Studii şi Cercetări Ştiinţifice
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

2019, **20** (1), pp. 021 – 028

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry

ISSN 1582-540X

ORIGINAL RESEARCH PAPER

EFFECT OF SODIUM HYDROXIDE CONCENTRATION, TEMPERATURE AND FRUIT SIZE ON THE PROCESSING TIME OF SEVILLIAN-STYLE GREEN TABLE OLIVES (*OLEA EUROPAEA* L.)

Enrique de Florio Ramírez[1], Omar Bellido-Valencia[2*], Luis A. Medina-Marroquín[2], Aníbal Vásquez-Chicata[2]

[1]*Universidad Nacional Jorge Basadre Grohmann, School of Food Industry Engineering, Avenida Miraflores s/n, Tacna, Peru*
[2]*Universidad Nacional de San Agustín de Arequipa, Department of Food Industry Engineering, Av. Independencia s/n, Arequipa, Peru*

*Corresponding author: obellido@unsa.edu.pe

Received: April, 16, 2018
Accepted: February, 05, 2019

Abstract: Sevillian-style green table olives are one of the main products derived from the olive (*Olea europaea* L.) tree on the market today. The way they are prepared involves the use of a sodium hydroxide solution to reduce the concentration of oleuropein, reducing the original bitter taste of the fruit. In this study we investigate the effect of hydroxide concentration, process temperature and fruit size on the time required to complete the process. Using a complete factorial design, these factors were evaluated at two levels. The shortest treatment time (180 min) was obtained using the highest levels of sodium hydroxide concentration and temperature (15 $g \cdot L^{-1}$ NaOH and 25 °C). The interaction of these two factors was significant, unlike the size of the fruits, which was not significant for the two levels tested (240 and 150 $units \cdot kg^{-1}$).

Keywords: *full factorial design, sevillana olive, sodium hydroxyde, tacna, penetration time*

INTRODUCTION

Table olives (*Olea europaea* L.) are a traditional product of the Mediterranean basin, with a great influence on the culture and diet of the countries of this area. The Phoenicians cultivated the olive tree in Greece and introduced it in Spain around 1050 BC, from where it spread to America [1]. There are more than 2500 olive cultivars, of which only a small amount have commercial value, that is, those that due to their characteristics can be used for the production of oil (oil content and ease of oil removal) and table olives (ratio of flesh to stone from 5 : 1 to 8 : 1, firmness and ease of removal of the stone), with some varieties having a dual purpose [2].

At the end of 2016, Peru was the third country in the Americas, after Argentina and the USA, in terms of table olive production (2.17 % of world production) [3]. The average olive production between 2012 and 2016 in Peru was 79361 metric tons. The southern region of Tacna is the main national producer (71 % of national production, followed by the Arequipa region with 21 %), and also has the highest yield (5360 $kg \cdot Ha^{-1}$) above the national average (4984 $kg \cdot Ha^{-1}$) [4]. However, in the same period only 5 % of the olives (4259 MT on average) entered a plant to produce olive oil [5], which indicates that most of the olives are destined for the production of table olives. Thus, it is reported that the average annual production (2012 - 2016) of table olives in Peru was 69 thousand metric tons [3]. Only 1.9 % of the olive production produced in the period 2012 - 2016 was exported, and only 0.03 % of the same olive production was exported as virgin olive oil [6].

The most economically important olive cultivar in the Tacna region, as well as in northern Chile [7], is the "*Sevillana*" olive, used in this area mainly for the production of table olives. Table olives can be prepared by four methods [2]: spontaneous fermentation (such as Greek-style black olives), processes for obtaining Greek-style Kalamon olives, oxidation treatment with lye (such as Californian-style black olives), and treatment with lye followed by fermentation (such as Spanish-style green olives).

