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Important factors to consider for acrylamide mitigation in potato crisps using pulsed electric fields

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1 **Highlights**

- 2 • PEF was applied for the reduction of acrylamide content in potato crisps
- 3 • PEF pre-treatment was compared to the conventional blanching pre-treatment
- 4 • PEF protocol and sample preparation were optimized for industrial application
- 5 • The quality of PEF-treated potato crisps (colour and texture) was evaluated
- 6 • A reduction of 30% of acrylamide content was achieved after applying PEF

7

1 **Industrial relevance**

2 The Commission Regulation (EU) 2017/2158 of 20 November 2017 has introduced new benchmark levels
3 and mitigation strategies for the reduction of the presence of acrylamide in foods, directing food businesses to
4 the research of measures to lower the acrylamide formation in foods. The actual industrial production process
5 of fried potato crisps involves the use of many mitigation strategies, such as a blanching of raw potatoes.
6 However, the traditional blanching treatment presents several practical drawbacks and leads to undesirable
7 changes of the product quality. The application of PEF as a pre-treatment could reduce the acrylamide content
8 in deep-fat fried potato crisps. This preliminary study gives important indications regarding the possibility of
9 combining a PEF pre-treatment on raw potato slices with subsequent industrial processing steps for the
10 production of potato crisps with low acrylamide concentration.

11

1 **Important factors to consider for acrylamide mitigation in potato crisps using** 2 **pulsed electric fields**

3

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16

17 **Abstract**

18 This preliminary study aimed to compare the application of pulsed electric field (PEF) with a traditional
19 blanching as pre-treatments before frying for the mitigation of acrylamide content in potato crisps.

20 Measuring the degree of cell disintegration index (p_o) and the changes in water electrical conductivity
21 during washing of potato slices, PEF protocol and sample preparation scheme were optimized. Peeled potato
22 slices (thickness 1.5 ± 0.2 mm) were subjected to PEF (1.5 kV cm^{-1} , pulse duration 10 μs , total treatment time
23 10 ms, pulse frequency 100 Hz) and to blanching (85°C for 3.5 min) pre-treatments and then to washing in
24 water, evaluating the reduction of acrylamide precursors (reducing sugars and free asparagine). After frying
25 (175°C , 3 min), product quality, in terms of colour, texture and acrylamide content were evaluated. Results
26 showed that PEF promoted acrylamide precursors leaching followed by a reduction of the final acrylamide
27 content of around 30%, significantly higher if compared to the reduction obtained with blanching, with only
28 slight modifications of the final quality of the product, in terms of colour and texture.

29 *Industrial relevance:* The Commission Regulation (EU) 2017/2158 of 20 November 2017 has introduced
30 new benchmark levels and mitigation strategies for the reduction of the presence of acrylamide in foods,
31 directing food businesses to the research of measures to lower the acrylamide formation in foods. The actual
32 industrial production process of fried potato crisps involves the use of many mitigation strategies, such as a
33 blanching of raw potatoes. However, the traditional blanching treatment presents several practical drawbacks
34 and leads to undesirable changes of the product quality. The application of PEF as a pre-treatment could reduce
35 the acrylamide content in deep-fat fried potato crisps. This preliminary study gives important indications

36 regarding the possibility of combining a PEF pre-treatment on raw potato slices with subsequent industrial
37 processing steps for the production of potato crisps with low acrylamide concentration.

38

39 **Keywords:**

40 Potato crisps; Acrylamide; electroporation; mass transfer; colour, texture

41

42 **1. Introduction**

43 Acrylamide has been identified as a contaminant in a range of fried and oven-cooked foods (e.g. French
44 fries, potato crisps, bread and cereal) and drinks (e.g. coffee); and its classification as probably carcinogenic
45 in humans has caused worldwide concerns (International Agency for Research on Cancer, 2014). Although
46 most epidemiologic studies examining the relationship between estimated dietary consumption of acrylamide
47 and specific cancer resulted inconclusive, experimental animal studies identified neurotoxicity,
48 carcinogenicity, adverse effects on male reproduction, as possible critical endpoints for acrylamide toxicity
49 (European Food Safety Authority, 2015).

50 Certain foods, more specifically certain food components such as asparagine and reducing sugars, could
51 lead to the formation of acrylamide during heat treatment at temperatures above 120 °C as a result of the
52 Maillard reaction (Mottram, Wedzicha, & Dodson, 2002). Among fried carbohydrate-rich foods, potato crisps
53 contribute to a substantial proportion of the estimated intake of acrylamide in the European adult population
54 (European Food Safety Authority, 2015). Statistical data revealed that in Europe the consumption of salty
55 snacks, in particular potato chips/crisps, stands to an average of 1.5 kg per capita in 2018 (Statista, 2018).

56 National authorities, together with research institutes and food industries reported many strategies of
57 controlling and minimizing the formation of acrylamide, with particular concern to fried potato-based
58 products, due to the presence of large concentration of acrylamide precursors in the potato. In fact, levels up
59 to 4000 ppb of this contaminant have been detected in potato crisps (Becalski, Lau, Lewis, & Seaman, 2003).

60 Recently, the Commission Regulation (EU) 2017/2158 of 20 November 2017 has established new
61 “mitigation measures and benchmark levels for the reduction of the presence of acrylamide in foods”, which
62 aim to ensure that food businesses put in place steps to mitigate acrylamide formation (European Union, 2017).
63 Powers, Mottram, Curtis, & Halford (2017) analysed European manufacturers’ data on acrylamide in potato
64 crisps from 2002 to 2016, and the study showed that, even though acrylamide levels in potato crisps in Europe
65 have been levelled off in recent years, more than 5% of samples exceeded the regulated benchmark level for
66 potato crisps (750 ng g⁻¹).

67 Several investigations have proposed mitigation ways to be applied at different stages of the manufacturing
68 process of potato crisps, in order to reduce the concentration of acrylamide precursors. Among conventional
69 mitigation strategies, hot water blanching of potato slices appeared to facilitate the extraction of Maillard
70 reaction substrates, in addition to enzyme inactivation, also improving the colour uniformity and texture, and
71 oil absorption reduction (Pedreschi, Kaack, & Granby, 2004; Mestdagh et al., 2008). However, this thermal

72 pre-treatment presents the drawbacks of being time-intensive, high energy-consuming and of promoting
73 considerable modifications of sensorial properties of final product.

