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**Processing of table olives with KOH and characterization of the wastewaters as
potential fertilizer**

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Abstract

In Mediterranean countries the table olive industry produces a huge volume of wastewaters that are phytotoxic due to their high sodium content. Olives intended for Spanish-style green olives are currently debittered with NaOH that generates lyes and washing waters that cannot be used for agronomic purposes. In this study, the substitution of NaOH with KOH during the debittering of Manzanilla and Hojiblanca cultivars was assessed as well as the vacuum evaporation of the olive wastewaters to comply with fertilizer requirements. Typical lactic acid fermentation occurred in brines of olives treated with KOH and a final product with similar color and flavor characteristics to those treated with NaOH was achieved. However, lower texture was found in olives debittered with KOH than those with NaOH, using them at the same molar concentration. Furthermore, the lyes and washing waters from the KOH treatment were concentrated up to 10% of their initial volume and they complied with Spanish requirements to be considered as organo-mineral fertilizers ($C_{\text{organic}} > 4\%$, $K_2O > 2\%$, $N_{\text{total}} + K_2O > 6\%$) although an external source of nitrogen would be needed. They also had a high content in phenolic compounds, particularly hydroxytyrosol. This research demonstrates that table olives can be processed with KOH and the generated waste streams could have potential applications in agriculture or being a source of bioactive substances.

Keywords: wastewater, table olive, fertilizer, bioactive, KOH

1. Introduction

Table olives (*Olea europaea* L.) are a food product, esteemed throughout the world, that are mainly produced in the Mediterranean area. Among the different types of commercial table olives, the Spanish-style green olives are the most popular. Fruits freshly picked from the tree cannot be eaten due to the presence of the bitter glucoside oleuropein, so olives are submitted to an alkaline treatment (lye) with NaOH to hydrolyze this substance (Brenes and De Castro, 1998). The alkali deactivates the enzymes polyphenoloxidase and β -glucosidase involved in the final color of the product and the formation of anti-lactic acid compounds in brines respectively (Ramírez et al., 2015; Medina et al., 2009). In addition, the lye affects the firmness of the olives to a large extent, causing the degradation and solubilization of the cell wall components, particularly the loss of pectic polysaccharide involved in cellular adhesion (Marsilio, Lanza and De Angelis, 1996; Mafra, Barros and Coimbra, 2006). After the alkali treatment, the fruit are subsequently washed for several hours in tap water and put in brine, where a lactic acid fermentation takes place over several months to yield a product with a pleasing yellow color and a characteristic flavor (Montaño et al., 2003; Lucena-Pradós et al., 2014).

The main drawback arising from alkaline treatment is the wastewaters accumulated in the olive factories from the lyes and the washing solutions (Papadaki and Mantzouridou, 2016), which are heavily polluted and difficult to handle (Brenes et al., 2000). Nowadays, they are a problem not only due to the high cost of treatment but also because in many cases treatment is either non-existent or ineffective. They are generated between September and November, due to the seasonally olive harvesting, and most of the olive factories discharge them into big open air evaporation ponds,

which is a temporary solution. The reuse of these alkaline solutions has also been proposed but the processors concerns have limited its application.

Despite organic contamination, the spent lyes and washing waters have a high concentration of sodium that causes high conductivity and limits their discharge into public streams, as well as restricts their use for agronomic purposes. Several studies have proposed the treatment of spent vegetable waste with KOH to valorize the residue as biofertilizer (Zhu et al., 2013; Jaffar et al., 2015). Hence, the substitution of NaOH with KOH during the debittering of Spanish-style green olives could allow the use of the wastewaters as organic fertilizers. It is worth noting that wastes from the olive oil extraction process (wastewaters mill and olive pomace) have been assessed for agricultural purpose (Koutsos, Chatzistathis and Balampekou, 2018; Muscolo et al., 2019).

To date, there has only been one study on the bioremediation of processing wastewaters from green olives treated with NaOH and KOH but the effect of KOH debittering on the sensory and physicochemical characteristics of the fermented product has not been reported (Lasaridi et al., 2010), despite it being permitted as coadyuvant for the elaboration of table olives (IOC, 2004). Additionally, it must be taken into account that the presence of potassium ions in olives may cause bitterness (Panagou et al., 2011; Ambra et al., 2017).

Likewise, the alkaline and subsequent washing steps give rise to a high loss of bioactive substances in olives such as phenolic compounds and triterpenic acids that diffuse from the fruit to the wastewaters (Romero et al., 2004; Romero et al., 2010; Alexandraki et al., 2014). The former have been attributed with antioxidant activity and the latter with anticancer activity (EC, 2012), among other beneficial properties for

human health (Lozano-Mena et al., 2014). Precisely, there are many recent reports about the recovery of these bioactive substances from olive by-products (Gullón et al., 2018; Nunes et al., 2018).

For these reasons, the purpose of this study was to evaluate the use of KOH during the debittering of Spanish-style green olives instead of NaOH. The characterization of vacuum concentrated solutions from the generated wastewaters was also assessed as potential organic fertilizers or a source of bioactive substances.

2. Materials and methods

2.1 Olive processing

Olives of the Manzanilla and Hojiblanca cultivar with a green-yellow color on the surface were supplied by a farmer cooperative located in Lora de Estepa (Seville, Spain). On arrival to the laboratory, olives were sorted in order to use those with uniform size. Five kilograms of Manzanilla fruit were put into 8.5 L cylindrical polyethylene vessels and covered with 3.4 L of 0.47 M NaOH (19 g/L) or KOH solution (26 g/L) until it penetrated two-thirds of the way to the pit of the olives, which was visually determined by cutting 10-20 fruits. Hojiblanca fruit were treated with 0.52 M NaOH (21 g/L) or KOH solutions (29 g/L). In all cases the duration of the alkaline treatment was around 7 hours, which is the currently time employed in most of the table olive factories to elaborate this product. Subsequently, olives were washed with tap water for seven hours and then covered with 100 g/L NaCl solution. Two identical vessels were prepared for each cultivar and treatment. Starter cultures were applied in order to assure the necessary lactic acid fermentation. According to the pH values at the end of the first week, different strategies were applied. Manzanilla brines were directly

inoculated on day 8 with *Lactobacillus pentosus* LP99, whereas a sequential inoculation, firstly with *Enterococcus casseliflavus* cc45 on day 7 and then *L. pentosus* LP99 the day after, was applied for Hojiblanca brines (De Castro et al., 2002). Both homofermentative LAB strains are held by our culture collection and were originally isolated from green olive brines. The reason for this difference between olive cultivars is the greatest fermentation difficulty of Hojiblanca olives. *Enterococcus casseliflavus* can stand and grow at the high initial pH values, favouring the subsequent *L. pentosus* development. Both starter species produce lactic acid as their main metabolic product. The preparation of inoculations was as follows. For propagation from stocks maintained at -80°C, the strains were cultivated twice in BHI (enterococci) or MRS (lactobacilli) broths, the second time with 45 g/L NaCl to allow adaptation to the saline environment. After incubation at 32°C for 48 hours, cultures were centrifuged, washed with saline (9 g/L NaCl), and the washed pellets were resuspended in brine from the corresponding fermenters. For enterococci, the initial population inoculated was ca. 8.45×10^6 cfu/mL into the Hojiblanca brines (Log 6.93), and for lactobacilli ca. 9.50×10^6 cfu/mL into the Hojiblanca and Manzanilla brines (Log 6.98).

2.2 Microbiological analysis

The viable and culturable populations of lactic acid bacteria (LAB), and yeast and molds were determined by plating the brines and their decimal dilutions (in 9 g/L NaCl) with a Spiral Plater (Don Whitley Sci. Ltd., Shipley, England). The culture media used were de Man, Rogosa, Sharpe MRS agar (Biokar diagnostics, Beauvais, France) with and without 0.2 g/L sodium azide (Sigma-Aldrich), and oxytetracycline-glucose-yeast extract (Oxoid Ltd., Basingstoke, England) agar for the aforementioned groups,

respectively. MRS plates were incubated under anaerobic conditions (AnaeroGen, Oxoid). Plates were incubated at 32°C for up to five days, and the numbers of colony forming units were counted with a Scan 500 (Interscience, St Nom la Bretèche, France) colony counter.

2.3 Sugars and organic acid analyses

Sugars in brines (glucose, fructose, sucrose and mannitol) were analyzed by HPLC. Samples were put into contact with resins Amberlite IR-120 and Amberlite IRA-93, shaken occasionally for 30 min, and the centrifuged solution was injected (20µL) into the chromatograph. A Rezex RCM-Monosaccharide Ca⁺ column was used and deionized water as eluent (De Castro et al., 2007). The major end-products of fermentation (lactic acid, acetic acid and ethanol) were analyzed by HPLC using a C18 column and deionized water (pH adjusted to 2.2 using concentrated H₃PO₄) as the mobile phase (De Castro et al., 2007).

2.4 Chemical characteristics of brines

The concentration of NaCl was analyzed by titration with a 0.86 N silver nitrate solution, using a potassium chromate solution as indicator. The pH, titratable acidity and combined acidity of brines were measured using a Metrohm 670 Titro Processor (Herisau, Switzerland). Titratable acidity was determined by titrating to pH 8.3 with 0.2 M NaOH and expressed as lactic acid. Combined acidity was measured by titration to pH 2.6 with 2 M HCl and expressed as eq/L (De Castro et al., 2002).

2.5 Assessment of olive quality

The color of olives was measured using a BYK-Gardner model 9000 Color-view spectrophotometer, equipped with computer software to calculate the CIE L^* (lightness), a^* (redness), and b^* (yellowness) parameters. Interference by stray light was minimized by covering samples with a box that had a matte black interior. The data from each measurement is the average of 20 olives (Ramírez et al., 2015).

The firmness of fruit was determined using a Kramer shear compression cell coupled to a Texture Analyzer TA.TX plus (Stable Microsystems, Godalming, UK). The crosshead speed was 200 mm/min. Firmness was the mean of 10 replicate measurements, each of which was performed on three pitted olives, and expressed as Newton/100 g pitted olives (De Castro et al., 2007).

Sensory characteristics of olives were tested according to the “Method for sensory analysis of table olives” in the normalized testing room of the Instituto de la Grasa (IOC, 2011). A total of 10 panelists (6 men and 4 women) participated in the study. They were recruited because of their participation in the habitual Instituto de la Grasa sensory analysis of table olives for decades and their role in the implementation of the IOC method. The IOC method uses descriptors related to the perception of negative sensations to classify olives commercially, particularly for “abnormal flavor”. Gustatory attributes (salty, bitter, acidic) and kinesthetic sensations (hardness, fibrousness, crunchiness) were also assessed. The statistic used to indicate the values of the attributes was the mean of the individual data of the 10 testers and the variability by robust standard deviation.

2.6 Phenolic compounds and triterpenic acids in olives

The extraction of phenolic compounds from the olive pulp was based on the methodology proposed elsewhere using dimethyl sulfoxide (DMSO) and syringic acid as the internal standard (Ramírez et al., 2015). Separation and quantification of each compound was carried out by HPLC by using a Spherisorb ODS-2 column, an elution gradient with acidified water and methanol, a flow rate of 1 mL/min and a temperature of 35°C. Chromatograms were recorded at 280 nm. The analysis of these substances in the liquids was made by mixing 0.25 mL of the liquid, 0.5 mL of deionized water and 0.25 mL of the internal standard. The mixture (20 µL) was filtered and injected into the chromatograph.

Triterpenic acids in olive pulp were analyzed following the procedure described elsewhere (Romero et al., 2010). One gram of dried olive was mixed in a 10 mL centrifuge tube with 4 mL of methanol/ethanol (1:1, v/v) and vortexed for one minute, centrifuged at 6000 g for five minutes at 20°C, and the solvent was separated from the solid phase. This step was repeated six times, and the pooled solvent extract was vacuum evaporated. The residue was dissolved in 4 mL of methanol, filtered and injected into the chromatograph. The column and the solvents were the same as used for the phenolic compounds determination. Separation of the peaks was achieved under isocratic conditions with the flow rate of 0.8 mL/min, and the compounds were monitored at 210 nm.

