

## Preservation of Paste Obtained from Picual Green Olives by High Hydrostatic Pressure Treatment

ANDREA NIETO, MARIA JOSE GRANDE BURGOS, ANTONIO GÁLVEZ\* and RUBÉN PÉREZ PULIDO

Área de Microbiología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales, Universidad de Jaén, Jaén, Spain

\*Corresponding author: [agalvez@ujaen.es](mailto:agalvez@ujaen.es)

### Abstract

Nieto A., Grande Burgos M.J., Gálvez A., Pérez Pulido R. (2017): Preservation of paste obtained from Picual green olives by high hydrostatic pressure treatment. *Czech J. Food Sci.*, 35: 246–250.

Olive paste can be a valuable source of phenolic compounds with antioxidant activity. High hydrostatic pressure (HHP) treatment (600 MPa, 8 min) was tested for the preservation of olive paste obtained from Picual unfermented green olives, a variety known for its high content of phenolic compounds. Microbiological load (total aerobic mesophilic counts, yeasts, and moulds), and antioxidant activity were measured during one-month refrigeration storage. At the end of the storage period, control samples maintained 27% of their initial antioxidant activity, showed browning, and had microbial loads (ca.  $10^5$  CFU/g) composed mainly of yeasts and moulds, while samples treated with HHP preserved 79% of their initial antioxidant activity, kept green colour and had no microbial counts. These results showed the potential of HHP treatment at 600 MPa for 8 min for the preservation of olive paste with strong antioxidant activity.

**Keywords:** antioxidant; preservation; olive paste; phenols; bacteria; yeasts; moulds

Olives and olive products have an important role in the Mediterranean diet. Thanks to its use for the production of table olives and olive oil, the olive tree is grown in many places of the world (GHANBARI *et al.* 2012). Spain is the leading exporter of olive oil with about 170 million trees, followed by Italy, Greece, Turkey, Tunisia, Portugal, and Morocco (ARSLAN & Özcan 2011). The olive fruit contains bioactive compounds (carotenoids, xanthophylls, chloroplasts, and polyphenols) which are able to act as chemical antioxidants with the ability to reduce oxidative damage associated with various degenerative diseases (CICERALE *et al.* 2012; KLEN *et al.* 2015).

The application of high hydrostatic pressure (HHP) can improve food safety with minimal changes in the organoleptic properties and nutrient quality (RENDUELES *et al.* 2011). A previous study on Manzanilla Aloreña table olives reported that when pressure

treatments at 300 MPa for 5 min were applied on the fermented olives, no viable yeast cells were detected, while the bacterial population, despite being lower than the yeast population, only decreased significantly after treatment at 600 MPa (ABRIOUEL *et al.* 2014). Other studies have also proven the efficacy of HHP for the preservation of fermented Cornezuelo dressed olives (PRADAS *et al.* 2012) and Halkidiki green table olives (ARGYRI *et al.* 2014). Furthermore, HHP treatments have also shown to confer microbiological stability on paste obtained from black table olives (TOKUSOGLU *et al.* 2010), paté made from fermented green olives of Manzanilla variety (SÁNCHEZ *et al.* 2012), and jam from fermented green olives of Carasqueña variety (DELGADO-ADAMEZ *et al.* 2013).

Pastes obtained from olives can be used as a food ingredient and are also interesting as a potential functional food with antioxidant properties. For this

purpose, green olives should be preferred compared to fermented table olives, since fermentation strongly decreases the levels of phenolic compounds in the olive fruit. However, there are no previous studies on the application of HHP treatments on paste from non-fermented green olives. Since olives of Picual variety are rich in phenolic compounds, and therefore seem to be a potential source for the preparation of olive pastes from non-fermented olives with a high antioxidant capacity, it seemed interesting to study the stabilisation of Picual olive paste by HHP. The aim of the present study was to obtain a microbiologically stable olive paste from raw Picual olives with preserved levels of antioxidant activity by the application of HHP processing.

## MATERIAL AND METHODS

**Sample preparation.** Green olives (*Olea europaea* L., Picual variety) were harvested from plantations located in the southern part of the Jaen province (Andalusia, Spain). Olives were harvested at a still green maturity stage on a single day in November 2014. Olives were stored at 4°C until processing and analysis (for not more than two days). Olives were manually pitted and then homogenised using a conventional kitchen blender to obtain a homogeneous paste consistency; aliquots (10 g each) of the resulting paste (pH 4.4) were packed in vacuum thermo-sealed polyethylene-polyamide plastic bags (oxygen and CO<sub>2</sub> transmission rates of 150 and 450 cm<sup>3</sup>/m<sup>2</sup>/day, respectively). Two control bags and two bags for treatment were prepared for each day of measurement. Taking into account oxidative damage suffered by olives, the preparation of samples was done on the same day of treatment (Day 0).

