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DIETARY ACRYLAMIDE: WHAT HAPPENS DURING DIGESTION

Sansano, M., Heredia, A. *, Peinado, I. and Andrés, A.

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

P.O. Box 46022 Valencia, Spain

* Corresponding author. Tel.: +34 963877365

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

Abstract

Acrylamide is a well-known potentially carcinogen compound formed during thermal processing as an intermediate of Maillard reactions. Three objectives were addressed: the impact of gastric digestion on acrylamide content of French Fries, chips, chicken nuggets, onions rings, breakfast cereals, biscuits, crackers, instant coffee and coffee substitute; the acrylamide content evolution during gastrointestinal digestion of French fries and chips; and the effectiveness of blanching and air-frying on acrylamide mitigation after gastrointestinal digestion.

A significant increase (p-value<0.05) in acrylamide content was observed for most of the products after gastric digestion (maximum registered for sweet biscuits, from 30±8 to 150±48 µg/kg,). However, at the end of the intestinal stage, acrylamide values were statistically similar (p-value=0.132) for French fries and lower than the initial values (before digestion) in potato chips (p-value=0.027). Finally, the low acrylamide content found in blanched and air-fried samples, remained still lower than for deep fried samples even after gastrointestinal digestion.

Key words: acrylamide, *in vitro*, digestion, kinetics, bioaccessibility

1. Introduction

Acrylamide is a soluble compound with low molecular weight formed during thermal processing as an intermediate product of the Maillard reactions, mainly through the reaction

between the amino acid asparagine and some reducing sugars (Stadler et al., 2002). It is considered a neurotoxic and potential carcinogenic compound (IARC, 1994) and glycidamide, an acrylamide metabolite, has also a genotoxic character (Blank, 2005). Temperature above 120 ºC is required to generate acrylamide, this occurring frequently in carbohydrates rich-foods subjected to frying, roasting or baking (Matthäus, Haase, & Vosmann, 2004; Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002). The highest concern about acrylamide intake comes from the cereal grains based products (such as biscuits, crackers or bread), breakfast cereals, coffee and more especially, potato products (French fries and chips).

Since the first announcement of acrylamide presence in foods, health institutions and food industries have put together some effort in order to reduce consumer exposure. For that purpose, the European Food and Drink Federation published the 'Acrylamide Toolbox'. A useful document where the last scientific and technological developments are gathered and periodically updated (Food Drink Europe, 2013). It summarizes the most effective low-acrylamide strategies for each defined food group ((I) potato based snacks, (II) French Fries & Other Cut Potato Products, (III) Cereal/Grain Based Products, (IV) Coffee, Roasted Grain & Substitutes and (V) Baby Biscuits, Infant Cereals & Baby Foods Other than Cereal Based Foods), and specifies in which step of the process they might be applied (raw material selection, recipe design or process design). Among the proposed strategies, blanching has been widely studied as potential procedure to mitigate acrylamide in French fries (Pedreschi, Granby, & Risum, 2010; Pedreschi, Kaack, & Granby, 2004), being its application strongly recommended at domestic and industrial levels. As an alternative to deep fryers, air fryers have recently been introduced domestically, due to its capability to produce healthier fried products (low fat). In fact, a recent study showed reductions in acrylamide formation up to

90 % in fried potatoes obtained by air frying compared to conventional deep oil-frying (Sansano, Juan-Borrás, Escriche, Andrés, & Heredia, 2015).

Besides the scientific evidence of the influence of process variables and food composition on acrylamide generation, numerous studies have been published in relation to acrylamide distribution, metabolism and excretion in animal and human assays (Doerge, Young, McDaniel, Twaddle, & Churchwell, 2005a, 2005b; Shipp et al., 2006; Zödl et al., 2007). Distribution studies showed that acrylamide is rapidly distributed to all tissues without evidence of accumulation; it might be found in breast milk being even capable of penetrating the placenta (Schettgen et al., 2004; Sörgel et al., 2003). In addition, the presence of other Maillard products should be considered because they could enhance or suppress acrylamide toxicity (Friedman, 2005; Somoza, 2005). Concretely, antiallergenic/allergenic, antibiotic, anticarcinogenic/carcinogenic, antimutagenic/mutagenic, antioxidative/oxidative, clastogenic, and cytotoxic activities have been attributed to certain Maillard products.

