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Using a continuous microwave system for postharvest almonds' disinfestation

Antonia Tamborrino[a], Antonio Berardi[a] Enrico de Lillo[a], Gianvito Ragone[a] Maria De Benedictis[b], Miriam Tufariello[b], Angelo Santino[b], Alessandro Leone[a]

[a]Department of Plant, Soil and Food Sciences, University of Bari Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy

[b]Institute of Sciences of Food Production, C.N.R. Unit of Lecce, 73100 Lecce, Italy

*Corresponding author: Antonio Berardi

Abstract

This paper reports on the development of a physical technology for the sanification of shelled almonds from *E. kuehniella* as an alternative to chemical treatments. A continuous microwave (MW) system was developed and a program of experimental trials of treating almonds by administering different increasing levels of specific energy from 7.3 to 18.1 kJ kg^{-1} was performed. Insect mortality in three different life stages: 24-hours old eggs, 24-hours old larvae and mature larvae was evaluated. Lipoxygenase (LOX) activity and sensory analysis was carried out to evaluate the quality of the almonds treated. One hundred percent mortality of mature larvae was achieved in all test conditions explored, while mortality of 24-hours old eggs and 24-hours old larvae was achieved by administering specific energy of at least 12.1 kJ kg^{-1} with a almonds surface temperature of 55,7 °C. No significant differences in the LOX activity and about the sensory analysis the authors asserted that either MW treated almonds and control sample retain all the main organoleptic properties. Furthermore, no off-flavors were detected either from the olfactory and gustatory points of view.

This study demonstrated that continuous microwave treatment can be used for an effective pest control treatment of almonds, with potential for use also for other foodstuffs, proving to be a valid alternative to chemical treatments.

Keywords: insect pests, *Ephestia kuehniella*, physical pest control method, specific energy, almonds' quality

1. Introduction

Several pests, including arthropods, can affect stored products (flours, almonds, seeds, dates, cereals, etc.) and cause, directly and indirectly, severe qualitative and quantitative damages (Ahmad et al., 2022). Effective control of stored food pests is the goal of operators around the world and chemical applications have been the main choice for long time and are still widely used. Chemical control of arthropods in warehouses may have harmful side effects, such as leaving pesticides on salvaged produces, less impact on some pest life stages and/or this approach may induce pest resistance development to the applied chemicals (Sinha & Watters, 1985; Moirangthem & Baik, 2021). The replacement of pesticides with alternative means, with more or similarly efficiency than the current authorized chemicals and less negative impacts on the physic-chemical properties of the treated stored products, human health and the environment, is strongly needed. To this aim in Grieshop et al., 2006 the biological control was attractive alternative to insecticides for reducing infestations and damage from the Indianmeal moth in retail and warehouse environments. Other alternative means to pesticides include conventional hot air or water heating, controlled atmospheres and dielectric heating (radio frequency (RF) and microwaves (MW)). The efficient use of RF and MW for disinfestation is still in the research stage either for the application parameters or the most appropriate and effective tools to be applied. MW heating is an emerging technology, mostly used in food processing by virtue of its low treatment length, operational simplicity and high heating rates, which result in lower maintenance requirements (Tamborrino et al., 2021). MW treatment may replace other techniques due to the selective heating, environmentally friendly application, energy minimization, equivalent or better quality maintenance of the treated food materials (Moirangthem & Baik, 2021). MW are electromagnetic waves produced by frequencies ranging from about 300 MHz to 300 GHz, corresponding to wavelengths from 1 to 0.001 m. MW heating is mainly due to the interaction of the electric field with free or bound charges and dipoles in the organic matter. Many molecules, such as water and fat, are electric bipolars, meaning that they have a positive charge at one end and a negative charge at the other. Therefore, the alternating electric field induced by the microwave beam provokes the rotation of these bipolars as they try to align themselves. The rapid movement of these molecules creates friction and results in heat dissipation in the material exposed to the microwave radiation (Das et al., 2022). This interaction causes volumetric heating with the consequence that materials can absorb microwave energy directly and internally, and convert it into heat. On the contrary, conventional heating occurs by convection or conduction where heat must diffuse from the surface of the material. An interesting feature of control with MW energy regards the insects that, due to their moisture content, are heated faster than the dry product they infest. Thus, a temperature lethal to the insects can be reached without affecting the organoleptic properties of the stored products (Vadivambal et al., 2006). In recent years, numerous studies have been conducted on MW disinfestation of cereals, especially wheat (Vadivambal et al., 2006), as well as other dry products such as almond, by using heating associated with radio frequency energy (Wang et al., 2013) and microwave (Patil et al., 2020). Some studies also on other infested substrates (woodworms) have combined several technologies, e.g., MW treatment in conjunction with RF (Andreuccetti et al., 1994) or with cold storage (Ayvaz et al., 2008). Power level and exposure time of MW are the two crucial parameters to provide 100 % insect mortality (Bedi et al., 1992). Insect mortality increases with increasing MW exposure time and power intensity or both (Singh et al., 2012). Little research has been conducted on the disinfestation of almonds using MW. Specifically, Patil et al. (2020) found that heating almonds with MW at a power of 120-600 W and with an exposure time of 30-90 s resulted in 100 % mortality of various life stages of *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae). That research referred to the use of domestic and static microwave oven, and exposures to different microwave power levels and times. The major disadvantage of MW heating is the uneven distribution of the electromagnetic field inside the cavity where MW heating takes place. This phenomenon could generate localized hot and cold spots (Mescia et al., 2022) especially if MW is statically provided, resulting in "hot spots" where the temperature at certain locations is significantly higher than the average temperature in the sample. In order to uniform the temperature of the product inside the treatment chamber, a metal spiral has been provided with a dual function: mixing and entraining of the product. Another major disadvantage of MW disinfestation is the low penetration capability of the MW. The intensity of MW decreases with increasing penetration. It has been reported that MW treatment of loose grain is not feasible when the depth exceeds 4 inches (Das et al., 2013). Regarding MW systems, many studies have been conducted with domestic microwave oven or static laboratory dryers. No specific systems have been projected for specific applications especially in a continuous way and with a reduced thickness of the treated product in order to minimize the negative effects of the microwave treatment.

