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Finite element simulation and practical tests on Pulsed Electric Field (PEF) for packaged food pasteurization: inactivating *E. coli*, *C. difficile*, *Salmonella spp*. and mesophilic bacteria

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Abstract

In this study we demonstrated the inactivation of *E. coli, C. difficile,* meshophilic bacteria and *Salmonella spp.* through Pulsed Electric Field (PEF) technology. First, a simulation using Finite Element Method Magnetics Mathematical Modeling was used to obtain the electric field values to be used in the electro-pasteurization tunnel. Then, practical tests were carried out by using an industrial scale apparatus set with the following parameters: pulse 20 s – 40 s, 40, 80 and 450 kV, radio frequency of 350 kHz and treadmill speed at 10 m/min. The results from practical tests shows a complete elimination of all microrganisms, thus proving that PEF technology significantly contributes to food safety and can be used on an industrial scale.

Keywords: electrolysis; food security; electro-pasteurization; pulsed electric field; simulation.

Practical Application: The practical application of this technology allows the pasteurization of beverages and packaged foods, sterilizing them and increasing their shelf life without the need to add salt or preservatives.

1 Introduction

During the last decades, many novel techniques of food processing have been developed in response to growing demand for safe and high quality food products. Nowadays, consumers have high expectations regarding the sensory quality, functionality and nutritional value of products. They also attach great importance to the use of environmentally-friendly technologies for food production (Nowosad et al., 2021).

PEF technology is an effective approach for the preservation and processing of a variety of food products without affecting their quality attributes. PEF technology involves the use of pulses, with high electric fields during only a few micro to milliseconds, with intensities ranging 10-80 kV/cm. The process depends on the number of pulses delivered to the product which is usually held between two electrodes. These electrodes have a specific gap between them, known as treatment gap of the chamber. During PEF processing, the high voltage applied results in the inactivation of microorganisms present in the food sample. The electric field is applied in different forms such as exponentially decaying waves, bipolar waves or oscillatory pulses. The process can also be carried at various temperature ranges such as ambient, sub-ambient and above-ambient. Food is treated with PEF and then stored under refrigerated conditions (Syed et al., 2017).

Table 1 summarizes a list of studies on conditions and effects of using PEF in food processing.

PEF is also used in meat processing, to increase cell membrane permeability or to form permanent pores in muscle cells. This cellular disintegration can increase the tenderness of low-value tough meat cuts, enhance mass transfer, and improve the efficiency and cost-effectiveness of subsequent unit operations such as sous vide, ageing, curing, drying, fermentation and maturation. The effects of PEF on meat structure and processing can be of great commercial value. The efficiency of PEF treatments depends on process parameters such as electric field strength, pulse frequency, treatment time and specific energy, as well as the intrinsic meat properties, since meat components such as muscle, fat, collagen and bone vary in both electrical and morphological properties. To ensure an effective application of PEF and the production of prime quality meat products, the processing parameters and instrumental design need to be optimised for each application (Karki et al., 2022).

PEF systems market and it is poised to grow by \$ 273.19 mn during 2021-2025, progressing at a Compound Annual Growth Rate (CAGR) of 25.09% (TechNavio, 2021).

In this context, we have investigated (in an industrial scale) the effect of electromagnetic fields added to high voltage modulated radiofrequency and pulsatile plasma to inactivate harmful microorganisms in foods. The advantages are that this technology can be used for both pre-packed and post-packed