In the organism, acrylamide is mainly metabolized through the cytochrome P450 2E1, action that catalyzes the formation of glycidamide, a reactive epoxide metabolite (Blank, 2005). Both, acrylamide and glycidamide, have genotoxic effects though only glycidamide forms adducts with proteins and DNA in vivo (Friedman, 2003). Nevertheless, few studies have been published about the effect of digestion process on acrylamide (Eriksson & Karlsson, 2006; Hamzalıoğlu & Gökmen, 2015; Schabacker, Schwend, & Wink, 2004). Eriksson & Karlsson (2006) studied the effect of digestive enzymes and pH on acrylamide extraction. Pepsin extraction in acid conditions at 37 ºC during 72 h (62.5 FIP-U/g) showed no differences in acrylamide content compared to normal water extraction. Schabacker, Schwend, & Wink, (2004) showed that acrylamide binds to egg albumin through Caco-2 cells (human intestine model), under simulated intestinal conditions, revealing that protein intake could attenuate acrylamide content in the organism. Hamzalıoğlu & Gökmen (2015)

studied the influence of gastrointestinal digestion of biscuits and fried potato products on the acrylamide content and reported different results depending on the food product. Though acrylamide content was notably reduced after the gastrointestinal digestion, acrylamide from French fries experimented a noteworthy increase after gastric digestion (Hamzalıoğlu & Gökmen, 2015).

Due to its solubility in water, acrylamide bioavailable content is assumed to be the same as the intake amount (Eriksson, 2005; Eriksson & Karlsson, 2006). However, as any other contaminant, the total amount present in the ingested food does not necessary correspond to the bioavailable content. After the intake of any food product, an alteration of its structure and chemical composition may take place by multiple mechanical and physicochemical factors, such as chewing, dilution, variation in pH and/or the action of the different enzymes present in the mouth, the stomach and intestine, among others. The effect of different digestive stages on the compounds present in food varies considerably between individuals, and *in vivo* studies would be the ideal ones. However, *in vivo* studies are intrusive, and imply ethical and costs constraints. Based on these limitations, *in vitro* models represent an alternative methodology scientifically validated to evaluate the influence of digestion on different compounds. Some of the advantages of *in vitro* models are the quick results, lower cost, lack of ethical constraints and reproducibility, selection of controlled conditions or to facilitate sampling at different times of the digestion process (Minekus et al., 2014).

In this scenario, there is a lack of information related to acrylamide changes during gastrointestinal digestion in different food matrices. The objective of this study was multiple: (a) to analyse the effect of gastric digestion on acrylamide content in nine food products with noteworthy levels of this toxic; (b) to study the kinetics of acrylamide variation along digestion (gastric and intestinal stages) of French fries and chips and (c) to

evaluate whether the effectiveness of blanching and air-frying as mitigating strategies of acrylamide persists during gastrointestinal digestion.

2. Materials and methods

2.1. Reagents

Potassium chloride, sodium chloride, magnesium chloride, hexane and methanol were purchased from Panreac (Barcelona, Spain). Ammonium bicarbonate, potassium dihydrogen phosphate, porcine pepsin (3200-4500 U/mg), α-amylase from human saliva (500 U), pancreatin (8 × USP) from porcine pancreas and bovine bile extract, were from Sigma-Aldrich (Deisenhofen, Germany). The standard acrylamide (≥ 99 %) was obtained from Merck (Darmstadt, Germany) and $^{13}C_3$-labelled acrylamide (99 %) from Cambridge Isotope Laboratories (Andover, MA). Sodium carbonate hydrogen was purchased from Scharlau (Barcelona, Spain). Acetonitrile, formic acid (99-100 % purity) and magnesium sulphate were purchased from VWR (Fontenay-sous-Bois, France). PSA (Primary Secondary Amine) was obtained from Supelco (Bellefonte, PA). All solvents used for the determination of acrylamide were HPLC grade and all other analytical grade. Bidistilled water was used for chromatographic analysis (Milli-Q, Millipore Corp., Bedford, MA). Acrylamide and $^{13}C_3$-acrylamide solutions (1 mg/mL) were prepared daily from stock solutions (100 mg/mL in acetonitrile). Standard solutions were stored at -20 ° C.

2.2. Food samples

Nine different food products representatives of the food groups defined in Acrylamide Toolbox (Food Drink Europe, 2013) were selected. Concretely, fried potatoes (French Fries), chicken nuggets and fried onions rings purchased in a fast food restaurant in Valencia (Spain); and chips, breakfast cereals (based on rice, wheat and barley), sweet biscuits, crackers, instant coffee and coffee substitute (cereals basis) bought in a supermarket. In all cases, except in coffee and coffee substitute, a visual selection was

performed to discard excessively brown samples, in as much as the relationship between the non-enzymatic browning degree and acrylamide content.

Potato of Agria variety was bought in a local market for the study of the effect of gastrointestinal digestion on acrylamide from French fries obtained by conventional deep-oil frying (control) or hot-air frying without pretreatment, and subjected to blanching and subsequent deep-oil frying.

2.3. In vitro digestion

The digestive process (oral, gastric and intestinal stages) and the simulated fluids (salivary, gastric and intestinal) were prepared as the internationally agreed protocol, published by Minekus et al., (2014) with slight modifications. The simulated salivary fluid (SSF), the simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared daily from stock solutions prepared weekly (Table 1).

