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

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### 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 ( $> 120\text{ }^{\circ}\text{C}$ ) 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  $1.5 \pm 0.2$  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 ( $18 \pm 2$  °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  $\mu\text{s}$ ), 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 ( $h^\circ$ ) 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 ( $\mu$ 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  $\alpha$ -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  $\beta$ -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  $h^\circ$  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

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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

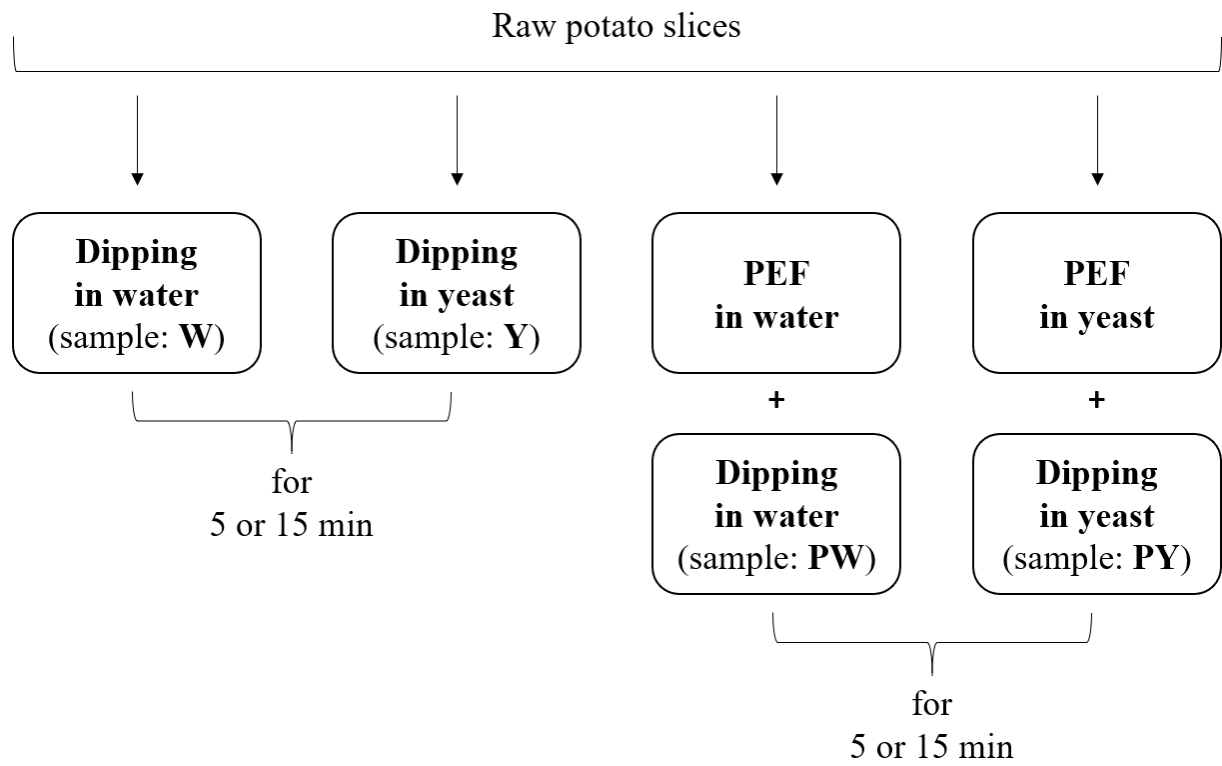


Fig. 2

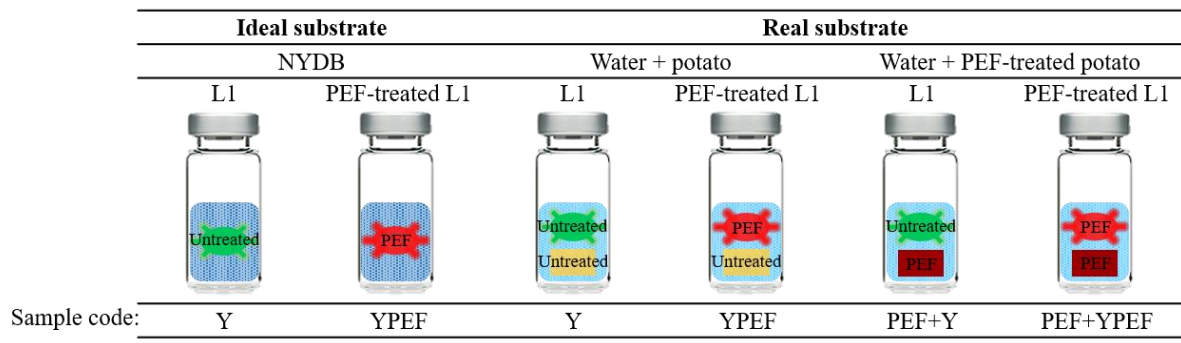


Fig. 3

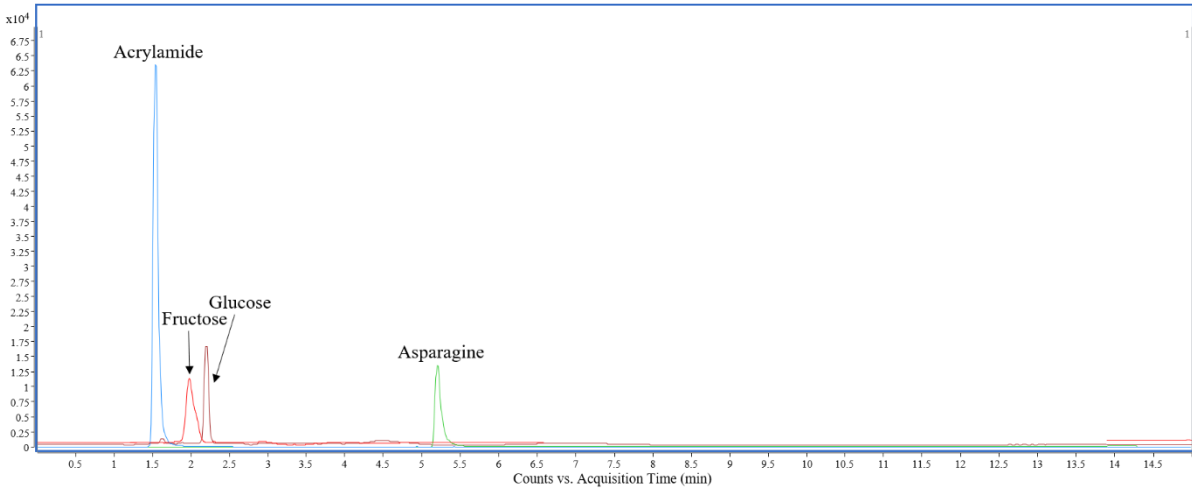


Fig. 4

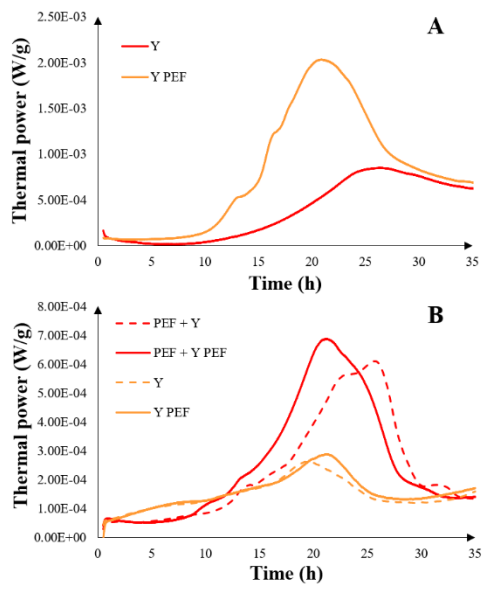
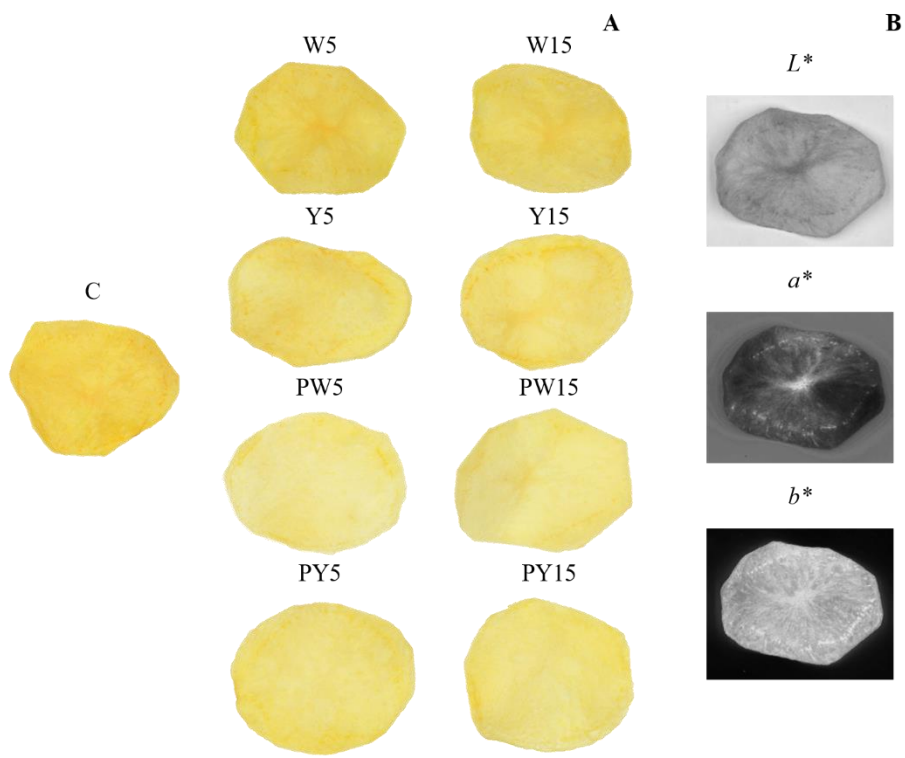


Fig. 5





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

<b>Compound</b>	<b>Precursor Ion (<i>m/z</i>)</b>	<b>Product Ion (<i>m/z</i>)</b>	<b>Fragmentor (V)</b>	<b>Collision Energy</b>	<b>Retention Time</b>	<b>Polarity</b>
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).

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

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

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

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: