Enrichment in phenolic compounds of black ripe olives through nano-filtration and vacuum evaporation techniques

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Abstract

The aim of this work is to obtain ripe olives enriched in bioactive compounds of phenolic nature by the use as cover brine in the packaging washing waters of Spanish-style green olives or previous preservation acid solutions of ripe olives partially purified.

More than five times the phenol content in commercial product can be obtained using washing water of Spanish-style green olives nano-filtered by 4,000 Daltons cut membrane with subsequent vacuum evaporation and tap water addition to reach de initial volume.

Less increase in phenolic content was observed when the same purification treatment was applied to the previous preservation solution of ripe olives or using as cover brine the washing water simply nano-filtered and diluted to 50% with tap water

In these packaging, surface color, texture and flavor are similar to traditional ripe olives and the concentration of phenols remains stable for at least 11 months

Key words:

Nano-filtration, Phenol, Ripe olives, Vacuum evaporation

Industrial relevance

The methodology developed in this work would allow the nutritional assessment of the ripe black olives, eliminating part of the wastewaters that are generated in the table olive processing industries.

1. Introduction

The olive is a fruit with a high content in substances of phenolic nature. Its concentration is estimated at 2-5% of the flesh. The major compound is the oleuropein that gives the characteristic bitterness to the fruits.

In addition to oleuropein, there are also hydroxytyrosol 4-glucoside (Romero et al., 2002a), the ester of caffeic acid bound to secologanoside (Innocenti et al., 2006), as well as other glucosides and oleuropein dimers (Cardoso et 2006) tyrosol, comelogósido (Karioti et al., 2006) and caffeic, p-coumaric and vanilic acids (Brenes-Balbuena, García-García & Garrido-Fernández, 1992). Also, flavonoids such as luteolin 7-glucoside, apigenin 7-glucoside and rutine (Vazquez, Graciani & Maestro, 1974), as well as different types of anthocyanins, such as cyanidin 3-rutinoside and cyanidin 3-glucoside (Romero et al., 2002b) were in the olives.

These substances have been associated with numerous beneficial properties for health of consumers, such as anti-inflammatory, antioxidant, antitumor and antimicrobial actions (Kountouri et al., 2009; Medina et al., 2009; Malheiro et al., 2011; Martínez-Peláez et al., 2013; Serreli et al., 2017).

The polyphenol compounds have a great importance in the color, flavor and nutritional properties of table olives. During the processing to produce any of the commercial preparations, besides the loss of phenolic compounds by dilution in the different solutions used an important variation in the phenol composition occurs. Thus, in the final product, qualitatively and quantitatively the phenolic compounds present are very different from those initially had.

In particular, the processing of black ripe olives involves a first stage of fruit preservation in an acidic solution during which diffusion of the polyphenolic compounds from the flesh to the solution occurs and the acid hydrolysis of the oleuropein, forming glucose, elenolic acid and hydroxytyrosol (de Castro et al., 2007; Brenes et al., 1993)

The darkening phase consists of successive treatments (2-5) of the olives with dilute solutions of NaOH, each was followed by washing with water for about 20-24 hours and air is injected during the whole process (Sánchez-

Gómez, García-García & Rejano-Navarro, 2006). During this phase the oleuropein and verbascoside residues are transformed, releasing compounds such as hydroxytyrosol and caffeic acid. Both orthodiphenols in the presence of oxygen oxidize and polymerize and the olives get the characteristic black color of this form of elaboration (Brenes-Balbuena, García-García & Garrido-Fernández, 1992).

To neutralize excess alkali, CO₂ is bubbled in the last wash, then the olives are placed in a ferrous solution to avoid discoloration and finally they are packed in containers that are sterilized to ensure proper preservation (Sánchez-Gómez, García-García & Rejano-Navarro, 2006).

The phenolic profile of the final product consists mainly of simple phenols, hydroxytyrosol and tyrosol, with a total concentration of about 200 mg/kg of olive which is the lowest concentration of all table olive commercial preparations (Romero et al., 2004; Melliou, Zweigenbaum & Mitchell, 2015).

It has recently been found that reducing the number of alkaline treatments to only one and/or using the previous preservation liquid in the first washing after NaOH treatment increases the phenol content to almost double in the final packaged product (Romero, García-García & Brenes, 2016; Brenes, Romero & García-García, 2017). However, this concentration remains low compared to the natural black olives (Romero et al., 2004).

As discussed above, solutions rich in phenols are produced in the various olive processes. If these liquids suitably treated for reuse retain a large part of these bioactive substances, they could be used as cover brine in the packaging of ripe olives and they could significantly increase the phenol content of the commercial final product. Two liquids present possibilities for this, the washing waters after the alkaline treatment in the preparation as Spanish-style green olives and the previous storage solution in the ripe olives (Brenes et al., 1993; de Castro et al., 2007).