In the "*Sevillano*" process of preparing green table olives, olives that are ripe green (green to yellow green in color [8]) are washed with potable water, sorted by size and treated with lye (debittering), then rinsed with potable water several times to remove excess lye and placed in a brine (NaCl, 8 to 10 %) to ferment the olives before they are sorted again, packed and pasteurized [2]. In the debittering process, a solution of lye (13 to 50 $g \cdot L^{-1}$ [1, 2, 8 – 11], alternatively 2.9 Baumé degrees (°Bé) [12]) is used for the time necessary for the lye to penetrate to 2/3 of the olive pulp [8, 10]. The treatment time is usually between 6 to 12 hours [11 – 13]. Debittering rapidly reduces the concentration of oleuropein in the fruit from approximately 11.6 (raw fruit) to 3.4 $g \cdot kg^{-1}$ [12]. The reduction of oleuropein through its hydrolysis into elenolic acid and hydroxytyrosol is the main objective of this process [1].

In recent years, technologies have been researched to replace all or part of the lye in the de-bittering process using biotechnological techniques [14], ultrasound [15, 16], vacuum impregnation [17] and scratching [18]. Although they have different advantages, it appears that the ultrasound process would have better prospects for reducing the consumption of NaOH, which is nevertheless necessary. Optimizing the use of NaOH is important to improve process efficiency.

The aim of this study is to determine the effect of the most important parameters (sodium hydroxide concentration, temperature) and the size of the fruit on the time required to complete the alkaline treatment used in the production of Sevillian-style green table olives.

MATERIALS AND METHODS

Raw material and preparation

The Tacna and Ilo valleys (latitude 17 - 18 °S) have an annual minimum temperature of 13.8 °C and a maximum of 23 °C. Annual rainfall is 26.2 mm [19]. Samples of olive fruit (yellow to green) from the "*Sevillano*" variety, from the Tacna region (Universidad Nacional Jorge Basadre Grohmann, INPREX germoplasm bank) were collected and transferred to the Food Technology laboratories of the Universidad Nacional Jorge Basadre Grohmann. Once received, the fruits were classified to discard those showing signs of damage, cuts or defects.

The fruits were washed with tap water and drained before being separated by size. Two groups were formed depending on the amount of fruit needed to complete one kilogram: 240 units ($U \cdot kg^{-1}$) and 150 units ($U \cdot kg^{-1}$).

Alkaline treatment (debittering process)

Alkaline treatment was performed at two levels of sodium hydroxide concentration (1 and 2 °Bé, measured with a Baumé densimeter, then converted to 6.0 and 15.0 $g \cdot L^{-1}$ NaOH, respectively [20]) and two levels of temperature (15 and 25 °C). Samples of fruits (300 g) from the two size groups were placed in plastic containers (1 L) containing NaOH solutions at the indicated temperatures using water baths. Temperature control is important because it has been reported that there is an increase in temperature in the containers with the lye solution during debittering, mainly due to alkaline hydrolysis reactions [21].

Hydroxide penetration

To verify the progress of the alkaline treatment, samples were taken from each tank and the fruits were sliced to observe the progress visually using a solution of phenolphthalein [13]. When NaOH reaches 2/3 of the distance between the skin and the stone fruit, the treatment was considered complete [22] and then time (in minutes) was recorded.

Experimental design

A full factorial design of three factors at two levels was used (Table 1). The runs were randomized prior to sample preparation and four measurements (repeated measurements) were performed for each run.

Table 1. *Experimental design with real values (levels)*

Run	Temperature [°C]	Concentration NaOH [$g \cdot L^{-1}$]	Size [$U \cdot kg^{-1}$]
1	15	6	240
2	25	6	240
3	15	15	240
4	25	15	240
5	15	6	150
6	25	6	150
7	15	15	150
8	25	15	150

Statistical analysis

The analysis of variance (ANOVA) and graphs were obtained using the version 3.4.3 of the R programming language and environment for statistical computation [23], the "*car*", "*agricolae*" and "*dae*" packages [24 – 26] and the version 1.1.442 of the Integrated Development Environment (IDE) RStudio [27] were also used.

