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Progress on green table olive processing with KOH and wastewaters reuse for agricultural purposes

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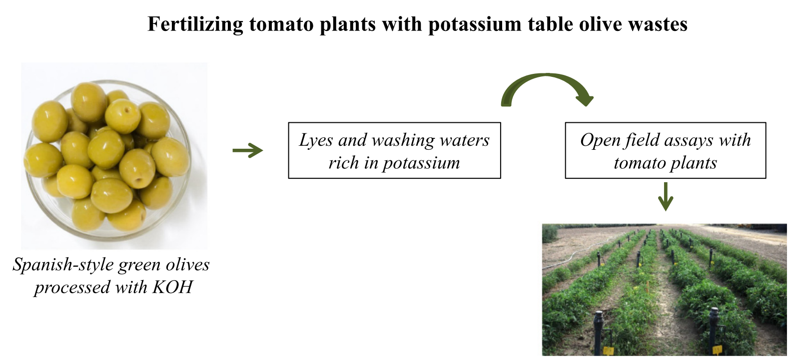
HIGHLIGHTS

- NaOH can be substituted by KOH for processing of green olives.
- Good olive texture can be achieved using low concentration of KOH.
- Potassium waste solutions had more than 50 g/L of this mineral.
- Tomato yield and quality was similar by using potassium olive waste and KNO_3.

GRAPHICAL ABSTRACT



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ABSTRACT

Table olive wastewaters are seriously polluting and a difficult to treat effluent worldwide, mainly due to their high content in sodium. An alternative approach could be the treatment of the olives with KOH instead of NaOH, in order to reuse the olive streams as biofertilizers. In this study, the debittering of olives with KOH was investigated at pilot plant scale in two olive seasons. The results indicated that a concentration between 1.7 and 2.0% of KOH (similar to that employed with NaOH) led to a fermented product with the same physicochemical and organoleptic characteristics than the traditional one. The spent lyes and washing waters from the KOH treatments were gathered and vacuum evaporated, giving rise to a concentrated solution rich in potassium (52 g/L) that was tested as biofertilizer in open tomato fields. Furthermore, the drip irrigation of the tomato plants with a combined olive solution and mineral fertilizer (NH_4NO_3) produced similar tomato yield and quality than the irrigation with only mineral fertilizer ($NH_4NO_3 + KNO_3$). Overall, it has been demonstrated that Spanish-style green olives can be processed with KOH and the effluents valorized to be used as biofertilizer.

1. Introduction

The two main food products derived from olives, olive oil and table olives, are of great economic importance for many countries, mainly in the Mediterranean basin. Nevertheless, solid and liquid wastes generated during processing of these foods make their production unsustainable in the future (Papadaki and Mantzouridou, 2016). A myriad of methods, patents, and studies have been carried out to manage this problem; although low-cost, feasible and robust technology is still necessary. Among them, the application of the olive wastes for agricultural purposes has been investigated extensively, particularly in olive oil wastes (Koutsos et al., 2018; Muscolo et al., 2019) despite the toxic

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effect of these by-products for plants found on many occasions (Sofiadou and Tzortzakis, 2012; García-Sánchez et al., 2014).

In the case of olive oil wastes, researchers have tried to solve their toxicity for plants by composting them alone or combined with other materials (Sánchez-Monedero et al., 2019; Brunetti et al., 2019), although the fertilization of the soil with high doses of these composts is not recommended (Ceglie et al., 2011; Killi and Kadvir, 2013). Moreover, olive oil wastewaters have shown growth inhibition against plant pathogens (Disciglio et al., 2018) and weeds (Vagelas et al., 2009), an antimicrobial activity that has also been attributed to table olive wastewaters (Medina et al., 2011).