The nano-filtration would be one of the techniques that could be used for the partial purification since it has been proven its use for the reuse of fermentation brines as cover brine in the packaging of Spanish-style green olives without

influencing the physical-chemical and organoleptic characteristics in the commercial product (Brenes, García & Garrido, 1988; Rejano et al., 1995; Romero Barranco et al., 2001).

Complementary to this technique, the vacuum evaporation could be used as it can be applied to purify the preservation liquids of ripe olives to prepare the acid preservation solutions for its use in a new campaign (Medina et al., 2011).

The aim of this work is to obtain ripe olives enriched in bioactive compounds of phenolic nature by the use as cover brine in the packaging of the washing waters of Spanish-style green olives or previous preservation acid solutions of ripe olives partially purified through nano-filtration and vacuum evaporation techniques.

2. Materials and methods

2.1. Olives

Pitted commercial ripe olives from Hojiblanca variety ready to be packaged were used to packing experiences. Fruits preserved for 6 months (batch 1) or 14 months (batch 2 and 3) were darkened by industrial process in Agrosevilla SCA packaging plant (La Roda de Andalucía, Seville, Spain) with single lye treatment, 2 washes and color fixation (Romero, García-García & Brenes, 2016).

Darkened pitted fruit were transported to the Instituto de la Grasa pilot plant where they were kept in a cold room (7 ± 1 °C) for about 18 h before packaging.

2.2. Liquids with high phenolic content

2.2.1. Washing waters of green Spanish style (WW)

In October 2014 and 2015 washing waters from the Spanish-style green olives of the Hojiblanca variety were collected at the Cooperativa de Casariche (Casariche, Sevilla, Spain) which were acidified with hydrochloric acid (E507)

until reaching a pH $\approx$ 1.5 to prevent the oxidation of phenolic compounds (García et al., 1992). They were transported to the Institute de la Grasa pilot plant and stored at room temperature.

2.2.2. Storage solution of ripe olives (SS)

Just before the packaging experiments began (April 2016), 120 L of preservation liquid was collected from a fermenter with olives of the Hojiblanca variety at the Agrosevilla SCA packaging plant (La Roda de Andalucía, Sevilla, Spain) and were transported to the Instituto de la Grasa and they were kept in a cold room (7 ± 1 °C) for 2 days.

2.2.3. Partial purification of liquids

The washing waters of Spanish-style green olives (WW) and storage solutions (SS) of ripe olives (30 L of each) were nano-filtered (NF) passing the solutions through a membrane of 4,000 Daltons of molecular cut (Xylem Water Solutions, NY, USA) in an ultrafiltration/reverse osmosis equipment Paterson Candy International (Whitchurch, Hants, UK) as it was done with fermentation brines of Spanish style green olives (Rejano et al., 1995). About 8 L of permeate of each liquid (WW-NF; SS-NF) was obtained.

Then, 4 L of each liquid were concentrated in a rotovapor Büchi R-220 (BÜCHI Labortechnik, Flawil, Suiza) until reaching a volume 25% of the initial (1 L) to avoid the presence of volatile substances which can affect to final taste of the ripe black olives. These concentrates were regenerated to the initial volume with the addition of 3 L tap water (WW-NF-ER; SS-NF-ER).

To nano-filtered solutions (WW-NF; SS-NF) and nano-filtered with subsequent evaporation volume regeneration solutions (WW-NF-ER; SS-NF-ER), NaOH (4 M) were added until reach a pH of 6.5-6.7 and they were stored in a refrigerator (7 °C) for about 12 h.

2.3. Design of the experiences

First, the effect of the use of partially purified solutions as cover brine in the packaging of ripe black olives previously preserved for 6 months (batch 1) was studied. The 4 liquids (WW-NF, WW-NF-ER, SS-NF and SS-NF-ER) were used in two proportions, undiluted (100%) and diluted to 50% with tap water (50%). Thus, the 8 different types of packaging are summarized in Table 1.

As control, the same olives were packaged using only tap water (C).

To check the previous results a new packaging was made with olives preserved 14 months (batch 2 and 3) with a partially purified new washing water of Spanish-style green olives collected in October 2016. Only 50% diluted nano-filtered liquid (WW-NF-50) and undiluted nano-filtered with subsequent evaporation and volume regeneration solution (WW-NF-ER-100) were used as cover brine.

2.4. Packaging, sterilization and control

Previously to packaging, NaCl (35 g/L) was added to all solutions to reach an equilibrium concentration on the bottle of about 20 g/L and also 0.1 g/L of ferrous gluconate to prevent discoloration of the olives.

The packaging was carried out in bottles A314 (Juvasa, Dos Hermanas, Spain) with about 145 g of pitted fruit and 170 mL of liquid. The closed jars are then sterilized in a computer-controlled Steriflow retort (Madinex, Barcelona, Spain) to reach a lethality value of 15 F_0 .

From each experiment, 12 bottles were canned and stored at room temperature in the pilot plant.

