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Pulsed light treatment of packaged pistachio seeds: impact on microbiological and quality parameters with storage

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ABSTRACT

Pistachios (*Pistacia vera* L.) are susceptible to microbial contamination, causing quality and safety issues. Heat treatments of pistachio provide effective reduction on microbial load, but they also cause undesirable changes in quality properties. Thus, this study explored pulsed light (PL) application to improve pistachio seeds safety and quality. PL treatment (10-30-60 s) of packaged pistachios (varying pistachio layer thickness) reduced bacterial counts, particularly at 30–60 s in monolayer samples, but had limited effect on yeasts and molds. PL did not significantly affect moisture, chlorophylls or color parameters. Total acidity increased significantly in samples treated for 30 and 60 s, reaching values in the range of 4.69–5.67 g/kg. However, after 12 months of storage, no significant differences were observed between treated and untreated samples. PL treated samples, after 12 months of storage, showed lower peroxide values (ranging from 1.99 to 6.78 meq O_2/kg) than the untreated ones (18.39 meq O_2/kg), indicating a protective effect against lipid oxidation. Total phenolic content increased in stored samples of about 1.44 and 1.73 fold, in untreated and treated pistachios respectively, suggesting defense activation. PL improved microbial safety without compromising quality also after long storage, demonstrating its potential as a non-thermal pistachio processing method.

1. Introduction

Pistachio (*Pistacia vera* L.) plant is adapted to regions experiencing hot, dry summers and cold winters, and exhibits a strong tolerance to salinity stress, with fruit production commencing approximately seven years after planting. Freshly picked pistachio kernels have a moisture content of about 35 %–40 % (Ballistreri et al., 2009; Khadivi et al., 2025), then it is stabilized, generally sun-dried in open fields, up to a moisture lower than 6 %. As a result of this process, environmental contamination with Enterobacteriaceae, molds and yeasts can occur, representing a safety concern (Pakdaman et al., 2021). Adequately dried pistachios (<7 % moisture) do not support the growth of bacterial foodborne pathogens, which instead can survive on these products for periods extended more than one year. Previous studies showed that the decline of Salmonella populations during storage of dried inshell pistachios is slow (approximately 0.15 log CFU/g/month) at 23 °C, or insignificant over more than a year at 4 °C (Kimber et al., 2012; Lambertini et al., 2017). While lower moisture levels inhibit most bacterial growth, some fungi can thrive, requiring lower moisture content for multiplication. Fungal contamination is a risk throughout the food chain, encompassing plant cultivation, post-harvest drying, transport, storage and processing, and key environmental factors, including temperature and humidity, alongside crop damage from insects and inadequate drying, promote fungal proliferation. A significant consequence of fungal contamination is the presence of mycotoxins in nuts, low-molecular-weight secondary metabolites produced by various fungal species, known for their toxicity in animals and humans (Mirabile et al., 2021).

Pulsed light (PL) technology is considered a non-thermal treatment that uses inert gas flash lamps to generate short-duration, high-power pulses of an intense broad spectrum of light within the frequency ranges of ultraviolet (UV), visible (VL), and infrared (IR) light (200–1000 nm). PL is approved by the FDA for treatment not to exceed a total of 12.0 J/cm^2 (FDA.U.S. Food and Drug Administration, 1996) and is commonly used as surface decontamination technology for pathogens (John & Ramaswamy, 2018 and references therein). It has been proposed as a promising minimal process for improving microbial safety or extending the shelf life of treated foods (Rowan, 2019 and references therein). The

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lethal effect of PL is due to the coexistence of three different mechanisms of action, resulting from the absorption of the energy provided by the radiation: photochemical, photothermal and physical (Elmnasser et al., 2007; Heinrich et al., 2015; Santamera et al., 2020). Disinfection efficiency of PL is closely associated with the pulse voltage, fluence, distance of the surface from the light source, frequency and number of pulses, and the treatment duration (Salehi, 2022). Even PL covers wavelengths from 200 to 1100 nm, the light radiation used for PL treatments belongs to the UV-C range (200–289 nm). It was reported a maximum inactivation at 270 nm for a reduction of 0.43 log/cm^2 in a suspension of *E. coli* (Wang et al., 2005), and the suppression of UV–C made the PL treatment ineffective on *Bacillus subtilis* and *Aspergillus niger* (Levy et al., 2012). Li et al. (2023) reports that PL can achieve over 99 % for planktonic molds and over 90 % for biofilm molds *Aspergillus niger* and *Penicillium glaucum* at room and refrigerator temperatures.

The effectiveness of PL treatment could be influenced by several factors: foods rich in protein and fat reduce the effective amount of radiation reaching the targets as both macronutrients absorb UV (Gómez-López et al., 2005). PL seems to be more effective on foods rich in water and carbohydrates such as fruits and vegetables (Gómez-López et al., 2005) generally also associated with other treatments as washing (Huang & Chen, 2019). Another variable that may affect the effectiveness of the PL treatment is the shape of the food: foods with a less homogeneous shape could cause a lower effectiveness of the PL (Koh et al., 2016). In addition, the potential adverse effects of PL applications on food quality, such as weight loss, texture and color changes, lipid peroxidation, aroma and sensory changes, vary greatly depending on the foodstuff treated, distance from the light source, fluence and treatment time (Rowan, 2019 and references therein).

According to Sánchez-Bravo et al. (2022), research into the use of PL in nuts has had three different goals: the foodborne pathogen inactivation, the reduction of allergenicity and the degradation of aflatoxins. Consequently, researchers investigated the potential consequences of PL treatment on the quality of nuts within the framework of the treatment conditions required to achieve one of these three goals. PL application on dry almonds was ineffective for surface inactivation of *Salmonella*, and water dipping improved *Salmonella* inactivation with no changes in surface colour and lipid peroxidation (Liu et al., 2021); surface browning of almonds was visually observed after different PL treatment times (Harguindeguy & Gómez-Camacho, 2021). Shelled walnut was PL treated and no effect on TBARS, peroxide value, total phenolics and antioxidant activity was reported, but the concentration of volatiles associated with green/herbaceous odours increased significantly while compounds related to fruity and citrus odours decreased (Gómez-López et al., 2022). PL was successfully used to reduce up to 91 % aflatoxin levels in contaminated peanuts, and no significant effects were observed in peanut oil quality parameters, but differences were determined in the colour of treated samples (Abuagela et al., 2018).

