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EFFECT OF PRE-TREATMENTS AND AIR-FRYING, A NOVEL TECHNOLOGY, ON ACRYLAMIDE GENERATION IN FRIED POTATOES

Sansano, M., Juan-Borrás, M., Escriche, I., Andrés, A., Heredia, A.*

Institute of Food Engineering for Development, Universitat Politècnica de València,

P.O. Box 46022 Valencia, Spain

* Corresponding author. Tel.: +34 963877365; fax: +34 963877369

E-mail address: anhegu@tal.upv.es

Short tittle: Acrylamide generation in air-frying

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14 **Abstract**

15 This paper investigated the effect of air-frying technology, in combination with a pre-

16 treatment based of soaking the samples in different chemical agent solutions (citric acid,

17 glycine, calcium lactate, sodium chloride or nicotinic acid (vitamin B3)), on the

18 generation of acrylamide in fried potatoes. The influence of reducing sugars on the

19 development of surface's color was also analyzed. The experiments were conducted at

20 180 ºC by means of air-frying and deep oil-frying, as a reference technology. Based on

21 the evolution of colour crust with frying time, it could be concluded that the rate of

22 Maillard reaction decreased as the initial reducing sugars content increased in the raw

23 material, and was also lower for deep-oil frying than for air-frying regardless of pre-

24 treatments applied. Air-frying reduced acrylamide content by about 90% compared with

25 conventional deep-oil frying without being necessary the application of a pre-treatment.

26 However, deep-oil fried potatoes pre-treated with solutions of nicotinic acid, citric acid,

27 glycine at 1% and NaCl at 2% presented much lower acrylamide levels (up to 80-90%

28 reduction) than non-pretreated samples.

29

30 **Keywords:** acrylamide, air-frying, additives, reducing sugars, color

31

32   **Introduction**

33   In June 2010, the European Union published recommendations for the control of

34   acrylamide content in food (2010/307/UE). This publication was the result of the

35   adventitious discovery of the excessive incidence of acrylamide in different foods,

36   mainly submitted to frying and baking, by researchers of the Stockholm University and

37   the publication of analytical data of various foods on the web site of the Swedish

38   National Food Agency in 2002 (Rosén and Hellenäs 2002). According to

39   epidemiological studies, the toxicity of acrylamide, a probable carcinogenic compound

40   for humans classified in Group 2A (IARC), it is not only due to its consumption, but

41   also to its role as a precursor in the development of other compounds during hepatic

42   metabolism e.g. glycidamide (Blank 2005).

43   Acrylamide is an early product in Maillard reaction which is produced by the reaction

44   between the amino acid asparagine and reducing sugars (glucose, fructose etc.) when

45   food is subjected to temperatures above 120 ° C in processes such as frying or baking

46   (Yaylayan and others 2003). Acrylamide generation is especially relevant in processed

47   potato products due to the high presence of the precursors (asparagine and reducing

48   sugars) in the matrix. The presence of the precursors can vary depending on the variety,

49   soil conditions and postharvest storage conditions (Kumar and others 2004).

50   The influence of the main frying variables (oil temperature, frying time, potato:oil ratio,

51   surface:volume ratio, etc.) on acrylamide generation has been extensively studied over

52   the past ten years (Bråthen, and Knutsen 2005; Gökmen, and Palazoglu 2009), as well

53   as its relationship with potato properties such as pH, water activity, capillarity and

54   porosity (Low and others 2006). It has been reported that the higher the frying

55   temperature and the lower the moisture content in the potato, the higher the acrylamide

56   content in the fried product (Pedreschi and others 2005). Some alternatives such as low

57 pressure-frying (Troncoso and Pedreschi 2009; Dueik and others 2012), microwave

58 assisted frying or microwave pre-thawing (Ngadi and others 2009; Tuta and others

59 2010), and pre-drying (Pedreschi and Moyano, 2005) have been studied in order to

60 obtain healthier fried products in terms of fat and/or acrylamide content. Air-frying is a

61 technology which permits the reduction of fat content in fried products by 90% (Andrés

62 and others 2012). The technology consists of direct contact between the product and a

63 dispersion of oil droplets in hot air in a frying chamber. The product is constantly in

64 motion to promote homogeneous contact between both phases. In this way, the product

65 is dehydrated and the typical crust of fried products gradually appears (Andrés and

66 others 2012). To the authors' knowledge, no publication provides scientific information

67 about the impact of this technology on acrylamide generation.

68 The application of a treatment prior to frying for acrylamide reduction has raised

69 interest in recent years. Some pre-treatments based on the immersion of the product in

70 solutions with chemical agents in order to mitigate acrylamide generation during frying

71 could be grouped according to the following objectives: (I) to reduce the content of

72 asparagine and reducing sugars in the raw material e.g. by blanching (Pedreschi and

73 others 2005); (II) to enzymatically hydrolize asparagine i.e. asparaginase (Zyzak and

74 others 2003, Ciesarova and others 2006, Pedreschi and others 2011); (III) to add amino

75 acids (e.g. glycine (Low and others 2006)) or cations (e.g. Ca^{2+} or Na^+: (Gökmen and

76 Senyuva 2007) able to compete with asparagine in Maillard reaction and (IV) to

77 kinetically control the rate of acrylamide formation by pH reduction (Stadler and Scholz

78 2004) or by lactic acid fermentation (Anese and others 2009; Bartkiene and others

79 2012). The criteria taken into account to select the substances employed in this study to

80 perform pre-treatments were mainly two: (i) the number of studies in which the

81 substance was used and its effectiveness. This is the case of blanching, citric acid or

82 sodium chloride; (ii) the functionality of the substance. In this sense, the incorporation

83 of a bioactive compound such as glycine, nicotinic acid (vitamin B3) or calcium during

84 pre-treatment could contribute to the functionality of the final fried product.

85 Moreover, it is important to point out that the majority of published studies concerning

86 the effect of pre-treatments by immersion in different additives to reduce acrylamide

87 have been carried out in model systems, but not in real foods in which the structure

88 could play an important role. In this study, the advantages of the application of these

89 different pre-treatments could accurately be established because of all of them are

90 applied at the same frying conditions and raw material.

91 The main objective of this study was to evaluate the effect of air-frying, in combination

92 with different chemical pre-treatments, on acrylamide reduction in a real food matrix

93 such as fried potato.

94

95 **Materials and Methods**

96 Reagents

97 Citric acid, nicotinic acid and acrylamide standard ($\geq$ 99%) were obtained from Merck

98 (Darmstadt, Germany), calcium lactate and sodium chloride from Scharlab (Barcelona,

99 Spain). Glycine, L (+) potassium sodium tartrate tetrahydrate, formic acid (99-100%

100 purity) and magnesium sulfate were purchased from VWR (Fontenay-sous-Bois,

101 France). 3.5-dinitrosalicylic acid (DNS) and hexane were from Panreac (Barcelona,

102 Spain). Primary secondary amine (PSA) was obtained from Supelco (Bellefonte).

103 Double distilled water was prepared for chromatographic use (Milli-Q, Millipore Corp.,

104 Bedford, MA). All chemicals used were analytical grade, and those used for

105 chromatographic analysis were HPLC grade. The acrylamide standard was purchased

106 from Merck (Germany), the stock solution (1mg mL^{-1}) was prepared by dissolving 100

107   mg of the acrylamide in 100 mL of acetonitrile, and was kept at 4ºC until use. All

108   working solutions were prepared daily by serial dilution in acetonitrile.

109   Raw material

110   The potatoes (*Solanum tuberosum* L., Frisia variety) were provided by a local supplier.

111   This variety was chosen because of its availability during all the year in Spain. This

112   variety is characterized by yellow skin, light yellow-white flesh and oval shape. The

113   potatoes were sorted, washed, peeled, cut into 7 mm thick slices and cored with a

114   stainless steel core borer (25 mm in diameter) to produce discs. The experiments were

115   performed in two different periods of time: March-April and May-June being necessary

116   to analyze the total reducing sugars content of potatoes used in each period due to the

117   relevance of these compounds in acrylamide generation as well as color changes along

118   frying.

119   Experimental Methodology

120   *Pre-treatments*

121   The potato discs were subjected to various treatments prior to frying. Specifically,

122   samples were blanched at 85 °C for 5 minutes or immersed in solutions of different

123   chemical agents: citric acid, glycine, calcium lactate, sodium chloride or nicotinic acid

124   at 1 and 2% for 60 minutes at room temperature. Likewise, a control consisting of

125   dipping the samples in distilled water at room temperature for 60 minutes was also

126   performed. In all cases, the potato:solution ratio was 1:5 (w/w).

127   The potato discs were removed from the solution and the excess liquid was soaked up

128   with absorbent paper for two minutes prior to frying.

129   *Frying Step*

130   The experiments were carried at a fixed frying temperature of 180 °C in a commercial

131   deep oil fryer (model: FM 6720 Ideal 2000 Professional, Solac with a nominal power of

132   2,000 W) and hot air frying equipment (model: AH-9000 Actifry, Tefal with a nominal

133   power of 1,400 W). For deep oil experiments, a potato-to-oil ratio of 1:20 (w/v) was

134   used. Therefore, a total amount of 2 L of oil was employed to deep-oil fry 100 g of

135   potatoes. Commercial sunflower oilseed was used in all frying experiments. This ratio

136   was large enough to avoid important changes in terms of product-to-oil ratio and

137   therefore in the oil composition and temperature. The oil was renewed every 2

138   experiments. For hot air frying experiments, 0.003 kg of oil per kilogram of potatoes

139   was added to the air chamber according to the specifications of the equipment, i.e. a

140   total amount of 0.3 g of oil per 100 g of potatoes. A constant frying temperature was

141   confirmed by means of two PT-100 temperature sensors (model: TF101K) located at the

142   top and the bottom of each fryer. Samples were immersed in the fryers when the initial

143   frying temperature of 180 °C was achieved. Each experiment was conducted in

144   triplicate.

145   The concentration of reducing sugars was determined in raw potatoes in order to

146   evaluate its influence on color development. Color development was based on the

147   CIEL*a*b* colorimetric characteristics of fried potatoes. A reference frying time was

148   complementary established based on a visual evaluation of color on the surface and the

149   external (crispy outer crust) and internal cooking level of the samples. For this purpose,

150   samples were removed from the fryers for color determination at different time intervals

151   depending on the frying technology and initial reducing sugars content. Concurrently,

152   samples with low reducing sugar content were removed every 2 minutes until 16

153   minutes and at minute 26 for deep oil-frying conditions, and every 2 minutes until 24

154   minutes and at minute 34 for air frying. Samples with high reducing sugar content were

155   removed every minute until minute 7 for deep oil-frying, and every 2 minutes until

156   minute 20 for air frying. The sampling time was chosen according to a previous study

157　on mass and heat transfer phenomena for both frying technologies (Andrés and others

158　2012). Before the analytical determinations, the excess oil was removed from the

159　samples with absorbent paper.

160　Determination of the acrylamide contents was only carried out at the reference frying

161　time.

162　Analytical Determinations

163　All determinations were carried out in triplicate.

164　*Optical properties*

165　The determination of the optical properties of the potato disks in each experiment and

166　frying time was carried out on the surface of the samples by means of a

167　spectrocolorimeter (MINOLTA, mod. CM-3600d). The color space coordinates

168　CIEL*a*b* were obtained from the absorption spectrum between 380 and 770 nm by

169　reflectance with the reference system: D65 illuminant and 10 ° observer, and a 7 mm

170　lens. Previously, samples were measured with both black and white calibration tiles in

171　order to study the possible translucency of the samples. Since the same spectrum was

172　obtained with the black and white tiles, the opacity of the samples was confirmed, and

173　only data corresponding to the black tile were analyzed.

