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# Efficacy of olive leaf extract (*Olea europaea* L. *cv* Gentile di Larino) in marinated anchovies (*Engraulis encrasicolus*, L.) process

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ABSTRACT

In this study, the antimicrobial activity and the preservative properties of olive leaf extract (OLE) *Olea europaea* L. "Gentile di Larino" cultivar, were evaluated. The antibacterial activity was performed *in vitro* against spoilage bacterial strains: *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas fragi* (ATCC 4973), *Pseudomonas putida* (ATCC 17514), *Brochotrix thermosphacta* (ATCC 11509), *Clostridium sporogenes* (ATCC 11437), and *Listeria innocua* (ATCC 33090). About the preservative properties of OLE, they were evaluated in the marinating process of anchovy fillets. During the process have been determined the change of sensory characteristics and monitored these chemical parameters: pH, aw, salt content (% NaCl), thiobarbituric acid index (mgMA/Kg), total volatile basic nitrogen (mg/100g), and trimethylamine nitrogen (mg/100g). Moreover, were determined the spoilage bacteria on raw material, after 7 days and at the end of marination process, 22 days. The OLE exhibited an inhibitory effect against the bacteria tested. In marinated anchovy fillets, showed that the extract improves their shelf life without modifying the organoleptic characteristics of the product; this suggests that it could be considered in the food industry as a natural antioxidant and antimicrobial food additive.

## 1. Introduction

In recent years, the consumer demand for natural foods with no preservative has increased; in particular the request of olive leaf extract (OLE) and its use as food additive, both for its high phenolic content and also for its antimicrobial and antioxidant activity (Lee and Lee, 2010; De Leonardis et al., 2008, 2018); like other phenolic compounds naturally occurring in numerous food and known for their beneficial biological and physiological properties, such as anti-inflammatory, antiallergenic, anticarcinogenic, antihypertensive, antiarthritic and antimicrobial activities (Lombardi et al., 2012; Micol et al., 2005; Liu et al., 2017). OLE is a dark brown, bitter-tasting liquid derived from the leaves of the olive tree (*Olea europaea* L., Oleaceae), contains many different compounds, specifically biophenols, which are thought to give the extract its varied therapeutic properties (Difonzo et al., 2017; Sahin et al., 2017). The most abundant biophenol is oleuropein, a secoiridoid composed from elenolic acid and hydroxytyrosol, which is considered the major bitter constituent in olive fruits with a high concentration especially in green olives up to 1–2 % (De Leonardis et al., 2016; Moudache et al., 2016). Other biophenols such as verbascoside, apigenin-7-glucoside, luteolin-7-glucoside and hydroxytyrosol are present in lower quantities (Japón-Luján et al., 2006; Iorizzo et al., 2016). Like many natural products, variation due to differences such as geographical location, plant nutrition, harvesting time, climate and cultivar, can influence the composition of the extracts which could influence the antibacterial and activities of the extracts (Korukluoglu et al., 2010). Phenolic compounds are known to inhibit the growth of *Escherichia coli, Klebsiella pneumoniae,* and *Staphylococcus aureus* (Aziz et al., 1998; Paster et al., 1988). Some authors (Tassou and Nychas, 1991; Holley and Patel, 2005) specified that Oleuropein inhibit sporulation of *Bacillus cereus*. Hydroxytyrosol also reported to be effective against clinical human pathogenic strains of *Haemophilus influenzae, Moraxella catarrhalis, Salmonella typhi, Vibrio parahaemolyticus, and Staphylococcus aureus* (Bisignano et al., 1999). Several reports have been published on olive leaf, especially its antimicrobial activity against microorganisms such as *Helicobacter pylori, Campylobacter jejuni, Staphylococcus aureus,* and others bacteria were studied from some authors (Sudjana et al., 2009). The anchovy (*Engraulis encrasicolus*, L.) represents an important economic resource for the Mediterranean region. Most of the catch is used for human consumption as fresh, salted, marinated, and freezed. Marinated fish are semi-preserved fish products, ready-to-eat

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with no heat treatment and are a high-value gastronomy product (Fuentes et al., 2010). Organic acid and salt are added to retard the action of bacteria and enzymes, resulting in a preserved product with a limited shelf life (Yeannes and Casales, 2008). The use of 6.5% of salt in combination with acetic acid are important because it makes the bacteria more sensitive in fact their growth is decelerated; moreover, the salt improves the texture and taste (Šimat et al., 2011). Some authors found that an increase in the vinegar content of the marinating solution to extend the shelf life may cause defects in the taste and odor of the final product (Yeannes and Casales, 1995; Kilinc and Cakli, 2004; Sallam et al., 2007).

The aim of this research has been to examine the activity of the OLE against a wide range of food spoilage bacteria, and to investigate the use of olive leaf extract as preservative in the marination process of anchovy fillet, in order to extend their shelf life and preserve their quality without the loss of their specific sensory properties.

## 2. Materials and methods

### 2.1. OLE preparation and phenolic composition

The olive leaves (*Olea europaea* L.) were randomly handpicked in mid-October 2014 from olive trees, "Gentile di Larino" cultivar, located in Larino, Molise region (Italy). After the harvest, the olive leaves were transported to the laboratory of the Department of Agriculture, Environment and Food Sciences of the University of Molise, where they were air-dried at room temperature, for 10 days before use. About 100 g of olive leaves were homogenized with an Ultra Turrax homogenizer; for the extraction was used a solution of methanol: water (80:20, v/v); after that solvent was evaporated using a rotary evaporator. Before using the extract, it was lyophilized to a dry powder, re-dissolved in water and then frozen. The total phenol contents were determined by Folin–Ciocalteu's method and the calculation of their content in OLE was carried out using the gallic acid calibration curve. The results were expressed as mg gallic acid per mL of extract (mg GA/mL); whereas the analysis of phenolic compounds was performed by HPLC (Iorizzo et al., 2014, 2016). The HPLC analysis was performed using a Varian ProStar 230 instrument (Mulgrave, AUS), equipped with a UV–VIS detector and set at a wavelength of 280 nm. The Chromatographic separation was carried out according to the IOC method (The International Olive Oil Council, 2009), using the ternary solvent system constituted by: $H_3PO_4$–bidistilled water 0.2% v/v (eluent A), methanol (eluent B), acetonitrile (eluent C) and with the following gradients (A/B/C): 0 min 96/2/2%; 24 min 50/25/25%; 27 min 40/30/30%; 36 min 0/50/50%; 49 min 96/2/2%. Identification of oleuropein in OLE was based on retention times in comparison with the corresponding standard.

### 2.2. Microorganisms and culture conditions

The microorganisms used for this study were six spoilage bacterial strains: *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas fragi* (ATCC 4973), *Pseudomonas putida* (ATCC 17514), *Brochotrix thermosphacta* (ATCC 11509), *Clostridium sporogenes* (ATCC 11437), and *Listeria innocua* (ATCC 330909). All microorganisms were sub-cultured on Muller-Hinton broth (Oxoid Ltd. CM0405, England) at the appropriate temperature for 24h. The OLE was frozen, re-dissolved in water at a concentration of 25.89 mg/mL, sterilized by filtrating through 0.22 μm Millipore filters, and analyzed for their antimicrobial activity.

### 2.3. Agar Well Diffusion Assay

The antimicrobial activity of OLE was performed by Agar Well Diffusion Assay (Azizollahi Aliabadi et al., 2012), using Muller-Hinton agar (Oxoid Ltd. CM0337, England) and under aseptic conditions. All the bacterial cultures were diluted to obtain a microbial suspension of $10^6$ cfu/mL. The Petri plates containing 20 mL of culture medium were inoculated with 200 μL of microbial suspension and allowed to dry in a sterile chamber. The plates with wells of 8 mm diameter were spotted with 100 μL of OLE. Sterile water was used as negative control and chloramphenicol (Sigma-Aldrich, USA) 100 μg/mL as a positive control. The plates were incubated at the appropriate temperature for 24h. The antimicrobial activity was evaluated by measuring the inhibition zone against the tested microorganisms.

### 2.4. Determination of minimum inhibitory concentration (MIC)

Determination of MIC was carried out according to EUCAST Definitive Document (The Definitive Document E.DEF 3.1, 2000). Each strain was tested with different concentrations of OLE, that it was serially diluted in water to obtain concentrations ranging from 1.62 mg/mL at 25.89 mg/mL, and before use sterilized by filtrating through 0.22 μm Millipore filters. All the microbial cultures were diluted to obtain a microbial suspension of $10^6$ cfu/mL. The petri plates containing 20 mL of Muller Hinton culture media (MHB, Merck, Germany), were inoculated with 200 μL of microbial suspension. The plates with wells of 8 mm diameter were spotted with 100 μL of various concentrations of OLE, from 1.62 mg/mL at 25.89 mg/mL, and then incubated at appropriate temperature for 24h. Sterile water was used as negative control and chloramphenicol (Sigma-Aldrich, USA) 100 μg/mL as positive control.