74 Recent studies have proposed alternative non-thermal technologies in food processing and preservation.
75 Pulsed electric fields (PEF) have been widely described as one of the most promising non-thermal novel
76 technologies in the last decades, stimulating intensive research in several fields such as biotechnology,
77 medicine and food processing. PEF treatment is based on the application of an external electric field applying
78 short and intensive electric pulses. The application of PEF for potato snacks pre-treatment has been extensively
79 studied, and many researchers have already reported high numbers of benefits that could be achieved by
80 applying electric pulses to raw potatoes. Fauster et al. (2018) have recently described the impact of PEF
81 treatment on potato structure and various potential advantages on quality and economic aspects of industrial
82 French fries production, including reductions of cutting force, starch loss and oil uptake. Furthermore, the
83 application of pulsed electric field treatments above a specific critical value of field strength is well known to
84 enhance mass transfer from plant tissues, increasing the cell membrane permeabilization (Donsi, Ferrari, &
85 Pataro, 2010). Following this principle, Jaeger, Janositz, & Knorr (2010) have stated that PEF treatment of
86 raw potatoes could assist and increase the release of sugars and amino acids that represent the main substrates
87 for the Maillard reaction, consequently leading to formation of lower amounts of acrylamide. Moreover,
88 Janositz, Noack, & Knorr (2011) have reported a significant increase in the release of reducing sugars
89 (fructose, glucose) and sucrose in potato slices after PEF application.

90 However, the effective reduction of acrylamide content in deep-fat fried potato products is still unclear,
91 although many researchers observed a significant increase of acrylamide precursors extractability on PEF
92 treated potatoes, reducing browning during frying (Ignat, Manzocco, Brunton, Nicoli, & Lyng, 2015). Besides,
93 lab-scale investigations followed different trials' schemes, applying PEF as a pre-treatment of whole potato
94 tubers before or after peeling, concentrating on the treatment itself, with low attention to the combination of
95 other process operational units that could influence the outcome. It is necessary to understand how the rate of
96 mass transfer promoted by applying certain electric field strengths could be influenced by other processes
97 operations, and how the quality of the final product will be preserved.

98 This preliminary study aimed to evaluate the effect of the application of PEF as a pre-treatment, and the
99 effect of time modulation of subsequent washing steps of treated potato slices, on the Maillard reaction
100 substrates and final acrylamide content, and on the quality of fried potato crisps. Measuring the degree of cell
101 disintegration index (p_o) (Angersbach, Heinz, & Knorr, 1999) and the changes in water electrical conductivity
102 during washing of potato slices, it is possible to optimise either PEF protocol and sample preparation scheme,
103 in order to maximise the release of acrylamide precursors from the raw tissue. The efficiency of PEF as a non-
104 thermal pre-treatment on the acrylamide reduction was compared to a conventional blanching in hot water
105 usually used as a pre-treatment in the fried potato industrial lines.

106

107 **2. Materials and Methods**

108

109 2.1 Sampling

110 Potato tubers (*Solanum tuberosum L.*) of the Lady Claire variety (suitable for industrial processing), were
111 purchased at a local market one month after harvesting and stored in the dark at 10 ± 2 °C for a maximum of
112 two weeks before trials. The storage temperature was chosen according to Pinhero et al. (2009). The initial
113 moisture content of potato tubers was 81.92 ± 0.58 %, evaluated by drying 5g of fresh potato tissue in a
114 convection oven at 105 °C until a constant weight was achieved. Before pre-treatment, only tubers of a similar
115 size and shape were selected, manually peeled and sliced (1.5 ± 0.2 mm in thickness) using a stainless-steel
116 electric slicer machine (Mod. KAFPL0922N CAD, Italy). Both whole tubers and slices were rinsed for 1 min
117 in tap water (water temperature: 18 ± 2 °C) and subsequently submitted to blanching or PEF treatment,
118 followed by washing as better detailed in section 2.4.

119

120 2.2 Pre-treatments

121

122 2.2.1 Blanching

123 Blanching was performed by immersing potato slices in hot distilled water at 85 °C and for 3.5 min stirring
124 with a product-to-water ratio of around 1:2 (w/w), according to the method used by Pedreschi et al. (2011).
125 The use of distilled water, according to the same authors, allowed to maximise the diffusion of acrylamide
126 precursors from potato tissue. Hence, although the use of distilled water is not industrially relevant, in this
127 study distilled water blanching was chosen in order to compare pulsed electric fields achievements with the
128 best performances of blanching.

129

130 2.2.2 Pulsed electric fields (PEF) treatment

131 PEF pre-treatments were performed using a lab-scale PEF unit delivering a maximum output current and
132 voltage of 60 A and 8 kV, respectively (Mod. S-P7500, Alintel, Italy). The generator provides monopolar
133 rectangular-shape pulses and adjustable pulse duration (5-20 μ s), pulse frequency (50-500 Hz) and total
134 treatment time (1-600 s). The treatment chamber (50 mm length x 50 mm width x 50 mm height) consisted in
135 two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. Output voltage and current were
136 monitored using a PC-oscilloscope (Picoscope 2204a, Pico Technology, UK). Samples were treated at room
137 temperature in tap water, with an initial electrical conductivity of 542 ± 2 μ S cm^{-1} at 25 °C, measured using
138 an EC-meter (Mod. Basic 30, Crison, Spain). Trials were conducted delivering $n = 1000$ pulses at fixed pulse
139 width (10 ± 1 μ s), frequency (100 Hz) and repetition time (10 ± 1 ms). An electric field strength of 1.5 kV
140 cm^{-1} was selected in order to achieve irreversible electroporation (Faridnia et al., 2015). Temperature changes
141 due to PEF treatments were negligible.

142 Fig. 1 shows a schematic diagram of the PEF apparatus used. The same PEF protocol was applied on
143 both whole tubers (Fig.1 A) and potato slices (Fig.1 B), in order to understand if the different exposed surface-
144 to-volume ratio could have affected the efficiency of mass transfer. In both cases, the treatment chamber was
145 filled with a product-to-water ratio of around 1:5 (w/w).

146

147 2.3 Determination of cell disintegration index (p_o)

148 Cell disintegration index (p_o) was analysed, according to Angersbach, Heinz, & Knorr (1999), for PEF
149 treated whole tubers and slices. The method is based on changes in the electrical properties of an intact and
150 permeabilized biologic membrane (considered equal to a resistor and a capacitor). The electrical conductivity
151 (σ) for intact and processed samples was obtained by impedance measurements at low (1 kHz) and high (5
152 MHz) frequencies. The impedance spectra were acquired using a precision impedance meter (Mod. LCR-
153 8105G, GW Instek, Taiwan) connected to a parallel plate measuring cell with adjustable gap. The cell
154 disintegration index (p_o) was calculated by the following equation:

155

$$156 \quad p_o = \frac{(\sigma_h^i / \sigma_h^s) \sigma_l^s - \sigma_l^i}{\sigma_h^i - \sigma_l^i} \quad (1)$$

157

158 where σ_l^i and σ_l^s indicate the electrical conductivity of untreated and treated cell material at low frequency (1
159 kHz), respectively; and σ_h^i and σ_h^s indicate the electrical conductivity of untreated and treated material at high
160 frequency (5 MHz), respectively. The parameter p_o ranges from 0 for intact tissue to 1 for complete disrupted
161 tissue.

162

163 2.4 Washing in water

164 Potato slices, either untreated or subjected to PEF or blanching were soaked and stirred in tap water (18
165 ± 2 °C), with an initial electrical conductivity of $319 \pm 4 \mu\text{S cm}^{-1}$ at 25 °C, and with a product-to-water ratio
166 around 1:1.5 (w/w). In order to select the optimal soaking time as a result of maximum release of intracellular
167 compounds into the external aqueous phase due to PEF-induced electroporation, washing times of 5, 10, 15,
168 20 min were tested on PEF-processed potato slices before frying. For each dipping time, changes in water
169 electrical conductivity were registered using an EC-meter (Mod. Basic 30, Crison, Spain). The selected
170 washing times, based on the highest water electrical conductivity variation recorded, were applied also to
171 untreated (control) and blanched potato slices before frying, in order to obtain comparable results for further
172 analysis.

173

174 2.5 Frying conditions

175 Untreated (control), blanched and PEF-treated potato slices were deep-fried in high-oleic sunflower oil
176 using an electrical fryer (Mod. MFR280R, Fama Industrie, Italy) at 175 °C as initial oil temperature. Potato-
177 to-oil weight ratio was around 1:10 and slices were fried for 3 min until a final moisture of ~2% (wet basis)
178 was reached. Temperatures of frying oil and frying potatoes were monitored using thermocouples sensors type
179 K connected to a data logging system (Mod. 9211A, National Instruments™, Texas).

180

181 2.6 Acrylamide precursors in raw potatoes

182

183 2.6.1 Reducing sugars analysis

184 The concentrations of glucose and fructose in raw potatoes were quantified using the method described
185 by Rodriguez-Saona et al. (1997) with few modifications. A 2g sample of freeze-dried potato slices was
186 dissolved in 20 ml of distilled water using ultrasonic bath (Elmasonic, Germany). The sample was centrifuged
187 (Centrifuge Thermo Electron, USA) 15 minutes at 3500 rpm, and the supernatant was collected. A 1 ml aliquot
188 of the solution was passed through C18 cartridges (1000mg, 6mL; Phenomenex) for purification.
189 Subsequently, the sample was resuspended in 0.25 ml of deionised distilled water before injection into HPLC.
190 Glucose and fructose were determined with HPLC Agilent Infinity 1260 (Agilent Technologies, Santa Clara,
191 CA, USA) coupled to ELSD PL-ELS 1000 (Agilent, Santa Clara, CA, USA) as detector. The analytical column
192 was a SphereClone NH2 (250 mm x 4.60 mm i.d.; 5 µm particle size) (Phenomenex, Torrance, CA, USA); the
193 elution was in isocratic mode using a mixture of water:acetonitrile 70:30 (v/v) as mobile phase at a flow rate
194 of 0.6 mL/min. The sample injection volume was 10 µL. All samples were analysed in triplicate.

195

196 2.6.2 Asparagine analysis

197 The concentration of free asparagine in raw potatoes was quantified dissolving 5g sample of freeze-dried
198 potato slices in 50 ml of distilled water using ultrasonic bath (Elmasonic, Germany) for 10 min. The sample
199 was centrifuged (Centrifuge Thermo Electron, USA) 15 minutes at 3500 rpm, the supernatant collected, micro-
200 filtered (0.22 µm) before HPLC analysis. Free asparagine was determined with HPLC Agilent Infinity 1260
201 (Agilent Technologies, Santa Clara, CA, USA) coupled to a diode array detector (UV wavelength set at 338
202 and 262 nm). In order to obtain the derivatization sample, a 200 µL sample was added to 400 µL borate buffer
203 with 50 µL o-phthalaldehyde-3-mercaptopropionic acid (OPA) reagent. Chromatographic conditions are
204 described in Plata-Guerrero et al. (2009). All samples were analysed in triplicate.

205

206 2.7 Analysis of fried potato crisps

207

208 2.7.1 Computer Vision System (CVS) for colour determination

209 The surface colour of potato crisps was measured using a Computer Vision System (CVS) consisting of
210 an illumination source, a colour digital camera (CDC), and an image processing software. Potato crisps
211 samples were placed inside a dark box to exclude external light, and RGB images were acquired by a CDC
212 (Mod. D7000, Nikon, Japan) with a 105 mm lens (Mod. AF-S Micro Nikkor), located vertically over the
213 sample at a distance of 35 cm and connected to a PC. The lighting system consisted of four daylight fluorescent
214 lamps (60 cm in length) connected to an electronic ballast to ensure uniform illumination, with a colour
215 temperature of 6500 K and sited at an angle of 45° with the CDC. For each sample, untreated, blanched and
216 PEF treated, 12 images were captured, each of one side of potato crisps. The pre-processing of RGB images,
217 segmentation and colour quantification were performed with ImageJ analysis software (NIH, USA). The

218 average value of the segmented pixels in the CIE L* a* b* colour space was registered as the colour of the
219 sample. From numerical values of a* (green/red) and b* (yellow/blue) chromatic parameters, hue angle (h°)
220 was calculated by the following equation and used to describe colour variations between samples:

$$222 \quad h^\circ = \tan^{-1}(b^*/a^*) \quad (2)$$

224 2.7.2 Texture

225 Texture analysis of crisps were performed at room temperature (~20 °C) using a Texture Analyser TA-
226 XT2 (Mod. HDi 500, Stable Micro System, Surrey, UK) equipped with a 5 kg load cell. A puncture test was
227 selected to evaluate samples firmness and crispness. Crisp samples selected on the basis of uniform size and
228 shape, were placed on a support rig (HDP/CFS, Crisp Fracture Support Rig and corresponding Heavy Duty
229 Platform) and compressed for 3 mm distance using a spherical probe (P/0.25S) of ¼ - inch diameter (Salvador,
230 Varela, Sanz, & Fiszman, 2009). Force vs distance curves were obtained using a test speed of 1.0 mm s⁻¹ and
231 the results obtained from 12 slices for each sample were expressed as firmness, calculated by means of
232 maximum force values and as crispness, calculated from means of linear distance (the length of a line joining
233 all fracture points in the force-deformation curve) between the first and the last fracture peaks registered.

235 2.7.3 Acrylamide

236 *Sample extraction and SPE purification.* Potato chips samples were finely ground before the extraction;
237 1 g of sample was weighted into a polypropylene conical tube, and 100 µL 10µg/mL internal standard solution
238 (¹³C₃-labelled acrylamide in MeOH) followed by 10 mL 0.1% (v/v) formic acid were added. After mixing with
239 Vortex for 10 min, the extract was centrifuged at 4500 rpm for 15 min. A 2 mL portion of clarified solution
240 was removed, avoiding collection of top oil layer when present, and filtered through paper filter. A solid-phase
241 extraction (SPE) was performed using C18 cartridges (1000mg, 6mL; Phenomenex). Cartridges were first
242 conditioned with 5 mL methanol followed by 5 mL water; 1 mL of filtered sample was loaded and washed
243 with 1 mL water. Elution was performed with 1 mL acetone. Acetone was removed under nitrogen flow and
244 sample was dissolved in 1 mL 0.1% (v/v) formic acid before injection. SPE clean-up was performed on 3
245 extracts for each sample.

246 *HPLC-ESI-MS/MS analysis.* LC-ESI-MS/MS in positive ion mode (ESI⁺) analyses were performed by an
247 Agilent 6420 triple quadrupole (Agilent, Santa Clara, United States) coupled to an Agilent 1290 Infinity LC
248 Pump equipped with an autosampler and a thermostated column oven, according to Calbiani, Careri, Elviri,
249 Mangia, & Zagnoni (2004) with some modifications. The analytical column was a Poroshell 120 C18, 3.0 x
250 100 mm, 2.7 µm (Agilent, USA) maintained at 20 °C. The elution was in isocratic mode using a mixture of
251 0.1% (v/v) aqueous formic acid and methanol (99.5/0.5, v/v) as mobile phase at a flow rate of 0.3 mL/min.
252 The sample injection volume was 10 µL. Full-scan analyses were performed in the 40-100 Da mass range,
253 acquiring the following transitions: extracted ion at m/z 55, due to the transition 72 > 55, and at m/z 58, due
254 to the transition 75 > 58 were used for the quantitative analysis. A calibration curve was made for the

255 quantification diluting stock solution of acrylamide with water in the 1.5-200 µg/L range. For acquisition and
256 processing data, the Agilent MassHunter Workstation software was used.

257

258 2.8 Statistical analysis

259 Significant differences between results were calculated by paired samples Student's t-test, parametric
260 analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95% ($p < 0.05$).
261 If Shapiro-Wilk test for normality and Levene's test for homoscedasticity of data resulted statistically
262 significant ($p < 0.05$), non-parametric multiple range test Kruskal-Wallis and Holm stepwise adjustment were
263 used, with a significant level of 95% ($p < 0.05$) (R Foundation for Statistical Computing, Austria). All
264 treatments were conducted in triplicate and results were expressed as mean \pm standard deviations of
265 replications.

266

267 3 Results and discussion

268

269 3.1 Experimental design set up

270

271 3.1.1 Effect of PEF treatment on cell disintegration index and degree of mass transfer

272 As reported by Angersbach et al. (1999), impedance measurements of plant tissues allow the evaluation
273 of cell membrane permeabilization after applying PEF treatments.

274 To understand the efficiency of selected PEF protocol on the degree of cell disruption, and so on the level
275 of mass transfer, cell disintegration index of PEF-treated potato tubers and potato slices was calculated from
276 Eq. 1. Results are shown in Fig. 2; as expected, higher surface dimension exposed to electric pulses, being
277 related to slices, resulted in higher cell disintegration, explaining the better efficiency of the pre-treatment if
278 applied after the slicing step of the experimental scheme.

279 It is well established that measurements of the changes in electrophysical properties of untreated and
280 treated cell tissues represent a reliable method to correlate PEF processing protocol and the cell damage degree
281 in biological systems. Moreover, it has been widely reported that PEF-induced membrane permeabilization
282 has the potential to effectively enhance mass transfer from the inner part of biological tissues, increasing the
283 diffusion of cell compounds/metabolites (Donsi et al., 2010).

284 Another useful method to assess the intactness of cell membranes, reported by many researchers, is the
285 measurement of ions/small molecules leakage from intracellular compartments. Washing of potato slices is a
286 common practice in potato crisps production and allows to remove any surface starch residue prior to frying.
287 In this work, different washing times of PEF-treated potato slices were evaluated, measuring the changes in
288 electrical conductivity of residual washing water. Results are shown in Fig. 3; the maximum water conductivity
289 variation was achieved after 5 min of washing, highlighting the period of time subsequent to PEF-treatment
290 that permitted the highest release of cell fluid into the aqueous media.

291 Faridnia et al. (2015) reported that by suspending a PEF-treated potato tuber into an isotonic solution of
292 mannitol (0.2 M) it was possible to monitor the electrolytes leakage from cell tissue by measuring changes in
293 electrical conductivity of the surrounding media. Furthermore, it has been demonstrated that cell fluid leakage
294 due to electroporation is function of time, as many transition processes induced by PEF (e.g. moisture and air
295 redistribution among microscopic extracellular channels, mass transfer, partially or completely resealing of
296 cell membranes) could last from seconds to hours (Oey et al., 2017). On the basis of this concept, it is clear
297 that subsequent unit operations for potato crisps manufacturing need to be assessed in order to maximise mass
298 transfers.

299 Thanks to the aforementioned introductory studies, in this work the experimental plan for the production
300 of lab-scale deep-fat fried potato crisps was designed applying the different pre-treatments on raw potatoes
301 directly after slicing and by selecting 5 min as the preferred time for potato slices washing step. A scheme with
302 the experimental processing steps is shown in Fig. 4.

303

304 3.2 Deep-fat fried potato crisps analysis

305

306 3.2.1 Colour

307 Colour is one of the most important parameters to control during frying, being strongly related to
308 consumer perception (Scanlon, Roller, Mazza, & Pritchard, 1994). Visual quality is associated with physical,
309 chemical and sensorial evaluation and it is an important driver for buying being associated by consumers with
310 flavour, safety, storage time, nutritional aspects and taste (Pedreschi, Kaack, & Granby, 2006a).

311 Colour of potato crisps is often measured using a colorimeter in $L^*a^*b^*$ units. According to Pedreschi,
312 León, Mery, & Moyano (2006b), the use of a computer vision system (CVS) technique instead of the
313 conventional colorimeter for monitoring the development of colour in potato crisps has different advantages,
314 such as the possibility to analyse the whole surface of the product and to identify the presence of brown spots
315 and other defects.

316 Fig. 5 shows images of control (5A), blanched (5B) and PEF treated (5C) potato crisps after frying. After
317 blanching and PEF treatment, changes are noticeable in the appearance of potato slices compared to the control.
318 The slices pre-treated by PEF showed a more uniform and lighter surface colour. Images have been converted
319 from RGB into $L^*a^*b^*$ channels. The calculated values of L^* and h° (Eq. 2) of the three samples are reported
320 in Fig. 6.

321 Development of colour during frying is the result of the Maillard reaction, that involves reducing sugars
322 and the amino acid asparagine and has been related to the formation of toxic compounds such as acrylamide.
323 The extent of the Maillard reaction depends on the presence of reaction substrates and on frying parameters
324 such as temperature and time (Romani et al. 2008; Romani et al. 2009).