174　*Reducing sugars content*

175　The reducing sugars content was determined by spectrophotometry (model V-630;

176　Jasco Inc., Tokyo (Japan) according to the protocol described by Miller (1959). This

177　colorimetric method is based on the reduction of acid 3.5-dinitrosalicylic by reducing

178　sugars. 90 g of Rochelle salt (L (+) potassium sodium tartrate tetrahydrate) were added

179　to 210 mL of dinitrosalicylic acid solution (2.3 % of NaOH and 1.4 % dinitrosalicylic

180　acid (w/w) in distilled water) and made up to 300 mL. In order to carry out the

181　determinations, 0.3 g of homogenized potato sample was mixed with 1 mL of distilled

182 water and 2 mL of dinitrosalicylic acid solution. A blank consisting of 1mL of distilled

183 water and 2 mL of dinitrosalicylic acid solution was also prepared. After that, the mix

184 was heated for 5 minutes in boiling water and then cooled at room temperature for 10

185 minutes. Finally, 1 mL of the mix was diluted in 4 mL of distilled water and the

186 absorbance determined at 546 nm. The quantification of reducing sugars content (g/100

187 g of potatoes) was calculated using equation 1:

188 Reducing sugars (g/100 g of potatoes)=(Absorbance - 0.00385) x 1.07893   eq. (1)

189 *Analysis of acrylamide*

190 The acrylamide content determinations were carried out using the method of dispersive

191 solid phase extraction called QuEChERS, originally designed for pesticide analysis in

192 food and modified by Mastovska and Lehotay (2006) for the extraction of acrylamide,

193 following the method described by Al-Tasher (2011) (Agilent Technologies, Note of

194 Application) with some modifications. Three potato disks were ground in a blender and

195 a sub-sample (2 g) of potato was placed in a 50mL Falcon tube and 5 mL of n-hexane

196 was added. The tube was shaken in a vortex for 30 seconds and after that 10 ml

197 bidistilled water, 10 mL acetonitrile, 4 g MgSO4 and 0.5 g NaCl were added and stirred

198 in the vortex for one minute. The suspension was then centrifuged at 2026 RCF

199 (Centronic BL II (Selecta, Spain)) for 5 minutes, the hexane layer (upper phase) was

200 discarded and 1mL of the acetonitrile phase, containing the acrylamide, was transferred

201 to a 2 mL polypropylene tube containing 50 mg PSA and 150 mg MgSO4, and stirred

202 for 30 seconds. The homogenate was centrifuged at 2697 RCF (Labofuge 200 (Heraeus,

203 Germany)) for 1 minute and the supernatant was transferred to a vial for analysis by

204 LC/MS/MS. The vials were amber type with a 1.5 mL of capacity.

205 The chromatographic analysis was performed with an Agilent 1200 Series HPLC

206 system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent

207   Technologies Inc., CA, USA) with an electrospray type ionization source. The column

208   used in this study was a Zorbax Eclipse XDB C-18 (2.1mmx50mm, 1.8μm). The mobile

209   phase used consisted of 2.5% methanol and 97.5% aqueous formic acid (0.1%) (A) and

210   methanol (B). The elution gradient was as follows: 0-4 min 100% of A; 4.1-6 min 70%

211   A; 6.1 min 100% A, with 2 min post-time to equilibrate the column and the total run

212   time was 8 min. The column oven temperature was set at 30°C, the flow was maintained

213   at 0.2 mL/minute, and the injection volume was 10μL; after each injection a needle

214   wash was performed.

215   The electrospray operated in positive ion mode. The conditions used in the ionization

216   source were: 350ºC at 12 L/min for the drying gas (N_2), a nebulizer pressure of 40 psi

217   and a capillary voltage of 4000 V. Identification and quantification of acrylamide in the

218   samples was performed using the multiple reaction monitoring (MRM) mode, and the

219   two most abundant ions, m/z 72 > 27 and m/z 72 > 55.2, were studied respectively.

220   Before analysis, the chromatography method was validated according to the directive

221   2002/657/CE. The validation included the determination of linearity, recovery,

222   precision (repeatability and reproducibility), LOD (limit of detection) and LOQ (limit of

223   quantification). The linearity and matrix effect of the analytical procedure were studied

224   using calibration standards prepared in neat solvent and in fortified samples submitted

225   to QuEChERS extraction. The five-point-calibration curves (10, 20, 50, 100, and 200 μg

226   kg^{-1}) in solvent and in fortified raw and fried potatoes were constructed and compared.

227   The slope ratios (slope matrix/slope solvent) were < 1 in all cases. This indicated that a

228   matrix effect existed with a suppression of ionization (Cuadros-Rodriguez and others

229   2003). Therefore, acrylamide content (μg kg^{-1}) in fried potatoes was quantified from the

230   "fried matrix curve"; whereas acrylamide content (μg kg^{-1}) in pre-treated samples was

231   quantified from the "raw matrix curve". The presence of Maillard reaction products in

232   fried potato matrices leads to a different MS/MS response than in raw potatoes samples

233   in which no Maillard reaction products exist (Zhang and others 2011).

234   The accuracy of both the fried and raw matrix was evaluated through recovery

235   experiments by fortifying an appropriate sample at three different levels (10, 50 and 100

236    $\mu g\ kg^{-1}$), with six replicates at each level (n=6).

237   Repeatability was calculated from the analysis of six blank samples fortified at each one

238   of the three specified levels of fortification (10, 50 and 100 $\mu g\ kg^{-1}$), and performed by

239   the same operator on the same day. To evaluate reproducibility the analyses were

240   repeated on three consecutive days by two different operators.

241   The LOD and LOQ of the method were obtained by fortifying samples with acrylamide

242   at different concentrations. The values were determined as the amount of analyte for

243   which signal-to-noise ratios (S/N) were higher than 3 and 10, respectively.

244   2.3.4. Statistical analysis

245   Statistical analysis of variance (ANOVA) was performed by Statgraphics Centurion to

246   estimate the effect of process variables (reducing sugars content in raw material, type of

247   pre-treatment and frying technology) on the obtained results. Evaluations were based on

248   a 95% significance level.

249

250   **Results and Discussion**

251   *Influence of frying technology, pre-treatment and initial reducing sugars content on*

252   *color changes during frying*

253   It is well known that browning reactions induce changes in the crust of fried products.

254   The kinetics of Maillard reaction depends on several factors such as precursor

255   concentrations in raw material, pre-treatments, frying technology and frying variables

256   (De Wilde and others 2005). Additionally, a correlation between acrylamide

257   development and reducing sugars content in raw material has been reported in previous

258   studies (Williams 2005; De Wilde and others 2005).