Today, the USA world's leading producer of pistachios accounting for around 47 % of world production, followed by Turkey (30 %) and Iran (19 %) (Mandalari et al., 2021). In Italy pistachios grown almost exclusively in province of Catania in Sicily, where it is recognized by the Protected Designation of Origin (PDO) 'Pistacchio Verde di Bronte'. According to the production specification (Official Journal of the European Union, 2016), the PDO is reserved for shelled, in-shell or peeled products belonging to the botanical species *Pistacia vera*, cultivar "Bianca", characterised by an intense green colour even when ripe, due to a high chlorophyll content (Bellomo & Fallico, 2007; Mandalari et al., 2021). The skin also shows the presence of red pigments, anthocyanins, whose concentration depends on the ripeness of the seed: the riper the seed, the higher the concentration (Ballistreri et al., 2009). Thanks to its peculiar characteristics, the Pistacchio Verde di Bronte is not only a key economic driver for the region, but also a symbol of its cultural heritage, reflecting a history of unique environmental and human interactions. This product, acknowledged for its superior taste for over a century, strengthens local identity through its use in recipes, festivals, and the region's self-image, with traditional harvesting remaining a vital community activity (Wilson et al., 2018).

To the best of our knowledge, no research has been carried out on the use of PL on pistachios seeds or on packaged nuts. Similarly, no literature was found on the effect of PL application on the quality parameters of foodstuffs during long-term storage. Therefore, the objectives of this work were: i) to evaluate the efficiency of different treatment times with PL on both the microbiological load and the main quality physicochemical characteristics of packaged shelled pistachio; ii) to evaluate the change of these parameters in the treated pistachio samples after one year of storage.

2. Materials and methods

2.1. Chemicals

Petroleum ether 40/60, methanol HPLC-analytical grade, ethanol 96° ACS grade, hydrochloric acid (34 %–37 %) RS-super pure, chloroform RPE grade, sodium sulfate anhydrous (>99 %), Folin-ciocalteu's reagent RS and phenolphthalein solution (1 %) in ethanol (RPE grade) were purchased from Carlo Erba reagents (Milan, Italy). Diethylether (EPR grade), were purchased from Labkem (Barcelona, Spain). Acetic acid (≥99.8 %), sodium hydroxide solution (1 N), potassium iodide extra pure (99.0–100.5 %), sodium chloride (≥99.5 %), were purchased from Honeywell (Germany). Starch solution (1 %) stabilized was from Titolchim (Rovigo, Italy). Sodium carbonate anhydrous (≥99.5 %) was from PanReac ApplyChem (Monza, Italy). Gallic acid monohydrate (min. 99 %) was purchased from Riedel-de Haën (Germany).

2.2. Preparation of pistachio samples

Pistachio Verde di Bronte PDO, supplied by the Cooperativa Produttori Pistacchio Smeraldo Bronte s.r.l., located in Bronte (Catania, Italy), was from the 2023 crop. It was harvested at the beginning of September, about 150 days after blossoming, when the hull changed colour (white or pale pink). It was packaged and PL treated five months after harvest.

A total of about 4.32 kg of shelled pistachios was used for the study. Different amounts of pistachio seeds were weighed in a round polyethylene terephthalate (PET) box (internal diameter 9 cm, height 4.5 cm) to obtain packaged samples with different filling levels (thickness of pistachio in the boxes). The quantities of pistachio seeds contained within the boxes were the following: 18 g for the thickness equivalent to one layer of pistachio in the tray (about 1 cm), 36 g for two layers (about 2 cm), 54 g for three layers (about 3 cm). The boxes were subsequently sealed with a semi-automatic heat sealer (JPack) prior to PL treatment. In total were prepared: 60 boxes for one layer samples (1.08 kg total), 30 boxes for two layers samples (1.08 kg in total), 20 boxes for three layers ones (1.08 kg) and 20 boxes for the untreated pistachio samples. Table 1 provides the coding of the samples according to the filling level of pistachios and the time PL treatment. The Control sample consists of pistachio kernels packaged in heat-sealed boxes and not subjected to PL

Table 1
Samples coding and time of PL treatment.

Code	Layer	Time of PL treatment
Control	–	–
1L10	One	10 s
1L30	One	30 s
1L60	One	60 s
2L10	Two	10 s
2L30	Two	30 s
2L60	Two	60 s
3L10	Three	10 s
3L30	Three	30 s
3L60	Three	60 s

treatment.

2.3. Pulsed light treatment (PL)

The PL treatment was conducted using a system for pilot or small production process (Z-1000, Xenon™, USA), equipped with a benchtop controller (Model RC-847 Controller) and an air cooled standalone enclosed lamp housing with flashlamp. The xenon flashlamp is fixed on a vibrating conveyor belt and emitted high-intensity light with the spectrum of 200–1100 nm (Xenon, USA). The highest pulse fluence was 1.27 J/cm^2 delivered at a distance of 1.93 cm and was equal to 0.9 J/cm^2 as the boxes containing pistachio seeds were treated at a constant distance of 5.0 cm from the lamp during the whole treatment (according to the manufacturer specifications). The system was equipped with an elliptical reflector installed above the lamp in its housing, which directed into the light downwards. Packaged pistachio samples were treated with PL for different times: 10, 30 and 60 s, respectively. The vibrating conveyor belt provides continuous shaking of the pistachios inside the boxes to ensure homogeneous exposure to PL.

After the treatment, the trays were divided into two equivalents batches, one was immediately subjected to analysis (T0), to evaluate the effect of PL on the main physico-chemical and microbiological quality parameters. The other batch was stored at a controlled temperature of 23 ± 2 °C for up to 12 months (T12) to evaluate the effect of the pulsed light during storage.