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Table 1. Studies on conditions and effects of us	sing PEF in food processing.
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Material	PEF parameters	Effect of PEF	References
Basil (Ocimum basilicum L.) leaves	Drying - 65 pulses of 650 V/cm, 150 µs pulse width, 760 µs between pulses. The research used Randomized Block Design (RBD) with two factors, factor 1: exposure time (10, 15, and 20 seconds) and factor 2: voltage (1000, 1500, and 2000 Volts).	Drying times reduced 57% for air drying, 33% for vacuum drying and 25% for freeze drying. PEF treatment before the extraction of basil leaves increased TPC by 1.5 times and antioxidant activity by 3.2 times.	Telfser & Gómez-Galindo, 2019; Sukardi et al., 2020
Parsnip and carrot	Drying - 20 μs, 50 Hz, 0.9 kV/cm, after 1000 pulses. PEF pre-treatment at 0.9 kV/cm and 1000 and 10,000 pulses.	Drying time reduced to 28% at 70 °C and to 21% at 60 °C, compared to the untreated samples. PEF is effective in reducing drying times.	Alam et al., 2018; Fratianni et al., 2019
Carrot	Drying - Pulse number 10, 50 and 100; 1.85 and 5 kV/ cm; 5.63, 8 and 80 kJ/kg.	Drying time reduced up to 8.2%. Decrease of sample lightness up to 25.3%	Wiktor et al., 2016
Potato tissue	Drying – 300-400 V/cm. Impact of PEF on relevant quality parameters for French fries validated in industrial scale.	Decreasing the drying temperature approximately on 20°. Beneficial effects already at low treatment intensities (E = 1,0 kV/cm, W = 0.2 kJ/kg). Improved cutting surface properties. Reduction of starch loss during cutting after PEF pre-treatment. Decrease of fat content for PEF treated pre-fried fries in comparison to untreated samples.	Lebovka et al., 2007; Fauster et al., 2021
Citrus fruits and peel (orange, pomelo and lemon)	Extraction - 3 kV/cm—fruits, 10 kV/cm — peel. Mild (Thermal-1) and intensive (Thermal-2) thermal treatments were applied for comparison. A pilot-scale PEF system, with a flow rate of 30 L/h and maximum field strength of 20 kV/cm, was used.	Increased yield of juice by 25% for oranges, 37% for pomelos and 59% for lemon, improved extraction of polyphenols to 50%. PEF treatment at a specific energy of 150 kJ/L resulted in 9.0 and 8.0 decimal reductions of <i>E. coli</i> and <i>S. cerevisiae</i> . PEF preserved the characteristic compounds associated with a fresh flavor.	Kantar et al., 2018; Lee et al., 2021; Salehi, 2020
Vegetables	Sterilization - Using a relatively small, low temperature, atmospheric, dielectric barrier discharge surface plasma generator. Samples were exposed to a fixed electric field strength of 2.15 kV/cm. The specific energy ranged from 0.6 kJ/ kg to 50.3 kJ/kg.	achieved ≥ 6 log reduction in concentration of vegetative bacterial and yeast cells within 4 minutes and ≥ 6 log reduction of Geobacillus stearothermophilus spores within 20 minutes. The best result for tomatoes at a specific energy of 1.2 kJ/kg induced a high score of peeling ability that led to less product loss and could therefore increase the yield by 33.84%-41.53%.	Mastanaiah et al., 2013; Koch et al., 2022
Fruit juice with the addition of stevia	Extraction - 30 kV/cm for 230 μs; 40 kV/cm for 230 μs; 21 kV/cm 300 μs with 2.5% stevia. 1.3 and 5 kV/cm, 10 kJ/kg.	The retention of ascorbic acid increased by over 74%. The enhancement of anthocyanins and carotenoids extraction. Increased the juice yield (+ 28%).	Carbonell-Capella et al., 2017; Nowosad et al., 2021