### 2.5. Marinating process

The fresh anchovy fillets were obtained from a local fisherman in Termoli (Molise). The process consisted of gutting, heading and filleting of the anchovies followed by washing to remove blood spots. The fillets were placed in the plastic containers, and they were added with marinade solution. The marinade solution used for the experimental trials consisted of 10% NaCl and 2% acetic acid. The experimentation was divided in two batch A and B; batch A (control) consisted by anchovy fillets and marinade solution and batch B by anchovy fillets, marinade solution and with the addition of OLE (10 mg/mL). The fillets were kept in marinade solutions in a ratio 1:1 (fish: marinade solution) for 22 days and the fish temperature was kept below 5 °C. This ratio according to Capaccioni et al. (2011) decreases the immersion marinating time without damaging their sensorial characteristics. During the entire production process as well as storage, fillets were completely immersed in the marination batch.

### 2.6. Microbiological assay of anchovy fillets

The microbiological assays they were made on fresh anchovy fillets and on samples taken after 7 days and at the end of the marination process, after 22 days. About 10 g of sample was mixed with 90 mL (0,1 %) of sterile peptone water, in a stomacher for 1 min at room temperature. Decimal dilutions were performed for plating. In addition to aerobic mesophilic bacteria and lactic acid bacteria counts, for fish samples, psychrotrophic bacteria, coliform, yeast, and mold counts were also monitored during storage. For psychrotrophic and mesophilic aerobic bacterial count, sample dilutions were plated in plate count agar (PCA, Oxoid CM325) and incubated at 7 °C for 10 days and 28 °C for 48/72h; for yeast and mold counts, potato dextrose agar (PDA, Oxoid CM139) was acidified to a pH value of 3.5 by tartaric acid, 0.1 mL of sample dilutions were spread on PDA and incubated at 30 °C for 5 days; Violet Red Bile Agar (VRBA, Oxoid CM107) by double layer poured plate method was used for coliform bacterial count, incubated at 37 °C for 24h, and De Man, Rogosa and Sharpe agar (MRS, Oxoid) by poured plate method was used for lactic acid bacterial counts, incubated at 30 °C for 72h.

### 2.7. Physical-chemical analysis of anchovy fillets

The fillets of each sample were analyzed for activity water (aw), sodium chloride, pH, and acetic acid. Water activity was determined using

aw meter AquaLab CX2 (Decagan Devices, Inc). Sodium chloride was determined by the Mohr method (Kirk et al., 1996) and the acidity determination was made by titration with sodium hydroxide (Kirk et al., 1996). The thiobarbituric acid (TBA), total volatile basic nitrogen (TVB-N), and trimethylamine nitrogen (TMA-N) were determined for marinated anchovy products after 0, 7, and 22 days of storage. The pH was measured using a digital pH meter (Crison basic 20), equipped with a glass electrode. The TBA was determined according to Weilmeier and Regenstein (2004) and Khan et al. (2006) and TBA values were expressed as milligram malonaldehyde equivalents per kilogram of muscle. The TVB-N was determined according to the method Antonocoupoulos and Vyncke (1989) and the TMA-N was determined by the method of AOAC (Horwitz, 1990). Results of TVB-N and TMA-N were expressed as mg per 100 g of muscle.

### 2.8. Sensory analysis of anchovy fillets

The sensory assessment of the anchovy fillets was done at the end of marination process, after 22 days, and was carried out by five panelists to evaluate the sensory attributes such as appearance, odor, structure, and flavor. The sensory analysis was conducted using the scoring test of Neuman, Molnar, and Arnold (Neumann et al., 1983) and according to Kilinc et al. (Kilinc et al., 2007). A score between 18.2 and 19.9 indicated very good quality, scores between 15.2 and 18.1 indicated good quality, scores between 11.2 and 15.1 indicated middle quality, scores between 7.2 and 15.1 indicated the limited of acceptability, and scores between 4.0 and 7.1 indicated spoiled samples.

### 2.9. Statistical analysis

All experiments and analytical determinations were carried out at least in triplicate. Statistical analyses were performed by using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). Significant of difference $p < 0.05$ was determined by one-way ANOVA (Friedman test) using the Duncan post hoc analysis.

## 3. Results and discussion

### 3.1. Chemical characteristics of OLE

The HPLC analysis of OLE extract allowed the identification of eight phenolic compounds (Table 1): oleuropein, verbascoside, luteolin-7-glucoside, rutin, vanillin, vanillic acid, catechin, and hydroxytyrosol. The total phenol content of the extract was 25.89 mg GA/mL. The retention times (min), the absolute peak area (%) and the main compounds present in OLE are shown in Table 1. The aqueous extract exhibited a profile in which oleuropein was the compound present in the highest quantity, with other biophenols such as verbascoside and luteolin-7-glucoside present in lower quantities.