RESULTS AND DISCUSSION

Interaction between temperature, sodium hydroxide concentration and fruit size

The interaction between the three factors is shown in Figure 1. The temperature and the concentration of the NaOH solution are seen to have the most important role, as the measurements obtained from the two size levels are very close to each other. On the other hand, the size of the fruits had very limited effect on the processing time.

Normally the olives are unbittered at a temperature between 15 and 25 °C, the concentration of NaOH varies depending on the variety, cultivar, degree of ripeness and ambient temperature, and the time required varies between 6 and 11 hours [8, 28].

The temperature of 25 °C provided the shortest processing times (180 minutes, 15 $g \cdot L^{-1}$ NaOH) in all cases. For the cultivars "*Manzanilla Cacereña*" and "*Carrasqueña*", using a more concentrated solution of NaOH (25 $g \cdot L^{-1}$), the processing times at 25 °C were 237 and 201 min respectively [29]. In comparison, when temperatures of 18 and 25 °C were used, with a NaOH concentration similar to the maximum level used in this study (15.6 $g \cdot L^{-1}$), the fruits of the "*Manzanilla*" variety took 420 to 427 minutes [30].

The reduction of bitter oleuropein by hydrolysis into hydroxytyrosol and oleoside-11-methyl ester (which is then hydrolyzed to oleoside) is the objective of debittering [8]. However, if the concentration of NaOH is very high, the pulp of the olive fruit becomes very soft, while if the concentration is very low the process takes longer [2]. Therefore, very low or high concentrations should not be used. In the Sevillian process, high concentrations of NaOH (40 $g \cdot L^{-1}$) have little effect on the fatty acid composition, but may cause a "soapy" taste that appears when the fruits are not rinsed sufficiently with water or are consumed immediately or after a short period of time after debittering [31].

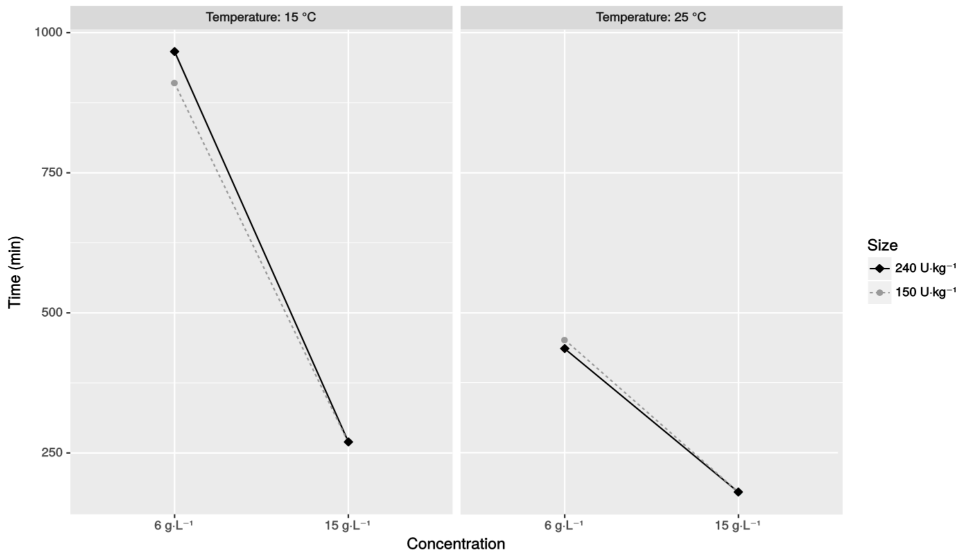

Figure 1. *Interaction plot for the three factors*

Time required to complete the alkaline treatment

The time required to complete the alkaline treatment (when a penetration of 2/3 of the distance between the skin and the stone fruit is reached) for each treatment is presented in Table 2. Two of the measurements of run 5 were not performed, so a type III sum of squares (for unbalanced designs) had to be used to perform the analysis of variance [25].

Table 2. *Average times measured for each run*

Run	Temperature [°C]	Concentration [$g \cdot L^{-1}$]	Size [$U \cdot kg^{-1}$]	Mean [minute]	SD
1	15	6	240	966.3^a	65.2
2	25	6	240	436.0^b	19.3
3	15	15	240	270.0^c	8.2
4	25	15	240	180.0^d	3.3
5	15	6	150	910.0^a	4.2
6	25	6	150	452.0^b	6.6
7	15	15	150	268.5^c	3.1
8	25	15	150	180.0^d	4.4

Different letters in one column indicate significant difference using Tukey's HSD test ($p < 0.05$)

The ANOVA table is presented in Table 3. Temperature and sodium hydroxide concentration were the significant factors ($p < 0.05$), along with their interaction. Fruit size did not have a significant influence on the time of alkaline treatment.

Table 3. *ANOVA table of the factorial experiment (type III sums of squares)*

Source	**Sum of squares**	**Df**	**F value**	**Pr (>F)**
(Intercept)	5962550	1	9134.99	< 2.2e-16
Temperature	605025	1	926.93	< 2.2e-16
Concentration	1547121	1	2370.28	< 2.2e-16
Size	775	1	1.18	0.29
Temperature : Concentration	291	1	446.47	4.2e-16
Temperature : Size	2417	1	3.70	0.07
Concentration : Size	667	1	1.02	0.32
Temperature : Concentration : Size	2225	1	3.41	0.08
Residuals	14360	22	-	-

The time required for the de-bittering process reported in previous studies varies significantly according to the concentration of NaOH and olive variety. Thus, the "*Manzanilla*" variety (*Olea europaea* L. *pomiformis*), treated with a 26 $g{\cdot}L^{-1}$ NaOH solution, took approximately 6 hours [32], while the same variety with a 22 $g{\cdot}L^{-1}$ NaOH solution took 5 hours [33]. In another study, it took seven hours for the "*Manzanilla*" and "*Hojiblanca*" varieties to complete the destemming using a 19 $g{\cdot}L^{-1}$ NaOH solution [34]. Longer times are reported for lower NaOH concentrations, although they vary even for the same variety. Thus, for the "*Nocellara del Belize*" variety, 12 hours are required using a 20 $g{\cdot}L^{-1}$ NaOH solution (equivalent to 2.9 °Bé) [35], while the same variety takes 8 hours using a NaOH solution at 2.6 °Bé [36].

CONCLUSION

The time required to complete the alkaline process in the production of Sevillian-style green table olives was measured. Using a full factorial design, the effect of process variables (temperature and NaOH concentration) and fruit size were evaluated at two levels. Higher levels of temperature and NaOH solution concentration (25 °C and 15.0 $g{\cdot}L^{-1}$ NaOH, respectively) resulted in shorter treatment times. The interaction of these two factors was also significant. However, the size of the fruits did not have a significant effect at the two levels tested. Therefore, further studies should be carried out to optimize these two factors (temperature and concentration of NaOH solution) and their interaction in order to reduce the alkaline treatment time in the processing of green table olives.

ACKNOWLEDGMENTS

The authors would like to thank Mr Juan José Alca Machaca, Department of Food Industry Engineering of the Universidad Nacional de San Agustín, for assistance with the statistical software and for comments that greatly improved the manuscript.