2.4. Evaluation of pistachio quality parameters

2.4.1. Microbiological analysis

For microbiological analysis, 10 g of samples were homogenized with 90 mL of sterile saline solution (NaCl 9 g/L) using a Stomacher (BagMixer®, Interscience, Cantal, France); serial tenfold dilutions of the homogenate were prepared using the same diluent, followed by surface plating (0.1 mL) onto suitable nutrient media. Plate Count Agar (PCA; CM0325, Oxoid, Basingstoke, UK) with cycloheximide solution (SR0222, Oxoid, Basingstoke, UK), incubated at 30 °C for 48 h, was used to enumerate total mesophilic bacteria; MacConkey Agar (CM0007, Oxoid, Basingstoke, UK), incubated at 35 °C for 24–48 h, was used to enumerate Enterobacteria; Potato Dextrose Agar (PDA; CM0139, Oxoid, Basingstoke, UK), incubated at 25 °C for 5 days, was used to enumerate yeasts and molds. All microbiological analyses were performed in triplicate and expressed as average $\log_{10}$ CFU/g.

2.4.2. Physicochemical properties

Before analysis for each sample (Table 1), were withdrawn a number of boxes to obtain about 500 g of pistachio, and the pistachio seeds were mixed to obtain a single batch. Colorimetric analysis was carried out on whole seeds, chemical analyses were run on ground pistachio samples, ground in a home grinder (Imetec CH 3000, Tenacta Group S.p.A, Italy). All analyses, unless otherwise stated, were conducted in triplicate.

Moisture content was determined on grounded pistachio samples by gravimetric method using an oven (Heathermoven, Thermo Fisher Scientific Inc., USA) at a temperature of 105 °C until dry weight was constant (Ballistreri et al., 2009).

The CIE L*a*b* color parameters (L*, a*, b*, C* and h) were measured using a colorimeter (Chroma Meter CR 400, Konica Minolta, Tokyo, Japan) using an illuminant D65. A total of 15 pistachio seeds were withdrawn from each sample. The color parameters were measured on the external skins of the seeds and after the reading of the parameters, each seed was cut with a scalpel to carry out the determination on the internal surface of the section.

2.4.3. Oil extraction and determination of oxidative parameters

Oil was extracted as follows: 30 g of pistachio powder was mixed with 50 mL of petroleum ether 40/60 and stirred for 30 min. The organic extract was recovered and filtered on filter paper (diam. 90 mm, Whatman, Grade 1). Then the solvent was evaporated using a rotary evaporator (BUCHI 461, Büchi, Switzerland) and the oil was placed in an oven at 60 °C for 20 min to evaporate any residual solvent. Total acidity expressed as g of oleic acid/kg of oil and peroxide value expressed as $meqO_2$/kg of oil were determined on the extracted oil according to Reg. (EU) 2022/2015.

2.4.4. Extraction and determination of total polyphenols

Polyphenols extraction was carried out on pistachio powder (2 g) mixed with 25 mL of acidified methanol (0.1 % hydrochloric acid) as solvent. Subsequently the mixture was shaked with a vortex (Minishaker MS2, IKA, USA) for 5 min, put into an ultrasonic bath (Ultrasonic cleaning instrument, FALC, Italy) for 10 min, and centrifuged (5478×*g*, 10 °C, 10 min) (Thermo Scientific SL16R, USA)(Ballistreri et al., 2009). The supernatant was collected, and total polyphenols was determined by Folin-Ciocalteu method:1 mL of extract was mixed with 5 mL of Folin-Ciocalteu (diluted at 10 % in water) and 4 mL of sodium carbonate (7.5 % in water), after the samples were kept for 2.5 h in the dark before spectrophotometer measurement.

The specific absorbance was measured at a wavelength of 765 nm (lambda 25 UV–VIS spectrometer, PerkinElmer, USA); blank for comparison was obtained by replacing methanolic extract with acidified methanol. The polyphenol content was expressed as mg of gallic acid equivalent per 100 g (mg GAE/100g) and was calculated using a calibration curve of external standards.

2.4.5. Extraction and determination of chlorophylls

Chlorophyll extraction and determination was carried out according to AOAC 942.04 method.

Briefly, 5 g of pistachio powder was mixed with 20 mL of 85 % acetone solution in aqueous solution and 5 mL of diethyl ether. The mixture was stirred for 10 min and after was filtered through Whatman No. 1 filter paper and collected in a flask. This extraction procedure was repeated 4 times until a colorless extractive mixture was obtained. All the extracts were collected into a 100 mL volumetric flask and diluted up to a volume with diethyl ether. After 25 mL of the filtrate solution was put into a separatory funnel containing 50 mL of diethyl ether. Then an aliquot of ca 100 mL of deionized water was added to wash ether solution, water was drained and discarded. The washing process was repeated 5 times, then ether solution was passed on Na_2SO_4, transferred to 100 mL volumetric flask and diluted to volume with diethyl ether. The spectrometer determination was performed using a Lambda 25 UV–VIS spectrophotometer (PerkinElmer Inc., USA) at the wavelengths of 660 and 642.5 nm against diethyl ether. All glassware used for chlorophylls extraction was covered with an aluminum sheet to avoid photodegradation of the chlorophylls.

Total chlorophylls and each a and b component concentration (mg/kg) were calculated as follows:

$$\text{Total chlorophylls (mg/kg)} = (7.12^{*}\text{Abs}_{660} + 16.8^{*}\text{Abs}_{642.5})\ ^{*}\ \text{df}$$

$$\text{Chlorophyll } a \text{ (mg/kg)} = (9.93^{*}\text{Abs}_{660} - 0.777^{*}\text{Abs}_{642.5})\ ^{*}\ \text{df}$$

$$\text{Chlorophyll } b \text{ (mg/kg)} = (17.6^{*}\text{Abs}_{642,5} - 2.81^{*}\text{Abs}_{660})\ ^{*}\ \text{df}$$

were "Abs_{660}" indicates the absorbance value at 660 nm, "$Abs_{642.5}$" indicates the absorbance value at 642.5 nm, and "df" indicates "dilution factor".

2.5. Statistical analysis

All the results are expressed as mean ± standard deviation. The chemical, physical and microbiological results were subjected to statistical analysis using the Minitab Statistical Software 2.0. Significant differences were assessed by one-way analysis of variance (ANOVA) and the mean separation was determined by Tukey's multiple comparison ($p < 0.05$).

Three-way ANOVA was used to determine the main effects and the interaction of PL treatment time, filling levels and storage on physical and chemical parameters of pistachio samples.

3. Results and discussion

3.1. Effect of the PL treatment on the microbiological parameters

The effect of PL treatment duration (10, 30 or 60 s) and pistachio sample thickness (one, two or three layers) on the microbial populations under study is depicted in Fig. 1. The total mesophilic bacterial count

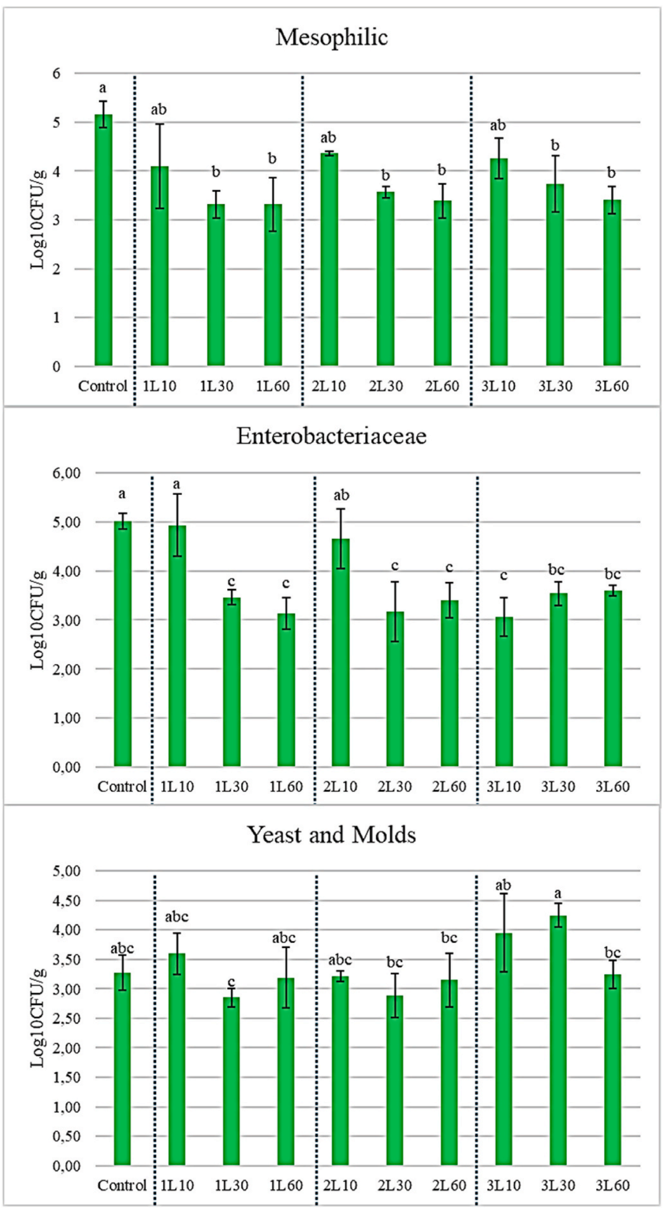

Fig. 1. Effect of Pulsed light treatment on mesophilic bacteria (A), enterobacteriaceae (B) and yeast and molds (C) in Control and in PL treated pistachio samples. Mean ± standard deviation bars are shown. Means with different letters are significantly different ($p < 0.05$) according to Tukey's least significant difference test. 1L10: 10 s treatment of one layer sample; 1L30: 30 s treatment of one layer sample; 1L60: 60 s treatment of one layer sample; 2L10: 10 s treatment of two layer sample; 2L30: 30 s treatment of two layer sample; 2L60: 60 s treatment of two layer sample; 3L10: 10 s treatment of three layer sample; 3L30: 30 s treatment of three layer sample; 3L60: 60 s treatment of three layer sample.

significantly decreased in samples treated for 30 and 60 s, regardless of number of layers (Fig. 1a): total count in Control sample was 5.16 ± 0.27 $\log_{10}$ CFU/g and had a reduction of 1.84 $\log_{10}$ CFU/g in the one layer sample treated for 30 and 60 s, 1.59 and 1.77 $\log_{10}$ CFU/g in the two layer sample treated for 30 or 60 s, respectively, and 1.42 and 1.75 $\log_{10}$ CFU/g in three layers sample for treatment duration of 30 and 60 s, respectively. Regardless of the treatment time, the greatest effectiveness in reducing the bacterial population occurred in the monolayer sample.

With reference to Enterobacteria (Fig. 1b), the untreated pistachio sample had a load of 5.02 $\log_{10}$ CFU/g; such value significantly decreased for one and two layer samples when exposure time to PL was maintained for 30 or 60 s; in one layer samples, Enterobacteria presence was reduced by 1.56 and 1.89 $\log_{10}$ CFU/g, respectively for exposure time of 30 and 60 s, and in two layer samples by 1.82 and 1.62 $\log_{10}$ CFU/g, respectively for exposure time of 30 and 60 s. In the three layer samples a significant decrease of Enterobacteria load was highlighted with PL treatment, particularly in sample treated for 10 s.

Fig. 1c shows that pulsed light did not have any effects on yeasts and molds, with the exception of the one layer sample treated for 60 s that had a fungal count reduction of 1.09 $\log_{10}$ CFU/g; indeed, the samples in three layers presented yeast and mold counts comparable with those of the Control, regardless of time of PL irradiation.

The effect of PL on the microbial inactivation has been explained by photophysical, chemical and thermal mechanisms (Elmnasser et al., 2007; Heinrich et al., 2015 and references therein; Santamera et al., 2020), which acting in parallel or in sequence make PL treatments more effective than UV treatment alone. The different levels of efficacy of PL obtained against the microbial groups under study are in line with previous studies, which have demonstrated that PL treatment is influenced by the microorganism, its intrinsic structure and cell dimension, population density, growth rate and phase. In general, the susceptibility of microorganisms to PL is higher for Gram-negative bacteria than Gram positive ones, because of their different cell wall structure. Furthermore, fungi seem to be more resistant than bacteria (Heinrich et al., 2015 and references therein), as confirmed in this study.

In a food processing plant, PL treatment can be conducted at various stages: for the decontamination of incoming raw materials, during processing for avoiding recontamination or for treatment of the final product prior or post-packaging (Fernández et al., 2009; Lyon et al., 2007; Rajkovic et al., 2010a, 2010b). While a direct comparison of decontamination efficiency of unpackaged to packaged products has been done for meats and associated products (Heinrich et al., 2015 and references therein), the extent of research studies dealing with nuts' decontamination is smaller and mainly focused on bacterial pathogens, such as *Salmonella* spp. (Gyawali et al., 2024; Harguindeguy & Gómez-Camacho, 2021); to the best of our knowledge, no study has been carried out on packaged nuts and on pistachio in particular.

3.2. Effect of pulsed light treatment on pistachio quality parameters

In Table 2 were reported the *p*-value for physico-chemical parameters determined on pistachio samples with respect to PL treatment duration x thickness x storage.

Table 3 reports the moisture content of the pistachio samples, and the quality parameters of the lipid fraction determined immediately after the PL treatment (T0) and after 12 months of storage (T12). Moisture is an important quality parameter for pistachio. The PDO "Pistachio verde di Bronte" fixed a moisture level between 4 % and 6 %. At T0 the Control sample had a moisture content of about 5.67 %, similar values were found in all pistachio samples independently from PL treatment time and thickness of the kernels in the trays. Izmirlioglu et al., 2020 reported an increase in walnut temperature from 3.8 to 28.5 °C after 1 and 15 s of PL treatment, respectively, carried out at a distance of 5 cm from the source, resulting in surface burns. Our data showed no change in moisture content with treatment, confirming a reduced temperature rise in the treated samples.

Table 2
Analysis of variance of the physical-chemical parameters of pistachio samples (*p*-values).

Variability factors	Degrees of freedom	Moisture	Total acidity	Peroxide value	Polyphenols	Total chlorophylls	External			Internal		
							L*	a*	b*	L*	a*	b*
Treatment (A)	3	0.769	0.000	0.000	0.000	0.002	0.541	0.149	0.080	0.127	0.000	0.000
Layer (B)	2	0.767	0.395	0.000	0.434	0.000	0.691	0.283	0.258	0.993	0.365	0.258
Storage (C)	1	0.000	0.002	0.028	0.000	0.000	0.000	0.000	0.000	0.705	0.000	0.000
A x B	6	0.828	0.187	0.000	0.000	0.000	0.000	0.029	0.196	0.998	0.184	0.036
A x C	3	0.494	0.099	0.000	0.000	0.000	0.038	0.027	0.159	0.706	0.000	0.000
B x C	2	0.030	0.162	0.000	0.630	0.000	0.022	0.407	0.769	0.638	0.107	0.044
A x B x C	6	0.577	0.098	0.000	0.001	0.000	0.021	0.008	0.340	0.000	0.003	0.001

Table 3
Moisture content and quality parameters of the oil extracted from pistachio seeds treated with PL immediately following the treatment (T0) and after 12 months (T12) of storage.

Samples	Moisture (%)	Total acidity (g/kg)	Peroxide value (meqO_2/kg)
T0			
Control	5.67 ± 0.98a	3.75 ± 0.02c	nd
1L10	6.16 ± 0.28a	4.21 ± 0.64c	nd
1L30	6.05 ± 0.41a	4.70 ± 0.00b	nd
1L60	5.99 ± 0.02a	3.72 ± 0.06c	nd
2L10	6.11 ± 0.21a	3.77 ± 0.02c	nd
2L30	5.73 ± 0.02a	4.71 ± 0.01b	4.44 ± 0.02c
2L60	5.57 ± 0.16a	4.69 ± 0.03b	nd
3L10	6.00 ± 0.28a	4.16 ± 0.57bc	5.47 ± 0.04b
3L30	5.70 ± 0.14a	5.67 ± 0.01a	6.31 ± 0.35a
3L60	6.30 ± 0.42a	4.71 ± 0.01b	4.00 ± 0.71d
T12			
Control	4.40 ± 0.34a	10.31 ± 0.94a	18.39 ± 0.49a
1L10	4.19 ± 0.20a	10.60 ± 1.36a	6.58 ± 0.81b
1L30	4.00 ± 0.35a	11.87 ± 1.43a	3.27 ± 0.55e
1L60	4.13 ± 0.31a	9.08 ± 1.99a	3.64 ± 0.31de
2L10	3.99 ± 0.20a	9.35 ± 0.02a	5.71 ± 1.11bcd
2L30	3.92 ± 0.31a	10.92 ± 2.18a	1.99 ± 0.49e
2L60	4.39 ± 0.01a	8.77 ± 1.12a	3.31 ± 0.58e
3L10	4.18 ± 0.53a	9.97 ± 0.55a	6.19 ± 0.31bc
3L30	4.39 ± 0.39a	9.70 ± 0.55a	6.78 ± 1.85b
3L60	4.26 ± 0.12a	10.56 ± 0.53a	4.04 ± 0.56cde

Means values in columns within storage time followed by different letters are significantly different (*p*-value <0.05).

As the shelf life of pistachios is approximately one year, untreated and treated pistachios were analysed after 12 months from treatment, to highlight the possible adverse effect of PL application on quality parameters with storage. After 12 months of storage, the moisture content of the control sample was about 4.40 %. Also, in this case pistachio samples treated with PL had similar moisture content to the untreated ones and no significant differences were found between samples. The lowest moisture levels determined in the stored samples were probably due both to a natural moisture evaporation which can normally occurred during storage (Fatemi et al., 2024; Tajeddin & Shakerardekani, 2022) mitigated by the permeability of the PET package (Shakerardekani & Karim, 2013). This trend was confirmed by the results of the two and three factors ANOVA, where storage significantly affected the moisture of pistachio as well as its interaction with layer.