259   Statistical analysis showed (Table 1) that frying technology, initial reducing sugars in

260   raw potatoes, and their interaction, had a significant influence on the evolution of

261   CIEL*a*b* parameters with frying time. Moreover, frying technology was the factor

262   which most influenced the evolution of color parameters during frying (higher value of

263   F-ratio), and especially on a*. Pre-treatment was not found to have a significant effect

264   which means that it is possible to apply pre-treatments without affecting the color of

265   fried potatoes. Figures 1, 2 and 3 show the evolution of lightness (L*), a* and b* with

266   regard to air and deep-oil frying time, of untreated, control and blanched samples as

267   well as after dipping them in 1 and 2% of citric acid. The authors do not consider it

268   necessary to show the results corresponding to all pre-treatments in Figures 1-3. On one

269   hand, the results showed that the higher the initial reducing sugars content, the faster the

270   rate of browning reactions. Color changes in potato products are the direct consequence

271   of non-enzymatic browning reactions which reducing sugars (i.e. glucose and fructose)

272   participate in (Manzoco and others 2011). On the other hand, frying technology clearly

273   affected the kinetic of color changes. In fact, Maillard reaction was much faster for

274   deep-frying than air-frying. This is due to differences between both technologies in

275   terms of mass and heat transport kinetics, even if the temperature of the external

276   medium (air or oil) was 180 ºC in both cases. The thermal properties of oil favor heat

277   transport by convection from the external medium to the potato surface, and lead to a

278   faster frying process (Andrés and others 2012).

279   For oil-frying, lightness (L*) increased initially but tended to decrease with time

280   resulting in a darkening of the fried potatoes; while it almost remained constant in

281   samples submitted to air-frying (Figure 1). Browning was directly reflected in a gradual

282   increase of the a* color parameter. As it can be observed in Figure 2, a* values

283   gradually increased from negative values to positive ones. Finally, the CIE b* parameter

284   (related to yellow tint when positive) experimented a notable increase during frying,

285   regardless of frying technology (Figure 3). Samples submitted to oil-frying reached

286   higher b* values than air-fried potatoes and the rate of changes of b* and a* was faster

287   in fried potatoes with high initial reducing sugars content. The frying time required to

288   achieve positive values of a* in fried potatoes with low initial reducing sugars content

289   was 26 and 34 min for oil and air-frying conditions; while the time at which samples

290   with high initial reducing sugars content was 7 and 20 min in oil and air-frying,

291   respectively. The reference frying times, those at which a* values were lower than 0,

292   were 16 and 24 minutes in samples with low reducing sugars content for oil and air-

293   frying, respectively; whereas 5 and 16 minutes were required in samples with high

294   reducing sugars content for oil and air-frying, respectively. It is important to point out

295   that the reference time varied depending on each technology and the initial reducing

296   sugars content, but not on the type of additives used for pre-treatment.

297   *Validation of the analytical method for acrylamide determination*

298   As there is no official methodology described for the analysis of acrylamide, validation

299   of the analytical procedure was carried out as first step in order to ensure the quality of

300   the obtained results. The calibration curves for raw and fried samples were linear in the

301   range 10-200 μg kg^{-1}, with a correlation coefficient (R^2) of 0.9926 and 0.9986,

302   respectively. The recoveries, performed by adding known quantities of acrylamide were

303   in the range 97 to 108 % for raw matrix and 98 to 116 % for the fried one. The relative

304   standard deviation for recovery data ranged from 4.4 to 15.5 % and 4.8 to 16.0 %,

305   respectively.

306 Repeatability and reproducibility, expressed as the relative standard deviation, were 0.5-

307 3.5 and 0.5-12.8 % for the raw matrix, whereas for the fried matrix they were 2.6-15.6

308 and 4.1-14.6 %, respectively. All the relative standard deviations are in agreement with

309 the 2002/657EC Directive, which permits up to 20 %. The limit of quantification of the

310 method assayed, in both cases, was 10 μg kg^{-1}.

311 The results of validation demonstrate that the method used is accurate and precise, and

312 therefore it can be concluded that this analytical procedure guarantees the quantification

313 of acrylamide in the samples.

314 *Influence of frying technology and pre-treatment on reducing sugars content and*

315 *acrylamide generation*

316 The effectiveness of pre-treatment in initial reducing sugars lixiviation as well as the

317 influence of chemical agents involved in the pre-treatments and the frying technology

318 on acrylamide reduction at reference frying time were also studied. This analysis was

319 only conducted in samples with high initial reducing sugars (0.203±0.005 %), as this

320 condition is the most disadvantageous for acrylamide formation.

321 Table 2 shows the reducing sugars content of the samples after pre-treatment. The

322 results have been expressed as g of reducing sugars per 100 g of fresh potatoes in order

323 to compare the effect of pre-treatment. For this purpose, the net mass variation

324 occurring under the different experimental conditions (data not shown) was taken into

325 account. The relative variation of initial reducing sugars content (%) taken place during

326 pre-treatment was also calculated.

327 On one hand, pre-treatment by immersion in different chemical agents, as well as

328 blanching, resulted in a statistically significant loss of the initial reducing sugars of the

329 raw potatoes. It is important to point out that placing the samples in water alone for 60

330 min (control) resulted only in a 6% reduction, therefore it could be considered that no

331 reduction took place under control conditions; while the presence of a solute in the

332 external immersion medium produced reductions of over 22%, excepting in 1% of

333 NaCl. In fact, the presence of a solute in the immersion medium generates a mild

334 chemical potential gradient acting as a driving force for mass fluxes, leading to an

335 exchange of soluble solutes between the potato tissue and the surrounding solution

336 (Pointing 1973). Concretely, there is an intake of the chemical agent, from the external

337 solution to the potato tissue, at the same time as a partial removal of native hydrosoluble

338 solutes, such as reducing sugars and asparagine, from the liquid phase of the potato to

339 the external solution (Wicklund and others 2006). However, an increase of solute

340 concentration in the external solution did not significantly affect the variation of

341 reducing sugars (Table 2); whereas some chemical agents such citric acid, glycine or

342 calcium lactate and sodium chloride at 2% improved the reducing sugars lixiviation.

