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Electrolyzed water and gaseous ozone application for the control of microbiological and insect contamination in dried lemon balm: Hygienic and quality aspects

Paola Dolci [a,*], Barbara Letizia Ingegno [a], Elena Mangia [a], Daniela Ghirardello [a], Lucia Zaquini [b], Selena Costarelli [a], Luciana Tavella [a], Sylvain Perrot [c], Bert Candaele [c], Olivier Bagarri [d], Elena Cerutti [b], Giuseppe Zeppa [a]

[a] Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University of Torino, Grugliasco, Torino, Italy
[b] Associazione Le Terre dei Savoia, Racconigi, Torino, Italy
[c] Centre Régionalisé Interprofessionnel d'Expérimentation en Plantes à Parfum Aromatiques et Médicinales (CRIEPPAM), Manosque, France
[d] Université européenne des Saveurs et des Senteurs, Forcalquier, France

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ABSTRACT

In this study, the sanitization efficacy of decontamination techniques was evaluated on lemon balm (*Melissa officinalis*). Dried aromatic herbs are minor food components with widespread use and, despite their low water activity, they are often contaminated with microorganisms, including toxigenic and pathogenic ones. Gaseous ozone (GO) and electrolyzed water (EW) treatments were applied on lemon balm after and before drying (traditional at 40 °C and cold at 20 °C), respectively. Microbiological and entomological decontamination aspects were assessed as well as the sensory quality and compositional properties of the product in terms of essential oil, total polyphenol and ascorbic acid contents, and antioxidant capacity. The most interesting results concerned EW treatment (400 ppm × 1 min dipping) reducing aerobic mesophilic bacteria, *Enterobacteriaceae*, moulds and yeasts of about 4.0, 2.5, 2.0 and 1.0 Log cfu/g, respectively, thus below law limits. Moreover, 200 ppm EW x 1 min dipping was enough for limiting *Bacillus cereus* growth. However, sanitization against the beetle species *Tribolium castaneum* and *Lasioderma serricorne* was less efficient with EW (less than 40% mortality) compared with GO (200,000 ppm × min) (more than 95% mortality). Noteworthy, sensory analysis highlighted that lemon balm exposed to EW treatment did not lose organoleptic quality, in particular the color even seemed to improve in brightness when the herbs were subsequently cold dried. Furthermore, the loss in essential oils was not significant in terms of the overall content as well as total polyphenol, ascorbic acid and antioxidant capacity in EW treated lemon balm then submitted to cold drying. Differently, the combination of EW treatment and traditional drying led to a significant detriment of compositional properties. The results obtained in this study are encouraging and deserve to be discussed with producers.

1. Introduction

Dried aromatic herbs (DAH) are minor food components with widespread use. They are receiving attention of researchers because, despite their low water activity, they are often contaminated with microorganisms, including pathogenic and toxigenic ones. That means they could be a potential health hazard when added to ready-to-eat meals or used for herbal teas (Kazi et al., 2017; Schaarschmidt et al., 2016). DAH contamination may be caused by natural epiphytic microbiota as well as by microorganisms occurring and developing during harvesting, drying, transporting and storing (Oner, 2017).

Microbial contaminants can cover fungi as well as bacteria, including both spore forming pathogens like *Bacillus cereus* and *Clostridium perfringens* (Akbas & Ozdemir, 2008; Fogele et al., 2018; Sagoo et al., 2009; Schaarschmidt et al., 2016), and non-spore formers like *Salmonella* spp. (Torlak et al., 2013; Wojcik-Stopczyńska et al., 2009). The Panel on Biological Hazards (BIOHAZ) of the European Food Safety Authority (EFSA) evaluated the microbiological risk related to foods of non-animal

* Corresponding author.
E-mail address: paola.dolci@unito.it (P. Dolci).

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origin in the EU, and ranked dried herbs and spices in combination with *Salmonella* spp. and *Bacillus* spp. within the top four food-pathogen combinations (EFSA BIOHAZ, 2013). Regarding microbiological safety concerns in low-moisture foods, DAH (and spices) were ranked in the top three (FAO & WHO, 2014).

Despite the real and probably underestimated risk related to these food components, no mandatory microbiological criteria specific for DAH are laid down by EU law. The criteria adopted to assess the safety and microbiological quality of DAH refer mainly to the European regulation Rec. 2004/24/EC concerning a coordinated program for the official control of foodstuffs, such as spices (Annex III, Section I, Bacteriological safety of spices). The recommended values concern *Enterobacteriaceae*, *Salmonella* spp., presumptive *B. cereus* and *C. perfringens*.

DAH can be also subject to damage and contamination by insect pests. DAH are harvested, dried and stored for various time before subsequent processing (Šveistytė et al., 2016), with infestations reported from various parts of the world (Mohapatra et al., 2015; Platt et al., 1998). Insect activity can cause a great deal of economic loss affecting the entire system and, ultimately, the consumer (Guo et al., 2019; Kenkel et al., 1994). Among insect pests reported on DAH, the cigarette beetle *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) by eating the stored products and defecating allows contamination with fungi and bacteria (Guo et al., 2019). In addition, the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) can cause serious economic losses and be harmful to human health, since it produces an unpleasant odor due to the secretion of benzoquinones from the abdominal glands, also compromising the taste of the attacked substrate (Hodges et al., 1996).

In order to limit microbiological hazards and entomological contamination, effective sanitization should be carried out and side effects minimized. DAH manufacturers should ensure maximum safety and quality of the product and, contemporary, preservation of valuable properties (antioxidant activity, polyphenol, total ascorbate and essential oil content) and sensory attractiveness in terms of color and aroma. Many physical and chemical treatments have been tested to sanitize herbs but, currently, there is no a uniform procedure and no single method has been shown to completely eliminate biological risks without affecting product quality (Issa-Zacharia et al., 2011).

Thus, the aim of this study was to analyze the effectiveness of decontamination techniques on lemon balm (*Melissa officinalis*) to improve the safety of the product before commercialization, without negatively affecting the quality. Lemon balm was chosen for its importance in organic farming in Piedmont region (northwestern Italy), with 20 tons/year (2018) of fresh herbs cultivated for drying (market survey, AlcotraItalia, 2014-2020).

In particular, electrolyzed water (EW) and gaseous ozone (GO) treatments were applied on fresh and dried lemon balm, respectively. The literature shows that GO treatments are the most promising, achieving high degrees of reduction on microbiological contamination (Kazi et al., 2017; Niveditha et al., 2021; Pandiselvam et al., 2019; Sivaranjani et al., 2021; Torlak et al., 2013), but data on the biological and sensorial properties of the processed DAH are few. GO treatment efficacy for controlling stored product insects has been investigated in several studies, however, experimental conditions, such as exposure time, ozone concentration and modalities of exposure, varied highly (Bonjour et al., 2011; Hansen et al., 2012, 2013; Ingegno & Tavella, 2022; Işikber & Öztekin, 2009; Kells et al., 2001; Mahroof et al., 2018; Tiwari et al., 2010; Yoshida, 1975). Available toxicity data for gaseous ozone treatments indicate a remarkable difference in susceptibility between insect species, life stages, and application times (Isikber & Athanassiou, 2015). Thus, we decided to focus on a fixed ozone concentration (100 ppm) at different exposure times to control two widely investigated species affecting the dried aromatic herb production chain.

Some authors have applauded EW as a potential and emerging nonthermal food sanitizer, included fruit and vegetable surfaces (Al-Haq et al., 2005; Issa-Zacharia et al., 2011). Scientific literature points out its potentiality for bacterial containment while studies evaluating the efficacy against fungi are few (Audenaert et al., 2012; Lyu et al., 2018) and often limited to *in vitro* studies (Lemos et al., 2020). To our knowledge, no studies have been yet published on EW efficacy on insect pests.

