Quantification of acrylamide in potato chips by hplc-ms

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ABSTRACT: A method of reversed-phase liquid chromatography coupled with mass spectrometry (LC-MS) in combination with a stable isotope dilution assay was used to quantify acrylamide in potato chips. This in-house procedure was evaluated for precision, and accuracy. LC-MS revealed as a cost-effective and robust technique for acrylamide analysis in potato chips. Acrylamide formation was studied during deep-frying in both sunflower and olive oil under domestic processing conditions at 170ºC and 190ºC for 3, 5 and 7 min. Although acrylamide increased with temperature and frying time regardless of the type of frying oil used, it was shown that for a similar time period (5 min), acrylamide levels were higher in olive oil than in sunflower oil at both temperatures.

INTRODUCTION

Acrylamide (AA) in thermally processed starchy food is primarily formed during a Maillard-type reaction of asparagine and carbonyl groups of reducing sugars such as glucose (1-3). Since AA has been shown to be carcinogenic and mutagenic both in animals and humans (4, 5), public health concerns have caused an increasing interest to be shown in this naturally formed substance. Maximum acrylamide levels have not been established yet by European Union legislation although considerable efforts have been made to lower AA levels in foodstuffs (CIIA Acrylamide Toolbox, 2009) (6). Many researchers have confirmed the presence of this compound in carbohydrate-rich foods, mainly in potato products like chips and have shown that the AA amounts formed are attributable to parameters such as time and temperature (1, 7).

Because of its chemical properties such as poor retention, high polarity or lack of a chromophore group, acrylamide has not been easy to detect. Analytical techniques used for acrylamide analysis are gas chromatography coupled with mass spectrometry (GC-MS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) to increase sensitivity. These methods have become powerful analytical techniques for AA quantification and identification, though rather expensive and requiring laboratory safety issues to be considered. LC-SQ is a rapid, robust and cost effective alternative to determine acrylamide content in a number of food commodities, but efficient clean-up steps like solid phase extraction (SPE) are necessary to prevent co-extractive interference and obtain good results.

By applying these analytical techniques and assessing exposure and risk from dietary intake, data bases were designed to compile results such as those of the Institute for Reference Materials and Measurements (IRMM) of the European Union (http://www.irmm.jrc.be/html/activities/acrylamide/database) and the FDA (http://www.cfsan.fda.gov). More recently, EFSA confirmed the need to continue collecting acrylamide data for foods to evaluate more thoroughly the effectiveness of the mitigation strategies launched (EC-Commission Recommendation, 2007) (8). The scientific report of EFSA (2009) (9) came to the conclusion that acrylamide levels, in particular those for potato chips and bread, seemed to have decreased over time. Reduction of acrylamide concentrations in potato products has been the subject of investigation by many researchers (10-12) but, in Spain this has not been the case, so the obtention of data for Spanish potato products is of particular interest. Therefore, the present study has determined AA levels in potato chips by a rapid in-house validated method based on solid-phase extraction and subsequent determination by LC-MS in the SIM mode. The influence of different parameters, such as oil type (sunflower and olive oil), time and frying temperature have also been studied. All the samples were fried at lab-scale.

MATERIAL AND METHODS

Potatoes

Fresh potato (Agria variety) was harvested and collected in Spain during the appropriate season and stored at 8ºC. The tubers were washed, drip dried, peeled and then sliced into pieces 2 mm thick with a slicer. The sliced potatoes (350 - 400 g) were soaked in tap water (20ºC / 3 L) for 5 minutes as pre-treatment and subsequently wiped off for frying.

Frying process

The frying experiments were performed with sunflower and olive oil at 170 and 190ºC for 3, 5 and 7 minutes. In the last minute before removing from the basket, a grid was placed in the basket to ensure complete immersion of the potato slices during frying. This step was done to mimic industrial-scale conditions where potato slices are immersed in the frying oil in the last stage of the continuous process. After frying, samples were allowed to stay in the basket to dry, and were then ground, vacuum-packed and stored at -20ºC for additional experiments. The experiments were performed in triplicate by using three different fryers at the same time. During the frying process a mass ratio potato:oil of about 3 percent was maintained.

