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Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Schouten M.A., Genovese J., Tappi S., Di Francesco A., Baraldi E., Cortese M., et al. (2020). Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips. *INNOVATIVE FOOD SCIENCE & EMERGING TECHNOLOGIES*, 64, 1-11 [10.1016/j.ifset.2020.102397].

Availability:

This version is available at: <https://hdl.handle.net/11585/765084> since: 2024-05-14

Published:

DOI: <http://doi.org/10.1016/j.ifset.2020.102397>

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(Article begins on next page)

Manuscript Details

Manuscript number	IFSET_2020_126
Title	Effect of innovative pre-treatments on the mitigation of acrylamide formation in potato chips
Article type	Research Paper

Abstract

The aim of this research was to evaluate the reduction of acrylamide (AA) formation in potato chips applying innovative pre-treatments. Raw potato slices were subjected for 5 and 15 min to: dipping in water; dipping in *Aureobasidium pullulans* L1 yeast water suspension; dipping in water or in yeast water suspension after pulsed electric fields (PEF) in order to investigate a possible synergistic effect of pre-treatments. The raw potato samples were analysed for AA precursors and, after frying, for AA by using HPLC-MS/MS. In addition, the final potato chips main quality parameters were evaluated.

Compared to untreated potato chips, yeast treatment promoted a reduction of AA formation mainly at the longest dipping time (15 min). PEF treatment followed by water dipping was the most effective in reducing AA for both the studied treatment times. The combination of PEF and yeast treatments led only a slight reduction of AA formation.

Keywords Potato chips; Acrylamide; Pulsed electric fields; *Aureobasidium pullulans* yeast

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Highlights

- PEF and yeast were applied for the reduction of acrylamide content in potato chips.
- Yeast activity can promote the reduction of acrylamide content in potato chips.
- PEF treatment was the most effective in reducing acrylamide in potato chips.
- The pre-treatments slightly influenced the quality characteristics of potato chips.

1 **Effect of innovative pre-treatments on the mitigation of acrylamide**
2 **formation in potato chips**

3

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23

24 **Abstract**

25 The aim of this research was to evaluate the reduction of acrylamide (AA) formation in potato
26 chips applying innovative pre-treatments. Raw potato slices were subjected for 5 and 15 min
27 to: dipping in water; dipping in *Aureobasidium pullulans* L1 yeast water suspension; dipping
28 in water or in yeast water suspension after pulsed electric fields (PEF) in order to investigate a
29 possible synergistic effect of pre-treatments. The raw potato samples were analysed for AA
30 precursors and, after frying, for AA by using HPLC-MS/MS. In addition, the final potato chips
31 main quality parameters were evaluated.

32 Compared to untreated potato chips, yeast treatment promoted a reduction of AA formation
33 mainly at the longest dipping time (15 min). PEF treatment followed by water dipping was the

34 most effective in reducing AA for both the studied treatment times. The combination of PEF
35 and yeast treatments led only a slight reduction of AA formation.

36

37 **Industrial relevance**

38 The Commission Regulation (EU) 2017/2158 has established new acrylamide (AA) benchmark
39 levels in different foods due to its negative classification as "probably carcinogenic to human".
40 For this reason, food industries are interested in developing different processing methods to
41 reduce the AA formation and at the same time to maintain an acceptable quality of final
42 products. Fried potatoes (French fries and chips) are the most vulnerable foods in terms of high
43 content of AA formation, being rich in the main Maillard reaction substrates, such as asparagine
44 and reducing sugars, and characterized by a high surface to volume ratio. Among the strategies
45 recently suggested for the reduction of AA in potato chips, pulsed electric fields (PEF) and
46 yeast pre-treatments are very promising, having the potentiality to reduce AA precursors in raw
47 potato tissues. In this study the possibility to apply yeast and PEF pre-treatments on raw potato
48 slices with suitable processing times for a possible industrial application were evaluated.

49

50 **Keywords**

51 Potato chips; Acrylamide; Pulsed electric fields; *Aureobasidium pullulans* yeast

52

53 **Chemical compounds studied in this article**

54 Acrylamide (PubChem CID: 6579); Asparagine (PubChem CID: 6267); Fructose (PubChem
55 CID: 2723872); Glucose (PubChem CID: 5793).

56

57 **1. Introduction**

58 Acrylamide (AA) is an undesired food toxic compound classified as “probably carcinogenic to
59 human” (group 2A), due to its neurotoxic and genotoxic proprieties on the basis of animal
60 studies (IARC, 1994). In the last few years, authorities and regulations have been more and
61 more restrictive concerning the maximum AA levels allowed in popular heat-treated foods and
62 beverages (European Commission, 2017; Food Drink Europe, 2019; Palermo et al., 2016). The
63 latest Commission Regulation (EU) 2017/2158, although not introducing a legal limit, contains
64 stricter measures to reduce AA levels in different food groups and subgroups such as fried
65 potato, bakery products and roasted coffee (European Commission, 2017).

66 AA is principally formed during food thermal processing (> 120 °C) by the reaction of free
67 amino acids (e.g. asparagine) with reducing sugars (D-glucose and D-fructose) as part of the
68 Maillard reactions, also known as non-enzymatic browning reactions (Stadler & Scholz, 2004).
69 On the other hand, Maillard reactions result in physical and chemical changes, important for
70 the development of some peculiar food characteristics like the desirable taste, colour and
71 flavour (Medeiros Vinci, Mestdagh, & De Meulenaer, 2012; O’Connor, Fisk, Smith, & Melton,
72 2001). For this reason, the challenge for food industries and researches is to develop methods
73 to reduce this toxicant, without changing the desired final properties.

74 Potato is the second major crop for human consumption in Europe and the fourth in the world,
75 being considered an excellent source of energy, carbohydrates and nutritional fibres (Dourado
76 et al., 2019). Nevertheless, fried potato products (French fries, chips, etc.) are highly susceptible
77 to AA formation due to asparagine and reducing sugars content as well as the high temperatures
78 applied during the frying process (Parker et al., 2012) and the high surface to volume ratio. Due
79 to the large consumption of fried potato products worldwide, the reduction of undesirable AA,
80 without compromising the sensory characteristics is essential (Dourado et al., 2019).

81 The principal strategies proposed to reduce AA in potato products are the selection of cultivar
82 and storage conditions, the control of time and temperature of heat treatment, the application of
83 different frying techniques (e.g. under vacuum), the use of asparaginase enzyme and hot water
84 blanching as pre-treatments (Amrein et al., 2003; Foot, Haase, Grob, & Gondé, 2007; Medeiros
85 Vinci et al., 2012; Pedreschi, Mariotti, Granby, & Risum, 2011; Romani, Bacchiocca, Rocculi,
86 & Dalla Rosa, 2009). However, these strategies present different disadvantages such as long
87 processing times, high costs, negative sensory modifications and difficult implementation for
88 industrial scale. Therefore, it is necessary to find alternative methods to reduce the formation
89 of AA in potato products.

90 Recently, the application of biocontrol agents as asparaginase producers (Di Francesco et al.,
91 2019) and non-thermal treatments such as pulsed electric fields (PEF) (Genovese et al., 2019)
92 were proven useful to reduce the AA precursors in the potato tissues and hence the subsequent
93 AA formation. The metabolic activity of microorganisms could reduce the asparagine
94 concentration through the activity of the asparaginase enzyme, which hydrolyses asparagine to
95 aspartic acid and ammonia. Di Francesco et al. (2019) reported for the first time that the yeast
96 *Aureobasidium pullulans* L1 strain can successfully assimilate asparagine in “Primura” var.
97 potato homogenate after 30 min of contact, leading to a great decrease in the AA content in the
98 final fried potato chips (- 85%). The yeast ability to produce enzymes has attracted considerable
99 biotechnological interest because these hydrolytic enzymes have a potential commercial value
100 in various industries (Deshpande, Rale, & Lynch, 1992). As demonstrated by Dunlop & Roon
101 (1975), *A. pullulans* showed a hydrolytic cleavage of L-asparagine with intact whole cells.
102 Generally, the liberation of enzymic activity from intact protoplast cells is considered the single
103 conclusive proof for the existence of an extracellular enzyme (Dunlop & Roon, 1975),
104 subsequently able to act on the potato tissue composition. Moreover, *A. pullulans* has
105 demonstrated to be able to ferment sugars, among which sucrose covers an important role
106 because proved as the carbon source for pullulan synthesis (Sheng, Tong, & Ma, 2016).
107 Furthermore, An, Ma, Chang, & Xue (2017) showed how potato starch can promote *A.*
108 *pullulans* enzyme production and pullulan biosynthesis. PEF technology consists in an
109 electrical treatment of short time (from nanoseconds to milliseconds) that promotes the
110 temporary or permanent loss of the semi-permeability of cell membranes in biological tissues,
111 favouring mass transfer phenomena (Fincan & Dejmek, 2003; Puértolas, Koubaa, & Barba,
112 2016; Vorobiev & Lebovka, 2010). Recently, Genovese et al. (2019) described the possibility
113 to reduce AA precursors in raw potatoes by the application of PEF followed by a 5 min water
114 dipping, which led to a reduction of around 30% of AA content in fried potato chips.
115 The aim of present research was to evaluate the possibility to reduce AA in potato chips by
116 applying for different times the above-mentioned innovative pre-treatments, *A. pullulans* L1
117 strain and PEF, alone or in combination.
118 The AA and its precursors (e.g. reducing sugar and asparagine) content, quality parameters such
119 as moisture, oil content, colour and texture of potato chips were also evaluated.

120

121 **2. Materials and methods**

122

123 **2.1. Potato**

124 Fresh potato tubers (*Solanum tuberosum* cv Lady Claire) were purchased at the local market
125 (Emilia-Romagna, Italy) and stored for a maximum of 15 days in the dark at 15 °C and 90%
126 relative humidity (RH). Potato slices were prepared by reproducing as much as possible the
127 main industrial production steps and conditions. Tubers were washed in running water,
128 manually peeled and cut in slices of 1.5 ± 0.2 mm thickness by using an electric cutter machine
129 mod. KAFPL0922N (CAD Italy, Italy). Potato slices were rinsed immediately after slicing for
130 approximately 1 min in tap water (18 ± 2 °C) in order to eliminate part of the starch material
131 on the surface.