2. Materials and Methods

2.1. Microbiological quality of fresh lemon balm and plant material collected for sanitization assays

Lemon balm was provided by two farmers located in Piedmont region (northwestern Italy) and harvested in 2018 and 2019, during summer and autumn time (Fig. 1). Two harvests per season were

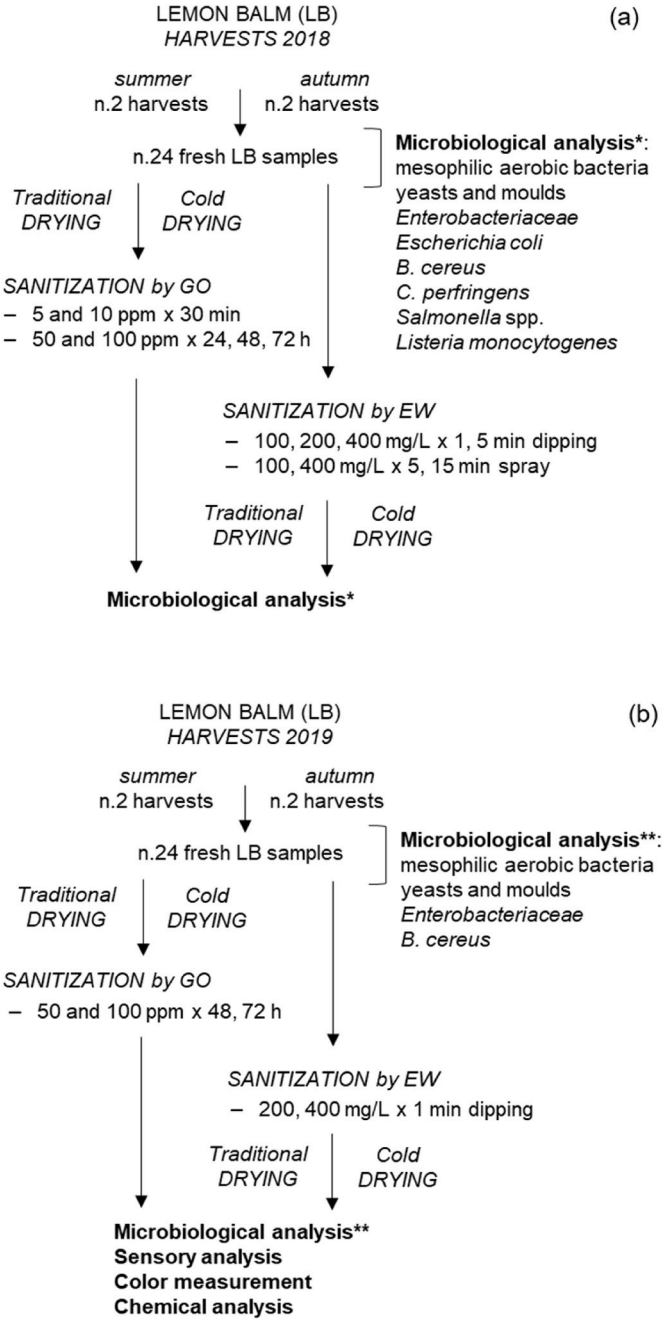

Fig. 1. Experimental setup of lemon balm sanitization treatments carried out in 2018 and 2019: preliminary trials (a) and application of optimized sanitization parameters (b).

managed and, after harvesting, fresh plant material (6 samples for harvest, totally 24 samples/year) was immediately transferred to the laboratory and submitted to microbiological analysis. The general level of contamination was specifically assessed for fresh lemon balm samples collected in 2018, with reference to European and Italian law limits (Racc. 2004/24/CE and D. D n.780, 2011), for the following microbial groups: mesophilic aerobic bacteria on Plate Count Agar (PCA, Oxoid, Milan, Italy) at 30 °C for 48 h; yeasts and moulds on Dichloran Glycerol Agar (DG18, Oxoid) at 25 °C for 96 h; *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBG, Oxoid) at 37 °C for 24 h; *Escherichia coli* on Tryptone Bile X-Gluc Agar (TBX, Oxoid) at 44 °C for 24 h; *B. cereus* on PEMBA (Oxoid) at 30 °C for 24 h; *C. perfringens* on Sulfhite Polymyxin Sulphadiazine Agar (SPS, Oxoid) at 37 °C for 24 h; *Salmonella* spp. on Xylose Lysine Desoxycholate Agar (XLD, Oxoid) and Brilliant Green Agar (BGA, Oxoid) at 37 °C for 24 h; *Listeria monocytogenes* on PALCAM (Oxoid) at 37 °C for 24 h. Three independent measurements were carried out for each analysis. Means and standard deviations were calculated. Based on the average microbiological quality of lemon balm observed in 2018, the microbial groups chosen as target for trials in 2019 were mesophilic aerobic bacteria, yeasts and moulds, *Enterobacteriaceae* and *B. cereus* (see Fig. 1).

2.2. Sanitization techniques

The sanitization treatments applied on lemon balm samples were gaseous ozone (GO) and electrolyzed water (EW). GO was used on herbs after drying while EW on fresh herbs before drying. Lemon balm was submitted to both traditionally drying, in oven (Memmert UF110, Schwabach, Germany) at 40 °C for 24 h, and cold drying by using a NWT-100 system (North West Technologies, Cuneo, Italy) that works through the dehumidification of plant material at low temperatures (20 °C for 48 h) and subsequent condensation of the extracted water. The time of the dehydration processes was chosen in order to reach a residual water content of the dried herbs lower than 12%, starting from an initial moisture content of approximately 78%.

2.2.1. Gaseous ozone

The system for GO treatment included (1) an oxygen condenser tank (model LM-5 Biofresh, Stocksfield, UK) concentrating the oxygen from the environment, (2) an ozone generator (model LM-5 Biofresh) producing ozone by means of a corona discharge and (3) a 63 L airtight testing chamber (440 × 640 × H 480 mm) (Melform AF7, Savigliano, CN, Italy) where the ozone was conveyed to the herbs on 3 perforated trays (approximately 200 g for each tray). The GO was injected into the chamber from below with 4 g/h flow rate; the time requested for chamber saturation was less than 1 min even for the highest concentration of 100 ppm used in this study. The GO concentration inside the chamber was maintained uniform by a fan, and measured and adjusted by an ozone sensor (Analyzer UV-100, Eco Sensors, Santa Fe, New Mexico, USA) monitoring the ozone-enriched air.

2.2.2. Electrolyzed water

EW solutions were produced by using an EVA SYSTEM® 100 equipment (Industrie De Nora S. p.A., Milan, Italy) according to the electrolysis method described by Cravero et al. (2016). The free chlorine concentration obtained in EW solution was 4000 mg/L at ≈ pH 9, as determined by iodometric titration (APHA, 1992). The starting solution was diluted with deionized water, and three different EW concentrations were freshly prepared before each experiment: 100, 200 and 400 mg/L of free chlorine solution checked by iodometric titration (APHA, 1992).

2.3. Experimental plan and optimization of sanitization parameters

Different parameters, in terms of treatment time and concentration of GO and EW solution, were initially tested on lemon balm samples collected in 2018 (2.3.1). Based on the results obtained from these preliminary trials, the most promising sanitization parameters were selected and used on samples collected in 2019 (2.3.2). The diagram of the experimental setup is shown in Fig. 1.

2.3.1. Optimization of GO and EW parameters: preliminary trials

Immediately after traditional and cold drying, lemon balm samples were placed into airtight chamber and subjected to GO treatment. The following GO concentration and treatment time combinations were tested: (a) 5 ppm × 30 min, (b) 10 ppm × 30 min, (c) 50 ppm x 24, 48 and 72 h, (d) 100 ppm x 24, 48 and 72 h. In order to evaluate the efficiency of GO treatments, microbiological analyses were carried out initially on fresh herbs, then on traditionally and cold dried samples, and, finally, immediately after GO treatment of dried lemon balm. The target microbial groups are reported in paragraph 2.1. Three independent measurements were carried out for each sample. Means and standard deviations were calculated.

Regarding the treatment with EW, two methodological approaches were used: (1) plant material (500 g) was dipped in trays (50 × 50 × 10 cm) containing 10 L of EW solutions; (2) 1 L EW solutions were sprayed onto plant material (250 g) placed in trays (100 × 50 × 2 cm) until leaf surfaces were abundantly moistened. The following parameters were used for method (1): (1a) 100 mg/L of free chlorine solution x 1 and 5 min stirring, (1b) 200 mg/L x 1 and 5 min stirring, (1c) 400 mg/L x 1 and 5 min stirring; and for method (2): (2a) 100 mg/L x 5 and 15 min spray, (2b) 400 mg/L x 5 and 15 min spray. Immediately after the treatments, plant material was rinsed by dipping twice in deionized sterile water for totally 2 min. In order to check the efficiency of EW treatments, microbiological analyses were performed initially on fresh herb samples, then immediately after EW treatment and water rinsing, and, finally, after traditional and cold drying of EW sanitized herbs. The microbial counts were compared with the values obtained from lemon balm samples treated exclusively with deionized water (negative control) by using both dipping and spraying methods. The target microbial groups are reported in paragraph 2.1. Three independent measurements were carried out for each sample. Means and standard deviations were calculated.

2.3.2. Application of optimized sanitization parameters on lemon balm

Based on the results obtained from preliminary trials, the following GO and EW parameters were selected and used on lemon balm harvested in summer and autumn 2019 (two harvests for season, 6 samples for harvest, totally 24 samples): (a) 50 ppm x 48 and 72 h, (b) 100 ppm x 48 and 72 h for GO treatment; (c) 200 mg/L x 1 min stirring, (d) 400 mg/L x 1 min stirring for EW dipping treatment. The microbiological analyses of herbs treated with GO were performed initially on fresh samples (FLB), then on traditionally (TLB) and cold dried (CLB) samples, and, finally, immediately after GO treatment of traditionally (TLBO) and cold (CLBO) dried herb samples (Table 1). Regarding EW treatment, the microbiological analyses of the herbs were performed initially on fresh samples (FLB), then immediately after EW treatment and subsequent deionized water rinsing (LBE), and, finally, after traditional (LBET) and cold drying (LBEC) of the EW sanitized herbs (Table 2). The microbial counts were compared with the values obtained from herb samples treated exclusively with deionized water (negative control). Based on the average microbiological quality of lemon balm observed in 2018, the microbial groups chosen as target were mesophilic aerobic bacteria, yeasts and moulds, *Enterobacteriaceae* and *B. cereus*. Three independent measurements were carried out for each sample. Means and standard deviations were calculated.

2.4. Sensory analyses

Sensory analyses were performed on lemon balm samples harvested in 2019 and submitted to both GO and EW treatments according to the optimized sanitization parameters (2.3.2). A 'duo-trio' overall difference test (ISO 10399, 2017) was performed by a group of 20 trained panelists,

Table 1
Mean microbial counts (±SD) of lemon balm (LB) samples treated with 50 and 100 ppm of gaseous ozone for 48 h and 72 h after cold or traditional drying, compared to LB samples treated with deionized water (negative control).

Sample	Mesophilic aerobic bacteria	*Enterobacteriaceae*	Moulds	Yeasts	*B. cereus*
FLB	7.1 ± 0.2	5.3 b ± 0.8	5.7 ± 0.7	5.1 b ± 0.1	2.7 ± 0.3
TLB	6.9 ± 0.2	5.1 b ± 0.3	5.4 ± 0.2	5.3 b ± 0.2	2.6 ± 0.4
TLBO 5048	6.5 ± 0.1	4.4 a ±0.2	5.5 ± 0.1	5.0 b ± 0.2	2.7 ± 0.2
TLBO 5072	7.0 ± 0.8	5.8 c ± 0.1	5.6 ± 0.3	4.5 a ±0.4	2.5 ± 0.2
	ns	***	ns	*	ns
FLB	7.3 b ± 0.2	6.3 ± 0.4	5.5 b ± 0.5	4.8 b ± 0.3	2.7 b ± 0.3
TLB	6.9 b ± 0.5	6.1 ± 0.1	5.6 b ± 0.5	4.9 b ± 0.4	2.6 b ± 0.4
TLBO 10048	7.4 b ± 0.1	5.9 ± 0.4	5.7 b ± 0.2	3.6 a ±1.3	2.8 b ± 0.2
TLBO 10072	**5.0 a ± 0.8**	6.0 ± 0.1	**4.8 a ± 0.3**	3.8 a ±1.6	**2.2 a ± 0.3**
	***	ns	**	***	**
FLB	7.7 ± 0.4	5.9 ± 0.8	5.2 ± 0.6	5.3 b ± 0.4	2.4 b ± 0.5
CLB	7.3 ± 0.6	6.0 ± 0.3	5.7 ± 0.2	5.1 b ± 0.2	2.3 b ± 0.4
CLBO 5048	7.8 ± 0.2	6.0 ± 0.3	5.9 ± 0.1	5.3 b ± 0.2	2.0 a ±0.0
CLBO 5072	7.4 ± 0.2	5.3 ± 0.7	5.8 ± 0.1	4.8 a ±0.1	2.5 b ± 0.1
	ns	ns	ns	**	**
FLB	7.9 ± 0.2	5.8 b ± 0.9	5.3 a ±0.5	5.4 b ± 0.2	2.4 ± 0.7
CLB	7.5 ± 0.3	6.0 b ± 0.4	5.8 b ± 0.2	5.3 b ± 0.4	2.4 ± 0.4
CLBO 10048	6.6 ± 0.6	5.9 b ± 0.4	5.4 ab ± 0.1	**1.0 a ± 0.0**	2.3 ± 0.3
CLBO 10072	7.1 ± 1.1	**5.2 a ± 0.7**	5.3 a ±0.4	**1.0 a ± 0.0**	2.3 ± 0.5
	ns	*	*	***	ns

*P < 0.05 **P < 0.01 ***P < 0.001.
FLB: fresh LB.
T/C: traditional/cold drying.
O: gaseous ozone treatment.
50/100 and 48/72: gaseous ozone concentration (ppm) and treatment time (h).

aged 20 to 65 and in a 12:8 female: male ratio, recruited from the staff of the University of Turin, using an $\alpha = 0.05$ and a $\beta = 0.05$. The three samples were arranged side by side in glass cups.

2.5. Color measurement

Color was evaluated on EW treated lemon balm samples according to the optimized parameters (2.3.2). Differences were evaluated between herbs treated by dipping in EW and deionized water (Table 3, negative control), and between herbs traditionally and cold dried following EW dipping. In order to identify perceivable color differences among the herbs, the CIE L*a*b* coordinates (where L* indicates lightness, a* the red/green coordinate, and b* the yellow/blue) were used (Śledź et al., 2013). The color was measured on ground herbs using a CM-5 spectrocolorimeter (Konica Minolta, Tokyo, Japan) on transmittance and in Specular Component Excluded (SCE) mode, using a circular measuring area of 8 mm in diameter. The CIE L*a*b* parameters were used to calculate the total difference between two colors, Delta E (ΔE*), by the CIE 1976 formula (Robertson, 1977):

$$\Delta E^{*}_{Lab} = \left[(\Delta L^{*})^2 + (\Delta a^{*})^2 + (\Delta b^{*})^2\right]^{1/2}$$

As reported by Mokrzycki and Tatol (2011), $2 < \Delta E < 3.5$ values suggest that unexperienced observer notices the difference, while a clear difference in color is noticed at ΔE*higher than 3.5.

2.6. Chemical analyses

2.6.1. Essential oil content

In order to evaluate a possible impact of EW treatments on the composition and quantity of essential oils, hydro-distillation of lemon balm samples was performed with a distillation apparatus (lab scale), according to the method described in ISO 6571–2008. For this purpose, 100 g of lemon balm leaves were placed in a 2000 mL round bottom flask and 800 mL of tap water were added. Extractions were carried out for 2 h. Three independent extractions were carried out for each sample. After distillation, the quantity of oil was read in the graduated tube of the apparatus (3 mL, divisions of 0.02 mL) and calculated per 100 g of distilled dry matter by placing 3 samples of 30 g each in an oven at 105 °C for 24 h. The essential oils were collected in 2.5 mL bottles and stored in the dark at 4 °C before sending to Pyrénessences Analyses Laboratory (Belcaire, France). The essential oils were analyzed by gas chromatography coupled to mass spectrometry (GC–MS) according to the general guidance NF ISO 11024 standard. The amount of the main essential oil components has been expressed as percentage (Table 4): the area of all identified peaks, corresponding to each component, was summed and made equal to 100. Each component was reported as percentage of its peak on the total.