2.3.1. Influence of gastric digestion on acrylamide from different food matrices

Samples of food products (French Fries, chips, chicken nuggets, fried onions rings breakfast cereals, sweet biscuits and crackers) were mixed with SSF (75 U α-amylase/mL SSF) in a ratio 50:50 w/v during 2 min (hand blender, Ufesa 600W, Slovenia). For instant coffee and coffee substitute, foods were dissolved with water in a ratio 50:50 (w/v) before and then mixed with SSF in the same ratio as the other foodstuff (50:50 w/v). 10 g of the simulated bolus was weighed in a Falcon tube and 10 mL of SGF (2000 U pepsin/mL in the final mixture) was added (ratio 50:50 w/v) and pH adjusted to 3.0 ± 0.1 with HCl 1M (pH- meter Mettler Toledo, Schwerzenbach, Switzerland). Porcine Pepsin activity was previously determined according to the EC 3.4.23.1, described by Minekus et al. (2014), to achieve 2000 U/mL in the final digestion mixture. Subsequently, the mixture was placed in a thermostatically controlled chamber (Selecta, Spain) at 37 °C coupled to a shaker (Intelli-Mixer RM 2, Elmi Ldt.,

149 Baltics and Russia) at a speed of 55 rpm and head-over-heels movements. The total
150 duration of this stage was 120 min, and every 30 min, the pH was measured and
151 adjusted to 3 (if necessary). To stop enzymatic reactions, right after the gastric
152 digestion, tubes were immersed in ice and samples were frozen to - 40 ºC (model CVN-
153 40/105, Matex, Barcelona) for subsequent freeze-drying (-40 °C and 1.25 mbar, Telstar,
154 Terrassa, Spain). Sampling was performed at the beginning (just after 5 min of the SGF
155 addition and pH adjustment) and at the end of the gastric stage (120 min). Samples were
156 always independent tubes and tested in triplicate.

157 *2.3.2. Kinetics of acrylamide variation during gastrointestinal digestion of commercial*
158 *French fries and chips*

159 Acrylamide content was determined along gastric (at 0, 15, 30, 60 and 120 min) and
160 intestinal digestion (15, 30, 60 and 120 min) of French fries and chips. Three
161 independent tubes for each sampling time were subjected to digestive reactions.
162 Simulation of oral and gastric stages was proceeded as for the previous section. For the
163 intestinal stage, 20 mL of SIF was added to the gastric chime (ratio 50: 50 (v/w)), and
164 pH was adjusted to 7.0 ± 0.1 with NaOH 1M, right after 120 min of gastric stage. The
165 final bile salts and pancreatin concentrations were 10 mM and 1260 LU (Lipase Units
166 or FCC), respectively, in each tube. To stop the enzymatic reactions (after the different
167 sampling times along the gastric and intestinal simulated digestion) the tubes were
168 placed in ice for 10 minutes, and then stored at -40 °C until they were freeze-dried.

169 *2.3.3. Influence of blanching and air frying on acrylamide changes along*
170 *gastrointestinal digestion of French fries.*

171 Potatoes were sliced with a commercial cutter (Taurus kitchenline, New Wulmstorf,
172 Germany) into strips (10 mm x 10 mm x 50 mm). Strips were introduced in tap water
173 until pretreatment or frying step, and they were divided in 3 groups that corresponded to

each treatment: A: control (deep oil-frying); B: blanching (85 ºC, 5 min) + deep oil-frying; C: hot air-frying. Frying step was carried at 180 °C during 6.5 min in a commercial deep-oil-fryer (model: FM 6720 Ideal 2000 Professional, Solac, with a nominal power of 2000W); and air-frying in a hot-air-frying equipment for 21 min (model: AH-9000 Actifry, Tefal with a nominal power of 1400 W). The frying time was that required to achieve the same superficial color as under deep-oil fried samples. Commercial sunflower oilseed was used with a potato-to-oil ratio of 1:20 (w/v) in deep oil-frying, and 0.3 g of oil per 100 g of potatoes in air-frying, according to the equipment specifications.

Samples were subjected to salivary, gastric and intestinal digestion as explained in the previous sections. Sampling was performed at the beginning (just after the SGF addition and pH adjustment) and at the end of the gastric stage (after 120 min), as well as after the intestinal stage (120 min more). Samples were always independent tubes and tested in triplicate.

2.4. Analytical determinations

Water content of the food products was analyzed by vacuum drying at 60 ºC until constant weight was achieved (AOAC, 2000).