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae), commonly called Mediterranean flour moth or mill moth, is considered one of the main and common pests on stored almonds in Southern Italy. Larvae of this moth may colonize stored grains, seeds, decorticated or split fruits and dried flowers after harvesting and, in general, nutritious substrates which remain in the fields, houses, farms or storage sheds (https://www.cabi.org/isc/datasheet/21412). The sensitiveness of *E. kuehniella* to the MW treatments was previously demonstrated in a few applications running in discontinuous ovens (Azizoglu et al., 2011; Pandir & Guven, 2014; Sadeghi et al., 2018) and it is highly expected in a continuous apparatus. Although MW heating has potential for the disinfestation applications in the dried seeds industry, MW can have some adverse effects on various quality parameters and they have to be studied. In 2020, Patil et al., studied the effects of the MW treatment on almond and they found that the quality attributes such as color difference, water activity, hardness, PV, FFA, IV were acceptable after microwave treatment.

This paper is aimed at investigating the setting of a specific continuous microwave pilot system for treating almond dried seeds, in order to establish the operating parameters to control the population of *E. kuehniella* at three different stages: eggs, 24-hours old and mature larvae. Finally, a quantitative descriptive sensory analysis of the almonds processed was performed.

2. Materials and methods

2.1. Microwave pilot system

A pilot system for the continuous treatment of shelled almonds with MW was built and tested in the current experiment (Fig. 1).

Fig. 1: Microwave pilot system scheme: a) loading hopper, b) microwave filter, c) treatment chamber, d) spiral, e) magnetrons (M) with the power generator (G), f) discharge point, g) Programmable Logic Controller (PLC), h) speed reducer, i) electric motor, j) sensor, k) thermometer, l) power generator

The system is equipped with a 40 L feed hopper, a microwave filter, a treatment chamber consisting of a polyethylene (PE) tube of 3000 mm length and 91 mm inner diameter with a metal spiral of circular section for the mixing and extraction of the product. The volume of the treatment chamber was 19.5 L without the volume occupied by the metal spiral. The microwave system was equipped with five magnetrons provided with a power generator of 1.5 kW each. The metal spiral was driven by an electric motor of 0.37 kW coupled at a reducer gear (reduction ratio 1/46). The electric motor operated in a range between 30 and 80 Hz drive the metal spiral (minimum and maximum limit). The system is controlled and adjusted by a programmable logic controller (PLC): ON/OFF switch of each magnetron, regulation of the total electrical power supplied by the magnetron and regulation of the residence time depending on the frequency of the electric motor (adjustment of rotation speed of the spiral inside the PE tube). A safety system, consisting of two product detection sensors (the first sensor mounted in the loading hopper and the second one in the final part of the treatment chamber), ensures that the treatment chamber is filled with almonds before the magnetrons are switched on. Switching on the magnetrons in the absence of product would irreversibly damage the treatment chamber. Two infrared thermometers are mounted in the loading hopper and at the product outlet from the treatment chamber to measure the temperature of the incoming and outgoing product.

2.2. Raw material

Almonds (*Prunus dulcis* Webb) of the cultivar Tuono were used for the experimental assays. Almonds were harvested in Apulia (Italy) in August 2021 and, afterwards, hulled, sun dried, shelled; the shelled almonds were stored at controlled temperature (18°C) at Coop Contado (Toritto, Bari, Apulia, Italy).

The moisture content of the shelled almonds (% wet basis) was calculated according to the method described in UNI EN ISO 18134-1:2015.

2.3. Experimental procedure to investigate the operative parameters of microwave pilot system

To study the operating parameters of the MW pilot system, the experimental tests were carried out depending on the frequency range of the electric motor driving the metal spiral. The throughput of the almonds processed the residence time and the surface temperature of the almonds after treatments by varying the motor frequency. Six different speeds of rotation of the spiral were defined in the frequency range 30-80 Hz (30 Hz - 40 Hz - 45 Hz - 50 Hz - 60 Hz - 80 Hz). Residence time was calculated by measuring the time the product samples took for crossing the treatment chamber. To this purpose, twenty shelled almonds were identified and dyed with grey colour to highlight the contrast in the bulk and allow a fast detection. Dyed shelled almonds were mixed in sample almonds, which were placed in the lower part of the hopper and in a blank trial, and their residence time was measured. Since it is a continuous process, the amount of product (kg) processed in the time interval set to one minute was determined for measuring the flow rate. For each test, at each frequency value, a homogeneous batch of shelled almonds weighing 200 kg each was used. In all tests, the temperature of the incoming almonds was kept constant at 16±1 °C and moisture of 5.0±0.2%. The measurement was repeated 10 times for each frequency value. For the measurement of the surface temperature of the product leaving the machine after treatment, an infrared thermometer (accuracy ±1°C), with which the machine is equipped was used and temperature was displayed on the PLC. The measured operating parameters allowed the calculation of the specific energy that the pilot system supplies to the product with the following formula:

$$SP = \frac{EP * RT}{M} \qquad (1)$$

Where:

SP = Specific Energy [kJ kg^{-1}]

EP = Electrical Power [kW]

RT = Residence Time [s]

M = Mass [kg]

2.4. *Ephestia kuehniella: stock culture and sample preparation*

A population of *E. kuehniella* was reared on wheat flour or shelled almonds in baskets (5 L) closed by a lid and maintained at 27±1ºC, 60±5% relative humidity and room photoperiod (from L12:D12 to L16:D8). Old substrates with silk secretions were removed and renewed almost every month. The eggs (24-hours old) and the larvae (24-hours old and mature) were chosen as targets of the MW application. Groups of 5-6 adults were took away from the rearing baskets and were isolated into Petri dishes of 10 cm of diameter whose bottom was provided with a transparent cellulose disk of 12 cm of diameter. After 24 hours, the cellulose disks were inspected, the eggs glued on the disk were collected and addressed to the assays. With the same procedures eggs were got, reared up to larval hatching and 24-hours old larvae were used for the assays. Finally, mature larvae were selected from the microcosmos on the basis of their tendency to look for refuges where they produce a thin cocoon. These larvae were addressed to the assays.