343 The results also demonstrated the advantage of applying short high-temperature

344 treatments (blanching) instead of long low-temperature ones (control). In this way,

345 blanching (5 min at 85 ºC) resulted in a 26% loss of reducing sugars instead of 6% for

346 control conditions (60 min at room temperature). Pedreschi and others (2004) reported

347 similar levels of reduction after blanching potatoes of *Tivoli* var.; whereas Mestdagh

348 and others (2008a) reported lower values of reduction. During blanching, the alteration

349 of amylaceous tissue takes place resulting in a higher migration of acrylamide

350 precursors (Pedreschi and others 2005).

351 Previous studies reported that lixiviation of reducing sugars in raw materials and

352 acrylamide generation on frying are linked, since reducing sugars content is one of the

353 main limiting acrylamide precursors in potato products (Amrein and others 2003; De

354 Wilde and others, 2005). In this study, the roles of different chemical agents and frying

355 technologies in inhibiting acrylamide formation were also studied. The acrylamide

356   content (μg Kg^{-1}) of pretreated deep-oil fried and air-fried potatoes at their respective

357   reference frying times (5 min and 16 min in deep-oil frying and air-frying, respectively)

358   is shown in Figure 4. Air-frying resulted in a 77% reduction in acrylamide formation for

359   unpretreated samples in comparison to deep-oil fried ones. Therefore, the application of

360   a pre-treatment with the aim of reducing this toxic compound is not necessary for air-

361   frying. The effectiveness of air-frying in limiting acrylamide formation could be related

362   to the higher relative humidity of the product/external medium interphase for air-frying

363   compared to deep-oil frying. Moreover, Gökmen and Palazoglu (2009) reported that a

364   certain amount of acrylamide formed in potatoes during frying is lost through

365   evaporation. In fact, the higher fluxes of water from the core of the product to the

366   surface and finally to the air, linked to the long frying time during air-frying (Andrés

367   and others 2012), prolonged the constant-rate frying step characterized by the saturation

368   of the product surface with water. On the other hand, deep-oil frying mainly occurs at a

369   falling-rate. It is well known that the presence of water on the potato surface prevents

370   acrylamide formation in fried products.

371   Surprisingly, samples pre-treated with 1% of NaCl or Calcium lactate presented higher

372   acrylamide content than the control and the unpretreated air-fried samples. The presence

373   of any of these mono o divalent cations, Ca^{2+} or Na^+, at the product surface could

374   diminish the water activity of the surface and favor acrylamide formation in the fried

375   product. This result revealed a strong interaction between the frying technology and

376   type of pre-treatment as these cations reduced acrylamide formation for deep-oil frying.

377   In addition, pre-treatments with blanching, nicotinic acid, citric acid, glycine at 1% and

378   NaCl at 2% limited acrylamide generation in deep-oil fried potatoes. Zeng and others

379   (2009) previously reported that nicotinic acid was the most effective water soluble

380   vitamin in the inhibition of acrylamide with up to 70% mitigation. Moreover, since no

381 undesirable flavor was found in fried potatoes after treatment, nicotinic acid could be a

382 promising inhibitor of acrylamide formation in food processing. The effect of citric acid

383 (77 and 91% reduction at 1 and 2% of solution concentration, respectively) could be

384 attributed to both an important pH drop and a spatial hindrance which hinder the

385 reaction between acrylamide precursors (Mestdagh and others 2008b). The ability of

386 glycine to reduce the acrylamide content in fried potatoes compared to other amino

387 acids was previously reported in model systems (Bråthen and Knutsen, 2005; Low and

388 others, 2006) and blanched potato crisps (Kim and others 2005). Unexpectedly, a

389 reduction of 80 and 55% was found in this study at 1 and 2% of glycine, respectively.

390 Alternatively, concentrations above 1% of glycine in the soaking medium, i.e. in the

391 pretreated potato, did not result in a higher acrylamide reduction. The effectiveness of

392 glycine, and other free amino acids or proteins, was attributed to the promotion of

393 competitive reactions with asparagine to react with reducing sugars and/or by covalently

394 binding the formed acrylamide through Michael type addition reactions (Mestdagh and

395 others 2008). The effect of mono and divalent cations Na^+ and Ca^{2+} at 2% gave as a

396 result significant acrylamide mitigation. A loss of 78 and 68% took place at this

397 concentration with NaCl and calcium lactate, respectively; whereas a reduction of ≈20%

398 occurred at 1% with both cations. Mestdagh and others (2008) reported a maximum

399 reduction of 10% and 49% by adding 50 μmol/ g mixture of NaCl and 100 μmol/ g

400 mixture of calcium lactate to a model system. However, Gökmen and Senyuva (2007)

401 found a complete prevention of acrylamide formation by Ca^{2+}, whereas monovalent

402 cations, such as Na^+, almost halved the acrylamide formed in the model system.

403 Calcium lactate and NaCl, as food additives, are widely used as firming and

404 preservative agents in commercial foods and they could be also used by the food

405 industry to control the formation of acrylamide.

406   Finally, a correlation between reducing sugars content at the beginning of frying (Table

407   2) and acrylamide content (Figure 4) at the reference frying time was separately

408   performed for both frying technologies (data not shown). Results showed that reducing

409   sugars content at initial frying time had lack of correlation with acrylamide content

410   (R^2=0.648 for deep-oil frying and 0.252 for air-frying). This fact again reflected that

411   acrylamide inhibition is a complex mechanism in which not only the amount of the

412   precursors (reducing sugars and asparagine) has a major role but also the interference of

413   additives in Maillard reaction and the kinetics and conditions of frying.

414

415   **Conclusions**

416   From the results obtained in this study, it could be concluded that the lower the initial

417   reducing sugars content the higher the time required for reaching the typical tonality of

418   fried potatoes. The rate of Maillard reaction during air-frying technology was much

419   lower than for deep oil-frying conditions leading to a drastically acrylamide reduction

420   (about ≈ 90%) with compared with conventional frying even in unpretreated samples.

421   This fact permits to affirm that air-frying technology is a promising technology for

422   obtaining healthy fried products.

423   The application of a pre-treatment became an important step for acrylamide mitigation

424   for deep-oil frying. In this sense, both the extraction efficiency of reducing sugars and

425   the penetration of additives during pre-treatment played a combining role for

426   acrylamide mitigation. Concretely, dipping the potatoes into a solution of nicotinic acid,

427   citric acid, glycine at 1% or NaCl at 2% might be a viable approach for the

428   minimization of acrylamide content. Nevertheless, the sensory repercussion of any

429   strategy to reduce acrylamide generation should be evaluated before application.