Acrylamide content was analyzed in food products previous digestion as well as in lyophilized samples after the different digestion conditions. Acrylamide extraction was carried out as Sansano et al., (2015), with minor modifications. Sample preparation procedure has been tested and resulted fitted for different food matrixes (coffee, chocolate, peanuts butter, crackers and potato chips). It includes a dispersive solid phase extraction (dSPE) cleanup, named QuEChERS, and uses an internal standard ($^{13}C_3$-acrylamide). Concretely, 1 g of food product or lyophilized (digested samples) was weighted in a Falcon tube. 0.5 mL of internal standard and 5 mL of n-hexane were

199 added and mixed in a vortex for 30 s. Then, 10 mL of Mili-Q water and 10 mL of
200 acetonitrile were added as well as the salt packet included in QuEChERS 1 (4 g of
201 $MgSO_4$ and 0.5 g of NaCl), and the mixture was stirred for 1 min in the vortex.
202 Centrifugation (2026 RCF; Centronic BL II, Selecta, Spain) for 5 min) was performed
203 to properly separate all the layers, and after discarding the hexane layer, 1 mL of
204 acetonitrile phase was transferred to a 2 mL tube that contained 50 mg of PSA (Primary
205 Secondary Amine) and 150 mg of $MgSO_4$ (QuEChERS 2). The acetonitrile phase was
206 then vortexed during 1 min and centrifuged (2697 RCF; Labofuge 200 Heraeus,
207 Germany). The supernatant was filtered and transferred to a vial for the LC/MS/MS
208 analysis.
209 The acrylamide analysis was performed with an Agilent 1200 Series HPLC system
210 coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent Technologies
211 Inc., CA, USA) with an electrospray type ionization source. The column used was a
212 Zorbax Eclipse XDB C-18 (2.1 mm x 50 mm, 1.8 μm). The mobile phase used
213 consisted of 2.5 % methanol/ 97.5 % of 0.1 % formic acid (A) and methanol (B). The
214 elution gradient was as follows: 0-3 min 100 % of A; 3.1-3.5 min 70 % A; 3.6 min 100
215 % A, with 1 min post-time to equilibrate the column. The column oven temperature was
216 set at 30 °C, the flow was maintained at 0.4 mL/minute and the injection volume was
217 10μL. The electrospray was operated in positive ion mode. The conditions used in the
218 ionization source were: 350 ºC at 12 L/min for the drying gas (N_2), a nebulizer pressure
219 of 40 psi and a capillary voltage of 4000 V. Acrylamide content was performed using
220 the multiple reaction monitoring mode (MRM), monitoring the ions m/z 72 > 27 and
221 m/z 72 > 55.2, and also m/z 75>58 for $^{13}C_3$-acrylamide (internal standard). Five
222 different levels of acrylamide (10, 20, 50, 100 and 200 μg/L) and 50 μg/L of internal

223 standard, with six replicates for each level (n=6) were studied, being 10 µg/L of
224 acrylamide the limit of quantification.

225 *2.5. Statistical analysis*

226 Analysis of variance (one-way-ANOVA), using the Statgraphics Centurion software,
227 with a confidence level of 95 % (p-value $\leq$ 0.05) was performed to analyze the
228 statistical influence of the digestion process on the changes undergone by dietary
229 acrylamide coming from the different studied food matrices.

230

231 **3. Results and discussion**

232 *3.1. Changes in acrylamide content of different food products because of gastric*
233 *digestion*

234 Figure 1 shows the acrylamide content (µg/kg) of the studied foods prior to digestion, as
235 well as at the beginning and at the end of their gastric stage. According to the obtained
236 results, the major contribution of acrylamide throughout the diet comes from instant and
237 substitute coffees together with potato-based products (chips and French fries), followed
238 by breakfast cereals, crackers, chicken nuggets, onion rings and sweet biscuits. This
239 could be due to the differences existing in terms of macronutrients, processing
240 technology and moisture content among the studied food products. It is well known that
241 besides the presence of native precursors in the food (reducing sugars and asparagine)
242 and/or the process temperature, the low availability of water in food represents a key
243 factor that triggers acrylamide formation (Matthäus et al., 2004). Acrylamide is mainly
244 formed on the external surface of the product where moisture content is much lower
245 than in the inner part of the food. This lower moisture favors an important increase on
246 the surface with an exponential acrylamide generation (Bråthen & Knutsen, 2005). In
247 this sense, foods with lower moisture content and rich in acrylamide precursors

(reducing sugars and free asparagine), and those subjected to the highest processing temperatures were the ones with the highest acrylamide content. Table 2 illustrates the influence of moisture and acrylamide precursors on acrylamide content for chips and French fries (Biedermann-Brem et al., 2003), while the effect of roasting in acrylamide content was evident in coffee-products.