As concern total acidity, both the PL treatment and storage had a significant impact on this parameter (Table 2). The initial (T0) total acidity of the Control pistachio samples was approximately 3.75 g/kg, according to the literature (Arena et al., 2013; Evrendilek et al., 2025). After application of PL, total acidity increases significantly in samples treated for 30 and 60 s, and samples treated in the triple layer appear to be more sensitive to this change (Table 2). After 12 months of storage, the total acidity increased up to 10.31 g/kg in the Control sample, but contrary to what was observed at T0, no significant difference was found between the Control sample and the treated pistachio samples, regardless of the PL treatment time and the thickness of the pistachio in the boxes. Similar acidity values were reported in pistachios stored at 20 °C (Arena et al., 2013). This trend suggests that even if the use of PL could induce an increase in this parameter, the treated samples were not more susceptible to an increase in total acidity during storage.

The peroxide value serves as an indicator of primary lipid oxidation. For the Control sample, no peroxide value was measured. In the T0 samples, this parameter exhibited an increase, beginning with the two layer treated samples subjected to extended treatment (4.44 meq O_2/kg). The greatest increase was observed in the three layer configuration, resulting in the highest peroxide value (5.4 meq O_2/kg). This suggests that the filling levels of pistachios in boxes subjected to PL treatment have an impact. Prolonged storage revealed a notable trend: treated pistachio samples demonstrated peroxide values ranging from 1.99 to 6.78 meq O_2/kg, which were significantly lower than the Control sample's peroxide value (18.39 meq O_2/kg). This indicates a protective effect of pulsed light against the rancidity process. Lower peroxide values were observed in samples treated for the longest duration (60 s), underscoring that the treatment provides a protective role during storage, minimizing peroxide formation and safeguarding the product from oxidative rancidity. The *p*-values reported in Table 2 confirm that the peroxide value is significantly affected by all three variability factors and their interactions, corroborating the observed findings.

Previous studies have shown that PL treatment was able to completely inactivate lipoxygenase (LOX), even when treatments were conducted at a distance greater than 5 cm from the light source and for durations as short as 8 s (Janve et al., 2014), and to inhibit lipid oxidation and generation of off-flavors in whole soybeans (Alhendi et al., 2018). Our findings confirm these results, and it appears that PL exerts a protective effect on pistachio kernels during storage. Notably, after 12 months of storage, the peroxide value is lower than that reported for comparable products (Bellomo et al., 2009).

3.2.1. *Total phenolic content (TPC)*

Pistachios are recognized by as one of the foods richest in antioxidant compounds. Mandalari et al. (2013), demonstrated that the polyphenols, xanthophylls and tocopherols contained in pistachios have a bio accessibility greater than 90 % during digestion. Therefore, it's important to preserve these bioactive compounds during drying and sanitization treatments.

Fig. 2 shows the effect of PL treatment and storage on TPC. At T0 Control sample showed a TPC about 391.78 mg GAE/100g of edible product. This value, also considering the crop year variability, is in accordance with previous studies (Ballistreri et al., 2009), or higher (Gentile et al., 2007) (349 and 175 mg GAE/100g, respectively). At T0, TPC was not influenced by application of PL (Fig. 2, T0). In fact, pistachios treated for the longest time showed a TPC similar to the untreated ones with the exception of 1L60 sample. It has been shown how, at a fluence of 3.82 J/cm^2, the PL does not cause a degradation of more than 10 % of gallic acid (Wiktor et al., 2019) and this suggests that PL can be used on foods without considerably affecting their antioxidant properties (Avalos-Llano et al., 2018; Boateng et al., 2024).

After 12 months of storage (T12), a notable increase, respect to T0, of TPC in all pistachio samples was observed. In Control samples TPC increased about 1.44 fold respect to T0; in PL treated samples Samples

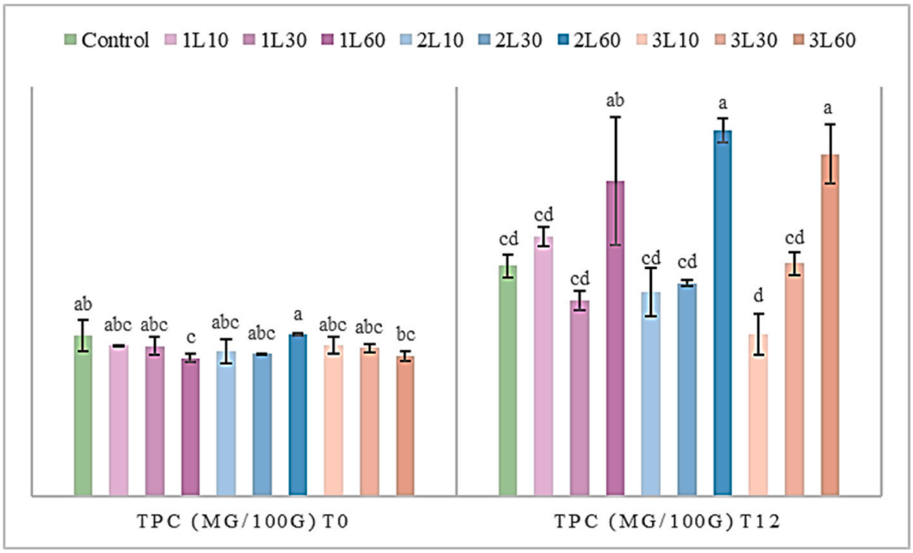

Fig. 2. Effect of PL treatment and storage on TPC of pistachio samples. Mean ± standard deviation bars are shown. Means belonging to T0 or T12 with different letters are significantly different ($p < 0.05$) according to Tukey's least significant difference test. T0 immediately after treatment, T12 12 months after PL treatment; Control: untreated samples; 1L10: 10 s treatment of one layer sample; 1L30: 30 s treatment of one layer sample; 1L60: 60 s treatment of one layer sample; 2L10: 10 s treatment of two layer sample; 2L30: 30 s treatment of two layer sample; 2L60: 60 s treatment of two layer sample; 3L10: 10 s treatment of three layer sample; 3L30: 30 s treatment of three layer sample; 3L60: 60 s treatment of three layer sample.