Blueberry fruits (Vaccinium myrtillus L.)	Extraction - 1, 3 and 5 kV/cm, 10 kJ/kg. Preservation-2 kV/cm, pulse width 1 μs and 100 pulses per second for 2, 4 and 6 min + disinfectant solution [60 ppm peracetic acid (PAA)].	Increasing the juice yield (+28%) compared to the untreated sample. The juice obtained had a significantly higher total phenolic content (+43%), total anthocyanin content (+60%) and antioxidant activity (+31%). Reduction of <i>E. coli</i> and <i>Listeria innocua</i> without changing the color and appearance of blueberries	Bobinaitė et al., 2015; Jin et al., 2017
Baby spinach leaves and juice	Freezing - Two trains of bipolar, rectangular pulses with amplitude of 350 V, with 10 s interval between trains. Each train consisted of 500 pulses of 200 μs pulse width and 1600 μs of space between the pulses (frequency 500 Hz). Combined effects of US and PEF treatment on spinach juice sonicated at a frequency of 40 kHz, radiating power 200 W, and temperature 30 °C for 21 min in an ultrasonic bath, followed by PEF treatment (pulse frequency: 1 kHz, flow rate: 60 mL/min, temperature: 30 °C, time: 335 μs, and electric field strength 9 kV/cm).	Improved freezing tolerance by applying vacuum impregnation and PEF in the presence of cryoprotectants. The combined treatment (US-PEF) has achieved the highest value of flavonoids, phenolic, flavonols, anthocyanin, carotenoids, total chlorophyll, vitamin C, DPPH, and total antioxidant capacity than single treatments of US and PEF as well as untreated sample. The inactivation of peroxidase and polyphenol oxidase was increased during US-PEF treatment from 0.85 and 0.025 Abs/min (untreated) to 0.18 and 0.011 Abs/min, respectively. There was a slightly visible variation in color values among all the treatments.	Demir et al., 2018; Manzoor et al., 2021
Apple tissue	Freezing-800 V/cm, pulse duration 1000 µs, time interval 100 ms and 10 pulses.	Acceleration of cooling processes; good preservation of the macro-shape, inhibition of shrinking, development of large pores in the electroporated tissue.	Parniakov et al., 2016; Wu & Zhang, 2019
Beef and Chicken meat	Freezing - 1.4 kV/cm, 20 μs, 50 Hz, 250 kJ/kg (combined with freezing and thawing). Pulses (150 vs. 300 and 450 vs. 600) and the electric field strength (0.60 vs. 1,20 kV/cm).	Microstructural changes in meat tissue, improved tenderness and purge loss. PEF to reduce the undesired liquid inside the package.	Faridnia et al., 2015; Baldi et al., 2021; Karki et al., 2022
Peptides isolated from pine and pecan nuts	Preservation - 1800 Hz and 15 kV/cm. PEF have been reported to increase the total oil extraction yield	No changes of the amino acid sequence. Fresh pecan nuts maintaining oil characteristics and increasing phenolic compounds	Lin et al., 2017; Rábago-Panduro et al., 2021
Milk	Preservation - 25.7 kV/cm for 34 μs after heating to 55 °C and maintained for 24 s and heat treatment at 63 °C for 30 min or at 73 °C for 15 min. Using two electrodes installed 0.1-1.0 cm apart in a treatment chamber separated by an insulator, with short pulses (1-10 μs) that are generated by a high voltage (5-20 kV) pulse generator.	Inactivation of alkaline phosphatase. Reduced xanthine (30%) and plasmin oxidase (7%) activity. PEF processing concurred that high voltage treatments produce a series of structural and functional changes in the cellular membrane that lead to microorganism death.	Sharma et al., 2018; Sampedro & Rodrigo, 2015