2.7 Minerals

Sodium and potassium content in liquids was determined by flame photometry. Solutions were diluted with deionized water in an automatic diluter (LIC Instruments Model 346, Sweden) to obtain a concentration lower than 200 meq/L of sodium or

potassium. Subsequently, the solutions were introduced into the flame photometer (Metheor model NAK-1, PACISA, Spain). The apparatus was calibrated with Na and K atomic absorption standards solutions (Aldrich Chem Comp, Milwaukee, USA) with the same dilution as the samples, adjusting the reading of apparatus to standard concentrations divided by 10.

Calcium and magnesium content in liquids was analyzed by atomic absorption. Solutions were diluted in the previously mentioned diluter with a lanthanum solution ($5000 \mu\text{g mL}^{-1}$) to remove the interferences. Solutions were introduced into a GBC model 932 AA (Victoria, Australia) atomic absorption spectrometer equipped with a hollow (Ca, Mg, Cu, and Zn) multi-element cathode lamp (Photometry, Victoria Australia).

Carbon and total nitrogen were analyzed by elemental analysis, using a LECO CHNS-932 analyzer (St. Joseph, MI, USA). Previously, the samples were dried at 105°C and the moisture was calculated.

2.8 Vacuum evaporation of lyes and washing solutions

The spent lyes and washwaters obtained from the alkaline treatment with KOH of Manzanilla and Hojiblanca olives were concentrated by evaporation in a rotary evaporator (Büchi Rotavapor model RE-114) under vacuum at 65°C . A 1:1 mixture of lye and washwater was also evaporated. All solutions were concentrated to 10% of their initial volume, and the pH dropped to 4 units by adding HNO_3 (65%, w/w). The chemical oxygen demand of the solutions (COD) and the total solids (TS) were determined according to standard procedures (APHA, 1998), the density of the liquids

was carried at 20°C with a 0.1 L volumetric flask, and the viscosity analyzed with a viscometer Ostwald at 20°C.

2.9 Statistical analyses

Statistical comparisons of the mean values for each experiment were carried out by one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test ($p < 0.05$) using SPSS software v. 23.0 (IBM Corp., Armonk, NY).

3. Results and discussion

The time needed for the alkaline solutions to reach two-thirds of the way to the pit of the Manzanilla and Hojiblanca olives was around seven hours, regardless of the type of alkali employed, NaOH or KOH. This was expected because the molecular concentration of the two alkalis was the same, but the content expressed as g/L of NaOH and KOH was obviously very different, being higher for the latter alkali. The combined acidity of the fermentation brines of the olives treated with NaOH and KOH was very similar, around 0.10 and 0.12 N for Manzanilla and Hojiblanca brines respectively after 2 months of brining, which also confirmed that the fruits were subjected to the same molecular alkali strength.

Similarly to processing in factories, fruits were washed after the alkaline treatment and put in brine to undergo lactic acid fermentation. LAB starters were applied in order to encourage the fermentation, and the evolution of microorganisms in the olive brines is depicted in Fig. 1. Growth of LAB was always above that of yeasts, which is expected for this type of table olive (De Castro et al., 2002). LAB counts, which

obviously include enterococci and lactobacilli, whether natural or inoculated, reached quantities between 10^8 and 10^9 cfu/mL for long enough to achieve a prompt and efficient fermentation in all cases (Table 1). Since lactic acid is the main metabolic product of the two LAB starters inoculated. Otherwise, yeast growth was around 10^4 and 10^5 cfu/mL most of the time, which is a normal result. Differences between treatments could hardly be made. Although higher yeast counts were observed in NaOH treated olives, and perhaps higher LAB in KOH treated olives, the differences are not significant and would require further assays. The evolution of free acidity and pH (Table 1) confirmed the rapid acidification of the brines that reached pH values lower than 4.5 before 15 days in the case of Manzanilla and between 23 and 43 days of fermentation in the case of Hojiblanca olives. Accordingly, free acidity was higher than 0.70% after 90 days of fermentation and pH below 4.2 units in all cases. This data, together with the LAB growth, indicated that the debittering of olives with a KOH solution gave rise to a typical lactic acid fermentation of Spanish-style green olives of both Manzanilla and Hojiblanca cultivars.