PL treated for 60 s showed the highest significant rise of TPC respect to the Control sample. Similarly, Hosseini et al. (2019) reported a higher TPC in soft-shell UV-C treated pistachios than the Control samples. This trend was probably caused by the activation of seed defence mechanisms in response to abiotic or biotic stress (Rivera-Pastrana et al., 2014). This positive effect could contribute to limit the rancidity process as observed in treated samples after 12 months of storage. *p*-value reported in Table 2 indicated that both PL treatment and storage significantly affected TPC, as well as their interaction. Also, for this parameter the thickness of pistachio in the boxes had no effect as well as its interaction with storage.

3.2.2. Chlorophylls

One of the quality parameters of pistachio kernels, particularly of the Bronte Pistachio PDO is the green colour. In fact, the a/b chlorophyll ratio, which must be higher than 1.3, has been included in the product specification (Official Journal of the European Union, 2016). Analogously to other food products, a PL treatment could lead to a change of colour due to the loss of pigments. Mohammadi et al. (2023), shown a decrease of anthocyanins in wine when treated at fluence upper than 2–4 J/cm^2, Tao et al. (2019) showed that lettuce treated with PL, at fluence of 4.0–12.5 J/cm^2, lost between 4.60 and 23.04 % of chlorophyll *a*, and 11.98–32.95 % of chlorophyll *b*, respectively, causing a variation in the colour of the product and the decrease in the colorimetric coordinates a* and b*.

The total chlorophylls levels determined on pistachio samples were significant to all the factors of statistic variability and their interaction (Table 2). In Control sample the total chlorophylls content at T0 was approximately 37.1 mg/kg. The PL treatment did not induce a significant change between Control and PL treated pistachios both on total and chlorophyll *a and b* content. Previous studies reported that chlorophyll *b* is thermally more stable than chlorophyll *a.* The degradation of this pigment is 12–18 times faster than that chlorophyll *b* (Pareek et al., 2017). At T0, the ratio fell within the acceptable range both for the Control sample and certain treated samples, while remaining near the threshold in the other samples.

After 12 months of storage (Table 4) the untreated pistachio showed the lowest levels of total chlorophylls (26.1 mg/kg). The trend in the content of total, chlorophylls *a and b*, as already observed at T0, was similar in all the samples after 12 months of storage and the a/b ratio ranging from 0.7 to 1.1, except for 2L10 s treatment sample (1.6). In any case all samples, even the Control, were out of the PDO limit, suggesting that the use of PL processing technology did not have a negative impact on this quality parameter during storage.

Table 4
Chlorophyll content pistachio seeds treated with PL immediately following the treatment (T0) and after 12 months (T12) of storage.

Samples	Total chlorophylls (mg/kg)	Chlorophyll *a* (mg/kg)	Chlorophyll *b* (mg/kg)	a/b ratio
T0				
Control	37.1 ± 9.0bc	21.2 ± 4.5 ab	15.8 ± 4.8bc	1.4
1L10	36.0 ± 3.3bc	20.6 ± 2.4 ab	15.4 ± 1.4c	1.3
1L30	53.3 ± 4.9a	28.7 ± 4.5a	24.7 ± 1.6a	1.2
1L60	48.2 ± 9.6 ab	24.1 ± 4.7 ab	24.1 ± 5.0 ab	1.0
2L10	31.6 ± 2.1c	19.2 ± 2.5b	12.5 ± 1.2c	1.5
2L30	35.2 ± 6.7bc	18.8 ± 3.0b	16.5 ± 3.8abc	1.2
2L60	32.2 ± 6.3c	17.8 ± 3.4b	14.4 ± 3.0c	1.2
3L10	27.3 ± 6.8c	17.6 ± 2.8b	11.7 ± 4.2c	1.8
3L30	28.6 ± 7.1c	16.9 ± 2.5b	11.6 ± 4.8c	1.4
3L60	35.5 ± 4.7bc	18.3 ± 2.7b	17.2 ± 2.7abc	1.1
T12				
Control	26.1 ± 5.7b	12.7 ± 2.1cd	13.9 ± 4.2bc	0.9
1L10	33.9 ± 5.4b	17.9 ± 4.1bc	16.0 ± 1.3bc	1.1
1L30	32.3 ± 2.4b	12.8 ± 2.3cd	19.6 ± 4.8b	0.8
1L60	28.4 ± 3.9b	13.2 ± 1.7cd	15.2 ± 3.4bc	0.9
2L10	35.1 ± 4.3b	19.9 ± 1.5b	12.1 ± 3.4bc	1.6
2L30	22.3 ± 8.8bc	9.9 ± 2.6d	15.5 ± 6.3bc	0.7
2L60	64.8 ± 7.1a	30.1 ± 2.4a	34.8 ± 6.2a	0.9
3L10	28.6 ± 6.9b	10.7 ± 0.5c	16.0 ± 6.8b	0.8
3L30	8.1 ± 2.4c	3.7 ± 1.5e	4.5 ± 3.0c	0.8
3L60	18.1 ± 5.7bc	12.9 ± 2.4cd	16.3 ± 1.3bc	0.8

Means values in columns within storage time followed by different letters are significantly different (*p*-value <0.05).

3.2.3. Colorimetric analysis

Table 5 reports the colorimetric parameters determined on the external surface of pistachio samples. Data showed that the PL treatment did not induce significant changes in the L*a*b*colour parameters, immediately after the treatment (T0). Only 2L60 showed a L* value (30.99 ± 2.95) significantly lower than Control sample, but similar to

Table 5
Colorimetric values determined on the external surface of pistachio seeds at different time of storage.