### 3.2. Agar well diffusion assay and MIC

Individual phenolic compounds present in the OLE were identified, but we choose to submit the entire extract to antimicrobial activity studies. In addition, extracts may be more beneficial than isolated constituents, since a bioactive individual component can change its properties in the presence of other compounds present in the extracts (Sahin et al., 2017).

Certainly, the chemical composition of OLE conditioned the antimicrobial effects observed. The high content of oleuropein and the other phenolic compounds identified in the extract might contribute for its antimicrobial properties.

The results of Agar Well Diffusion Assay and MIC of OLE against the tested microorganisms was reported in Table 2. This extract, had an inhibitory effect against the spoilage bacterial strains tested: *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas putida*, *Brochotrix thermosphacta*, *Clostridium sporogenes*, and *Listeria innocua* with a diameter of the inhibition zones varying from 12 to 17 mm. Inhibition zones with a diameter of less than 12 mm were considered as having low antibacterial activity; between 12 mm and 16 mm moderately active and higher of 16 mm were considered as highly active (Azizollahi Aliabadi et al., 2012; Indu et al., 2006). According to this, the OLE was highly active against (Sudjana et al., 2009) *Brochotrix thermosphacta* and was moderately active against the other bacteria that were used in this study. As regard MIC, the OLE values against the tested microorganisms, ranged from 2,00 mg/mL to 5,00 mg/mL. By considering the results as reported in Table 2, one Gram-positive bacteria *Brochotrix thermosphacta* and one Gram-negative bacteria *Pseudomonas putida* were the most sensitive tested microorganisms.

### 3.3. Microbiological analysis of marinated anchovy fillets

As regards to the use of OLE for the marination process of anchovy fillets, the results of the microbiological analysis were reported in Table 3. After 7 days of the marination process, in batch B that contain OLE, psychrophilic bacteria counts were <10 cfu/g instead in the batch A (control) was $4.5 \times 10^2$ cfu/g, so this result shows that OLE had inhibited in only seven days these microorganisms. At the end of the marination process, after 22 days, we have found for all microorganisms tested a microbiological count <10 cfu/g both in the sample of batch A (control) and both in batch B (OLE treated fillets), as also found in other studies (Fuselli et al., 1994). So, this suggests that the high concentration of sodium chloride 10% and the presence of acetic acid 2% had an inhibitory effect against many spoilage and pathogen bacteria as reported by several authors (Sen and Temelli, 2003; Gökoğlu et al., 2004).

### 3.4. Chemical analysis of anchovy fillets during the marination process

Results of the chemical analysis were reported in Table 4. TVB-N and TMA-N are used as a measure of deterioration of fish and fish products, so represent a quality index of this product. The normal level of TVB-N in fresh fish range from 5 to 20 mg/100g (Yeannes and Casales, 2008). We have found at the end of marination process (22days) in the sample of

**Table 1**
Retention times, absolute peak area and the main phenolic compounds present in OLE.

| Phenolic compounds | Retention times (min) | Absolute peak area (%) |
|---|---|---|
| Hydroxytyrosol | 4.80 | 1.46 |
| Catechin | 8.40 | 0.04 |
| Vanillic acid | 14.08 | 0.62 |
| Vanillin | 14.69 | 0.04 |
| Rutin | 17.20 | 0.05 |
| Luteolin-7-glucoside | 18.05 | 1.36 |
| Verbascoside | 20.03 | 1.10 |
| Oleuropein | 22.70 | 24.53 |

**Table 2**
Antimicrobial activity of Olive leaf extract (OLE).

| Agar Well Diffusion Assay | | MIC |
|---|---|---|
| Microorganisms | OLE[a] Inhibition zone (mm) | OLE (mg/mL) |
| *Pseudomonas fluorescens* ATCC 13525 | 12.5 ± 0.70 | 5.00 ± 0.25 |
| *Pseudomonas fragi* ATCC 4973 | 12.0 ± 0.61 | 3.00 ± 0.18 |
| *Pseudomonas putida* ATCC 17514 | 13.0 ± 0.90 | 2.50 ± 0.15 |
| *Brochothrix thermosphacta* ATCC 11509 | 17.0 ± 1.11 | 2.00 ± 0.10 |
| *Clostridium sporogenes* ATCC 11437 | 13.0 ± 0.91 | 3.24 ± 0.19 |
| *Listeria innocua* ATCC 33090 | 13.0 ± 0.90 | 3.00 ± 0.18 |

MIC: minimum inhibitory concentration.
Each value is expressed as means ±standard deviation (n = 3) ($p < 0.05$).
[a] The concentration of OLE was 25.89 mg GA/mL.

