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Simultaneous determination of acrylamide and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone in baby food by liquid chromatography–tandem mass spectrometry

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ABSTRACT

A liquid chromatography triple quadrupole mass spectrometry method was developed and validated for the simultaneous determination of acrylamide and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) in baby food. The sample preparation involves acetonitrile-based extraction combined with dispersive primary secondary amine (PSA) cleanup and cation-exchange solid-phase extraction (SPE), which promotes efficient removal of matrix interferences. Analytical selectivity and sensitivity were achieved for the quantification of acrylamide and HDMF in complex matrices such as fruit, cereal and milk-based baby foods; furthermore, adequate linearity (range 10–300 $\mu g\,kg^{-1}$) in solvent and matrix-matched calibration curves, and appropriate recoveries (94–110%) and precision (RSD $\leq$ 10%), under repeatability and within-laboratory reproducibility conditions, were also obtained. Expanded measurement uncertainty was estimated at the 20 $\mu g\,kg^{-1}$ level (limit of quantification) on the basis of data obtained from in-house validation, with values of 25.5 and 16.5% for acrylamide and HDMF, respectively. The fitness for purpose of developed method was verified by analyzing 15 commercial baby foods available in the Brazilian market. Acrylamide was detected in one plum-based baby food (35 $\mu g\,kg^{-1}$) while HDMF in 67% of the samples analyzed (levels between 25 and 262 $\mu g\,kg^{-1}$).

1. Introduction

Recently, the European Food Safety Authority (EFSA) published its scientific opinion about acrylamide in foods [1]. On the basis of evidences from animal studies and analytical results reported by 24 European countries and six food associations, it was concluded that acrylamide potentially increases the cancer development risk for the consumers. Although evidences from human studies between cancer and dietary acrylamide exposure have been limited and inconclusive [1]. Additionally, acrylamide has been classified under group 2A as a probably carcinogenic to humans by the International Agency for Research on Cancer (IARC) [2]. It is known that *in vivo* this compound is metabolized to the epoxide glycidamide, a reactive metabolite with potential genotoxicity [3]. Animal experimental studies have also demonstrated adverse effects of acrylamide on the male reproductive system as well as its neurotoxicity [3]. The Maillard reaction between the amino acid asparagine and reducing sugars is the major chemical process described for acrylamide formation in foods during heating at temperatures above 120 °C; however, other mechanisms involving acrolein, acrylic acid, wheat gluten and 3-aminopropionamide have also been proposed [4,5]. Carbohydrate-rich foods have been the main products investigated for acrylamide occurrence, with the highest levels found in solid coffee substitutes and coffee, and potato fried products [1]. Nonetheless, few reports on acrylamide in baby food are available in the literature [6–8].

Other compound generated by the Maillard reaction is 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF), an aroma compound with attractive sensory properties [9]. In addition, HDMF can be biosynthesized by certain microorganisms and plants, thus occurring naturally in some fruits and, wines, cheeses, soy sauce, cocoa and chocolate [9,10]. HDMF has also been added as flavouring agent in some foodstuffs [9,11]. However, positive results for genotoxicity tests have attracted attention for the levels of this compound in foods [11,12]. In 2011, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)

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reviewed HDMF studies and concluded that there are evidences for genotoxicity *in vitro* and *in vivo*, although absence of carcinogenic potential was observed in a chronic study in rats [13]. *In vitro*, HDMF induced reverse gene mutations, whose genotoxic effects have been associated with the generation of reactive oxygen species as a result of redox cycling in the presence of metal ions and oxygen, contributing to an increase in cellular oxidative stress and DNA-breaking activity [11–14]. Genotoxicity was also observed *in vivo* after HDMF administration via intraperitoneal and oral in mice [11–13,15]. A no-observed-adverse-effect level (NOAEL) of 200 mg kg^{-1} of body weight per day was reported for HDMF from a study in rodents [11,13].

Due to the increasing knowledge on health risks associated to compounds generated during high temperature cooking, several analytical methods have been proposed for food control. Nonetheless, very little works have explored simultaneous determination of these heat-induced compounds in foodstuffs. For instance, Nielsen et al. [16] and Zhang et al. [17] described simultaneous liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis of acrylamide and its precursors asparagine and sugars in carbohydrate-rich matrices. In other study, acrylamide and 3-monochloropropane-1,2-diol were simultaneously analyzed by gas chromatography–tandem mass spectrometry (GC–MS/MS) in bread, powered milk, soy sauce and others [18]. High polarity, low molecular weight, lack of sufficiently strong chromophore group in chemical structure, and low volatility has been critical factors for acrylamide analysis [19,20]. Furthermore, complex and time-consuming sample preparation methods have been reported, including stages of defatting with *n*-hexane or petroleum ether [7,8], rotatory evaporation [7], solvent exchange [7,21], filtration on paper [22], extract clarification using Carrez reagents [19,23] and derivatization reaction [24]. Alternatively, environmental-friendly sample preparations based on QuEChERS extraction have been optimized for the determination of acrylamide in some food matrices [25,26].

