

$$B = B_{max}c\frac{t}{(b+t)(c+t)} \tag{2}$$

(iii) The inverse power function was used to explain the changes of Cl concentrations in brines, and a_W, total acidity, reducing sugars and TPC in olives, *D*, (Eq. 3), where D_0 is the initial concentration estimated by the model and *d* the power parameter,

$$D = D_0 t^{-d} \tag{3}$$

The half-life ($t_{1/2}$), calculated as follows (Eq. 4), is the time required to attain half of the initial values of Cl concentrations in brines, or initial values of a_W in olives, as a

result of salt diffusion from the brines into the olive flesh. It is also the time required to attain half of the initial values of total acidity, reducing sugars and TPC due to nutrients leaching from the olives,

$$t_{1/2} = 2^{1/d} \quad (4)$$

(iv) The Naperian logarithmic function was fitted to the data to model the changes of the color parameter $-a/b$ for the olive's surface (Eq. 5), where f and g are the models' parameters,

$$-a/b = f \ln(t) + g \quad (5)$$

The fit of the models to experimental data were carried out using the "Solver" application from Excel, version 14.0.7162.5000, from Microsoft Office Professional Plus 2010 (Faro, Portugal), by minimizing the residual sum-of-squares (ΣSQ) between the experimental data points and those estimated by the respective models and using the following options: Generalized Reduced Gradient method; 100,000 iterations, precision of 10^{-6} and 10^{-4} convergence. The kinetic constants were obtained using this non-linear regression analysis for the above-mentioned models. The coefficient of determination and the residual sum-of-squares were used to evaluate the goodness of the models fit. The higher and the lower values of these respectively parameters, the better the goodness of the fit [18, 27–30].

Results and discussion

Cobrançosa split olives in different maturation stages, green (unripe) and turning color (semi-ripe) were naturally fermented in 8% salt brines. After 225 days, the brines' pH dropped down from near neutral to 4.54 ± 0.01 or 4.33 ± 0.01, whether olives were green or turning color. The drastic decrease in brines' pH occurred mainly during the first 3 days of fermentation, after which there was only a very slight downward trend. The relative difference observed between the initial and final values of olives' pH depends on the ripeness degree, being approximately 2% (unripe) or 9% (semi-ripe). According to the Trade Standard Applying to Table Olives [31], olive fermentations should lead to brines' pH values of 4.3 or less which was only obtained for turning color table olives. The decreasing of the pH value results from the increasing of organic acids in the brines, which play an important role during fermentation, contributing to the preservation and aroma characteristic of the final product. Nevertheless, these acids in equilibrium with their salts may act as buffers limiting the decrease of the pH of the brines. This buffering ability is not desirable as it does not permit the pH values reaching reasonable levels during fermentation [32]. Although the pH in the brines had not dropped as much as would be desirable, especially in green olives, total acidity increased steadily throughout the fermentation period of green or turning color olives (Fig. 1A), reaching values of maximum asymptotic acidity of 0.87 or 0.92 (%, w/v) and half saturation constants of 2.50 or 2.30 days (Table 1), respectively. The increasing of the acidity values results from the diffusion of drupe acids and the production of acids by the microorganisms after the metabolization of reducing sugars. The lower A_{max} and higher a values obtained with unripe olives may be explained by a lower diffusion rate of organic acids from the olive pulp through the skin into the brine due to the hardness of the cell wall structures in this maturation stage [13]. A similar trend was observed for brines' acidity changes during fermentation

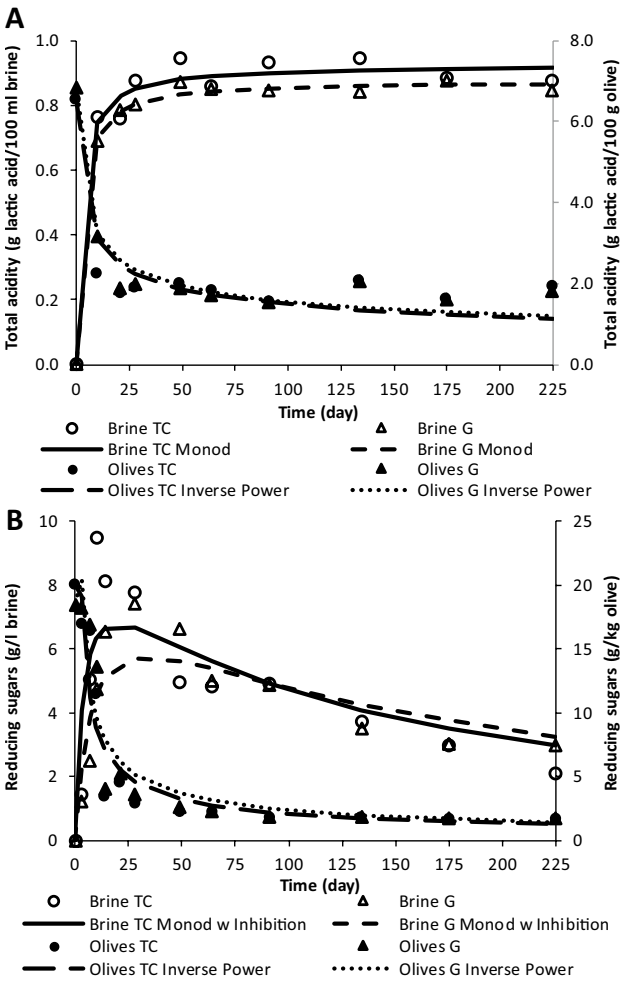

Fig. 1 Evolution of olives' and respective brines' total acidities (**A**) and reducing sugars (**B**) throughout the fermentation of split Cobrançosa olives in different ripeness degree (green, G and turning color, TC) and the kinetic models (lines) fitted to the experimental data (markers)

Table 1 Kinetic parameters estimated by the Monod model fitted to the experimental data for total acidity, total phenolic content and a_W on the brines, Monod with inhibition model fitted to the data of reducing sugars content on the brines, and, inverse power model fitted to the data of salt content on the brines fermentation of olives with different ripeness degrees (green or turning color) and the respective coefficients of determination (r)

	Model parameters	Green degree	Turning color degree
Total acidity	A_{max} (%, w/v)	0.874	0.924
	a (day)	2.50	2.39
	r	0.996	0.959
Reducing sugars content	B_{max} (g/l)	9.0	9.6
	b (day)	8.52	3.84
	c (day)	135.4	103.6
	r	0.999	0.998
Total phenolic content	A_{max}(g/l)	2.41	2.68
	a (day)	28.0	27.0
	r	0.944	0.976
a_W	A_{max}	0.955	0.958
	a (day)	0.038	0.043
	r	0.994	0.950
Salt content	D_0 (%NaCl, w/v)	4.81	4.63
	d	0.077	0.071
	r	0.988	0.999

Table 2 Kinetic parameters estimated by the inverse power model fitted to the experimental data for total acidity, reducing sugars and total phenolic contents, and a_W on the olives and Naperian logarithmic model fitted to color of the olive's surface ($-a/b$) data with different ripeness degrees (green or turning color) and the respective coefficients of determination (r)

	Model parameters	Green degree	Turning color degree
Total acidity	D_0 (%, w/w)	6.83	6.54
	d	0.32	0.32
	r	0.930	0.856
Reducing sugars content	D_0 (g/kg)	40.2	38.3
	d	0.615	0.634
	r	0.988	0.995
Total phenolic content	D_0 (g/kg)	4.31	4.03
	d	0.228	0.225
	r	0.984	0.999
a_W	D_0	0.97	0.97
	d	0.005	0.004
	r	0.840	0.886
Color	f	−0.083	−0.834
	g	0.285	2.66
	r	0.999	0.928

of Manzanilla-Aloreña cracked green table olives [33]. These authors stated that the best fit for brine acidification among several kinetics of diverse orders tested was a third order formation. However, in the present work, the evolution of total acidity in the brines was better explained by a Monod model, with the residual sum-of-squares between the experimental data points and those estimated by the respective models (ΣSQ) of 0.003 or 0.016 and the coefficients of determination (r) of 0.996 or 0.959, depending on whether olives were green or turning color. The increase of total acidity in the brines was accompanied by its decrease in the olive pulp (Fig. 1A), according to an inverse power model, with similar estimated initial values of 6.83 or 6.54 (%, w/w), whether olives were green or turning color and equal power constants of 0.322 (Table 2), corresponding to a half-life time of 8.6 days, despite the maturation olives' degree. The residual sum-of-squares between the experimental data points and those estimated by the respective models were 1.49 or 2.64 and the coefficients of determination were 0.930 or 0.856, showing a good fit of this inverse power model to the experimental data of olives' total acidity. Although the difference observed in the evolution of brines' total acidity, whether olives were green or turning color, justified by the easiness with which the diffusion of acids occurs due to the softening of the skin tissues of the semi-ripe olives compared with the unripe ones, the evolution of total acidity in the olives of both ripeness degree was nearly the same, translated by almost equal inverse power models.