The limiting factor for the agricultural application of table olive wastewaters is their high content in sodium, a proceed from the NaCl and NaOH used during olive processing (Murillo et al., 2000). However, salt-free effluents from the elaboration of this product such as the washing waters from the processing of Spanish-style green olives and the preservation solutions of olives derived from black olives have not shown any toxic effect when used for the growth of several Mediterranean food plants such as tomato and pepper cultivated in greenhouse conditions (De los Santos et al., 2019, 2020. Recently, the substitution of NaOH by KOH during the alkaline treatment of green and black olives has been researched with the aim of providing potassium rich effluents (García-Serrano et al., 2019, 2020). Nevertheless, Spanish-style green olives debittered with NaOH and KOH, at the same molecular concentration, showed lower texture in the case of the latter alkali (lye), which is a drawback for the industrial implementation of this new technology (García-Serrano et al., 2019). Consequently, the objective of this work was to investigate the fertilization of tomato plants under open field conditions with alkaline effluents from the processing with KOH of Spanish-style green olives. However, trials were previously carried out to optimize the use of KOH instead of NaOH in order to get fermented olives with similar characteristics than the traditional ones.

2. Materials and methods

2.1. Olive processing and experimental design

Olives of the "Manzanilla" and "Hojiblanca" cultivars with a greenish-yellow color on the surface were supplied by a farming cooperative located in Lora de Estepa (Seville, Spain) during the 2018/2019 and 2019/2020 seasons. On arrival to the laboratory, olives were sorted in order to use those more uniform in size. Five kilograms of fruit were put into 8.5 L cylindrical polyethylene vessels and covered with 3.4 L of the different alkali (Table 1) until it penetrated two-thirds of the way to the pit of the olives, which was visually determined by cutting 10–20 fruits. In all cases the duration of the alkaline treatment was around 7 ± 0.5 h, which is the current time to elaborate this product employed in most of the table olive factories. Subsequently, olives were washed with tap water for 7 h and then covered with 100 g/L NaCl solution. Two identical vessels were prepared for each cultivar and treatment. In some cases, the alkali solution was previously refrigerated (15–18 °C) for the debittering stage.

2.2. 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.

2.3. 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^* (lighteness), 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 with a Texture Analyzer TA.XT 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).

The 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 for decades in the habitual Instituto de la Grasa sensory analysis of table olives, 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 the robust standard deviation.

2.4. Minerals

For the determination of sodium and potassium in liquids and olives, 1 mL or 1 g was digested with HNO_3 at 120 °C during 8 h in a DigiPREP (Canada). Then, 5 mL of $HClO_4/HNO_3$ (4/1) solution was added and HNO_3 evaporated at 140 °C for 3 h. The solution was put into a 25 mL graduated flask with deionized water. They were diluted with deionized water in an automatic diluter (LIC Instruments Model 346, Sweden) to

Table 1
Experimental design for the alkali treatment of olives.

	Treatment			
	A	B	C	D
Season 2018/2019				
"Manzanilla" cultivar	NaOH 1.7%[a] (25 ± 1 °C)[b]	KOH 2.0% (25 ± 1 °C)	KOH 2.4% (25 ± 1 °C)	KOH 2.4% (18 ± 1 °C)
"Hojiblanca" cultivar	NaOH 2.0% (25 ± 1 °C)	KOH 2.4% (25 ± 1 °C)	KOH 2.8% (25 ± 1 °C)	KOH 2.8% (18 ± 1 °C)
Season 2019/2020				
"Manzanilla" cultivar	NaOH 1.9% (23 ± 1 °C)	NaOH 1.9% (15 ± 1 °C)	KOH 1.9% (23 ± 1 °C)	KOH 1.9% (15 ± 1 °C)

[a] Concentration is expressed as w/v.
[b] Initial temperature of the lye.

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.

Nitrogen was 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.

2.5. Vacuum evaporation of lyes and washing solutions

The mixture of spent lyes and washwaters obtained from the alkaline treatment with KOH of Hojiblanca olives from the 2018/2019 season were concentrated by evaporation in a rotary evaporator (Büchi Rotavapor model RE-114) in a vacuum at 60 °C. This alkaline mixture was concentrated to 10% of their initial volume, and the pH dropped from 13.1 to 5.7 units by adding HNO_3 (65%, w/w). The concentrated solution had 52 g/L of K and 15 g/L of N. This concentrated solution was employed for the fertilization experiments commented below.

2.6. Open field assays with tomato plants

The field experiment was conducted in an open field located at the IFAPA Las Torres Center (Alcalá del Río, Seville, Spain, 37°30′ N, 5°58′ W). The soil used for the experiment was divided into 4.5 × 1.2 m plots, each separated by a 0.5 m buffer zone. Plots were distributed in a completely randomized block design with four replications for each treatment, for a total of 16 plots. Fertilization was performed by drip irrigation every fifteen days; the first treatment being 15 days after transplanting, and a total of 5 fertilization treatments were carried out. The recommended fertilization made by local farmers was 210 g NH_4NO_3 (34% N) + 36 g KNO_3 (13% N, 46% K_2O) per plot and irrigation. It must be noted that NH_4NO_3 was substituted by 210 g of $(NH4)_2SO4$ (21% N) per plot for the last three fertilization stages. The treatments were: (i) Control, fertilization with NH_4NO_3 + KNO_3 at recommended quantities; (ii) olive solution, each plot was fertilized with 30 mL of the concentrate described above (K, 52 g/L; N, 15 g/L); (iii) olive solution + NH_4NO_3, 30 mL of the olive solution + 210 g NH_4NO_3; and (iv) water, only tap water without any fertilizer. The variety of tomato used in the experiments was 'Malpika'. Five weeks tomato seedlings were transplanted into the plots in rows spaced at 1.2 m, with plants spaced at 0.3 m along each single row. The transplanting was performed mid-May, and the tomatoes were harvested twice in August.

Before transplanting, weed control was carried out with herbicide containing the active ingredient glyphosate. At the end of June, *Tuta absoluta* damage was detected on tomato plants, so they were treated with *Bacillus thuringiensis*, following recommendations on the label.

2.7. Tomatoes analyses

For each plot, 5 fruits from 3 plants were taken during the two harvesting cycles in August and analyzed. Fruit size (equatorial and longitudinal diameter) and firmness (shore) were evaluated. The pH, sweetness (°Brix) and acidity (% of citric acid) were measured in tomato puree (100 g) (De los Santos et al., 2020).

The yield of each plot was measured taking into consideration the two harvesting cycles carried out in August. Fruits per plant and grams per plant of marketable fruits were recorded.

2.8. Statistical analyses

Statistical comparisons of the mean values for each experiment were carried out by one-way analysis of variance (ANOVA) using SPSS software v. 23.0 (IBM Corp., Armonk, NY). In the sensory analysis, differences were considered significant when confidence intervals of the medians ($P < 0.05$) did not overlap.

3. Results and discussion

3.1. Assays to process Spanish-style green olives with KOH

The duration of the alkaline stage was rather similar (c.a. 7 h) for all the treatments assayed during the 2018/2019 season despite the alkali employed, and a retard of half an hour occurred during the 2019/2020 season for the treatments with colder initial NaOH and KOH (Table 1).

Moreover, the pH and free acidity in all the fermentation brines of the 2018/2019 season followed a similar trend (Fig. 1), the pH drastically decreased after 20 days, achieving a final value below 4.3. In parallel, the production of lactic acid due to the growth of lactic acid bacteria, gave rise to a high increase of the free acidity during the first 20 days of brining. Thereafter, it continued up to reach values above 0.7% at 70 days of fermentation. Similar results were obtained during the 2019/2020 season. Therefore, the alkaline treatment of the olives with KOH and the residual potassium in the brines, did not affect the course of the spontaneous lactic acid fermentation, as has also been found in a previous work (García-Serrano et al., 2019). In fact, it has been reported that Spanish-style green olives can be fermented with a very high concentration of KCl without any problem detected, up to 4% KCl has been employed to substitute NaCl (Zinno et al., 2017; Mantzouridou et al., 2020).