**Table 3**
Microbiological analysis during marination process of anchovy fillets.

| Microrganisms (cfu/g) | Raw material | Marination process | | | |
|---|---|---|---|---|---|
| | | Batch A day 7 | day 22 | Batch B day 7 | day 22 |
| Total viable counts | $4.5 \times 10^4$ | $3.0 \times 10^2$ | <10 | $2.6 \times 10^2$ | <10 |
| Lactic acid bacteria | $4.3 \times 10^3$ | $2.8 \times 10^2$ | <10 | $2.1 \times 10^2$ | <10 |
| Psychrophilic bacteria | $7.0 \times 10^4$ | $4.5 \times 10^2$ | <10 | <10 | <10 |
| Moulds | $1.0 \times 10^2$ | <10 | <10 | <10 | <10 |
| Yeasts | $1.0 \times 10^2$ | <10 | <10 | <10 | <10 |
| Total coliforms | $<1.0 \times 10^1$ | <10 | <10 | <10 | <10 |

Batch A (control): anchovy fillets and marinade solution.
Batch B: anchovy fillets, marinade solution and with the addition of Olive leaf extract (10 mg/mL).

**Table 4**
Chemical analysis during marination process of anchovy fillets.

| | Marination process | | | | | |
|---|---|---|---|---|---|---|
| | days 0 | | days 7 | | days 22 | |
| | Batch A | Batch B | Batch A | Batch B | Batch A | Batch B |
| pH | $6.10^a \pm 0.45$ | $6.12^a \pm 0.38$ | $3.81^b \pm 0.23$ | $3.74^b \pm 0.31$ | $3.61^b \pm 0.19$ | $3.54^b \pm 0.21$ |
| aw | $0.994^a \pm 0.01$ | $0.995^a \pm 0.02$ | $0.856^b \pm 0.05$ | $0.816^c \pm 0.06$ | $0.848^b \pm 0.03$ | $0.803^c \pm 0.04$ |
| Sodium chloride (%) | $3.72^a \pm 0.40$ | $3.60^a \pm 0.41$ | $4.86^b \pm 0.41$ | $4.10^b \pm 0.38$ | $6.10^c \pm 0.45$ | $5.14^d \pm 0.31$ |
| Acetic acid (%) | $0.74^a \pm 0.05$ | $0.69^a \pm 0.04$ | $0.78^a \pm 0.07$ | $0.75^a \pm 0.04$ | $0.74^a \pm 0.07$ | $0.70^a \pm 0.08$ |
| TBA (mg MA/Kg) | $1.60^a \pm 0.22$ | $1.62^a \pm 0.02$ | $8.10^b \pm 0.90$ | $4.12^c \pm 0.51$ | $10.42^d \pm 0.98$ | $5.68^e \pm 0.35$ |
| TVB-N (mg/100g) | $10.20^a \pm 0.15$ | $10.30^a \pm 0.18$ | $11.80^b \pm 0.37$ | $9.64^c \pm 0.21$ | $15.81^d \pm 0.11$ | $11.40^b \pm 0.19$ |
| TMA-N (mg/100g) | $1.10^a \pm 0.07$ | $1.15^a \pm 0.08$ | $3.30^b \pm 0.36$ | $2.40^c \pm 0.29$ | $4.54^d \pm 0.45$ | $2.72^c \pm 0.31$ |

Batch A (control): anchovy fillets and marinade solution.
Batch B: anchovy fillets, marinade solution and with the addition of OLE (10 mg/mL).
$^{a-e}$ values in the same column labelled with different letters are significantly different ($p < 0.05$).

batch A, a TVB-N level <15.81 mg/100g instead in the batch B, that containing also OLE, was much lower, 11.40 mg/100g. Formation of TMA-N is caused by the reduction of trimethylamine oxide by bacterial activity and partially by enzymes, and in fresh fish range 0.93–1.11 mg/100g (Cadun et al., 2005), and this values increased during refrigerated storage while 5–10 mg/100g is considered the limit for acceptability (Kilinc and Cakli, 2004). Although results obtained at the end of marination process are below this limit, in particular in batch B, that contains also OLE, the value is much lower exactly 2,72 mg/100g. Some authors such as Kilinc and Cakli (2004) found that an increasing amount of acetic acid is effective on reduction of TMA formation, but in this case is low the concentration of acetic acid 2%, so the reduction of TMA it could be due to the effect of OLE added at the marination solution. TBA is a remarkable index of quality indicating lipid oxidation of unsaturated fatty acids and was expressed as malondialdehyde (MA) content, mg MA/Kg. In fish, the TBA values should be less than 3 mg MA/Kg. The fishes that have TBA values < 3 mg/kg are accepted as good quality and consumption level of TBA is 8 mg MA/Kg of fish, as reported by Cadun et al. (2005). As regards marinated anchovy fillets, the TBA values are increased in sample of batch A (control), at the end of the marination process was 10,42 mg MA/Kg so exceeded the consumption level in 22 days, that is of 8 mg MA/Kg, instead sample from batch B, that contain

**Table 5**
Sensory analysis results of marinated anchovy fillets.

| Sensorial attributes | Batch A | Batch B |
|---|---|---|
| Appearance | $2.80 \pm 0.18$ | $4.54 \pm 0.21$ |
| Texture | $2.45 \pm 0.08$ | $4.23 \pm 0.21$ |
| Odour | $2.38 \pm 0.22$ | $4.50 \pm 0.28$ |
| Flavour | $2.98 \pm 0.05$ | $3.50 \pm 0.07$ |
| Total result | $10.61 \pm 0.25$ | $16.77 \pm 0.39$ |

Batch A (control): anchovy fillets and marinade solution.
Batch B: anchovy fillets, marinade solution and with the addition of olive leaf extract (10 mg/mL). $n = 3$, mean $\pm$ SD ($p < 0.05$).

OLE, the TBA value at the end of marination process, was 5,68 mg MA/Kg, below the consumption level, so a good quality of anchovy fillets. The salt content in anchovy fillets at the beginning of the marination process it was 3.72% and it was increased during the process both into the control (batch A), it was 6.10% after 22 days, and both in OLE treated fillets (batch B) that it was 5,14%, due to diffusion of salt from marinade solution to fillets. The acidity content (% acetic acid) of the control (batch A) and OLE treated anchovy fillets (batch B) at the beginning of the marination process were 0.74% and 0.69% respectively. At the middle stage of the process, after 7 days, it's increased due to the diffusion of acetic acid through tissue. At the end of the marination process, after 22 days, the acidity values of both control and OLE treated anchovy fillets, were almost remained the same as the initial values.

### 3.5. Sensory analysis of marinated anchovy fillets

The results of sensory analysis of anchovy fillets, after the marination process (22 days), for each batch A (control) and B (with the addition of OLE), were reported in Table 5. The highest result has been obtained for the batch B 16.77 points against 10.61 points of batch A, so this suggests that OLE also has a positive role in preserving texture, appearance, and the organoleptic characteristics of the product.

## 4. Conclusion

Safety problems related to increasing use of chemical substances in food preservation are receiving growing attention (Neumann et al., 1983). The use of natural products can be a possible and desirable alternative to the use of chemical preservatives in the food industry. In the marination process of anchovy fillets, OLE has delayed the oxidative deterioration, TVB-N and TMA formation and had a positive effect on texture, appearance, and organoleptic characteristics of the fillets. These results show that OLE could be considered in the food industry as a natural preservative and antimicrobial additive (Sudjana et al., 2009).

Like many natural products, variation due to differences such as geographical location, plant nutrition, and cultivar can influence the composition of the extract. In fact, the future prospective will be to evaluate the OLE properties using leaves harvested in different period of the year, not only in autumn, and in different geographical location, to evaluate the different composition and characteristics that the extract can have based on the seasonality, location, and harvest period of the leaves.

## Declarations

### *Author contribution statement*

Bruno Testa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Silvia Jane Lombardi, Vincenzo Macciola: Performed the experiments.

Mariantonietta Succi: Contributed reagents, materials, analysis tools or data.

Patrizio Tremonte: Analyzed and interpreted the data.

Massimo Iorizzo: Conceived and designed the experiments; Wrote the paper.

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*Additional information*

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## References

Antonocoupoulos, N., Vyncke, W., 1989. Determination of volatile basic nitrogen in fish. Z. Lebensm. Unters. Forsch. 189, 309–316.

Aziz, N.H., Farag, S.E., Aziz, N.H., Farag, S.E., Mousa, L.A., Abo-Zaid, M.A., 1998. Comparative antibacterial and antifungal effects of some phenolic compound. An. Microbiol. 93, 43–54.