The effect of gastric digestion on acrylamide was marked, with a significant increase in all digested samples excepting in breakfast cereals. The magnitude of this increase seems to depend on the food product. It is worthy of note that the highest increase of acrylamide during the gastric digestion took place in those products previously fried and baked. Particularly, a relative increase of acrylamide content was registered in French fries (32 ± 3 %), potato chips (62 ± 5 %), crackers (83 ± 1 %), onion rings (87 ± 16 %), chicken nuggets (94 ± 11 %) and sweet biscuits (410 ± 163 %). Acrylamide content in heated foodstuffs is the net result of complex reactions leading to the formation and degradation of this compound during Maillard reactions (Luning & Sanny, 2016). Both, intermediate products such as Schiff bases and final acrylamide are accumulated in heated products and their final content depends on the heating conditions among other factors. Gastric pH seems to favor the conversion of intermediate products (Schiff bases formed during thermal process) into acrylamide (Hamzalıoğlu & Gökmen (2015). Moreover, the proteolysis occurring due to the pepsin activity together with the mechanical forces in the stomach might favor the release of acrylamide because of matrix degradation. Additionally, acrylamide increase rapidly occurred at the beginning of the gastric stage in some products (coffee substitute, crackers, sweet biscuits, breakfast cereals and onion rings), while it was observed after a longer gastric digestion time for other products (instant coffee, potato chips, French fries and nuggets) the acrylamide increase. This, suggests that the different kinetics of acrylamide changes

might be related to the matrix degradation rate. The enzymatic activity of pepsin, which contributes to the food matrix degradation, plays an important role in acrylamide' release from the food matrix into the gastric fluid. It can be said therefore, that the acrylamide increase observed at short times is related to the acid pH (enhancing Schiff bases conversion to acrylamide), while matrix degradation would explain the acrylamide increase observed at the end of the gastric stage.

Other mechanisms can be implied in acrylamide increase during digestion. Pastoriza, Rufián-Henares, & Morales, (2012) reported the formation of melanoidin-bound-acrylamide based on Michael addition reactions. This melanoidin-bound-acrylamide can be formed during heating processes (such as roasting of coffee-grains), and probably broken later on, under the acid conditions during the gastric stage explaining the observed acrylamide increase.

Finally, it is important to point out that the highest final content of soluble acrylamide (µg/kg) in the stomach is achieved after the complete gastric digestion of French fries, chips, instant coffee and substitute coffee, in spite of the partial increase after gastric digestion was higher in other products, such as sweet biscuits, crackers and battered foods.

3.2. Kinetics of acrylamide changes during gastrointestinal digestion of commercial French fries and potato chips

Due to the important consumption of potato products among populations of different ages and their contribution to acrylamide in the diet, a kinetic study of acrylamide changes during gastrointestinal digestion of French fries and chips was performed. Figure 2 gathers acrylamide content (µg/kg) at different times of the gastric and intestinal digestion. Results showed that the kinetics of acrylamide conversion from Schiff bases during gastric stage took place faster for chips, compared to French fries; a

maximum increase of acrylamide was reached after 60 min of gastric digestion for French fries and after 15 min for chips. From this point onwards an acrylamide decrease was observed for both products. These results evidenced that solubilization and conversion of Schiff base intermediates into acrylamide occurred mainly at the beginning of the gastric stage, being the acidic pH responsible for the observed changes. In order to confirm the role of gastric pH on the acrylamide increase, a parallel *in vitro* digestion was performed without pepsin addition. This time, sampling took place only after 120 min of gastric digestion. The relative variation of acrylamide after 120 min of gastric digestion with and without pepsin was similar, this confirming the role of pH on this phenomenon. Hamzalıoğlu & Gökmen (2015) reported an increase in acrylamide of 295 % and 20-45 % after gastric digestion of French fries and chips, respectively. However, in this work 45 % and 35 % increase, referred to acrylamide content before digestion, was found in French fries and chips respectively. Since these differences could be due to the different pH used in the *in vitro* simulation, (pH 3 in this work and pH 2 in Hamzalıoğlu & Gökmen (2015) study), an additional experiment was performed. The obtained results confirmed that the differences found between our study and the one carried out by Hamzalıoğlu & Gökmen (2015) could be attributed to differences in gastric pH used in the simulation. Acrylamide content was found to be higher at pH 2 when compared to pH 3 reaching values of 534 ± 36, 736 ± 41 and 556 ± 39 µg/kg at 0, 60 and 120 min of gastric digestion of French fries.

During the subsequent intestinal stage, acrylamide content decreased to a minimum value after 15 minutes of digestion, and remaining constant till the end of the experiment. This reduction could be explained by the formation of Michael adducts in which acrylamide would be involved. This in fact, is a mechanism proposed as a potential strategy to reduce acrylamide content in foods (Hamzalıoğlu & Gökmen,