132

133 **2.2. *Aureobasidium pullulans* L1 strain**

134 *Aureobasidium pullulans* L1 strain is a potential biocontrol agent mainly active against fruit
135 postharvest pathogens, representing a promising alternative to common fungicides in the
136 control of postharvest diseases (Di Francesco et al., 2018; Di Francesco, Ugolini, D'Aquino,
137 Pagnotta, & Mari, 2017). The yeast was maintained on Nutrient Yeast Dextrose Agar (NYDA),
138 consisting of nutrient broth 8 g, yeast extract 5 g, dextrose 10 g and technical agar 15 g (all from
139 Oxoid, Basingstoke, UK) in 1 L of distilled water, and stored at 4 °C until use. Two days before
140 the experiments, the yeast was cultured in 250 mL conical flasks, containing 50 mL of Nutrient
141 Yeast Dextrose Broth (NYDB: NYDA without Agar) on a rotary shaker at 200 rpm for 48 h at
142 25 °C. Cell suspension was prepared by centrifugation of the liquid culture samples at 6,000
143 rpm for 10 min at 4 °C. Yeast cells were suddenly washed twice with sterile distilled water to
144 remove the growth medium and suspended in sterile distilled water to reach a concentration of
145 10^8 cells/mL by using a hemocytometer. Yeast suspension was used for the pre-frying
146 treatments and isothermal calorimetry assay.

147

148 **2.3. Chemicals and reagents**

149 Acrylamide (for molecular biology, $\geq 99\%$ (HPLC), C_3H_5NO , molecular weight 71.08 g/mol,
150 CAS No 79-06-1) and its precursors namely, L-asparagine ($\geq 98\%$ (HPLC), $C_4H_8N_2O_3$,
151 molecular weight 132.12 g/mol, CAS No 70-47-3), D-(-)-fructose ($\geq 99\%$, $C_6H_{12}O_6$, molecular
152 weight 180.16 g/mol, CAS No 57-48-7) and D-(+)-glucose (analytical standard, $C_6H_{12}O_6$,
153 molecular weight 180.16 g/mol, CAS No 50-99-7) were purchased from Sigma-Aldrich (St.
154 Louis, MO, USA). Individual stock solutions of AA, fructose and glucose, at a concentration
155 of 1,000 mg/L, and asparagine, at 500 mg/L, were prepared by dissolving the pure standard
156 compounds in water and storing them in glass-stoppered bottles at -18 °C. Afterwards, standard
157 working solutions at various concentrations were prepared daily by appropriate dilution of the

158 stock solution with water. HPLC-grade acetonitrile was supplied by Sigma-Aldrich (Milano,
159 Italy). HPLC-grade formic acid (99%) was obtained from Merck (Darmstadt, Germany). C18
160 octadecyl silica sorbent was purchased from Phenomenex (Castelmaggiore, Bologna, Italy).
161 Deionized water ($> 18 \text{ M}\Omega \text{ cm}$ resistivity) was further purified using a Milli-Q SP Reagent
162 Water System (Millipore, Bedford, MA, USA). All solvents and solutions were filtered through
163 a $0.2 \mu\text{m}$ polyamide filter from Sartorius Stedim (Goettingen, Germany). Before HPLC
164 analysis, all samples were filtered with Phenex™ RC 4 mm $0.2 \mu\text{m}$ syringeless filter,
165 Phenomenex (Castelmaggiore, Bologna, Italy).

166

167 **2.4. Pre-treatments**

168 The raw potato slices were subjected to different pre-treatments before frying, obtaining
169 different samples as showed in Figure 1. In detail, the raw potato slices were subjected to:
170 dipping in water for 5 and 15 min (samples named respectively W5 and W15); dipping in *A.*
171 *pullulans* L1 yeast water suspension for 5 and 15 min (Y5 and Y15 samples); dipping in water
172 after PEF in water (PW5 and PW15 samples) and dipping in yeast water suspension after PEF
173 in the same yeast suspension (PY5 and PY15 samples). Raw potato slices that have not
174 undergone any pre-treatment were considered as control sample (C).

175

176 *2.4.1. Aureobasidium pullulans L1 treatments*

177 The pre-treatment by using L1 strain suspension was carried out according to the patented
178 procedure No. WO2019058248A1 (authors: M. Mari, A. Di Francesco and L. Ugolini, *Alma*
179 *Mater Studiorum*, Bologna and CREA), with slight modifications. Each treatment was carried
180 out at room temperature ($\sim 25 \text{ }^\circ\text{C}$) by dipping 80 g of potato slices in 200 mL of yeast suspension
181 concentrated 10^8 cells/mL for 5 (Y5) and 15 (Y15) min. The yeast untreated samples were
182 represented by 80 g of potato slices dipped in 200 mL of tap water at room temperature for the
183 same times (5 min: W5, 15 min: W15). After dipping, the potato slices were collected, rinsed
184 with tap water and carefully dried with absorbent paper. Each treatment for each sample (W5,
185 W15, Y5, Y15) was performed in triplicate.

186

187 *2.4.2. Pulsed electric fields (PEF) treatments*

188 PEF pre-treatments were performed using a lab-scale PEF unit delivering a maximum output
189 current and voltage of 60 A and 8 kV respectively mod. S-P7500 (Alintel, Italy). The generator
190 provides monopolar rectangular-shape pulses and adjustable pulse duration (5-20 μs), pulse
191 frequency (50-500 Hz) and total treatment time (1-600 s). The treatment chamber (50 mm

192 length × 50 mm width × 50 mm height) was a prototype built by Alintel (Italy) and consisted
193 of two parallel stainless-steel electrodes (3 mm thick) with a 47 mm fixed gap. The outputs of
194 the generator, tension and current, were monitored using a PC-oscilloscope mod. Picoscope
195 2204a (Pico Technology, UK). Raw potato slices (20 g) were treated at room temperature in
196 tap water (100 mL) with an initial electrical conductivity of $536 \pm 23.2 \mu\text{S}/\text{cm}$ at $25 \text{ }^\circ\text{C}$ (EC-
197 meter mod. Basic 30, Crison, Spain) and delivering $n = 1,000$ pulses at fixed pulse width ($10 \pm$
198 $1 \mu\text{s}$), frequency (100 Hz), time interval between pulses ($10 \pm 1 \text{ ms}$) and applying an electric
199 field strength of $1.5 \text{ kV}/\text{cm}$, resulting in a specific energy input of $105 \pm 5.5 \text{ kJ}/\text{kg}$, calculated
200 according to Raso et al. (2016). For more details regarding the PEF-treatment protocol and the
201 measured cell disintegration refer to Genovese et al. (2019).

202 The PEF treatment was repeated four times in order to obtain one batch (80 g) of treated product
203 for each sample; temperature changes due to PEF treatment, measured with a temperature probe
204 mod. TESTO 445 (Testo GmbH & Co, Milano, Italy), were negligible. After the PEF
205 treatments, 80 g of potato slices were collected and left dipped in 200 mL of water for 5 (PW5)
206 and 15 (PW15) min. After dipping, the potato slices were collected, rinsed with tap water and
207 carefully dried with absorbent paper. Each dipping treatment was performed in triplicate for
208 each sample (PW5, PW15), consequently the preliminary PEF treatment was repeated 12 times
209 for each sample ((20 g x 4) x 3)).

210

211 *2.4.3. Combination of treatments*

212 The combination of PEF and yeast pre-treatments was performed filling the PEF treatment
213 chamber with about 100 mL of yeast aqueous suspension (10^8 cells/mL) and 20 g of raw potato
214 slices. The initial electrical conductivity of the yeast aqueous suspension measured using an
215 electrical conductivity meter (EC-meter mod. Basic 30, Crison, Spain), was $536 \pm 23.2 \mu\text{S}/\text{cm}$
216 at $25 \text{ }^\circ\text{C}$ (comparable with the tap water initial electrical conductivity used for PEF treatment
217 in water). The selected PEF conditions and applied energy input were the same as mentioned
218 in section 2.4.2. After the PEF treatments, 80 g of potato slices were collected and left immersed
219 in 200 mL of the PEF-treated yeast suspension for 5 (PY5) and 15 (PY15) min. Subsequently,
220 the potato slices were collected, rinsed with tap water and carefully dried with absorbent paper.
221 Each dipping treatment in the PEF-treated yeast suspension was performed in triplicate for each
222 sample (PY5, PY15); consequently the preliminary PEF treatment in the yeast aqueous
223 suspension was repeated 12 times for each sample ((20 g x 4) x 3)).

224

225 *2.5. Frying conditions*

226 Untreated (C) and pre-treated potato slices were deep-fried in 6 L of high-oleic sunflower oil
227 (potato to oil ratio 1:20 w/w) at 175 °C for 3 min, by using an electrical fryer mod. MFR280R
228 (Fama Industrie, Italy). Temperatures of frying oil were monitored using K-type thermocouples
229 sensors with an accuracy of 0.1 °C (Chromel/Alumel, Tersid Came, Italy), connected to a data
230 logging system (mod. 9211A, National Instruments™, Texas).

231

232 **2.6. Analytical determinations**

233

234 *2.6.1. Isothermal calorimetry*

235 Isothermal calorimetry was used to evaluate the best combination of PEF pre-treatment and
236 dipping in aqueous yeast suspension, by monitoring and determining the development of
237 metabolic heat of the *A. pullulans* L1 yeast. The effect of PEF was investigated in different
238 substrates. NYDB medium was used as ideal substrate for the yeast growth, while tap water
239 and potato tissue in a 1:1 (w/w) ratio was used to simulate a real substrate. Moreover, because
240 PEF could also increase the release of solutes from the potato tissue that could influence the
241 yeast activity, tap water with potato tissue subjected to PEF was also considered. A schematic
242 representation of the combination of samples and substrate is reported in Figure 2.

243 In each vial, 2 g of potato (raw or PEF-treated), 1 mL of yeast (10^8 cells/mL) (control and PEF-
244 treated) and 2 mL of substrate (NYDB or tap water) were placed. Vials were sealed with Teflon
245 caps and aluminium screw lids.