The color and texture are two of the main quality attributes of Spanish-style green olives. As recorded in Table 2, the use of KOH instead of NaOH did not affect the superficial color of the Manzanilla and Hojiblanca olives. None of the color parameters (L^* , a^* and b^*) were significantly different between Manzanilla olives treated either with NaOH or KOH, and the same results were found for Hojiblanca fruits, except the a^* parameter that was higher in Hojiblanca olives treated with KOH than NaOH. As expected (Ramírez et al., 2015), the lightness (L^*) and the b^* parameter were higher in Manzanilla than Hojiblanca fruits, but it is a cultivar dependent factor.

In contrast, the texture of the fruits was affected by the type of alkali employed during the debittering step (Table 2). For the same molar concentration, the KOH

solution led to significantly lower firmness in olives of both Hojiblanca and Manzanilla cultivars than those treated with NaOH. The alkali treatment is a crucial step for the final firmness of fermented Spanish-style green olives since the cell wall is degraded by the loss of polysaccharides (Marsilio et al., 1996), particularly pectic substances involved in cellular adhesion, and a decrease in olive firmness is achieved (Brenes, García and Garrido, 1994). It must be noted that most fermented olives are currently destoned in factories before packing, and they have to maintain sufficient firmness to be successful during this destoning step. Hence, the loss of texture during debittering of olives with KOH is a handicap for the use of this alkali instead of NaOH, which must be a matter of investigation in the future.

Consumers are demanding healthier food products rich in bioactive substances, such as those present in table olives: phenolic compounds and triterpenic acids, which are lost to a large extent during the processing of Spanish-style green olives. The concentration of both phenolic compounds and triterpenic acids in fermented olives of the Manzanilla and Hojiblanca cultivars was not significantly influenced by the type of alkali used during the debittering step (Table 2) despite a higher content of phenolic compounds were extracted from the pulp of the olives treated with KOH than NaOH (Table 4). It must also be stressed that the bitter glucoside oleuropein was not found in any of the fermented olives, and hydroxytyrosol was the major phenolic compound in olive pulp, so a complete debittering process was achieved.

Regarding minerals, the high concentration of sodium in fermented olives proceeded mainly from the NaCl solution in which lactic acid fermentation took place, thereby it was the same for all treatments. By contrast, olives treated with KOH retained a higher concentration of potassium in the pulp after fermentation than those debittered with NaOH, irrespective of the olive cultivar (Table 2). This residual content of

potassium could be a problem from a sensory point of view due to the bitter sensation that this metal may impart to olives (Panagou et al., 2011). However, this bitter sensation was not detected by panellists in olives treated with KOH (Table 3), probably due to the low content of the metal in the fermented olives. Abnormal fermentation or other defects were not found in any of the fermented olives, and the acid and salty sensations were equilibrated. Moreover, panellists confirmed the lower texture in olives treated with KOH than NaOH, regardless of the cultivar: hardness, fibrousness and crunchiness were scored lower in the former than the latter olives.

With regard to the wastewaters, the lyes and the washing waters of olives treated with NaOH had a high concentration of sodium that may limit their use for agronomic purposes (Table 4). Around 4000-4840 mg/L and 2430-2480 mg/L of sodium were found in the lyes and washing waters respectively of olives of the Manzanilla and Hojiblanca cultivars. Due to the high concentration of potassium in raw olives, the residual lyes and washing waters of olives treated with NaOH were also enriched by around 1200-2740 mg/L of potassium. By contrast, the solutions from Manzanilla and Hojiblanca olives treated with KOH only had 50-130 mg/L of sodium, whereas they contained around 9600-11520 mg/L and 5280-6040 mg/L of potassium in the lyes and washing waters respectively. In addition to minerals, these solutions also have a high concentration in sugars and phenolic compounds. Glucose and mannitol were the main sugars found in both the lyes and the washing waters, the solutions from the KOH treatment being in general more concentrated in these substances than those from NaOH, particularly glucose (Table 4). The same trend was observed for phenolic compounds, particularly hydroxytyrosol, whose content ranged between 1186-3523 mg/L and 1790-2733 mg/L in the lyes and washing waters respectively so that they can be considered a good source of these bioactive substances if comparing with other olive

