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Dietary acrylamide: What happens during digestion

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

Institute of Food Engineering for Development, Universitat Politècnica de València, P.O. Box 46022, Valencia, Spain

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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.

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 °C 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

* Corresponding author.
E-mail address: anhegu@tal.upv.es (A. Heredia).

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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 and Karlsson (2006) studied the effect of digestive enzymes and pH on acrylamide extraction. Pepsin extraction in acid conditions at 37 °C during 72 h (62.5 FIP-U/g) showed no differences in acrylamide content compared to normal water extraction. Schabacker et al. (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 and 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 analyze 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 600 W, Slovenia). For

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 mmol/ L	SGF mmol/ L	SIF 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
$MgCl_2(H_2O)_6$	0.15	0.1	0.33
$(NH_4)_2CO_3$	0.06	0.5	–
$CaCl_2(H_2O)_2$	1.5	0.15	0.6