Two bottles of each experience were analyzed at 0, 2, 5 and 11 months of packaging. Also, at 4 months an organoleptic analysis of the olives was carried out to determine the influence of the different liquids used in the packaging on the olive taste.

2.5. Physicochemical Analysis

The analysis of liquids for pH and NaCl concentration was carried out using the routine methods applied in table olives and the color was measured in a spectrophotometer Cary 60 UV-VIS (Agilent Technologies, Ca, USA) and expressed in terms of CIE L^* , a^* , b^* parameters (García, Sánchez & Garrido, 2014).

The superficial color of ripe olives was expressed as reflectance at 700 nm (R_{700}) using a BYK-Gadner Model 9000 Color view spectrophotometer (Silver Spring, MD, USA). Lower reflectance values indicate darker fruit. In addition, color was measured in terms of the CIE L^* a^* b^* parameters. Results were the means of 10 determinations.

A Kramer shear compression cell coupled to an Instron Universal Testing Machine (Canton, MA, USA) was used to measure the firmness. The cross-head speed was 200 mm/min and the firmness was expressed as N/100 g pitted olives. The result was the mean of 10 measurements, each of which was performed on three pitted olives.

The extraction of phenolic compounds from the olive flesh was based on the methodology proposed by Ramírez et al. (2014) using dimethyl sulfoxide (DMSO) and syringic acid as the internal standard. The separation of phenols was performed using a Spherisorb ODS-2 (5 μ m, 25cm, 4.6mm i.d., Waters Inc.) column at 35 °C passing 1mL/min of a gradient elution with an initial composition of 90% water (pH adjusted to 2.7 with phosphoric acid) and 10% methanol, that was increased progressively (Medina et al., 2007). The chromatographic system was: a Waters 717plus autosampler, a Waters 600E pump, a Waters heater module, and a Waters 996 diode array detector (Waters Inc., Milford, MA, USA). Phenolic compounds were monitored at 280nm.

2.6. Sensory analysis

The olives were tested according to the "Method for sensory analysis of table olives", COI/OT/MO 1/Rev.2 No 1 (IOC, 2011) in the normalized testing

room of the Instituto de la Grasa. Fruits were presented in a standardized glass according to the standard COI/T.20/Doc. No 5 (glass for oil tasting). This method classifies olives commercially through the use of descriptor related to the perception of negative sensations (“abnormal flavor”). Also, gustatory attributes (salty, bitter, and acidic) and kinesthetic sensations (hardness, fibrousness, crunchiness) were also assessed. The values of the attributes were expressed by the median of the individual data (8 testers) and the variability by robust standard deviation.

Also, triangular comparisons were achieved to detect differences in flavor between control olives and packed with different solutions. Three samples (two the same and one different) were presented simultaneously to each taster who was asked to distinguish the unpaired one. Each panelist did six tests with the samples presented in all possible positions (Lawless & Heymann, 2010). Then, for each comparison (8 tasters x 6 positions) 48 determinations were made.

2.7.- Statistical analyses

Statistica software version 7.0 was used for data processing (Statistica for Windows, Tulsa, OK, USA). Comparison between means was made by the Duncan’s multiple range tests and the differences were considered significant when $p < 0.05$. In sensory analysis differences were considered significant when confidence intervals ($p < 0.05$) did not overlap.

3.- Results & discussion

3.1. Effect of purification treatments on the characteristics of liquids

As can be seen in Table 2, nanofiltration produced a significant loss of phenolic compounds in Spanish-style green olive washing water (WW) (almost 2000 mg/L), which should be attributed to retention in the membranes, especially the compounds with highest molecular weight such as luteolin-7-glucoside that it reduces its concentration 82% or hydroxytyrosol-4-glucoside (53%) almost 1,000 mg/L. The same occurred with previous ripe olive storage liquid (SS, Table 2) in which the decrease in total phenolic compounds was about 1600 mg/L.

The subsequent evaporation and regeneration at the initial volume gave rise to a slight decrease in the total phenol content of about 100 mg/L for both the washing water (WW-NF-ER) and the storage liquid (SS-NF-ER).

Also, the nano-filtration produced a slight decrease in the concentration of acetic acid and ethanol in the two liquids as already shown in the application of this technology to the fermentation brines of Spanish-style green olives (Rejano et al., 1995). However, vacuum evaporation reduced 60% the acetic acid concentration and the total disappearance of the ethanol due to its lower boiling point (Table 2).

The nano-filtration of the two liquids and the subsequent increase of the pH produced a loss of color, that changes from red to slightly yellow tonality as it was observed by the decreasing of CIE parameters a^* and b^* values (data not shown). The color of solutions does not change significantly after subsequent evaporation and regeneration.