246 For each sample three replicas were performed. The extent of metabolic heat production by the
247 yeast was measured continuously with a TAM Air isothermal calorimeter (TA
248 Instruments/Thermometric, Sweden) with a sensitivity of $\pm 10 \mu\text{W}$. This instrument contains
249 eight twin calorimeters, each of which has its own reference to increase sensitivity and
250 accuracy. As a reference, an ampoule with distilled water was used. The amount of distilled
251 water was determined according to Panarese, Tylewicz, Santagapita, Rocculi, & Dalla Rosa
252 (2012). Isothermal calorimetric measurements were performed at a constant temperature of 25
253 °C, chosen as the optimal one for yeast growth, for 48 hours. Baselines were recorded before
254 and after each measurement for 30 min. The heat produced was continuously recorded with
255 dedicated software (TAM Air assistant, TA Instruments/Thermometric, Sweden). The
256 thermograms obtained were normalized for the weight of the sample.

257

258 *2.6.2. HPLC-MS/MS analysis: quantification of asparagine, reducing sugars and*
259 *acrylamide*

260 The freeze-dried raw potatoes and potato chips samples were crumbled finely in mortar and 2
261 g of sample were weighed in a 50 mL conical flask. The extraction was performed with 20 mL
262 of Milli-Q water firstly by 1 min of agitation in a vortex mixer and secondly by 10 min of
263 ultrasound-assisted extraction at room temperature. After pouring into a 50 mL centrifuge
264 plastic tube, the sample was centrifuged at 5,000 rpm for 10 min and the supernatant was
265 collected and stored overnight at -18 °C to precipitate starch and facilitate the separation and
266 removal of fat fraction. Later, the sample was thawed at room temperature, once again
267 centrifuged at 5,000 rpm for 10 min and then 1 mL of water supernatant was transferred to a
268 1.5 mL microcentrifuge tube containing 100 mg of C18 sorbent. Before centrifugation at 13,300
269 rpm for 15 min, the sample was vortexed for 1 min. Finally, an aliquot of supernatant was
270 collected and diluted 1:100 in mobile phase for asparagine analysis and 1:2 in acetonitrile for
271 AA, fructose and glucose analysis. Before high performance liquid chromatography-tandem
272 mass spectrometry (HPLC-MS/MS) injection, the diluted samples were filtered with a 0.2 µm
273 syringeless filter.

274 The quantitation of AA, asparagine, fructose and glucose, is frequently performed by different
275 methods and using various analytical instruments. For instance, sugars are often analysed by
276 high performance liquid chromatography-refractive index detector (HPLC-RID) (Coelho et al.,
277 2018; Vivanti, Finotti, & Friedman, 2006), asparagine using high performance liquid
278 chromatography with fluorescence detection (HPLC-FD) (Amrein et al., 2003) and AA by high
279 performance liquid chromatography-mass spectrometry (HPLC-MS) (Elmore et al., 2015;
280 Zhou, Wang, Chen, & Zhang, 2015). Few works have reported the simultaneous analysis, in
281 starchy foods, of these four molecules using a single approach (Nielsen, Granby, Hedegaard, &
282 Skibsted, 2006). In the present research we introduced a simple method for simultaneous
283 quantification of AA, asparagine, glucose and fructose by using HPLC-MS/MS.

284 The extraction process was optimized by taking the cue from previous data (Nielsen et al., 2006)
285 with some modifications. Water was used as the extraction solvent for the high solubility of
286 target molecules and a dispersive solid-phase extraction (DSPE) using C18 sorbent was chosen
287 for sample clean-up, because was a simple and fast technique able to remove non-polar
288 molecules which could act as interferences (Anastassiades, Lehotay, Štajnbaher, & Schenck,
289 2003).

290 HPLC-MS/MS studies were performed using an Agilent 1290 Infinity series and a Triple
291 Quadrupole 6420 from Agilent Technology (Santa Clara, CA) equipped with an electrospray
292 ionization (ESI) source operating in positive ionization mode. The HPLC-MS/MS parameters
293 of each analyte were optimized in flow injection analysis (FIA) (1 µL of a 10 mg/L individual

294 standard solution) by using optimizer software (Agilent). The separation of target compounds
295 was achieved on a Kinetex Hilic analytical column (100 mm × 4.6 mm i.d., particle size 2.6
296 μm) from Phenomenex (Torrance, CA, USA) preceded by a KrudKatcher ULTRA HPLC In-
297 Line Filter (2.0 μm Depth Filter x 0.004in ID). The mobile phase for HPLC-MS/MS analysis
298 was a mixture of 15% water (A) and 85% HPLC-grade acetonitrile (B), both with 0.1% formic
299 acid. The separation was obtained by flowing at 0.8 mL/min with this gradient elution: isocratic
300 condition until 2.5 min (85% B), 3.5 min (70% B), 5.5 min (70% B), 6.5 min (85% B) and then
301 constant until the end of the run (15 min). All solvents and solutions were filtered through a 0.2
302 μm polyamide filter from Sartorius Stedim (Goettingen, Germany). The injection volume was
303 2 μL. The temperature of the column was 25 °C and the temperature of the drying gas in the
304 ionization source was 350 °C. The gas flow was 12 L/min, the nebulizer pressure was 45 psi
305 and the capillary voltage was 4,000 V. Detection was performed in the multiple reaction
306 monitoring (MRM) mode. The MRM peak areas were integrated for quantification and the most
307 abundant product ion was used for quantitation, and the rest of the product ions were used for
308 qualification. The selected ion transitions and the mass spectrometer parameters are reported in
309 Table 1.

310 As an example, Figure 3 shows the HPLC-MS/MS chromatogram of a standard mixture of the
311 monitored compounds plotted as overlapped multiple reaction monitoring (MRM) transition of
312 each compound. Results were expressed as μg/kg (dry matter) for AA and mg/kg (dry matter)
313 for AA precursors.

314

315 *2.6.3. Moisture*

316 The moisture content was determined on all types of raw and fried potato samples by
317 gravimetric method. The samples were dried to constant weight in a thermo-regulated
318 laboratory stove (UF110, Memmert, Schwabach, Germany) at 70 °C (around 48 h) and 105 °C,
319 (around 24 h) respectively for raw and fried potatoes. Results were expressed as percentage of
320 water. The analysis was carried out in triplicate for each sample.

321

322 *2.6.4. Oil content*

323 The oil content of potato chips after frying was determined by Soxhlet extraction, performing
324 the procedure with petroleum ether as a solvent at 60 °C for 3 h (AOAC, 1990). Oil content
325 was reported as a percentage on dry matter. The analysis was carried out in triplicate for each
326 sample.

327

328 2.6.5. *Colour*

329 The surface colour of whole potato chips was determined using a computer vision system
330 (CVS). The samples were placed inside a dark chamber over a white background in controlled
331 lighting conditions that consisted in four daylight fluorescent lamps (TL-D Deluxe, Natural
332 Daylight, 18W/965, Philips, USA) with a colour temperature of 6500 K (D65 standard). The
333 fluorescent tubes (60 cm long) were located 35 cm above the sample and inclined at an angle
334 of 45°. The RGB images of the samples were acquired using a colour digital camera mod.
335 D7000 (Nikon, Japan) equipped with 105 mm lens (mod. AF-S Micro Nikkor, Nikon, Japan),
336 positioned vertically and connected to display and capture the images directly by the computer.
337 For each sample, untreated and pre-treated, 10 images were captured on both sides of potato
338 slices.

339 The pre-processing of RGB images and colour quantification in CIE $L^*a^*b^*$ scale was
340 performed with ImageJ analysis software (NIH, USA). From numerical values of a^* (green-
341 red) and b^* (yellow-blue) chromatic parameters, hue angle (h°) was calculated by the following
342 equation (McGuire, 1992) and used to describe colour variations between samples:

343

$$344 \quad h^\circ = \tan^{-1}(b^*/a^*) \quad (1)$$

345

346 The colour measurements were carried out on the two surfaces of 5 potato chips for each
347 sample.

348

349 2.6.6. *Texture*

350 The texture measurement on chips samples was performed at room temperature using a Texture
351 Analyser mod. TA-HDi500 (Stable Micro System, UK) equipped with a 5 kg load cell and a
352 spherical probe in stainless steel with a diameter of 6 mm at 1.0 mm/s test speed. The samples,
353 selected on the basis of uniform size and shape, were placed on a support rig (HDP/CFS) and
354 compressed for 3 mm distance. The acquired results were expressed as hardness, calculated by
355 means of maximum force values, and as index of crispness, calculated by means of linear
356 distance between the first and the last peaks registered (Tylewicz et al., 2019). Force vs distance
357 curves were obtained from 12 potato chips for each sample.

358

359 **2.7. *Data analysis***

360 The results were reported as mean value \pm standard deviation of replications. The software
361 STATISTICA 8.0 (Statsoft Inc., UK) was used for the statistical analysis of the data. Significant

362 differences between results were calculated by parametric analysis of variance (ANOVA) and
363 Tukey multiple post-hoc comparison, with a significance level of 95% ($p < 0.05$).

364

365 **3. Results and Discussion**

366

367 ***3.1. Preliminary tests on ideal substrate for the optimization of combined treatments***

368 Isothermal calorimetry, that has been successfully used in the past to describe the growth ability
369 of microorganisms (Braissant, Wirz, Beat, & Daniels, 2009), was employed to measure
370 continuously and in real time the heat flow produced in different substrates by the yeast *A.*
371 *pullulans* L1, untreated and subjected to the chosen PEF treatment protocol.

372 At first, the ideal growth substrate (NYDB) was used to evaluate the only effect of the selected
373 PEF conditions on the yeast activity, in terms of heat produced. Figure 4A shows the heat flow,
374 recorded at 25 °C during 35 h, produced by *A. pullulans* after PEF treatment (YPEF) compared
375 to the untreated one (Y). The thermograms, as suggested by Morozova et al. (2017), are relative
376 to the kinetic profile of the fermentative process. The heat flow signal has been considered as
377 an index of the yeast growth rate. The shape of the signal suggests the presence of a series of
378 consecutive processes that occur in the vial.

