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OLIVE LEAF EXTRACT AS NATURAL PRESERVATIVE

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ABSTRACT

Olive leaves, a waste from olive oil production, represent a good source of bioactive compounds, exploitable as natural preservatives in foods for their antioxidant and antimicrobial activities. In this frame, olive leaf extract (OLE) was added in non-thermally stabilized olive-based-paste at two different concentrations (0.5 and 1 g kg^{-1}), and the samples were stored for 90 days. Antioxidant and antimicrobial activity were evaluated by means of ABTS-TEAC assay and microbiological analyses. The samples added with OLE showed the highest value of antioxidant activity. The main microbial groups registered a significant loss (of about 0.5-1 logarithmic cycles) when OLE was added.

Keywords: olive leaves, antioxidant, polyphenols, shelf-life

1. INTRODUCTION

Shelf-life is usually defined as the time during which a food product remains safe, comply with label declaration of nutritional data and retain desired sensory, chemical, physical, and microbiological characteristics when stored under the recommended conditions (IFST, 1993).
Modified atmosphere packaging (MAP) is an efficient means of extending the shelf-life of foods as well as the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated-hydroxytoluene (BHT), propyl gallate (PG), and *tert*-butylhydroquinone (TBHQ), although recent reports reveal as these compounds may be implicated in many health risks, including cancer and carcinogenesis (UMEMURA *et al*., 2001) causing a general consumer rejection of synthetic food additives. Among natural antioxidants, extracts rich in phenolic compounds have been reported as good alternatives since they are readily available as industrial wastes and maintain a potential preservative effect (LORENZO *et al*., 2014) and present many positive effects on human health including anti-inflammatory, anti-carcinogenic, cardioprotective, and vasodilatory properties (BONILLA *et al*., 1999). Olive leaves are a waste from olive oil industry (10% of the total weight of the olives) and accumulate during pruning of the olive trees (TABERA *et al*., 2004). The most abundant phenolic component of olive leaves extract (OLE) is oleuropein (DIFONZO *et al*., 2017), which along with the bitter taste given to olives and olive oil, shows an in vitro inhibitory effect against many foodborne pathogens such that it could be suitable for use in the food industry as natural preservative.
In this framework, the aim of this work was to investigate the effect of OLE addition in modulating the antioxidant activity and the microbiota of non-thermally stabilized olive-based paste stored in MAP at refrigerated conditions.

2. MATERIAL AND METHODS

2.1. Formulation and manufacture of olive-based paste

Olive-based paste was produced with 840 g kg^{-1} of fermented table olives and 160 g kg^{-1} of extra-virgin olive oil. All ingredients were mixed using a homogenizer (WFP16SE, Waring Commercial, Torrington, USA) for 5 min to produce a homogeneous creamy paste. Three kinds of olive-based paste were produced: (i) control olive paste without any supplementary antioxidant (CTR); (ii) OLE 0.5, added at the concentration of 0.5 g kg^{-1}; (iii) OLE 1, added at the concentration of 1.0 g kg^{-1}. After homogenization, approximately 70 g of each mixture was transferred into plastic trays (95×10 mm), and a stainless steel heat sealer (VGP 25n, Orved, Musile di Piave, Veneto, Italy) was used to pack under argon-based atmosphere for a total of 96 samples. After packaging, the products were stored at 4°C, and then sampled after 1, 15, 30, 45, 60, 75, and 90 (T0, T15, T30, T60, T90). Three independent production trials were carried out for each sampling time and for each batch.

2.4. Antioxidant activity evaluation and microbiological analyses

The antioxidant activity was assayed by means of ABTS-TEAC as reported in DIFONZO et al. (2018). The results were expressed as μmol TE g^{-1} of olive-based paste.
Microbiological analyses were carried out as reported by COSMAI et al. (2017) and CAPONIO et al. (2019). Briefly, olive-based paste samples (5 g) were diluted with 45 mL of sterilized physiological solution, homogenized using a Stomacher 400 lab blender (Seward Medical, London) for 3 min, serially diluted and plated in triplicate in selective media

according to methods previously described (De Angelis et al., 2015; Difonzo et al., 2019). Counts were expressed as log cfu g^{-1}. The microbiological counts were preliminary confirmed by taking representative colonies for each medium which were analyzed for morphology, motility Gram staining reaction and catalase test.

2.5. Statistical analysis

Analysis of variance (one-way ANOVA) was carried out on the experimental data and significant differences among the values of all parameters were determined at $p<0.05$. All data were processed by Minitab (Minitab Inc., State College, PA, USA).

3. RESULTS AND CONCLUSIONS

Fig. 1 shows the effect of OLE addition on the antioxidant activity measured by ABTS-TEAC. The samples OLE showed values significantly higher than CTR ($p<0.05$), and only the samples added with OLE at the highest concentration (1 g kg^{-1}) kept this trend until T60.