REFERENCES

1. Arroyo-López, F.N., García-García, P., Rodríguez-Gómez, F., Garrido-Fernández, A.: Olives: Types and Consumption in: *Encyclopedia of Food and Health* (Editors: Caballero, B., Finglas, P. M. and Toldrá, F.), Academic Press, Oxford, **2016**, Volume 4, 167-170;
2. Kailis, S.G., Kiritsakis, A.: Table Olives: Processing, Nutritional, and Health Implications in: *Olives and Olive Oil as Functional Foods*, (Editors: Shahidi, F. and Kiritsakis, A.), John Wiley & Sons, Chichester, **2017**, 295-324;
3. http://www.internationaloliveoil.org/documents/viewfile/4248-production3-ang/, World Table Olive Figures, accessed March 14, **2018**;
4. http://siea.minag.gob.pe/siea/?q=actividades-estadísticas-del-sistema/agrícola, Sistema Integrado de Estadística Agraria, accessed March 14, **2018**;
5. http://siea.minag.gob.pe/siea/?q=estadistica-agroindustrial, Anuario Estadístico de Producción Agroindustrial Alimentaria, accessed March 14, **2018**;
6. http://www.siicex.gob.pe/promperustat/, Estadísticas de exportaciones peruanas, accessed March 14, **2018**;
7. Contreras, R., Tapia, F.: Identificación genética de la variedad de olivo (*Olea europaea* L.) Sevillana y su relación con variedades productivas existentes en la provincia del Huasco, *Idesia (Arica)*, **2016**, **34** (3), 17-24;
8. Charoenprasert, S., Mitchell, A.: Factors Influencing Phenolic Compounds in Table Olives (*Olea europaea*), *Journal of Agricultural and Food Chemistry*, **2012**, **60** (29), 7081-7095;
9. Sahan, Y., Cansev, A., Gulen, H.: Effect of processing techniques on antioxidative enzyme activities, antioxidant capacity, phenolic compounds, and fatty acids of table olives, *Food Science and Biotechnology*, **2013**, **22** (3), 613-620;
10. Cortés-Delgado, A., Sánchez, A.H., Castro, A., López-López, A., Beato, V.M., Montaño, A.: Volatile profile of Spanish-style green table olives prepared from different cultivars grown at different locations, *Food Research International*, **2016**, **83**, 131-142;
11. Mettouchi, S., Sacchi, R., Ould Moussa, Z.E.D., Paduano, A., Savarese, M., Tamendjari, A.: Effect of Spanish style processing on the phenolic compounds and antioxidant activity of Algerian green table olives, *Grasas y Aceites*, **2016**, **67**, e114;
12. Ambra, R., Natella, F., Bello, C., Lucchetti, S., Forte, V., Pastore, G.: Phenolics fate in table olives (*Olea europaea* L. cv. Nocellara del Belice) debittered using the Spanish and Castelvetrano methods, *Food Research International*, **2017**, **100**, 369-376;
13. Collado-González, J., Moriana, A., Girón, I.F., Corell, M., Medina, S., Durand, T., Guy, A., Galano, J.M., Valero, E., Garrigues, T., Ferreres, F., Moreno, F., Torrecillas, A., Gil-Izquierdo, A.: The phytoprostane content in green table olives is influenced by Spanish-style processing and regulated deficit irrigation, *LWT - Food Science and Technology*, **2015**, **64**, 997-1003;
14. De Leonardis, A., Testa, B., Macciola, V., Lombardi, S.J., Iorizzo, M.: Exploring enzyme and microbial technology for the preparation of green table olives, *European Food Research and Technology*, **2016**, **242** (3), 363-370;
15. Habibi, M., Golmakani, M.T., Mesbahi, G., Majzoobi, M., Farahnaky, A.: Ultrasound-accelerated debittering of olive fruits, *Innovative Food Science and Emerging Technologies*, **2015**, **31**, 105-115;
16. Habibi, M., Golmakani, M.T., Farahnaky, A., Mesbahi, G., Majzoobi, M.: NaOH-free debittering of table olives using power ultrasound, *Food Chemistry*, **2016**, **192**, 775-781;
17. Tamer, C.E., Incedayi, B., Yildiz, B., Çopur, Ö.U.: The Use of Vacuum Impregnation for Debittering Green Olives, *Food and Bioprocess Technology*, **2013**, **6** (12), 3604-3612;
18. Savaş, E., Uylaşer, V.: Quality improvement of green table olive cv. 'domat' (*Olea europaea* L.) grown in turkey using different de-bittering methods, *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **2013**, **41** (1), 269-275;
19. Torres, M., Pierantozzi, P., Searles, P., Rousseaux, M.C., García-Inza, G., Miserere, A., Bodoira, R., Contreras, C., Maestri, D.: Olive Cultivation in the Southern Hemisphere: Flowering, Water Requirements and Oil Quality Responses to New Crop Environments, *Frontiers in Plant Science*, **2017**, **8**, 1-12;
20. Japan Soda Industry Association, *Safe handling of caustic soda (Sodium hydroxide)*, Soda Industry Association, Tokyo, **2006**;