Determination of acrylamide content

A powdered sample (0.450 g) was weighed and mixed with 5 mL water and placed in polypropylene centrifugal tubes. The mixture was then spiked with 100 µL of a 10 µg/mL [13C3]-acrylamide methanolic solution, an internal standard procedure, and later homogenized for 1 min. Afterwards, the sample was treated with 750 µL of both Carrez I and Carrez II solutions and centrifuged (9000 g for 10 min) at 4ºC. For sample clean-up, Oasis-HLB cartridges were used. Cartridges were preconditioned with 1 mL methanol, and 1 mL water. An aliquot of the clear supernatant (1 mL) was loaded onto the cartridge at a flow rate of 2 mL/min and first drops were...
discharged. The solution was filtered through a 0.45 µm filter into an amberlite LC-MS vial. Sample extracts and calibration standards were analysed on an Agilent 1100 liquid chromatographic system coupled to an Agilent Quadrupole MS detector (Agilent Technologies, Palo Alto, CA, USA). Analytical separation was performed with an Inertsil column (25 x 0.46 cm, 5 µm, GLC-Sciences, Tokyo, Japan) at 32°C. Isocratic elution was achieved with a mobile phase of 0.1 percent formic in water at a flow rate of 0.6 mL/min. Electrospay ionization in the positive ionization mode was used. The MS detector operated in the selected ion monitoring (SIM) mode at m/z ratios of 72.1 and 75.1 for acrylamide and [13C3]-acrylamide respectively. Under these chromatographic conditions, acrylamide eluted at 11.7 min. A delay time of 8 minutes was selected to prevent the introduction of co-extracted matrix components into the MS instrument prior to acrylamide elution. The needle and cone voltages were set at 3.0 kV and 100 V respectively. Nitrogen was used as nebulizer gas (12.0 L.h⁻¹) and the source temperature was set at 300°C. Acrylamide was quantified using a linear calibration function that was established with standard solutions of acrylamide and [13C3]-acrylamide in Milli-Q water (5 to 1000 µg/L). The acrylamide content in sample extracts was calculated from the calibration slope and intercept value, taking into account the recovery calculated by means of the value, taking into account the calibration slope and intercept value. The limit of recovery calculated by means of the value, taking into account the calibration slope and intercept value. Sample extracts was calculated from µg/L). The acrylamide content in potato chips with satisfactory results. Furthermore, the analysis was integrated within the scope of a certified laboratory controlled by the Tukey test.

**RESULTS AND DISCUSSION**

Generally, liquid chromatography tandem mass spectrometry (LC-MS/MS) is used extensively for acrylamide analysis in food. However, most of these methods are highly expensive. This paper describes a simple and cost-effective method based on liquid chromatography coupled to a single quadrupole mass detector capable of analysing acrylamide with high sensitivity, repeatability and recovery. Satisfactory results for this method have been reported internally (in-house validation) and externally by participation in proficiency tests. On an industrial scale, the most common frying temperature for potato products was 180-190°C before the “acrylamide issue” arose. In this work we have analysed acrylamide levels in potato chips which were fried at lab scale at 170 and 190°C for different periods of time. The influence that the oil type and acrylamide formation was also studied. Olive oil and sunflower oil were selected as frying medium since these oils are used by Spanish industry.

Acrylamide formation is a surface phenomenon that depends on temperature and time and requires temperatures of 120°C for its formation in food during cooking (14). The acrylamide concentration in potato chips fried at 170 and 190°C in sunflower and olive oil is presented in Figure 1. It is obvious that under conditions of constant temperature, the frying time affected the amount of acrylamide formed during the process. In both experiments, the highest concentrations were detected at 7 min showing that the levels increased with the frying time. While this increase at a temperature of 170°C followed an almost linear function, at 190°C there was a drastic increase between 3 and 5 min, and from then on the amounts of acrylamide continued to rise slowly. Remarkably, Figure 1 also indicated an increase in the formation of acrylamide by increasing the temperature from 170 to 190°C. From this it can be concluded that time and temperature are key factors in determining AA levels in potato products as has been repeatedly demonstrated (7, 15). This is due to the fact that during the final stages of the frying process, the surface becomes drier and the temperature rises above 120°C.

With regard to the oil used, Figure 2 shows the acrylamide concentration in potato chips fried with sunflower and olive oil and takes into account all the variable factors, time (3, 5 and 7 min) and temperature (170 and 190°C). Statistical analysis showed no significant differences though it should be stressed that results differed significantly for 5 min, which was the best frying time for colour and texture. When potato chips were fried for 5 min at 170 and 190°C in olive oil, acrylamide values were higher and significantly different from those of sunflower oil (Figure 3). Publications dealing...
with this subject have generated controversy between researchers. Previously, Mestdag et al., 2005 (16) demonstrated that the origin of the deep-frying oils did not seem to affect acrylamide concentrations in potato during frying, and Williams (2005) (17) came to the same conclusion. In contrast, Gertz and Klostermann (2002) (18) reported that different oils could affect acrylamide formation in French fries and Becalski et al. (2003) (14) also found that acrylamide concentration was influenced by the choice of frying oil.

CONCLUSION

In summary, it can be concluded that frying at a lower temperature for a shorter time will reduce the amount of acrylamide produced in fried potato chips. But, it is important to stress that other parameters such as colour or overall sensorial attributes will also inevitably be affected, and would produce a negative impact on product quality and consequently on consumer acceptance. So, a compromise between reduction of acrylamide and quality attributes is worthy of consideration. Furthermore, the influence of the oil used was specific to a determined time, since in general acrylamide formation was not favoured by using either sunflower oil or olive oil. Additionally, the sugar content in the raw material, the storage temperature of the potato and pre-treatments with citric acid, water or asparaginase also play a part in determining acrylamide concentration, but these factors were not the objective of the present investigation.

REFERENCES AND NOTES

6. Confederation of the Food And Drink Industries in the EU (CIAA), The CIAA Acrylamide Toolbox, Rev.12. Available at: