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New pulsed electric fields approach to improve the blanching of carrots

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ABSTRACT

Pulsed Electric Fields (PEF)-ohmic is one of the most recently proposed alternatives for the application of ohmic heating in order to provide a more uniform and faster heating of food. In this study, the application of PEF-ohmic (1.33 kV/cm, 100 Hz) for the blanching of carrot cylinders was explored, and the inactivation of the peroxidase enzyme was mathematically simulated and experimentally validated. The effect of this PEF-ohmic treatment on carrot texture and its effect on the *in vitro* bioaccessibility of β-carotene were also determined. The best heating uniformity was achieved when PEF-ohmic was applied to carrots immersed in water at a starting temperature of 80 °C and after applying brief heating-up treatments (90 s). However, a holding heating phase (85 °C for 50 s) was required to achieve complete peroxidase inactivation after the heating-up PEF phase. The blanching time was reduced by 60 % without negatively affecting the texture of the carrots and slightly increasing (3.9 %) the bioaccessibility of the β-carotene. Overall, PEF could be an effective system for rapid and volumetric blanching of vegetables.

1. Introduction

Blanching is a thermal process widely used in the food industry, applied to vegetables and certain fruits prior to freezing, heat sterilization, or dehydration (Fellow, 2017). The main goal is the inactivation of natural spoiling enzymes (lipoxygenase, polyphenol oxidase, peroxidase, polygalacturonase, chlorophyllase, etc.) that negatively affect the sensory quality of food during storage, i.e., undesirable color, texture, flavor, and/or nutritional modifications (Gonçalves et al., 2010; Vámos-Vigyázó, 1981; Xiao et al., 2017).

Peroxidase (POD) is used as an indicator of blanching efficiency due to its high thermo-resistance and abundance in most vegetables (Guida et al., 2013). Traditional food blanching technologies include the use of boiling water and steam, which, due to the conventional heat transfer phenomenon, require time-consuming and energy-intensive treatments that can affect the sensory quality of the product (Xiao et al., 2017). Moreover, large vegetable pieces present the challenge of thermal uniformity since heat transfer occurs from the hot medium to the vegetable core, resulting in slow heat penetration. Therefore, high temperatures (70–100 °C) and long periods of time are required to ensure that the core of the food receives the proper heat treatment (Fellow, 2017). This often results in overtreatment of the food surface, which negatively affects sensory properties and leads to the loss of nutritional compounds (Ismail et al., 2004; Mizrahi, 1996).

Carrots are an important source of β –carotene; however, due to the high temperatures and long times applied in conventional heating, this nutrient could be heat degraded. β-Carotene is an important nutrient: it is the carotenoid with the highest theoretical vitamin A activity (Dias et al., 2021). Nevertheless, more important than the total content of carotenoids in the food, is their bioaccessibility, defined as the percentage of carotenoids released from the matrix during digestion and incorporated into micelles (Meléndez-Martínez et al., 2021). Micelles are small, water-soluble structures that help transport fat-soluble substances, like carotenoids, across the watery environment of the digestive tract and facilitate their absorption by the intestinal cells. The efficiency of this process can vary depending on factors such as food preparation methods, the presence of dietary fat, and individual variations in digestion and metabolism. By using an *in vitro* digestion model, it is possible to measure how much of the carotenoids in a carrot are incorporated into micelles in the intestinal phase. Such measurement of *in vitro* bioaccessibility of carotenoids could be used as a way of evaluating the impact of a blanching process on food quality.

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Ohmic heating has been evaluated as a very interesting alternative to conventional heating because of the fast and volumetric heating it produces (Sastry, 2005; Sastry et al., 2014). Ohmic heating consists in the conversion of electrical energy into thermal energy inside the food, due to the Joule effect:

$$W = \int_0^{\omega} \sigma E^2 dt \quad (1)$$

where σ is the electrical conductivity of the treated medium or product (S/m), E is the electric field strength (V/m); and dt is the time (s) during which the field strength is applied (Sastry & Li, 1996).

Most studies dealing with ohmic blanching focus on treatments at low electric fields (10–40 V/cm) and frequencies in the order of 50–100 Hz (Bhat et al., 2017; Gomes et al., 2018; Guida et al., 2013). However, the electric field is a highly critical parameter, since, according to the Joule equation, it quadratically increases the transferred heat energy. Recently, several studies have reported a new application of Pulsed Electric Fields (PEF) as an ohmic heating system of agar cylinders by applying high electric fields (>1 kV/cm) (Ariza-Gracia et al., 2020; Astráin-Redín et al., 2022). PEF consists in the application of electric fields of high intensity (>0.1 kV/cm) and short duration (from milliseconds to microseconds) to a product placed between two electrodes (Barbosa-Cánovas et al., 2001).

Precisely because this technology leads to the permeabilization of cell membranes, a phenomenon called electroporation, most research conducted so far on its application in the food industry has focused on exploitation of this electroporation phenomenon, i.e. as a non-thermal technology. Thus, it has been demonstrated that it can be used as non-thermal technology for liquid pasteurisation (Delso et al., 2022; Ortega-Rivas, 2011; Zulueta et al., 2013), for improving the extraction of compounds (Kumari et al., 2018; Parniakov et al., 2014) and also as a pre-treatment before drying for improving the drying rates (Andreou et al., 2021; Liu et al., 2020) in order to improve mass transfer phenomena and for meat processing (Alahakoon et al., 2018, 2019; Suwandy et al., 2015). However, there is an area of study that has not yet been explored in depth, which is the application of PEF as an ohmic heating system. As explained above, the current flow through a food causes its heating. Furthermore, the application of PEF for the heating of food results in cellular electroporation and the consequent outflow of extracellular material to the medium that increases its electrical conductivity (Blahovec & Kouřím, 2023). This phenomenon, together with the application of high electric field strengths, would lead, at least theoretically, to more rapid and more uniform heating. The difference between the application of PEF to achieve ohmic heating and the application of PEF to achieve only the non-thermal effect of electroporation lies in the amount of energy applied per unit of time. Therefore, this new PEF-ohmic application aims to apply large amounts of energy in very short periods, achieving a double effect, volumetric and fast ohmic heating of solid foods together with electroporation of the cells.

Some research has already been conducted on the application of PEF as an ohmic heating system. Ariza-Gracia et al. (2020) evaluated the thermal inactivation of *Salmonella* Typhimurium 878 in agar cylinders by applying a PEF treatment of 2.5 kV/cm and 50 Hz. After 49 and 60 s of treatment, 5-Log_{10} reductions were obtained in the center and in the area close to the electrode, respectively. This difference in inactivation time was due to the fact that a 10 °C gradient was observed between the center (hot zone) and the area in contact with the electrodes (cold zone). Similar results were observed in traditional ohmic heating systems (Choi et al., 2020; Ito et al., 2014; Marra, 2014). To overcome this problem, Astráin-Redín et al. (2022) proposed the use of treatment chambers with tempered electrodes in order to minimize the presence of cold spots in the area surrounding the electrodes when PEF ohmic treatment was applied. They successfully validated this approach for the inactivation of *Listeria monocytogenes* 5672, using technical agar cylinders as a model food heated via the application of PEF (2.5 kV/cm; 50 Hz) by direct contact.

Another feasible strategy to avoid such cold spots could be immersion systems, in which the food is surrounded by a conductive medium. This also facilitates the application of these (PEF) treatments since food would not be limited to a specific shape unlike direct contact systems as it has been described for high pressure high temperature treatments (Ramos et al., 2018). However, to the best of our knowledge, this approach of immersing the solid sample in a conductive medium for PEF -ohmic heating has not yet been investigated.

The application of PEF prior to blanching of vegetables to a product immersed in water offers additional advantages. In some cases, calcium chloride (1–2 % w/v) is added to the blanching water with the aim of creating insoluble calcium pectate complexes that help to maintain firmness in the tissues (Siliha et al., 1996). PEF has been investigated as a pretreatment to the blanching process, since it can favor Ca^{2+} uptake by producing cell electroporation (Leong et al., 2018). Structural changes caused by PEF may also improve the release of carotenoids from the food matrix, thereby enhancing their bioaccessibility, although results are conflicting (Bot et al., 2018; González-Casado et al., 2018; López-Gámez et al., 2021). Nevertheless, these advantages for blanching due to the application of PEF have not been investigated when PEF is applied as a heating system (PEF-ohmic). In other words, there are still many knowledge gaps regarding the application of PEF technology as an ohmic heating system and its effect on food. No previous studies have been carried out to evaluate the impact of electroporation together with heating on both the heat process itself and the quality of the final product.

Therefore, in this article the potential of PEF-ohmic heating (high electric field strengths and high frequencies) for the blanching of carrots when immersed in a $CaCl_2$ solution was evaluated. First, the influence of PEF parameters (electric field, frequency and electrical conductivity and temperature of the $CaCl_2$ solution) on the uniformity of heating was assessed. Secondly, the impact of PEF-ohmic heating on texture, calcium absorption and β-carotene bioaccessibility was studied.

2. Materials and methods

2.1. Raw materials

The carrots (*Daucus carota* L. var. Nantes) used in this study were grown on the southeastern coast of Spain and purchased at a local supermarket. They were stored at 4 °C. One hour prior to the experiments, carrots were tempered at room temperature up to a starting temperature of 15 ± 1 °C. For the experiments, cylinders (2 × 2 cm, 7 ± 0.5 g) were punched out of the central part of the carrots using a borer, so that the samples were completely skin-free.

2.2. PEF device

PEF treatments were applied with a Scandinova 6 MW apparatus (Modulator PG, ScandiNova, Uppsala, Sweden). This device applies square wave pulses of 3 μs pulse width at a frequency ranging from 0.5 to 300 Hz and works at maximum output voltage and current of 30 kV and 200 A, respectively. For safe manipulation of the PEF device, and in order to obtain rectangular 3 μs-pulses, the electric current delivered in the treatment chamber has to lie within the range of 80 and 150 A.

The treatment chamber was a parallel square chamber (30 × 30 × 40 mm) with a gap of 30 mm. Additionally, the walls of the electrodes were covered with thermal insulation material to prevent heat loss, although the top of the chamber was open.

2.3. Blanching treatments

For blanching treatments, distilled water solutions at 850 mg/kg $CaCl_2$ were used as treatment media in which the carrots were immersed. The ratio (vol/vol) between treatment medium and carrot

applied in our experiments was 3.8 (24 cm^3 of treatment medium/6.28 cm^3 carrot cylinder). During blanching treatments, the temperatures of the samples and the treatment medium were recorded by means of fiber optic probes (Fiberoptic Components, USA). Resulting data were used to obtain heat penetration curves that plotted temperature against time; the blanching rate (°C per unit of time) was obtained from the slope of the curve.

2.3.1. PEF-assisted blanching

The carrot cylinders were minimally prodded with a plastic stick to ensure that the sample remained in the center of the chamber surrounded by the treatment medium and none of its parts were resting on the insulating material. Then, carrot samples were heated up by applying PEF, then immersed in hot water at 85 °C until complete POD inactivation was achieved. The treatment was determined after characterizing the thermoresistance of the POD enzyme and testing its inactivation, as explained in the Results and Discussion section.

To evaluate the uniformity of the treatment, the central zone of the carrot (P1) and the treatment medium zone next to it (P2) were chosen as measurement points (Fig. 1).

A series of PEF parameters were studied to determine their influence on the heating-up rate until the blanching temperature was achieved: frequency, electric field strength, electrical conductivity of the treatment medium. The parameters selected are based on the working limitations of the equipment and on the fact that electroporation of a plant cell is achieved at electric fields above 0.7 kV/cm (Toepfl et al., 2006). The PEF treatments applied are presented in Table 1.

These treatments were applied at room temperature of 20 °C; however, tests were carried out to evaluate the influence of the initial temperature of the treatment medium. For this purpose, PEF treatments of 1.33 kV/cm and 100 Hz were applied when the treatment medium had an initial temperature of 40, 60 and 80 °C.

In order to adjust the electrical conductivity of the $CaCl_2$ solutions and the required initial temperature, the following equation was experimentally developed:

$$K_T = -0.3846 + 0.0112 \cdot C + 0.03023 \cdot T \quad R^2 = 0.99 \tag{2}$$

where K_T is the electrical conductivity of the treatment medium (mS/cm) at a specific temperature, C is the concentration of $CaCl_2 \cdot 2H_2O$ (mg/100 mL), and T is the temperature of the solution in °C. The concentrations of $CaCl_2$ used ranged from 830 to 2056 mg/kg.

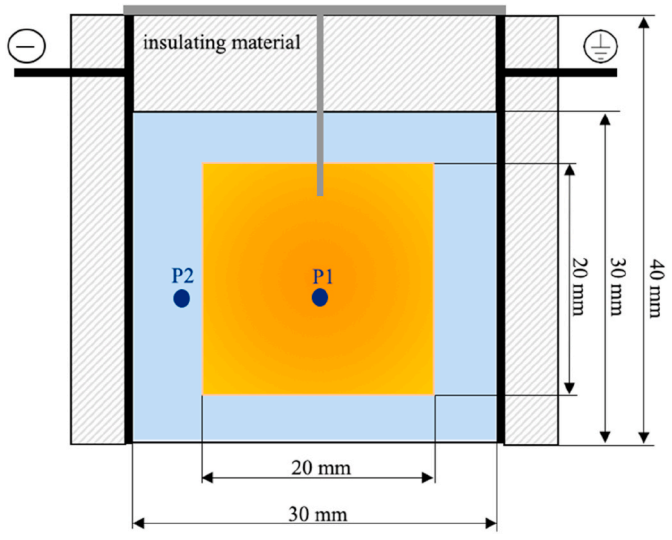

Fig. 1. Schematic of a longitudinal section of the treatment chamber together with the carrot sample during application of the PEF treatment. P1 and P2 refer to the area where the carrot and treatment medium temperature were measured, respectively.

Table 1
Processing parameters evaluated for PEF-ohmic assisted blanching of carrot samples.

Frequency (Hz)	Electric field strength (kV/cm)	Electrical conductivity of the treatment medium (mS/cm)
50	1.33	2.25
100		
150		
100	1	2.25
	1.33	
	1.67	
100	1.33	1.5
		2.5
		3.5

This equation was determined using the indirect method for measuring electrical conductivity (σ, S/m), based on Equation (3) (Olivera et al., 2013):

$$\sigma = \frac{I \cdot L}{A \cdot V} \tag{3}$$

where I is the intensity of the current (A), V is the voltage (V), L is the gap between the electrodes (m), and A is the surface area of the electrodes (m^2).

Equation (3) was also used to determine the electrical conductivity of the carrots. In this case, cylindrical carrot samples of 2 × 2 cm were introduced into a cylindrical PEF chamber with parallel electrodes of the same dimensions and 10 pulses of 1.0 kV/cm were applied at 1 Hz.

2.3.2. Conventional blanching

Conventional blanching was carried out by immersing the carrot samples in the treatment medium at 85 °C until complete POD inactivation was achieved. Similarly, to the PEF-assisted blanching, treatment time was determined based on POD heat resistance.

2.4. Determination of peroxidase (POD) enzyme inactivation

2.4.1. Qualitative technique

The qualitative test paper Peroxtesmo KO for Peroxidase in food (Macherey-Nagel, Germany) was used to evaluate the effectiveness of blanching. This test is commonly used in the canned industry to verify the correct application of vegetable blanching. It consists of strips of paper that turn blue when the enzyme is present, whereas no color change occurs when the enzyme is inactive (99 % enzyme inactivation). After blanching, the carrots were immersed in ice. Once the temperature reached 20 °C, the carrots were cut to break up the cells; two drops of the liquid thereby obtained were added to the test paper.

2.4.2. Mathematical prediction of POD enzyme inactivation

Raw carrot juice extracted with a blender (Slow Juicer, Juissen, Spain) was used for the characterization of the POD enzyme thermoresistance. 500 μl of the juice was added in glass tubes that were placed in a thermostatic bath at different temperatures (80, 82.5, 85, 87.5 °C).

Throughout the blanching process, samples were collected and Peroxtesmo KO tests were conducted to determine the required inactivation time for each temperature, known as *TDT* (thermal destruction time). These values were used to calculate the thermal destruction curve by plotting the $\log_{10}$ *TDT versus* temperature. In addition, the *z-value* (i.e. temperature increase required to reduce the *TDT* value by 99 %) was calculated as the inverse of the slope, or by applying the following equation:

$$Z = \frac{T_{ref} - T}{log(TDT) - log\left(TDT_{ref}\right)} \tag{4}$$

where T_{ref} = 85 °C. Once the thermoresistance of the POD enzyme

characterized, the mathematical prediction of its inactivation in the carrot cylinders according to the heat penetration curves obtained during the blanching processes was performed. For this purpose, the *lethality* parameter (L) was determined, which is an equivalent of the time that would be required at reference temperature (T_{ref} = 85 °C) to inactivate the same amount of enzyme as is inactivated at the temperature of the sample during 1 min (Equation (5)).

$$L = \int 10^{\frac{T - T_{ref}}{z}} dt \quad (5)$$

From the *L-values*, the *accumulated inactivation* (AI_{Tref}, expressed in seconds) at a reference temperature (i.e. T_{ref} = 85 °C) was calculated as the sum of lethalities over 1 s intervals.

$$AI_{T_{ref}} = \sum_0^t L \quad (6)$$

The above mathematical prediction was validated by determining the inactivation of the POD enzyme in carrot cylinders as explained in section 2.4.1.

2.5. Influence of the addition of calcium to the treatment-medium on blanched carrot texture

To evaluate the effect of calcium intake during blanching using non-treated (conventionally blanched) and PEF-treated carrots, four blanching treatments were applied: 1) non-PEF-treated carrots blanched in water at 85 °C with 850 mg/kg $CaCl_2$; 2) non-PEF-treated carrots blanched in water at 85 °C with 940 mg/kg NaCl; 3) PEF-treated carrots blanched in water with 850 mg/kg $CaCl_2$ (3.5 mS/cm at 80 °C), and 4) PEF-treated carrots blanched in water with 940 mg/kg NaCl (3.5 mS/cm at 80 °C).

After blanching, samples were cooled by immersing them in ice, after which they were vacuum-packed and frozen at −21 °C for 24 h. Subsequently, the vacuum-packed and frozen carrots were thawed in a room at 4 °C for 17h.

2.5.1. Texture analyses

For texture analyses, a TA-XT2i texture analyzer (Stable Micro Systems, Surrey, England) was used, applying the methodology described by Leong et al. (2018) with certain modifications. Thus, a loading cell, a cylindrical flat-head aluminum probe (38 mm diameter, Stable Micro Systems), and a heavy-duty platform were employed to perform a double compression test. The probe was set at 4 mm distance from the sample. The compression test started with a constant probe speed of 60 mm/min, and then the probe returned to its starting position and repeated the compression test on the same sample. At least 5 samples were analyzed for each blanching treatment combination, at least 5 samples were analyzed. From each test, the hardness value (N) represented as the maximum peak force detected during the first compression was chosen in order to assess the effect on the carrot texture.

2.5.2. Calcium analysis

The amount of calcium was measured by means of ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy). 0.03 g of dehydrated carrot was diluted in 50 mL MilliQ water in microwave-resistant tubes (MarsX-press Plus Wessel, CEM Corporation). Then, 6 mL of HNO_3 and 2 mL of H_2O_2 were added to the tubes, which were incubated at room temperature for 20 min before sealing. Samples were digested in a microwave system using a 15-min heating ramp at 1600 W and maintained at 200 °C for 15 min. Then, the samples were cooled, filtered (0.45 μm), and adequately diluted prior to injection with Ar gas in the ICP-OES equipment (iCAP PRO XP Duo, ThermoFisher Scientific, Waltham, MA, U.S.A.). The analysis condition was as follows: R.F. (Radiofrequency) power was 1150 W, auxiliary gas flow rate was 0.50 L/min, plasma flow rate was 12.5 L/min, nebulizer gas flow was 0.50 L/min, and the pump rate was 15 rpm.

2.6. Determination of β-carotene bioaccessibility

The effect of PEF-assisted blanching (90s/80 °C followed by 50s at 85 °C) *versus* traditional blanching (85 °C for 6 min) on the bioaccessibility of β-carotene in carrots was assessed by performing *in vitro* digestion experiments. Carrot samples were shipped the same day as processed from Zaragoza, Spain, to Ås, Norway, as an overnight express package, chilled with CO_2 solid pellets. Boiled carrots (100 °C for 15 min) and raw carrots were included as references. Carotenoid standards used in the bioaccessibility study were; echinenone (CAS Number 432-68-8) from CaroteNature GmbH and β-carotene (CAS Number 7235-40-7) from Sigma-Aldrich Co (St. Louis, MO, USA). Pepsin (porcine, P7000), pancreatin (porcine, P1750), and bile extract (bovine/ovine, B8381) used in the *in vitro* digestion model were likewise obtained from Sigma-Aldrich Co (St. Louis, MO, USA).

The procedure for estimating *in vitro* bioaccessibility of β-carotene is described in detail in sections 2.6.1-2.6.4. Samples were withdrawn from the intestinal phase during *in vitro* digestion for determination of micellarized β-carotene (available for intestinal absorption), as well as for the determination of the amount of free fatty acids (FFA), reflecting the rate of lipid digestion (micellarization). Bioaccessibility was estimated as the percentage of β-carotene released from the food matrix into the micellar phase:

$$Bioaccessibility(\%) = \frac{content\ of\ \beta - carotene\ in\ micellar\ phase}{ontent\ of\ \beta - carotene\ in\ whole\ carrot} \times 100\% \quad (7)$$

2.6.1. In vitro digestion

Carrot samples were digested using an *in vitro* static digestion model based on the INFOGEST standardized protocol (Brodkorb et al., 2019), with some modifications. The model uses standardized electrolyte solutions for the preparation of salivary, gastric, and intestinal fluids, and simulates the breakdown of food in the gastrointestinal tract using commercial enzymes, porcine pancreatin, and bile. All digestion experiments were performed in triplicate and were conducted in the dark to ensure minimal loss of β-carotene. Chewing was simulated using a kitchen blender (6720 Mini Chopper, OBH Nordica, Sweden) and aliquots (30 g) of the finely chopped carrot were freeze dried for analyses of dry matter and carotenoid content (section 2.6.2.). Aliquots of 1.8 g of the finely chopped carrot was digested together with 0.2 g rapeseed oil (Rema 1000, product no 1258961, Norway) to ensure adequate micellarization of lipids in the intestinal phase. Each sample (2 g) was added 2 mL of an electrolyte solution containing salivary amylase (5 U/mL) and kept at 37 °C under continuous shaking for 2 min to simulate the oral phase. The gastric phase was simulated by adding 4 mL of an electrolyte solution containing pepsin (4000 U/mL) and the pH was adjusted to 3.0 before incubation in a rotary incubator (Innova® 40/40R, New Brunswick Scientific, Edison, NJ, USA) at 37 °C and 215 rpm for 120 min. To simulate the intestinal phase, 8 mL of simulated duodenal fluid containing 0.07 mM $NaHCO_3$, porcine pancreatin, and bile, was added to each sample, resulting in a pancreatin concentration of 1.25 mg/mL and a bile salt concentration of 10 mM in the final volume (16 mL total volume). After adjusting the pH to 7, the samples were incubated at 37 °C and 215 rpm (rotary incubator) for 80 min in intestinal phase. After withdrawal, samples were centrifuged at 39800×*g* for 10 min at 4 °C and the aqueous phase in the middle (the micellar phase) was isolated using a glass pipette. An aliquot (2 mL) was filtered for further determination of β-carotene content in micelles (section 2.6.3), whereas another aliquot (5 mL) was prepared for analysis of FFA to determine the rate of lipid digestion (release of fatty acids in the intestine) by a method described in Kirkhus et al. (2019). In short, the enzyme activity was stopped by adding 15 ml $CHCl_3$:MeOH (2:1), after which the FFA fraction was isolated by solid phase extraction (SPE), and the FFA content was measured by gas chromatography with flame ionization detection (GC-FID) as described in Kirkhus et al. (2019). A

sample consisting of water containing 10 % rapeseed oil was used as control.

2.6.2. Analysis of β-carotene in carrot

Finely chopped raw carrot was freeze-dried under vacuum in a Christ Gamma 1–16 LSCplus apparatus (Osterode am Harz, Germany). The lyophilized carrot samples were extracted as triplicates in a room with no daylight (work under red light). Subsequently, 25 ± 0.1 mg freeze-dried carrot was added with 2 mL methanol:tetrahydrofurane (1:1) containing 0.1 % butylated hydroxy toluene (BHT) and 0.1 mL internal standard Echinenone (80 μg/mL) in a polystyrene tube. Samples were flushed with nitrogen and whirlmixed for 5 s, then placed on ice for 10 min in darkness before they were centrifuged at 5346×*g* for 10 min at 4 °C (Heraeus Multifuge 4 KR centrifuge, Thermo Scientific, Waltham, MA, USA). The supernatant was transferred to a new polystyrene tube and the extraction was repeated four times (8 mL in total). An aliquot of 1 mL supernatant was filtered (0.2 μm, 13 mm filter with PTFE membrane, Millex ® LG), into a vial, flushed with nitrogen, and capped before immediate HPLC analysis.

2.6.3. Analysis of β-carotene in the micellar phase of digested samples

The aliquot of 2 mL micellar phase was sequentially filtered through 0.8 μm (Acrodisc® 25 mm Syringe Filters with Versapor® membrane from Pall Corp.) and 0.2 μm (Phenex™-RC 15 mm Syringe Filters from Phenomenex Inc.) filters into a glass tube with cap (16 × 100 mm, 12 mL, round bottom, from Kimax). The filtered micellar phase (1.0 mL) was added with ethanol (3.0 mL) and internal standard (Echinenone, 1.67 μg/mL), flushed with nitrogen, and mixed (30 s in whirlmixer), before left in refrigerator (4 °C) until the next day. Placed on ice, the sample was added with 4.0 mL hexane and vortexed (1 min in whirlmixer) before centrifugation (500×*g*, 10 min, 4 °C). The upper hexane phase was transferred to a new glass tube, and the water/ethanol phase was re-extracted with 4 mL hexane, mixed, and centrifuged as above. The supernatants were combined and mixed, and a defined volume was evaporated under a nitrogen flow at 37 °C (RapidVap Vertex Dry, Labconco Corp., Kansas City, MO) until dryness, after which the sample was dissolved in 0.5 mL methanol:tetrahydrofuran (1:1, v/v) containing 0.1 % BHT before immediate HPLC analysis.

2.6.4. HPLC analysis

Samples (20 μL) were analyzed in an Agilent 1200 HPLC system (Agilent Technologies) equipped with a diode array detector. Separation was performed on a YMC Carotenoid C30 column (5 μm particle size, 250 × 4.6 mm i.d., product No. CT99S05-2546WT, YMC Europe GmbH) with a guard column (product No. CT99S05-0104 GC, YMC Europe GmbH). Mobile phase consisted of methanol (HiPerSolv CHROMANORM®, VWR International) (solvent A) and *tert*-butyl methyl ether (L14030, Thermo Scientific Chemicals) (solvent B), using the following gradient: 30–70 % B for 35 min, 70 % B for 18 min, 70-30 % B for 1 min, and 30 % B for 4 min. The solvent flow rate was 1.0 mL/min and column temperature was 23 °C. β-Carotene was identified by retention time and spectral characteristics at 452 nm; quantification was carried out by integrating the peak area and calculating by 5-point calibration curve. The amount of β-carotene was analyzed as mg/g dry matter (DM) and calculated to mg/100 g FW based on DM contents.

2.7. Determination of cell disintegration index (Z_p)

In order to determine if the application of PEF-ohmic causes the electroporation of carrot cells, the cell disintegration indez (Z_p) was carried out. This parameter measures the proportion of permeabilized cells based on the frequency dependence of the electrical conductivity of intact as well as permeabilized plant tissues (Angersbach et al., 1999). Cell disintegration index analysis was carried out using an impedance measurement equipment (DIL, Quakenbrück, Germany). Carrot cylinders of 2 × 2 cm were placed in a methacrylate chamber with a diameter of 2 cm and a gap of 2 cm, which was placed in the equipment's measuring cell. Z_p was calculated using the following equation:

$$Z_p = 1 - \left(\frac{K_h}{K'_h}\right) \cdot \frac{(K'_h - K'_l)}{K_h - K_l}; 0 \leq Z_p \leq 1 \quad (8)$$

where K_l, K'_l are the electrical conductivities of untreated and treated material, respectively, in a low-frequency field (1–5 kHz), and K_h, K'_h are the electrical conductivities of untreated and treated material, respectively, in a high-frequency field (3–50 MHz). The Z_p value varies between 0 for intact tissues and 1 for a tissue with all the cells permeabilized.

Z_p index was determined throughout the blanching treatments (PEF-assisted blanching consisted in applying PEF for 90s at 80 °C followed by water immersion at 85 °C, whereas conventional blanching was applied by immersion in water at 85 °C) at 4 points in time (when achieving 40 °C, 60 °C, and 80 °C at the center of the carrot, and then at the end of the blanching treatment). The applied specific energies required to reach those temperatures by PEF treatment were 56, 112, and 168 kJ/kg, respectively. In all cases, after reaching the targeted temperature, the carrot samples were immersed in ice and the Z_p was measured at 15 °C.

2.8. Statistical analyses

GraphPad PRISM software was used for statistical analyses. Significant differences between means were estimated by either Student's *t*-test or by one-way analysis of variance (ANOVA) followed by the Tukey method. P values < 0.05 denoted significance. Error bars in the figures correspond to the mean standard deviation. All runs were carried out in triplicate.

3. Results and discussion

3.1. Influence of PEF parameters on heating uniformity

Two of the main parameters that characterize PEF treatments are the electric field strength and the frequency at which the pulses are applied (Álvarez, et al., 2006). Electrical conductivity is also critical, in the same way it is in ohmic heating (Varghese et al., 2014). Therefore, the first step of this research was to evaluate the effect of the electric field strength (1.0–1.67 kV/cm), of the pulse frequency (50–150 Hz), and of the electrical conductivity of the treatment medium (1.5–3.5 mS/cm) on the heating kinetics of both treatment medium and carrot (Fig. 2).

As can be observed, the heating curves of carrot and the treatment medium when applying PEF showed the same linear heating kinetics that are characteristic of ohmic heating (Gally et al., 2016). In the case of the electric field and the electrical conductivity of the treatment medium (Fig. 2A and C), the higher the values, the higher the heating rates, as can be deduced from the Joule equation (Eq. 1). Thus, at an electric field of 1 kV/cm (100 Hz and 2.25 mS/cm), heating rates of 7.6 and 15.4 °C/min were obtained, while at 1.67 kV/cm, the rates were 28.2 and 42.5 °C/min for the treatment medium and the carrot sample, respectively (Fig. 2A). When assessing the influence of the electrical conductivity of the treatment medium (1.33 kV/cm and 100 Hz) (Fig. 2C), low values of 1.5 mS/cm resulted in rates of 13.48 and 21.47 °C/min for the medium and carrot, while an increase to 3.5 mS/cm resulted in rates of 17.90 and 36.82 °C/min, respectively. Similarly, heating at 150 Hz (1.33 kV/cm and 2.25 ms/cm) (Fig. 2B), resulted in higher heating rates than at frequencies of 50 Hz, with values of 22.5 °C/min and 8.7 °C/min for the treatment medium and 33 °C/min and 15.5 °C/min for the carrot, respectively.

Moreover, in order to evaluate the uniformity of heating under different electric fields (100 Hz and 2.25 mS/cm) (Fig. 2A), and considering an initial temperature of 20 °C, the carrot would take 1.25 min, 0.75 min, and 0.5 min to reach 40 °C for 1, 1.33 and 1.67 kV/cm, respectively. By the end of those durations, the medium would reach a

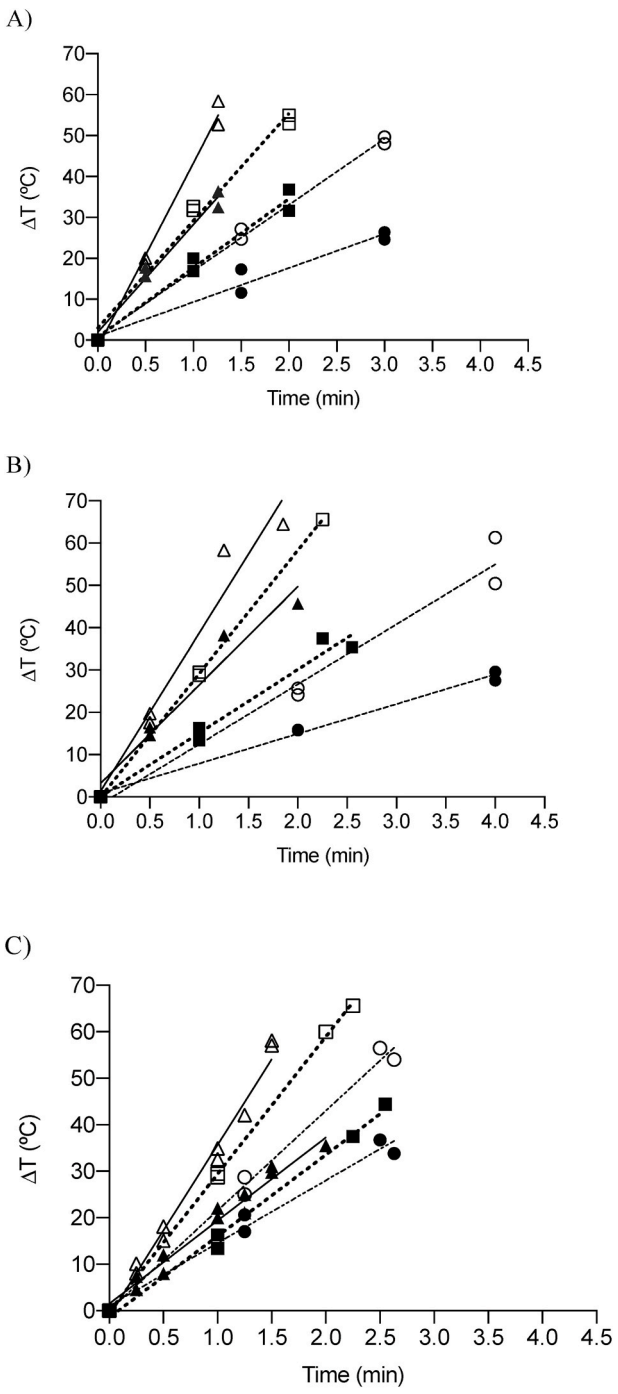

Fig. 2. Temperature difference (temperature – initial temperature) throughout the heating process in carrot and treatment medium. A) PEF-ohmic treatment of 100 Hz and initial medium conductivity of 2.25 mS/cm at 1, 1.33 and 1.67 kV/cm for the medium (●, ■, and ▲) and for the carrots (○, □, △), respectively; B) PEF-ohmic treatment of 1.33 kV/cm and initial medium conductivity of 2.25 mS/cm at 50, 100 and 150 Hz for the medium (●, ■, and ▲) and for the carrots (○, □, △), respectively; C) PEF-ohmic heating of 1.33 kV/cm and 100 Hz at initial medium conductivity of 1.5, 2.5 and 3.5 mS/cm for the medium (●, ■, and ▲) and for the carrots (○, □, △), respectively.

temperature of 31, 32 and 36 °C, respectively; thus, the temperature difference between the two matrices would be 10, 8, and 6 °C for 1, 1.33 and 1.67 kV/cm, respectively. Regarding conductivity (1.33 kV/cm and 100 Hz) (Fig. 2C), the carrot would reach 40 °C after 0.9, 0.75 and 0.55 min when medium conductivity was 1.5, 2.5 and 3.5 mS/cm. In the course of those time intervals, the temperature gradient generated would be 8, 10, and 9 °C, respectively. Regarding the frequency parameter (1.33 kV/cm and 2.25 mS/cm) (Fig. 2B), the medium would reach 40 °C after 1.5 min, 0.7 min and 0.5 min for 50, 100, and 150 Hz, resulting in temperature gradients of 9, 9.5 and 6 °C, respectively. Therefore, the electric field, frequency, and electrical conductivity of the treatment medium allowed for the application of faster heating rates, but did not achieve optimal heating uniformity, as the carrot heated up more rapidly regardless of the parameters applied.

Since we are dealing with an ohmic heating process, the electrical conductivity of the medium has to be well adjusted to achieve uniform velocities in both matrices. The literature shows that the electrical conductivities of the treatment medium and the food have to match in order for both matrices to heat up in a similar way (Varghese et al., 2014). For this purpose, the electrical conductivity of the raw carrot was measured and a value of 2.66 ± 0.77 mS/cm was determined. As explained above, when the conductivity of the medium is around 2.66 mS/cm, both the carrot and the treatment medium should be heated in a similar way. However, as shown in Fig. 2C, at a conductivity of 2.5 mS/cm, heating rates of 15.1 °C/min for the medium and 26.3 °C/min for the carrot were obtained. This behaviour could be due to the electroporation achieved by applying PEF to the carrot, which would increase its electrical conductivity to over 2.5 mS/cm. Following this approach, in order to achieve uniform heating, the electrical conductivity of the treatment medium should be made similar to the conductivity of the electroporated carrot.

To verify this hypothesis, a standard PEF treatment (4 kV/cm, 125 pulses, 10 kJ/kg) was applied to carrot samples, and the electrical conductivity of the carrots after treatment of 6.00 ± 0.32 mS/cm was determined. Then, PEF heating (100 Hz, 1.5 kV/cm) was applied to carrots immersed in a treatment medium at 6 mS/cm. The heating curves obtained for both carrot and treatment medium are shown in Fig. 3, and, as observed, the carrot and the treatment medium were heated at a similar rate. These results indicate that heating unformity between carrot and treatment medium would be obtained at high electrical conductivities. However, the use of high-conductivity treatment medium implies an increase in energy consumption and the use of powerful PEF equipment, which would make the process difficult to scale up to an industriall level. The use of high electrical conductivities could thus be a limitation.

Based on the obtained results in which the heating rate of carrots is higher than the treatment medium, a way to compensate this difference would be by treating the product in media of low electrical conductivities, but at high temperatures. This would be also in accordance with previous research, in which the high temperature of the area

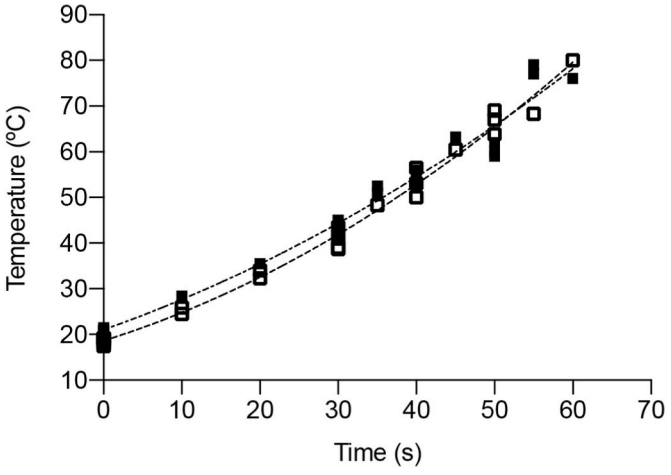

Fig. 3. Heating curves of carrot (empty squares) immersed in treatment medium (filled squares) of 6 mS/cm at 30° C at PEF heating of 1.5 kV/cm and 100 Hz.

surrounding the heated sample was used (i.e. increasing the temperature of the electrodes or the surrounding medium) in order to assess heating uniformity in a PEF-ohmic heating process (Ariza-Gracia et al., 2020; Astráin-Redín et al., 2022; Farahnaky et al., 2012; Gratz et al., 2021). Moreover, this strategy would be in line with the practical application of traditional blanching, since conventional blanchers operate with water at high temperatures. Additionally, in view of the difference between electrical conductivities, the application of PEF in water at high temperatures would make it possible to heat up only the product saving the energy required to heat up the blanching medium. Therefore, this article has been focused on applying PEF as an assisting heating process in treatment media at high temperatures and low electrical conductivities.

3.2. *Influence of the temperature of the treatment medium on PEF heating uniformity*

Many different combinations of electrical conductivity, electric field strength, and frequency can be applied to heat food via PEF, depending on the characteristics of the PEF equipment, the dimensions of the chamber, and the properties of the food itself. In this case, field strengths and frequencies of an intermediate level among all those previously tested (1.3 kV/cm; 100 Hz) were applied. Moreover, in order to add the same concentration of $CaCl_2$ as industry (0.5–1.5 %, Rastogi et al., 2008) to the treatment medium, an electrical conductivity of 3.5 mS/cm was chosen and the influence of the initial temperature (40, 60 and 80 °C) was studied.Thus, the $CaCl_2$ concentrations of the treatment medium were 1600 mg/kg, 1200 mg/kg, and 850 mg/kg for working temperatures of 40, 60, and 80 °C, respectively.

For methodological reasons (including preventing water from boiling) a target temperature of 85 °C at the end of the treatment, both inside the carrot and in the treatment medium, was fixed for the application of the blanching treatment. Fig. 4 shows the temperature evolution of both carrot and treatment medium when applying PEF at an electric field of 1.3 kV/cm, and at a frequency of 100 Hz for an electrical conductivity of the treatment medium of 3.5 mS/cm at different temperatures (40, 60, and 80 °C).

It should be noted that increasing the temperature of the treatment medium favours convective heat transfer from the treatment medium to the surface of the carrot, which is the limiting factor (cold points) of this process (Choi et al., 2020), since the carrot heats rapidly on the inside. As observed in Fig. 4, at all investigated temperatures, the heating rate of the carrot was 30 °C/min, 34.2 °C/min, and 42.5 °C/min: thus higher than heating rates obtained at room temperature, and up to 13 fold faster than the heating rate of the treatment medium (3.1 °C/min). On the other hand, results indicated that the higher the initial temperature of the treatment medium, the more uniform the final temperature distribution of the carrot would be. At treatment medium starting temperatures of 40 and 60 °C (Fig. 4A and B), when the carrot was at 85 °C, the medium was at 73 °C and 75 °C, respectively. However, with a treatment medium starting temperature of 80 °C (Fig. 4C), both the carrot and the treatment medium reached 85 °C after 90s of treatment. These results indicated that the application of PEF-ohmic treatments at high initial temperatures of the treatment medium could be a new way to heat up vegetables rapidly while achieving a uniform final temperature of the whole product.

The strategy of increasing the initial temperature of the treatment medium to improve heating uniformity has been scarcely investigated. Gratz et al. (2021) investigated the uniformity of ohmic cooking of potato by applying high frequencies (12 kHz, 55.6–111 V/cm; 300 kHz, 10–40 V/cm), under initial temperatures of 20 °C and 90 °C with different electrical conductivies. They observed that the treatments with the treatment medium starting at 90 °C presented lower heating uniformities than when starting at 20 °C, and they attributed this to the mismatch of conductivities between the treatment medium and the potato, since at 90 °C the electrical conductivity of the treatment medium was considerably higher than at 20 °C. This clearly indicates – and our results also support this view – that all the variables involved in ohmic heating must be precisely controlled in order to optimize treatments.

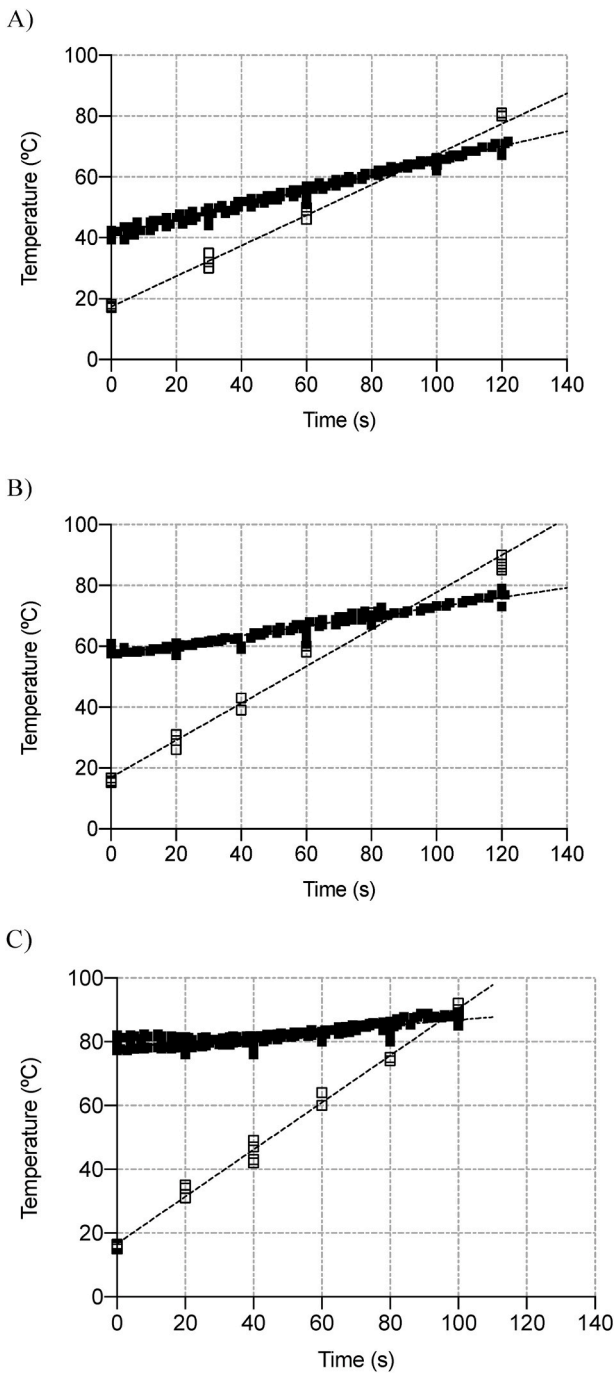

Fig. 4. Heating curves of carrot (empty squares) immersed in treatment medium (filled squares) of 3.5 mS/cm at different initial temperatures: 40 °C (A), 60 °C (B), 80 °C (C).

3.3. *Characterization of the thermoresistance of POD enzyme*

Although the literature already provides data concerning POD heat resistance (Gonçalves et al., 2010; Güneş & Bayindirli, 1993; Soysal & Söylemez, 2005), its D_t and z values vary across a wide range ($D_{75°C}$ = 4.87–12.00 min; z = 15.9–22.17 °C). Therefore, in order to properly evaluate POD inactivation after PEF-assisted blanching of carrots, and in order to compare those results with those obtained after conventional

water-inmmersed blanching, it was necessary to characterize the thermorersistance of POD in the carrots used in our study.

Fig. 5 shows the activity of the POD present in the juice obtained from carrots after applying several heating times at different temperatures (80, 82.5, 85, 87.5 °C). A linear relationship can be observed between the Log_{10} of the time required for the complete inactivation of the enzyme and the treatment temperature (*TDT* line). Based on this *TDT* curve, a *z-value* of 16.44 ± 1.40 °C was determined. This z value is within the range of those reported for carrots (Gonçalves et al., 2010). Therefore, this *z* value and a *TDT* value of reference at 85 °C ($TDT_{85°C}$ = 1.26 min) was used to estimate the degree of POD inactivation after PEF-assisted-blanching using Equations (5) and (6).

3.4. PEF-assisted blanching

Fig. 6 shows the carrot heating curve represented by thin lines (real values) obtained by applying a PEF treatment of 1.33 kV/cm at a frequency of 100 Hz and an initial temperature of the treatment medium of 80 °C with an electrical conductivity of 3.5 mS/cm (measured at 80 °C), followed by a holding heating time at 85 °C. Dotted lines represent the 95 % confidence interval of temperatures in the center of the carrot. The figure also includes the mathematical prediction of the accumulated inactivation of POD (*AI*) at the reference temperature (85 °C). The time required to inactivate the POD enzyme at 85 °C is 76 s based on the obtained D_t and *z* values (see above). Thus, when the *AI* during the blanching treatment equals 76 s ($AI_{85°C}$ = 76s), the enzyme would already be inactivated.

As observed in Fig. 6A, the PEF heating-up phase up to 85 °C, requiring a processing time of 90 s, hardly affected POD activity, accounting for an $AI_{85°C}$ of only 8 s. These results showed the need for a subsequent holding heating phase at a certain temperature, in this case at 85 °C. Therefore, after 90s of treatment, a holding phase was added in which the carrot was immersed in a thermostatic bath with the same treatment medium at 85 °C. In this way, 68s of holding time at 85 °C would be necessary to completely inactivate the POD enzyme. To validate this mathematical calculation, Fig. 6A also shows the experimental results, with enzyme active (red crosses) or inactive (green hyphen) after several different treatment times. As can be seen, at treatment times up to 140s, the POD test result showed that there was still POD activity, while longer treatment times led to its complete inactivation (at least below the detection limit of the test). These results once more confirmed that the PEF treatment producing such rapid heating (1.33 kV/cm, 100 Hz, initial temperature of the medium of 80 °C, during 90s) was not sufficient to inactivate the POD enzyme, and that a subsequent heat maintenance phase was necessary.

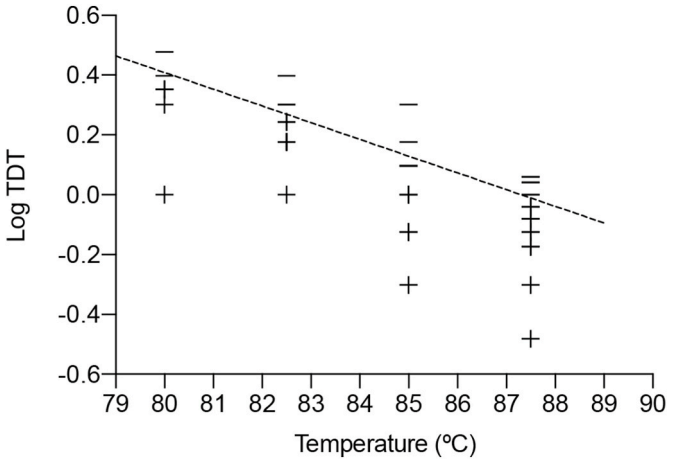

Fig. 5. Thermal destruction curve of POD enzyme in carrot juice samples. + indicates that the enzyme is active and - indicates that the enzyme is inactivated.

In order to compare PEF-assisted blanching *versus* conventional blanching for carrot, we also calculated the thermal inactivation of POD immersed in a water bath at 85 °C (Fig. 6B). In this case, according to the heating curve, it took 5 min for the core (cold spot) of the carrot to reach 85 °C, whereas with PEF the same state was achieved in 90s (center spot). The prediction of POD inactivation estimated that 5.7–6.8 min of blanching were necessary to completely inactivate the enzyme in carrot cylinders by conventional means. This result was experimentally validated, since up to 6 min were required to completely inactivate POD by this conventional heating method. Therefore, results indicated that PEF-assisted blanching would reduce processing time by 60 % as compared to the conventional blanching method based on immersion in water.

In addition, *in silico* simulations were also performed in order to predict what would occur if higher treatment temperatures were applied (and consequently higher carrot temperatures were attained). This was not experimentally validated, since it was not possible to apply higher temperatures with the PEF equipment used in this study. Fig. 7 shows the results obtained (heating curves and POD inactivation) after a PEF-assisted blanching treatment that reached 95 °C. As can be observed, it would take 108 s to reach 95 °C in the center of the carrot, and 15 s of holding phase would be required to ensure the complete inactivation of POD enzyme. In this case, the improvement in processing time would be 65.8 % compared to the conventional blanching (Fig. 6A), and hardly any holding heating phase would be necessary. Thus, 123 s of blanching treatment are necessary to achieve inactivation of the POD enzyme.

Although the application of PEF for ohmic heating has not been extensively investigated, studies of POD enzyme inactivation by ohmic heating have been widely reported in the literature. Mannozzi et al. (2019) studied the inactivation of POD enzyme in carrot juice obtained from mash after: a) preheating to 80 °C and applying a PEF treatment (exponential decay pulses, 0.8 kV/cm, 0.5 kJ/kg); b) applying ohmic heating (114 V/cm, 12 kHz) to raise temperature from 20 to 80 °C; c) heating up to 80 °C. They did not observe any statistically significant differences among any of the heating treatments in terms of inactivation of the POD enzyme. Guida et al. (2013) applied ohmic blanching (24 V/cm, 80 °C) to artichoke heads and reduced the blanching time by 25 % compared to conventional blanching in water at 100 °C. The cause of the improvement in POD enzyme inactivation was explained by the improved uniformity of the heating during ohmic blanching, compared to large differences between central and peripheral zones in conventional blanching. Other authors have nevertheless reported that the application of ohmic heating can accelerate the inactivation of enzymes due to non-thermal effects (Icier et al., 2006; Jakób et al., 2010).

3.5. Impact on texture

Texture is one of the most affected properties in vegetables after blanching and the subsequent freeze-thawing process. Because of this, a pre-treatment is usually carried out prior to blanching by soaking the vegetables in a solution containing calcium salts with the aim of promoting the formation of cross linkages between the pectins and the new calcium ions in order to minimize softening. In the industry, however, this pretreatment with calcium solution tends to be prolonged due to slow diffusion of calcium into the plant tissue. PEF might be a good alternative that could aid in improving this mass transfer process thanks to the electroporation phenomenon (Vorobiev & Lebovka, 2022); very few studies, however, have explored this possibility.

In this study, calcium was added to the treatment medium in order to sidestep the previous soaking phase. Thus, in a single blanching phase, the incorporation of calcium would be encouraged thanks to the application of PEF heating. For this purpose, the hardness value of the carrot samples was determined after the application of four different blanching treatments (adding or not, NaCl or $CaCl_2$, and applying PEF-assisted blanching or conventional blanching).

The results are shown in Fig. 8. No significant differences ($p > 0.05$)

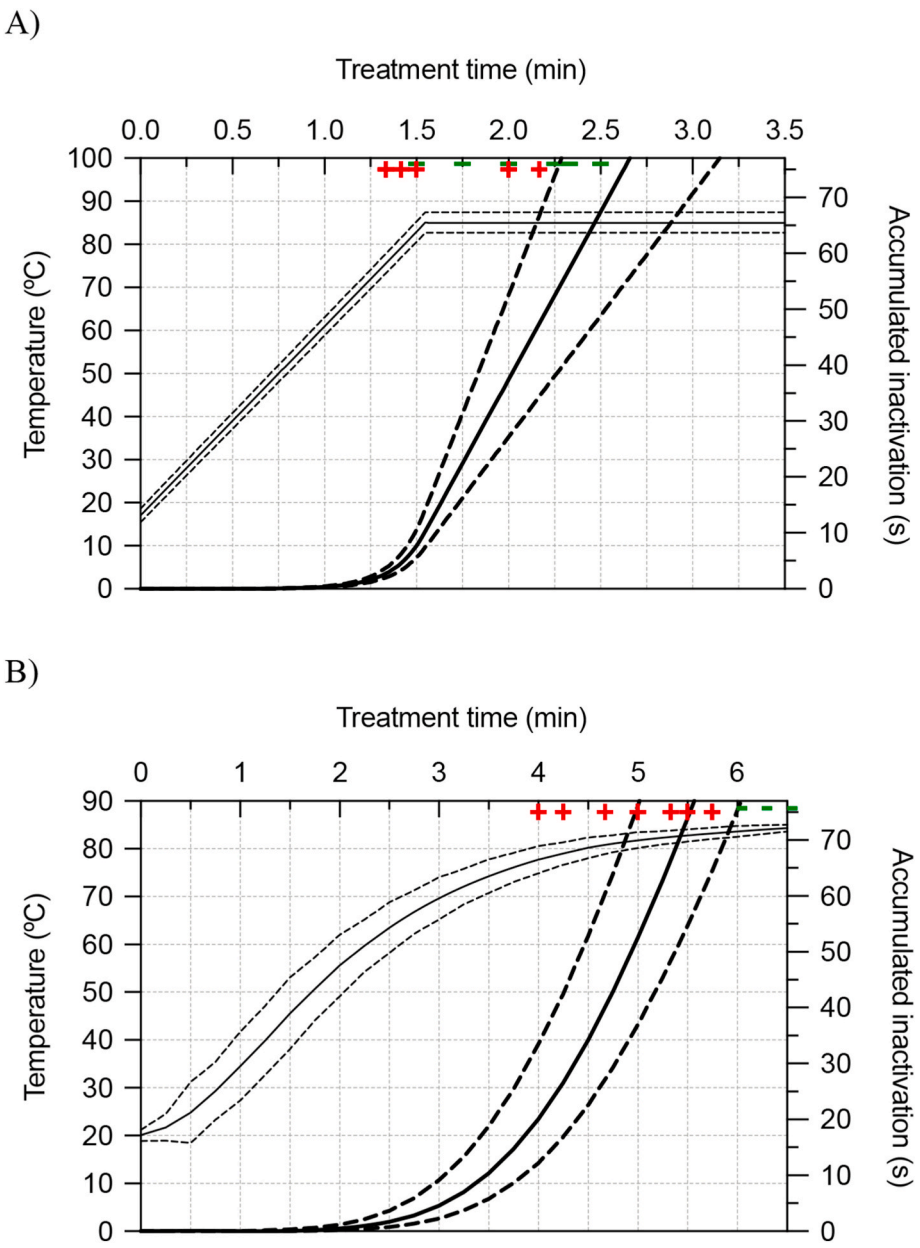

Fig. 6. Mathematical prediction of POD enzyme inactivation in carrot cylinders when applying a treatment of: A) PEF blanching (1.33 kV/cm; 100Hz; 3.5 mS/cm at an initial treatment medium temperature of 80 °C + immersion in water at 85 °C). B) Conventional blanching in water at 85 °C. The thin lines represent the carrot heating and the thick lines the predicted enzyme inactivation. Solid and dotted lines represent the mean and 95 % confidence interval. Validation of the inactivation of the enzyme is represented by red + for POD activity and green - for POD inactivation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

were observed between the PEF-assisted blanched samples in the presence of calcium compared to those blanched by conventional means. Nevertheless, a significant increase of 33 % in hardness was observed compared to raw carrots when PEF-assisted blanching was applied with calcium in the treatment medium. Moreover, comparing the effect of the two PEF treatments, samples treated in a medium containing calcium displayed 36.8 % greater hardness than PEF samples with no calcium in the medium.

To determine if there was any relationship between these hardness values and the degree of calcium absorption, the amount of calcium in the carrot samples was analyzed after blanching-freezing-thawing. Results were 1.35 (±0.06) mg Ca/g d.e. for raw carrots, 2.06 (±0.19) mg Ca/g d.e. for conventionally blanched samples, and 2.00 (±0.10) mg Ca/g d.e. for carrots that had undergone PEF-assisted blanching. PEF-assisted blanched samples thus did absorb calcium, although not to a greater degree than standard blanched carrots. The improvement in carrot hardness due to calcium uptake has been described in the literature, but with calcium as a pre-treatment prior to blanching. Leong et al. (2018) studied the application of a PEF pre-treatment (1.9 kV/cm; 20 Hz; 191 kJ/kg) to carrots immersed in a solution with 300 mg/kg $CaCl_2$ in comparison with the conventional treatment of overnight soaking with a solution of 5000 mg/kg $CaCl_2$. They observed that the hardness of pulsed carrots after blanching (100 °C/5 min) was 57 % higher than that of non-blanched ones, and similar to that of carrots pretreated with the overnight 5000 mg/kg $CaCl_2$ solution. This increase in calcium content might partially explain the increased hardness of freeze-thawed carrots observed when they were blanched using PEF in a solution containing calcium, but, as the results obtained for carrots conventionally blanched demonstrate, an increase in calcium content alone was not enough to improve carrot texture. Thus, it can be

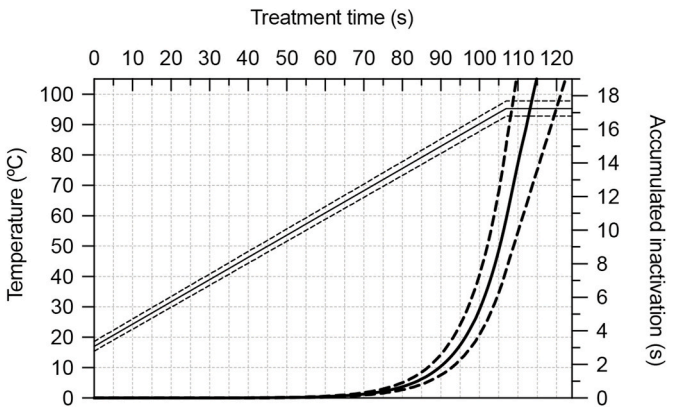

Fig. 7. Mathematical prediction of POD enzyme inactivation in carrot cylinders when applying a PEF blanching treatment (1.33 kV/cm; 100Hz; 3.5 mS/cm at an initial treatment medium temperature of 80 °C + immersion in water at 95 °C). The thin lines represent the carrot heating and the thick lines the predicted enzyme inactivation. Solid and dotted lines represent the mean and 95 % confidence interval.

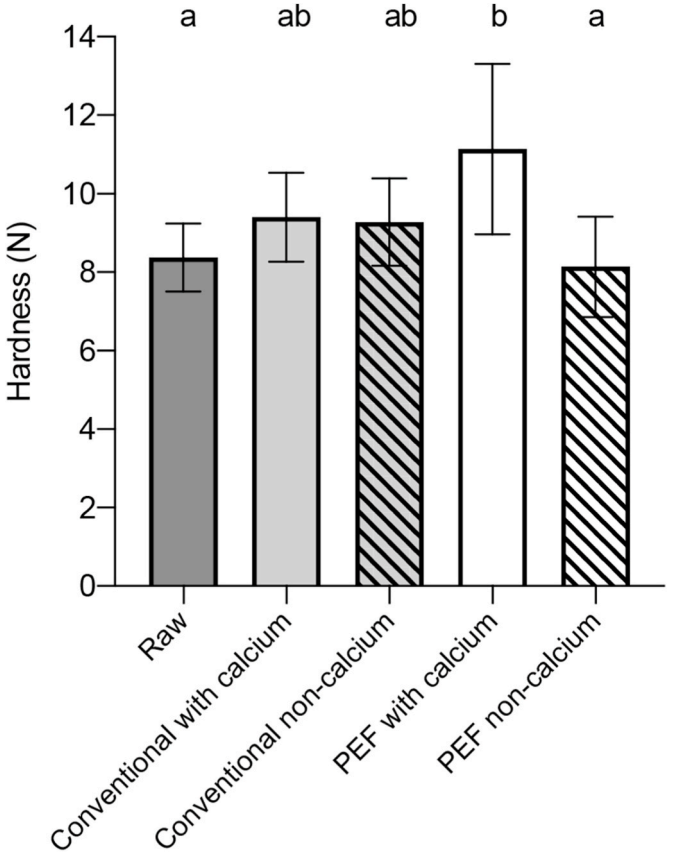

Fig. 8. Texture analysis results after blanching – freezing – thawing of carrot cylinders. The same letter above the bars means that there are no significant differences ($p > 0.05$) between them.

hypothesized that this phenomenon might be related to the fact that PEF-blanching would involve less intense treatments (at least to the external parts of the carrots) or that PEF might be promoting calcium interactions with different or more relevant structures/components -from the point of view of texture-than conventional heat treatments. Further study should therefore be undertaken in the future in order to fully elucidate this point.

3.6. Impact on β-carotene bioaccessibility

The impact of PEF assisted blanching on the bioaccessibility of β-carotene in carrot was investigated using *in vitro* digestion experiments. Results are shown in Fig. 9A and Table 2.

Seen from Table 2, the amount of β-carotene per g of fresh weight varied only slightly between the carrot samples, and there was no significant difference between the PEF blanched sample and the other samples. They had an average content of 10.5 ± 0.7 mg β-carotene per 100 g carrot sample.

Estimations showed that β-carotene bioaccessibility in PEF-blanched carrot (3.9 %) increased significantly (1.2-fold) compared to conventionally blanched carrot (3.2 %) ($p = 0.048$) (Fig. 9A). Boiled carrots had the highest bioaccessibility (4.4 %), significantly higher ($p = 0.000$) than raw carrots (Fig. 9A), which is in accordance with the literature showing that cooking increases the release and bioaccessibility of β-carotene in carrots (Hedrén et al., 2002; Hornero-Méndez & Mínguez-Mosquera, 2007). No significant difference in β-carotene bioaccessibility was found in the present study between raw carrots and conventionally blanched carrots, indicating that the heating during the blanching process was not sufficient to soften and disrupt food matrix structures for enhanced release of β-carotene (Fig. 9A). Moreover, since overall lipid digestion, i.e. release of free fatty acids (FFA) in the intestinal phase, was unaffected (Fig. 9B), the observed increase in bioaccessibility when blanching was combined with PEF was most likely due to increased release of β-carotene from the carrot matrix, resulting in increased incorporation of β-carotene into micelles. This result indicates that the application of PEF for assisting blanching may electroporate the cells of the plant tissue, thereby favouring the release of a greater amount of β-carotene from the carrot matrix compared to conventional blanching.