**High hydrostatic pressure treatment.** High hydrostatic pressure treatment was performed using a HHP system (Stansted Fluid Power LTD, Essex, UK), equipped with a container capacity of 2.5 l and operating pressure range of 0–700 MPa. Increase of pressure was set to 75 MPa/minute. Pressure was held at 600 MPa for 8 min followed by almost instant decompression. These treatment conditions were selected based on previous experience with fermented table olives (ABRIOUEL *et al.* 2014). Water with 5% propylene glycol was a pressurisation fluid. Untreated control and HHP-treated olive pastes were stored under refrigeration (4 ± 0.5°C) until further analysis. The temperature inside the vessel before treatment was 21°C, and it increased to 27°C during treatment.

In the process of decompression, the temperature decreased to the initial value. The temperature of samples was between 5 and 8°C before treatments, and between 17 and 19°C after treatments.

**Extract preparation.** In order to assess the antioxidant activity and microbial load in olive paste in the course of one month, extracts from treated and control samples were obtained on 0, 3, 7, 15, and 30 days of storage. Extracts were prepared under aseptic conditions by adding 10 ml of sterile peptone water to each sample bag and homogenising manually. The liquid phase was recovered and distributed in 2 aliquots: one for immediate use in microbiological testing, and the other was stored at –20°C, protected from light until use in quantifying antioxidant activity.

**Antioxidant activity.** The antioxidant activity was measured by a Trolox equivalent antioxidant capacity assay (TEAC) as described by BEN OTHMAN *et al.* (2008). Extracts were subjected to successive centrifugation steps at 5000 rpm for 5 min to ensure the removal of suspended solids. ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); Sigma-Aldrich, Spain] was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS<sup>+</sup>) was produced by reacting the ABTS stock solution with 2.45 mM (final concentration) potassium persulfate (Sigma-Aldrich, Spain) and allowing the mixture to stand in the dark at room temperature for 12 to 16 h before use. Before use, the ABTS<sup>+</sup> solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. Ten µl of clarified extract and 1 ml of diluted radical ABTS<sup>+</sup> were mixed in a spectrophotometer cuvette and maintained in darkness for 6 min, and then absorbance was measured at 734 nm using a SmartSpec TM spectrophotometer Plus (BioRad, Spain). The calibration curve was obtained using concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Sigma-Aldrich, Spain) in ethanol between 0.1 and 2.1 mM. The results were expressed as µmol Trolox/g of sample. Two separate extracts were made for each assay and the measurements were performed in triplicate. Thus, each result was calculated as the mean of six determinations.

**Microbiological analyses.** Samples were serially diluted in a sterile saline solution and plated on Tryptone Soy Agar – TSA (Scharlab) for estimation of total aerobic mesophiles and on Yeast Malt Agar – YMA (Scharlab) supplemented with chloramphenicol (150 mg/l; Sigma) for yeasts and moulds. Viable counts were obtained after 48 h of incubation at 30°C

and converted to  $\log_{10}$  CFU/g. The inactivation associated with treatment was established according to the difference in the counts obtained for each day of storage between control and treated samples. From each extract, two aliquots were serially diluted employing a sterile saline solution and spread separately in duplicate. Thus, each count was calculated as the mean of four determinations.

**Statistical analysis.** Mean and standard deviation were determined for each assay by using Excel (Microsoft Corp., USA). An ANOVA test was performed with a confidence level of 95% followed by Tukey's test in order to establish the existence of significant differences in antioxidant activity during storage. A paired *t*-test was carried out to detect significant differences between control and HHP-treated samples.

## RESULTS AND DISCUSSION

Variations in antioxidant activity for control and HHP-treated samples are shown in Table 1. On the first day, controls and treated samples had the same antioxidant activity. Since the third day, antioxidant activity in the control samples was significantly lower ( $P < 0.05$ ) compared to the HHP-treated samples, and showed a decline of about 65% of the initial value. On the last day of storage, the antioxidant activity of control samples decreased to 27% of its initial value. By contrast, the samples subjected to HHP treatment maintained the initial antioxidant activity during the first 15 days of storage, falling barely to 79% of its

Table 1. Antioxidant activity in extracts from olive paste treated or not with high hydrostatic pressure (600 MPa, 8 min) and stored under refrigeration

| Storage (days) | Antioxidant activity ( $\mu\text{mol Trolox/g}$ ) |                        |
|----------------|---|------------------------|
|                | control   | HHP treatment          |
| 0              | $39 \pm 1^{\text{aA}}$                            | $33 \pm 4^{\text{aA}}$ |
| 3              | $23 \pm 2^{\text{bA}}$                            | $37 \pm 2^{\text{aB}}$ |
| 7              | $25 \pm 2^{\text{bA}}$                            | $37 \pm 4^{\text{aB}}$ |
| 15             | $20 \pm 2^{\text{bA}}$                            | $34 \pm 3^{\text{aB}}$ |
| 30             | $10 \pm 1^{\text{cA}}$                            | $26 \pm 2^{\text{bB}}$ |

<sup>a-c</sup>values in the same column with different letters differ significantly ( $P < 0.05$ ); <sup>A,B</sup>values in the same row with different letters differ significantly ( $P < 0.05$ )

initial value on the last day of measurement. That means a loss of only 21% of the initial antioxidant activity after one month of storage.

Moreover, it was observed that treated samples preserved the green colour until the end of the month, while control samples showed the brown coloration typical of enzymatic oxidation since the third day. Figure 1 presents the sequence of pictures where the difference in coloration between control and treated samples can be noticed.

Altogether, the results obtained indicated that the progressive loss of antioxidant capacity and browning observed in the control olive paste during storage was significantly reduced in the samples treated with HHP at 600 MPa. The green olive fruits are a complex substrate rich in phenolic compounds

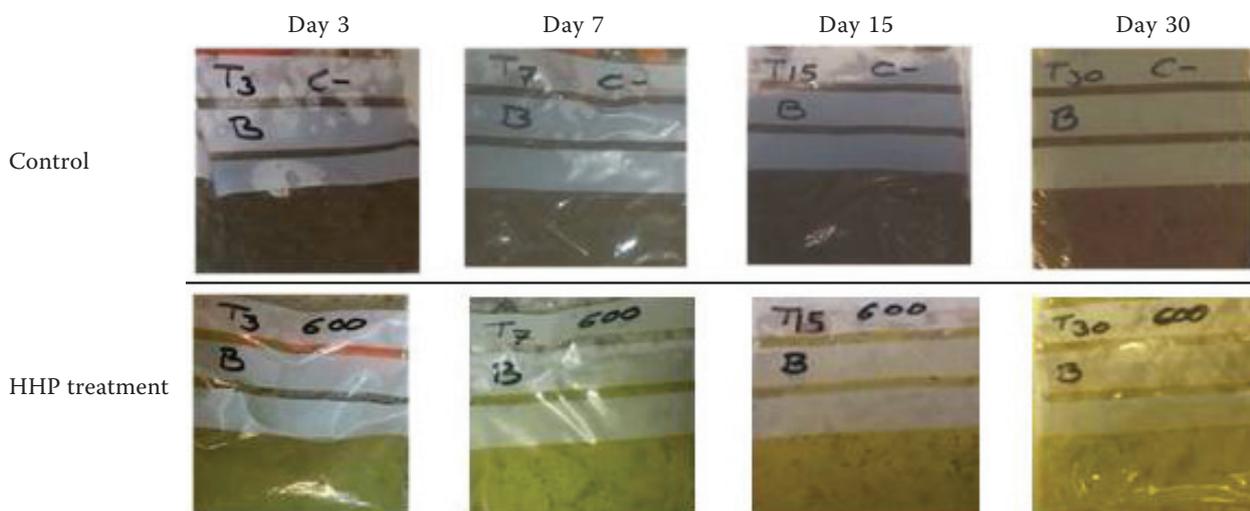


Figure 1. Colour variation during one-month storage under refrigeration of control olive paste samples and samples treated with high hydrostatic pressure (HHP) (600 MPa, 8 min)

doi: 10.17221/358/2016-CJFS

(BIANCO & UCCELLA 2000), and in which various endogenous enzymes such as pectinases, lipases, hydroperoxide lyase,  $\beta$ -glucosidases, peroxidase, and polyphenol oxidase (PPO) catalyse deteriorative reactions (HACHICHA HBAIEB *et al.* 2015). Oxidation of phenols by PPO results in vegetable tissue darkening (HACHICHA HBAIEB *et al.* 2015). Natural control of PPO activity is mainly produced by compartmentalisation. The loss of enzyme compartmentalisation by mechanical damage such as cutting or grinding facilitates the browning reaction. We suggest that the phenol oxidase activity could explain both the browning and loss of antioxidant activity observed in the control olive paste.

Previous studies have shown that HHP can inactivate degradative enzymes associated with food quality, including polyphenol oxidase activity (HENDRICKX *et al.* 1998; CAO *et al.* 2011). SÁNCHEZ *et al.* (2012) reported that the application of HHP treatments at 600 MPa for 5 or 10 min improved the oxidative stability of paté obtained from Manzanilla fermented olives compared to heating at 80°C for 20 min, and DELGADO-ADAMEZ *et al.* (2013) reported similar effects on jam obtained from Carrasqueña fermented olives. In these previous studies, the fermented olives were previously treated with lye and immersed in brine, which might influence phenol oxidase activity and phenolic content. By contrast, this is the first study on paste obtained from non-fermented olives.

Results of microbiological analyses for each day of measurement of control and treated samples are shown in Table 2. In control samples, viable counts of the total aerobic mesophiles and of yeasts and moulds were almost identical. During the first seven days of measurement, the microbiological load remained below  $10^2$  CFU/g, while from day 15 it was above  $10^5$  CFU/g, which means an increase by at least three logarithmic cycles in that period of time. By contrast, in the samples

treated with HHP, no viable cells were detected from the day of treatment until the last day of measurement.

Comparing viable counts and antioxidant activity shows that antioxidant activity also remained stable in control samples for the first 7 days and then dropped significantly on days 15 and 30. There could be two possible explanations for the observed coincidence of decrease in antioxidant activity and increase in viable cell counts: (1) microorganisms (together with the fruit PPO activity) could contribute to degradation or transformation of antioxidant compounds, and/or (2) since green olives contain a variety of antimicrobial compounds (MEDINA *et al.* 2007), these could be responsible for inhibition of microbial growth during the first week of storage, but they possibly lost their inhibitory activity upon oxidation. By contrast, the HHP treatment yielded a microbiologically stable paste where viable counts were reduced below detectable levels. Each group of microorganisms has different resistance to HHP; moulds and yeasts have a greater sensitivity, followed by Gram negative bacteria, complex enveloped viruses, Gram positive bacteria, and bacterial spores, which finally cannot be affected by this type of treatment (RENDUELES *et al.* 2011). Vegetative forms of eukaryotes, such as yeasts and moulds, are inactivated by pressures between 200 and 300 MPa, an aspect that has been confirmed in studies with fermented green table olives (PRADAS *et al.* 2012; ABRIOUEL *et al.* 2014; ARGYRI *et al.* 2014). Therefore it is expected that a 600 MPa treatment would yield a product free of detectable yeasts and moulds, especially when the initial cell concentrations were very low. However, according to previous results reported by ABRIOUEL *et al.* (2014), HHP treatments below 600 MPa did not prevent the browning of brined green table olives unless oxygen was excluded by purging with  $N_2$  (results not shown). Based on these previous data, we chose

Table 2. Microbiological analyses (viable cell counts –  $\text{Log}_{10}$  CFU/g) of control and HHP-treated (600 MPa, 8 min) samples

|                            | Time (days)     |                 |                 |                 |                 |
|----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                            | 0               | 3               | 7               | 15              | 30              |
| <b>Untreated controls</b>  |                 |                 |                 |                 |                 |
| Total aerobic mesophiles   | $1.6 \pm 0.4^A$ | $1.5 \pm 0.2^A$ | $1.5 \pm 0.4^A$ | $5.6 \pm 0.0^B$ | $5.4 \pm 0.1^B$ |
| Molds and yeasts           | $1.8 \pm 0.1^A$ | $1.6 \pm 0.1^A$ | $1.7 \pm 0.1^A$ | $5.5 \pm 0.1^B$ | $5.4 \pm 0.2^B$ |
| <b>HHP-treated samples</b> |                 |                 |                 |                 |                 |
| Total aerobic mesophiles   | < 1.0           | < 1.0           | < 1.0           | < 1.0           | < 1.0           |
| Molds and yeasts           | < 1.0           | < 1.0           | < 1.0           | < 1.0           | < 1.0           |

<sup>A, B</sup> values in the same row with different letters differ significantly ( $P < 0.05$ )

to apply a 600 MPa treatment to the non-fermented olive paste, with satisfactory results.

## CONCLUSIONS

The results obtained suggest that HHP at 600 MPa for 8 min can be an effective method for the short-term preservation of paste obtained from non-fermented Picual green olives. The applied treatment achieves a significant reduction of microbial load, by at least 2 logarithmic cycles, keeping it free from mesophilic microorganisms, enhancing the preservation of antioxidant activity and preventing enzymatic browning possibly by prevention of degradation or transformation of phenolic compounds. HHP is an attractive food preservation technology for olive industries to provide food products with possibly enhanced functional quality, thus increasing their added value. The special bitter taste of the paste from non-fermented olives and its antioxidant activity make it an attractive non-thermally processed food product with potential applications as an ingredient in appetisers and also as a ready-to eat ingredient for the preparation of special dishes in catering services and innovative cuisine.

## References

- Abriouel H., Pérez Pulido R., Benomar N., Gálvez A. (2014): Preservation of Manzanilla Aloreña cracked green table olives by high hydrostatic pressure treatments singly or in combination with natural antimicrobials. *LWT-Food Science and Technology*, 56: 427–431.
- Argyri A.A., Panagou E.Z., Nychas G.J., Tassou C.C. (2014): Nonthermal pasteurization of fermented green table olives by means of high hydrostatic pressure processing. *BioMed Research International*, 2014, Article ID 515623. doi: 10.1155/2014/515623
- Arslan D., Özcan M.M. (2011): Phenolic profile and antioxidant activity of olive fruits of the Turkish variety 'Sarıulak' from different locations. *Grasas y Aceites*, 62: 453–461.
- Ben Othman N., Roblain D., Thonart P., Hamdi M. (2008): Tunisian table olive phenolic compounds and their antioxidant capacity. *Journal of Food Science*, 73: 235–240.
- Bianco A., Uccella N. (2000): Biophenolic components of olives. *Food Research International*, 33: 475–485.
- Cao X., Zhang Y., Zhang F., Wang Y., Yi J., Liao X. (2011): Effects of high hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps. *Journal of the Science of Food and Agriculture*, 91: 877–885.
- Cicerale S., Lucas L.J., Keast R.S.J. (2012): Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. *Current Opinion in Biotechnology*, 23: 129–135.
- Delgado-Adamez J., Franco M.N., Sanchez J., De Miguel C., Ramirez M.R., Martin-Vertedor D. (2013): Comparative effect of high pressure processing and traditional thermal treatment on the physicochemical, microbiology, and sensory analysis of olive jam. *Grasas y Aceites*, 64: 432–441.
- Ghanbari R., Anwar F., Alkharfy K.M., Gilani A.H., Saari N. (2012): Valuable nutrients and functional bioactives in different parts of olive (*Olea europaea* L.) – a review. *International Journal of Molecular Sciences*, 13: 1291–1340.
- Hachicha Hbaieb R., Kotti F., García-Rodríguez R., Gargouri M., Sanz C., Pérez A.G. (2015): Monitoring endogenous enzymes during olive fruit ripening and storage: Correlation with virgin olive oil phenolic profiles. *Food Chemistry*, 174: 240–247.
- Hendrickx M., Ludikhuyze L., Van den Broeck I., Weemaes C. (1998): Effects of high pressure on enzymes related to food quality. *Trends in Food Science and Technology*, 9: 197–203.
- Klen T.J., Wondra A.G., Vrhovšek U., Vodopivec B.M. (2015): Phenolic profiling of olives and olive oil process-derived matrices using UPLC-DAD-ESI-QTOF-HRMS analysis. *Journal of Agricultural and Food Chemistry*, 63: 3859–3872.
- Pradas I., del Pino B., Peña F., Ortiz V., Moreno Rojas J.M., Fernández Hernández A., García Mesa J.A. (2012): The use of high hydrostatic pressure (HHP) treatments for table olives preservation. *Innovative Food Science and Emerging Technologies*, 13: 64–68.
- Rendueles E., Omer M.K., Alvseike O., Alonso-Calleja C., Capita R., Prieto M. (2011): Microbiological food safety assessment of high hydrostatic pressure processing: a review. *LWT-Food Science and Technology*, 44: 1251–1260.
- Sánchez J., De Miguel C., Ramírez M.R., Delgado J., Franco M.N., Martín D. (2012): Effect of high hydrostatic pressure versus thermal pasteurization on the microbiological, sensory aspects and oxidative stability of olive paté. *Grasas y Aceites*, 63: 100–108.
- Tokuşoğlu Ö., Alpas H., Bozoğlu F. (2010): High hydrostatic pressure effects on mold flora, citrinin mycotoxin, hydroxytyrosol, oleuropein phenolics and antioxidant activity of black table olives. *Innovative Food Science and Emerging Technologies*, 11: 250–258.

Received: 2016–09–15

Accepted after corrections: 2017–06–06