3.2. Characteristics of packages with the different solutions

3.2.1. pH and NaCl of the cover brines

As shown in Table 3, the pH of the liquids in the first sampling was in the normal range of 6.8-7.0 (García-García et al., 2014). Throughout the preservation a slight decrease (0.3-0.4 units after 11 months) was observed in

all cases as in commercial samples (García-García et al., 2014), which may be related to the non-enzymatic β -elimination and demethoxylation that could occur in low acidity conditions in other vegetables and fruits (Sila et al., 2006; De Roeck et al., 2009).

The NaCl concentration in cover brines was the expected (1.9-2.1%, w/v), no differences were observed between the different liquids used and the samples during shelf life (data not shown).

3.2.2. Color and firmness of ripe olives

During preservation, an increase in reflectance values at 700 nm of fruits was observed (Table 3) which means a loss of black color as observed in commercial samples (García-García et al., 2014). This may be related to the decrease in pH values which gives rise to a change of tonality in Fe-phenol compounds (Brenes et al., 1995). The loss of the reflectance value at 700 nm is related to a slight increase in luminosity (L^*), red (a^*) and yellow (b^*) tonalities (data not shown), the evolution was similar in all samples, including the control (C).

During studied shelf life, for each experience no significant differences ($p < 0.05$) in the firmness of the fruits was observed with the storage time (Table 3). Also, there were no significant differences ($p < 0.05$) between the different types of liquid used as cover brine; only, there was a tendency to have a greater firmness in the olives packaged with storage solution (SS).

3.2.3. Content in phenolic compounds

The control samples (C) have a total phenol concentration of about 375 mg/kg of olive and remained stable during the studied time (Table 4). This value was higher than the average found by Romero et al. (2004) in commercial samples. This may be due to the characteristics of the darkening process with a single lye treatment and only two washes (García-García et al., 2016).

In the rest of the experiences, the total phenol in the olive flesh (Table 4) was according the concentration of phenolic compounds in the used liquid as cover brine (Table 2). Thus, for the same type of purification treatment and packaging process (dilution), there are greater quantities of these compounds

when the washing water (WW) was used. The highest content of phenolic substances in the flesh (about 1,300 mg/kg) was reached when the nano-filtrated washing water was used (WW-NF-100). In this case, the concentration was almost 3.5 times higher than in the control sample (C).

In all cases, the most abundant compounds were hydroxytyrosol-4-glucoside and the simple hydroxytyrosol, followed by tyrosol, its glucoside (salidroside) and luteolin-7-glucoside.

In most experiences (except in control) the concentration of phenols just after the packaging and sterilization (0 months) was higher than at 2, 5 and 11 months, both in the flesh (Table 4) and in the cover brine (data not shown). It appears that degradation in these substances after packaging was produced. Afterwards, no reaction should occur since the concentration does not change statistically ($p < 0.05$) with the storage time.

To verify if this is true, a balance in each flask of the total content in phenolic compounds (olive and liquid) was made. The study starts from the time before packaging (Initial) taking into account the total concentration of phenolic compounds in the ripe olive (750 mg/kg) and in the different liquids used as cover brine (Table 2).

As can be seen in Table 5, in experiments with higher phenol content (more than 367 mg/bottle) during the manipulation to realize the packaging and in the heat treatment a decrease of these substances occurred since the differences between the initial concentration and in the time 0 months (just after sterilization) are statistically significant ($p < 0.05$).

The phenol reaction should be continued during few days after storage, since in the 2-month analyzes all contents in the bottles (Table 5) were statistically ($p < 0.05$) lower than just after packaging and sterilization (time 0 months). Subsequently, in the measurement performed at 5 months, no changes were observed in the total content according the 2-month analyzes.

Therefore, it can be stated that total phenol concentration in ripe olives from 2 months after packaging remain unchanged in the bottles during for at least 11 months (Tables 4 and 5).

3.2.4. *Organoleptic assessment of final product*

Ripe olives from control experience (C) can be considered as "extra" category since the median of "negative sensations" has a value of 1.0 (Table 6) (IOC 2011).

A higher value of the median of this parameter, although statistically the same ($p < 0.05$), was obtained for the samples corresponding to the experiences in which diluted (50%) nano-filtered washing water of Spanish-style green olives (WW-NF-50), nano-filtered with subsequent evaporation and regeneration to the initial volume (WW-NF-ER-100) and, logically, the last same diluted to 50% (WW-NF-ER-50) was used as cover brine. In these cases the fruits can be considered as "extra" category.

When storage liquid was used for packaging only in cases SS-NF-ER-100 and SS-NF-ER-50 have statistically equal scores ($p < 0.05$) that the control samples.

In the cases with high score in abnormal flavor, the tasters also score higher the bitter gustatory sensation. Thus the median values of the bitter taste sensation, presents a good linear correlation ($R^2 = 0.95$) with the median values of the negative sensations.

It seems that the use of the previous conservation solution of ripe olives (SS) communicates a greater final bitterness to the fruits that Spanish-style washing water (WW).