As was expected, none of the KOH treatments led to significant differences in the color of the fermented olives, in comparison with NaOH treatments (Table 2) (García-Serrano et al., 2019), confirmed for both the "Manzanilla" and "Hojiblanca" cultivars during the two evaluated seasons.

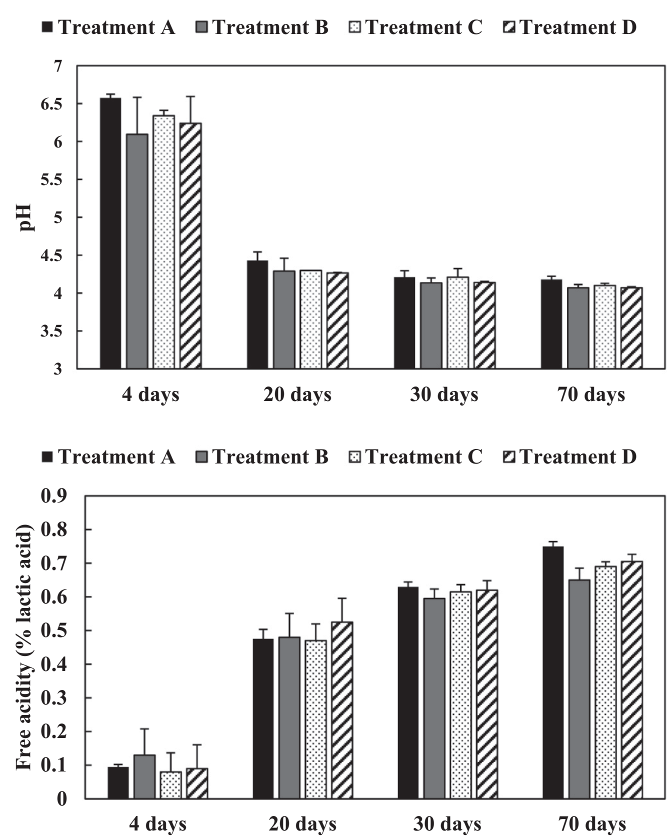


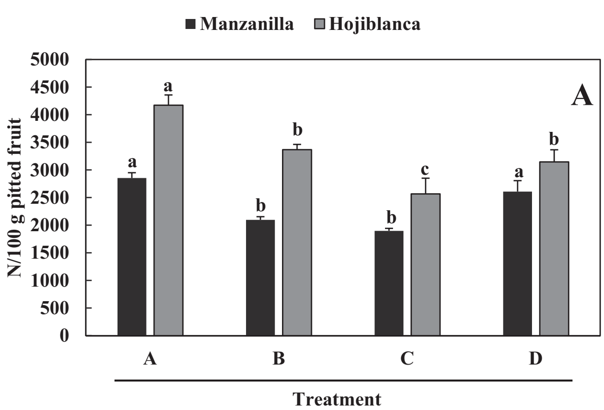
Fig. 1. Evolution of the pH and free acidity in brines during fermentation of "Manzanilla" olives from the 2018/2019 season.

Table 2
Influence of the type of alkali treatment on the color of fermented olives from the 2018/2019 season.

	Color parameters		
	*L**	*a**	*b**
"Manzanilla" cultivar			
Treatment A	56.6 (0.7)a	2.7 (0.1)a	36.1 (0.1)b
Treatment B	55.8 (0.5)a	2.7 (0.3)a	39.8 (1.4)a
Treatment C	55.0 (0.1)a	3.0 (0.2)a	36.9 (0.5)b
Treatment D	55.3 (1.3)a	3.3 (0.3)a	39.0 (0.3)a
"Hojiblanca" cultivar			
Treatment A	51.8 (0.2)a	3.4 (0.2)a	33.1 (0.5)a
Treatment B	51.2 (1.2)a	3.3 (0.1)a	34.5 (2.3)a
Treatment C	50.8 (0.8)a	3.2 (0.2)a	33.7 (1.4)a
Treatment D	50.3 (0.5)a	3.0 (0.4)a	33.3 (0.5)a

Note. For each cultivar, values followed with different letter in the same column means significant difference according to Duncan's test ($P < 0.05$).