LC–MS achieves adequate performance characteristics for the identification and quantification of acrylamide in foodstuffs [19]; although, some authors have demonstrated the applicability of LC with diode array detection (DAD) [22,27]. The most of LC–MS methods includes electrospray interface (ESI) and triple quadrupole (QqQ) mass analyzer, which has contributed to acrylamide detection at low levels [20]. In addition, atmospheric pressure chemical ionization (APCI) and ion trap mass analyzer has also been related [23]. An advantage associated to LC methods includes acrylamide determination without derivatization step resulting in a shorter sample preparation [20]. On the other hand, underivatized acrylamide analysis by GC–MS presents some drawbacks such as unselective detection of the compound due to lack of characteristic ion in mass spectrum as well as high interference caused by matrix composition and high limits of detection [20].

For the HDMF analysis, LC–MS/MS with APCI ionization and QqQ mass analyzer has also been applied to apple cider and wines, after solid-phase extraction (SPE) [28]; while LC coupled to DAD set at wavelength of 280 nm was described for strawberries employing aqueous extraction and clarification of extracts with Carrez solutions [29]. Additionally, GC coupled to flame ionization detection (FID), olfactometry or MS has been reported for HDMF analysis [30–33]. For this purpose, sample preparations involving liquid–liquid or solid-liquid extractions with large volumes of extracting solvent such as dichloromethane or diethyl ether, as well as solid-phase microextraction (SPME) and headspace extraction techniques have been used for several foodstuffs [30–33]. Therefore, the development of straightforward sample pre-treatments has been encouraged, particularly focusing minimal reagent consumption and lower waste generation.

In the present work, an accurate and selective method based on solid-phase extraction and LC-QqQ-MS/MS was developed and validated for the simultaneous determination of acrylamide and HDMF in baby food. The proposed method meets the Commission Decision 2002/657/EC criteria for quantitative method of analysis [34] as well as the Commission Recommendation 2010/307/EU for the monitoring of acrylamide in food [35]. In addition, the expanded measurement uncertainty was estimated from in-house validation data and the developed method was successfully applied to commercial samples of baby food available in the Brazilian market.

2. Experimental

2.1. Chemicals and consumables

Analytical standards of acrylamide (99.9%), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (99%) and the internal standard (IS) methacrylamide (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock standard solutions at concentrations of 1087, 1081 and 1061 μg mL^{-1} were prepared in 10 mL of deionized water for acrylamide, methacrylamide and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, respectively; working standard solutions were obtained by diluting of these stock solutions with deionized water to obtain concentrations of 10 and 100 μg mL^{-1}. A multi-compound standard solution was prepared in deionized water at 1 μg mL^{-1} by combining appropriate aliquots of individual working solutions. All standard solutions were stored at −18 °C in glass flasks and protected from light. Acetonitrile (J.T. Baker, USA) and methanol (Panreac, Spain) were HPLC grade; and formic acid (85%) was acquired from Synth (Diadema, SP, Brazil). Primary secondary amine (PSA) sorbent was purchased from Supelco (Bellefonte, PA, USA). The deionized water was obtained using a Milli-Q system (Millipore, Milfold, MA, USA). PVDF syringe filters (0.22 μm pore size, 13 mm i.d.) were purchased from Merck Millipore, and the glass vials, all LC–MS certified, from Waters. Bond Elut SCX (100 mg) SPE cartridges were supplied by Agilent.

2.2. Sample collection

A total of fifteen samples of ready-to-eat baby foods, which included fruit purées, cereal flour, starch, milk and/or other dairy ingredient in their composition, was purchased in the city of Campinas, located in the South-Eastern region of Brazil, between January and February 2016. The samples were randomly collected from 3 supermarkets and all they were kept in their original packaging, glass jars (120 g each) or stand up pouch (113 g each), at room temperature until analysis.

2.3. Simultaneous determination of acrylamide and HDMF

2.3.1. Sample preparation

Five grams of baby food sample, previously homogenized, were weighed into a polypropylene centrifuge tube and 5 mL of acetonitrile were added. The mixture was shaken in a vortex for 2 min. After centrifugation at 4000 rpm for 30 min, the supernatant was transferred to a volumetric glass flask and the volume was completed to 10 mL with acetonitrile. Then, 7 mL of acetonitrile extract were added to a polypropylene centrifuge tube containing 350 mg of PSA sorbent, and each tube was vortexed for 1 min and centrifuged at 4000 rpm for 15 min. Five milliliters of the clarified extract were transferred to a volumetric glass tube and this was carefully evaporated to 2 mL under a stream of nitrogen, followed by addition of 1 mL of 0.01% formic acid solution. Finally, 1 mL of the acidified extract was passed through a Bond Elut SCX (100 mg) cartridge in a 20-port vacuum manifold at approximately 15 drops per minute rate. The eluate was collected, filtered through a 0.22 μm PVDF