Effect of Pulsed Electric Fields (PEF) combined with natural antimicrobial by-products against *S.* Typhimurium

Maria Sanz-Puig[1], Leonor Santos-Carvalho[2], Luís Miguel Cunha[2], M. Consuelo Pina-Pérez[1], Antonio Martínez[1], Dolores Rodrigo[1*]

[1]Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Carrer del Catedràtic Agustín Escardino Benlloch, 7, 46980, Paterna, València, España.

[2]LAQV, REQUIMTE/DGAOT, Faculdade de Ciências. Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, 7, 4485-661 Vairão, Vila do Conde, Portugal.

*corresponding author: lolesra@iata.csic.es

Abstract

The effect against *Salmonella enterica* serovar Typhimurium of PEF treatment combined with cauliflower and mandarin by-product infusions at several concentrations (0, 1, 5, and 10% (w/v)) was evaluated at various incubation temperatures (10, 22, and 37 °C). The possible synergistic antimicrobial action of the combined process of Pulsed Electric Field (PEF) technology followed by exposure to the by-product infusions and the occurrence of sublethal cellular damage were also studied. Antimicrobial kinetics of by-product infusions alone or following PEF treatment were fitted to a Weibull model. Both mandarin and cauliflower by-product infusions showed a maximum antimicrobial effect against *S.* Typhimurium after 10 hours at 37 °C when the microorganism was exposed to 10% of by-product infusion, achieving reductions of initial bacterial load up to undetectable levels. The effect of the PEF treatment (20 kV – 900 μs) caused a reduction of 4 log cycles of the initial cell population (10^8 cfu/mL) of *S.* Typhimurium and 1 log cycle (90%) of cellular damage. Moreover, when the PEF pre-treated *S.* Typhimurium population was subjected to subsequent incubation in the presence of both by-product [10%] infusions, the microbial inactivation was faster, achieving a reduction of the initial bacterial load (4 $\log_{10}$ cycles) up to undetectable levels in 2 hours. The kinetic values of the Weibull model were obtained. The higher the concentration of by-product infusion, temperature, and PEF treatment applied, the greater the kinetic parameter "*b*" values, which are related to the microbial inactivation rate. Therefore, the addition of cauliflower and mandarin by-product infusions could be a good additional control measure contributing to ensure bacterial counts below recommended limits in pasteurized PEF products during their storage at refrigeration temperatures.

Keywords: *S.* Typhimurium, cauliflower by-product, mandarin by-product, PEF treatment, antimicrobial effect, sublethal damage

1. INTRODUCTION

In the last few years, international organizations such as the World Health Organization (WHO) and Food Agricultural Organization (FAO) have shown their concern about microbiological contamination in the food chain, because population mobility and food globalization have led to an increase in food outbreaks (WHO, 2008; EFSA, 2010).

One of the most important foodborne pathogens is *Salmonella*, which causes approximately 93.8 million foodborne illness outbreaks and 155,000 deaths per year (Majowicz et al., 2010). *Salmonella enterica* serovar Typhimurium is especially related to meat, eggs, and fresh fruits and vegetables (EFSA, 2011). In the last few years, the incidence of these foodborne outbreaks has been greater, and has increased people's concern about them (Pui et al., 2011). Therefore, one of the aims of current food research is to avoid outbreaks caused by foodborne pathogens such as *Salmonella*.

Traditionally, thermal treatment was the most used mechanism to guarantee the microbial safety of food products. Now, however, new non-thermal treatments have been developed to preserve food products, maintaining their organoleptic and nutritional properties (Knorr et al., 2011; Barret & Lloyd, 2011). Among the most validated non-thermal treatments applied to food preservation, a notable tendency is the addition of natural antimicrobial compounds from plants (Cava et al., 2007; Ferrer et al., 2009) or the application of new non-thermal technologies such as High Hydrostatic Pressure or Pulsed Electric Fields (PEF) (Aymerich et al., 2005; Mosqueda-Melgar et al., 2012).

The development of non-thermal technologies such as PEF for food preservation has increased in recent years, mainly because of the demand for potential methods to ensure not only the microbiological harmlessness of products but also the preservation of their organoleptic and nutritional properties. In this respect, PEF technology appears to be a good alternative to thermal pasteurization processes, only applied to liquid products but with good prospects for being used in the dairy and juice industries (Pina-Pérez et al., 2012). In fact, there are several studies that show that the antimicrobial reduction achieved by PEF treatments both in reference media and in food products with various bacteria (Saldaña et al., 2011; Pina-Pérez et al., 2012; Monfort et al., 2012) could be up to 6 $\log_{10}$ cycles. Moreover, recently many studies have tested a wide variety of hurdle combination technologies that reduce the intensity of treatments through the synergistic effect of combinations (Iu et al., 2001; Pina-Pérez et al., 2009).

Many research studies have also demonstrated the antimicrobial properties of compounds such as polyphenols, carotenoids, and flavonoids, which we can find in some fruits and vegetables (Djilas et al., 2009; O'Shea et al., 2012). Among them, both *Citrus* and *Brassicaceae* families have been shown to contain bioactive compounds with antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial capacity (Ghafar et al., 2010; Igual et al., 2013). These bioactive compounds are usually in the peel, pulp, or leaves of these fruits and vegetables. Consequently, we can also find them in agro-industrial by-products, large amounts of which are generated in the food industry. Moreover, the revalorization of food by-products can avoid the economic and environmental costs that they create for producers (Martin-Luengo et al., 2011) and help to meet the requirements of the European Union (EUROSTAT, 2010).