While olives undergo fermentation, water-soluble constituents pass from the flesh through the skin into the processing medium. These nutrients include reducing sugars, amino acids, vitamins and minerals. Along the 225 days of split Cobrançosa olive fermentations, the changes of reducing sugars were monitored in the olive pulp and respective brines (Fig. 1B). Throughout the fermentation period, brines' reducing sugar concentrations may be explained by Monod models with substrate inhibition, as it was also observed by Saúde et al. [18] with fermentation of cracked Maçanilha Algarvia table olives. The first phase is characterized by an increase to similar maximum values of 9.00 or 9.61 g/l, with half constants of 8.52 or 3.84 days, and a second phase corresponding to a decrease, with half constants (c–b) of 126.88 or 99.76 days, depending on whether olives were green or turning color (Table 1). The half constant values estimated by the model for green olives were higher (8.52 days and 126.88 days) than those for turning color ones, which means that: (i) it takes longer to reach half of the maximum reducing sugars' concentration in the increasing phase, due to its greater diffusion difficulty from the green olive tissues into the brine, and (ii) it takes more time, in the inhibition or disappearance phase, for the reducing sugars' concentration in the brine to attain half of the maximum

value reached before as a result of the physiological activity of microorganisms. The residual sum-of-squares (ΣSQ) and the coefficients of determination (r) were 19.1 or 26.5 and 0.999 or 0.998, respectively, depending whether olives were green or turning color, showing a very good fit of this kinetic model to the experimental data.

The increase of the reducing sugars' concentration observed in the brines in the first phase, resulted from the olive tissues diffusion and their decrease in the second phase may be explained by the microorganisms' metabolism, either for growth or maintenance purposes. Simultaneously, olives' reducing sugar concentrations decreased according to an inverse power model, with similar estimated initial values of 40.2 or 38.3 g/kg, power constants of 0.615 or 0.634 and half-life time values of 3.09 or 2.98 days, whether olives were green or turning color, respectively (Table 2). A slightly higher d value, corresponding to a lower half-life time observed for turning color olives is also justified, as mentioned previously, by their skin tissue softening in this maturation stage, which facilitates the diffusion [13]. Though sugars are consumed by the microbes, at the end of fermentations they were still detected, 1.79 ± 0.01 or 1.76 ± 0.02 g/kg, whether olives were green or turning color, which may result in over-fermentations during the following shelf life period, as occurs in other table olives, especially if there are viable microorganisms in the fermentations and if the pH attained is slightly high [34, 35]. Again, a very good fit of the inverse power model to the experimental data of olives' reducing sugar concentrations was obtained, with ΣSQ and r of 63.5 or 57.8 and 0.988 or 0.995, respectively, depending whether olives were green or turning color.

During ripening, from green to black, cell walls in the olive flesh become thinner, and there is a greater cell separation because of partial solubilization of pectin, hemicellulose and cellulose polysaccharides within the cell wall matrix. This weakens the cell wall structures resulting in the softening of the olive skin and an easier diffusion of nutrients into the brine during fermentation [12, 36, 37]. On the other hand, black-ripe olives are less bitter than the green ones, because of the lower oleuropein and other polyphenols levels and overripe olives require little processing to debitter [36]. Phenolic compounds are among the most important components of olive drupes, and due to their several biological properties and effects, they could also play an important role in the prevention of chronic degenerative diseases [38]. These health effects depend on their type and amount, which is strongly influenced by the degree of ripeness of the drupes at the time of harvest [39]. The total phenolic contents of Cobrançosa olive drupes, before brining, were not significantly different whether they were unripe (3.90 ± 0.17 g/kg) or semi-ripe (4.27 ± 0.22 g/kg), which suggest *a priori* that the time for the debittering process could be nearly the same for both stages of maturation. Indeed, throughout the fermentation period, total phenolic contents of green and turning color Cobrançosa olives (Fig. 2A) decreased 64% (relative difference of TPC for both ripeness degree) according to inverse power models. Similar estimated initial values of 4.31 or 4.03 g/kg and power constants of 0.228 or 0.225, corresponding to half-life time values of 20.9 or 21.8 days were obtained, whether olives were green or turning color (Table 2). A very good fit of this model to the experimental data was obtained, as it is shown by the ΣSQ and r values of 1.48 or 3.15 and 0.984 or 0.999, respectively, depending on the ripeness degree. Simultaneously, the brines' TPC increased steadily (Fig. 2A) according to Monod models, reaching similar maximum asymptotic values of 2.41 or 2.68 g/l and half saturation constants of 28.0 or 27.0 days (Table 1), when olives were unripe or semi-ripe, respectively. The longer time to attain the half maximum content of total phenols is explained by the less maturation stage.

