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

(Article begins on next page)

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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

Industrial relevance

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

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

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

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

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

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

2. Materials and Methods

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 μs), 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 μS 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 μs), 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).

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   disintagration index (p_o) was calculated by the following equation:

155

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

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   $\pm$ 2 °C), with an initial electrical conductivity of 319 ± 4 µS 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-phtalaldehyde-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:

221

222    $h° = tan^{-1}(b^*/a^*)$ (2)

223

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 facture peaks registered.

234

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   ($^{13}C_3$-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. Eluition 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

7

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 ± 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 (Donsì 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

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

3.2.2 Firmness & crispness

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

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

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

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

Recently, some studies have been performed showing the effect of PEF pre-treatment on texture and other quality parameters of potato tissue before frying. Fauster et al. (2018) observed softening of the potato tissue 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

11

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 kV/cm; n = 1000). Results are

430   expressed as means ± 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 ± 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 ± 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 ± standard deviations (error bars) of n=20. Values with different letters differ significantly ($p < 0.05$).

452

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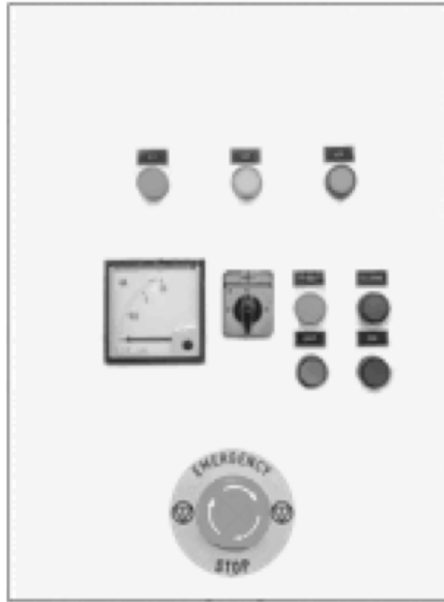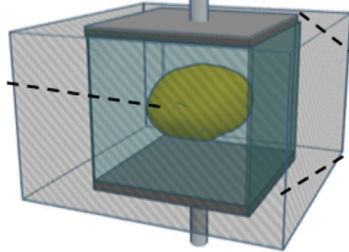
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PEF Generator

HV
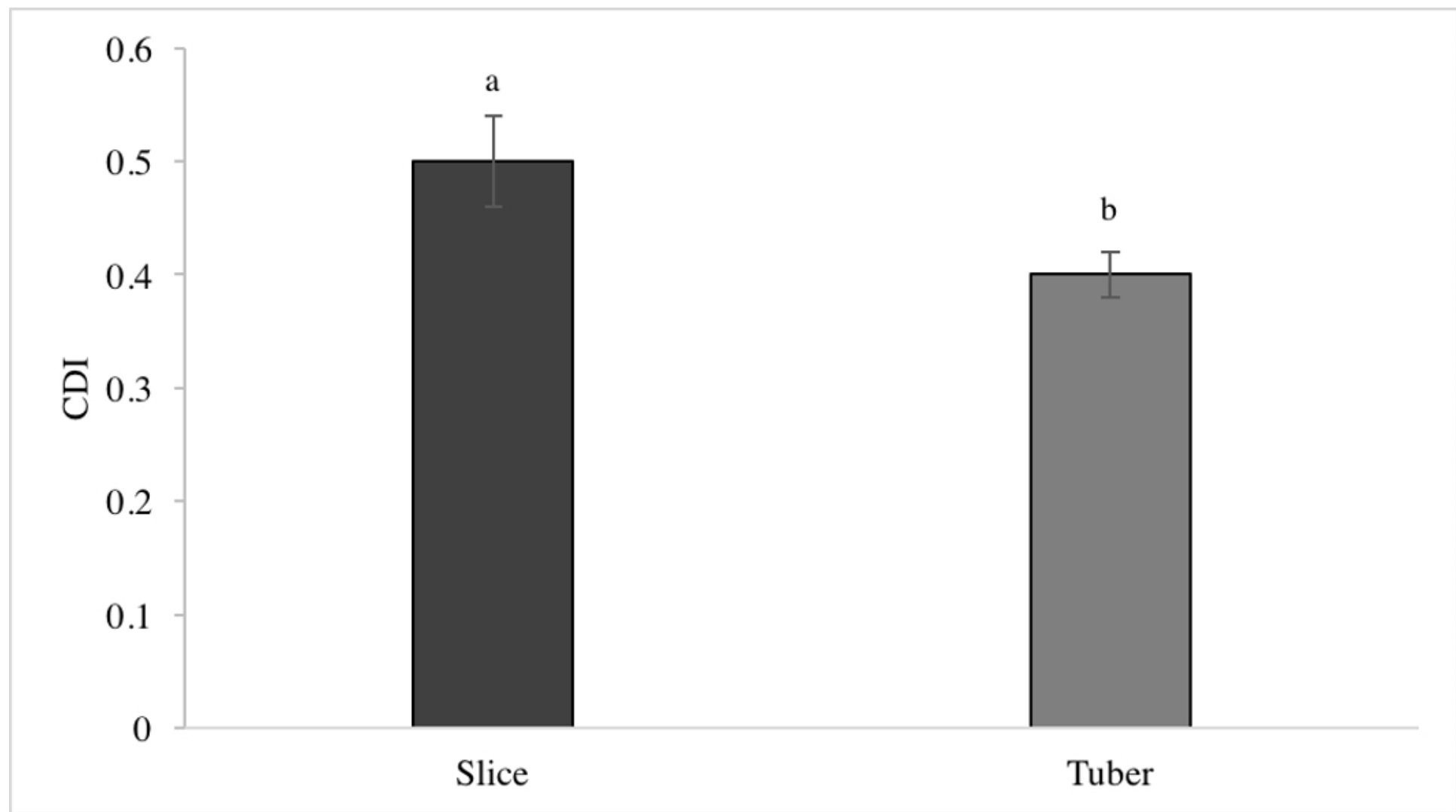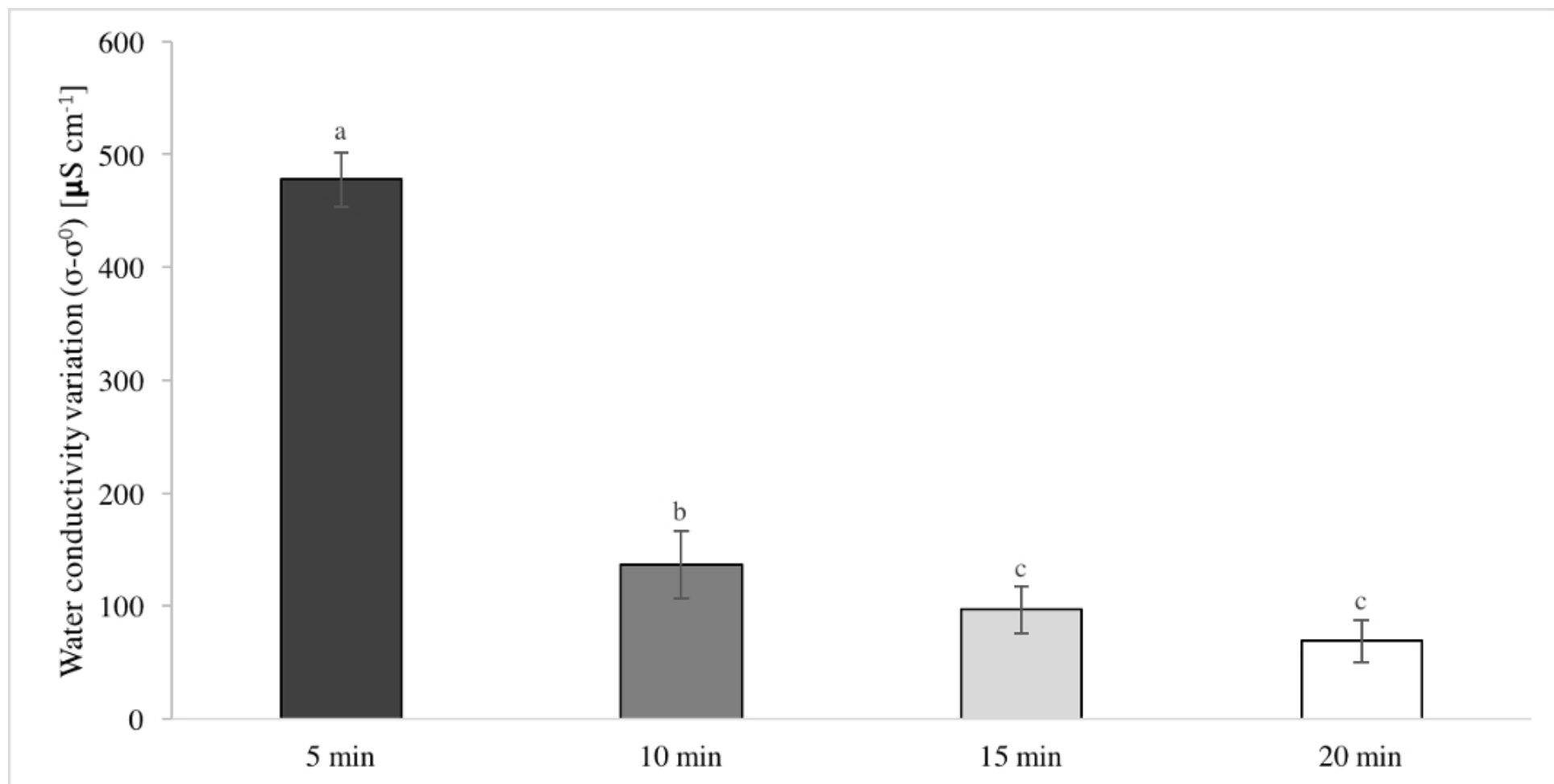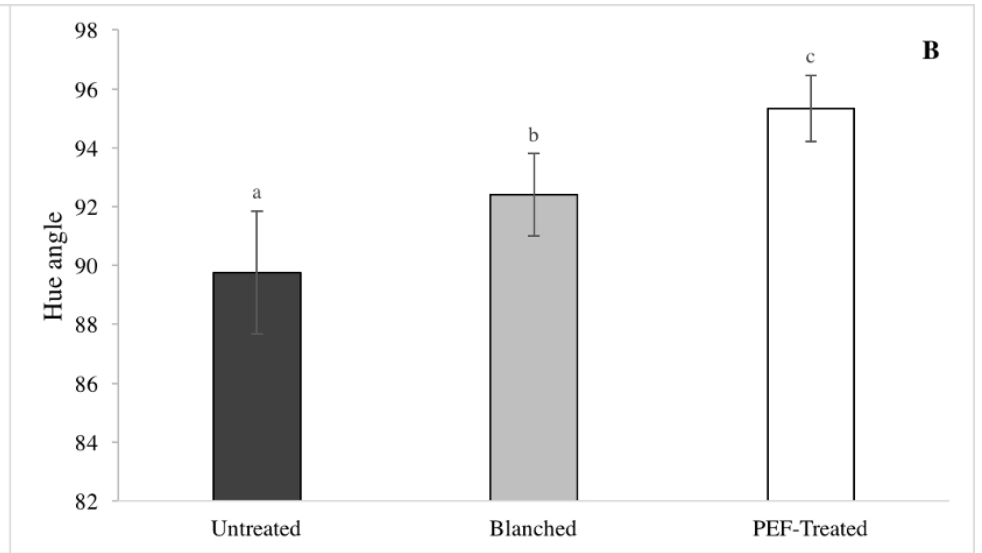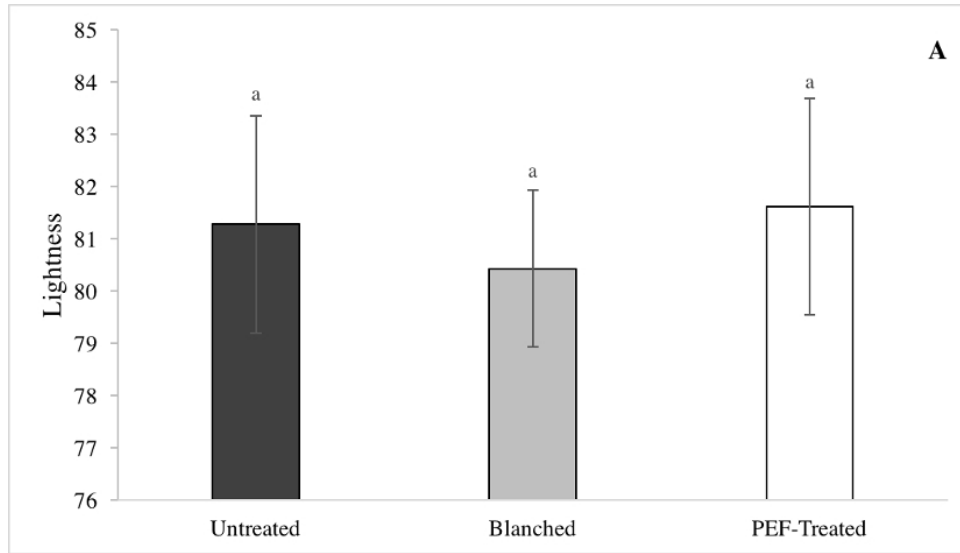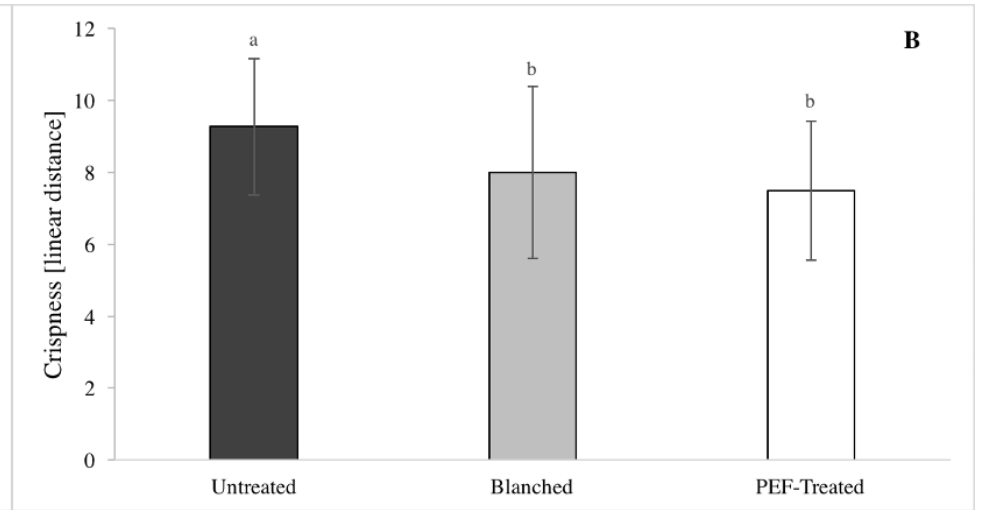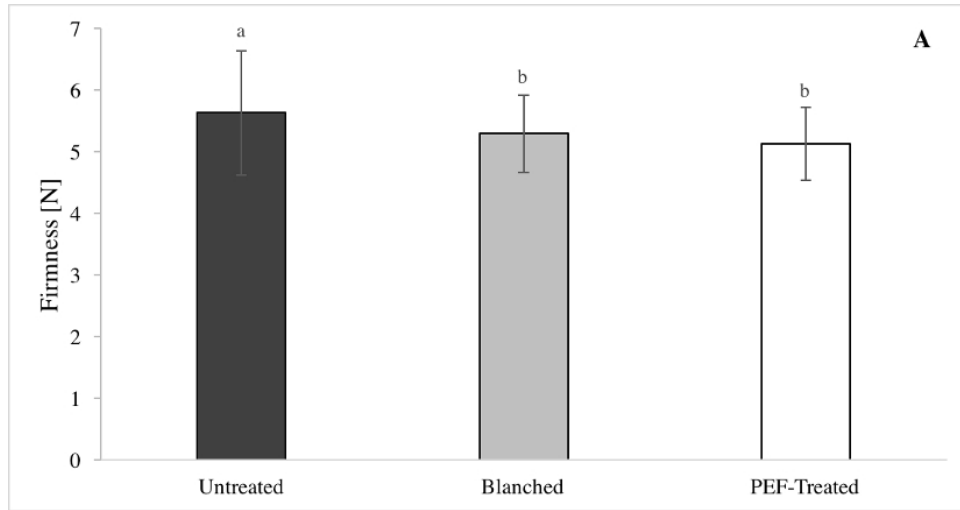
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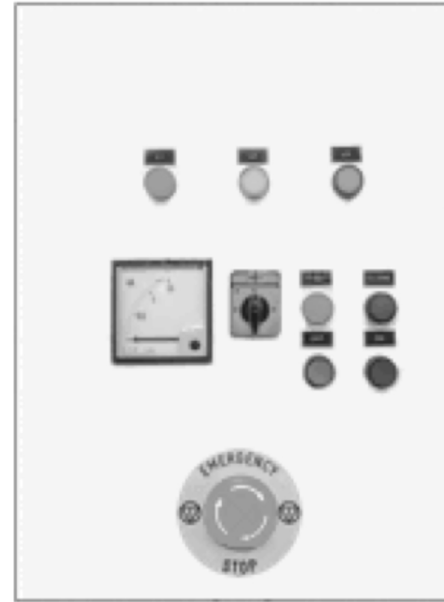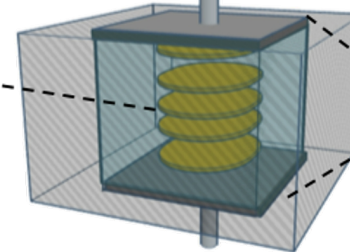
Sample

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
9
10
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14
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