For the salty and acid gustatory sensations, the values of the median below 6 (center of the scale) can be considered normal for this type of elaboration due to the low salt concentration (about 2 %) and the pH values close to neutrality.

With regard to kinesthetic sensations, the olives have a low firmness, lower than center value of the scale, similar in all cases, with a slight tendency, not significant, to be higher when storage liquid of ripe olives (SS) was used as

cover brine. This was also observed in the objective measures by shear-compression (Table 3).

Also, no differences among samples were found in fibrousness and crunchiness sensations with normal values for this commercial preparation as described García-García et al. (2014).

3.3. New packaging experiences

The washing water used in these experiments (2016) gives rise to partially purified liquids with slightly higher phenol content than in the first experiment (Table 2). Thus, the nano-filtered liquid presents 3,491 mg of total phenols/L and the nano-filtered plus vacuum evaporation and volume regeneration, 3,410 mg/L.

As occurred in the first experiment, the total phenol concentration was statistically the same ($p < 0.05$) for the samplings performed at 5 and 11 months. Therefore, Table 7 only includes the results obtained on the last date.

As can be seen in Table 7, by each batch (2 and 3) the total phenols concentration in the control samples (C) was the third part that had in the batch 1 (about 375 mg/kg, Table 4). This is due to the longer preservation time that produces a dilution of the phenols in the preservation solution (de Castro et al., 2007). Thus, these fruits have before packing 188 and 157 mg/kg for batch 2 and 3 respectively, while in batch 1 had 429 mg/kg.

In spite of the lower phenol content in the darkened fruit ready for packaging, for the same type of liquid used as cover brine there was a total phenol concentration in the final product (Table 7) similar to those of the first experience (Table 4). This is due to the already mentioned higher concentration in phenolic compounds of the solutions used as cover brine.

As in the first packaging, the most abundant compounds were also hydroxytyrosol and its glucoside (data not shown).

At 11 months of packaging, surface color and firmness were statistically the same ($p < 0.05$) for each batch (2 and 3) regardless of the liquid used as cover brine (Table 7).

In the triangular comparisons made to detect differences in flavor between the control ripe olives (C) and when nano-filtered washing water of Spanish-style green olives plus vacuum evaporation and volume regeneration (WW-NF-ER-100) or simply nano-filtered and diluted to 50% (WW-NF-50) was used as cover brine the tasters identify the sample that was different from the other two (Table 3) in in less than the 22 recognitions that are necessary to confirm that the two samples are different ($p < 0.05$) (Lawless & Heymann, 2010).

So it was concluded again that ripe olives packing with nano-filtered washing water of Spanish-style green olives plus vacuum evaporation and volume regeneration (WW-NF-ER-100) or simply nano-filtered and diluted to 50% (WW-NF-50) presented the same flavor, firmness and superficial color that when tap water was used to prepare the cover brine,.

4. Conclusion

According to the above, it can be stated that it is possible to obtain ripe olives with high concentration of phenolic compounds (more than five times the content in commercial product) using as cover brine in the packaging washing water of Spanish-style green olives nano-filtered by 4,000 Daltons cut membrane with subsequent vacuum evaporation to obtain a quarter of the initial volume to remove the volatile compound and tap water addition to reach de initial volume.

When the same purification treatment was applied to the preservation liquid of ripe olive for later use in the packaging there are less increase in phenolic content on final product (three-four times the content in commercial product).

Similar final increase in phenols was obtained when the washing water simply nano-filtered and diluted to 50% with tap water was used as cover brine.

In these cases no statistically significant differences ($p < 0.05$) in surface color, texture and flavor were found with traditional ripe olives. In addition, the new product evolves during the shelf life as the commercial samples keeping the concentration of phenols stable for at least 11 months.

Therefore the methodology developed in this work would allow the nutritional assessment of the ripe black olives, eliminating part of the wastewaters that are generated in the table olive processing industries.

Acknowledgments

This work was supported by the Spanish Government (project AGL2013-42739-R), partially financed by European regional development funds, ERDF), and the Junta de Andalucía (through financial support to group AGR-125). The authors thank Alejandra Expósito de la Cerda for her assistance with the experimental work.

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Table 1.

Design of packaging experiences to increase the content in phenolic compounds on ripe black olive.

Liquid	TREATMENT	CONCENTRATION IN COVER BRINE ^f (%)	ACRONYM
Tap Water	Control samples	100	C
Washing Water ^a (WW)	Nano-filtration ^c	100	WW-NF-100
		50	WW-NF-50
	Nano-filtration + Vac. Evap. ^d + Vol. Reg. ^e	100	WW-NF-ER-100
		50	WW-NF-ER-50
Storage Solution ^b (SS)	Nano-filtration	100	SS-NF-100
		50	SS-NF-50
	Nano-filtration + Vac. Evap.+ Vol. Reg.	100	SS-NF-ER-100
		50	SS-NF-ER-50

Notes: ^a Washing waters of the Spanish-style green olive processing; ^b storage solutions of ripe olives; ^c Through a membrane of 4,000 daltons pore size; ^d Until concentrating to 25% of the initial volume; ^e tap water addition until initial volume; ^f 35 g of NaCl / L and 0.1 g of ferrous gluconate / L were added in all cases.

Table 2.

Content (mg/L) in acetic acid, ethanol, individual and total phenolic compounds of the washing water of green olives (WW) and the storage solution of ripe olives (SS) used in the experiences and after the different treatments in order to use them as cover brine in the packaging of ripe olives

	PHENOLIC COMPOUNDS (mg/L)							TOTALS	Acetic Acid (mg/L)	Ethanol (mg/L)
	Hydroxy-tyrosol	Hydroxy-tyrosol-4-glucoside	Tyrosol	Verbascoside	Oleuropein	HyEDA ^e	Other ^f			
WW initial	2668 (30) ^c a	1995 (42)a	208 (18)a	ND ^e	ND	ND	235 (9)	5104 (42)a ^c	550 (70)a	250 (40)a
WW-NF-100^a	2012 (36)c ^d	909,4 (53)c	168 (24)b	ND	ND	ND	94 (5)	3183 (105)b	500 (70)a	200 (30)a
WW-NF-ER-100^b	1910 (30)d	867 (50)c	156 (19)b	ND	ND	ND	89 (1)	3022 (73)b	210 (20)b	ND
SS initial	1052 (50)a ^h	1115 (84)a	85 (8)a	370 (30)a	199 (11)a	302 (44)a	145 (6)	3280 (208)a	7920 (20)a	580 (90)a
SS-NF-100^a	866 (62)b	393 (25)b	84 (6)a	78 (1)b	39 (5)b	111 (8)b	65 (4)	1642 (102)b	7040 (60)b	550 (80)a
SS-NF-ER-100^b	822 (12)b	358 (22)b	85 (7)a	73 (1)b	25 (2)c	109 (3)b	53 (2)	1531 (22)b	3350 (60)c	ND

Notes: ^a NF: Nano-filtered through a 4000 daltons pore size membrane and corrected to pH≈6,5; ^b NF-ER: Nano-filtered and evaporated up to 25% of the volume with subsequent addition of 75% of water and corrected to pH≈6,5; ; ^c Standard deviation between parenthesis; ^d For each liquid (WW or SS), column values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple-range test; ^e ND, no detected; ^e Di-carboxymethylated dialdehyde form of elhenolic acid linked to hydroxytyrosol; ^f sum of hydroxytyrosol glicol, salidroside, vanillic, caffeic and *p*-coumaric acids, hydroxytyrosol acetate and luteolin-7-glucoside concentrations.

Table 3.

Influence of liquid used as cover brine in the packaging on the characteristics of ripe olives during shelf life.

EXPE-RIENCES^a	Time (months)	pH	Superficial Color (<i>R</i>₇₀₀)	Firmness (N/100g pitted fruit)
C	0	7.00	5,2 (0,4) ^b	1934 (153)
	2	6.88	5,7 (0,6)	1932 (13)
	5	6.81	6,9 (0,5)	1911 (11)
	11	6.80	7.5 (0.2)	1820 (21)
WW-NF-100	0	6.90	5,5 (0,5)	1704 (58)
	2	6.65	6,0 (0,4)	1751 (8)
	5	6.61	7,2 (0,1)	1722 (20)
WW-NF-50	0	6.97	5,5 (0,2)	1825 (8)
	2	6.73	5,8 (0,1)	1792 (33)
	5	6.66	7,2 (0,1)	1798 (27)
	11	6.61	7.5 (0.2)	1743 (104)
WW-NF-ER-100	0	6.81	5,5 (0,2)	1749 (55)
	2	6.68	5,8 (0,1)	1766 (14)
	5	6.63	7,2 (0,1)	1746 (47)
	11	6.61	7.1 (0.1)	1710 (54)
WW-NF-ER-50	0	6.90	5,5 (0,3)	1795 (11)
	2	6.69	6,5 (0,1)	1853 (105)
	5	6.66	7,0 (0,4)	1723 (110)
	11	6.62	7.3 (0.1)	1710 (20)
SS-NF-100	0	6.85	5,4 (0,3)	1866 (10)
	2	6.68	6,4 (0,1)	2008 (48)
	5	6.62	6,3 (0,4)	1990 (16)
SS-NF-50	0	6.88	4,9 (0,6)	1908 (30)
	2	6.80	6,1 (0,4)	1987 (10)
	5	6.68	6,4 (0,2)	1909 (27)
SS-NF-ER-100	0	6.88	4,9 (0,6)	1914 (33)
	2	6.65	6,1 (0,4)	1935 (177)
	5	6.61	6,4 (0,2)	1895 (92)
	11	6.60	6.9 (0.1)	1825 (70)
SS-NF-ER-50	0	6.86	5,1 (0,2)	1875 (10)
	2	6.75	6,3 (0,2)	1871 (168)
	5	6.70	6,4 (0,2)	1876 (74)
	11	6.68	6.7 (0.5)	1839 (52)

Note: ^a Acronyms correspond to what is shown in Table 1; ^b Average value of duplicates and standard deviation are shown in parentheses;

Table 4.