The main drawback found in the previous work on the use of KOH for the processing of Spanish-style green olives, was the softer olives found when treated with the same molecular concentration than those with NaOH (García-Serrano et al., 2019). It must be noted that the lye treatment is the key stage for the final firmness of the fermented olives, because cell wall undergoes degradation mainly due to loss of pectic substances and hemicellulosic polysaccharides (Mafra et al., 2007). In the first olive season studied (2018/2019), fruits of the "Manzanilla" cultivar were treated with 0.425 M of NaOH (1.7%, w/v) and KOH (2.4%, w/v), but also with a lower concentration of the latter alkali (2%, w/v) at ambient temperature (25 °C) or 0.425 M of KOH at 18 °C



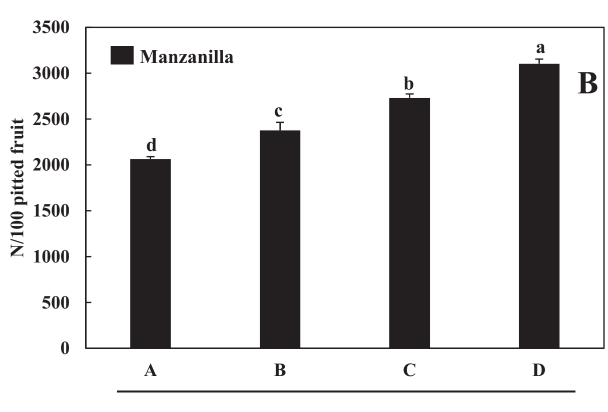


Fig. 2. Influence of the type of alkali treatment on the firmness of fermented olives. A, season 2018/2019; B, season 2019/2020. See Table 1 for treatment characteristics. For each cultivar, bars with the same letter do not differ at the 5% level of significance according to Duncan's multiple-range test.

Table 3
Content in sodium and potassium (mg/kg) in the pulp of fermented olives of the 2018/2019 season.

Treatment	"Manzanilla cultivar"		"Hojiblanca cultivar"	
	Na	K	Na	K
A	13500a	341c	14250a	306c
B	12450a	2719b	14650a	3688b
C	12650a	3313a	14800a	3844a
D	12660a	3625a	14200a	4094a

For each cultivar, values followed with different letter in the same column means significant difference according to Duncan's test ($P < 0.05$).

(Table 1). The treatment of olives with KOH at similar molar concentration than NaOH, gave rise to softer olives (Fig. 2A) and slightly better texture with lower KOH concentration (1.7%). In contrast, the use of cold lye (0.425 M KOH at 18 °C) led to olives with similar texture than those treated with NaOH at room temperature (23–25 °C), and this trend was also observed for the assays with olives of the "Hojiblanca" cultivar (Fig. 2A). Processing of Spanish-style green olives with cold lye has previously been studied for the reduction of skin sloughing in the fermented fruit (Jaramillo et al., 2011), and it has been adopted by many Spanish table olive factories in their elaboration methods.

In the second season studied, the concentration (1.9%, w/v) of both alkalis was the same, and the initial cold temperature of the lye tested was even lower than the first season (15 °C). In this case, the texture of the olives treated with KOH at room temperature was significantly higher than those fruits debittered with NaOH (Fig. 2B). Again, the use of cold lye led to higher texture despite the alkali employed, thereby the highest firmness was achieved with olives treated with KOH solution initially refrigerated to 15 °C. It must be remembered that the debittering stage with KOH and the further fermentation process occurred without any remarkable drawback. In addition, the color and the texture of the olives was similar to the commercial product (Sánchez-Gómez et al., 2013).