430

435 **Author Contributions**

436 M. Sansano collected experimental data and drafted the manuscript

437 M. Juan-Borrás and I. Escriche performed method validation for LC/MS/MS

438 acrylamide analysis

439 A. Andrés planned the study and interprets the results

440 A. Heredia planned the study, interprets the results and draft the manuscript

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553 **Table 1.** Values of F-ratio and level of significance from ANOVA multifactor of the
554 influence of the main effects (pre-treatment, frying technique and initial reducing
555 sugars) on the CIEL*a*b* changes along frying.

	F-ratio		
Main effects	**L***	**a***	**b***
A: Pre-treatment	1.77 NS	1.60NS	1.76NS
B: Frying Technique	19.61**	50.09**	192.47**
C: Initial Reducing sugars	4.29*	7.17**	81.36**
Interactions			
AB	1.42NS	1.64NS	3.99**
BC	3.80NS	10.45**	35.32**

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557 ** Statistical significance ≥99% (p-value ≤ 0.01); * Statistical significance ≥95% (p-value ≤
558 0.05); NS (not statistical significance, p-value > 0.05)

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Table 2. Reducing sugar content (g/ 100 g fresh potato) of the samples after pre-treatments and variation of initial reducing sugars (%) as a consequence of pre-treatments.

Pre-treatment	Reducing sugars content (g/100g fresh potato)	Variation of initial Reducing sugars (%)
Raw potatoes	0.203(0.005)d	
Control	0.20(0.03)d	-6.54(1.03)
Blanching	0.15(0.03)bc	-26(5)
Nicotinic acid 1%	0.146(0.016)bc	-31(6)
Nicotinic acid 2%	0.156(0.009)bc	-23(5)
Citric acid 1%	0.133(0.013)abc	-35(6)
Citric acid 2%	0.1237(0.0112)abc	-39(6)
Glycine 1%	0.13(0.02)bc	-39(5)
Glycine 2%	0.118(0.013)ab	-42(6)
NaCl 1%	0.21(0.02)d	1.3(1.4)
NaCl 2%	0.097(0.015)a	-55(5)
Calcium lactate 1%	0.158(0.006)c	-22(3)
Calcium lactate 2%	0.130(0.005)abc	-36(2)

[abcd] different letters indicate differences between homogeneous groups at a 95% of significance level (p-value ≤ 0.05))
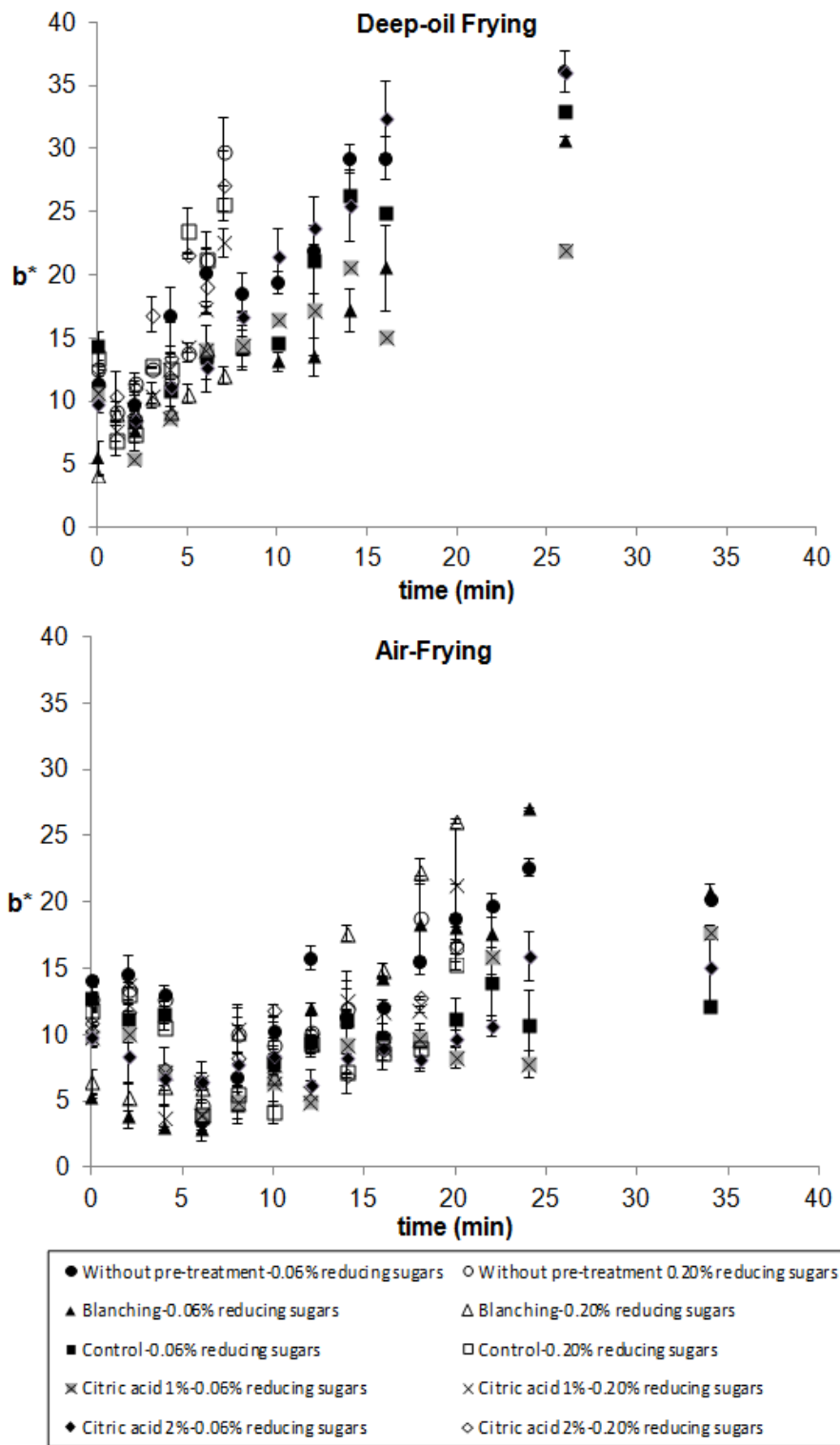Mean (standard deviation)