323 2015; Hidalgo, Delgado, & Zamora, 2010; Zamora, Delgado, & Hidalgo, 2010). During
324 gastric digestion, pepsin hydrolyses the protein releasing short peptides and free amino
325 acids such as cysteine or lysine with nucleophilic character (-SH and -NH_2). These
326 small peptides and amino acids are capable to interact with acrylamide, forming adducts
327 and causing thus, an apparent reduction in its bioavailability during the intestinal stage
328 (Hamzalıoğlu & Gökmen, 2015). Hamzalıoğlu & Gökmen, (2015) studied model
329 systems composed of acrylamide and lysine or cysteine and obtained slight reductions
330 of the acrylamide content after simulating gastric digestion, compared to the control
331 (without amino acids). In other studies, a rapid decrease of acrylamide content was
332 observed after a heating process in the presence of N-acetyl-cysteine or lysine, resulting
333 from Michael addition, between the nucleophilic groups of these amino acids (-SH and-
334 NH_2) and the double bond (C = C) of acrylamide (Hidalgo et al., 2010; Zamora et al.,
335 2010). Hoenicke & Gatermann, (2005) observed an acrylamide reduction after long
336 storages in specific foodstuff, reporting the apparent reactive effect of SH-group-
337 containing substances. The results obtained in this study are in agreement with those
338 found in literature, confirming the possible interaction of amino acids from the
339 proteolytic activity and acrylamide in the gastrointestinal tract, specifically under
340 intestinal conditions.

341 *3.3. Acrylamide bioaccessibility of French fries subjected to blanching and air-frying*

342 French fries were prepared in lab-scale to specifically study the influence of blanching
343 and air frying on acrylamide changes during *in vitro* digestion. Figure 3 shows the
344 acrylamide content (µg/kg) corresponding to the different French fries samples, at the
345 beginning and at the end of the gastric stage (5 and 120 min) and after the intestinal
346 stage (120 min), compared to acrylamide content before digestion. As expected,
347 acrylamide content was lower in blanched and air fried samples (Pedreschi, Kaack,

348 Granby, & Troncoso, 2007; Pedreschi, Mariotti, Granby, & Risum, 2011; Sansano et
349 al., 2015). Air-frying was the most effective technique to reduce acrylamide formation,
350 (88 % reduction), similarly to the results reported in a previous study, (Sansano et al.
351 (2015). Blanching has been commonly used not only due to its effectiveness in reducing
352 acrylamide content, but also to improve the final color and texture of French fries
353 (Pedreschi et al., 2004). This pre-treatment promotes the lixiviation of the main
354 precursors of acrylamide formation (reducing sugars and asparagine),
355 After gastric digestion (120 min) a high acrylamide increment was observed in all
356 samples. This increment was observed since the beginning of the gastric stage and
357 progressed especially in French fries obtained from air-frying. These results prove again
358 the important role of pH on acrylamide conversion. Blanching lead to lower levels of
359 acrylamide, from the beginning to the end of the gastric stage. This suggests that the
360 decrease of reducing sugars and asparagine during the pre-treatment also reduces the
361 formation of Schiff bases and thus, less acrylamide appears at the end of the gastric
362 stage. On the other hand, air-frying was used as a strategy to obtain French fries with
363 low acrylamide content. However, these samples were the ones that experienced the
364 higher increase of acrylamide after gastric digestion. These results suggest that air
365 frying would enhance the formation of Schiff basis, this explaining the acrylamide
366 conversion observed in gastric digested samples. After that, a reduction of acrylamide
367 content was observed at the end of the intestinal digestion (values below 100 µg/Kg in
368 all samples), although blanched and air-fried samples showed lower values than the
369 control samples. These results suggest that regardless the different increments of
370 acrylamide during gastric digestion, the strategy to reduce acrylamide is useful after the
371 intestinal stage. Specific products originated from digestive enzymes reactions, such as
372 amino acids (cysteine or lysine), sulfhydryl compounds, or melanoidins, with

nucleophilic properties, could react with acrylamide, reducing its bioavailability (Hoenicke & Gatermann, 2005; Lineback, Coughlin, & Stadler, 2012). Acrylamide content in lab-scale French fries was found to decrease after intestinal stage, compared to French fries from the fast food restaurant.

4. Conclusions

Our findings showed that the gastric *in vitro* simulation of the studied food matrices resulted into a significant increase of the soluble fraction of acrylamide, for all the tested products except for breakfast cereals. The maximum increase was found in crackers and sweet biscuits, followed by battered foods and potato products (Chips and French fries). Despite the acrylamide increment observed after gastric digestion, acrylamide bioaccessibility (estimated from acrylamide content after the intestinal digestion), was similar in French fries and lower in potato chips than before digestion. Finally, the evaluated mitigation strategies, blanching and air-frying, were effective in reducing acrylamide content, even after the gastrointestinal digestion, compared to control.

In vitro simulated digestion can be considered then as a useful tool to obtain the bioavailable acrylamide content. It takes into account not only the influence of food intrinsic factors (structure, composition, nutrients interactions, etc.) but also extrinsic factors associated to the physiological process (gastric and intestinal pH, transit time, enzyme activities, etc.).

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503 **Figure captions**

504 **Figure 1**. Acrylamide average content and standard deviation (µg/kg in wet basis) of
505 food products before digestion and at 5 and 120 min of gastric digestion. Homogenous
506 groups are represented by the same letter in each food product (One way ANOVA).