The essential oil analysis was carried out on lemon balm samples to compare their content after EW treatment and subsequent cold drying (LBE4C) with the one after deionized water treatment and subsequent cold drying (negative control, LBWC), and, finally, with the samples subject to cold drying only (blank, LBC) (Table 4); furthermore, oil content was compared in herbs after EW treatment and subsequent traditional drying (LBE4T) with the one after deionized water treatment and subsequent traditional drying (negative control, LBWT), and, finally, with the samples subject to traditional drying only (blank, LBT) (Table 4). In addition, 6 independent lemon balm fresh samples were analyzed for their average content in essential oil.

2.6.2. Total polyphenols and ascorbic acid content, antioxidant capacity, residual chlorate/perchlorate

Lemon balm bioactive extracts were analyzed for total polyphenol content and antioxidant capacity ($DPPH^{\bullet}$ scavenging activity). In addition, in order to evaluate the effect of EW treatment and drying techniques on antioxidant bioactive compounds, total ascorbic acid content was estimated as vulnerable nutrient to food processing and storage. In this regard, herb samples after EW treatment and subsequent cold drying (LBE4C) were compared with the ones after deionized water treatment and subsequent cold drying (negative control, LBWC), and, finally, with the samples subject to cold drying only (blank, LBC) (Table 5); similarly, herbs after EW treatment and subsequent traditional drying (LBE4T) were compared with the ones after deionized water treatment and subsequent traditional drying (negative control, LBWT), and, finally, with the samples subject to traditional drying only (blank, LBC) (Table 5). In addition, 6 independent lemon balm fresh samples were analyzed for their average content in total polyphenols, antioxidant

Table 2
Mean microbial counts (±SD) of lemon balm (LB) samples after 1-min immersion into electrolyzed water at 200 and 400 ppm followed by cold or traditional drying, compared to LB samples treated with deionized water (negative control).

Sample	Mesophilic aerobic bacteria	*Enterobacteriaceae*	Moulds	Yeasts	*B. cereus*
FLB	9.0 c ± 0.6	5.2 b ± 0.2	9.0 b ± 0.2	1.0 a ±0.0	3.4 c ± 0.3
LBE2	8.5 c ± 0.5	5.0 b ± 0.7	8.4 b ± 0.5	1.0 a ±0.0	**1.0 a ± 0.3**
LBE2C	6.5 b ± 0.8	4.9 b ± 0.6	3.6 a ±0.1	3.1 b ± 0.4	**1.0 a ± 0.0**
LBE2T	5.7 a ±0.4	3.4 a ±0.4	3.4 a ±1.2	3.4 b ± 1.2	2.4 b ± 0.3
	***	***	***	***	***
FLB	7.7 c ± 0.2	5.6 ab ± 0.4	5.7 b ± 0.3	5.9 b ± 0.1	1.0 a ±0.0
LBW2	7.6 bc ± 0.2	6.1 b ± 0.3	5.8 b ± 0.1	6.0 b ± 0.1	1.0 a ±0.0
LBW2C	7.2 a ±0.1	5.2 a ±0.7	5.3 a ±0.2	5.1 a ±0.1	2.2 b ± 0.3
LBW2T	7.4 ab ± 0.2	5.5 a ±0.2	5.8 b ± 0.2	5.5 a ±0.3	2.6 b ± 0.4
	**	*	ns	***	***
FLB	7.8 c ± 0.5	3.7 c ± 1.1	4.6 c ± 0.2	4.8 b ± 0.1	<100
LBE4	5.6 b ± 0.4	2.9 bc ± 0.8	3.4 b ± 0.2	3.3 a ±0.5	<100
LBE4C	**3.8 a ± 0.4**	**1.0 a ± 0.0**	**2.6 a ± 0.4**	**3.6 a ± 0.5**	<100
LBE4T	5.3 b ± 0.5	2.6 b ± 1.0	3.3 b ± 1.1	3.6 a ±0.2	<100
	***	***	***	***	
FLB	7.2 b ± 0.3	3.8 b ± 0.7	4.4 c ± 0.2	4.6 d ± 0.1	<100
LBW4	7.9 c ± 0.1	4.0 b ± 0.5	4.1 b ± 0.2	4.2 c ± 0.4	<100
LBW4C	6.1 a ±0.1	5.6 c ±0.2	3.5 a ±0.1	3.2 a ±0.3	<100
LBW4T	5.6 a ±0.9	1.0 a ±0.2	3.6 a ±0.3	3.7 b ± 0.2	<100
	***	***	***	***	

*P < 0.05 **P < 0.01 ***P < 0.001.
FLB: fresh LB.
E/W: electrolyzed/deionized water treatment.
2/4: 200/400 ppm EW concentration.
C/T: cold/traditional drying.

capacity and total ascorbic acid content.

Lastly, lemon balm samples treated with 400 ppm EW (1 min dipping and stirring) were sent to AgriParadigma Company (Ravenna, Italy) to check the level of residual chlorate/perchlorate and, thus, the compliance with law limits of 0.05 mg/kg and 0.07 mg/kg in herbs for perchlorate (Regulation EU 2020/685) and chlorate (Regulation EU 2020/749), respectively.

Analytical data were expressed on dry weight of 5 g of herbs, measured in triplicate at 105 °C, by using a Gibertini Eurotherm electronic moisture balance (Gibertini Elettronica, Novate Milanese, MI, Italy).

2.6.2.1. Solvent extractions. To extract the bioactive compounds, a 1:1 water-ethanol mixture was chosen as the best clean solvent assessed for phenolic compound extraction (Tavares et al., 2010). Half a gram of sample was suspended in a 50 mL centrifuge tube with 20 mL of solvent and homogenized until uniform consistency using an Ultra-Turrax homogenizer (T25, Ika Works Inc., USA). The homogenates were centrifuged (4500×*g*, 10 min, 4 °C), filtered (0.45 μM PTFE filter) and stored at −20 °C until analysis. All samples were prepared and assessed in triplicate.

For total ascorbic acid (TAsA) determination, 0.5 g of herbs were extracted using 10 mL of cold extraction solvent (3% *m*-Phosporic acid in ultrapure water) in a 50 mL polypropylene tube. The mixture was mixed well using an Ultra-Turrax homogenizer (3 min, 3000 rpm), vortexed for 30 s, then centrifuged (4500×*g*, 10 min, 4 °C) and filtered (0.45 μM CA filter). Tris (2-carboxyethyl)phosphine hydrochloride (TCEP, 5 mM) was used as reducing agent of dehydroascorbic acid, the oxidized form of vitamin C (Chebrolu et al., 2012). An aliquot (500 μL) of the sample was treated with an equivalent volume of TCEP solution. After 30 min, the samples were injected in HPLC for TAsA analysis. All samples were prepared and injected in triplicate.

2.6.2.2. Folin-ciocalteu assay. The total phenolic content (TPC) of the extracts was estimated according the procedure describe by Singleton et al. (1999) by using 96-well microplates (total volume 195 μL) and a BioTek Synergy HT spectrophotometric multi-detection microplate reader (BioTek Instruments, Milan, Italy). The absorbance of the reaction mixture was recorded at 740 nm against a reagent blank. For quantitative purpose, an analytical curve was prepared using gallic acid as a stable phenolic compound (100–500 μM). The amount of the total phenolic compounds was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample.

2.6.2.3. DPPH radical-scavenging activity assay. The DPPH radical-scavenging activity (RSA) assay was performed according to the method of von Gadov et al. (1996) with some modifications to adapt it to microplate system. A 120 μM radical solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was prepared in 80% ethanol daily. Trolox was used as a standard at 12.5–300 μM to construct a calibration curve. The radical scavenging activity values of each sample were expressed as micromolar Trolox equivalents (TE) per gram of dry sample.

2.6.2.4. Total ascorbic acid determination. The total ascorbic acid content (TAsA) was determined by the HPLC method developed and validated by Valente et al. (2014) using a Thermo Scientific SpectraSYSTEM HPLC system (Thermo Fisher Scientific, San Jose, CA, USA), equipped with a SpectraSYSTEM SCM1000 degasser, a binary gradient pump system (SpectraSYSTEM P2000), an autosampling injector (SpectraSYSTEM AS100) and a UV-DAD detector (SpectraSYSTEM

Table 3
Color differences (ΔE) between lemon balm (LB) samples after 1-min immersion into electrolyzed (200 and 400 ppm) or deionized water followed by cold or traditional drying.

LBW2T	LBW4T	LBW2C	LBW4C	LBE2T	LBE4T	LBE2C	LBE4C	delta E
X				X				4.03
	X				X			1.50
		X				X		6.90
			X				X	2.88
				X		X		7.44
					X		X	4.85

LBE/W: electrolyzed/deionized water treated LB.
2/4: 200/400 ppm EW concentration.
C/T: cold/traditional drying.

Table 4
Total essential oil content (mL/100 g dw) and percentage of the 5 main components in lemon balm (LB) samples treated with 400 ppm electrolyzed water (and subsequently dried) versus the negative control (treated with deionized water and subsequently dried) and the blank (dried only).

Sample	Total essential oil content (mL/100 g dw)	Citronellal %	Beta-Caryophyllene %	Neral %	Germacrene D %	Geranial %	Others %
LBT	0.16 ± 0.02 a	0.4	5.3	34.3	0.6	44.1	15.3
LBE4T	0.09 ± 0.01 b	2.3	6.6	16.2	1.0	21.2	52.7
LBWT	0.12 ± 0.04 a *	2.6	6.9	18.9	1.8	25.2	44.6
LBC	0.16 ± 0.02	4.9	13.9	17.1	11.4	22.5	30.2
LBE4C	0.12 ± 0.03	3.9	12.4	20.0	8.8	25.0	29.9
LBWC	0.13 ± 0.05 ns	4.2	12.8	20.5	9.8	23.0	29.7

*P < 0.05 **P < 0.01 ***P < 0.001.
a, b: oil contents labelled with different letter are significantly different (Duncan test, P < 0.05).
C/T: cold/traditionally dried LB.
E/W: electrolyzed/deionized water treatment.
4: 400 ppm electrolyzed water.

Table 5
Total polyphenol (mg GAE/g dw) and ascorbic acid (μu/g dw) content, and antioxidant activity (μM Trolox/g dw) in lemon balm (LB) samples treated with 400 ppm electrolyzed water (and subsequently dried) versus the negative control (treated with deionized water and subsequently dried) and the blank (dried only).

Sample	TPC (mg GAE/g dw)	RSA (μM Trolox/g dw)	TAsA (μu/g dw)
LBT	75.39 ± 7.11 b	645.29 ± 41.75 b	647.58 ± 92.27 b
LBE4T	25.68 ± 6.97 a	189.75 ± 5.60 a	451.70 ± 97.05 ab
LBW4T	61.06 ± 6.66 b ***	592.99 ± 53.38 b ***	362.31 ± 76.70 a *
LBC	82.70 ± 11.51	702.17 ± 29.40	619.94 ± 189.27
LBE4C	81.23 ± 3.76	842.88 ± 179.90	744.49 ± 47.82
LBW4C	73.41 ± 3.48 ns	737.92 ± 30.77 ns	854.71 ± 68.83 ns

*P < 0.05 **P < 0.01 ***P < 0.001.
a, b: Different letters in the same column indicate significant statistical differences (Duncan test, p < 0.05).
C/T: cold/traditionally dried LB.
E/W: electrolyzed/deionized water treatment.
4: 400 ppm electrolyzed water.

UV6000LP). Separation was achieved using a SynergiTM Hydro-RP (150 × 4.6 mm I.D., 4 μm particle size) analytical column from Phenomenex (Phenomenex, Castel Maggiore, BO, Italy) protected with a securityGuard Cartridge AQ C18 (40 × 2.0 mm I.D., 5 μm particle size) from Phenomenex. The AsA peak was detected at 245 nm, and ChromQuest 4.2 software (Thermo Fisher Scientific) was used for data processing. The identification of AsA peak was achieved by comparing retention time (RT) and spectra with those of pure standard (L (+)-ascorbic acid), and the TAsA amount was calculated by comparing the obtained values with those from a standard curve (AsA, 1–100 μg/mL). Data were expressed as AsA micrograms per gram of dry sample.

2.7. Evaluation of sanitization treatments on contamination by insects

2.7.1. Insect supply

Colonies of the two beetle species *L. serricorne* and *T. castaneum* were started from individuals obtained from infested flour after their identification using morphological keys (Bousquet, 1990, pp. 1–215; Ferrer, 1995; Papadopoulou & Buchelos, 2002). Beetles were reared by species in cubic Plexiglas cages (30 cm side) on a mixed substrate of organic whole meal flour, dry bread, rice and dried herbs. Ventilation was possible through an inspection net sleeve on one side (Ø 15 cm, mesh size 80 μm). One to 2 month-old adults were used in the experiments. All cultures were kept in climatic chambers at 25 ± 1 °C, 70 ± 5% RH, 16:8 L:D.

2.7.2. Sanitization by GO

In the sanitization trials with GO, the experiments were performed by applying a fixed ozone concentration (C) of 100 ppm at different exposure times (T) (0.5, 2.4, 4.8, 24, 48, 72 h) on mixed substrate of dried herbs and flour. In particular, six C × T GO categories (3,000, 14,400, 28,800, 72,000, 144,000, 432,000 ppm × min) were evaluated by comparison with the untreated control (0 ppm × min), on adults of *L. serricorne* and *T. castaneum*. Ten replicates were made for each C × T combination, for a total of 70 experimental units [10 replicates × (6 C × T + control)] per species. The experimental unit consisted in a plastic container (height 80 mm, Ø 55 mm) closed with a cap equipped with a net (mesh size 0.3 mm). In each container, five adults of *T. castaneum* or *L. serricorne* were introduced on a thin layer of flour mixed with dried lemon balm. The experimental units, thus set up, were then placed for treatment on three shelves into a 63 L airtight testing chamber (2.2.1), where they were subjected at the predetermined GO concentration, and left for the required exposure time following the C × T combinations. The chamber was connected to an ozone generator (Biofresh LM-5) and an ozone sensor (Ozone Analyzer Uv-100) as described in paragraph 2.2.1. The exposure time started after reaching at least 5 min-stable concentration.

The mortality of the two beetle species was checked, with the help of a tiny brush and a magnifier, by counting dead adults after 48 h. Since Hansen et al. (2013) found that ozone treatment is efficient at both high and low temperatures (7.3–31.6 °C), the experiments were conducted at the temperature of 24 ± 1 °C. The untreated controls were placed in similar chamber under the same climatic conditions for the same corresponding durations.

2.7.3. Sanitization by EW

In the sanitization trials with EW, the experiments were performed by dipping together herbs and pests, following the same concentration of free chlorine solution indicated in paragraph 2.2.2. In particular, fresh leaves of lemon balm with adults of *L. serricorne* or *T. castaneum* were dipped for 1 min in EW, diluted at different concentrations (100, 200, 400 ppm). The experimental unit consisted in a plastic container (height 80 mm, Ø 55 mm) closed with a cap equipped with a net (mesh size 0.3 mm), with three adults of *L. serricorne* or *T. castaneum* on 1 g of lemon balm leaves. In each container 10 mL of EW diluted at different concentrations (0, 100, 200, 400 ppm) were added. After 1 min of agitation, the container was turned upside-down for EW removal. The mortality of the two beetle species was checked 48 h after treatment with the help of a tiny brush and a magnifier. The untreated controls were dipped in deionized water following the procedure described above. Six replicates were made for each concentration per species.

2.8. Statistical analysis

The microbiological and chemical results are presented as mean values ± standard deviation of triplicate measurements. Data were subjected to a one-way analysis of variance (ANOVA) with Duncan's post hoc test at a 95% confidence level.

In insect sanitization trials with GO, ANOVA was performed to analyze data mortality per C × T category with $P < 0.05$ as threshold for significance. After testing for normality and homogeneity (Shapiro Wilk and Levene tests at $P > 0.05$), in case of significant differences, means were separated using Tamhane's post hoc test. Mortality data by C × T of GO were also subjected to probit regression analysis to determine lethal doses × time resulting in 50% (LDT_{50}) and 99% (LDT_{99}) of insect mortality, 95% upper (UCL) and lower (LCL) confidence limits, regression equations, and Chi square (χ^2) values for both species. In insect sanitization trials with EW, after testing for normality and homogeneity (Shapiro Wilk and Levene test at $P > 0.05$), mortality data were analyzed with ANOVA; in case of significant differences, means were separated using Tukey's post hoc test.

The Kruskal-Wallis H-test (95% confidence level) with a multiple comparison test was used to evaluate consumer acceptance.

All statistical analyses were performed by means of the IBM SPSS version 27 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Microbiological quality of lemon balm

Lemon balm samples collected and analyzed in 2018 showed a microbiological quality of compliance or non-compliance with the law criteria, depending on the target microbial group. The average counts of mesophilic aerobic bacteria, *Enterobacteriaceae* and moulds were higher than microbiological law requirements. Specifically, they reached mean values of $1.2*10^8$ cfu/g ($\pm 1.1*10^2$), $5.1*10^5$ cfu/g ($\pm 3.3*10^2$) and $3.5*10^6$ cfu/g ($\pm 9.1*10$), respectively, in spite of law limits of $5.0*10^6$ cfu/g (D.D n.780, 2011), $1.0*10^2$ cfu/g (Racc. 2004/24/CE) and $1.0*10^4$ cfu/g (D.D n.780, 2011), respectively. *E. coli*, *C. perfringens*, *Salmonella* spp. and *L. monocytogenes* were never detected in the samples analyzed while *B. cereus* was found to comply with law limits ($1.0*10^4$ cfu/g, Racc. 2004/24/CE) in 7 out of 12 samples collected in summer and 10 out of 12 samples collected in autumn.

Based on the obtained results, four microbial groups were chosen as target for sanitization trials in 2019: mesophilic aerobic bacteria, *Enterobacteriaceae*, moulds and *B. cereus* together with yeasts that reached, on average, counts of $8.8*10^4$ cfu/g but for which a law threshold was missing.

3.2. Effects of GO and EW treatments on lemon balm microbial contamination

Preliminary trials performed in 2018 allowed to fix the most promising GO and EW parameters (concentration x time). Thus, (a) 50 ppm x 48 and 72 h, (b) 100 ppm x 48 and 72 h for GO treatment, and (c) 200 mg/L x 1 min stirring, (d) 400 mg/L x 1 min stirring for EW dipping treatment were used on lemon balm samples harvested in 2019, with the purpose of lowering microbial counts below law limits. Based on the results collected in 2018 (data not shown), GO concentration of 5 and 10 ppm (30 min treatment) were excluded because totally ineffective with the exception of *Enterobacteriaceae* and moulds which were lowered by 2 logarithmic units in few random samples. Regarding EW data, the spray method (5 and 15 min treatment) was excluded because it gave inconsistent results probably due to the not uniform EW coverage of the herbs, and to the unfavorable ratio grams of herbs/Liters of EW (250 g/L) compared to the dipping method (50 g/L) even if the spray time was longer (5 and 15 min instead of 1 min dipping).

According to 2019 trials, treatments with GO (Table 1) showed a partial efficacy only at 100 ppm after 72 h. In particular, 100 ppm GO made mesophilic microbial counts compliant with law limits in the samples sanitized after traditional drying (TLBO 10072), lowering mean counts by 2 logarithmic units. A significant lowering of counts was also observed with regard to moulds (TLBO 10072) and *Enterobacteriaceae* (CLBO 10072), even if not below law limits, and *B. cereus* (TLBO 10072), which was already present in fresh lemon balm samples with satisfactory count values. Finally, a marked efficacy was observed on yeasts which showed counts lowered by 4 logarithmic units in the samples sanitized after cold drying at both 48 h and 72 h (CLBO 10048 and CLBO 10072).

The EW dipping treatments (Table 2) showed interesting results at 400 ppm for 1 min stirring, when moulds, *Enterobacteriaceae* and mesophilic microbial counts were lowered below the legal limits, in cold dried samples (LBE4C). In particular, mesophilic microbial population decreased of 4 logarithmic units compared to fresh lemon balm, *Enterobacteriaceae* of 2 logarithmic units, and moulds and yeasts of 2 and 1 logarithmic units, respectively. Moreover, the growth of *B. cereus* was kept under law limits with 200 ppm EW treatment (LBE2 and LBE2C).

3.3. Effects of GO and EW treatments on sensorial characteristics of lemon balm

Overall, lemon balm samples sanitized with GO were found, to the taster panel, different from the untreated ones and with a much lower approval rating; moreover, the color test highlighted a noticeable browning of samples treated with GO (data not shown). Samples sanitized with EW gave different results: (a) no differences were detected between treated and untreated samples (LBE4T vs LBW4T), (b) differences were detected and the EW treated samples were preferred (LBE2C vs LBW2C), (c) differences were detected and the untreated samples were preferred (LBE2T vs LBW2T; LBE4C vs LBW4C) (Table 3). Finally, color analysis highlighted marked differences between lemon balm samples sanitized with EW and negative control (deionized water treated), and also between samples submitted to cold and traditional drying (Table 3). In addition, samples treated with EW were greener and more yellow and, among those, the ones exposed to cold drying were brighter than the ones submitted to traditional drying.

3.4. Effects of EW treatments on essential oil, total polyphenol and ascorbic acid content in lemon balm, and on antioxidant capacity and residual chlorate/perchlorate

Based on the results obtained from microbiological and sensorial assays, chemical analyses were focused on lemon balm samples submitted to EW dipping treatment, 400 ppm for 1 min stirring.

The total content in essential oils, expressed as milliliters of essential oils per 100 g of dried herb (Table 4), was significantly lower in lemon balm samples treated with EW and subsequently traditionally dried (LBE4T) compared to the negative control (LBW4T, treated with deionized water and subsequently traditionally dried) and the blank (LBT, traditionally dried only); differently, the essential oil content was not significantly different between herbs treated with EW and, then, cold dried (LBE4C) compared to the negative control (LBW4C, treated with deionized water and subsequently cold dried) and the blank (LBC, cold dried only) (Table 4). The average content of essential oils in fresh lemon balm was 0.19 ± 0.05 mL/100 g dw. The contents of the 5 main components did not show marked differences, except for neral and geranial, which were halved in samples treated with both EW and deionized water and, then, traditionally dried (LBE4T and LBW4T) compared to the blank submitted to traditionally drying only (LBT) (Table 4).

In Table 5, the total polyphenol and ascorbic acid contents, and the antioxidant activity of lemon balm samples treated with 400 ppm EW (and subsequently dried) were compared with the negative control (treated with deionized water and subsequently dried) and the blank (dried only). The lowest values were observed in samples 400 ppm EW sanitized and then traditionally dried (LBE4T), which were significantly

different to LBW4T (negative control) and LBT (blank) for both total polyphenol content and antioxidant activity. Ascorbic acid content was also significantly lower in LBW4T compared to the blank (LBT, traditionally dried only). On the contrary, no significant differences were detected between samples EW treated (LBE4C) and not (LBW4C and LBC) exposed to cold drying, for all three chemical indicators. The content of total polyphenols and ascorbic acid, and the antioxidant capacity in fresh lemon balm was on average 111.05 mg GAE/g dw, 6506.29 μg/g dw, and 806.07 μM Trolox/g dw, respectively.

Minimum and maximum residual perchlorate values of 0.055 mg/kg and 0.178 mg/kg, and minimum and maximum residual chlorate values of 2.79 mg/kg and 10.2 mg/kg were detected in lemon balm samples immediately after treatment with 400 ppm EW; the residual values dropped to 0.033 mg/kg (minimum) and 0.069 mg/kg (maximum), and 0.022 mg/kg (minimum) and 0.123 mg/kg (maximum) for chlorate and perchlorate, respectively, after herb treatment with 400 ppm EW and subsequent washing in deionized water. A total of 7 out of 12 samples complied with the legal limits.

3.5. Effects of sanitization treatments on Lasioderma serricorne and Tribolium castaneum

In the sanitization trials with GO, adults of both *L. serricorne* and *T. castaneum* showed a survival rate of 100% in the untreated controls. Mortality ratios from C × T of 3000 to 432,000 ppm × min raised from 4.0 ± 2.67% to 100.0 ± 0.0% for *L. serricorne*, and from 6.0 ± 3.06% to 100.0 ± 0.0% for *T. castaneum*. GO treatments were found to be effective with C × T of 144,000 on both beetle species, causing a mortality higher than 80% with a significant difference from the untreated control (ANOVA: *L. serricorne*, df = 4, 15; F = 40.306; P = 0.001; *T. castaneum*, df = 4, 15; F = 78.337; P = 0.001 and between C × T values (Fig. 2).

Lethal dose × time estimates for adults of *L. serricorne* and *T. castaneum* after exposure to the six tested combinations of C × T (3,000, 14,400, 28,800, 72,000, 144,000, 432,000 ppm × min; plus control 0 ppm × min) resulting in 50% (LDT_{50}) and 99% (LDT_{99}) of mortality, upper and lower confidence limits, regression equations and χ^2 are reported in Table 6. LDT_{50} estimates were very similar for adults of both species, as well as upper and lower confidence limits, while LDT_{99} estimates, as well as upper and lower confidence limits, were slightly higher for *L. serricorne* than *T. castaneum*. A C × T value of 200,000 ppm × min was effective to cause 96.5% mortality of *L. serricorne* and 98.5% mortality of *T. castaneum*.

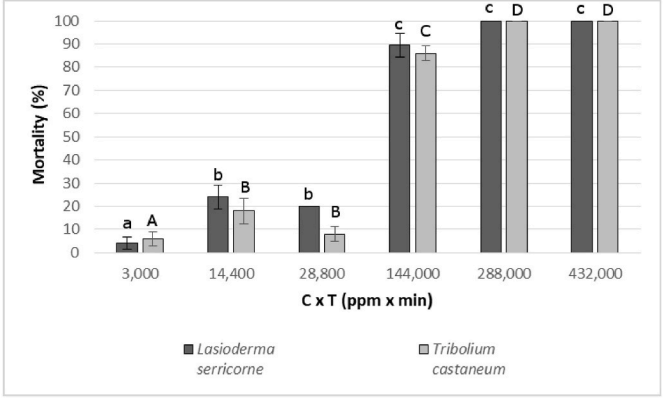

Fig. 2. Mean percentages (±SE) of mortality of adults of *Lasioderma serricorne* and *Tribolium castaneum* exposed to a concentration of 100 ppm (C) of gaseous ozone for different durations (T, min) in laboratory conditions. Mortality was assessed 48 h after treatment. For each species, bar with different letters are significantly different (Tamhane test, P < 0.05).

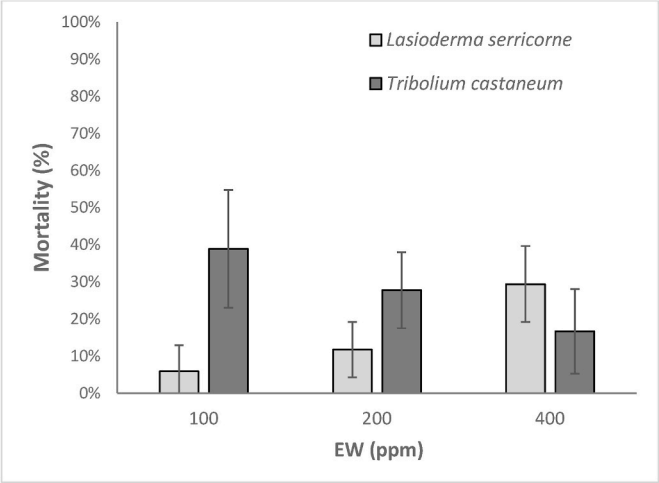

Fig. 3. Mean percentages (±SE) of mortality of adults of *Lasioderma serricorne* and *Tribolium castaneum* after 1-min immersion into electrolyzed water (EW) at different concentration (100, 200, 400 ppm). Mortality was assessed 48 h after treatment.

In the sanitization trials with EW, although the mean mortality at growing concentration (from 100 to 400 ppm) increased from 6 to 29% in *L. serricorne* and decreased from 39 to 17% in *T. castaneum*, no significant differences were found between treatments (Fig. 3).

4. Discussion

Over the years, consumer awareness has increased significantly in food sector. The consumers have become increasingly demanding and looking for quality products as well as safe. Aromatic herbs are currently subject of considerable interest, and their use as minor food components has led the scientific community to pay great attention to their organoleptic quality and microbiological safety as well as the possibility of extending their shelf life. Researchers have been investigating different technologies to prevent microbial contamination and ensure high quality in minimally processed herbs. The present study aims to contribute to the knowledge of new approaches to prevent the growth of microorganisms as well as the contamination by insects without affecting quality in terms of sensorial and chemical attributes. In this frame, the focus was on GO and EW which may represent economically convenient, environmentally friendly and safe technologies for sanitization.

According to different authors (Akbas et al., 2008; Kazi et al., 2017; Torlak et al., 2013), a marked reduction of mesophilic aerobic bacteria,

Table 6
Lethal dose × time estimates for adults of *Lasioderma serricorne* and *Tribolium castaneum* after exposure to six combinations (C × T: 3,000, 14,400, 28,800, 72,000, 144,000, 432,000 ppm × min) of fixed ozone concentration (100 ppm) at different exposure time (30, 144, 288, 720, 48, 1440, 4320 min). LDT_{50} and LDT_{99} represent lethal dose × time that cause 50% and 99% of mortality, respectively. LCL and UCL represent lower confidence limit and upper confidence limit, respectively.

Species	No.	Slope±SE	LDT_{50} ppm × min		LDT_{99} ppm × min		χ^2	DF
			Estimate	(LCL – UCL)	Estimate	(LCL – UCL)		
Lasioderma serricorne	70	−10.804 ± 0.131	87,475	(73,911–104,787)	231,073	(197,125–282,155)	36.11	68
Tribolium castaneum	70	−11.324 ± 0.146	87,164	(74,447–103,115)	209,472	(180,603–251,750)	43.83	68

yeasts and moulds, *B. cereus* and *E. coli*, in herbs and spices as dried oregano, thyme and red pepper, were reached by using GO concentration in the range of 1–5 ppm for 5–360 min. Differently, Brodowska et al. (2014) used 160 ppm for 30 min to limit microbial growth in cardamom. The results obtained in this study on lemon balm support the need for concentrations above 100 ppm to reach a moderate efficacy, with long treatment time (72 h), which presumably do not suit with lemon balm production practices. Furthermore, the loss of organoleptic quality in GO treated samples led to exclude this technique as a possible alternative in the sanitization of DAH.

To kill insect pests, the increase in the GO exposure period led to higher mortality rates for both tested species. Effective mortality percentages (95–99%) were obtained after 24 h and 48 h GO treatment, with CxT values higher than 200,000 ppm × min. The triatomic form of oxygen is a natural agent and a strong oxidizer, highly toxic to living organisms at high concentrations (James, 2011). It is known to affect plants, insects, soil microbial communities, and their interactions (Evgenios et al., 2020). Negative GO effects on insects have been attributed to oxidative stress. In fact, GO has been shown to induce molecular damages such as oxidation of proteins, lipid peroxidation, and damage to DNA; it also causes the deregulation of intracellular signal transduction, which could disrupt the whole organism and lead to death (Dalibor et al., 2015; Vanderplanck et al., 2021).

On the contrary, the use of EW deserves attention when applied to lemon balm. The most common pollutants in herbal raw materials include the natural habitat of the plant, and the soil itself can become the main source of contamination of the raw material (Guentzel, Lam, & Callan, 2008). The practice of washing herbs in EW before drying could take on the dual role of mechanically washing away contaminating microorganisms (Gram-positive bacteria, spore-forming bacteria, yeasts and moulds), and detrimentally acting on their cells by oxidation of membrane-bound respiratory enzymes and lipids and perturbation of cellular electrical charge maintenance (Veasey & Muriana, 2016).

As far as we know, EW has been used on fresh fruits and vegetables but not yet on aromatic herbs. Izumi (1999) obtained reduction of total microbial counts up to 2.6 Log cfu/g in fresh-cut vegetables by treatment with 20 ppm EW without any significant effect on tissue pH, surface color, and general appearance of vegetables. More recently, Issa et al. (2011) reduced the total aerobic mesophilic bacteria from Chinese celery, lettuce and daikon sprouts by 2.7, 2.5 and 2.45 Log cfu/g, respectively, by dipping in 100 ppm EW for 5 min. Stefanello et al. (2020) studied the efficiency *in vitro* of 121 ppm EW (5 min treatment) on heat-resistant moulds obtaining reduction up to 3 Log cfu/g. In the present study, we reached reduction of about 4.0, 2.5, 2.0 and 1.0 Log cfu/g of aerobic mesophilic bacteria, *Enterobacteriaceae*, moulds and yeasts, respectively, by 400 ppm EW for 1 min treatment, while 200 ppm EW was enough for limiting *B. cereus* growth. In addition to available chlorine concentration, microorganism, and vegetable type, it is known that exposure time affects the action of EW (Deng et al., 2020). Other authors obtained comparable results by treatment ranging from 1 to 10 min. Chinchkar et al. (2022) reviewed the application of EW on fruits and vegetables and reported interesting results, for instance, on lettuce, in terms of reduction of both total aerobic bacteria and pathogens by using electrolyzed oxidized water: aerobic bacteria decreased between 2 and 4 log CFU/unit in 10 min by 30 mg/L free chlorine; *E. coli* 0157:H7 was reduced of more than 4 log CFU/unit in 1 min and *L. monocytogenes* between 2 and 4 log CFU/unit in 1 min by 22 mg/L free chlorine (Huang et al., 2008). Similarly, Lee et al. (2014) reported a reduction of 2.7 log CFU/g of total bacteria count on carrot by using neutral electrolyzed water (200 mg/L, 10 min).

Noteworthy, in the present trials, the target microorganisms were reduced below law limits after only 1 min. It could be hypothesized that stirring guarantees adequate disinfectant mixing, as reported by other authors (Gil et al., 2015), despite it is known that agitation leads to a decrease in free chlorine content.

In general, although the scientific literature reports GO and EW as two powerful tools to contain microbial contamination in food matrices, the results of the present study suggest an important contribution due to the treatment modality; in particular, the mixing of the herbs dipped in EW could have promoted detachment of microbial cells and favored their contact with free chlorine, more than GO conveyed to herbs lying statically on trays.

Finally, the results highlighted that microbial reduction by EW was kept stable in cold dried samples more than in traditionally dried ones, suggesting the influence of drying temperature as a key element in achieving a good microbiological quality of the product.

However, sanitization by EW was not efficient enough against insects, killing low percentage of both beetle species (less than 40%). To our knowledge, no studies have been published yet on EW efficacy on insect pests of dried aromatic herbs. Previous studies on EW revealed that it has no effect on living organisms (Bodas et al., 2013), but it has been efficiently applied on major types of pathogenic agents including bacteria, fungi, viruses, protozoa, algae, and nematodes (Al-Haq et al., 2005). EW reacts with any oxidizable matter with different mode of actions (Attia et al., 2021). Possible negative effects on insects could be attribute to oxidative stress. Since no literature is currently available on this subject, it is not possible to compare the data obtained here with those of other studies. The low mortality could probably be due to low concentration or short exposure time, and further studies are certainly needed. Nevertheless, EW can be considered a food safety agent, and also reduce health hazards for workers by eliminating the need to handle concentrated chemicals, attention should be paid for any chlorate and perchlorate residual on the treated herbs. Our data underline the importance of the rinse step in deionized water, after treatment with EW, which allowed to reach residual chlorate and perchlorate values below law limits in 7 out of 12 lemon balm samples. On the basis of these results, it is possible to hypothesize an increase in the rinsing time or the use of a different washing method: by percolation rather than by immersion to further improve the reduction of residuals and definitely keep them under law limits in order to assure a safe product from both microbiological and chemical points of view.

The results of the sensory analysis are also quite encouraging. In terms of color, lemon balm samples were not negatively affected by EW treatments; on the contrary, they appeared greener and more yellow than untreated control. In addition, the EW treated samples submitted to cold drying were brighter than the ones submitted to traditional drying, according to previous results by Vallino et al. (2021, Fig. 1). Finally, the taster panel did not highlight neither an improvement nor a marked deterioration of lemon balm organoleptic quality following EW treatment; actually, it was not possible to detect a specific trend based on sensory tests.

Regarding essential oils, the contents found in lemon balm samples were comparable with what reported by other authors (Moradkhani et al., 2010; Nurzyńska-Wierdak et al., 2014; Seidler-Łożykowska et al., 2017). In EW treated samples, then traditionally dried, it was observed a decrease in essential oils in particular of the two main components, neral and geranial; differently, in EW treated and cold dried samples, the loss in essential oils was not significant in terms of the overall content, and the most representative aromatic molecules showed slight variations with both higher or lower percentages compared to untreated samples. With regard to total polyphenols, ascorbic acid and antioxidant capacity, EW treatment did not cause any significant negative variation in lemon balm. In particular, EW immersion seemed not to affect their concentration in cold dried lemon balm. Differently, the combination of EW treatment and traditional drying led to a significant decrease of total polyphenols content and antioxidant capacity. The decrease in total ascorbic acid content in all treated samples compared to the fresh sample would suggest an active role of this antioxidant in preserving other bioactive compounds from oxidation.

5. Conclusion

The use of EW for the sanitization of aromatic herbs could offer interesting implications. The data obtained in this study suggest that the optimization of EW concentration and treatment time parameters, as well as rinsing, can lead to a reduction of both microbiological and chemical risk while new assessments will be necessary to improve the control of contamination by insects. The best combination EW concentration x time was 400 mg/L for 1 min dipping and stirring while the long treatment time (48–72 h) and the loss of sensory quality of the herbs excluded GO as a potential tool in their sanitization. Regarding EW, the results are also encouraging as lemon balm exposed to the treatment did not lose organoleptic quality, in particular the color even seemed to improve in brightness when the herbs were subsequently cold dried. It is likely that low temperatures of cold drying help to preserve the peculiar characteristics of lemon balm as well as some nutritional aspects of no less importance.

Finally, the introduction of EW treatment as further step in lemon balm processing will have to be carefully evaluated with the farmers. Surely, despite an effort in terms of production practices and timing, aromatic herbs with higher microbiological quality would allow an extension in terms of shelf-life of the product, an aspect certainly of interest for producers.

CRediT authorship contribution statement

Paola Dolci: Conceptualization, Methodology, Investigation, Writing – original draft, Formal analysis, Supervision, Visualization. **Barbara Letizia Ingegno:** Conceptualization, Methodology, Investigation, Writing – original draft. **Elena Mangia:** Investigation, Resources. **Daniela Ghirardello:** Methodology, Investigation, Writing – original draft. **Lucia Zaquini:** Resources. **Selena Costarelli:** Investigation. **Luciana Tavella:** Conceptualization, Supervision. **Sylvain Perrot:** Conceptualization, Methodology, Writing – original draft. **Bert Candaele:** Supervision, Project administration, Funding acquisition. **Olivier Bagarri:** Project administration, Funding acquisition. **Elena Cerutti:** Project administration, Funding acquisition. **Giuseppe Zeppa:** Conceptualization, Formal analysis, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

We declare that none of the authors who contributed to this article have any conflicts of interest to disclose.

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