21. Tarrado-Castellarnau, M., Domínguez Ortega, J.M., Tarrado-Castellarnau, A., Pleite Gutiérrez, R.: Estudio de la transferencia de calor en la etapa de cocido en la elaboración de aceitunas verdes al estilo sevillano, *Grasas y Aceites*, **2013**, **64** (4), 415-424;
22. Smyth, J.D.: *Table olive production manual. A practical guide for all table olive producers*, Rural Industries Research and Development Corporation, Canberra, **2012**;
23. R Development Core Team. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, **2017**;
24. Fox, J., Weisberg, S.: An R companion to applied regression, (2nd ed.), Sage, Thousand Oaks, **2011**;
25. https://cran.r-project.org/web/packages/agricolae/index.html, Agricolae: Statistical procedures for agricultural research, accessed March 14, **2018**;
26. https://cran.r-project.org/web/packages/dae/index.html, dae: Functions useful in the design and ANOVA of experiments, accessed March 14, **2018**;
27. RStudio Team.: RStudio: Integrated development environment for R. RStudio Inc., Boston, **2016**;
28. Cillidag, S.I.: Table olive processing technologies, *Options Méditerranéennes. Séries A: Mediterranean Seminars*, **2013**, **106**, 67-74;
29. Cabrera-Bañegil, M., Pérez-Nevado, F., Montaño, A., Pleite, R., Martín-Vertedor, D.: The effect of olive fruit maturation in Spanish style fermentation with a controlled temperature, *LWT - Food Science and Technology*, **2018**, **91**, 40-47;
30. Jaramillo Carmona, S., de Castro, A., Rejano Navarro, L.: Proceso tradicional de aderezo de aceitunas verdes de mesa. Racionalización del cocido, *Grasas y Aceites*, **2011**, **62** (4), 375-382;
31. Lanza, B., di Serio, M.G., di Giacinto, L.: Fatty-acid alkyl esters in table olives in relation to abnormal fermentation and poorly conducted technological treatments, *Grasas y Aceites*, **2016**, **67** (2), 1-11;
32. Rodríguez-Gómez, F., Romero-Gil, V., Arroyo-López, F.N., Roldán-Reyes, J.C., Torres-Gallardo, R., Bautista-Gallego, J., García-García, P., Garrido-Fernández, A.: Assessing the challenges in the application of potential probiotic lactic acid bacteria in the large-scale fermentation of Spanish-style table olives, *Frontiers in Microbiology*, **2017**, **8**, 1-15;
33. Rodriguez-Gomez, F., Romero-Gil, V., Bautista-Gallego, J., García-García, P., Garrido-Fernández, A., Arroyo-Lopez, F.N.: Production of potential probiotic Spanish-style green table olives at pilot plant scale using multifunctional starters, *Food Microbiology*, **2014**, **44**, 278-287;
34. Ramírez, E., Gandul-Rojas, B., Romero, C., Brenes, M., Gallardo-Guerrero, L.: Composition of pigments and colour changes in green table olives related to processing type, *Food Chemistry*, **2015**, **166**, 115-124;
35. Zinno, P., Guantario, B., Perozzi, G., Pastore, G., Devirgiliis, C.: Impact of NaCl reduction on lactic acid bacteria during fermentation of Nocellara del Belice table olives, *Food Microbiology*, **2017**, **63**, 239-247;
36. Martorana, A., Alfonzo, A., Gaglio, R., Settanni, L., Corona, O., La Croce, F., Vagnoli, P., Caruso, T., Moschetti, G., Francesca, N.: Evaluation of different conditions to enhance the performances of Lactobacillus pentosus OM13 during industrial production of Spanish-style table olives, *Food Microbiology*, **2017**, **61**, 150-158.