Phenolic compounds (mg/kg) during shelf life in ripe olives canning with partially purified washing waters of Spanish-style green olives and previous storage solution of ripe olives

EXPE-RIENCES ^a	Time (months)	Hydroxy-tyrosol	Hydroxy-tyrosol-4-glucoside	Tyro-sol	Other ^c	TOTAL PHENOLS
C	0	33 (6) ^b	230 (14)	25 (1)	110 (7)	398 (29) a ^d
	2	35 (4)	213 (21)	24 (3)	100 (5)	372 (28) a
	5	41 (2)	222 (4)	25 (1)	91 (1)	379 (3) a
	11	40 (6)	225 (15)	25 (5)	95 (8)	385 (30) a
WW-NF-100	0	476 (18)	650(57)	72 (4)	139 (82)	1337 (39) a
	2	484 (66)	545 (30)	67 (3)	129 (1)	1225 (26) b
	5	642 (20)	430 (32)	65 (1)	132 (4)	1269 (62) b
WW-NF-50	0	237 (17)	445 (7)	58 (2)	114 (5)	881 (30) a
	2	225 (25)	373 (1)	45 (2)	107 (1)	748 (15) b
	5	263 (34)	315 (5)	40 (1)	113 (5)	731 (7) b
	11	250 (20)	388 (12)	42 (4)	90 (15)	740 (30) b
WW-NF-ER-100	0	504 (20)	650 (34)	79 (4)	156 (5)	1389 (4) a
	2	546 (12)	554 (2)	71 (1)	140 (5)	1311 (31) b
	5	570 (13)	473 (2)	62 (3)	138 (2)	1243 (9) c
	11	566 (21)	487 (22)	63 (2)	154 (11)	1270 (21) bc
WW-NF-ER-50	0	258 (13)	507 (55)	55 (1)	115 (2)	935 (35) a
	2	294 (21)	387 (7)	46 (1)	114 (1)	841 (16) b
	5	262 (27)	392 (10)	43 (2)	110 (1)	807 (23) b
	11	275 (25)	397 (15)	46 (7)	90 (9)	808 (25) b
SS-NF-100	0	84 (6)	370 (59)	46 (4)	128 (5)	628 (47) a
	2	70 (38)	366 (28)	43 (2)	120 (5)	599 (12) a
	5	86 (25)	337 (11)	43 (2)	103 (2)	569 (3) b
SS-NF-50	0	57 (13)	342 (32)	38 (2)	119 (1)	556 (16) a
	2	56 (1)	283 (11)	32 (1)	93 (4)	464 (15) b
	5	50 (4)	295 (2)	33 (1)	107 (4)	485 (2) b
SS-NF-ER-100	0	129 (5)	466 (40)	50 (1)	138 (2)	783 (36) a
	2	74 (39)	388 (13)	45 (1)	123 (6)	630 (41) b
	5	101 (18)	371 (14)	40 (2)	121 (4)	633 (18) b
	11	120 (20)	381 (15)	50 (9)	105 (9)	656 (31) b
SS-NF-ER-50	0	65 (6)	344 (38)	40 (1)	124 (3)	573 (19) a
	2	60 (24)	321 (4)	36 (1)	122 (6)	539 (10) b
	5	65 (1)	293 (16)	33 (3)	124 (7)	515 (34) b
	11	71 (18)	315 (15)	38 (7)	105 (12)	529 (31) b

Note: ^a Acronyms correspond to what is shown in Table 1; ^b Average value of duplicates and standard deviation are shown in parentheses; ^c sum of salidroside, vanillic, caffeic and *p*-coumaric acids, hydroxytyrosol acetate, rutin and luteolin-7-glucoside concentrations; ^d Standard deviation between parenthesis; ^d For each packaging type, Total phenol values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple-range test.

Table 5.