Our results are in agreement with a recent bioaccessibility study by López-Gámez et al. (2021), who reported that β-carotene content in micellar fraction from PEF-treated (0.61 kJ/kg) whole carrots (*Daucus carota* cv. Nantes) was significantly higher than in untreated carrots (4.2 mg vs 2.3 mg/100 g); i.e., the authors observed enhanced carotenoid bioaccessibility, and similar to our study PEF did not affect the overall carotenoid content. Other nonthermal processing techniques that have been investigated for potential effect on *in vitro* bioaccessibility of β-carotene in carrot are high-pressure homogenization (HPH) and high-pressure processing (HPP) (Cilla et al., 2018). Studies have shown that HPH may increase *in vitro* β-carotene bioaccessibility in carrot emulsions (Svelander et al., 2011) and carrot juice (Liu et al., 2019). In the study of Svelander et al. (2011) Svelander et al. (2011) HPH caused a significant 1.6-fold increase in the micellar incorporation of β-carotene. Knockaert et al. (2011) compared mild pasteurisation with equivalent high pressure process (HPP) pasteurisation and found that the *in vitro* bioaccessibility increased 1.2 fold in HPP treated carrot, an increase in the same range as observed after PEF in our study. However, the authors concluded that the effect of HPP was dependent on the intensity of the process and that in contrast to thermal processing, HPP does not always result in improved β-carotene bioaccessibility. In conclusion, alternative preservation techniques to thermal processing, such as high pressure and PEF, may affect the microstructure of carrot and the release of β-carotene, but the impact of varying processing conditions on the capacity to increase β-carotene bioavailability needs further investigation.

3.7. Cell disintegration index (Z_p)

After observing an improvement in β-carotene release, we attempted to differentiate between the electroporation effect and the thermal effect by determining the percentage of cell damage caused by PEF blanching *versus* conventional blanching.

The degree of "tissue damage" or "cellular membrane permeabilization" caused by PEF-assisted blanching treatments to carrots was determined by calculating the cell disintegration index (Z_p) reached

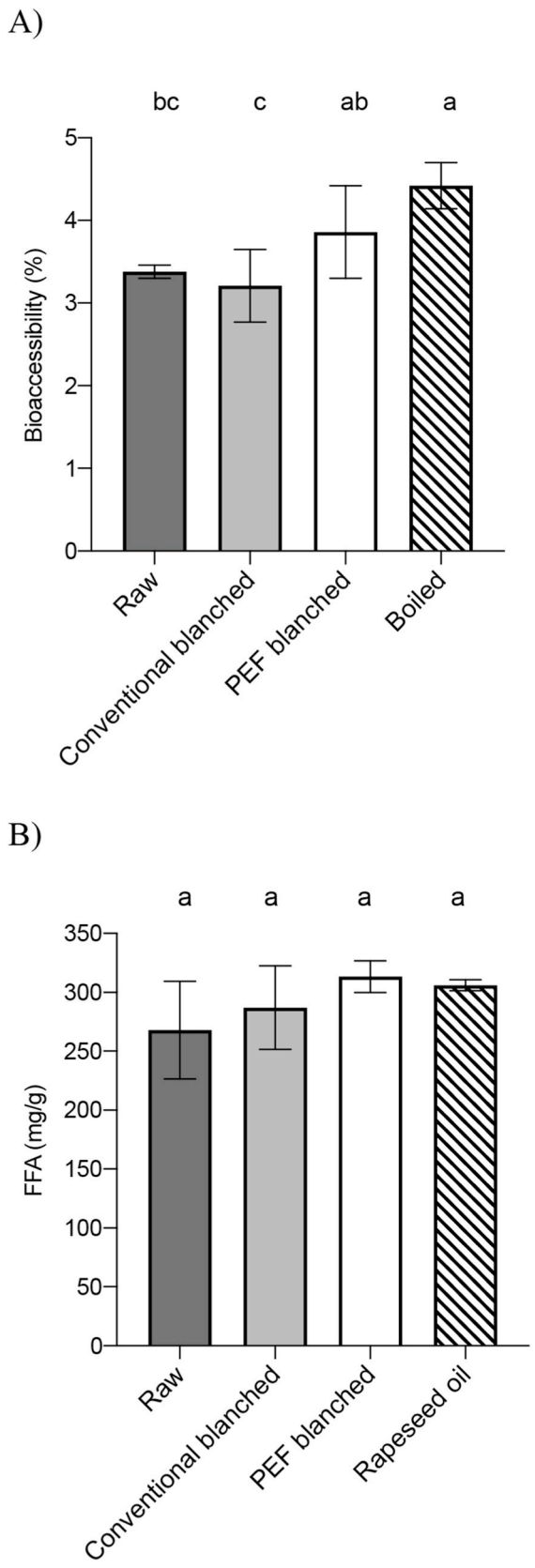

Fig. 9. *In vitro* digestion results. A) the bioaccessibility of β-carotene (%) and B) the level of free fatty acids (FFA) (mg/g fat) after 80 min intestinal phase. Means that do not share a letter (a, b, c) are significantly different (one-way ANOVA).

Table 2
β-Carotene content (mg/100 g) in raw, conventionally blanched, PEF blanched, and boiled carrots.

	β-carotene (mg/100 g)
Raw	9.5 ± 1.14 [a]
Conventionally blanched	10.8 ± 0.05 [a,b]
PEF blanched	10.6 ± 0.17 [a,b]
Boiled	11.1 ± 0.04 [b]

*Values with the same letter are not significantly different ($p > 0.05$).

after those treatments, and was also compared to that attained after conventional blanching treatments. As observed in Fig. 10, the samples blanched by PEF displayed a higher Z_p (>50 %) than those treated only by heat, regardless of the final temperature that had been attained. In conventional heating, increasing the final temperature reached in the center of the sample from 40 to 80 °C resulted in an increase in the cell Z_p from 0.12 to 0.36, whereas for PEF-assisted blanching the Z_p was already 0.77 when samples reached 40 °C and increased further up to 0.87 when the final temperature was 80 °C. This result demonstrates the capacity of PEF for the permeabilization of carrot cells, which would likewise work in favor of the outflow of β-carotene.

Shorstkii et al. (2022) studied the evolution of carrot Z_p when applying a PEF treatment (1 kV/cm; 2 Hz) at specific energies ranging from 0.25 to 10 kJ/kg. They observed that above 5 kJ/kg, cell disintegration did not increase, with a Z_p of approximately 0.8 (the maximum permeabilization reached), which is in accordance with the values obtained in Fig. 10.

4. Conclusions

This study demonstrates the potential of PEF as a new way of applying ohmic heating for rapid heating-up processes of solids, in this case as an assisting agent in the blanching of vegetables.

Under the evaluated PEF-ohmic treatment conditions (frequencies of 50–150 Hz; electric fields of 1–1.67 kV/cm and electrical conductivities

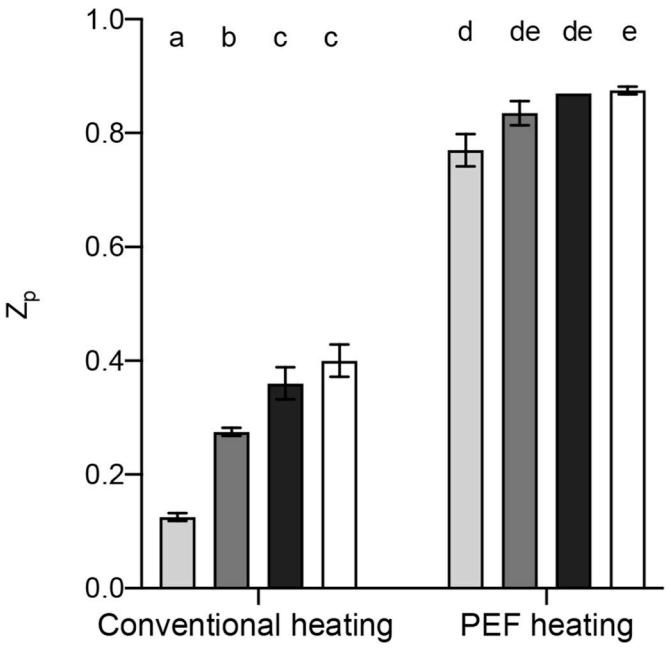

Fig. 10. Disintegration index (Z_p) of the carrot tissue after heating up to different temperatures (20–40 °C (■), 20–60 °C (■), and 20–80 °C (■), and until the end of blanching (□)) by immersion in water at 85 °C or by PEF (1.33 kV/cm; 100Hz; 3.5 mS/cm at an initial treatment medium temperature of 80 °C). The same letter above the bars means that there are no significant differences ($p > 0.05$) between them.

of the treatment medium of 1.5–3.5 mS/cm) the heating rate of the carrot was 1.5–2 times higher than that of the treatment medium. Therefore, the initial temperature of the heating medium had to be set at 80 °C in order to achieve a homogeneous heating of the carrot samples. This new approach ensured rapid, volumetric heating up to the blanching temperature, after which a heat maintenance stage at 85 °C was necessary in order to completely inactivate carrot polyphenol oxidase. Despite this, a 60 % reduction in blanching time was achieved compared to conventional blanching.

Moreover, application of this PEF-ohmic treatment increased the bioaccessibility of β-carotenes compared to conventional blanching due to the electroporation caused by PEF. However, PEF treatment did not negatively affect carrot texture.

In conclusion, a new way of applying PEF is proposed to promote the ohmic blanching of vegetables in the early stages of heating. However, further research is needed in order to assess the influence of this treatment on the nutritional and sensory quality of vegetables and, particularly, to assess the scalability of this ohmic-PEF treatment considering the currently available PEF generators/systems. In this sense, it would be of the highest interest to study the possibility of using heating media of lower electrical conductivity than the one used in this investigation, since this would facilitate the industrial implementation of this process.

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CRediT authorship contribution statement

Leire Astráin-Redín: Methodology, Validation, Investigation, Writing – original draft. **Javier Raso:** Review. **Ignacio Álvarez:** Conceptualization, Writing – review & editing. **Bente Kirkhus:** Methodology, Writing – review & editing. **Ane Meisland:** Investigation, Writing – review & editing. **Grethe Iren A. Borge:** Methodology, Writing – review & editing, Resources. **Guillermo Cebrián:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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