Azizollahi-Aliabadi, M., Kazemi-Darsanaki, R., Laleh-Rokhi, M., Nourbakhsh, M., Raeisi, G., 2012. Antimicrobial activity of olive leaf aqueous extract. Ann. Biol. Res. 3, 4189–4191.

Bisignano, G., Tomaino, A., Lo Cascio, R., Crisafi, G., Uccella, N., Saija, A., 1999. On the in vitro activity of oleuropein and hydroxytyrosol. J. Pharm. Pharmacol. 51, 971–974.

Cadun, A., Cakli, S., Kisla, D., 2005. A study of marination of deepwater pink shrimp (*Parapenaeus longirostris*, Lucas, 1846) and its shelf life. Food Chem. 90, 53–59.

Capaccioni, M.E., Casales, M.R., Yeannes, M.I., 2011. Acid and salt uptake during the marinatig process of *Engraulis anchoita* fillets influence of the solution: fish ratio and agitation. J. Food Sci. Technol. 31, 884–890.

De Leonardis, A., Macciola, V., Iorizzo, M., Lombardi, S.J., Lopez, F., Marconi, E., 2018. Effective assay for olive vinegar production from olive oil mill wastewaters. Food Chem. 240, 437–440.

De Leonardis, A., Testa, B., Macciola, V., Lombardi, S.J., Iorizzo, M., 2016. Exploring enzyme and microbial technology for the preparation of green table olives. Eur. Food Res. Technol. 242, 363–370.

De Leonardis, A., Aretini, A., Alfano, G., Macciola, V., Ranalli, G., 2008. Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea Europaea* L.) and evaluation of its antioxidant properties and bioactivity. Eur. Food Res. Technol. 226, 653–659.

Difonzo, G., Russo, A., Trani, A., Paradiso, V.M., Ranieri, M., Pasqualone, A., Summoa, C., Tamma, G., Silletti, R., Caponio, F., 2017. Green extracts from Coratina olive cultivar leaves: antioxidant characterization and biological activity. J. Funct. Food. 31, 63–70.

EUCAST Definitive Document E.DEF 3.1, 2000. June 2000: Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. Clin. Microbiol. Infect. 6 (9), 509–515.

Fuentes, A., Fernadez-Segovia, I., Barat, J.M., Serra, J.A., 2010. Physicochemical characterization of some smoked and marinated fish products. J. Food Process. Preserv. 34, 83–103.

Fuselli, S.R., Casales, M.R., Fritz, R., Yeannes, M.I., 1994. Microbiology of the marination process used in anchovy (*Engraulis anchoita*) production. LWT – Food. Sci. Tech. 27, 214–218.

Gökoğlu, N., Cengiz, E., Yerlikaya, P., 2004. Determination of the shelf life of marinated sardine (*Sardina pichardus*) stored at 4°C. Food Control 12, 1–4.

Holley, R.A., Patel, D., 2005. Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiol. 22, 273–292.

Horwitz, W., 1990, Fifteenth ed.. Official methods of Analysis of the AOAC Volume 2, pp. 685–1298

Indu, M.N., Hatha, A.A.M., Abirosh, C., Harsha, U., Vivekanandan, G., 2006. Antimicrobial activity of some of the south Indian spices against serotypes of *Escherichia coli, Listeria monocytogenes* and *Aeromonas hydrophila*. Braz. J. Microbiol. 37, 153–158.

Iorizzo, M., Lombardi, S.J., Macciola, V., Testa, B., Lustrato, G., Lopez, F., De Leonardis, A., 2016. Technological potential of lactobacillus strains isolated from fermented green olives: in vitro studies with emphasis on oleuropein-degrading capability. Sci. World J. 1–11.

Iorizzo, M., Macciola, V., Testa, B., Lombardi, S.J., De Leonardis, A., 2014. Physicochemical and sensory characteristics of red wines from the rediscovered autochthonous Tintilia grapevine grown in the Molise region (Italy). Eur. Food Res. Technol. 238, 1037–1048.

Japón-Luján, R., Luque-Rodríguez, J., Luque de Castro, M.D., 2006. Dynamic ultrasound-assisted extraction of oleuropein and related biophenols from olive leaves. J. Chromatogr., A 1108, 76–82.

Khan, M.A., Parrish, C.C., Shahidi, F., 2006. Effects of mechanical handling, storage on ice and ascorbic acid treatment on lipid oxidation in cultured Newfoundland blue mussel (Mytilus edulis). Food Chem. 99, 605–614.