Content of total phenols (flesh fruit plus cover brine) in the bottles (mg/bottle) of ripe olives packaged using Spanish-style green olives washing water (WW) and storage solution of ripe olives (SS) before bottling (INITIAL) and after 0, 2 and 5 months of preservation

EXPERIENCE ^a	INITIAL	TOTAL PHENOLS (mg/bottle)		
		PRESERVATION TIME (<i>months</i>)		
		0	2	5
C	145 ^b (4) a ^c	152 (11) a	131 (4) b	138 (5) ab
WW-NF-100	637 (9) a	570 (10) b	493 (3) c	515 (13) c
WW-NF-50	386 (9) a	362 (3) b	333 (1) c	334 (2) c
WW-NF-ER-100	632 (6) a	593 (9) b	524 (5) c	525 (14) c
WW-NF-ER-50	361 (4) a	359 (7) a	345 (2) b	340 (7) b
SS-NF-100	376 (5) a	340 (8) b	296 (11) c	288 (2) c
SS-NF-50	254 (4) a	248 (11) a	210 (10) b	228 (10) b
SS-NF-ER-100	367 (3) a	350 (3) b	292 (6) c	306 (12) c
SS-NF-ER-50	240 (7) a	254 (8) a	220 (5) b	229 (4) b

Note: ^a Acronyms correspond to what is shown in Table 1; ^b Average value of duplicates and standard deviation are shown in parentheses; ^c For each experience (row), values followed by different letters indicate that they are different with $p < 0.05$ according to the Duncan test.

Table 6.

Sensory evaluation of ripe olives after 4 months canning according IOC method (results are medians of 8 observations).

EXPERIENCE^a	NEGATIVE SENSATIONS	GUSTATORY SENSATIONS			KINAESTHETIC SENSATIONS		
	Abnormal flavor	Salty	Bitter	Acid	Hard- ness	Fibrous- ness	Crunchi- ness
C	1,0 (0,4) ^b a ^c	4,1 (1,7) a	1,0 (0,8) a	1,2 (0,4) a	3,7 (1,2) a	3,3 (1,8) a	2,5 (1,4) a
WW-NF-100	7,2 (2,2) c	3,0 (1,6) a	5,5 (0,5) b	1,9 (1,2) a	3,3 (0,8) a	3,0 (0,9) a	3,0 (0,6) a
WW-NF-50	2,0 (0,7) a	3,0 (1,3) a	2,0 (0,6) a	1,7 (0,9) a	3,2 (1,3) a	2,9 (1,7) a	2,6 (1,7) a
WW-NF-ER-100	1,7 (0,6) a	2,6 (1,8) a	1,8 (1,1) a	1,8 (0,8) a	3,2 (1,3) a	2,5 (1,6) a	2,7 (1,2) a
WW-NF-ER-50	1,2 (0,6) a	3,1 (1,6) a	1,0 (0,9) a	1,6 (0,9) a	3,6 (1,9) a	2,9 (1,7) a	2,3 (1,8) a
SS-NF-100	7,0 (1,8) c	4,2 (1,7) a	6,1 (1,0) c	1,3 (0,9) a	3,6 (1,1) a	3,3 (1,2) a	1,8 (1,4) a
SS-NF-50	3,5 (0,5) b	3,8 (1,3) a	4,1 (0,6) ab	2,3 (1,2) a	4,0 (0,8) a	3,8 (1,3) a	3,0 (1,5) a
SS-NF-ER-100	2,0 (1,0) a	3,9 (2,1) a	2,2 (1,3) a	1,0 (0,8) a	3,7 (1,0) a	3,9 (1,4) a	3,0 (1,7) a
SS-NF-ER-50	1,5 (0,4) a	3,8 (12) a	1,4 (1,3) a	1,4 (0,8) a	3,7 (1,9) a	2,8 (1,4) a	2,0 (1,8) a

Notes: ^a Acronyms correspond to what is shown in Table 1; ^b Robust standard deviation in parenthesis (n=8); ^c In each column median values do not differ at the 5% level of significance because superimposing their corresponding confidence intervals

Table 7.

Superficial color, firmness and total phenol concentrations of ripe olives (Batch 2 and 3) after 11 months of packaged and number of correct judgments in each of the 48 triangular comparisons made to detect differences in flavor between the olives control and when reusing purified washing water of Spanish-style green olives.

Experiences ^a	Batch 2				Batch 3			
	COR- RECT JUG- MENTS	SUPERFICIAL COLOR (<i>R</i> ₇₀₀)	FIRMNESS (<i>N</i> /100 g pitted fruit)	TOTAL PHENOLS (mg/kg)	COR- RECT JUG- MENTS	SUPERFICIAL COLOR (<i>R</i> ₇₀₀)	FIRMNESS (<i>N</i> /100 g pitted fruit)	TOTAL PHENOLS (mg/kg)
C	-	5.2 (0.1) ^b a ^c	1575 (98) a	131 (7) a	-	5.5 (0.2) a	1475 (105)a	111 (8) a
WWb-NF-50	19	5.2 (0.3) a	1568 (35) a	847 (95) b	20	5.5 (0.1) a	1398 (84)a	824 (133)b
WWb-NF-ER-100	14	5.4 (0.3) a	1494 (35) a	1179 (72)c	16	5.5 (0.4) a	1498 (98)a	1303 (35)c

Notes: : ^a Acronyms correspond to what is shown in Table 1; ; ^b Average value of duplicates and standard deviation are shown in parentheses; ^c For each column values followed by the same letter do not differ at the 5% level of significance according to Duncan's multiple-range test.