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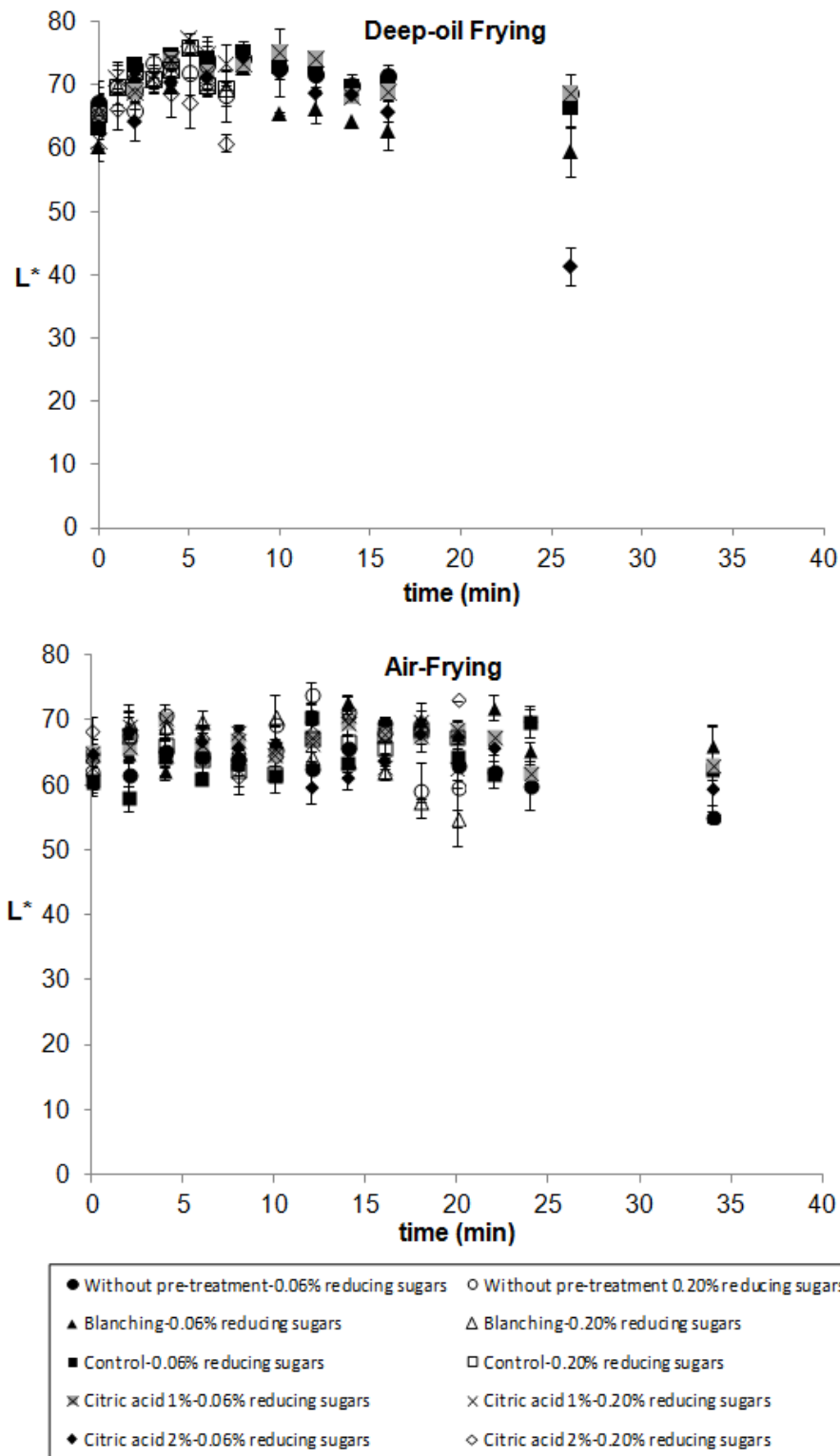
Figure 1. Evolution of lightness (L*) along deep-oil and air-frying of unpretreated (without any pre-treatment), control (60 min at room temperature in distilled water), blanched and dipped in citric acid at 1 and 2% samples with low and high-medium initial reducing sugars content in raw potatoes.
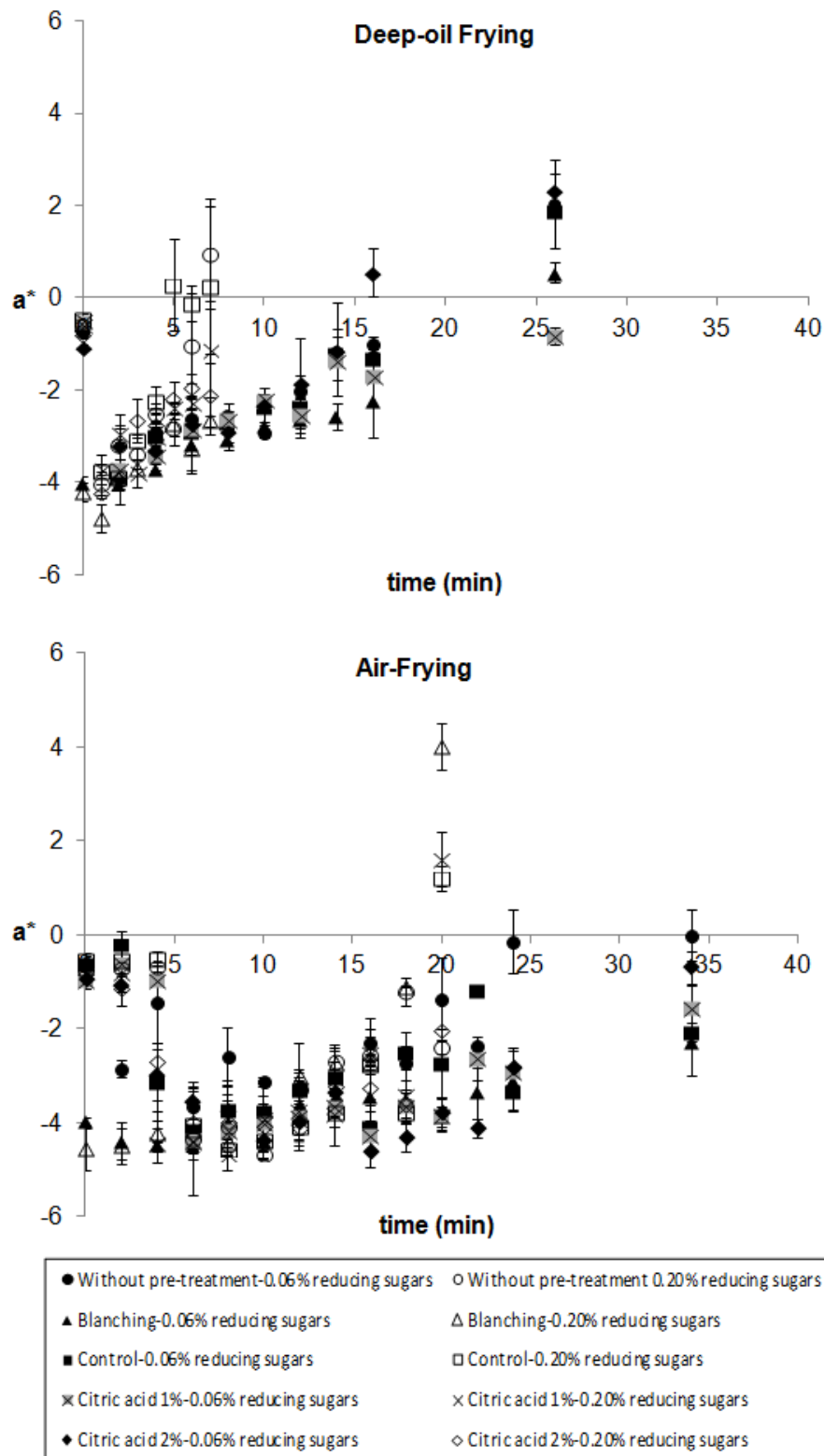
**Figure 2.** Evolution of a* colorimetric parameter along deep-oil and air-frying of unpretreated (without any pre-treatment), control (60 min at room temperature in distilled water), blanched and dipped in citric acid at 1 and 2% samples with low and medium-high initial reducing sugars content in raw potatoes.

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Figure 3. Evolution of b* colorimetric parameter along deep-oil and air-frying of

unpretreated (without any pre-treatment), control (60 min at room temperature in

distilled water), blanched and dipped in citric acid at 1 and 2% samples with low and

medium-high initial reducing sugars content in raw potatoes.

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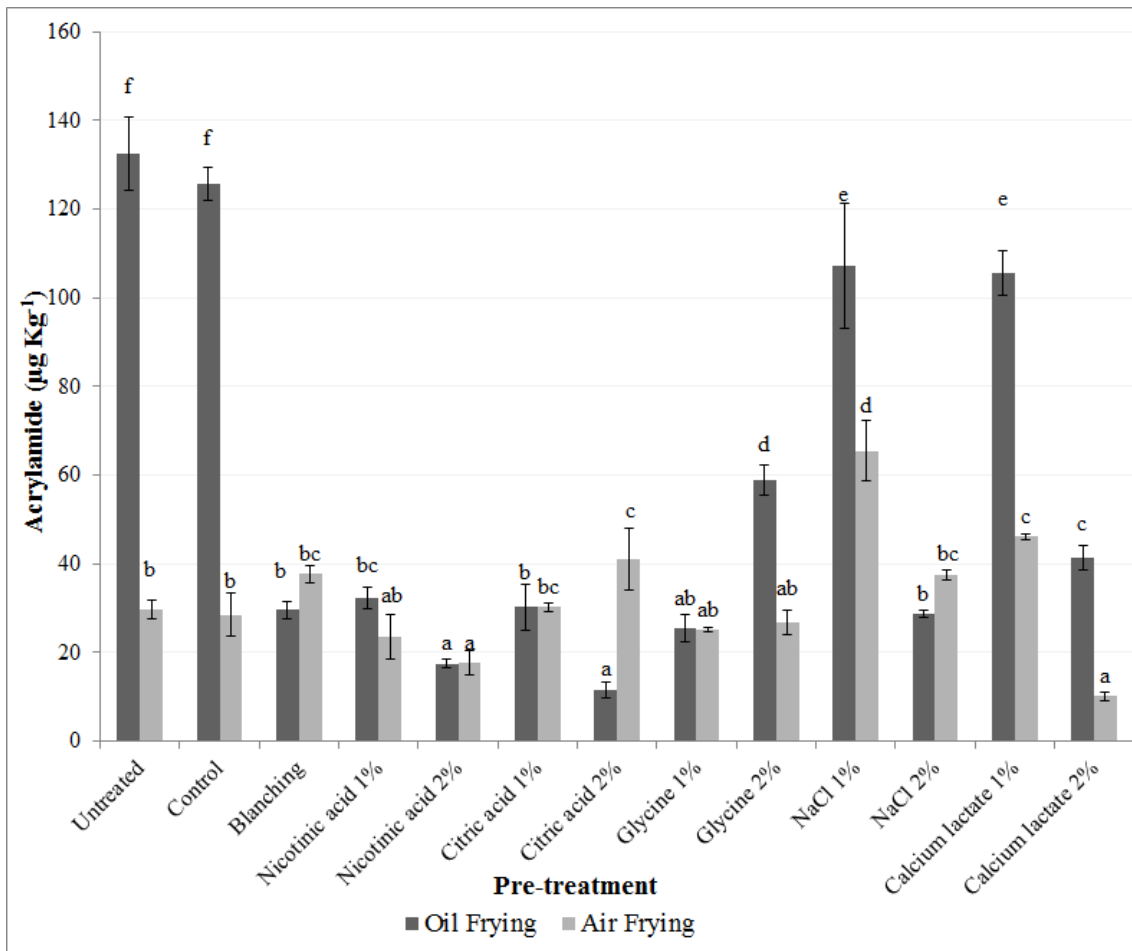
Figure 4. Acrylamide content (μg kg^{-1}) of fried potatoes submitted to the different studied pre-treatments and at the reference frying time of deep-oil frying and air-frying. These values correspond to fried potatoes with medium-high initial reducing sugars content in raw potatoes.