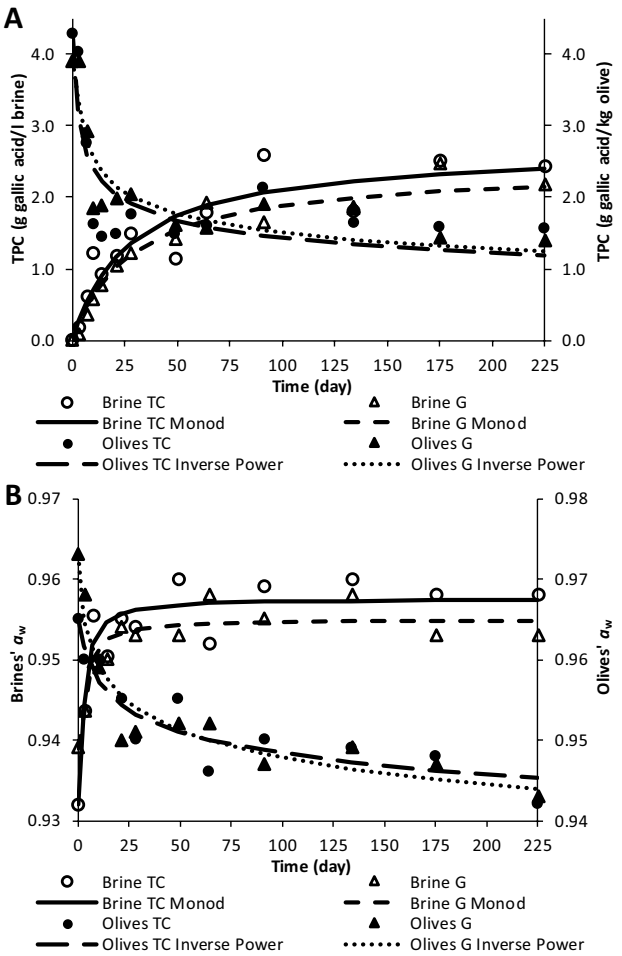

Fig. 2 Evolution of olives' and respective brines' total phenolic content, TPC (**A**) and a_W (**B**) throughout the fermentation of split Cobrançosa olives in different ripeness degree (green, G and turning color, TC) and the kinetic models (lines) fitted to the experimental data (markers)

The fit of the Monod models was very good, with residual sum-of-squares and coefficients of determination of 0.95 or 0.80 and 0.944 or 0.976, for green and turning color olives, respectively.

Throughout the olives' fermentation, salt in the brine draws water-soluble components, sugars, organic acids and minerals out of the flesh into the brine. The effective water activity of olives decreased (Fig. 2B) due to the reducing moisture and increasing salt content in the olive pulp. Reversely, brines' a_w increased due to the reduction of salt concentration in the solution and its absorption into the olive flesh (Fig. 2B), as it was also referred by Bautista-Galego et al. [33]. The changes of a_w in brines can be explained by Monod type models for both fermentation olives, whether green or turning color, with similar maximum asymptotic values of 0.955 or 0.958 and half saturation constants of 0.038 or 0.043 days (Table 1). The fit of the models was very good, as it is shown by ΣSQ and r values of 3.9E-5 or 8.3E-5 and 0.994 or 0.950, for green or turning color olives, respectively. On the other hand, inverse power models were well fitted (6.5E-5 or 7.2E-5 for ΣSQ and 0.840 or 0.886 for r) to the olives' a_w changes, with equal estimated initial values of 0.97 and power constants of 0.005 or 0.004, whether olives were green or turning color (Table 2).