In this study, the antimicrobial capacity of two infusions of agro-industrial by-products, cauliflower and mandarin, alone or combined with several PEF treatments, against *Salmonella enterica* serovar Typhimurium was evaluated.

2. MATERIAL AND METHODS

2.1 Microorganism

A freeze-dried pure culture of *Salmonella enterica* serovar Typhimurium (CECT 443) was provided by the Spanish Type Culture Collection. It was rehydrated with 10 mL of tryptic soy broth (TSB) (Scharlab Chemie). After 20 min, the rehydrated culture was transferred to 500 mL of TSB and incubated at 37 °C, with continuous shaking (Selecta Unitronic) at 200 rpm for 14 h. The cells were centrifuged (Beckman Avanti J-25) twice at 4000 rpm at 4 °C for 15 min and then resuspended in TSB. After the second centrifugation, the cells were resuspended in 20 mL of TSB with 20% glycerol and then dispensed in 2 mL vials to a final concentration of 7.6×10^9 cfu/mL obtained by plate count. The 2 mL samples were immediately frozen and stored at -80 °C until needed for the kinetic inactivation studies.

2.2 Antimicrobial substances

Cauliflower and mandarin by-products from agro-industrial raw materials were provided as dehydrated residues from primary production of TRASA S.L. and INDULLEIDA S.A., respectively. The raw by-products were washed in sterile water to eliminate contaminating substances, dried, triturated, and homogenized using a

105 laboratory grinder (Janke & Kunkel Ika-Labortechnik) to obtain a powder with a
106 particle size of 40 μm, which was used to perform the experiments (Brandi et al., 2006).

107 **2.3 Preparation of by-product infusions**

108 Infusions at 10% (w/v) from dried cauliflower and mandarin by-products were obtained
109 by boiling in buffered peptone water (0.1% (w/v)) for 30 min. After this, the infusions
110 were centrifuged at 4000 rpm – 15 min at 4 °C for cauliflower and at 3000 rpm – 5 min
111 in the case of mandarin. Then the infusions were filtered three times, using filters with a
112 pore size of 11 and 2.5 (Whatman), and 0.45 μm (PVDF syringe filter) to sterilize the
113 infusions before use.

114 Finally, from the 10% infusions of cauliflower and mandarin by-products it was
115 obtained 1 and 5% infusions by diluting them with buffered peptone water (0.1%
116 (w/v)). For the control sample, buffered peptone water (0.1% (w/v)) without addition of
117 infusion was used.

118 **2.4 Pulsed Electric Field treatment (PEF)**

119 Initially, one sample of pure culture prepared and stored frozen (2 mL), was diluted in
120 18 mL of buffered peptone water (Scharlab Chemie, Barcelona, Spain) 0.1% (w/v).
121 Later, 1 mL of this dilution with approximately 10^8 cfu/mL initial concentration of *S.*
122 Typhimurium was inoculated in buffered peptone water (Scharlab Chemie, Barcelona,
123 Spain) (0.3% (w/v)) and was then treated by PEF. The PEF equipment (OSU-4D,
124 designed by Ohio State University) consists in eight chambers connected in series with
125 a diameter of 0.23 cm. Between chambers there was connected cooling coils and
126 submerged in a refrigerated bath (20± 0.5 °C). The intensity, voltage and pulse of
127 treatment were recorded by an oscilloscope (Tektronic TDS 210, Tektronic, OR). The
128 pulses are square-wave bipolar, with a duration of 2.5 μs, the flow was 30 mL/min (set
129 using a gear pump (Cole-Parmer 75210-25, Cole-Instruments Parmer, IL)) and the
130 medium was buffered peptone water 0.3% (w/v) because its conductivity (2,57 mS/cm
131 at 25 ºC) was optimal to applied PEF treatment. The pulse frequency was in the range
132 164 – 904 Hz and the temperature increased from 13 to 45 ºC during the treatment.

133 First we applied a screening of 20 PEF treatments ([10–40] kV/cm; [40–220 μs]) to the
134 sample and from all of them we chose a treatment of 20 kV/cm – 900 μs, an

intermediate treatment that is able to produce 4 log cycles of microbial inactivation and 1 log cycle of cellular damage.

2.5 Evaluation of antimicrobial capacity

Both treated and untreated *S.* Typhimurium samples were inoculated in tubes with cauliflower and mandarin infusions (1, 5, and 10% (w/v)) and incubated at 10 and 37 °C. During the incubations, the *S.* Typhimurium population was determined by plate count in Tryptic Soy Agar (TSA) (Scharlab Chemie, Barcelona, Spain) at regular time intervals after serial dilution with 0.1% (w/v) buffered peptone water The initial counts in the samples without PEF treatment were 10^7 cfu/mL and in the samples with previous PEF treatment were 10^3 cfu/mL. The plates were incubated at 37 °C for 24 hours. All analysis was done in triplicate.

2.6 Evaluation of cellular damage

In the same way as with the antimicrobial capacity evaluation, cellular damage was evaluated by plate count after several decimal dilutions of 1 mL of sample in buffered peptone water at regular time intervals, in TSA and in TSA with 3% of NaCl,. The addition of 3% of salt converts TSA (general medium) into a selective medium in which only intact cells will grow, while in the TSA medium all viable cells (damaged and intact) will grow. The damaged and dead cell counts were obtained by using the following equations:

$$Damaged\ cells = log\left(\frac{CFU/mL\ nonselective}{CFU/mL\ selective}\right) \quad (1)$$

$$Dead\ cells = log\left(\frac{CFU/mL\ \ nonselective\ t2}{CFU/mL\ nonselective\ t1}\right) \quad (2)$$

where CFU/mL selective is the count in selective medium (TSA with 3% NaCl); CFU/mL nonselective is the count in non-selective medium (TSA) and t is the time. Differences in damaged cell counts lower than 0,5 log cycles were not considered.

2.7 Total polyphenol content

158 The total phenol content of the cauliflower and mandarin by-product infusions was
159 determined spectrophotometrically according to the Folin–Ciocalteu colorimetric
160 method (Singleton & Rossi, 1965). We prepared gallic acid calibration standards with
161 concentrations of 0, 100, 200, 300, 400, 500, 600, 700, 800, and 1000 ppm. Three mL
162 of sodium carbonate solution (2% (w/v)) (Sigma-Aldrich Co. LLC, USA) and 100 µL of
163 Folin–Ciocalteu reagent (1:1 (v/v)) (Sigma-Aldrich Co. LLC, USA) were added to an
164 aliquot of 100 µL from each gallic acid standard (Sigma-Aldrich Co. LLC, USA) or
165 sample tube. The mixture was shaken and allowed to stand at room temperature in the
166 dark for 1 h. Absorbance was measured at 750 nm using a Lan Optics Model PG1800
167 spectrophotometer (Labolan, Spain), and the results were expressed as mg of gallic acid
168 equivalents (GAE)/L.

169 ### 2.8 Mathematical modelling of *S.* Typhimurium inactivation

170 The microbial behavior of *S.* Typhimurium was fitted to a Weibull equation (Peleg &
171 Cole, 1998):

$$\log_{10}\left(S(t)\right) = -b \times t^{n} \quad (3)$$

172 where t is the time (hours), S is the survival fraction, i.e., the quotient between the cell
173 concentration at time t (Nt) (CFU/mL) and the initial cell concentration (N_0) (CFU/mL);
174 *b* is the scale factor, and *n* is the form factor.

175 ### 2.9 Statistical analysis

176 The statistical analysis was performed with STATGRAPHICS Centurion XV (version
177 15.1.03; STATGRAPHICS, Warrenton, VA).

178 Also, average and standard deviation calculations for the three repetitions and an
179 ANOVA analysis to test significant differences between samples were carried out. The
180 goodness of fit of the model was assessed by using the adjusted regression coefficient
181 (adjusted-R^2) (López et al., 2004). Assumptions regarding the application of the
182 Weibull model to fit the data were performed in accordance with Cunha et al. (2006).

183

184 ## 3. RESULTS AND DISCUSSION

3.1 Antimicrobial effect of cauliflower and mandarin by-product infusions against *S.* Typhimurium

The antimicrobial effect of mandarin and cauliflower by-product infusions against *S.* Typhimurium was evaluated under different incubation conditions combining concentrations of infusion in the range [0–10]% and temperatures of 10, 22, and 37 °C. The samples were incubated until the population of *S.* Typhimurium became stable, in case of growth , or until it reached the method detection limit in the cases in which it was inactivated.

When *S.* Typhimurium was exposed to cauliflower infusion (Figure 1), the 1% concentration did not produce an antimicrobial effect and the microorganism grew, showing a behavior similar to that of the control sample without by-products (0%). However, the 5 and 10% cauliflower concentrations had an antimicrobial effect against *S.* Typhimurium, achieving complete bacterial reduction at 10% of cauliflower infusion at all the temperatures tested (10, 22, and 37 °C). Obviously, at lower incubation temperatures the time necessary for microbial inactivation was longer, probably owing to the reduction of its metabolic activity and also the low permeability of the cell membranes at cold temperatures, which would slow down the effect of antimicrobials. These results are in agreement with other studies, such as McDonald et al., 1999, or Swinnen et al., 2004.

In previous studies (Sanz-Puig et al., 2015), the antimicrobial potential of cauliflower by-product infusion obtained with buffered peptone water at ambient temperature was tested against *S.* Typhimurium and other bacteria, and now, if we compare the antimicrobial effect of cauliflower by-product infusion obtained at ambient temperature and 100 °C, we can conclude that the infusion obtained at 100 °C exerts a higher antimicrobial effect than the infusion obtained at ambient temperature, achieving total inactivation in a shorter period of time. Jaiswall et al. (2012) also reported the antimicrobial properties of different extracts from several brassicas against Gram – and Gram + bacteria. Also, Burris et al. (2012) tested the antimicrobial activity of aqueous extracts of yerba mate against *E. coli*, achieving approximately 4–5 log cycle reductions in apple juice with 40 mg/mL extract.

The bactericidal effect of the mandarin by-product infusion (Figure 2) was only exerted at 37 and 10 °C. At 22 °C, the infusion only showed a bacteriostatic effect, slowing

down the *S.* Typhimurium growth, at all the concentrations studied. In contrast, at 37 °C, the bactericidal effect of the mandarin infusion was effective at all concentrations, and the higher the concentration, the higher the antimicrobial effect, achieving total inactivation of the initial bacterial load with 10% of mandarin by-product infusion after 96 hours of exposure. Finally, at 10 °C, the concentrations of 1 and 5% exerted a bacteriostatic effect (slowing down the growth) against *S.* Typhimurium, while 10% was a bactericidal concentration, achieving complete inactivation of the bacterial inoculum after 264 hours of incubation. Our results are in agreement with Espina et al. (2011), who showed the antimicrobial effect of mandarin and other citrus fruits using the agar disc diffusion technique.

Previous studies have indicated the antimicrobial properties of species of both families studied, brassica and citrus, mainly due to the fact that they are rich in various phytochemicals with antimicrobial properties.

Polyphenols are among the most important phytochemicals with antimicrobial potential (Daglia, 2012). The high antimicrobial effect produced by both cauliflower and mandarin by-product infusions might be related to their total polyphenol contents, which are shown in Table 1. Although there are no statistical differences between the total polyphenol contents of the cauliflower and mandarin by-product infusions, the fact that the cauliflower infusion has a higher value than the mandarin infusion or their different polyphenolic profile could be the main causes of the greater antimicrobial effect of cauliflower infusion against *S.* Typhimurium. Our results are in agreement with those obtained by Adámez et al. (2012), who showed that aqueous extracts from grape seeds (*Vitis vinifera* L.) had a total polyphenol content of 6000 mg/L gallic acid, approximately, and exerted an antimicrobial effect against Gram-positive and Gram-negative bacteria, achieving a maximum microbial reduction of 10^5 cfu/mL with the highest extract concentration tested (100 µL/mL).

3.2 Antimicrobial effect of pulsed electric fields (PEF) followed by exposure to cauliflower and mandarin by-product infusions against *S.* Typhimurium

Results of the effect of the exposure of bacterial cells to PEF and the cauliflower and mandarin infusions can be seen in Figures 3 and 4. When the samples inoculated with 10^8 cfu/mL of *S.* Typhimurium were treated by PEF, reductions of 4 log cycles were achieved in the *S.* Typhimurium bacterial load.

PEF-treated samples were incubated in the presence of cauliflower and mandarin by-product infusions at concentrations of 0, 1, 5, and 10% and at different temperatures (10, 22, and 37 °C).

When PEF-treated samples of *S.* Typhimurium were incubated with cauliflower infusion (Figure 3), both 5 and 10% concentrations exerted a bactericidal effect, while the concentration of 1% had a bacteriostatic effect, slowing down the microbial growth at all the temperatures tested. The treated bacterial population was reduced completely after exposure to 5 and 10% of cauliflower for (i) 2 hours at 37 °C, (ii) 24 hours at 22 °C, and (iii) 48 hours at 10 °C.

Also, when the treated samples were incubated with mandarin by-product infusion (Figure 4), at 22 and 37 °C the 1% concentration had a bacteriostatic effect against *S.* Typhimurium and concentrations of 5 and 10% exerted a bactericidal effect. However, all concentrations of mandarin by-product infusion had a bactericidal effect at the temperature of 10 °C. The time necessary for inactivation by mandarin by-product infusion of bacteria surviving the PEF treatment was (i) 2 hours at 22 and 37 °C, and (ii) 32 hours at 10 °C.

The results obtained are in agreement with other research studies with PEF and other natural compounds (Pina- Pérez et al., 2012; Mosqueda-Melgar et al., 2012).

3.3 Evolution of *S.* Typhimurium different cell populations (intact, damaged and dead) under exposure to by-product infusions combined or not with PEFpre-treatment

The *S.* Typhimurium cellular damage caused by the addition of cauliflower and mandarin by-product infusions to the media, alone or following the application of PEF treatment was evaluated during incubations 10, 22 and 37ºC and at different infusion concentrations (0, 1, 5, 10 %).

As an example, Figure 5 shows the intact, damaged, and dead cells of *S.* Typhimurium during their incubation at 37 °C with/without 5% of cauliflower infusion and with/without PEF pre-treatment (20 kV/cm – 900 μs). The control sample (a), 0% cauliflower infusion without PEF treatment, grew during incubation and the damaged cells were maintained at low levels. However, for the PEF-treated sample (b), in addition to the 4 log cycles of inactivation due to the treatment, an additional percentage

of damage was observed due to the effect of PEF treatment, which was higher than in the control sample and decreased after 2h incubation time due to these damaged cells were recovered. In contrast, when the initial population of *S.* Typhimurium was incubated with 5% of cauliflower infusion (c) the number of intact cells decreased and the death of bacterial cells increased during the incubation period. The amount of damaged cells increased during incubation owing to the effect of the cauliflower infusion addition. Finally, in the sample that was treated by PEF and then exposed to 5% of cauliflower infusion (d), the cellular damage was the highest, approximately 1.5 log cycles of the PEF survival population (4 $\log_{10}$ cycles). During incubation of this sample, intact cells (selective medium) seem to be constant with the incubation time, but the counts in non-selective medium were reduced, therefore, dead cells increased, and damaged cells decreased progressively up to undetectable limits. This situation was reached in a shorter period of time than in the other samples (1.5 hours), owing to the combined effect of PEF treatment and addition of the cauliflower infusion. In fact, if we focused in hour 1,5-2, we can see that when the microorganism was incubated with cauliflower infusion there was 7'13 log cycles of intact cells, in contrast, when PEF treatment was applied produced a reduction of intact cells until 3,27 log cycles and 0,5 log cycles of cellular damage and, finally, the combination of PEF treatment and cauliflower infusion caused a reduction until 1,99 log cycles of intact cells and 0,65 log cycles of cellular damage.

Figure 6 shows the results obtained for *S.* Typhimurium treated/not treated by PEF and incubated at 10 °C with/without 10% of mandarin by-product infusion. With regard to the mandarin by-product infusion, for example in a concentration of 10% incubated at 10 ºC, the control sample (a) showed growth behavior again. When the initial *S.* Typhimurium population was treated by PEF (b), it was reduced by 4 log cycles and some of the survival cells were damaged. During the incubation period the damaged population remained static, because 10 °C is a refrigeration temperature that slows down microbial metabolic activity (Belda-Galbis et al., 2014; Okada et al., 2013). When *S.* Typhimurium was incubated with 10% of mandarin by-product infusion (c), the intact cells decreased, the dead cells increased, and the sublethal damage increased with the incubation time, achieving complete bacterial inactivation at 240 hours. However, the effect of PEF treatment and subsequent exposure to addition of the 10% mandarin infusion (d) caused (i) a reduction of 4 log cycles in the initial cell population, and

approximately 1.5 log cycles of damaged cells owing to the PEF treatment, (ii) a reduction of survival cells during the incubation period, achieving total inactivation (undetectable limits) in a shorter time (24 hours) than the sample incubated only with mandarin infusion, owing to the combined hurdle effect and (iii) the proportion of sublethal damage cells increased. Also, we can compare the microbial population at 24 hours: when the microbial cells were incubated with mandarin infusion there was 5,52 log cycles of intact cells at 24 hours, when PEF treatment was applied there was 2,62 log cycles of intact cells and 0,29 log cycles of damaged cells at the same time and when PEF treatment was combined with cauliflower infusion only there was 1,43 log cycles of damaged cells and the population of intact cells was below the detection limit of the skill.

The cellular damage produced by PEF treatment has already been tested in other research studies with other *Enterobacteriaceae* such as *E. coli* (Rivas et al., 2012), but the present study also demonstrates its synergistic effect with the antimicrobial effect of infusions from agro-industrial by-products.

3.4 Mathematical modelling of *S.* Typhimurium inactivation

The effect of the treatments producing inactivation was also evaluated by fitting the experimental results to the Weibull distribution function. The values of *b* (scale factor), also considered as the kinetic parameter (Cunha et al., 1998), and *n* (form factor) obtained for the various conditions are shown in Tables 2, 3, 4 and 5. There are *n* values higher and lower than 1, indicating that the survival pattern for *S.* Typhimurium has a concave or convex form, depending on the conditions. Tables 2 and 4 show the *b* values obtained for *S.* Typhimurium inactivation with different concentrations (0, 1, 5, and 10%) of mandarin and cauliflower by-products, respectively. It can be observed that, at all temperatures, when the mandarin and cauliflower by-product concentration was increased, the microbial inactivation rate was greater. Tables 3 and 5 show the *b* values obtained for *S.* Typhimurium inactivation, previously treated by PEF and incubated in the presence of different concentrations of the infusions. It can be seen that the higher the by-product concentration, the greater the microbial inactivation rate. Finally, if we compare Tables 2 and 3 or Tables 4 and 5 (with and without PEF treatment), we can see that the rate of *S.* Typhimurium inactivation was higher in the samples that had been

344 treated by PEF before exposure to mandarin and cauliflower than in the samples without
345 PEF treatment. Higher *b* values mean less resistance of the cells to the treatments given.

346 4. CONCLUSIONS

347 Both mandarin and cauliflower by-product infusions showed a substantial antimicrobial
348 capacity against *S.* Typhimurium directly related to the concentration, and probably due
349 to the polyphenol content.

350 The results of the present study reveal that the addition of infusions from by-products
351 could be a good option to ensure food safety in PEF-treated products, exerting a higher
352 antimicrobial effect against *S.* Typhimurium than when they are applied separately. It
353 could also be an additional control measure when problems with the refrigeration chain
354 arise. Accordingly, mandarin and cauliflower by-product infusions appear to be tasty
355 alternative antimicrobial ingredients that could contribute to the food safety of PEF-
356 treated products by application of the hurdle technology concept.

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Table 1. Total polyphenol content of cauliflower and mandarin by-product infusions at 10%.

By-product Infusion	Total Polyphenol Content (mg gallic acid/L)
Mandarin 10%	3958.75 ± 185.62
Cauliflower 10%	4560.00 ± 433.90

Table 2. Weibull kinetic parameters (scale factor "b" and form factor "n") for *S.* Typhimurium inactivation with different concentrations of mandarin by-product (0, 1, 5, and 10%) at different incubation temperatures (10, 22, and 37 °C). R^2 and MSE values are indicators of goodness of fit. --- Microbial cells grew.

T °C	% Mandarin	*b* (t^{-1})	*n*	R^2 *adjusted*	*MSE*
10 °C	0	---	0.520±0.034	0.959	0.063
	1	---	0.492±0.145	0.957	0.094
	5	---	0.978±0.862	0.965	0.163
	10	0.190±0.098	0.564±0.131	0.986	0.100
T °C	**% Mandarin**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
22 °C	0	---	0.149±0.018	0.979	0.062
	1	---	0.462±0.191	0.984	0.130
	5	---	0.458±0.070	0.987	0.681
	10	0.007±0.060	0.602±0.187	0.976	0.659
T °C	**% Mandarin**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
37 °C	0	---	0.110±0.042	0.977	0.0134
	1	0.003±0.004	1.768±0.443	0.984	12.778
	5	0.065±0.037	0.913±0.091	0.958	9.434
	10	0.780±0.398	0.369±0.209	0.998	7.662

Table 3. Weibull kinetic parameters (scale factor "b" and form factor "n") for *S.* Typhimurium inactivation with different concentrations of mandarin by-product (0, 1, 5, and 10%) at different incubation temperatures (10, 22, and 37 °C) after PEF treatment. R^2 and MSE values are indicators of goodness of fit. --- Microbial cells grow.

T °C	% Mandarin	*b* (t^{-1})	*n*	R^2 *adjusted*	*MSE*
10 °C	0	0.005±0.005	1.388±0.371	0.953	0.083
	1	0.038±0.042	1.187±0.655	0.972	0.377
	5	0.857±0.197	0.212±0.066	0.977	0.204
	10	1.348±0.531	0.159±0.093	0.974	1.604
T °C	**% Mandarin**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
22 °C	0	---	1.625±0.102	0.986	0.043
	1	0.234±0.076	0.490±0.097	0.992	0.007
	5	0.777±0.148	0.447±0.136	0.986	0.007
	10	1.734±0.355	0.338±0.103	0.998	4.693
T °C	**% Mandarin**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
37 °C	0	---	1.233±0.067	0.990	0.025
	1	---	2.542±0.396	0.989	0.047
	5	0.992±0.019	0.321±0.067	0.993	0.009
	10	1.391±0.252	0.277±0.050	0.977	0.018

508 **Table 4.** Weibull kinetic parameters (scale factor "b" and form factor "n") for *S.*
509 Typhimurium inactivation with different concentrations of cauliflower by-product (0, 1,
510 5, and 10%) at different incubation temperatures (10, 22, and 37 °C). R^2 and MSE
511 values are indicators of goodness of fit. --- Microbial cells grow.

512

T °C	% Cauliflower	*b* (t^{-1})	*n*	R^2 *adjusted*	*MSE*
10 °C	0	---	0.510±0,153	0.977	0.361
	1	---	1.119±0,406	0.989	0.851
	5	0.060±0.007	0.802±0,062	0.960	1.647
	10	0.095±0.018	0.811±0.029	0.981	0.084
T °C	**% Cauliflower**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
22 °C	0	---	0.433±0,010	0.992	0.339
	1	---	0.439±0,015	0.972	0.845
	5	0.034±0.003	0.831±0,019	0.953	0.345
	10	0.170±0.032	0.695±0,027	0.978	0.510
T °C	**% Cauliflower**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
37 °C	0	---	0.341±0,002	0.958	0.058
	1	---	0.263±0,137	0.999	0.225
	5	0.022±0.002	1.985±0,016	0.960	0.094
	10	0.382±0.037	0.970±0,158	0.991	0.429

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514

515 **Table 5.** Weibull kinetic parameters (scale factor "b" and form factor "n") for *S.*
516 Typhimurium inactivation with different concentrations of cauliflower by-product (0, 1,
517 5, and 10%) at different incubation temperatures (10, 22, and 37 °C) after PEF
518 treatment. R^2 and MSE values are indicators of goodness of fit. --- Microbial cells grow.

519

T °C	% Cauliflower	*b* (t^{-1})	*n*	R^2 *adjusted*	*MSE*
10 °C	0	---	0.362±0.107	0.992	0.287
	1	---	0.349±0.001	0.968	0.023
	5	0.047±0.009	0.859±0.047	0.965	0.079
	10	0.350±0.033	0.321±0.150	0.983	0.225
T °C	**% Cauliflower**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
22 °C	0	---	1.637±0.072	0.968	0.133
	1	---	1.480±0.016	0.951	0.332
	5	0.124±0.013	0.875±0.030	0.986	0.033
	10	0.272±0.052	0.706±0.062	0.970	0.053
T °C	**% Cauliflower**	***b* (t^{-1})**	***n***	***R^2 adjusted***	***MSE***
37 °C	0	---	0.538±0.219	0.980	0.007
	1	---	0.259±0.225	0.963	0.013
	5	0.875±0.022	0.156±0.081	0.972	0.005
	10	1.459±0.068	0.254±0.050	0.995	0.003

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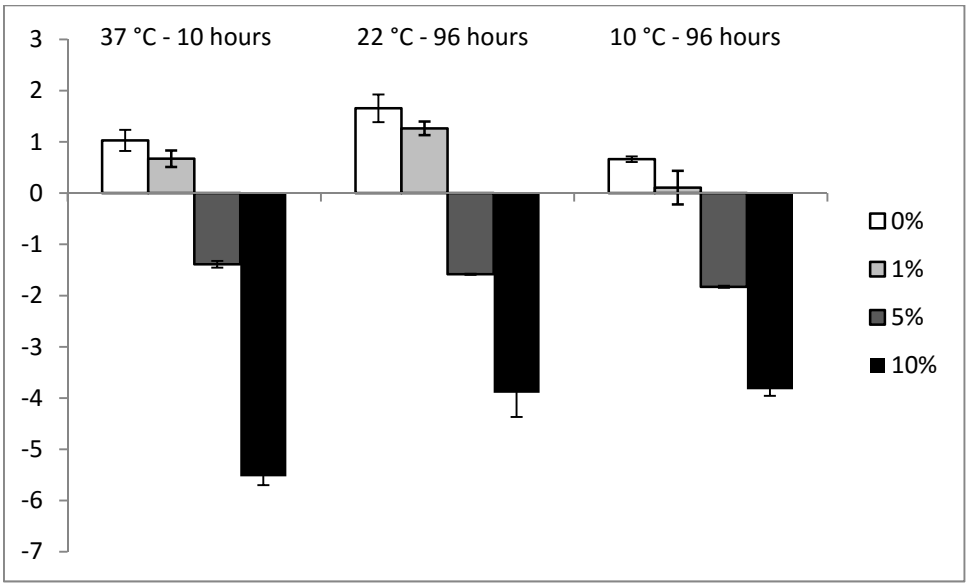
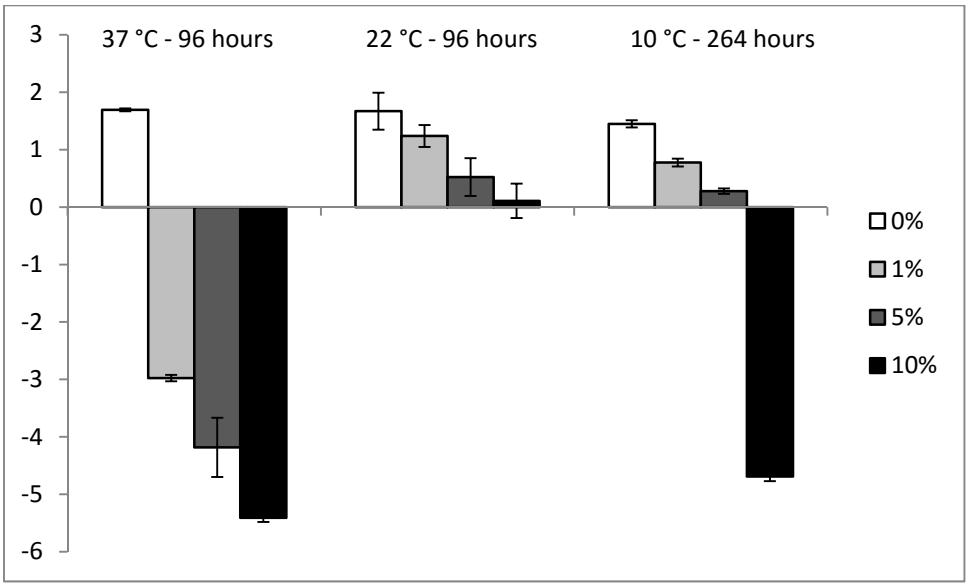
Figure 1: *S.* Typhimurium inactivation levels achieved with different concentrations of cauliflower by-product infusion (0, 1, 5, 10%) and various incubation temperatures (10, 22, 37 °C).



528 **Figure 2:** *S.* Typhimurium inactivation levels achieved with different concentrations of
529 mandarin by-product infusion (0, 1, 5, 10%) and various incubation temperatures (10,
530 22, 37 °C).



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534 **Figure 3:** Inactivation levels of *S.* Typhimurium cells treated by PEF and incubated
535 with different concentrations of cauliflower by-product infusion (0, 1, 5, 10%) and
536 various incubation temperatures (10, 22, 37 °C).

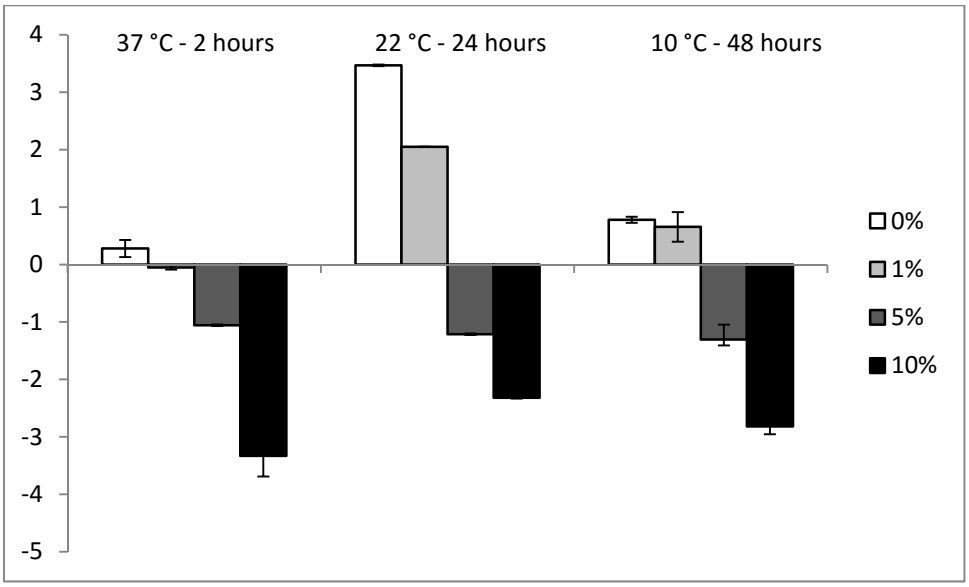

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Figure 4: Inactivation levels of *S.* Typhimurium cells treated by PEF and incubated with different concentrations of mandarin by-product infusion (0, 1, 5, 10%) and various incubation temperatures (10, 22, 37 °C).

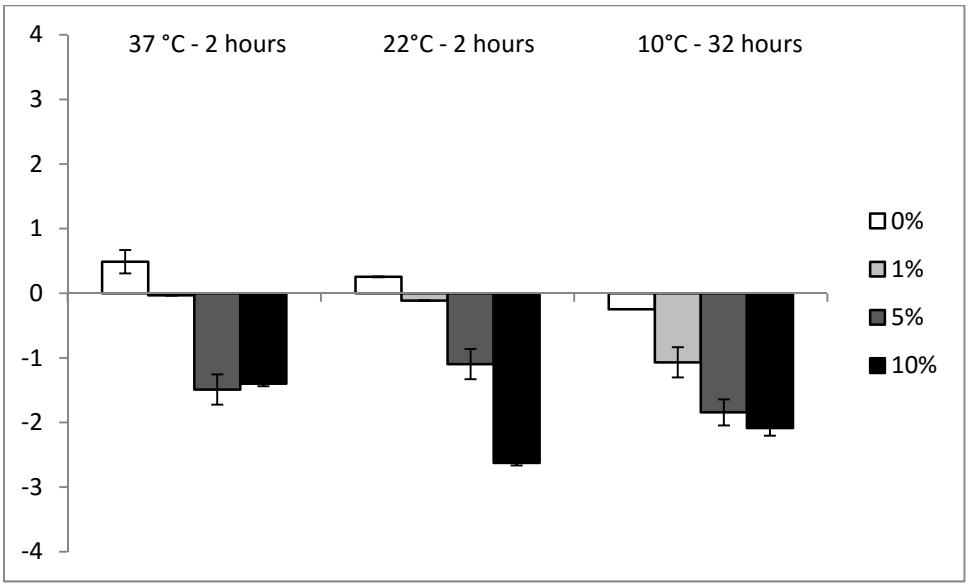
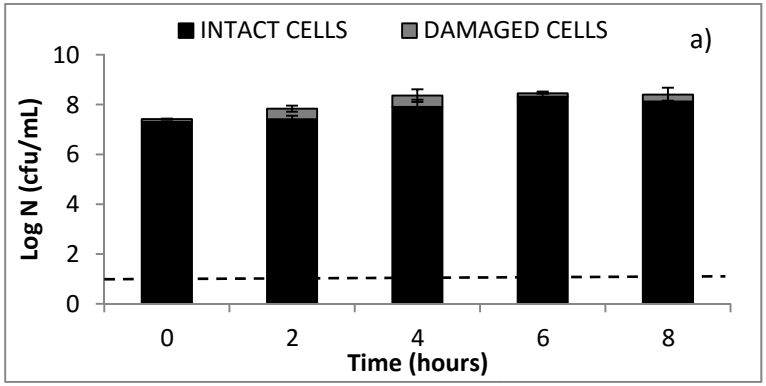
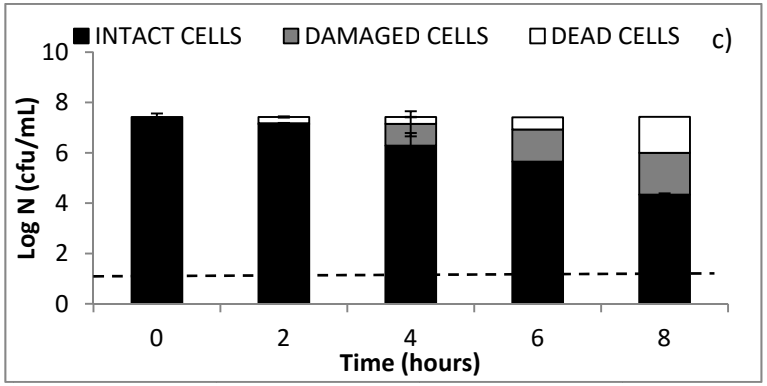
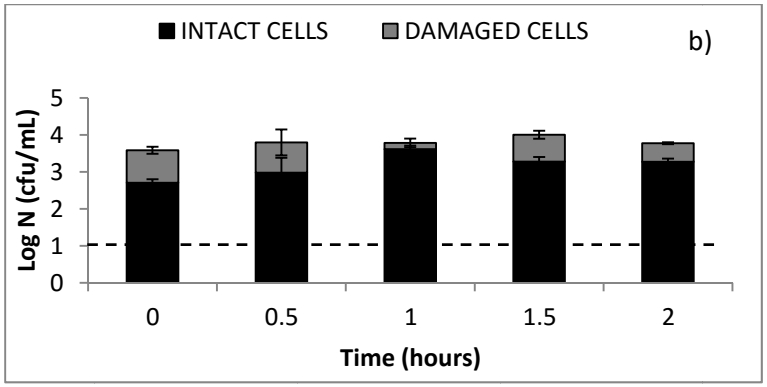


Figure 5: Cellular damage of *S.* Typhimurium caused by Pulsed Electric Field treatment (20 kV/cm – 900 µs) combined/not combined with the addition of 5% cauliflower by-product infusion at 37 °C. a) 0% cauliflower by-product infusion – without PEF treatment, b) 0% cauliflower by-product infusion – with PEF treatment, c) 5% cauliflower by-product infusion – without PEF treatment, d) 5% cauliflower by-product infusion – with PEF treatment. - - - Detection limit.







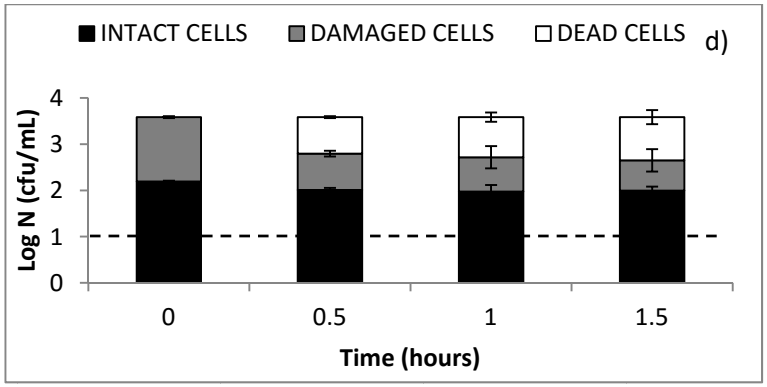


Figure 6: Cellular damage of *S.* Typhimurium caused by Pulsed Electric Field treatment (20 kV/cm – 900 µs) combined/not combined with the addition of 10% mandarin by-product infusion at 10 °C. a) 0% mandarin by-product infusion – without PEF treatment, b) 0% mandarin by-product infusion – with PEF treatment, c) 10% mandarin by-product infusion – without PEF treatment, d) 10% mandarin by-product infusion – with PEF treatment. - - - Detection limit.