507 **Figure 2.** Acrylamide content (µg/kg in wet basis) before digestion and during *in vitro*
508 gastric and intestinal digestion of French fries and chips. Different letters mean
509 significant differences (95% confidence level) between digestion times for each food
510 matrix (One-way ANOVA).

511 **Figure 3.** Acrylamide content (µg/kg in wet basis) of deep-oil fried French fries with or
512 without blanching pretreatment (control), and fried by air-frying, before digestion and
513 after 5 and 120 min of gastric digestion and after 120 min of intestinal digestion.
514 Different letters mean significant differences in each digestive stage (One-way
515 ANOVA).

Table 1. Electrolyte composition of salivary, gastric and intestinal fluids prepared from stock solutions (salivary, gastric and intestinal). Final volume was adjusted with distilled water after adjusting the pH.

	SSF	**SGF**	**SIF**
	mmol/ L	**mmol/ L**	**mmol/ L**
KCl	15.1	6.9	6.8
KH_2PO_4	3.7	0.9	0.8
$NaHCO_3$	13.6	25	85
NaCl	-	47.2	38.4
$MgCl_2(H_20)_6$	0.15	0.1	0.33
$(NH_4)_2CO_3$	0.06	0.5	-
$CaCl_2(H_20)_2$	1.5	0.15	0.6

Table 2. Moisture (%) of studied food products (wet basis). Homogenous groups are represented by the same letter.

Food product	**Moisture content (%)**
Potato chips	1.04 ± 0.02 ab
Instant coffee	2.69 ± 0.07 bc
Coffee substitute	2.72 ± 0.06 bc
Sweet biscuits	0.20 ± 0.02 a
Crackers	0.20 ± 0.03 a
Breakfast cereals	3.2 ± 0.3 c
Onion rings	40 ± 3 d
Chicken nuggets	51 ± 1 g
French fries	43.7 ± 0.9 e

Figure 1

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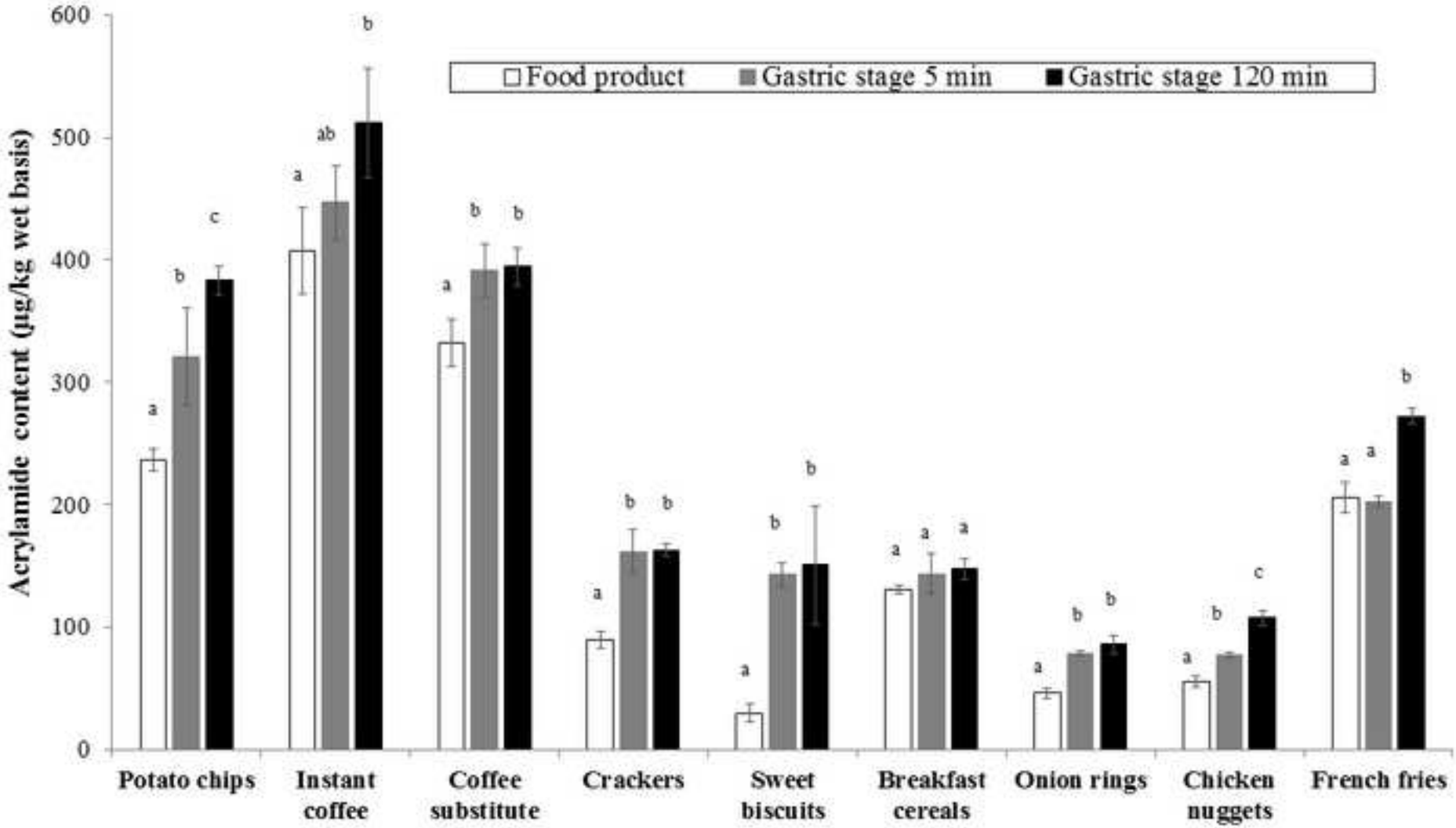