When olives are debittered, fermentable substrates diffuses out of the olive flesh into the brine and salt passes into the olives. The disappearance of Cl in the brines was due to its diffusion into the olive pulp until an equilibrium was established. In fact, a sharply decrease of brines' Cl concentrations was observed in the first 7 days (more than half of the total relative decrease), followed by a very slight reduction in the remaining fermentation period (Fig. 3), from initial values of 5.71 ± 0.01 or 5.47 ± 0.07%NaCl equivalents, to final values of 3.29 ± 0.02 or 3.19 ± 0.04%NaCl equivalents, for green or turning color olives', respectively. The progress of the Cl concentrations in both brines are explained by a kinetic model of an inverse power type with nearly the same parameter values for both ripeness degree. The estimated initial Cl concentrations were 4.81 or 4.63% NaCl equivalents and the power parameters 0.077 or 0.071 (Table 1), corresponding to half-life time values of 7832 or 18,369 days, depending whether olives were green or turning color. These very high $t_{1/2}$ values are justifiable because after the day 134 quasi-equilibrium states were achieved between the Cl concentration of olives and their brines. A very good fit of this model to the experimental data is shown by the ΣSQ and r values of 0.693 or 0.397 and 0.988 or 0.999, for green or turning color olives, respectively.

The color of the olive's surface gives a measure of their quality, so it is commonly monitored during fermentation. According to the results, this parameter changed during fermentation, probably due to the chlorophylls and carotenoid pigments being transformed and oxidation of polyphenol compounds. In fact, table olives were considerably different from fresh ones, as they became less green (higher a values) during fermentation. The ratio $-a/b$ changed along fermentation (Fig. 4) showing a decreasing trend, explained by the Naperian logarithmic model, with the parameter values of -0.083 or -0.834 and 0.285 or 2.66 for f and g, respectively

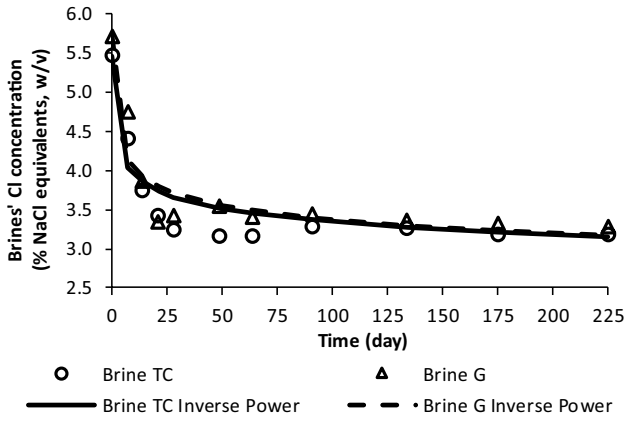

Fig. 3 Brine´s Cl concentration (% NaCl equivalents, w/v) throughout the fermentation of split Cobrançosa olives in different ripeness degree (green, G and turning color, TC) and the kinetic models (lines) fitted to the experimental data (markers)

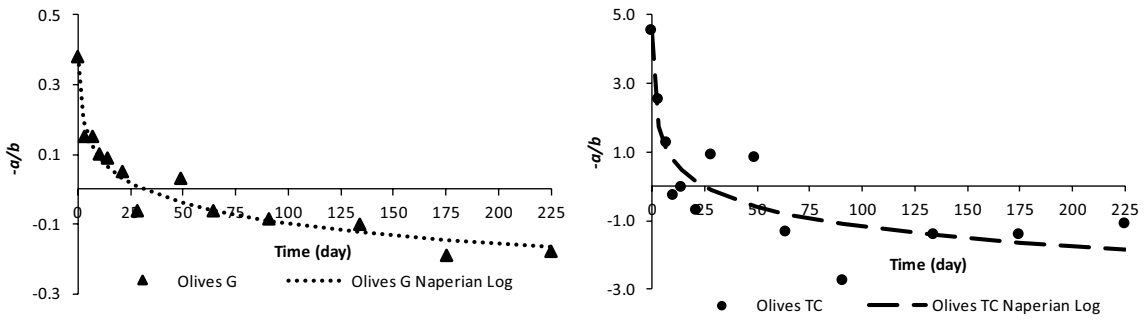

Fig. 4 Color parameter ($-a/b$) of olive surface of split Cobrançosa cultivar in different ripeness degree (green, G and turning color, TC) throughout the fermentation and the kinetic models (lines) fitted to the experimental data (markers)

(Table 2), depending on whether olives were green or turning color. The residual sum-of-squares, ΣSQ were 0.016 or 9.44 and the coefficients of determination, *r*, were 0.999 or 0.928, showing a very good fit of this kinetic model to the experimental data. This tendency was also observed by Bautista-Gallego et al. [40] in fermented cracked Aloreña olives and Saúde et al. [18] with cracked Maçanilha Algarvia olives. These observations, namely the loss of the green color ($-a/b \geq 0$ for fresh olives to $-a/b \leq 0$ for all fermented olives) is due to the chlorophyll degradation and the appearance of brown pigments during the fermentation process [36].