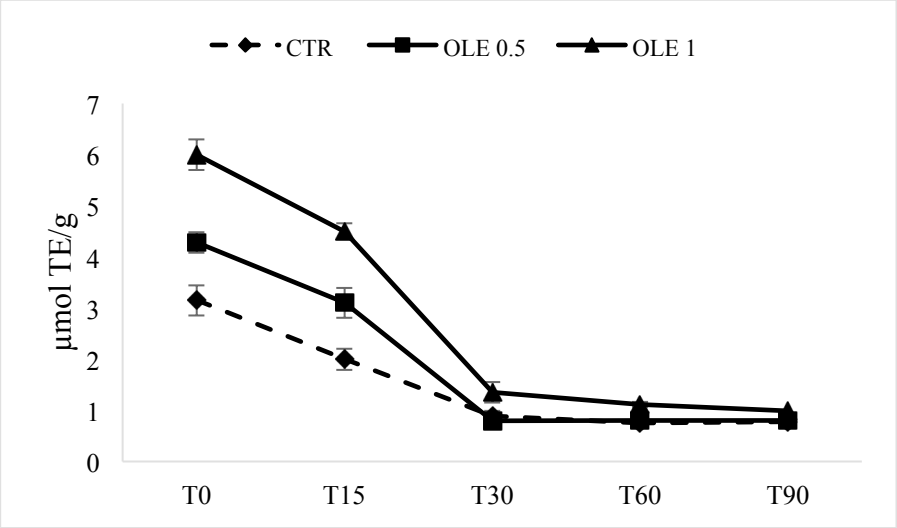

Figure 1. Trend of antioxidant activity assayed by ABTS-TEAC during storage. CTR: control samples; OLE 0.5: olive-based paste added of 0.5 g/kg of olive leaves extract; OLE 1:olive-based paste added of 1 g/kg of olive leaves extract; T0: production day; T15, T30, T60, and T90: 15, 30, 60, and 90 days of storage.

Several studies have reported the application of OLE in improving the antioxidant activity of olive oil (BOUAZIZ *et al*., 2008; DIFONZO *et al*., 2017), cooked meat products (HAYES *et al*., 2009), and bakery products (DIFONZO *et al*., 2018). The antioxidant activity improvement could result in stability oxidative improvement, thus the extension of shelf-life and in some cases is also related to the improvement of the nutritional value of foods (SHAHIDI *et al*., 2015).
Fig. 2 reports the results of the microbiological analysis related to the growth of microorganisms in olive-based pâté under study. Spoilage and pathogen bacteria were not detected. The cultivable bacteria, yeasts and moulds gradually decreased in all samples during storage. The addition of OLE and the refrigeration storage affected the cultivable

microbiota, as reported in previous research activities (CAPONIO et al., 2019) using the olives leaf extract.

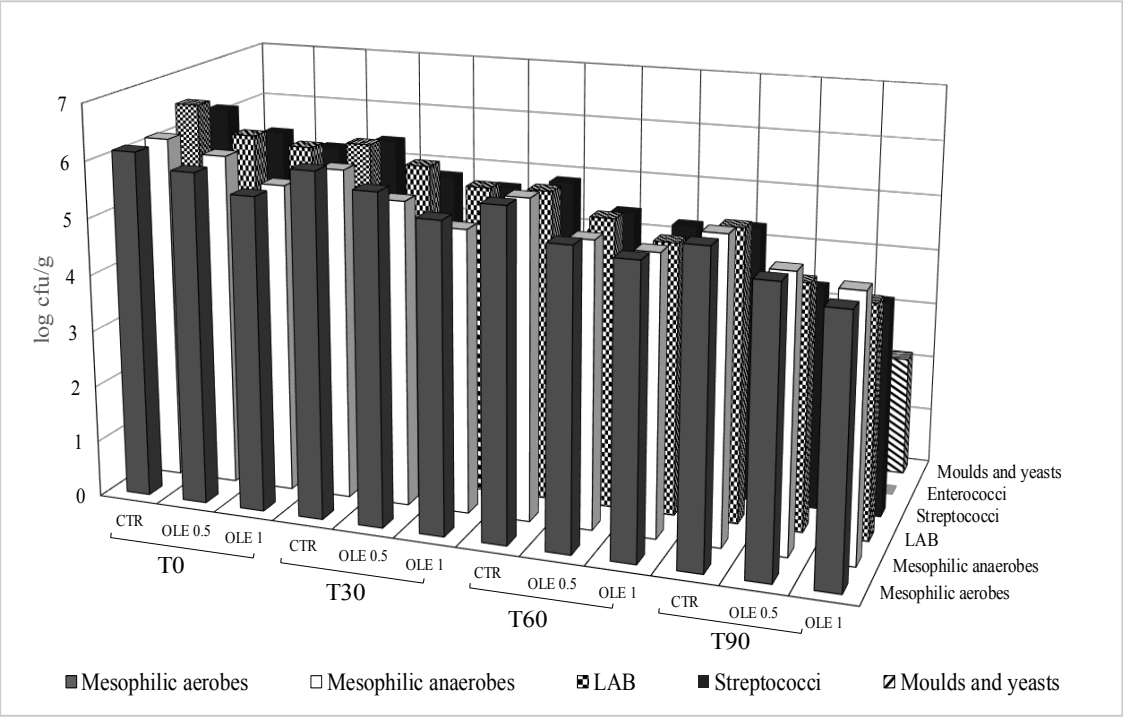

Figure 2. Cell densities (log cfu g^{-1}) of microbial groups detected in olive-based paste during storage time. CTR: control samples; OLE 0.5: olive-based paste added of 0.5 g kg^{-1} of olive leaves extract; OLE 1: olive-based paste added of 1 g kg^{-1} of olive leaves extract; T0: production day; T30, T60, and T90: 30, 60, and 90 days of storage.

The authors reported that the OLE addition affected the fermentative and oxidative processes of table olives and their nutritional properties. When 1 g kg^{-1} of OLE (OLE 1) was added to olive-based paste, the main microbial groups registered a loss (p<0.05) of ca. 0.5-1 logarithmic cycles. DIFONZO *et al*. (2019) showed that olive leaves are a waste of the olive oil processing industry and represent a good source of phenolic compounds. Those ones possess potential anti-microbial properties that can inhibited the microorganisms (HURTADO *et al*., 2012).
To conclude, the results of the present study indicate the potential use of OLE as natural preservative in non-thermally stabilized foods.

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