Samples	L*	a*	b*
T0			
Control	38.94 ± 5.61a	15.35 ± 4.52a	6.28 ± 2.93 ab
1L10	34.59 ± 4.66 ab	16.11 ± 4.63a	5.53 ± 2.81 ab
1L30	36.34 ± 7.50 ab	12.22 ± 2.9a	6.07 ± 2.3 ab
1L60	36.46 ± 5.05 ab	12.27 ± 3.63a	8.15 ± 3.33a
2L10	36.77 ± 5.87 ab	12.50 ± 4.76a	6.89 ± 2.20 ab
2L30	37.62 ± 7.47 ab	13.72 ± 5.00a	6.86 ± 2.68 ab
2L60	30.99 ± 2.95b	14.54 ± 5.13a	4.24 ± 1.55b
3L10	40.05 ± 7.54a	12.35 ± 5.68a	7.45 ± 3.60 ab
3L30	34.88 ± 5.08 ab	15.22 ± 3.57a	6.41 ± 3.63 ab
3L60	39.46 ± 6.83a	13.95 ± 6.24a	7.78 ± 2.88a
T12			
Control	41.76 ± 8.21b	8.81 ± 3.41 ab	9.21 ± 4.44 ab
1L10	39.73 ± 9.13b	8.18 ± 3.70 ab	8.20 ± 5.60 ab
1L30	45.20 ± 10.50b	9.40 ± 3.20a	8.90 ± 5.50 ab
1L60	45.80 ± 11.10 ab	11.30 ± 4.70a	10.00 ± 8.00 ab
2L10	53.90 ± 10.50a	5.72 ± 3.00b	6.95 ± 4.20b
2L30	42.97 ± 10.00b	9.30 ± 3.70a	10.50 ± 6.40 ab
2L60	42.20 ± 9.10b	9.60 ± 3.40a	10.90 ± 5.30 ab
3L10	41.90 ± 9.50b	10.40 ± 4.00a	9.70 ± 5.70 ab
3L30	41.40 ± 7.40b	10.40 ± 4.30a	8.50 ± 5.00 ab
3L60	44.10 ± 8.80b	8.80 ± 3.40 ab	12.90 ± 7.50a

Means values in columns within storage time followed by different letters are significantly different (*p*-value <0.05).

Table 6
Colorimetric values determined on the internal section of pistachio seeds at different time of storage.

Samples	L*	a*	b*
T0			
Control	72.87 ± 4.88 ab	−6.39 ± 2.83 ab	32.83 ± 4.72 ab
1L10	74.45 ± 2.45 ab	−6.88 ± 1.81b	33.34 ± 5.21a
1L30	73.99 ± 2.4 ab	−5.57 ± 2.45 ab	28.7 ± 6.72 ab
1L60	70.87 ± 2.80b	−6.39 ± 1.78 ab	29.92 ± 5.81 ab
2L10	71.92 ± 2.27 ab	−6.50 ± 1.35 ab	31.52 ± 5.27 ab
2L30	73.21 ± 2.64 ab	−5.65 ± 1.94 ab	29.08 ± 5.88 ab
2L60	75.50 ± 3.14a	−5.00 ± 1.83 ab	29.05 ± 4.69 ab
3L10	73.45 ± 3.91 ab	−5.75 ± 1.93 ab	30.76 ± 6.98 ab
3L30	74.01 ± 2.71 ab	−4.32 ± 1.55a	26.69 ± 5.44b
3L60	71.62 ± 3.64b	−6.28 ± 2.49 ab	32.06 ± 5.65 ab
T12			
Control	72.22 ± 4.44a	−3.21 ± 1.82abc	22.28 ± 5.19bcd
1L10	72.90 ± 5.60a	−2.77 ± 1.80 ab	17.10 ± 8.26cd
1L30	73.80 ± 5.30a	−2.60 ± 2.40 ab	16.60 ± 7.20cd
1L60	74.80 ± 3.00a	−4.10 ± 2.30bc	23.20 ± 7.00bc
2L10	74.70 ± 4.70a	−2.10 ± 1.74 ab	15.00 ± 9.20d
2L30	74.60 ± 4.30a	−2.90 ± 1.80abc	20.80 ± 6.60bcd
2L60	70.40 ± 4.20a	−7.00 ± 1.80d	33.20 ± 3.80a
3L10	72.70 ± 3.80a	−3.40 ± 1.60abc	21.80 ± 5.80bcd
3L30	74.10 ± 6.00a	−1.60 ± 1.40a	18.80 ± 6.80bcd
3L60	74.80 ± 4.40a	−4.90 ± 1.80cd	24.90 ± 5.80b

Means values in columns within storage time followed by different letters are significantly different (*p*-value <0.05).

the other PL treated samples. This difference could be attributed more to the non-uniformity of the external skin of the pistachio seed, rather than the PL treatment. A similar trend was observed after 12 months of storage: pistachio samples both untreated and PL treated showed no significant differences on colorimetric parameters. *p*-value reported in Table 2 indicated that only storage significantly affected L*a* and b* parameters determined on the surface of the pistachio seed; the interaction between treatment and layer and storage significantly affected the parameters L* and a*.

The a* and b* color parameters determined on the internal section of pistachio samples were significant to treatment and storage factors of variability and for their interaction for a*, while for b* parameters also interaction with layer were significant (Table 2). L* parameter was significant only for the interaction of the three factors of variability (Table 2). Colour parameters determined on the internal section of the pistachio seed samples were similar and no significant differences were highlighted regardless of the treatment, layer and storage (Table 6). In the last case only 2L60 showed a* and b* values significantly different from the Control sample. These results could be explained with the highest levels of chlorophylls *a and b* determined in this sample (Table 4).

4. Conclusions

The present study demonstrates for the first time the efficacy of pulsed light (PL) on packaged pistachio kernels, even at different fill levels. The treatment effectively improved the microbiological safety of the product. Specifically, PL treatment significantly reduced the total mesophilic and Enterobacteria load after 30 and 60 s of exposure, particularly in single-layer samples. However, no effects were observed on molds and yeasts, likely due to the limited penetration of UV rays and the higher resistance of these microorganisms to pulsed light.

PL treatment, applied at different exposure times (10, 30, and 60 s) showed minimal or no adverse effects on moisture, total polyphenols, colour and chlorophylls. Furthermore, the treatment appeared to exert a protective effect on the lipid fraction, which was maintained for up to 12 months of storage.

CRediT authorship contribution statement

Sebastiano Maria Caldarella: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Antonia Grasso:** Investigation, Formal analysis, Data curation. **Cristina Restuccia:** Writing – review & editing, Writing – original draft, Validation, Investigation, Conceptualization. **Biagio Fallico:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Elena Arena:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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