Figure 2

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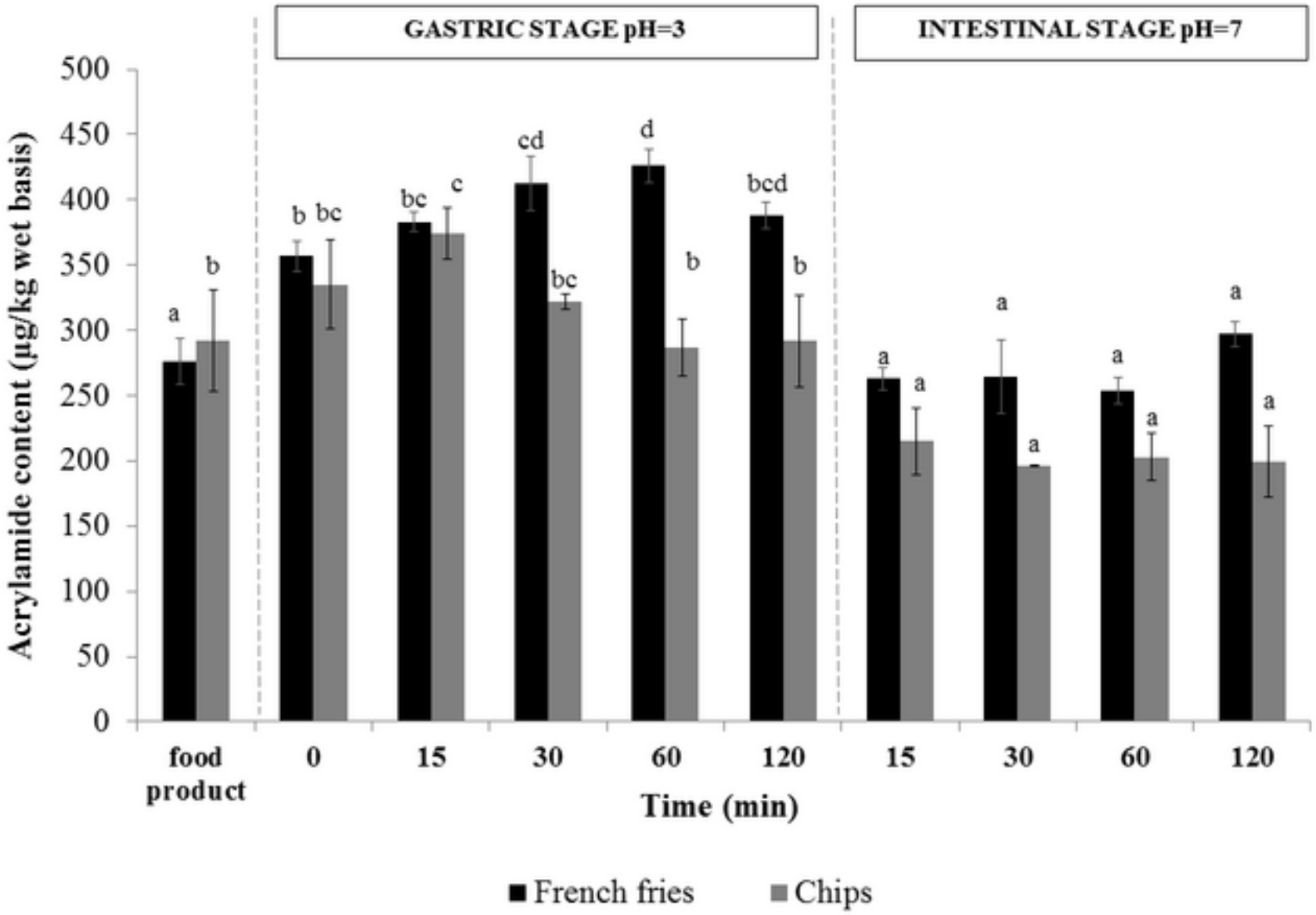

Figure 3

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