379 Initially, in both samples, a constant thermal power was recorded, of about $8.4 \cdot 10^{-4}$ and $2.0 \cdot 10^{-3}$
380 W/g for sample Y and YPEF respectively, indicating the lag phase. Typically, the lag phase
381 corresponds to the period of time in which yeasts synthesize the enzymatic pool necessary for
382 their catabolism during which the multiplication is neglectable (Morozova et al., 2017). After
383 10 h, the signal started to increase exponentially. This sudden change is due to the exponential
384 growth phase of the yeast. The signal increased until reaching a maximum, in different times
385 for the 2 samples, and then, because of various factors limiting the growth (e.g. reduced oxygen
386 concentration in the head space, reduction of carbon and nitrogen sources in the growth
387 medium, production of ethanol or increased pressure in the vial), the signal started decreasing,
388 reaching almost a stationary phase, the extension of which depends on the ability of the yeast
389 to survive (Morozova et al., 2017).

390 Table 2 reports the average values of the heat produced by the yeast according to the different
391 conditions investigated, expressed as either total heat (integral of the heat flow) and slope of
392 the curve related to the exponential growth phase. Results obtained suggest that PEF treatment
393 had a positive effect on the yeast metabolism stimulating both its entity and rate. Different
394 literature reports indicated that low intensity electrical fields (0.1-1 kV/cm) and short treatment
395 times (μ s-s) did not bring damage to the cell membranes functionality, but actually enhanced

396 microbial reactions and activities (Mattar et al., 2014; Schottroff, Krottenthaler, & Jaeger,
397 2017). According to these authors, low intensity PEF allows to improve enzymatic synthesis,
398 frequency of cell division, probability of survival of daughter-cells, increases tolerance to
399 inhibitors (e.g. ethanol) and yeasts fermentation ability. Moreover, electroporation induced by
400 PEF can modify the cytoplasmic membrane and hence the nutrients transportation due to the
401 formation of pores or the activation of transport proteins. However, such mechanisms have not
402 been fully clarified yet and PEF effect depends also on other factors such as growth substrate,
403 dimension and specific resistance of microbial cells (Mattar et al., 2014). In relation to the
404 different susceptibility of different microorganisms and to the entity of PEF treatment applied,
405 the cells can be in three different states: intact, dead or damaged (sub-lethal stress) (Wang et
406 al., 2018).

407 Figure 4B shows the heat flow signals recorded at 25 °C during the yeast growth process,
408 subjected to PEF (YPEF) compared to the control (Y), placed in a substrate simulating the real
409 conditions, consisting of water and untreated potatoes or water and potatoes treated with PEF
410 (PEF + Y and PEF + YPEF). In Table 2 the corresponding data of heat produced, and slope of
411 the curve were reported. As expected, the extent of yeast growth in water-potato medium was
412 significantly reduced compared to its optimal growth medium (NYDB). However, when placed
413 in water-potato medium, no difference was observed for the yeast treated by PEF (PEF + YPEF)
414 compared to its untreated control (PEF + Y) for both heat and slope produced. However, when
415 the potato in the substrate was pre-treated by PEF (PEF+Y and PEF+YPEF), the heat produced
416 almost doubled and the slope increased 5-6-fold in comparison to water-potato medium not
417 treated with PEF (Y and YPEF). While total heat is an index of the metabolism entity, the slope
418 indicates the rate of metabolic reactions. Our hypothesis is that PEF pre-treatment increased the
419 membrane permeability of the potato tissue inducing a higher release of cell content into the
420 water, that in turn became richer in nutrients exploitable by the yeast metabolism.

421 In addition, in the water-potato medium (real substrate) no differences in heat and slope were
422 observed between PEF-treated (YPEF) and control (Y) yeast in both potato medium treated
423 with PEF or not (Table 2). It seems that the positive effect of PEF on *A. pullulans* metabolism
424 is visible only when the growth substrate is NYDB (Figure 4A), while in the case of the water-
425 potato substrate, the limiting nutrient availability flattened the differences (Figure 4B).

426 Following these preliminary results, it was decided to PEF-treat the potato slices in the yeast
427 water suspension in order to verify the combined effect of the two pre-treatments on AA
428 formation.

429

430 **3.2. Application on real substrate**

431

432 *3.2.1. Acrylamide and precursors analysis*

433 The levels of AA in fried potato chips and the concentrations of AA precursors in untreated raw
434 potato (control C) and in differently pre-treated raw potato samples are reported in Table 3. The
435 AA concentration of untreated potato chips was 1,384.3 µg/kg, value that exceeds the reference
436 level set by the Commission Regulation (UE) 2017/2158 (750 µg/kg). The untreated potato
437 tubers were characterized by a glucose, fructose and asparagine content of 33.6 mg/kg, 6.8
438 mg/kg and 8,826.3 mg/kg respectively.

439 Each applied treatment, especially after 15 min of dipping, has led to a reduction of AA in final
440 potato chips; however, not always proportional to the content of evaluated AA precursors.

441 Dipping in water (W5, W15) had a positive effect in reducing AA levels in fried potatoes due
442 to the promotion of precursors release from the food matrix to the water for leaching effect.
443 Potato samples dipped in water for 5 and 15 min (W5, W15) have presented a significant AA
444 reduction respectively of 6.6% and 44.0%. The decrement of AA in these samples can be
445 attributed to the reduction of glucose and fructose, while the asparagine content did not undergo
446 any reduction. It is worth to note that the sample W15 resulted in AA content in compliance
447 with the limit set by the EU Regulation. However, the concentration of AA precursors, and so
448 the potential concentration of AA formation, is linked to intrinsic properties of the food matrix,
449 i.e. potato cultivar, post-harvesting conditions. Therefore, AA levels in fried potatoes can be
450 much higher than those measured in this study, explaining the necessity of assessing new AA
451 mitigation measures suitable for industrial processing conditions.

452 The yeast treatment after 5 min (Y5) did not lead to a significant reduction in AA compared to
453 the control (C) and to the water dipped sample for the same time (W5). On the other hand, after
454 15 min (Y15) a significant AA reduction of 51.1% was reached, highlighting that the yeast
455 requires contact times greater than 5 min. Nevertheless, in comparison to the sample dipped in
456 water for the same time (W15) the additional reduction after 15 min yeast treatment (Y15) was
457 only 7%. This slight, but significant reduction of AA after 15 min yeast dipping could be
458 attributed to enzymatic and metabolic activity of the yeast able to reduce the levels of glucose,
459 fructose and asparagine. In a recent study with the same yeast strain, a reduction of AA in potato
460 chips, equal to 83.7%, was obtained after a dipping of 30 min (Di Francesco et al., 2019).
461 However, industrial application often requires shorter times.

462 The samples subjected to PEF treatment followed by dipping in water (PW) led to the highest
463 AA reduction for each treatment time, equal to 35.9% and 58.7% respectively for 5 and 15 min.

464 The cell electroporation phenomena induced by the PEF treatment on raw potato slices resulted
465 in a reduction of AA precursors leading to lower AA formation. This result confirms a recent
466 study in which a significant reduction in the AA content (30%) was found in potato chips treated
467 with PEF, compared to untreated samples (Genovese et al., 2019). Moreover, it is interesting to
468 notice that, although precursors concentration was similar for Y15 and PW15 samples, AA
469 levels were lower in PW15. This could be attributed to the possible modification of other
470 molecules and pathways which can participate to AA formation. In fact, it is known that AA is
471 mainly formed by Maillard reaction from asparagine and α -carbonyl sources such as reducing
472 sugars, but other mechanisms can contribute to the final AA level. For example, the acrolein,
473 which can be formed from lipid oxidation and degradation of amino acids, carbohydrate and
474 protein, the acrylic acid derived from aspartic acid, and the β -alanine can participate to AA
475 formation (Stadler & Studer, 2016).

476 The combination of PEF and yeast treatments (PY) resulted in a slight AA reduction (12.4%
477 after 5 min and 13.8% after 15 min), similar to the results obtained with 5 min of dipping in
478 water, but significantly lower compared to the reductions obtained with the singular pre-
479 treatments (Y15, PW5 and PW15 samples). This result could be related to the direct effect of
480 PEF on yeast cells that can notably influence their propriety and activity. In fact, as reported by
481 Stirke et al. (2014) and Mattar et al. (2015) PEF can influence yeast cell viability, dimensions,
482 and consequently their enzymatic activity. Although isothermal calorimetry results showed an
483 increase of the overall yeast metabolic activity, the specific alterations of metabolic pathways
484 induced by PEF are still unknown (Mattar et al., 2015). In order to elucidate how PEF can affect
485 *A. pullulans* L1 strain activity additional experiments have to be performed in the future.

486

487 3.2.2. Qualitative analysis

488 The intense deep fry heat treatment is responsible for the development of non-enzymatic
489 browning reaction (Maillard reaction) that can lead to undesired AA formation, but also to
490 desired changes in physical attributes, such as colour, texture, flavour, moisture and oil content
491 (Anese et al., 2009; Yang, Achaerandio, & Pujolà, 2016; Pedreschi, Moyano, Kaack, & Granby,
492 2005; Romani et al., 2009).

493 The main quality characteristics of fried potato samples were analysed in order to evaluate the
494 possible effect of different AA mitigation pre-treatments applied (Table 4).

495 The development of a brown-gold colour during potato frying is one of the most important
496 quality parameters demanded by customers (Medeiros Vinci et al., 2012). The use of the CVS
497 allowed to evaluate the colour of whole potato chips surface. Example of RGB images of potato

498 chips samples untreated and differently treated are shown in Figure 5A, each RGB image was
499 processed to extract the numerical CIE $L^*a^*b^*$ data (Figure 5B). The W15, Y15, PW5, PW15
500 and PY15 potato chips presented significant ($p<0.05$) higher L^* values compared to the other
501 samples, highlighting a brighter colour (Table 4). The h° values increased significantly for
502 W15, Y15, PW5 and PW15 samples, underling a colour variation from the red-orange (dark)
503 zone to the green-yellow (light) one compared to untreated sample (C).

504 The texture is another important sensorial characteristic of potato chips that should be
505 monitored (Yee & Bussell, 2007). The various pre-treatments studied led to a slight and not
506 always significant reduction of hardness and crispness index of potato chips compared with the
507 untreated sample (C). To our knowledge, very few studies were carried out on the structural
508 characteristics of the potatoes after similar pre-treatments. Zhou et al. (2015), using a different
509 potato variety, found that the untreated potato samples were darker than yeast-treated ones
510 characterized by a low AA content. However, no significant ($p>0.05$) differences in texture was
511 found among the treated fried potato samples and untreated ones. The colour and texture results
512 were also confirmed by the study of Di Francesco et al. (2019) who found that the potato
513 untreated samples appeared darker in colour than yeast treated ones. Regarding the effect of
514 PEF treatment, Ignat, Manzocco, Brunton, Nicoli, & Lyng (2015) observed significant
515 differences only in L^* and a^* chromatic values for PEF-treated fried potato cubes, while the
516 hardness was similar to the control sample. These results were confirmed also by Genovese et
517 al. (2019) who found similar colour development during frying of PEF-treated potato slices.
518 Moreover, the authors observed a slight and significant reduction of texture parameters in PEF-
519 treated samples in comparison to the untreated one as in this case.

520 In the raw potato samples the water dipping treatments led to a significant moisture content
521 increase for all treatment times (W5, W15) compared to untreated control sample (C). This
522 increase is probably due to capillary and osmotic phenomena. No significant ($p>0.05$)
523 differences were found between the moisture values of the samples subjected to dipping in
524 water and in yeast water suspension for all treatment times. The raw samples subjected to PEF
525 pre-treatment and subsequent dipping (PW5, PW15, and PY5, PY15) showed similar and lower
526 moisture values than those treated only in dipping (W5, W15 and Y5, Y15). This result is
527 probably explained by the increase of mass transfers from the sample to the dipping solution
528 due to the modification of the cell membrane permeability induced by the PEF treatment.
529 Similarly, in the fried potato chips, all PEF pre-treated samples showed a lower moisture
530 content than the only dipping pre-treated ones. All fried potatoes subjected only to dipping pre-
531 treatment presented moisture value not significantly different compared to the untreated one

532 (C). Nevertheless, the reduction of moisture due to frying was similar between the samples
533 when compared with the respective raw samples and equal to 95-96%. This and the low values
534 of standard deviation data demonstrate the uniformity of frying process conducted on the
535 different samples and repetitions.

536 Regarding the oil content, all treatments (W, Y, PW and PY) promoted an increase of oil uptake
537 (between 34-41%) compared to the fried untreated sample (C). This increase is probably due to
538 the slightly higher water content in the treated raw samples, especially for W and Y ones. In
539 fact, during frying the evaporation of the water leads to an absorption of frying oil (Aguilera &
540 Gloria-Hernandez, 2000). However, in the PW and PY samples, subjected to PEF pre-
541 treatment, which before frying showed the lowest water content compared to those subjected
542 only to dipping (W and Y), the highest oil content was found (between 38-41%). This result
543 suggests that in the PEF-treated samples the absorption of oil does not depend only on the initial
544 water content, but also on the induced tissue electroporation phenomenon.

545 In the literature there are conflicting results regarding oil absorption in potato after PEF pre-
546 treatments. According to Fauster et al. (2018), Ignat et al. (2015) and Janositz, Noack, & Knorr
547 (2011), PEF treatment on potatoes led to a reduction in oil content of the final fried product in
548 the shape of cubes or sticks (French fries). This result was ascribed to the transfer of water and
549 intracellular substances to the potato surface due to the PEF-induced electroporation that,
550 creating a barrier, reduced the oil uptake. This phenomenon is probably less evident in fried
551 potato chips than in French fries. Moreover, in the present study the frying phase was not
552 modulated as a function of the final reached moisture, while in the cited researches frying times
553 were reduced after PEF pre-treatment and thus allowed to obtain a reduction in the oil
554 absorption.

555

556 **4. Conclusions**

557 From the results obtained in this research work, the following conclusions can be drawn:

- 558 - the use of the yeast *A. pullulans* L1 strain in water suspension (patented procedure No.
559 WO2019058248A1) as pre-treatment of potatoes to be fried as chips, was confirmed to be able
560 to promote the reduction of AA in the finished product at dipping time longer than 5 minutes;
- 561 - the electroporation induced on raw potato slices by PEF treatment led to the highest reduction
562 of AA formation in potato chips at each tested dipping time (5 and 15 min);
- 563 - the effect of the PEF treatment on the reduction of the AA formation in frying has been
564 reduced when combined with dipping in yeast-water suspension for both tested dipping times;

565 - all the studied pre-treatments did not substantially influence the main final quality
566 characteristics of potato chips.

567 The proposed strategies seem promising for the reduction of AA formation in fried potato
568 products that will allow to comply with the current EU legislation, without causing detrimental
569 effects on their final quality. However, further optimizations of tested pre-treatments are needed
570 for industrial applications.

571 Moreover, the effect of PEF on the yeast activity should be further elucidated, in order to better
572 exploit the yeast metabolism for AA reduction. Particularly, the time needed for the metabolic
573 response of yeast to PEF treatment has to be carefully considered in order to optimize the
574 studied pre-treatments.

575

576 **References**

577 Aguilera, J. M., & Gloria-Hernandez, H. (2000). Oil absorption during frying of frozen
578 parfried potatoes. *Journal of Food Science*, *65*(3), 476–479.
579 <https://doi.org/10.1111/j.1365-2621.2000.tb16031.x>

580 Amrein, T. M., Bachmann, S., Noti, A., Biedermann, M., Barbosa, M. F., Biedermann-Brem,
581 S., ... Amadó, R. (2003). Potential of acrylamide formation, sugars, and free asparagine
582 in potatoes: A comparison of cultivars and farming systems. *Journal of Agricultural and*
583 *Food Chemistry*, *51*(18), 5556–5560. <https://doi.org/10.1021/jf034344v>

584 An, C., Ma, S. jian, Chang, F., & Xue, W. jiao. (2017). Efficient production of pullulan by
585 *Aureobasidium pullulans* grown on mixtures of potato starch hydrolysate and sucrose.
586 *Brazilian Journal of Microbiology*, *48*(1), 180–185.
587 <https://doi.org/10.1016/j.bjm.2016.11.001>

588 Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy
589 multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-
590 phase extraction” for the determination of pesticide residues in produce. *Journal of*
591 *AOAC International*, *86*(2), 412–431. <https://doi.org/10.1021/jf00110a037>

592 Anese, M., Bortolomeazzi, R., Manzocco, L., Manzano, M., Giusto, C., & Nicoli, M. C.
593 (2009). Effect of chemical and biological dipping on acrylamide formation and sensory
594 properties in deep-fried potatoes. *Food Research International*, *42*, 142–147.
595 <https://doi.org/10.1016/j.foodres.2008.09.008>

596 AOAC. (1990). *Official methods of analyses*. Association of Official Analytical Chemists
597 (15th ed.). Washington, DC.

598 Braissant, O., Wirz, D., Beat, G., & Daniels, A. U. (2009). Use of isothermal

599 microcalorimetry to monitor microbial activities. *FEMS Microbiology Letters*, 303(1), 1–
600 8. <https://doi.org/10.1111/j.1574-6968.2009.01819.x>

601 Coelho, E. M., da Silva Padilha, C. V., Miskinis, G. A., de Sá, A. G. B., Pereira, G. E., de
602 Azevêdo, L. C., & dos Santos Lima, M. (2018). Simultaneous analysis of sugars and
603 organic acids in wine and grape juices by HPLC: Method validation and characterization
604 of products from northeast Brazil. *Journal of Food Composition and Analysis*,
605 66(December 2017), 160–167. <https://doi.org/10.1016/j.jfca.2017.12.017>

606 Deshpande, M. S., Rale, V. B., & Lynch, J. M. (1992). *Aureobasidium pullulans* in applied
607 microbiology: A status report. *Enzyme and Microbial Technology*, 14(7), 514–527.
608 [https://doi.org/10.1016/0141-0229\(92\)90122-5](https://doi.org/10.1016/0141-0229(92)90122-5)

609 Di Francesco, A., Calassanzio, M., Ratti, C., Mari, M., Folchi, A., & Baraldi, E. (2018).
610 Molecular characterization of the two postharvest biological control agents
611 *Aureobasidium pullulans* L1 and L8. *Biological Control*, 123(February), 53–59.
612 <https://doi.org/10.1016/j.biocontrol.2018.05.005>

613 Di Francesco, A., Mari, M., Ugolini, L., Parisi, B., Genovese, J., Lazzeri, L., & Baraldi, E.
614 (2019). Reduction of acrylamide formation in fried potato chips by *Aureobasidium*
615 *pullulans* L1 strain. *International Journal of Food Microbiology*, 289, 168–173.
616 <https://doi.org/10.1016/j.ijfoodmicro.2018.09.018>

617 Di Francesco, A., Ugolini, L., D’Aquino, S., Pagnotta, E., & Mari, M. (2017). Biocontrol of
618 *Monilinia laxa* by *Aureobasidium pullulans* strains: Insights on competition for nutrients
619 and space. *International Journal of Food Microbiology*, 248, 32–38.
620 <https://doi.org/10.1016/j.ijfoodmicro.2017.02.007>

621 Dourado, C., Pinto, C., Barba, F. J., Lorenzo, J. M., Delgadillo, I., & Saraiva, J. A. (2019).
622 Trends in Food Science & Technology Innovative non-thermal technologies affecting
623 potato tuber and fried potato quality. *Trends in Food Science & Technology*, 88(March),
624 274–289. <https://doi.org/10.1016/j.tifs.2019.03.015>

625 Dunlop, P. C., & Roon, R. J. (1975). L-Asparaginase of *Saccharomyces cerevisiae*: an
626 extracellular enzyme. *Journal of Bacteriology*, 122(3), 1017–1024.
627 <https://doi.org/10.1128/jb.122.3.1017-1024.1975>

628 Elmore, J. S., Briddon, A., Dodson, A. T., Muttucumaru, N., Halford, N. G., & Mottram, D.
629 S. (2015). Acrylamide in potato crisps prepared from 20 UK-grown varieties: Effects of
630 variety and tuber storage time. *Food Chemistry*, 182, 1–8.
631 <https://doi.org/10.1016/j.foodchem.2015.02.103>