2.5. *Microwave treatments*

In order to recover the samples submitted to the treatments (eggs and larvae), taking in count their small size in respect to the almonds, the assays were carried out introducing the target stage into transparent gelatine capsules for medical preparation of size 1 (Capsulinem, by Dania Beach, USA) with the capacity of 0.48 mL, 19.4 mm long and 6.39 mm of diameter. Five 24-hours old eggs were introduced into each capsule and five capsules (each one represents a replica) were prepared per each treatment. Each treatment was repeated three times. Five 24-hours old larvae were introduced into each capsule and five capsules (each one represents a replica) were prepared per each treatment. Each treatment was repeated three times.

One mature larva was placed into the capsule and five capsules (each one represents a replica) were prepared per each treatment. Each treatment was repeated three times.

The experimental design provides MW treatments on homogeneous batches of shelled almonds at different speed of rotation of the spiral in the frequency range 30-80 Hz (30 - 40 - 45 - 50 - 60 - 80 Hz) and a total electrical power of 7.5 kW (1.5 kW for each magnetron) for each frequency range.

During each test (one for each moth stage), capsules containing various life stages of *E. kuehniella* were mixed with shelled almonds and poured into the loading hopper of the microwave pilot system. After treatment, the capsules were manually recovered, separated from the almonds and their contents were inspected in the laboratory to determine the mortality rate of each sample.

In the case of 24-hours old eggs, capsules were kept at 27±1ºC and checked daily for a week. The number of hatched larvae was recorded. In the case of 24-hours old and mature larvae, they were checked soon after the treatment for larval mobility and the number of immobile (dead) larvae was recorded.

2.6. Protein extraction and lipoxygenase activity

From a sample of shelled almonds taken for each test, protein samples were extracted as reported in Mita et al. (2001). In detail, almonds treated in the same conditions were pooled, grounded with mortar and pestle. 1 g of powder was homogenized in 7 mL of grinding medium containing 0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM MgCl2, 2 mM DTT, 0.15 M Tricine buffer pH 7.5 and proteinase inhibitor cocktail. Extracts were filtered and placed in a new tube before the addition of the flotation buffer (grinding medium containing 0.25 M sucrose instead of 0.6 M sucrose), which was layered on top. Samples were centrifuged at 5000 g for 20 min at 4°C.

After the removal of lipid bodies from the top of the tube, total proteins were recovered and quantified using the Bradford's protein assay (Bradford, 1976). Lipoxygenase (LOX) activity was measured spectrophotometrically, monitoring the increase in A234 of the conjugated-diene structures according to Santino et al. (2003), using 0.3 mM linoleic acid as substrate in a reaction mixture (1.0 mL) consisting of 100 mM sodium phosphate buffer pH 6.0 and 2 μL of each protein samples.

2.7. Sensory analysis

A quantitative descriptive sensory analysis (QDA) of the almond samples for each test was performed. The sensory profiles of the treated samples were obtained recruiting a panel composed of 8 judges. About 60 g of each treated sample coded with random three-digit numbers were served in cups to panellists. An untreated sample of almonds was added to the sensory analysis. Panellists tasted at least three almonds per treated sample and rated several attributes of appearance, texture, taste and odour on a 5-point scale. The sensory test was repeated twice.

2.8. Statistical analysis

Statistical analysis was performed by subjecting the data to one-way analysis of variance (ANOVA), significant differences between the different assays conditions were searched by means of Tukey's test. Differences were considered significant when $p<0.05$. Statistical analysis was performed using Statistica 6.0 software package.

Linear regression analysis was used to determine the trend of the operative parameters of the microwave pilot system. Second order polynomial regression analysis was used to determine the trend of mortality versus specific energy.

3. Results and discussion

3.1. Operative parameters of microwave pilot system

In table 1 operating parameters of the continuous microwave pilot system *versus* electric motor frequency are reported. Mass flow rate increases proportionally to the motor frequency from 1.91 to 5.39 kg min^{-1} (fig. 2). A linear regression was detected and the correlation resulted relatively strong with R-squared equal to 0.99. Consequently, residence time decreases proportionally in respect to the frequency of the electric motor following a linear progress from about 202 to 82 seconds. Also in this case, the correlation is relatively strong with R-squared equal to 0.86.

Figure 2: Mass flow rate and residence time versus frequency of the electric motor

As the residence time increases, the specific energy increases from 7.3 to 18.1 kJ kg^{-1} according to eq. (1) and the almonds' surface temperature increases from 42.8 to 65.1 °C, showing a linear regression with R-squared of 1 and 0.95 respectively (fig. 3).

Figure 3: Trend of the specific energy and surface temperature versus residence time

3.2. Mortality results

Concerning the mortality of three different life stages of *E. kuehniella* (24-hours old eggs, 24-hours old larvae and mature larvae), the recorded average percentages are represented in Figure 4 as a function of the frequency of the electric motor and of the specific energy supplied, respectively. Eggs and 24-hours old larvae exhibited one hundred percent mortality from 30 Hz until 45Hz of frequency motor, by administering a specific energy equal to 18.1 kJ kg^{-1} at 30 Hz, until 12.1 kJ kg^{-1} at 45Hz. Frequencies from 50 to 80 Hz, with specific energy administered from 10.8 to 7.3 kJ kg^{-1}, caused the mortality of the 24-hours old eggs ranging from 89.33 % to 10.00 % and that of the 24-hours old larvae varying in the range 88.90 % - 75.56 %. Vice versa, all mature larvae did not survive at the entire operating treating values.

Figure 4: The average mortality percentage recorded as a function of frequency of the electric motor and the related specific energy

Figure 5 shows the mortality trend as a function of the specific energy administered. A second order polynomial regression was applied for 24-hours old eggs and 24-hours old larvae obtaining the R-squared equal to 0.83 for both.

Figure 5: Trends of mortality versus specific energy

The results demonstrated that the complete disinfestation of the almonds was achieved by administering an optimal specific energy value of at least 12.1 kJ kg^{-1}. This value is much lower than that found in Patil et al. (2020) to kill the various stages of *T. castaneum* in almonds by a static oven. These different results can depend on the different pest species and on the different microwave technology used for the experimental tests (continuous in semi-industrial scale and discontinuous in laboratory scale).

It is worthy of interest to point out that the aforementioned optimal specific energy value (12.1 kJ kg^{-1}) can also be achieved by lowering the electrical power of the system to MW and increasing the residence time (reducing the frequency of the electric motor) according with equation 1. The trend of the specific energy per electric power equal to 7.5 kW, 6.75 kW and 5.25 kW corresponding to 100 %, 90 % and 70 % of the maximum electric power of the system has been represented in figure 6. Graphically, the horizontal line in correspondence with the specific energy value considered optimal, equal to 12.1 kJ kg^{-1}, intersects the three trends at frequency value that can guarantee the complete mortality of the infesting pest. Additional test had been carried out on eggs and 24-hours old larvae with 6.75 kW of electrical power at 40 Hz and with 5.25 kW at 32 Hz to verify their mortality by lowering the electrical power of the MW system and increasing the treatment time; as hypothesized, one hundred percent mortality of the individuals was recorded.

Figure 6: Trends of specific energy versus frequency of the electric motor at different electrical powers involved

3.3. Quality results

Moisture loss of almonds in the range of 0.08-0.7 % was detected. This data is in accordance to Patil et al. (2020) who recorded less than 1 % moisture loss by treating shelled almonds with MW.

Lipoxygenase (LOX) is considered the main enzyme involved in polyunsaturated fatty acids peroxidation and it is considered a reliable marker of processing and storage of nutty species

(Mita et al., 2001; Santino et al., 2003). We did not observe any significant variation in the LOX activity either in samples treated with low or high energy treatment (Fig. 7).

Also organoleptic properties of almonds might be impacted by MW treatments. The descriptive sensory analysis did not display any difference among almond samples subjected to different treatments. The only exception is represented by the salty descriptor for which panellists appreciated significant intensity in samples treated with 80 Hz (Tab. 2). However, the whole panellists' judgment was not affected by the treatments. In particular, sweetness, a key parameter of almond quality, was judged with not statistically different intensities among all samples and no *off*-flavours were reported either from the olfactory and gustatory points of view.

The sensory result confirms how reported by Patil et al. (2020) who did not find significant difference between control and microwave treated almonds.

4. Conclusions

In this study a continuous microwave pilot system was used for shelled almonds disinfestation by *E. kuehniella*.

The applied specific energy of 12.1 kJ kg^{-1} and the almonds' surface temperature of 55.7 °C effectively achieved 100 % mortality for eggs and 24-hours old larvae. In all tested process conditions, 100 % mortality of mature larvae was always achieved. The different results against the three stages at the three lowest specific energy values applied in these trials can depend on the lower water and fat content of eggs and neonate larvae than mature larvae. The MW system made possible to reach the optimal specific energy value by modulating the electrical power used and the residence time of the almonds in the treatment chamber. No significant differences in LOX activity was recorded either in samples treated with low energy MW or with higher energy treatments. Similarly, sensory analysis showed not differences among almond samples subjected to different treatments compared to the control and no olfactory and gustatory *off*-flavours were also detected. Finally, a very low moisture loss of almonds during the MW process was detected.

This study indicates the potential application of the continuous microwave system in the effective treatment of almonds for the control of *E. kuehniella*. It seems that this method can be also used for an effective treatment of almonds in order to control other pests, with a significant reduction of the treatment time, proving to be a valid alternative to chemical applications enhancing sustainability and competitiveness of the almond industry. The MW system applied in the present research is quite compact occupying little space, allowing a continuous operative running and can be installed in almond processing industries before packaging. Similarly, further foodstuffs commonly infested by *E. kuhniella* like raisin, dried fig (Sadeghi et al., 2018), many cereals, roots (dried), biscuits, dried human food and animal feed (https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.21412) could be managed by means of MW after verifying potential negative effects on the quality of the treated materials.

It should be emphasized that the continuous MW system currently applied has also the potential to be utilized for disinfestation and disinfection of other foodstuffs from more pests (research in progress), and it appears a valid alternative treatment to the chemicals applications. Obviously, more specific trials need to be carried out in order to identify the optimal operating parameters and possible critical and particular aspects to be overcome.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Author contributions

Conceptualization: A.B., A.L., A.T., E.d.L., G.R.; Methodology: A.L., A.S., A.T., M.T.; Software: A.B.; Data curation: A.B., A.L., A.T.; Writing- Original draft preparation: A.L., A.S., A.T., M.D.B., M.T,; Visualization: A.B., A.L., A.T., E.d.L., G.R.; Investigation: A.L., A.T., G.R.; Supervision: A.L., A.T; Validation: A.L., A.T., E.d.L.. A.S.; Writing-Reviewing and Editing: A.L., A.S., A.T., E.d.L.

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Table 1 – Operating parameters of the continuous microwave pilot plant

Electric motor frequency [Hz]	Mass flow rate [kg*min^{-1}]	Residence time [s]	Specific energy [Wh*kg^{-1}] at Pe 7.5kW	Almonds surface temperature [°C]
30	1.91±0.13	202.2±21.4	5.02	65.1±1.21
40	2.76±0.11	148.9±18.7	3.68	58.3±1.05
45	3.00±0.11	135.4±17.5	3.36	55.7±0.82
50	3.28±0.10	121.3±15.6	3.01	53.2±0.92
60	4.2320.11	100.7±13.2	2.49	50.4±0.85
80	5.39±0.11	82.2±10.1	2.04	42.8±0.88

Mean ± standard deviation (SD)

Table 2: Descriptive sensory analysis of almond samples

	Appearence		Texture			Taste Descriptors					Odour Descriptors			
	Color intensity	Roughness	Hardness	Friability	Crunchiness	Sweet	Salty	Marzipan taste	Hay taste	Earthy taste	Marzipan odour	Earthy odour	Woody	Hay odour
30Hz	3.1±0.1	2.8±0.2	2.8±0.02	3.0±0.3	3.5±1	2.5±0.1	3.3±0.4 a	3.3±0.4	1.6±0.1	2.4±0.1	2.5±0.02	2.1±0.2	2.1±0.1	1.5±0.7
40Hz	2.8±0.1	2.6±0.4	3.1±0.8	2.7±0.2	2.7±0.5	2.1±0.7	2.0±0.02 a	2.8±0.02	1.5±0.02	2.4±0.1	3.0±0.8	1.8±0.3	2.1±0.2	1.3±0.4
45 Hz	2.4±0.1	2.0±0.02	2.3±0.4	1.9±0.4	2.2±0.02	2.7±0.1	2.0±0.02 a	2.6±1.1	1.5±0.7	2.3±0.5	2.4±0.5	1.8±0.4	1.7±0.1	1.5±0.02
50 Hz	2.8±0.6	2.7±0.4	2.6±0.1	2.5±0.3	2.7±0.2	2.5±0.4	2.0±0.02 a	2.9±0.2	1.3±0.5	2.0±0.4	2.6±0.2	1.8±0.02	2.2±0.3	1.5±0.7
60 Hz	3.0±0.01	2.8±0.5	2.5±0.2	2.5±0.4	2.8±0.4	2.3±1.1	1.8±1.1 a	2.9±0.5	1.3±0.4	2.1±0.1	2.3±0.7	1.7±0.7	1.7±0.7	1.2±0.2
80 Hz	2.3±0.2	2.6±0.1	2.4±0.8	2.7±0.02	2.3±0.1	2.7±0.1	1.0±0.02 b	2.4±0.8	1.3±0.4	2.5±0.7	2.0±0.7	1.8±0	2.4±0.5	1.0±0.02
CTRL	3.2±0.5	2.8±0.6	2.7±0.2	1.9±0.1	2.1±0.1	2.7±0.1	1.5±0.2 a	2.5±0.1	1.0±0.02	2.1±0.5	2.2±0.3	1.9±0.9	1.8±0.2	1.3±0.4

Fig. 1: Microwave pilot plant scheme: a) loading hopper, b) microwave filter, c) treatment chamber, d) spiral, e) magnetrons (M) with the power generator (G), f) discharge point, g) Programmable Logic Controller (PLC), h) speed reducer, i) electric motor, j) sensor, k) thermometer

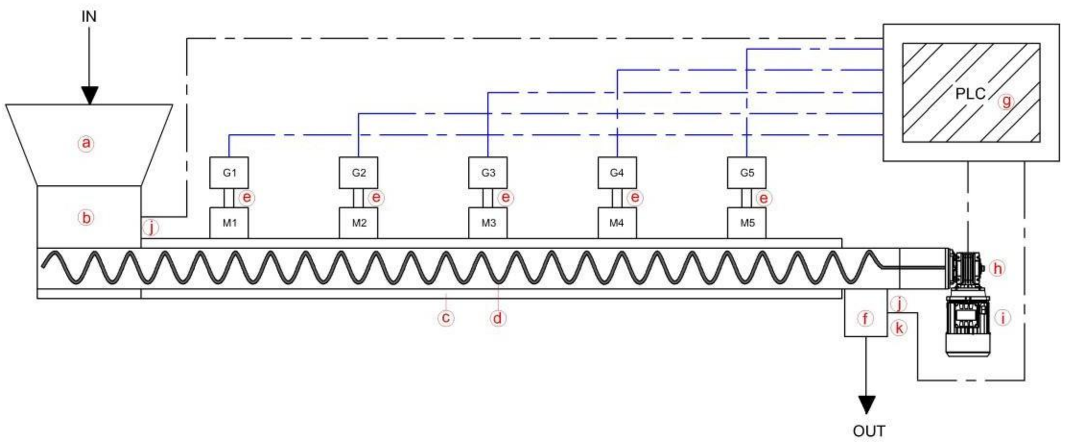
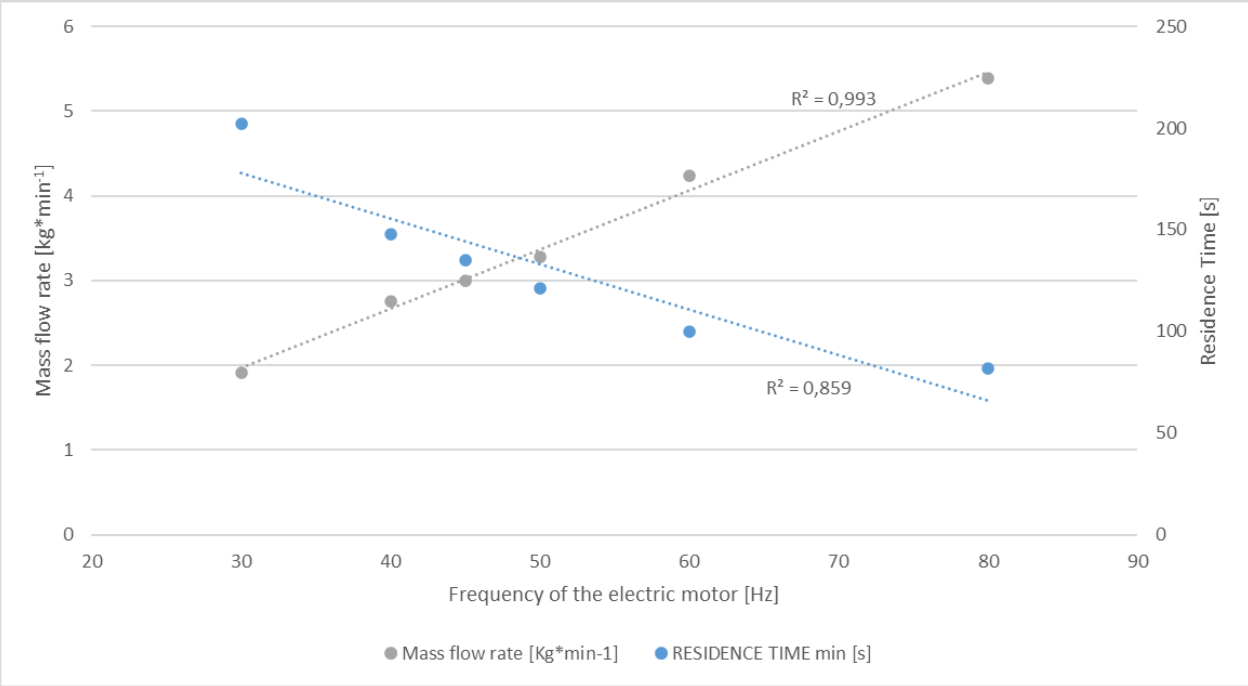


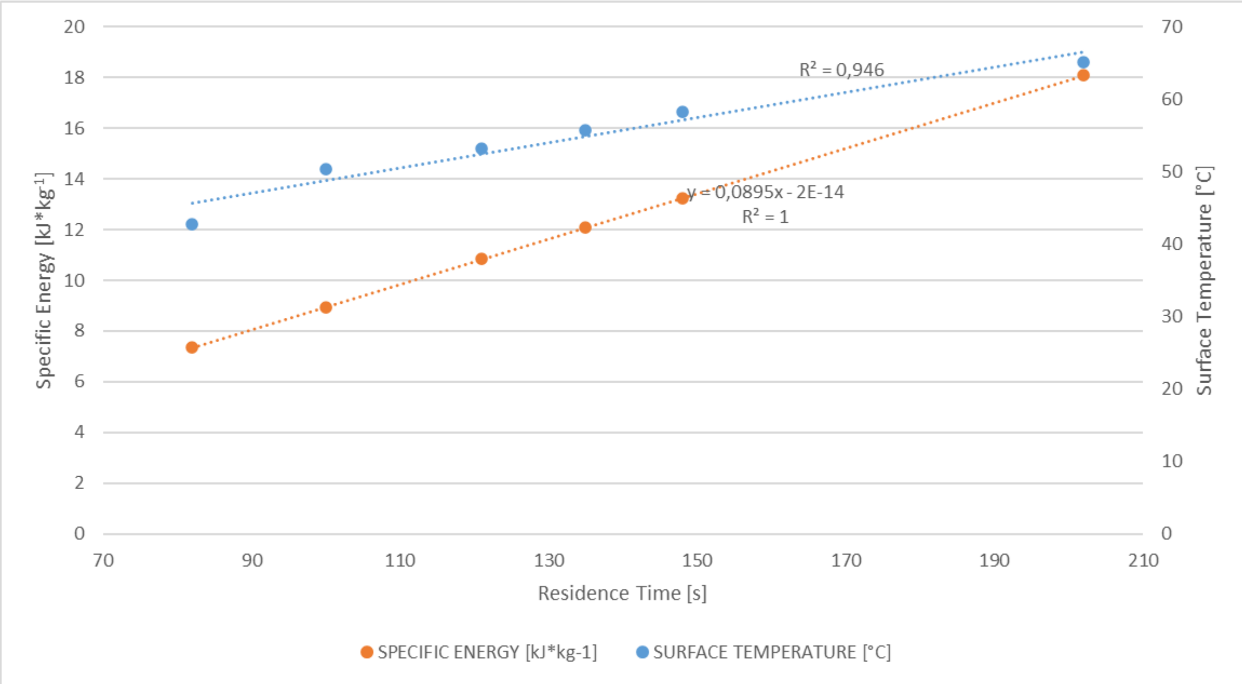
Figure 2: Mass flow rate and residence time versus frequency of the electric motor



Commento [?1]: Bisogna sostituire la virgola con il punto

Commento [AT2]: Deve provvedere Antonio

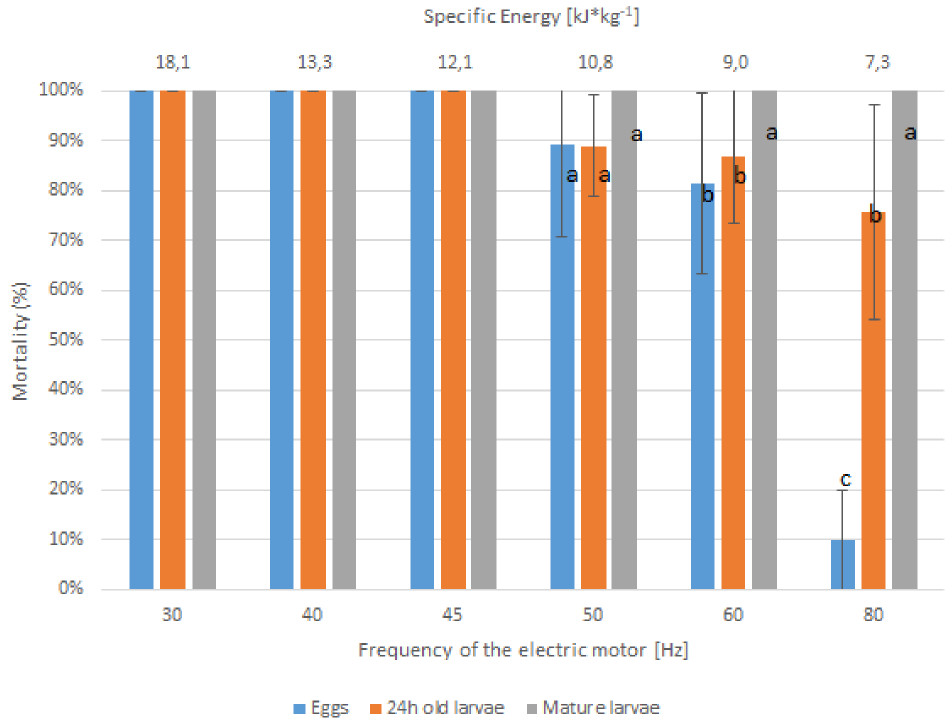
Figure 3: Trend of the specific energy and surface temperature versus residence time



Commento [?3]: Bisogna sostituire la virgola con il punto

Commento [AT4]: Deve provvedere antonio

Figure 4: The average mortality percentage recorded as a function of frequency of the electric motor and the related specific energy



Commento [?5]: Bisogna sostituire la virgola con il punto

Figure 5: Trends of mortality versus specific energy

Commento [?6]: Bisogna sostituire la virgola con il punto

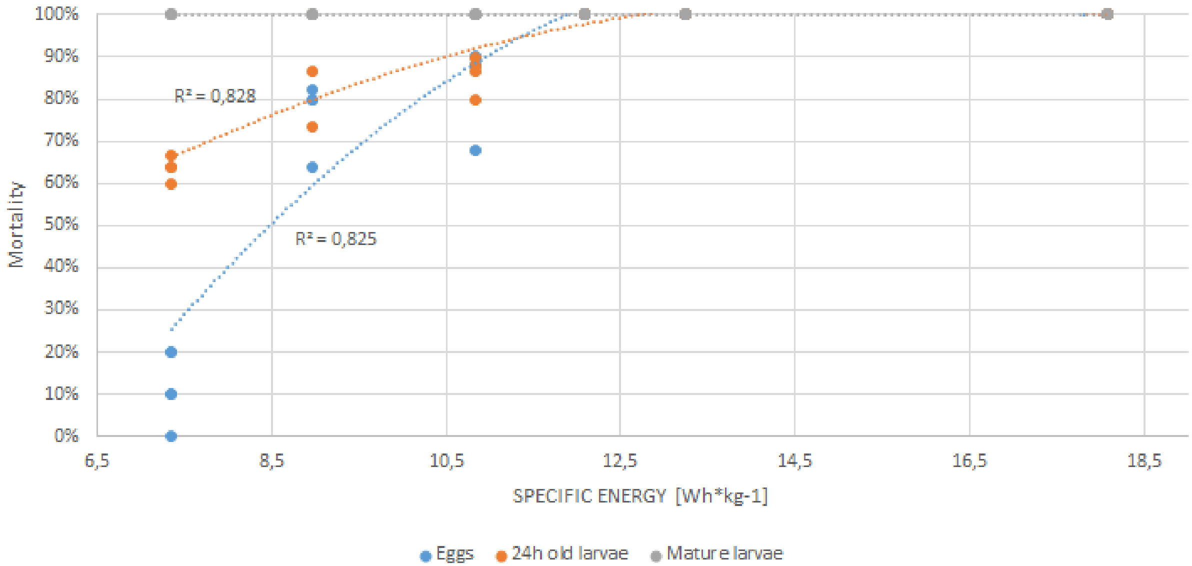
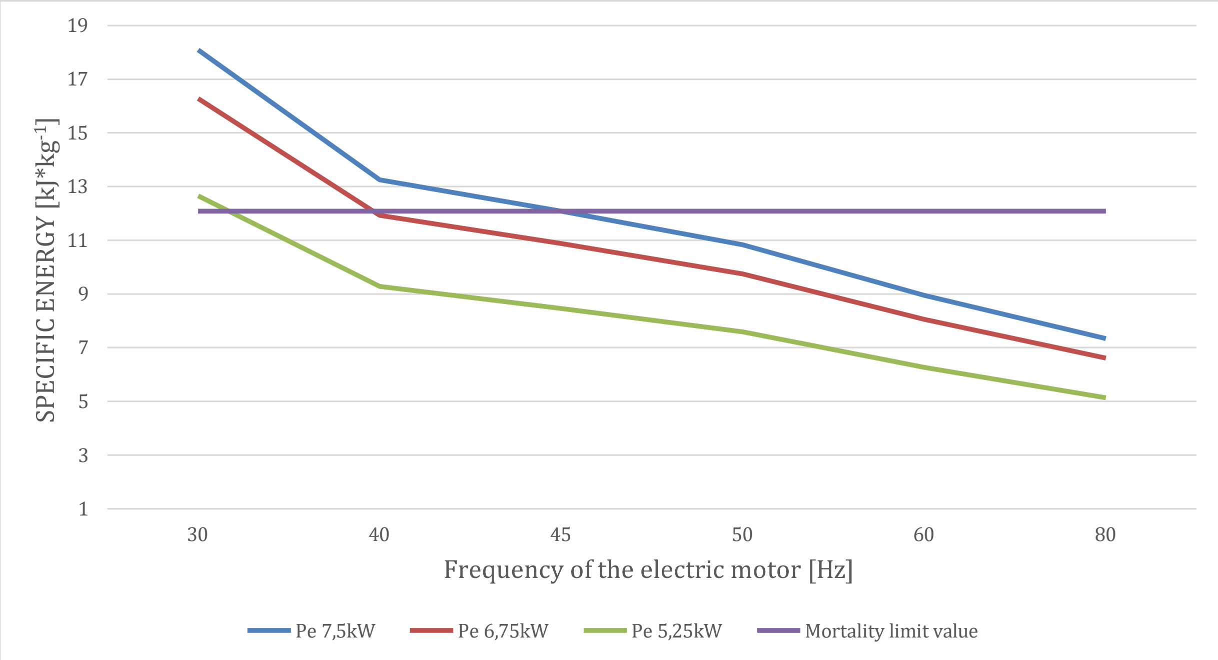
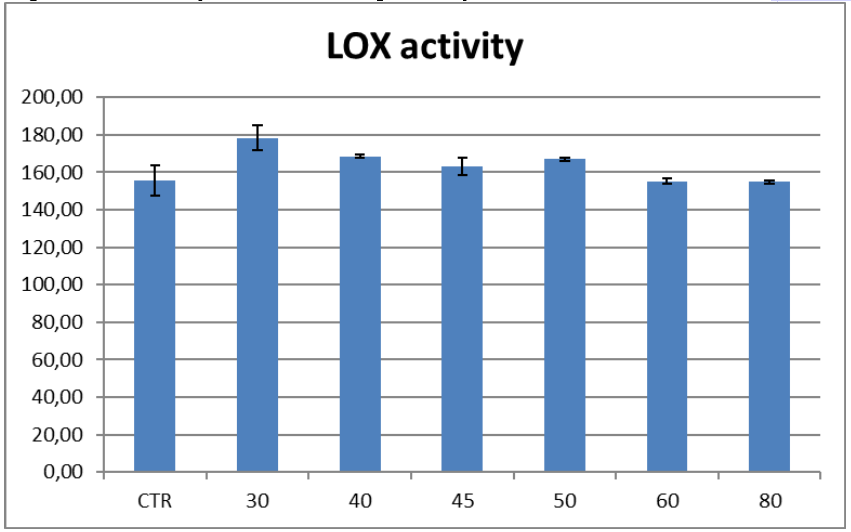


Figure 6: Trends of specific energy versus frequency of the electric motor at different electrical powers involved



Commento [?7]: Bisogna sostituire la virgola con il punto

Fig. 7. Lox activity in almond samples subjects to different microwave conditions.



Commento [?8]: Bisogna sostituire la virgola con il punto o, meglio ancora, cancellare i decimali

Responses to reviewers

Reviewer #1: In this manuscript investigations were conducted on the use of a physical technology for the disinfestation of shelled almonds from *E. kuehniella* as an alternative to chemical treatments. To this aim a continuous MW plant has been used. The manuscript reads very well, and it is interesting for possible useful industrial applications in the physical disinfestation process. In my opinion, the manuscript could be accepted after minor revisions.

INTRODUCTION

1) The introduction is well written. However, a better treatment of some references could be done (line 39-40 and 68-69).

A better treatment of the references has been done at lines 43-45 and 70-71.

2) Check all the references in the text and in the references chapter, there seem to be some discrepancies as an example: Das et al 2022 is not in the references, Azizoglu et al., 2010 is 2011 in the references; Stejskal, V. et l 2015 is not in the text.

Das et al 2022 added, Azizoglu revised, Stejskal, V. et l 2015 deleted.

3) Lines 106-109: specify better the research focus pertaining the stages of the pest and the qualitative parameters investigated.

The population stages of *E. kuehniella* have been specified. Lines 11-113

4) Line 107: Deleting the reference seems to be a mistake.

The reference reported by mistake has been deleted.

MATERIALS AND METHODS:

5) Figure 1: Delete M1, M2,… and G1, G2,… PLC and replace them with letters.

Figure has been replaced and description revised

6) How long is the treatment chamber? Please solve the weakness.
The diameter of 91 mm refers to the internal or external of the tube.
Does the volume of 20.4 L include the volume of the spiral or not?
What section does the spiral have?
Why did authors choose the frequency range between 30 and 80Hz? Please specify

The technical characteristics of the treatment chamber have been specified in chapter 2.1. Lines 126-132

8) Line 160: replace 5% in 5.0%.

Done.

9) Line 167: delete. Seems to be a useless repetition.

The sentence has been deleted.

10) Line 178: replace 5% in 5.0%.

Done

RESULTS

11) Figure 2, 3 and 5: please report the R-squared data with two decimals.

The R-squared value has been corrected

12) In table 1 specific energy is reported in Wh kg-1, please replace in kJ kg-1.

The unit has been corrected

13) Line 254: replace "from 202 to 82 seconds" with "from about 202 to 82 seconds".

The word has been added. Line 261

14) Line 260: in the test 60.2°C is reported but in table 1 60.1°C. Please check.

The correct value has been reported in the text. Line267.

15) Line 261: replace "….with R-squared of 0.95…." in ….. with R-squared of 1 and 0.95 respectively.

The sentence has been corrected. Line 268

16) Figure 4: please report specific energy data to one decimal place.
The value has been corrected

17) Figure 5: on the X-axis specific energy is capitalized, please standardize.
Done

18) Figure 5: please correct X-axis units to kJ kg-1
Done

19) Line 292: please show R-squared with two decimal places
The R-squared value has been corrected

20) Line 287-290: Please better specify the difference between the two microwave technologies used in the two experiments.
A sentence was added at line 297-298

Reviewer #2: The Ms by Tamborrino et al. entitled "Using a continuous microwave system for postharvest almonds' disinfestation" reports on laboratory experiments aiming at the development of a continuous microwave (MW) system and a proper treatment program of shelled almonds infested by eggs and larvae of the Mediterranean flour moth, Ephestia Kuehniella, one of the most damaging pests of stored almonds. By applying increasing levels of specific energy from 7.3 to 18.1 kJ kg-1 the authors achieved 100% mortality of fourth-instar larvae while 100% mortality of 24-h-old eggs and 24-h-old larvae was observed by administering specific energy of at least 12.1 kJ kg-1. Moreover, no significant differences in the LOX, organoleptic properties and off-flavors production were detected between MW-treated and control samples were detected. Rational for the study is clear. Methodology is appropriate. Results support conclusions. The use of MW as a safe pest control mean is not completely new however this paper adds new interesting information that can contribute to the development of an effective MW treatment program of shelled almonds to control the main postharvest insect pest. Therefore, in my opinion, after minor revision the MS deserves publication in Postharvest Biology and Technology.

I offer the following points for consideration.

1. In general, in the MS there are several sentences that are too long and not immediately understandable. Thus, the reading fluency of the MS could greatly benefit from a careful revision with the help of a native English speaker.
English proofreading by a native speaker has been done

2. To broaden the importance of the work, it would be appropriate to add further details on the pest status of E. kuehniella, for example, citing other foodstuffs attacked by this pest that could be treated with the same MW system.
A sentence was added. Lines 356-360.

3. Linear regression analysis reported in the text should also be mentioned in the materials and methods section. In addition, authors should indicate in the text and in the table the F, df and P values of ANOVA they performed.
Two sentences have been added to the chapter 2.8. Lines 250-252.

4. Lines 176-178. The meaning of "microcosmos" seems unclear. I suggest: *E. kuehniella* were reared on wheat flour (0.5 L) or shelled almonds in baskets (5 L) closed by a lid and maintained at 27±1ºC, 60±5% relative humidity and room photoperiod (from L12:D12 to L16:D8).
The sentence was modified according to reviewer's suggestion. Lines 183-185.

5. Line 181. "because they can be more probably present into the shelled almond samples" This sentence is unclear. In this species eggs are laid on the foodstuff surface and first-instar larvae are unlikely to penetrate almonds 24 hours after egg hatching. If I understand the meaning of this sentence correctly, in my opinion this is not a valid justification. I suggest removing this sentence from the MS.
The sentence was deleted according to reviewer's suggestion.