extracts (Nunes et al., 2018; Gullón et al., 2018). The high organic contamination originated elevated COD values that ranged between 20000 and 40000 mg/L, higher in the lyes from olives debittered with KOH than with NaOH. These findings suggest that the KOH treatment was more intense than the NaOH, even when using the same molecular concentration, which is in agreement with the lower texture found in olives treated with the potassium than with the sodium alkali.

The lyes and washwaters obtained from the alkaline treatment of olives with KOH were concentrated by evaporation up to 10% of their initial volume to produce a solution able to be used as fertilizer. According to Spanish legislation for fertilizer production (RD, 2013), a liquid organo-mineral fertilizer NK must contain 6% $N_{\text{total}} + K_2O$, 2% N_{total} , 1% N_{organic} , 2% K_2O , and 4% C_{organic} . From the data presented in Table 5, it can be deduced that the concentrated lyes, washing waters and the mixture of both complied with some of the legal requirements. The potassium and carbon content in these solutions was even much higher than the limits. Moreover, the pH of these concentrated solutions was very alkaline, and an external addition of nitric acid to drop this parameter by up to 4 units was studied. It was clear that 100, 50 and 80 mL of nitric acid was needed (65% w/w) per liter of concentrated lye, washing water and the mixture respectively to reach pH 4, but it was insufficient to reach the 2 % total nitrogen required by law due to the low content of organic nitrogen in these solutions, which can be deduced from the difference between N_{total} and $N_{\text{inorganic}}$ (Table 5). Therefore, the concentrated solutions must be spiked with an external source of organic nitrogen or olive processing must be modified in order to comply with current fertilizer legislation. It must also be noted that these concentrated solutions had a high content of total solids that led to high density and viscosity, although they could flow as a liquid.

4. Conclusions

The results indicated that the substitution of NaOH with KOH during the debittering step did not influence the lactic acid fermentation, and the final products had a similar color and flavor regardless of the alkali used. By contrast, the texture of olives treated with KOH was of lower quality than those debittered with NaOH, even though a similar molar concentration was used in both cases. Therefore, it is necessary to study in depth the strength of the KOH alkali that is able to debitter olives and at the same time gives rise to a final product with similar physico-chemical characteristics to the traditional version. Otherwise, the lyes and washing waters generated during the new process were shown to be a good source of phenolic compounds, particularly hydroxytyrosol. Moreover, these solutions were concentrated to 10% of their initial volume and they could comply with Spanish requirements to be used as organo-mineral fertilizer, although some external addition of organic nitrogen could be necessary.

Overall, the lyes and washing waters represent a big environmental and economic problem for the table olive factories that currently discharge them into evaporation ponds at a relatively low cost. However, public administrations are demanding for a permanent solution that will require a very high cost. The results of this study revealed that processing of Spanish-style green olives with KOH is possible although the loss in firmness of olives must be solved. In addition, the higher concentration of KOH needed as well as the higher cost of this alkali than that of NaOH must be taken into consideration together with the use of nitric acid to comply with fertilizer product requirements. Hence, an economic balance must be done before NaOH is replaced by KOH.

Notes

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Figure captions

Fig. 1. Growth of lactic acid bacteria and yeasts in the fermenting brines of (A) Manzanilla and (B) Hojiblanca olives. Arrows mean the dates when the starters were inoculated. Points are the means of duplicate fermenters. Where error bars (range of data) are not visible, determinations were within the symbols on the graph. $\diamond$ LAB in NaOH treated; $\square$ LAB in KOH treated; Δ yeasts in NaOH treated; $\circ$ yeasts in KOH treated olives.

Table 1

Evolution of pH and free acidity in brines of Spanish-style green olives debittered with NaOH and KOH.

	Manzanilla cultivar		Hojiblanca cultivar	
	NaOH	KOH	NaOH	KOH
<i>pH</i>				
7 days	6.53 (0.11) ^a	6.81 (0.12)	8.20 (0.15)	8.82 (0.25)
15 days	4.27 (0.08)	4.27 (0.11)	5.23 (0.13)	5.09 (0.08)
23 days	4.21 (0.04)	4.23 (0.06)	5.00 (0.11)	4.85 (0.04)
43 days	4.09 (0.10)	4.03 (0.03)	4.34 (0.04)	4.33 (0.11)
90 days	4.06 (0.10)	3.96 (0.01)	4.14 (0.01)	4.17 (0.01)
<i>Free acidity</i> (g lactic acid/100 mL)				
7 days	0.09 (0.00)	0.07 (0.00)	nd ^b	nd
15 days	0.51 (0.06)	0.52 (0.04)	0.23 (0.03)	0.23 (0.03)
23 days	0.60 (0.04)	0.57 (0.04)	0.27 (0.04)	0.28 (0.04)
43 days	0.77 (0.08)	0.76 (0.04)	0.64 (0.04)	0.66 (0.13)
90 days	0.77 (0.09)	0.79 (0.06)	0.82 (0.01)	0.75 (0.01)

^aValues are the mean of duplicates and the standard deviation is shown in parenthesis. ^bnot detected.

Table 2

Physicochemical characteristic of olives debittered with NaOH and KOH after 90 days of fermentation.

	Manzanilla cultivar		Hojiblanca cultivar	
	NaOH	KOH	NaOH	KOH
Color				
<i>L</i> *	58.4±0.8a ^y	58.8±0.1a	50.8±0a	51.4±0.1a
<i>a</i> *	2.3±0.2a	2.6±0.6a	1.3±0a	2.1±0.2b
<i>b</i> *	40.1±0.7a	40.0±1.0a	31.6±2.0a	33.4±1.0a
Firmness (N/100 g pitted fruit)				
	2133±196a	1482±20b	3347±328a	2544±11b
Triterpenics (mg/kg)				
Maslinic acid	393±68a	463±49a	918±161a	949±66a
Oleanolic acid	321±41a	285±49a	688±69a	713±2a
Phenolic compounds (mg/kg)				
Hydroxytyrosol	1052±33a	865±13b	1079±40a	1064±4a
Others ^x	718±220a	514±232a	616±53a	646±239a
Total polyphenols	1770±186a	1379±219a	1695±13a	1711±235a
Minerals (mg/kg)				
Na	15262±157a	15070±219a	15154±1120a	14732±622a
K	228±3a	557±25b	226±4a	678±14b

^xHydroxytyrosol-4-glucoside, tyrosol, verbascoside, rutin, comselogoside and luteolin.

^yFor each cultivar, column values followed by the same letter (a, b) do not differ at the 5% level of significance according to Duncan's multiple-range test.

Table 3

Sensory evaluation of olives debittered with NaOH and KOH after 90 days of fermentation.

	Manzanilla cultivar		Hojiblanca cultivar	
	NaOH	KOH	NaOH	KOH
Abnormal fermentation	1.0 (0.1) ^a	1.1 (0.1)	1.0 (0.1)	1.0 (0.1)
Other defects	1.2 (0.1)	1.1 (0.2)	1.0 (0.1)	1.0 (0.1)
Salty	5.7 (0.4)	5.7 (0.3)	6.6 (0.4)	6.4 (0.5)
Bitter	5.4 (0.8)	5.7 (0.7)	4.6 (0.4)	4.6 (0.6)
Acid	5.4 (0.7)	5.6 (0.6)	5.6 (0.8)	5.8 (0.8)
Hardness	5.4 (0.5)	4.2 (0.5)	7.1 (0.5)	5.8 (0.2)
Fibrousness	4.7 (0.6)	3.3 (0.3)	6.7 (0.6)	5.3 (0.2)
Crunchiness	5.2 (0.5)	4.2 (0.5)	6.1 (0.5)	5.3 (0.3)

^aValues are the median score of ten panelists and the robust standard deviation is shown in parenthesis according to the IOC sensory analysis method (IOC, 2011).

Table 4

Chemical characteristics of the residual alkali and washing water solutions used for the debittering of olives with NaOH and KOH.

	Manzanilla cultivar				Hojiblanca cultivar			
	Residual alkali		Washing water		Residual alkali		Washing water	
	NaOH	KOH	NaOH	KOH	NaOH	KOH	NaOH	KOH
Minerals (mg/L)								
Na	4840±113a	130±14b	2480±10a	100±2b	4000±170a	70±42b	2430±14a	50±14b
K	2740±141a	11520±85b	1740±57a	5280±113b	1780±10a	9600±170b	1260±85a	6040±170b
Ca	86±3a	64±6b	29±5a	27±3a	56±4a	65±7b	37±8a	37±4a
Mg	34±6a	31±11a	27±2a	25±1a	25±5a	25±6a	29±2a	33±5a
Sugars (mg/L)								
Glucose	3190±243a	4120±402b	4444±361a	5079±438b	1794±96a	3207±253b	4304±308a	4609±245a
Mannitol	1665±52a	1602±205a	1989±127a	1957±133a	3066±184a	4477±307b	4025±226a	4377±149b
Total sugars	7193±323a	8476±814b	9596±679a	10273±654a	6033±297a	9489±637b	9949±606a	10895±469b
Polyphenols (mg/L)								
Hydroxytyrosol	2588±67a	3523±45b	2491±82a	2733±74b	1186±99a	1991±196b	1790±190a	2454±23b
Others ^x	403±76a	425±17a	279±17a	274±23a	92±6a	164±13b	187±37a	250±27b
Total polyphenols	2991±142a	3948±35b	2770±92a	3007±95b	1278±105a	2155±210b	1977±227a	2704±47b
Acetic acid (mg/L)	294±37a	324±17a	239±17a	243±12a	286±18a	445±50b	403±50a	448±16a
Ethanol (mg/L)	187±21a	181±15a	275±49a	220±17a	142±15a	114±8a	225±23a	127±7b
COD (mg/L)	32099±135	39887±272b	42230±964a	43682±1634a	19961±1665	31046±3041	42890±1388	42626±998
	2a				a	b	a	

^x Hydroxytyrosol glycol, salidroside, tyrosol, caffeic and *p*-coumaric acids, verbascoside, luteolin-7-glucoside, rutin, and comselogside.^y For each cultivar and waste solution, column values followed by the same letter (a, b) do not differ at the 5% level of significance according to Duncan's multiple-range test.

Table 5

Physicochemical parameters of the concentrated solutions obtained from the evaporation of lyes, washing waters and a mixture 1:1 of them. These solutions were generated from the treatment of olives with KOH and they were concentrated to 10% of their initial volume.

	Manzanilla cultivar			Hojiblanca cultivar		
	Lye	Washing water	Mixture 1:1	Lye	Washing water	Mixture 1:1
pH	12.0±1.2 ^a	9.6±0.1	10.7±0.1	13.5±0.1	9.9±0.1	11.0±0.2
Density (g/mL)	1.22±0.01	1.15±0.01	1.19±0.01	1.16±0.01	1.15±0.01	1.16±0.01
Viscosity (g/m · s)	6.3±0.1	4.7±1.1	5.8±0.7	3.2±0.9	9.2±0.7	7.2±2.5
Total solids (g/kg)	372±2	293±2	348±1	348±1	301±1	314±1
K (g/kg)	80±7	39±4	60±1	59±7	39±3	60±1
C _{total} (g/kg)	129±7	95±1	111±1	101±8	102±1	90±6
N _{total} (g/kg)	16±3	11±0	13±0	15±1	10±1	14±2
N _{inorganic} (g/kg) ^b	13±0	6±0	11±0	12±1	7±0	11±1

^aValues are the mean of duplicates ± standard deviation. ^bCalculated from the HNO₃ needed to reach pH 4.