It is well-known that potassium may impart bitterness on fermented vegetables, in particular table olives (Panagou et al., 2011; Ambra et al., 2017), but this negative effect has been found at concentrations of this mineral higher than 2–3% in the fruit. Table 3 shows the content of Na and K in the pulp of fermented olives of the 2018/2019 season. Firstly, the rather similar and high concentration of sodium in all olives due to the fermentation of the product in a brine with initial NaCl of 10% (w/v) must be noticed. Secondly, the residual potassium in the pulp of the olives treated with KOH ranged between 2719 and 4094 mg/kg, a much lower amount than reported by researchers to produce bitterness in olives (Panagou et al., 2011). Obviously, most of the residual potassium after the lye treatment was lost in the further washing water and fermentation brine stages. In fact, this bitter sensation was not detected by panelists in "Manzanilla" and "Hojiblanca" olives treated with KOH, in comparison with those treated with NaOH (Table 4), as it was also

Table 4
Sensory evaluation of olives debittered with NaOH and KOH after 120 days of fermentation. Olives were of the 2018/2019 season, and it was compared fruit from treatments A and D (Table 1).

	"Manzanilla" cultivar		"Hojiblanca" cultivar	
	NaOH	KOH	NaOH	KOH
Abnormal fermentation	1.0 (0.1)[a]	1.0 (0.1)	1.0 (0.2)	1.0 (0.1)
Other defects	1.0 (0.2)	1.0 (0.1)	1.0 (0.0)	1.0 (0.0)
Salty	6.6 (0.5)	6.1 (0.6)	6.0 (0.8)	7.3 (0.7)
Bitter	4.3 (0.7)	4.6 (1.3)	3.6 (0.5)	3.3 (1.1)
Acid	5.9 (0.6)	6.5 (0.8)	6.8 (1.0)	6.5 (0.8)
Hardness	5.3 (0.5)	5.2 (0.4)	7.9 (0.2)	7.9 (0.6)
Fibrousness	4.6 (0.5)	4.4 (0.6)	7.8 (0.5)	6.9 (0.2)
Crunchiness	5.1 (0.6)	3.9 (0.5)	6.9 (0.9)	5.8 (1.3)

[a] Values 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).

observed in a previous work (García-Serrano et al., 2019). Abnormal fermentation or other defects were not found in any of the fermented olives, and the acid and salty sensations were balanced. Hardness was similar in "Manzanilla" olives treated with NaOH and KOH but higher in "Hojiblanca" cultivar treated with NaOH, which is contradictory with data reflected in Fig. 2. It is worthy of mention that the "Hojiblanca" cultivar is characterized by a fibrous texture.

3.2. Open field assays with tomato plants

Data on the quantity and quality of tomato fruits obtained from the open field assay is shown in Table 5. First it is noteworthy to say, that the drip irrigation with the concentrated olive solution did not exert any toxic effect on tomato plants at the dose tested, which is in agreement with previous studies conducted under greenhouse conditions and salt-free table olive solutions (De los Santos et al., 2019, 2020). Although the table olive wastewaters studied in this work are different than those generated during the olive oil processing, many components of the olive fruit are the same in both olive wastes. Thus, the importance of the absence of phytotoxic effects on tomato plants treated with table olive solution was paramount, given the toxicity reported by olive mill wastewaters, not only during germination and seedling but also during the whole stages of the tomato plant development (Ouzounidou et al., 2008).

As can be expected, the yield of tomato increased significantly in plants irrigated with inorganic fertilizer in comparison with only tap water, and the latter treatment presented similar results than irrigation with olive solution (Table 5). However, the combined use of the olive solution with NH_4NO_3 produced similar number of fruit/plant and grams/plant than the application of only inorganic fertilizer (NH_4NO_3 + KNO_3). It has been indicated that organic waste application as a substitute of conventional mineral fertilization is sometimes problematic, because some crops have nutrient needs throughout their growth cycle, which for example make the combined use of the organic compost with a nitrogen mineral fertilizer necessary, in order to meet tomato plant N needs (Hernández et al., 2014). In this study, KNO_3 was substituted by the olive solution and the results were similar despite the olive solution supplied being 8.8 times lower in the amount of potassium than the mineral KNO_3.

With respect to the tomato quality, fruit fertilized with NH_4NO_3 + KNO_3 and NH_4NO_3 + olive solution had a significantly lower ratio between the longitudinal and equatorial diameter, than those irrigated with only tap water or olive solution (Table 5). Hardness was also significantly lower for tomatoes from the two former treatments in comparison to the two latter. In contrast, tomato fruit from the tap water and olive solution treatments, had significantly lower total soluble solids (°Brix) and titratable acidity than those irrigated with mineral fertilizer and olive solution + N mineral fertilizer. Hence, it can be asserted that the organoleptic quality of the tomatoes obtained with the combined use of olive solution + NH_4NO_3 was similar to that achieved with only mineral fertilizer (NH_4NO_3 + KNO_3). Studies on the amendments of soil with olive oil waste composts did not find differences on tomato quality with respect to mineral fertilization (Hernández et al., 2014; Brunetti et al., 2019). Although the table olive concentrates were applied in this work by drip irrigation and they were free of sodium, it could be recommended in the future to study the effect of their use on the soil.

Table 5
Influence of the type of fertilization on the production and quality of tomato.

	Type of fertilization			
	NH_4NO_3 + KNO_3	Olive solution	Olive solution + NH_4NO_3	Water
Production data				
Fruit per plant	27.0a	14.8b	27.5a	14.6b
Grams per plant	2282a	1419b	2360a	1352b
Tomato quality				
Size (mm/mm) longitudinal/equatorial	1.94b	2.05a	1.99ab	2.03a
Hardness (shore)	65.5b	68.4a	66.1b	68.5a
° Brix	4.2a	3.7b	4.1a	3.7b
pH	4.20a	4.28a	4.22a	4.24a
Titratable acidity (%)	0.43a	0.33b	0.39a	0.32b

Values followed with different letter in the same row means significant difference according to Duncan's test ($P < 0.05$).

4. Conclusions

Processing of Spanish-style green olives with KOH can be performed successfully by using a concentration with similar percentage (%, w/v) than that currently employed with NaOH, and an even better texture is obtained if cold alkali is used during the debittering stage. The quality of the final product and the fermentation process was the same with both alkalis tested, KOH and the traditional NaOH. In addition, the effluents generated (lyes and washing waters) were rich in potassium, so a vacuum concentrate of these solutions can be used as biofertilizer. Thus, the combined application of this olive waste concentrate with mineral fertilizer (NH_4NO_3) during the irrigation of tomato plants in an open field assay, provided similar yield and fruit quality than the fertilization with only mineral NH_4NO_3 + KNO_3. Consequently, these findings open the possibility of reusing the wastewaters produced during the elaboration of table olives for agricultural purposes, thereby alleviating the environmental problem that they represent for the table olive factories worldwide.

Notes

The authors declare no competing financial interest.

CRediT authorship contribution statement

Pedro García-Serrano: Methodology, Investigation, Formal analysis. **Berta de los Santos:** Methodology, Investigation, Formal analysis. **Antonio H. Sánchez:** Methodology, Investigation, Formal analysis. **Concepción Romero:** Methodology, Investigation, Formal analysis. **Ana Aguado:** Methodology, Investigation, Formal analysis. **Pedro García-García:** Methodology, Investigation, Formal analysis. **Manuel Brenes:** Methodology, Investigation, Formal analysis, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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