325 The evolution of colour during frying is generally indicated by a decrease in L^* and/or an increase of the
326 redness parameter (a^*) and of the hue (h°). Variation of colour parameters observed in the present study
327 allowed the discrimination among samples. The three samples did not show significant differences in terms of

328 L*. On the other side, hue values increased for both pre-treated samples compared to the control, the highest
329 values being related to the PEF pre-treated sample. Ignat et al. (2015) found similar results comparing the
330 colour development during frying of blanched and PEF-treated potato slices. The increase of hue values
331 testified a change from red to yellow colour, indicating the decrease of non-enzymatic browning reactions
332 entity. Pre-frying treatments, such as blanching or dipping, are steps generally used in potato snacks
333 manufacturing to stabilise the colour (Pedreschi et al., 2004). In fact, hot water blanching of raw potatoes could
334 ease the leach out of superficial reducing sugars, main substrates or Maillard browning reactions. Moreover,
335 the evolution of potato chips colour during frying has shown a good correlation ($R^2 = 0.9569$) with the
336 acrylamide concentration, as reported by Pedreschi et al. (2005). Lighter and less red chips are related to a
337 lower concentration of non-enzymatic browning reactions substrates and so to a lower content of acrylamide.
338 The possibility of the release of the Maillard substrates from potato tissue treated with PEF has been already
339 observed by various authors (Jaeger et al., 2010; Janositz et al., 2011). The increase of mass transfer upon PEF
340 treatment is due to the effect of electroporation and cell permeabilization, that allow an increasingly diffusion
341 of intracellular components across the membranes, as confirmed by the increase of conductivity of the washing
342 water observed above.

343

344 3.2.2 Firmness & crispness

345 Texture is one of the main characteristics that influence the sensorial properties of potato-based products,
346 and a delicate and crispy texture is recommended for potato crisps (Kita, 2014). Texture changes in potato
347 crisps during frying result in the initial tissue softening and further crust development (Pedreschi, Moyano,
348 Santis, & Pedreschi, 2007).

349 The changes of firmness and crispness of untreated, blanched and PEF treated potato crisps subjected to
350 frying are shown in Fig. 7A and Fig. 7B, respectively. A slight, but significant reduction of both parameters
351 was observed for blanched and PEF treated samples in comparison to the untreated one.

352 Structural changes of potato crisps could be influenced by many factors, e.g. firmness depends on the
353 degree of starch gelatinization, on changes in the cell walls structure, mainly related to an increase in their
354 permeability and on the reduction of intercellular adhesion between neighbouring cells (Moyano, Troncoso,
355 & Pedreschi, 2007). Crispness instead is influenced mainly by dry matter content and oil uptake during frying
356 (Abong, Okoth, Imungi, & Kabira, 2011).

357 Blanching at high temperature (80-100 °C), contrary to that at low temperature, has been already reported
358 to promote potato tissue softening, by starch modification (hydration, swelling and gelatinization) along with
359 β eliminative cleavage and pectin solubilization (Botero-Uribe, Fitzgerald, Gilbert, & Midgley, 2017).
360 Moreover, high temperature blanching decreases polyphenol oxidase activity, responsible for enzymatic
361 browning (Bingol et al., 2014).

362 Recently, some studies have been performed showing the effect of PEF pre-treatment on texture and other
363 quality parameters of potato tissue before frying. Fauster et al. (2018) observed softening of the potato tissue
364 and therefore the improvement of cutting behaviour (smoother surface and lower feathering). PEF treatment

365 (0.3- 1.2 kV/cm) caused also a significant softening of the ground tissues of sweet potato, resulted into lower
366 force necessary for cutting (Liu et al., 2017). The softening of the tissue upon PEF treatment is probably due
367 to the cell structure modification, mainly the increase in the membrane permeability and the irreversible cell
368 breakdown, that in turn increase the water transfer, which is very important during potato-based product frying
369 (Botero-Uribe et al., 2017). However, there is a lack of information in the literature about the effect of PEF
370 pre-treatment on final products texture. Ignat et al. (2015) observed no differences in texture of PEF treated
371 potato cubes (0.75 kV/cm and 2.50 kV/cm) in comparison to blanched and untreated ones. In our work, PEF-
372 treated samples presented lower firmness and crispness in comparison to the untreated one, while no significant
373 differences were observed between PEF-treated and blanched samples. The discrepancy could be due to the
374 different shape of potato samples, indeed Ignat et al. (2015) performed their study on potato cubes ($2 \times 2 \times 2$
375 cm), while the present work was focused on 1.5 mm potato slices, as well as to different PEF process
376 parameters and frying temperature.

377

378 3.2.3 Maillard reaction substrates and acrylamide content

379 As previously demonstrated, reducing sugars and asparagine represent the main limiting substrates of
380 acrylamide formation in potato products (Amrein et al., 2003).

381 Table 1 shows the glucose, fructose and free asparagine content (mg kg^{-1}) in raw potato slices untreated
382 and submitted to conventional and innovative pre-treatments (blanching and PEF, respectively), all followed
383 by a washing step of 5 min.

384 Free asparagine was found at concentrations more abundant than reducing sugars, and its reduction in
385 treated potato slices were higher than those found for glucose and fructose. In fact, PEF treatment allowed a
386 reduction of 48% of free asparagine compared to the untreated sample, higher than the reduction reached by
387 blanching (40%). Both pre-treatments, blanching and PEF, allowed just a slight reduction of the fructose initial
388 content (4.9% and 5.4%, respectively). No glucose reduction was observed in PEF-treated potato slices; on the
389 contrary the 27% of its reduction was shown in blanched potato samples.

390 It is well established that acrylamide precursors, reducing sugars and amino acids, are leached out by
391 blanching treatment of raw potatoes (Zhang et al., 2018); on the other hand, PEF treatment has been mentioned
392 as a potential alternative method to assist the removing of Maillard reaction substrates, and consequently
393 reducing the acrylamide content in cooked potato-based products (Jaeger et al., 2010). While according to
394 Janositz et al. (2011), PEF promoted a significant decrease in the reducing sugars content, the results of the
395 present study seem to indicate that the main reduction is related to free asparagine.

396 The acrylamide content (mg kg^{-1}) of untreated, blanched and PEF-treated potato crisps is displayed in
397 table 1. For the potato crisps pre-treated by PEF, the acrylamide content appeared lower compared with those
398 pre-treated by conventional blanching. The PEF pre-treatment protocol and experimental conditions applied
399 in this study resulted on a reduction of around 30% of acrylamide content compared to control (untreated),
400 while only around the 17% of reduction was observed on blanched samples compared to control (untreated).
401 Similar results of acrylamide reduction due to hot water blanching of potato slices were previously reported

402 by other authors (Pedreschi et al., 2011). The cell electroporation phenomenon induced by the application of
403 the selected PEF protocol on raw potato slices resulted in a further improvement of the diffusion of Maillard
404 reaction substrates and so of acrylamide reduction in fried potato crisps compared to the applied conventional
405 pre-treatment.

406

407 **4. Conclusions**

408 Overall this preliminary study confirmed the high potentiality of the application of pulsed electric fields
409 as a pre-treatment to improve the release of acrylamide precursors in raw potatoes and so to reduce the
410 acrylamide content in deep-fat fried potato crisps. Moreover, important indications regarding the possibility
411 of industrial application of PEF pre-treatment for the production of potato crisps were given. By monitoring
412 PEF treatment parameters and other manufacturing steps, it was possible to achieve a consistent reduction of
413 acrylamide due to its precursors leaching during the washing step, with only slight modifications of the final
414 quality of the product, in terms of colour and texture.

415 Although PEF pre-treatment led to a significant reduction of acrylamide content in potato crisps if
416 compared to untreated and blanched samples, the final amounts found were still higher than recommended
417 legislative limits (0.75 mg kg^{-1}). In this direction other possible combined strategies need to be developed for
418 industrial applicability. The combination of PEF and a mild blanching of raw potatoes and the monitoring of
419 subsequent manufacturing operational units could enhance the extraction of reducing sugars and free
420 asparagine and consequently the reduction of the acrylamide formation in potato crisps.

421

422

423 **Figure captions**

424 **Fig. 1.**

425 Schematic diagrams of experimental apparatus: (A) whole potato tuber PEF treatment; (B) potato slices PEF
426 treatment.

427

428 **Fig. 2.**

429 Cell disintegration index (CDI) of PEF treated potato slices and tubers ($E = 1.5 \text{ kV/cm}$; $n = 1000$). Results are
430 expressed as means \pm standard deviations (error bars) of $n=20$. Values with different letters differ significantly
431 ($p < 0.05$).

432

433 **Fig. 3.**

434 Variations of water electrical conductivity affected by different washing times of PEF treated potato slices.
435 Results are expressed as means \pm standard deviations (error bars) of $n=5$. Values with different letters differ
436 significantly ($p < 0.05$).

437

438 **Fig. 4.**

439 Scheme of experimental processing steps.

440

441 **Fig. 5.**

442 Examples of RGB images of untreated (a), blanched (b) and PEF-treated (c) potato crisps (left), and image
443 conversion from RGB into L*a*b* channels (right). Pixels areas analysed are highlighted in yellow.

444

445 **Fig. 6.**

446 Lightness (A) and hue angle (B) of untreated, blanched and PEF-treated potato crisps. Results are expressed
447 as means \pm standard deviations (error bars) of n=20. Values with different letters differ significantly ($p < 0.05$).

448

449 **Fig. 7.**

450 Firmness (A) and crispness (B) of untreated, blanched and PEF-treated potato crisps. Results are expressed as
451 means \pm standard deviations (error bars) of n=20. Values with different letters differ significantly ($p < 0.05$).

452

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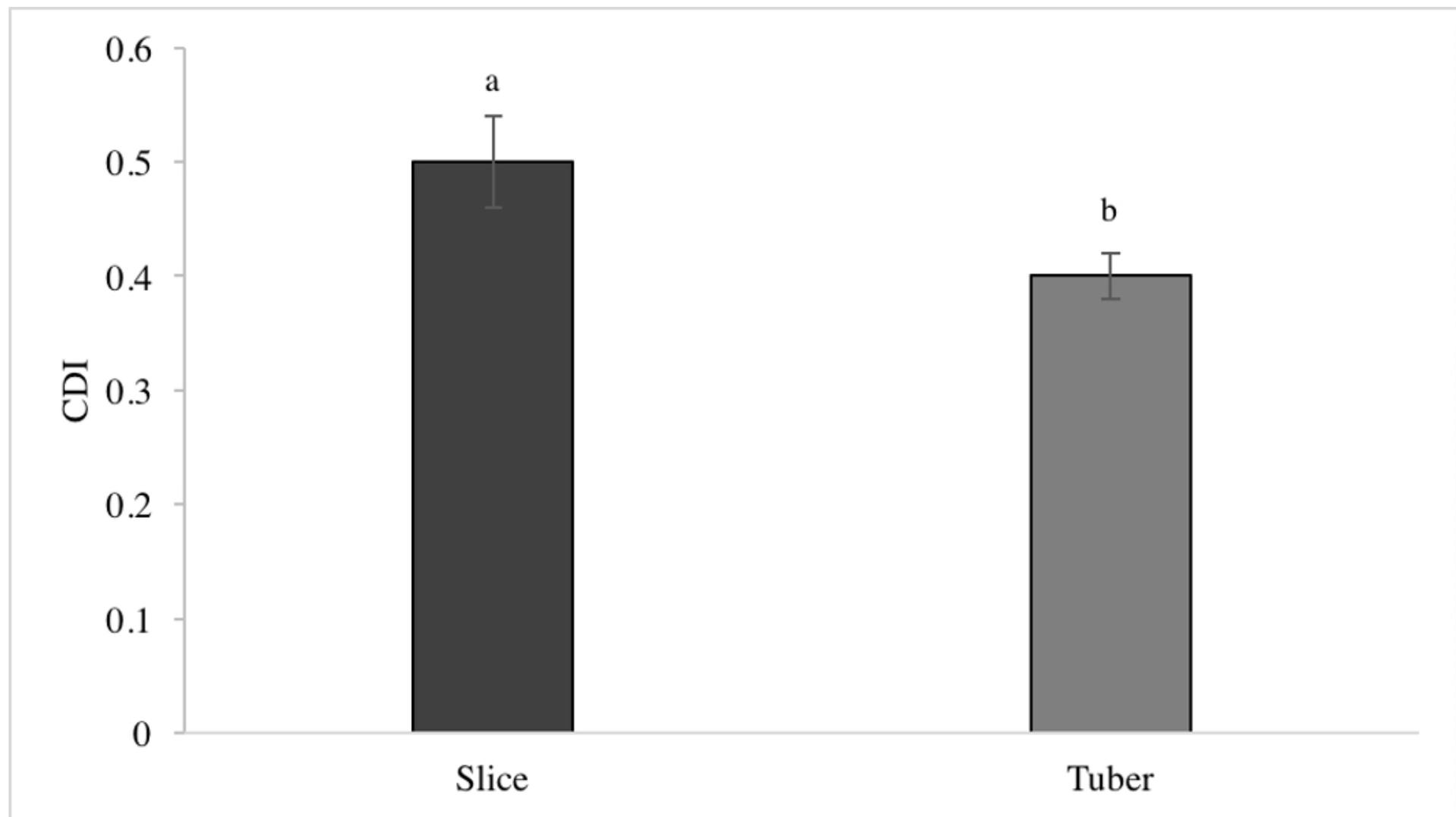
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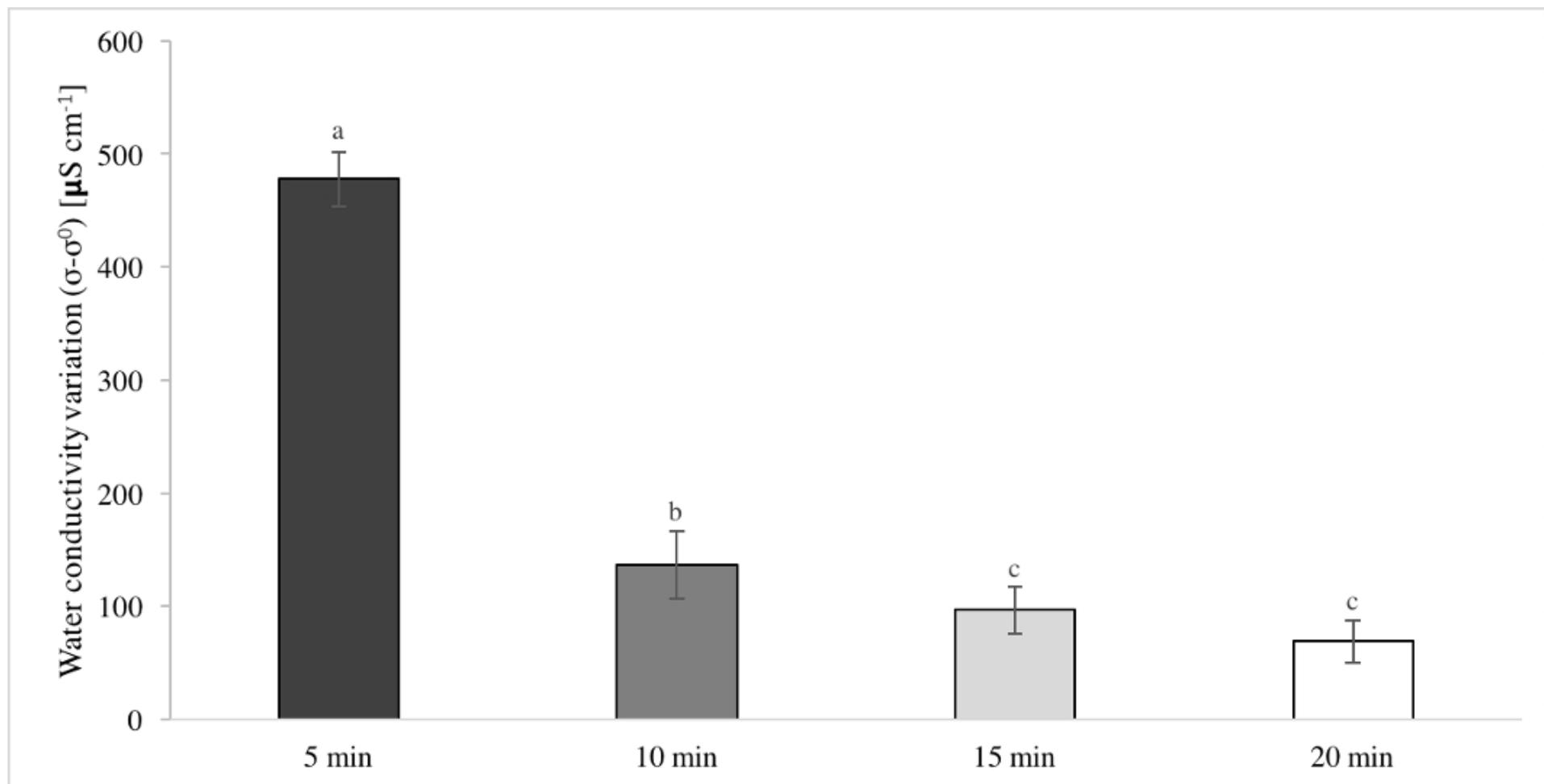
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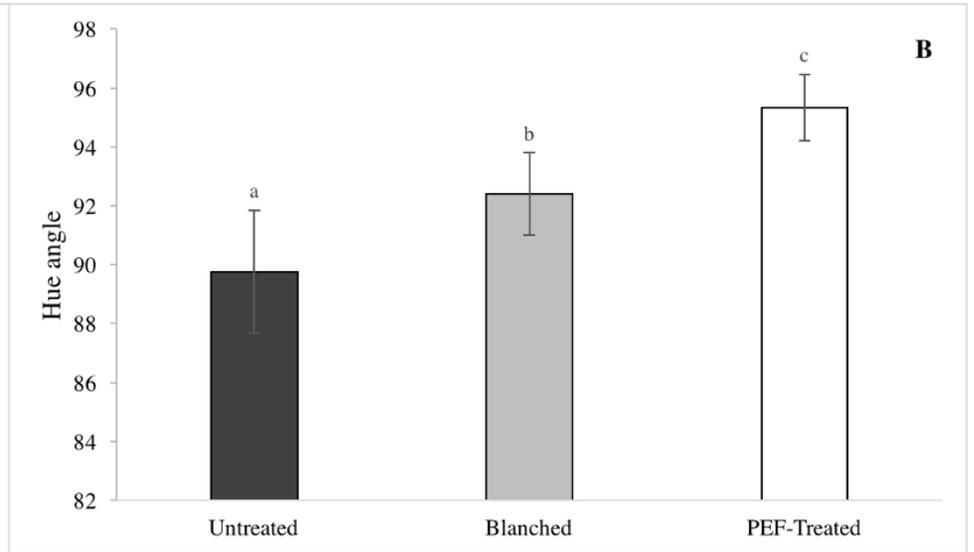
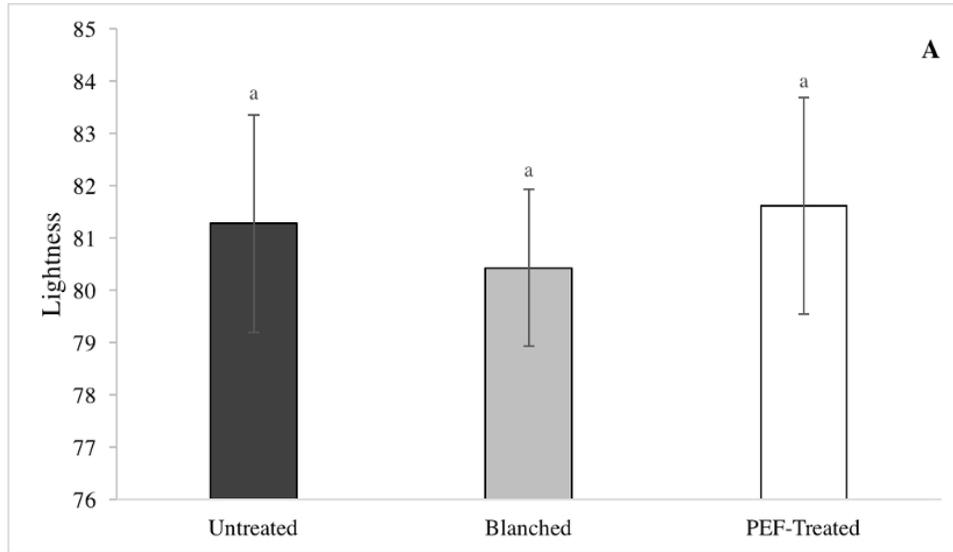
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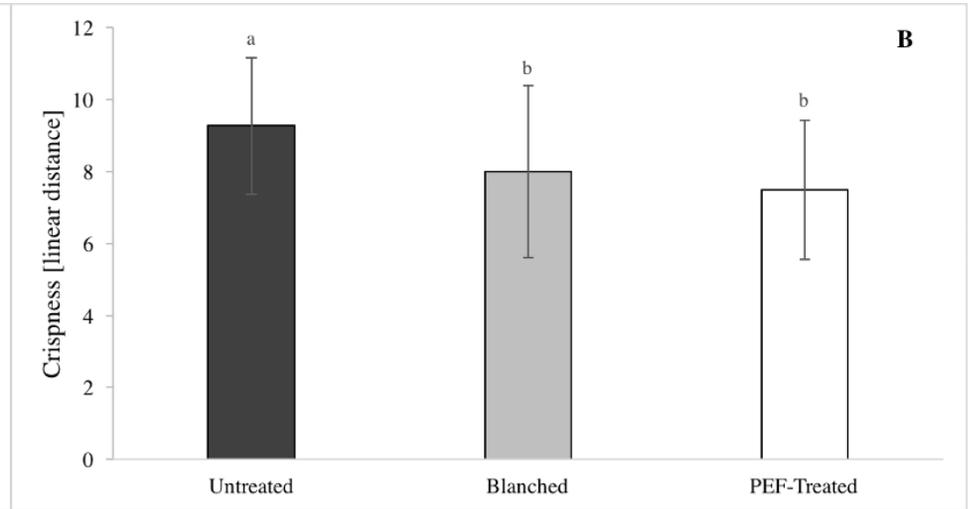
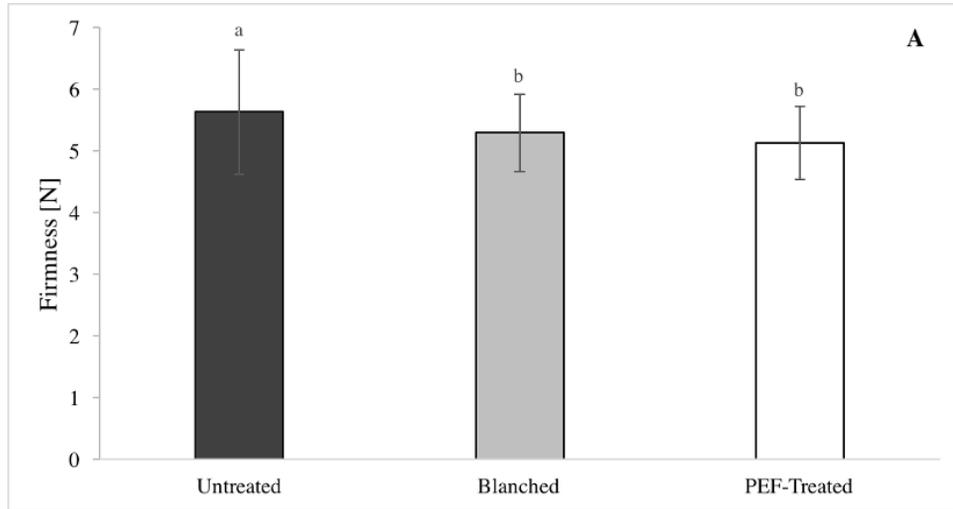
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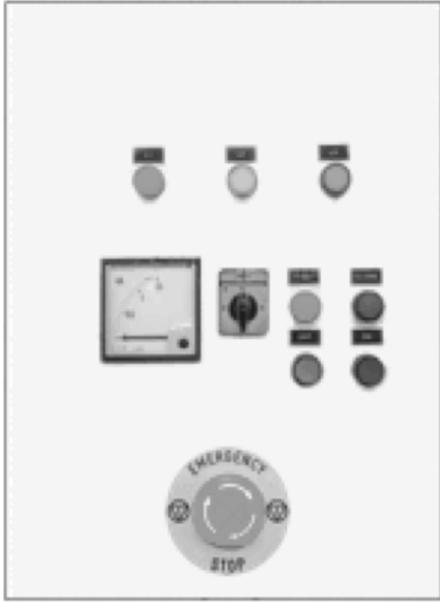








PEF Generator

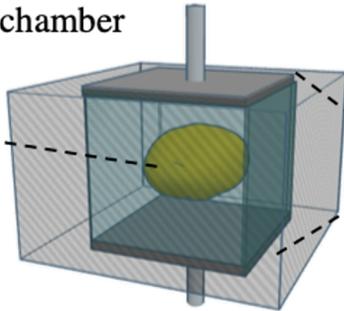


HV

Treatment chamber

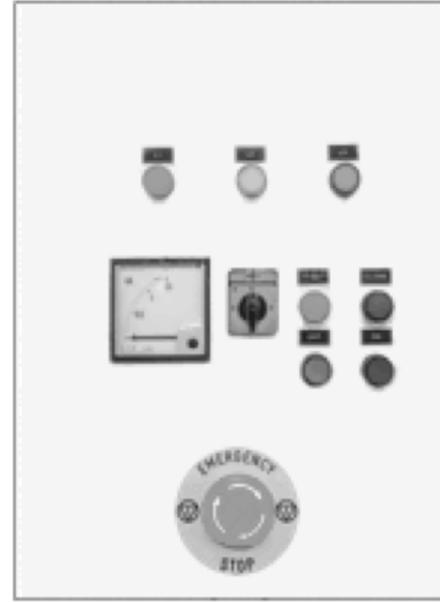
Sample

Electrodes



A

PEF Generator

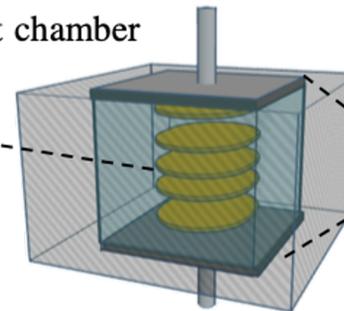


HV

Treatment chamber

Sample

Electrodes



B

1 **Table 1**

2

3 Maillard substrates (glucose, fructose and free asparagine) contents of raw potato slices untreated, after blanching and after PEF treatment, and acrylamide
 4 content of untreated, blanched and PEF-treated potato crisps. Results are expressed in mg kg⁻¹ of dry weight. Reduction percentages are calculated in relation to
 5 the untreated sample.
 6

Sample	Glucose			Fructose			Asparagine			Acrylamide		
	Mean	%RSD*	Reduction (%)	Mean	%RSD	Reduction (%)	Mean	%RSD	Reduction (%)	Mean	%RSD	Reduction (%)
Untreated	70.0	14.1	-	58.9	1.7	-	10487.8	8.7	-	2.0	3.3	-
Blanched	50.9	11.9	27	56.1	3.5	4.9	6296.1	6.2	40	1.6	9.1	17
PEF-treated	75.4	10.6	n.a.**	55.8	3.1	5.4	5416.9	9.3	48	1.4	7.5	31

7

*percent relative standard deviation (n=3)

8

**not applicable

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