The microbial stability and safety of table olives is only guaranteed if appropriate pH and acidity are attained [31]. In the case of the fermentations described in the present study, the slightly high pH values and the residual sugars detected in brines at the end of fermentation of Cobrançosa table olives, either green or turning color olives, may result in non-stable final products during the following shelf life period resulting from the growth of viable microorganisms. At the same time pathogenic microorganisms may find conditions to survive and growth. This way, at the end of the fermentations, the olives produced were characterized in relation to various microbial parameters. The predominant microorganisms in the olives were enumerated in the group of aerobic mesophilic and fungi, namely yeasts, while filamentous fungi were not detected. The levels found of aerobic mesophilic and yeasts were 6.5 and 6.7 Log CFU/g in both, green and turning color, table olives, respectively. These values were within the limits expected for fermented foods and defined in the Trade Standard Applying to Table Olives [31] and were in the range of those described by Mateus et al. [41] and in the review of Arroyo-López et al. [35]. Regarding the other microbial groups investigated, psychrotrophic microorganisms, BAL, coliforms, pseudomonas, coagulase positive staphylococci, *E. coli, Salmonella* sp., and *L. monocytogenes*, were not found either in the green or turning color olives produced. According to the European Commission Regulation [42], both maturation state table olives obtained met the microbiological criteria for food safety. The occurrence of mesophilic microorganisms and yeasts, especially when reducing sugars are present and the pH is slightly high, is a warning that the product may be spoiled resulting in gas pockets, swollen containers, leakage of brines, cloudy brines, off-flavors, off-odors and softening, indicating a non-stable product with a limited shelf life [33, 35]. In these situations, the application of pH regulators and/or the development of any alternative preservation strategy is usually recommended.

The elaboration of table olives by natural processing generally takes a long time, which is one of the reasons why table olive industries do not produce them on a very large scale. Although the splitting may quicken the osmotic changes between the flesh and the surrounding medium, olives from the Cobrançosa cultivar were left to ferment during 225 days, which is quite long. The rate of leaching decreased as time progressed but most of the nutrient compounds passed into the brine during the first fifteen days. For all the studied parameters in the olive flesh, a steady-state was achieved after the 175th day, with no changes until the end of fermentation. This behavior suggests that, at this time, the olives already have the characteristics suitable for consumption, both physicochemical and organoleptic. The processing time for olives' fermentation is also dependent on the maturation stage and, supposedly, green olives can take up more time to debitter than turning color olives. However, there was no difference in the fermentation profile of the green or turning color olives, probably because the olives were previously split, a process that promotes the diffusion of the nutrients during the fermentation. Whether they were green or turning color olives, the type of kinetic model adjusted to the experimental data of each physicochemical parameter studied on olives and on the correspondent brines was the same, which shows a similar tendency in the alterations that occurred in both olive fermentations in the two maturation stages. The ripeness degree of the olives affects the diffusion rate of nutrients due to the epidermis of the fruits, which acts as a barrier, namely through the unripe olive skin. This phenomenon was mainly observed in the alteration of the physicochemical parameters in the fermentation brine mentioned above. characterized by a typical bitter taste.

Conclusions

Split green and turning color table olives of the Cobrançosa cultivar are "natural olives" that ferment due to their own microbiota as well as the environments. This results in final brines with the required acidity values according to the Trade Standard. Although there are several studies on table olive fermentation and the effect of their ripeness degree on their composition, this work focuses on studying and modeling the progression of some physicochemical parameters in the olive pulp as well as their brines in two maturation stages throughout the fermentation period. Splitting the olives favored the solubilization of organic acids, sugars and phenolic compounds and their accumulation in the brines of green and turning color olives. In both olives, the various kinetic models adjusted to the experimental data were the same, showing a similar trend in the physicochemical profiles during the processing fermentations. However, different values of model parameters were estimated, depending on the ripeness degree, namely for total acidity, reducing sugars and total phenolic contents of the brines. In both table olives, at the end of fermentation, the conditions reached assured

the absence of coliforms, *E. coli, Salmonella* and *L. monocytogenes*. A better knowledge of the fermentation profile will allow the industry to improve the processing preparations of table olives and the reduction in the time needed for the debittering process.

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