632 European Commission. (2017). Commission Regulation (EU) 2017/2158 establishing

633 mitigation measures and benchmark levels for the reduction of the presence of acrylamide
634 in food. [https://doi.org/http://eur-](https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf)
635 [lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf](https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf)

636 Fauster, T., Schlossnikl, D., Rath, F., Ostermeier, R., Teufel, F., Toepfl, S., & Jaeger, H.
637 (2018). Impact of pulsed electric field (PEF) pretreatment on process performance of
638 industrial French fries production. *Journal of Food Engineering*, 235, 16–22.
639 <https://doi.org/10.1016/j.jfoodeng.2018.04.023>

640 Fincan, M., & Dejmek, P. (2003). Effect of osmotic pretreatment and pulsed electric field on
641 the viscoelastic properties of potato tissue. *Journal of Food Engineering*, 59, 169–175.
642 [https://doi.org/https://doi.org/10.1016/S0260-8774\(02\)00454-5](https://doi.org/https://doi.org/10.1016/S0260-8774(02)00454-5)

643 Food Drink Europe. (2019). Acrylamide Toolbox 2019. Retrieved September 20, 2019, from
644 [https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Ac-](https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf)
645 [rylamide_Toolbox_2019.pdf](https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf)

646 Foot, R. J., Haase, N. U., Grob, K., & Gondé, P. (2007). Acrylamide in fried and roasted
647 potato products: A review on progress in mitigation. *Food Additives and Contaminants*,
648 24(S1), 37–46. <https://doi.org/10.1080/02652030701439543>

649 Genovese, J., Tappi, S., Luo, W., Tylewicz, U., Marzocchi, S., Marziali, S., ... Rocculi, P.
650 (2019). Important factors to consider for acrylamide mitigation in potato crisps using
651 pulsed electric fields. *Innovative Food Science and Emerging Technologies*,
652 55(February), 18–26. <https://doi.org/10.1016/j.ifset.2019.05.008>

653 IARC. (1994). *Acrylamide. IARC monographs on the evaluation of carcinogenic risk to*
654 *humans. Some industrial chemicals* (Vol. 60). Retrieved from
655 <http://monographs.iarc.fr/ENG/Monographs/vol60/volume60.pdf>

656 Ignat, A., Manzocco, L., Brunton, N. P., Nicoli, M. C., & Lyng, J. G. (2015). The effect of
657 pulsed electric field pre-treatments prior to deep-fat frying on quality aspects of potato
658 fries. *Innovative Food Science and Emerging Technologies*, 29, 65–69.
659 <https://doi.org/10.1016/j.ifset.2014.07.003>

660 Janositz, A., Noack, A. K., & Knorr, D. (2011). Pulsed electric fields and their impact on the
661 diffusion characteristics of potato slices. *LWT - Food Science and Technology*, 44(9),
662 1939–1945. <https://doi.org/10.1016/j.lwt.2011.04.006>

663 Mattar, J. R., Turk, M. F., Nonus, M., Lebovka, N. I., El, H., & Vorobiev, E. (2015). S .
664 cerevisiae fermentation activity after moderate pulsed electric field pre-treatments.
665 *Bioelectrochemistry*, 103, 92–97. <https://doi.org/10.1016/j.bioelechem.2014.08.016>

666 Mattar, J. R., Turk, M. F., Nonus, M., Lebovka, N. I., Zakhem, H. El, & Vorobiev, E. (2014).

667 Stimulation of *Saccharomyces cerevisiae* Cultures by Pulsed Electric Fields. *Food and*
668 *Bioprocess Technology*, 7(11), 3328–3335. <https://doi.org/10.1007/s11947-014-1336-4>

669 McGuire, R. G. (1992). Reporting of Objective Color Measurements. *Horticultural Science*,
670 27(12), 1254–1255. <https://doi.org/10.21273/hortsci.27.12.1254>

671 Medeiros Vinci, R., Mestdagh, F., & De Meulenaer, B. (2012). Acrylamide formation in fried
672 potato products - Present and future, a critical review on mitigation strategies. *Food*
673 *Chemistry*, 133, 1138–1154. <https://doi.org/10.1016/j.foodchem.2011.08.001>

674 Morozova, K., Andreotti, C., Armani, M., Cavani, L., Cesco, S., Cortese, L., ... Mimmo, T.
675 (2017). Indirect effect of glyphosate on wine fermentation studied by microcalorimetry.
676 *Journal of Thermal Analysis and Calorimetry*, 127(2), 1351–1360.
677 <https://doi.org/10.1007/s10973-016-5891-y>

678 Nielsen, N. J., Granby, K., Hedegaard, R. V., & Skibsted, L. H. (2006). A liquid
679 chromatography–tandem mass spectrometry method for simultaneous analysis of
680 acrylamide and the precursors, asparagine and reducing sugars in bread. *Analytica*
681 *Chimica Acta*, 557, 211–220. <https://doi.org/https://doi.org/10.1016/j.aca.2005.09.077>

682 O'Connor, C. J., Fisk, K. J., Smith, B. G., & Melton, L. D. (2001). Fat Uptake in French Fries
683 as Affected by Different Potato Varieties and Processing. *Journal of Food Science*,
684 66(6), 903–908.

685 Palermo, M., Gökmen, V., De Meulenaer, B., Ciesarová, Z., Zhang, Y., Pedreschi, F., &
686 Fogliano, V. (2016). Acrylamide mitigation strategies: Critical appraisal of the
687 FoodDrinkEurope toolbox. *Food and Function*, 7(6), 2516–2525.
688 <https://doi.org/10.1039/c5fo00655d>

689 Panarese, V., Tylewicz, U., Santagapita, P., Rocculi, P., & Dalla Rosa, M. (2012). Isothermal
690 and differential scanning calorimetries to evaluate structural and metabolic alterations of
691 osmo-dehydrated kiwifruit as a function of ripening stage. *Innovative Food Science and*
692 *Emerging Technologies*, 15, 66–71. <https://doi.org/10.1016/j.ifset.2012.04.001>

693 Parker, J. K., Balagiannis, D. P., Higley, J., Smith, G., Wedzicha, B. L., & Mottram, D. S.
694 (2012). Kinetic model for the formation of acrylamide during the finish-frying of
695 commercial French fries. *Journal of Agricultural and Food Chemistry*, 60(36), 9321–
696 9331. <https://doi.org/10.1021/jf302415n>

697 Pedreschi, F., Mariotti, S., Granby, K., & Risum, J. (2011). Acrylamide reduction in potato
698 chips by using commercial asparaginase in combination with conventional blanching.
699 *LWT - Food Science and Technology*, 44, 1473–1476.
700 <https://doi.org/10.1016/j.lwt.2011.02.004>

701 Pedreschi, F., Moyano, P., Kaack, K., & Granby, K. (2005). Color changes and acrylamide
702 formation in fried potato slices. *Food Research International*, 38(1), 1–9.
703 <https://doi.org/10.1016/j.foodres.2004.07.002>

704 Puértolas, E., Koubaa, M., & Barba, F. J. (2016). An overview of the impact of
705 electrotechnologies for the recovery of oil and high-value compounds from vegetable oil
706 industry: Energy and economic cost implications. *Food Research International*, 80, 19–
707 26. <https://doi.org/10.1016/j.foodres.2015.12.009>

708 Raso, J., Frey, W., Ferrari, G., Pataro, G., Knorr, D., Teissie, J., & Miklavčič, D. (2016).
709 Recommendations guidelines on the key information to be reported in studies of
710 application of PEF technology in food and biotechnological processes. *Innovative Food
711 Science and Emerging Technologies*, 37, 312–321.
712 <https://doi.org/10.1016/j.ifset.2016.08.003>

713 Romani, S., Bacchiocca, M., Rocculi, P., & Dalla Rosa, M. (2009). Influence of frying
714 conditions on acrylamide content and other quality characteristics of French fries.
715 *Journal of Food Composition and Analysis*, 22, 582–588.
716 <https://doi.org/10.1016/j.jfca.2008.10.001>

717 Schottroff, F., Krottenthaler, A., & Jaeger, H. (2017). Stress Induction and Response,
718 Inactivation, and Recovery of Vegetative Microorganisms by Pulsed Electric Fields. In
719 D. Miklavcic (Ed.), *Handbook of Electroporation* (pp. 1–19). Cham: Springer
720 International Publishing. https://doi.org/10.1007/978-3-319-26779-1_183-1

721 Sheng, L., Tong, Q., & Ma, M. (2016). Why sucrose is the most suitable substrate for pullulan
722 fermentation by *Aureobasidium pullulans* CGMCC1234?. *Enzyme and Microbial
723 Technology*, 92, 49–55. <https://doi.org/10.1016/j.enzmictec.2016.06.016>

724 Stadler, R. H., & Scholz, G. (2004). Acrylamide: An update on current knowledge in analysis,
725 levels in food, mechanisms of formation, and potential strategies of control. *Nutrition
726 Reviews*, 62(12), 449–467. <https://doi.org/10.1301/nr.2004.janr.449-467>

727 Stadler, R. H., & Studer, A. (2016). Acrylamide Formation Mechanisms. In V. Gökmen (Ed.),
728 *Acrylamide in Food: Analysis, Content and Potential Health Effects* (1st ed., pp. 1–17).
729 Elsevier Inc. <https://doi.org/10.1016/B978-0-12-802832-2.00001-2>

730 Stirke, A., Zimkus, A., Ramanaviciene, A., Balevicius, S., Zurauskiene, N., Saulis, G., ...
731 Ramanavicius, A. (2014). Electric field-induced effects on yeast cell wall
732 permeabilization. *Bioelectromagnetics*, 35(2), 136–144.
733 <https://doi.org/10.1002/bem.21824>

734 Tylewicz, U., Mannozi, C., Romani, S., Castagnini, J. M., Samborska, K., Rocculi, P., &

735 Dalla Rosa, M. (2019). Chemical and physicochemical properties of semi-dried organic
736 strawberries enriched with bilberry juice-based solution. *LWT - Food Science and*
737 *Technology*, 114(February), 108377. <https://doi.org/10.1016/j.lwt.2019.108377>

738 Vivanti, V., Finotti, E., & Friedman, M. (2006). Level of Acrylamide Precursors Asparagine ,
739 Fructose , Glucose , and Sucrose in Potatoes Sold at Retail in Italy and in the United
740 States. *Food Chemistry and Toxicology*, 71(2), 81–85.

741 Vorobiev, E., & Lebovka, N. (2010). Enhanced extraction from solid foods and
742 biosuspensions by pulsed electrical energy. *Food Engineering Reviews*, 2(2), 95–108.
743 <https://doi.org/10.1007/s12393-010-9021-5>

744 Wang, M., Wang, L., Bekhit, A. E. A., Yang, J., Hou, Z., Wang, Y., ... Zeng, X. (2018). A
745 review of sublethal effects of pulsed electric field on cells in food processing. *Journal of*
746 *Food Engineering*, 223, 32–41. <https://doi.org/10.1016/j.jfoodeng.2017.11.035>

747 Yang, Y., Achaerandio, I., & Pujolà, M. (2016). Influence of the frying process and potato
748 cultivar on acrylamide formation in French fries. *Food Control*, 62, 216–223.
749 <https://doi.org/10.1016/j.foodcont.2015.10.028>

750 Yee, N. G., & Bussell, W. T. (2007). Good Potatoes for Good Potato Crisps , a Review of
751 Current Potato Crisp Quality Control and Manufacture. *Food*, 1(2), 271–286.

752 Zhou, W., Wang, M., Chen, J., & Zhang, R. (2015). The effect of biological (yeast) treatment
753 conditions on acrylamide formation in deep-fried potatoes. *Food Science and*
754 *Biotechnology*, 24(2), 561–566. <https://doi.org/10.1007/s10068-015-0073-4>

755

756

757 **Figure 1.** Scheme of processing pre-treatments steps studied for the acrylamide reduction in
758 fried potato chips and corresponding samples codes.

759

760 **Figure 2.** Schematic representation of the combinations of sample and substrate evaluated by
761 isothermal calorimetry as preliminary study to assess the possibility of combining PEF and
762 yeast dipping pre-treatments (NYDB: dark blue; Water: light blue; Untreated Yeast: green;
763 PEF-treated Yeast: light red; Untreated Potato: yellow; PEF-treated Potato: dark red).

764

765 **Figure 3.** HPLC-MS/MS chromatogram of a standard mixture of acrylamide, asparagine and
766 sugars plotted as overlapped multiple reaction monitoring (MRM) transition of each compound.

767

768 **Figure 4.** Thermal profiles measured by isothermal calorimetry during growth of *A. pullulans*
769 L1 strain subjected to PEF treatment, compared to its control growth in an ideal (A) and in real
770 substrate (B).

771

772 **Figure 5.** Examples of RGB images of potato chips samples untreated and differently treated
773 (A) and of images conversion from RGB into CIE $L^*a^*b^*$ channels (B).