food and beverages (without use heat or cold) with effective conservation of their organoleptic properties, with low energy expenditure, fast speed capacity, as well as the softening of meat since the binding of actin-myosin is broken by the process.

2 Materials and methods

2.1 Finite element simulation

The Finite Element Method Magnetics (FEMM) software version 4.2 was used in for the simulation of electric fields in the post-filling electro-pasteurization tunnel. The cross-sectional view is shown in Figure 1, where it is possible to observe the construction and arrangement of the electrodes (304 stainless steel) and dielectric barriers (20 mm acrylic).

To simulate the electric field in the software, equations for verification of parameters were used in according to Ganea (2017). These equations are used in sizing cells for ozone production using parallel planes. The equation, however, considers some points: the electric field is uniform inside the cell; the electric field strength value outside the cell is zero; the electric charge density is constant and uniform over the entire surface of the electrodes; the dimensions of the electrodes are much larger compared to the distance between them, in according to the Equation 1 presented below:

 $\mathbf{E} = \varepsilon 2 \times \mathbf{U} / \varepsilon \mathbf{1} \times \mathbf{d2} + \varepsilon 2 \times \mathbf{d1} \tag{1}$

Where:

E - electric field strength in the effective area,

E1 – air permittivity,

E2 - permittivity of the dielectric material,

d1 - distance between dielectrics,

d2 - total thickness of the dielectrics.

The simulations were carried out using the Finite Element Method Magnetics (FEMM) software 4.2, considering an electrical voltage of 120 kVolts and a change in the distance between the dielectric barriers of 5 cm, 10 cm, 15 cm and 20 cm, respectively. The permittivity of the materials considered were: air = 1/polypropylene = 2.2 and acrylic = 3.4. From simulations it was possible to calculate the pasteurization electric fields in practical tests.

Figure 2 presents graphically the modeling from FEMM software, regarding the electric field intensity, flux density, and equipotential electric field lines for the simulations performed:

2.2 Electro-pasteurization essay

For each microorganism tested (including E. coli, C. difficile and mesophilic bacteria) 1.0 x 106 CFU/mL was diluted in protein broth transferred to 100 mL of sterile saline solution and was inoculated in plastic packages containing milk (samples 1-16) and meat samples (17-30). In tests we used E. coli + C. difficile spores and mesophilic bacteria for milk samples, and Salmonella spp. for meat samples (packed sausages). For results reliability 10 samples were tested for each bacteria in the electropasteurization process essay. For the quantitative counting of bacteria the tests were carried out in accordance with the ISO-15213: (International Organization for Standardization, 2003) and AOAC-OMA 991.14. 20th ed. 2016, and for the qualitative determination of Salmonella spp. using by the Presence/Absence (P-A) test based on ISO 6579-1:2017 (International Organization for Standardization, 2017) being validated by the certified Microbiological Laboratory - Eireli EPP, Florianópolis - SC, Brazil.

In tests, 1 sample was contaminated with each microorganism being left as initial reference, with perfect conditions of vitality to test the bacterial spread. The PEF apparatus is protected under World Intellectual Property Organization – WIPO (Duvoisin, 2017) and follows the same principles of



Figure 1. Sectional view of the post-filling electro-pasteurization tunnel. Having as dimensions: width: 646 mm, Height: 400 mm; Electrodes (50 mm x 400 mm).



Figure 2. Graphical simulation 1: DDP = 120 kV/Distance = 5 cm. Image A1 shows Electric field strength (V = 120 kV/d = 5 cm); Image A2 - Electric field flux density (V = 120 kV/d = 5 cm); Image A3 - Equipotential electric field lines (V = 120 kV/d = 5 cm). Simulation 2: DDP = 120 kV/Distance = 10 cm. Image B1 - Electric field strength (V = 120 kV/d = 10 cm); Image B2 - Electric field flux density (V = 120 kV/d = 10 cm); Image B3 - Equipotential electric field lines (V = 120 kV/d = 10 cm); Image B3 - Equipotential electric field lines (V = 120 kV/d = 10 cm); Simulation 3: DDP = 120 kV/Distance = 15 cm. Image C1 - Electric field strength (V = 120 kV/d = 15 cm); Image C2 - Electric field flux density (V = 120 kV/d = 15 cm); Image C2 - Electric field flux density (V = 120 kV/d = 15 cm); Image C3 - Equipotential electric field lines (V = 120 kV/d = 15 cm). Simulation 4: DDP = 120 kV/Distance = 20 cm. Image D1 - Electric field strength (V = 120 kV/d = 20 cm); Image D2 - Electric field flux density (V = 120 kV/d = 20 cm); Image D2 - Electric field flux density (V = 120 kV/d = 20 cm); Image D2 - Electric field flux density (V = 120 kV/d = 20 cm); Image D3 - Equipotential electric field lines (V = 120 kV/d = 20 cm).

eletrons trap described by Duvoisin et al. (2020). With the purpose of microbiologically testing this electro-pasteurizer technique/equipment, we carried out 40 tests using packed milk and meat. Figure 3 shows the microbiological essay of packaged foods.

The electrical discharges were placed on a rolling treadmill with speed advance set at 10 m/min. Stainless steel 316L electrodes (round to avoid undesirable sparks) with width of 40 cm and were placed between 15 cm, the pulse was applied each 20-40 s. The frequency of pulsed electrical fields was 20 to 350 kilo/Hertz using double polarity electrodes with the possibility of inversion if needed. All samples were packed using plastic polyethylene with 5 layers to hold vacuum (standard packaging for vacuum).

Duvoisin et al.



Figure 3. Legend: A) packing contaminated samples; B) sausage samples before and after test; C) electro-pasteurization tunnel; D) plasma beam over the sample during essay.

enough to justify an efficient electro-pasteurization process. Figure 4 shows in details the industrial-scale PEF apparatus built.

3 Results and discussion

3.1 Electric field simulations

The comparison between the values calculated using the Ganea (2017) method and those simulated using the FEMM 4.2 software is shown in Table 2.

Table 3 presents the microbiology results obtained from the electro-pasteurization process, the results of the microbiological inactivation are the average result from 10 samples each (p < 0.05).

For *C. difficile* + *E. coli* contaminated meat samples, after pasteurization by PEF the microbiological count showed values < 0.1 CFU/mL thereby indicating absence. In relation to *Salmonella spp.* and mesophilic bacteria, the treatments of 10 X electrolysis + UV + 450 kVolts, radiofrequency of 350 kHz and treadmill speed of 10m/min, also showed the total absence of microorganisms.

In the 40 samples, a total inactivation of each microorganism occured, evidencing this technology as a rapid method for pasteurization of foods and beverages. Regarding the 10 samples of mesophilic bacteria, after PEF we also achieved total absense in concentration of this microorganism in practical testes.

Through the scientific evidence of previous works, as well as the results present here, it is clear that this technology can be applied to the large scale food industry, with promising perspectives to the progress of food security, since plasma pasteurization offers a faster, less toxic and versatile alternative to conventional methods.

The literature demonstrates that PEF can be used in food processing either in a singular, pure way or also as a summation method to the trivial systems already used in a classical way for the treatment of pasteurization of food itself. The great advantage of PEF is that this system does not use heat for food processing and thus conserving its organoleptic properties, as well as the process of death of microorganisms generated by PEF are quickly and thus PEF has been a great promise for the contemporary food industry (Nowosad et al., 2021).

By using PEF, a targeted cell disruption of membranes of biological cells and microorganisms takes place. The principle of the innovative technology is so-called electroporation, with which the product is subjected to electric pulses by applying a voltage. Cell disruption of biological materials encourages the mass transport of water, however also of valuable substances (pigments) out of the cells. In the potato processing industry, advantages result from implementation of this technology with regard to processing and product quality. The induced structural modification enables energy savings, less raw product waste and the ability to develop new products. In the area of drying as well, energy savings can be achieved, just as an improved structural preservation and a more intensive flavour of various fruit and vegetable products. The cell disruption achieved with PEF



Figure 4. PEF industrial-scale apparatus showing: A) chamber with automated control panel; B) isolated treadmill for pasteurization of packed foods; C) eletrical font; D) coil with ceramic insulation on the treadmill.

Simulation D (cm)	Calculated value [19]		Simulated value FEMM 4.2	
	E (V/m)	E (kV/cm)	E (V/m)	E (kV/cm)
1	5,513.514	55.14	-	_
2	3,777.778	37.78	-	-
3	2,873.239	28.73	-	-
4	2,318.182	23.18	-	-
5	1,942.857	19.43	1,942.850	19.42
6	1,672.131	16.72	-	-
7	1,467.626	14.68	-	-
8	1,307.692	13.08	-	-
9	1,179.191	11.79	-	-
10	1,073.684	10.74	1,073.670	10.73
11	985.507	9.86	-	-
12	910.714	9.11	-	-
13	846.473	8.46	-	-
14	790.698	7.91	-	-
15	741.818	7.42	741.800	7.41
16	698.630	6.99	-	-
17	660.194	6.60	-	-
18	625.767	6.26	-	-
19	594.752	5.95	-	-
20	566.667	5.67	565.534	5.65

Table 2. Pulsed electric field simulations used in this study.

Table 3. Results of el	ectro-pasteurization	process in this	study.
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Sample	Microorganism	Electric field	*Count (CFU/mL)	p-value
1	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
2	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
3	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
4	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
5	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
6	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
7	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
8	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10°] Absense	< 0.05
9	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10°] Absense	< 0.05
10	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
11	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10°] Absense	< 0.05
12	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
13	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10°] Absense	< 0.05
14	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
15	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10 [°]] Absense	< 0.05
16	E. coli + C. difficile	Pulse 20 s/40 s, 450 kV	[0.0 x 10°] Absense	< 0.05
17	Salmonella spp.	RF 50 kHz/10 m/min	Absence	< 0.05
18	Salmonella spp.	RF 350 kHz/10 m/min	Absence	< 0.05
19	Salmonella spp.	RF 350 kHz/10 m/min	Absence	< 0.05
20	Salmonella spp.	RF 350 kHz/10 m/min	Absence	< 0.05
21	Salmonella spp.	RF 350 kHz/10 m/min	Absence	< 0.05
22	Salmonella spp.	RF 350 kHz/10 m/min	Absence	< 0.05
23	Salmonella spp.	RF 350 kHz/10 m/min	Absence	< 0.05
24	Salmonella spp.	PEF 1 X 450 kVolts + UV	Absence	< 0.05
25	Salmonella spp.	PEF 10 X 450 kvolts + UV	Absence	< 0.05
26	Salmonella spp.	PEF 5 X 450 kvolts + UV	Absence	< 0.05
27	Salmonella spp.	PEF 5 X 450 kvolts + UV	Absence	< 0.05
28	Salmonella spp.	PEF 10 X 450 kvolts + UV	Absence	< 0.05
29	Salmonella spp.	PEF 10 X 450 kvolts + UV	Absence	< 0.05
30	Salmonella spp.	PEF 10 X 450 kvolts + UV	Absence	< 0.05

*Initial cout of each microorganism was 1.0 x 106 CFU/mL.

enables a rapid discharge of water from the cells. In addition, valuable substances, such as cell sap, fatty acids, amino acids or pigments can also be more easily extracted.

Moreover, this is used in juice production, in particular to supporting the pressing process for the extraction of ingredients from microalgae and in wine production (Deutsche Landwirtschafts-Gesellschaft, 2018).

Using 450 kvolts, high frequency and alternate current during short periods of time, demonstrated effectiveness to sterilize harmful microorganisms in hermetically packaged foods and beverages.

4 Conclusion

From the simulations carried out, it was possible to obtain the values used as main parameters in the practical electropasteurization tests.

The PEF procedure used was: pulses 20 s - 40 s, using 40, 80 and 450 kV, and radio frequency of 350 kHz and 10 m/min treadmill speed. The electrical pulses (20 to 350 kilohertz) were discharged using double polarity 316L electrodes connected

to the source, with the possibility of inversion as needed, the constant advancement of the treadmill was 10 m/min.

The results from tests shows a reduction from 1.0×10^6 CFU/mL to < 1.0 CFU/mL resulting in a complete elimination of all microrganisms tested including *E. coli*, *C. difficile*, meshophilic bacteria and *Salmonella spp*.

Excellent results were obtained with designed dimensions, having packed samples. This electro-pasteurization technology significantly contributes to food safety, beneficial for both prefilling and post-filling liquid and solid foods.

In future work we intend to study the role of this technology in the denaturation of food proteins.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Availability of data and material

The data used to support the findings of this study are available from the corresponding author upon request.

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