Kilinc, B., Cakli, S., Tolasa, S., 2007. Quality changes of sardine (*Sardina pichardus*) patties during refrigerated storage. J. Food Qual. 31, 366–381.

Kilinc, B., Cakli, S., 2004. Chemical, microbiological and sensory changes in thawed frozen fillets of sardine (*Sardina pilchardus*) during marination. Food Chem. 88, 275–280.

Kirk, R., Sawyer, R., Egan, H., 1996. Composición y Análisis de Alimentos de Pearson, II, pp. 10–34. México.

Korukluoglu, M., Sahan, Y., Yigit, A., Ozer, E.T., Gucer, S., 2010. Antibacterial activity and chemical constitutions of *Olea europaea L.* leaf extracts. J. Food Process. Preserv. 34, 383–396.

Lee, O.H., Lee, B.Y., 2010. Antioxidant and antimicrobial activities of individual and combined phenolics in *Olea europea* leaf extract. Bioresour. Technol. 101, 3751–3754.

Liu, Y., McKeever, L.C., Malik, S.A.N., 2017. Assessment of the antimicrobial activity of olive leaf extract against foodborne bacterial pathogens. Front. Microbiol. 8, 1–8.

Lombardi, S.J., Tremonte, P., Succi, M., Testa, B., Pannella, G., Tipaldi, L., Sorrentino, E., Coppola, R., Iorizzo, M., 2012. Effect of phenolic compounds on the growth and L-malic acid metabolism of *Oenococcus oeni*. J. Life Sci. 6, 1225–1231.

Micol, V., Caturla, N., Perez-Fons, L., Mas, V., Perez, L., Estepa, A., 2005. The olive leaf extract exhibits antiviral activity against viralhaemorrhagic septicaemia rhabdovirus (VHSV). Antivir. Res. 66, 129–136.

Moudache, M., Colon, M., Nerín, C., Zaidi, F., 2016. Phenolic content and antioxidant activity of olive by-products and antioxidant film containing olive leaf extract. Food Chem. 212, 521–527.

Neumann, R., Molnar, P., Arnold, S. (Eds.), 1983. Sensorische Lebensmitteluntersuchung. VEB Fachbuchverlag, Leipzig.

Paster, N., Juven, B.J., Harshemesh, H., 1988. Antimicrobial activity and inhibition of aflatoxin B1 formation by olive plant tissue constituents. J. Appl. Microbiol. 64, 293–297.

Sahin, S., Samli, R., Tan, A.S.B., Barba, F.J., Chemat, F., Cravotto, G., Lorenzo, J.M., 2017. Solvent-free microwave-assisted extraction of polyphenols from olive tree leaves: antioxidant and antimicrobial properties. Molecules 22, 1–13.

Sallam, K.I., Ahmed, A.M., Elgazzar, M.M., Eldaly, E.A., 2007. Chemical quality and sensory attributes of marinated Pacfic saury (Cololabis saira) during vacuum-packaged storage at 4 C. Food Chem. 102, 1061–1070.

Sen, M.K.C., Temelli, S., 2003. Microbiological and chemical qualities of marinated anchovy prepared with different vegetable Additives and sauce. Rev. Med. Vet. (Sao Paulo) 154, 703–707.

Šimat, V., Bogdanović, T., Bulić, M., 2011. The effect of different marinating baths on sensory properties and shelf life parameters of cold marinated anchovies (*Engraulis encrasicolus, L.*). Meso 2, 80–88.

Sudjana, A.N., D'Orazio, C., Ryan, V., Rasool, N., Ng, J., Islam, N., Riley, T.V., Hammer, K.A., 2009. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. Int. J. Antimicrob. Agents 33, 461–463.

Tassou, C.C., Nychas, G.J.E., 1991. Effect of phenolic compounds and oleuropein on the germination of *Bacillus cereus* T spores. Biotechnol. Appl. Biochem. 13, 231–237.

The International Olive Oil Council, 2009. Determination of Biophenols in Olive Oils by HPLC, COI/T.20/Doc. 2009; No. 29, Madrid.

Weilmeier, D.M., Regenstein, J.M., 2004. Cooking enhances the antioxidant properties of polyphosphates. J. Food Sci. 69, 16–23.

Yeannes, M.I., Casales, M.R., 2008. Modifications in the chemical compounds and sensorial attributes of *Engraulis anchoita* fillet during brining and marinating stages. Ciênc. Tech. Alim. 28, 798–803.

Yeannes, M.I., Casales, M.R., 1995. Estudio de las variables de proceso de marinados de anchoíta (*E. anchoíta*). Rev. Tech. Hig. Alim. 262, 87–91.