Fig. 1

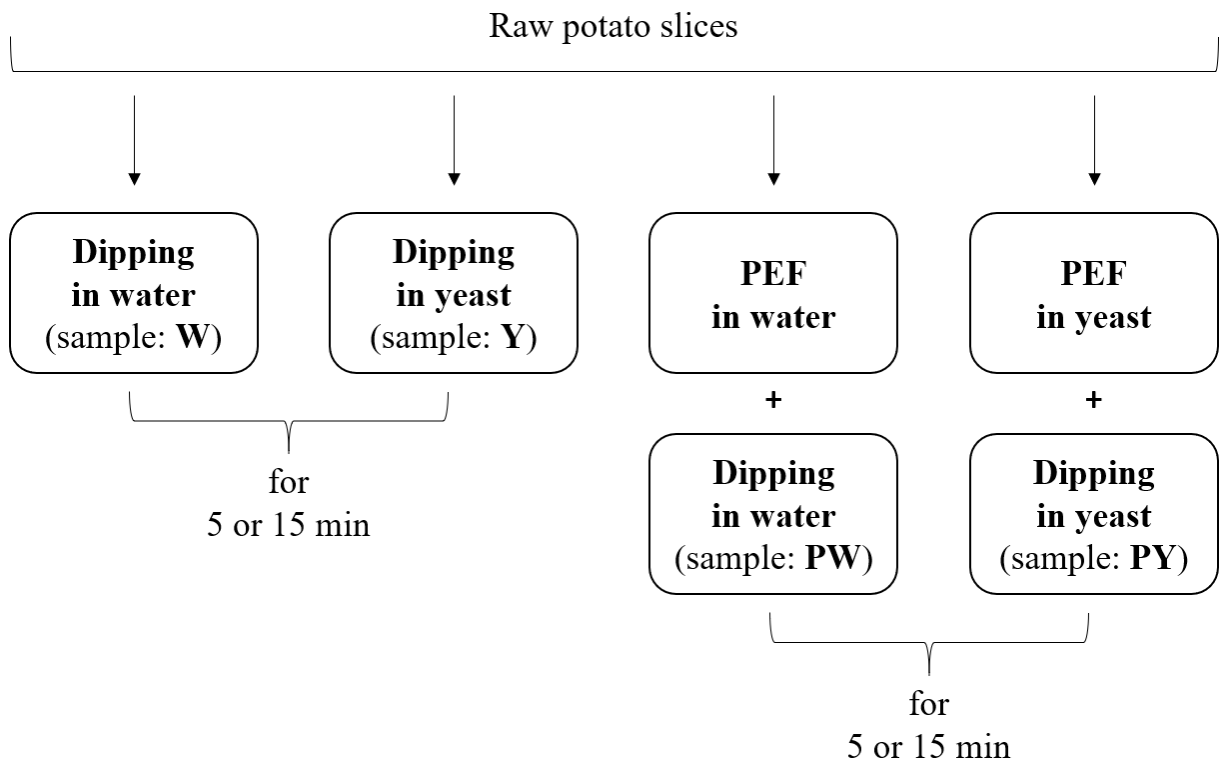


Fig. 2

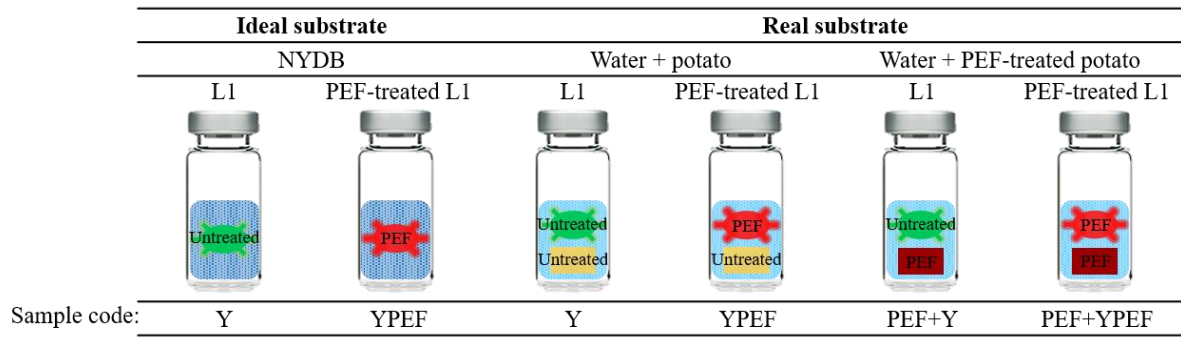


Fig. 3

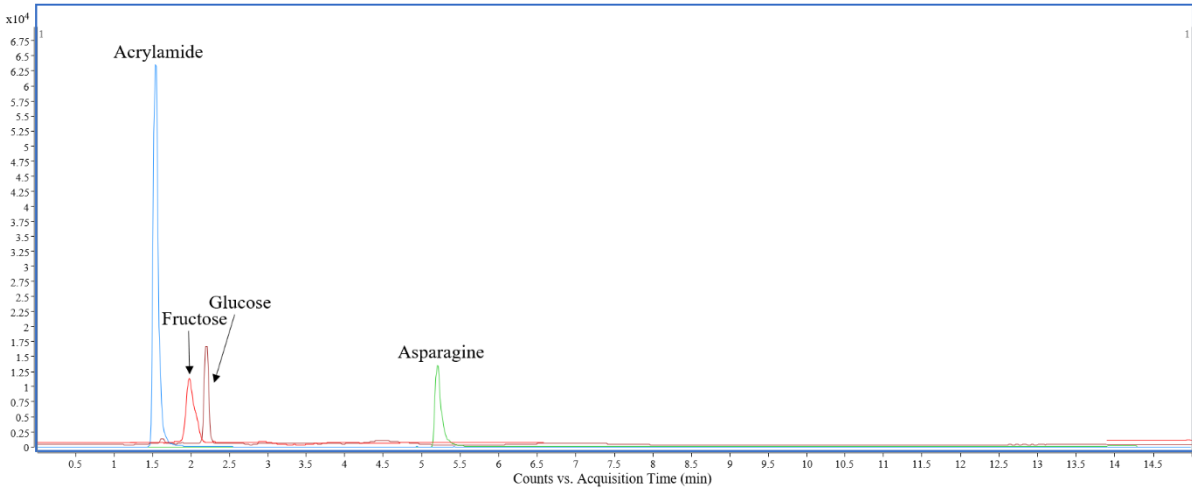


Fig. 4

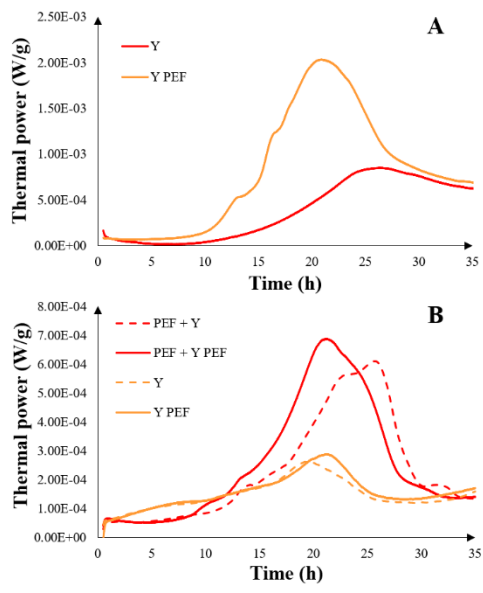


Fig. 5

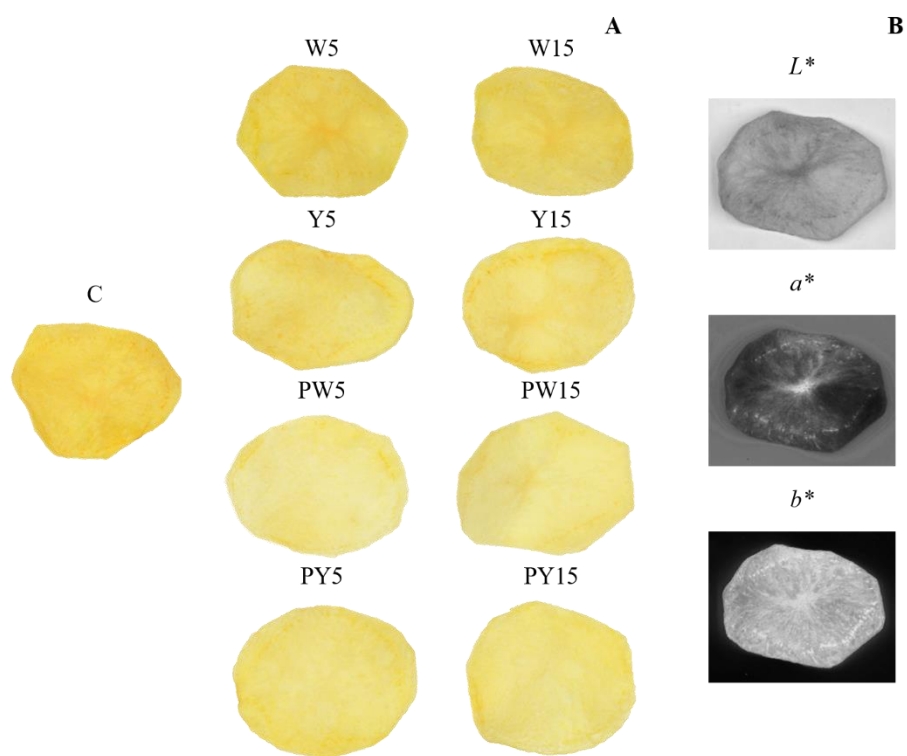


Table 1. HPLC-MS/MS acquisition parameters (MRM mode) adopted for the quantification of acrylamide, asparagine, and sugars.

Compound	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	Fragmentor (V)	Collision Energy	Retention Time	Polarity
Acrylamide	72	55	45	8	1.52	Positive
Fructose	203	203	80	0	1.96	Positive
Glucose	203	203	80	0	2.18	Positive
Asparagine	133	74* 98	64	16 16	5.17	Positive

*This product ion was used for quantitative analysis; the rest for confirmatory analysis.

Table 2. Calorimetric parameters (heat and slope) measured during *A. pullulans* L1 growth in ideal (NYDB) and real (water+potato) substrates after PEF treatment compared to the control (Y: untreated yeast; YPEF: PEF-treated yeast; PEF: PEF-treated potato).

		Ideal substrate			
		Y	YPEF		
Heat (J/g)		24.7 ± 2.8 ^a	45.8 ± 0.7 ^b		
Slope (W/g·h)		5.5 ± 0.7·10 ^{-5a}	2·10 ⁻⁴ ± 0.1 ^b		
		Real substrate			
		Y	YPEF	PEF + Y	PEF + YPEF
Heat (J/g)		17.9 ± 0.7 ^a	19.0 ± 0.9 ^a	31.6 ± 4.0 ^b	33.2 ± 2.3 ^b
Slope (W/g·h)		1·10 ⁻⁵ ± 0.1 ^a	1.5 ± 7·10 ^{-5a}	6.5 ± 4·10 ^{-5b}	5.0 ± 1·10 ^{-5b}

Different letters in the same line indicate significant differences among samples according to Tukey multiple post-hoc comparison ($p < 0.05$).

Table 3. Acrylamide levels in fried potato samples and acrylamide precursors contents in raw potato samples, untreated (C: control) and differently pre-treated at both 5 and 15 min (W: dipping in water; Y: dipping in yeast aqueous suspension; PW: PEF in water + dipping in water; PY: PEF in yeast aqueous suspension + dipping in yeast aqueous suspension).

Sample	Time (min)	Acrylamide ($\mu\text{g}/\text{kg}$)	Glucose (mg/kg)	Fructose (mg/kg)	Asparagine (mg/kg)
C		$1384.3 \pm 65.0^{\text{a}}$	$33.6 \pm 6.8^{\text{abc}}$	$8.4 \pm 1.0^{\text{c}}$	$8826.3 \pm 576.0^{\text{b}}$
W	5	$1292.4 \pm 96.2^{\text{bc}}$	$19.6 \pm 0.4^{\text{d}}$	$6.9 \pm 0.7^{\text{d}}$	$12952.0 \pm 1341.9^{\text{a}}$
	15	$775.3 \pm 81.5^{\text{d}}$	$29.50 \pm 5.0^{\text{c}}$	$7.8 \pm 1.1^{\text{cd}}$	$12036.4 \pm 2142.5^{\text{a}}$
Y	5	$1375.9 \pm 9.9^{\text{ab}}$	$39.5 \pm 4.1^{\text{a}}$	$16.4 \pm 0.7^{\text{a}}$	$12945.3 \pm 441.7^{\text{a}}$
	15	$676.4 \pm 42.3^{\text{e}}$	$18.6 \pm 0.9^{\text{d}}$	$3.8 \pm 0.2^{\text{f}}$	$5957.5 \pm 135.6^{\text{c}}$
PW	5	$886.8 \pm 9.9^{\text{d}}$	$36.7 \pm 0.88^{\text{ab}}$	$5.0 \pm 0.1^{\text{ef}}$	$4108.9 \pm 571.3^{\text{c}}$
	15	$572.0 \pm 8.8^{\text{f}}$	$17.8 \pm 3.0^{\text{d}}$	$5.4 \pm 0.4^{\text{e}}$	$5875.0 \pm 695.9^{\text{c}}$
PY	5	$1211.9 \pm 4.2^{\text{c}}$	$33.53 \pm 1.2^{\text{abc}}$	$5.7 \pm 0.2^{\text{e}}$	$4188.0 \pm 69.9^{\text{c}}$
	15	$1193.2 \pm 20.1^{\text{c}}$	$31.1 \pm 1.2^{\text{bc}}$	$14.1 \pm 0.5^{\text{b}}$	$4360.8 \pm 87.9^{\text{c}}$

Different letters in the same columns indicate significant differences among samples according to Tukey multiple post-hoc comparison ($p < 0.05$).

Table 4. Colour parameters (L^* , h°), texture values (hardness, index of crispness), moisture and oil content of untreated (C: control) and treated potato chips (W: dipping in water; Y: dipping in yeast aqueous suspension; PW: PEF in water + dipping in water; PY: PEF in yeast aqueous suspension + dipping in yeast aqueous suspension).

Sample	Time (min)	Raw potato	Fried potato					
		Moisture (%)	Moisture (%)	L^*	h°	Hardness (N)	Index of crispness (linear distance)	Oil (%)
C		74.1 ± 0.5 ^b	4.1 ± 0.1 ^{ab}	84.5 ± 1.1 ^b	93.2 ± 1.7 ^{ef}	3.9 ± 1.1 ^a	9.3 ± 2.6 ^a	30.3 ± 1.3 ^d
W	5	78.9 ± 0.4 ^a	4.2 ± 0.1 ^a	84.5 ± 1.2 ^b	94.6 ± 0.9 ^{de}	2.9 ± 0.4 ^{bc}	8.0 ± 1.7 ^{ab}	37.3 ± 0.1 ^b
	15	79.9 ± 0.8 ^a	4.1 ± 0.1 ^{ab}	87.1 ± 0.2 ^a	94.9 ± 0.6 ^{cd}	3.6 ± 0.8 ^{ab}	8.8 ± 2.6 ^{ab}	34.3 ± 0.6 ^c
Y	5	78.1 ± 0.7 ^a	4.0 ± 0.4 ^{ab}	84.3 ± 1.2 ^b	92.8 ± 0.8 ^f	3.7 ± 0.8 ^{ab}	9.7 ± 1.5 ^a	34.7 ± 0.8 ^c
	15	79.2 ± 0.2 ^a	3.7 ± 0.1 ^b	86.0 ± 1.3 ^{ab}	96.4 ± 1.1 ^{bc}	3.3 ± 0.6 ^{abc}	9.7 ± 2.4 ^a	33.5 ± 0.6 ^c
PW	5	74.5 ± 1.3 ^b	2.8 ± 0.1 ^c	86.1 ± 0.7 ^{ab}	98.9 ± 0.3 ^a	2.6 ± 0.7 ^{bc}	6.6 ± 2.1 ^b	41.3 ± 0.9 ^a
	15	75.6 ± 0.9 ^b	2.7 ± 0.3 ^c	86.4 ± 1.1 ^{ab}	97.8 ± 0.9 ^{ab}	3.1 ± 0.9 ^{bc}	9.1 ± 2.2 ^a	37.5 ± 0.8 ^b
PY	5	74.5 ± 0.9 ^b	2.5 ± 0.2 ^c	84.7 ± 1.3 ^b	93.5 ± 0.2 ^{def}	2.9 ± 0.7 ^{bc}	7.6 ± 2.9 ^{ab}	41.9 ± 0.8 ^a
	15	75.0 ± 0.4 ^b	2.5 ± 0.4 ^c	85.2 ± 0.8 ^{ab}	93.4 ± 0.2 ^{def}	3.0 ± 0.8 ^{bc}	8.8 ± 2.8 ^{ab}	38.4 ± 0.5 ^b

Different letters in the same columns indicate significant differences among samples according to Tukey multiple post-hoc comparison ($p < 0.05$).

Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: