DEVELOPMENT OF THE ANALYTICAL METHOD TO DETERMINE ACRYLAMIDE IN STRAW POTATO: VALIDATION AND DETERMINATION

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Acrylamide is not naturally present in food. One of the mechanisms of its formation in foods occurs through the Maillard reaction between amino acids, especially asparagine, and reducing sugars, when exposed to high temperatures. Foods rich in these two precursors are derived mainly from products of plant origin such as potatoes and cereals. The formation of acrylamide is observed in the cooking, frying, roasting or carbohydrate-rich foods baked with temperatures above 120°C, which tends to increase with cooking time and with the rising of the temperature. According to the International Agency for Research on Cancer (IARC), acrylamide is classified as a likely carcinogenic substance in humans (group 2A). Furthermore, it can be toxic to the nervous and reproductive systems of men and animals at certain doses. The World Health Organization (WHO) has established a tolerable daily intake of acrylamide of 12 μgkg $^{-1}$ body weight (BW) / day. Numerous studies around the world have facilitated the emergence of more specific research, allowing to evaluate the exposure to acrylamide in a certain context on a determined food. The objective of this study is to validate an analytical method by high performance liquid chromatography (HPLC) for the evaluation and quantification of the presence of acrylamide in straw - type fried potatoes marketed in the city of Rio de Janeiro. The analytical method used for the detection and quantification of acrylamide in straw potato was HPLC with ultraviolet detection. The validation of the method was supported by linearity tests, repeatability, matrix effect, intermediate precision, determination of the limit of quantification and limit of detection. The content of acrylamide found in the potatoes varied from 134.27 μgkg $^{-1}$ to 427.42 μgkg $^{-1}$. The effective determination of the acrylamide levels in different foods consumed by the Brazilian population may contribute to future public policies in order to establish guidelines for the manufacturing of processed foods. So that the content of this contaminant would be limited objectively.

KEYWORDS: MAILLARD REACTION; HIGH PERFORMANCE LIQUID CHROMATOGRAPHY; FOOD

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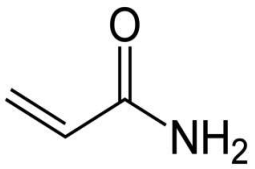
INTRODUCTION

Acrylamide (Figure 1) is a white and crystalline solid, with molecular weight 71, soluble in water, ethanol, ether, dimethyl ether, acetone and insoluble in benzene. Also known as 2-propenamide, its chemical structure consists of a polar amide function that grants its high water solubility and the function vinyl, which enables the polymerization (US EPA, 94).

FIGURE 1- ACRYLAMIDE CHEMICAL STRUCTURE



Acrylamide is not naturally present in food. One of the mechanisms of its formation in foods occurs through the Maillard reaction between amino acids, especially asparagine, and reducing sugars, when exposed to high temperatures. (CHAROENPAINICH et al, 2012). Foods rich in these two precursors are derived mainly from products of plant origin such as potatoes and cereals (FRIEDMAN; LEVIN, 2008). The formation of acrylamide is observed in the cooking, frying, roasting or carbohydrate-rich foods baking with temperatures above 120°C (TAREKE et al, 2000; 2002). This tends to increase with cooking time and raising the temperature (JACKSON, AL TAHER, 2005). This route seems to be more relevant in the formation of acrylamide in foods (FRIEDMAN, 2003). Since the formation of acrylamide in the process of heating amide-contained foods was observed and high contents of this substance were encountered in industrialized foods, there is a debate involving the potential human health risks related to its exposure (LOFSTEDT, 2003).

Being rapidly absorbed by the gastrointestinal tract and widely absorbed by tissues, acrylamide, which is glycidamide oxidized through catalyzing reaction to cytochrome enzyme P450 2E1, is also rapidly excreted in the urine of testing animals. Both, acrylamide and glycidamide, are reactive substances that form adducts with proteins (SEGERBÄCK et al, 1995). However, glycidamide is more reactive to DNA than acrylamide (FAO/WHO, 2005), thus the metabolite glycidamide is considered a key genotoxic factor of the exposure to acrylamide (PAULSSON, et al 2001). These substances have carcinogenic, mutagenic and clastogenic effects in mammals cells (JOHNSON et al, 1986; IARC, 1994), and may impair the fertility of male rodents by affecting the morphology and number of their sperms (FAO/WHO, 2005).

In humans, there is inconclusive data until now (VINHAS, 2011). A study conducted by Dutch researchers showed increase in risks of endometrial cancer in postmenopausal women, specially in non-smokers (HOGERVORST, et al, 2007). The same group of researchers in 2010 pointed out some epidemiologic indications that acrylamide may cause cancer in humans, which would be more evident in postmenopausal cancers (HOGERVORST, et al, 2010). Nevertheless, the authors emphasize the necessity of further investigation on the topic (HOGERVORST, et al, 2010). In a study on the acrylamide- and glycidamide-induced genotoxic damages in culture of human lymphocytes, it was concluded that glycidamide caused more damages to chromatin than the acrylamide, which needed to be tested in higher concentrations than glycidamide to present the harmful effect (PINGARILHO et al, 2013). After all, the main question remains, and how the acrylamide content in food may indeed impact the human health must still be clarified (ARISSETO; TOLEDO, 2006). Therefore, further researches should address the genotoxic and carcinogenic potential of these substances in humans prior to arriving at conclusions (ARISSETO; TOLEDO, 2006).

In view of the potential risks of the acrylamide ingestion, several countries have been developing researches to mitigate the formation of this contaminant during the process of producing food. Food Drink Europe Federation has recently launched a type of primer with guidelines and tools to manufacturers in order to reduce the formation of acrylamide in food products (EFSA, 2015). Apparently, the most effective strategy for the mitigation of acrylamide in foods is choosing raw materials with as less acrylamide content as possible and discouraging high temperatures in cooking or frying. For straw potatoes it would be the selection of varieties of potatoes with less sugars. But there is also the possibility of using the enzyme asparaginase, farming in specific soils, increasing storage process control, in addition to blanching the potatoes to help mitigate the production of this contaminant. It is important to notice that there is a huge difficulty of adherence to some proposed strategies, since they generate significant changes in the sensorial profile of foods. But given the risks of exposure to acrylamide to human health, practical measures should be adopted to diminish this exposure. (FOGLIANO et al, 2016; PEDRESCHI; MARIOTTI; GRANBY, 2014).

Multiple advances worldwide are observed in studies with acrylamide, facilitating the appearance of more specific researches where it is possible to concentrate in one food and with this, to obtain a world scenario of the presence of this contaminant in several products from different countries. In 2012, it was conducted a study in China to quantify the acrylamide present in instant noodles (YAN HE et al, 2012), and in the same year, in Thailand, another study was conducted to quantify the acrylamide in local-manufactured snacks (CHAROENPANICH et al., 2012). Another recent study in Switzerland reported the quantity of acrylamide in *rostie* potato (PFEFFERLE et al, 2016). In Mexico, researchers evaluated the quantity of acrylamide present in several types of corns (SALAZAR et al, 2016). In Colombia it was also determined the content of acrylamide in brown sugar. (LASSO et al, 2014).

The World Health Organization (WHO) has established a value of daily intake of acrylamide tolerable of 12 μgkg^{-1} BW / day. And fixated a NOAEL (No Observed Adverse Effect Level) for neuropathy in 0.5 $mgkg^{-1}$ BW / day and a NOAEL for changes of fertility four-fold bigger: 2 $mgkg^{-1}$ (JÁUREGUI, 2016). The National Sanitary Surveillance Agency (ANVISA) has not yet determined a restriction limit to acrylamide in foods, however, Directive number 2914 dated December 12, 2011 of the Brazilian Ministry of Health (Brazilian Ministry of Health published in the Public Daily Gazette of December 14, 2011) establishes the limit of acrylamide in 0.5 μgkg^{-1} in drinking water for human consumption (BRASIL, 2011) and the Commission Regulation Number 10/2011 established the SML (SPECIFIC MIGRATION LIMIT) = ND (non-detected) for foods and the Detection Limit (reliability) of the analytical method of 10 μgkg^{-1} (Comunidade Europeia, 2011).

Innumerous methods to determine acrylamide in foods have already been published (ARISSETO; TOLEDO, 2006), showing the advances in relation to the development of analytical methodologies. Coupled-chromatography mass spectrometry in series is used, for example, to avoid a long preparation of the sample and to obtain faster methods for larger number of different matrixes (ARISSETO; TOLEDO, 2006), though some investigators discourage the use of liquid chromatography with ultraviolet detection (UV), claiming that acrylamide does not present a strong spectrum in UV (CASTLE; ERIKSSON, 2005). A study of PALEOLOGOS and KONTOMINAS conducted in 2005 to detect acrylamide in foods by UV-detection high performance liquid chromatography, demonstrated applicability and similar results to that reported in the literature. This is a simple, rapid and cheap technique for possible routine analysis in foods (ARISSETO; TOLEDO, 2006).

The validation of the analytical methodology ensures the quality of the results and trustworthiness of the analysis performed. According to the Guidelines Validation of Analytical Procedures, the laboratories must have objective criteria and means to demonstrate, by means of validation, that the testing methods they adopt lead to reliable and appropriate results to the intended quality. The validation consists in the proof through demonstration of objective evidence that the demands for an application or specific use were complied with (INMETRO, 2016). In order to ensure the efficiency of a validation process, it is crucial that the parameters of validation and its criteria of

acceptance are correctly specified. There are several protocols to validate a method, and the matrix to be analyzed determines the choice of which protocol better matches the research, as well as which parameters are more applicable to this matrix (BAZILIO et al, 2014). Among the main parameters, the following are the most applied: working range, linearity, recovery, repeatability and intermediate precision, selectivity, robustness and detection limits and quantification (BAZILIO et al, 2014).

The control of acrylamide levels in food is one of the greatest challenges of Public Health and it is of crucial relevance because of the toxicological risks associated with the intake of this substance. Therefore, the current paper intends to contribute with Sanitary Surveillance by determining the levels of this contaminant in foods in order to prevent harms to the population health.

OBJECTIVE

To validate the analytical method by high performance liquid chromatography and to quantify the presence of acrylamide in straw fried potatoes traded in the city of Rio de Janeiro.

MATERIAL AND METHODS

SAMPLES

Three samples of three different brands of straw potato (Figure 2) traded in several markets of Rio de Janeiro were purchased. For each one of the three brands, three samples were bought in three different sales points.

The three different types of straw potato were selected byfollowing the criteria: most popular, cheaper and intermediate brand. These samples were coded with letters A to I (nine samples).

FIGURE 2 – STRAW POTATO

STANDARD

- Acrylamide P.A – standard – Bio-Rad Laboratories – 99,9 % purity.

EXPERIMENTAL PROCEDURE

Analysis of the samples

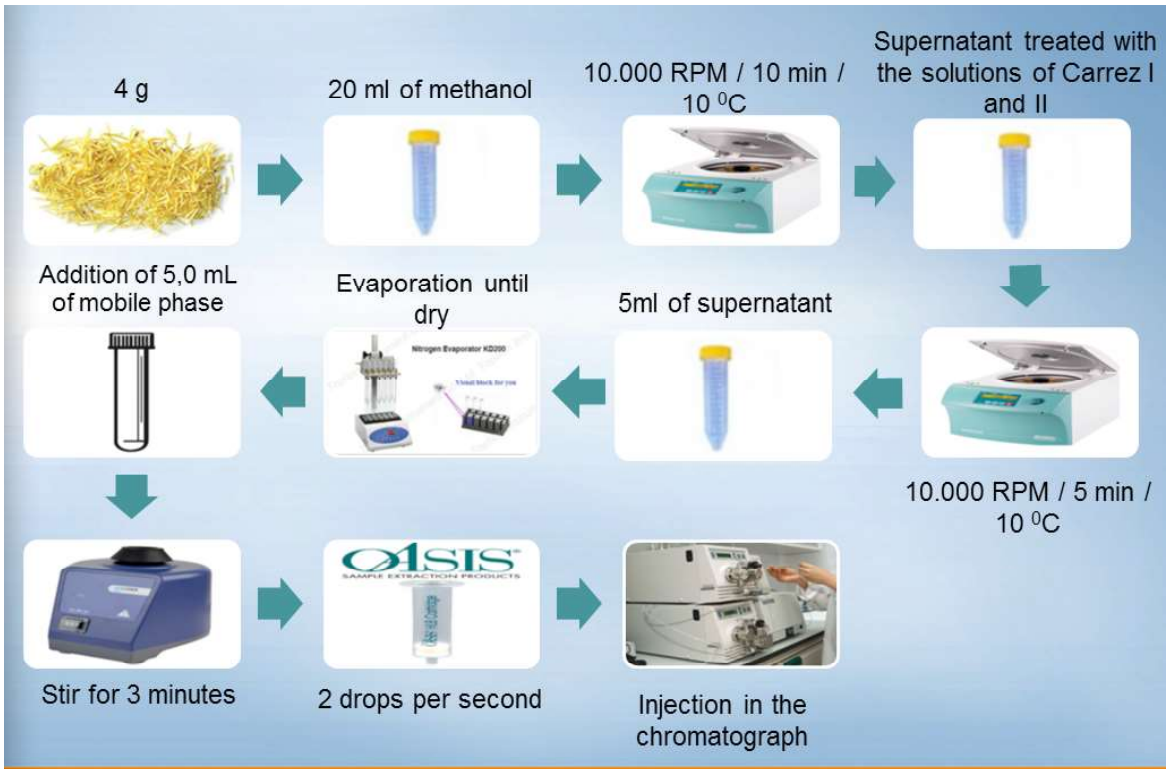
In a blender, 50 g of straw potato were ground. The whole process is displayed in Figure 3 and described in details in the following. A 4 g sample was weighted, and then homogenized in 20 mL of methanol for 3 minutes. Then, the solution was centrifuged for 10 minutes at 10.000 RPM, at 10^0 C. For clarification, the supernatant was transferred and treated with 100 µl of each one of the

solutions of Carrez I and II (Solution of Carrez I - 15 g of potassium hexacyanoferrate in 100 mL of water and Solution of Carrez II - 30 g of sulfate of zinc in 100 mL of water) and again centrifuged for 5 minutes at 10.000 RPM, at 10^0C. After, 5,0 mL of the limpid supernatant was withdrawn and evaporated until dry in smooth nitrogen current. In sequence, 5,0 mL of mobile phase was added: water/acetonitrile (95:5, v/v) and stirred in a mixer for 3 minutes. This solution was passed to a C18 OASIS HLB cartridge (packed with 1 mL of methanol + 1 mL of water at a flow of 2 drops per second), discarding the 7 initial drops. An aliquot of 150 µL of the final solution was injected in the chromatograph. This procedure was performed in true triplicate. For each analysis, standards and samples were solubilized in mobile phase; water/acetonitrile (95:5, v/v) to diminish the noise provoked by the baseline in the chromatogram.

The solutions were analyzed under the following chromatographic conditions:

- mobile phase: water/acetonitrile (95:5, v/v);
- flow: 1.0 mL/min;
- wave-length: 200 and 226 nm;
- “loop”: 500 µL;
- volume of injection: 150 µL.
- high performance liquid chromatography (HPLC), with isocratic elution mode, detector UV/vis WATERS – 2487 (variable and with double wave length), automatic injector WATERS 717 PLUS AUTOSAMPLER and computer for data acquisition and processing - Column C18 reversed phase (25 cm length and 4 mm diameter) SYMETRY® C_{18} – WATERS.

FIGURE 3 – FLOW FOR SAMPLE ANALYSIS OF THE STRAW FRIED POTATO.



Development and Validation of the Analytical Method

The analytical method to detect and quantify acrylamide in straw potato was validated intralaboratorially and was based on linearity analysis, effect matrix, detection limit, quantification limit, repeatability, intermediate precision and robustness. The procedure to intra-lab validation followed

the Standard Operational Procedure (SOP) 65.3120.126 (INCQS, 2015), based on documents of the "Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO)", DOQ-CGCRE-008 (2016); Thompson, Ellison and Wood (2002); Horwitz and Albert (2006) and Souza (2007).

INTRA-LAB VALIDATION

Working Range

The working range used for acrylamide was determined based on the concentration of the final aliquot of analysis (LM), considering the isolation procedure of acrylamide. This working range was based on Directive Number 10/2011 of the European Commission, which establishes that acrylamide must be undetectable by an analytical method with detection limit of until 10 μgL^{-1} (EUROPEAN COMMUNITY, 2011). The working range was also determined based on preliminary studies that present the contents of acrylamide per food. The applicable minimum range of concentration (AMR) through the formula (*Codex Alimentarius*, 2016) is:

$$AMR = LM \pm 0.44 \times LM$$

Where:
AMR – applicable minimum range;
LM – concentration of the final aliquot of analysis.

Linearity

Three stock solutions of acrylamide were prepared, weighting in analytical scale calibrated 3 mass approximately of 0.1 g of the standard of acrylamide. The masses were transferred quantitatively to 3 calibrated volumetric balloons of 100 mL and were solubilized in solution of water/acetonitrile (95:5, v/v). From each stock solution were taken 2 mL and completed the volume to 100 mL with water/acetonitrile (95:5, v/v). The final concentration of the solutions was 20 mgL^{-1} (S_1; S_2; S3).

A series of 18 working solutions were prepared, measuring the appropriate volumes, as presented in Table 1 (with the support of calibrated and certified pipettes by INCQS metrology laboratory) of the solutions of 20 mgL^{-1} and, diluted with solution water/acetonitrile (95:5, v/v) in calibrated volumetric balloon of 100 mL (INCQS, 2015).

After the preparation of the working solutions, each one of them was analyzed in the following chromatographic conditions:

mobile phase: water/acetonitrile (95:5, v/v);
flow: 1.0 mL/min;
wave length: 200 and 226 nm;
"loop": 500 µL;
volume of injection: 150 µL.

Under the aforementioned conditions, each concentration point of the analytical curve was analyzed 3 times and a chart was created from the height obtained in the chromatographic peaks related to the concentration of the work solutions.

Further, an assessment of the regression results was performed through the method of minimum ordinary squares. It was based on the assumption that the residuals follow the normal distribution, have homogenous variance and are independent. Such assumptions related to the analysis of the regression were evaluated for normality by the statistical method of Ryan and Joiner (1976); homogeneity of variances as proposed by Levene (1960) and Brown and Forsythe (1974); and independence of the residuals of regression as indicated by Durbin and Watson (1951). Test F was conducted to verify the adjustment to the linear model through evaluation of the significance of the regression (DRAPER E SMITH, 1998).

TABLE 1 – ACRYLAMIDE WORK SOLUTIONS

Concentration (μgL^{-1})	Acrylamide 20 mgL^{-1}					
	Volumes take S_1		Volumes taken S_2		Volumes taken S_3	
30	$S_{1.1}$ ←	150 µL	$S_{2.1}$ ←	150 µL	$S_{3.1}$ ←	150 µL
45	$S_{1.2}$ ←	225 µL	$S_{2.2}$ ←	225 µL	$S_{3.2}$ ←	225 µL
60	$S_{1.3}$ ←	300 µL	$S_{2.3}$ ←	300 µL	$S_{3.3}$ ←	300 µL
75	$S_{1.4}$ ←	375 µL	$S_{2.4}$ ←	375 µL	$S_{3.4}$ ←	375 µL
90	$S_{1.5}$ ←	450 µL	$S_{2.5}$ ←	450 µL	$S_{3.5}$ ←	450 µL
105	$S_{1.6}$ ←	525 µL	$S_{2.6}$ ←	525 µL	$S_{3.6}$ ←	525 µL

Repeatability

Pursuant to the method used to elaborate the analytical curve, 6 genuine repetitions of one type of straw potato were performed. The extreme values were excluded through the Grubbs test.

In 2002, Thompson, Ellison and Wood reduced the equation τ = 0,22 C, to estimate the standard deviation of reproducibility for food matrixes with concentration of analyte lower than 120 μgkg^{-1}, in in-lab tests of proficiency (THOMPSON; ELLISON; WOOD, 2002). The value of the coefficient of predicted reproducibility variation is deducted of 22% when it is replaced in the equation of the coefficient of variation.

Horwitz and Albert (2006) established one value of acceptance criteria lower or equal to two for the relation (HorRat) between experimental reproducibility ($CV_{R.exp.}$) of collaborative validation studies using analytical method and the predicted theoretical reproducibility ($CV_{R.pred.}$). Therefore, equal or lower values than 44% for coefficient of experimental reproducibility are acceptable.

Horwitz concluded, in 1982, that the values of coefficient variation of repeatability varied from one half to two thirds in relation to the coefficient of variation of repeatability. Thus, values lower than 29 % for the coefficient of variation of repeatability are appropriate to that purpose.

The standard deviation for repeatability (s_r) (Equation 1) and the coefficient of variation of repeatability (CV_r) (Equation 2) were calculated and it estimated the variation of repeatability in 29% according to the theory of Thompson, Ellison and Wood (THOMPSON; ELLISON; WOOD, 2002). Based on the criteria established from the equation of Horwitz and Albert (2006), the value of $HorRat_r$ (Equation 3) was calculated. Values of $HorRat_r$ lower or equal to 2 indicate appropriate repeatability.

$$s_r = \sqrt{\frac{\sum(x_i - \bar{x}_i)^2}{n-1}} \quad (1)$$

$$CV(\%)_r = \frac{s_i \ x\ 100}{\bar{x}} \quad (2)$$

$$HorRat_r = \frac{CV(\%)_r}{29\%} \quad (3)$$

Where,
S_r = standard deviation of repeatability
$CV(\%)_r$ = coefficient of variation of repeatability
$\dot{x}$ = average of the concentrations measured for the repetitions
x_i = value of each *i determination*

Limit of Quantification (LOQ) and Limit of Detection (LOD)

The determination of LOQ and LOD was carried out after the evaluation of the most appropriate analytical curve through the equations suggested by MILLER (1993).

Matrix Effect

For the determination of the matrix effect, 6 genuine solutions of the acrylamide standard and 6 genuine solutions of each one of the straw potatoes samples were prepared. Both types had the concentration of 75 μgL^{-1} acrylamide. From the results obtained with the chromatograms, a test was performed to check whether the results encountered in the standard and in the matrix are statistically equal, and consequently, if there was no matrix effect.

Intermediate Precision

The column used in the chromatograph was the variation chosen to be assessed to check the intermediate precision. The test was then performed under all equal chromatograph conditions, just with the variation of the column. For this test, 2 different batches of column C18 of reversed phase (25 cm) SYMETRY® C_{18} – WATERS were used.

The standard deviation for intermediate precision (s_i) (Equation 4) and the coefficient of variation of intermediate precision ($CV_{(\text{intermediate precision})}$) (Equation 5) were calculated and estimated the variation of reproducibility of 44% according to the theory of Thompson, Ellison and Wood (THOMPSON; ELLISON; WOOD, 2002). Based on the criteria established from the equation of Horwitz and Albert (2006), the value of $HorRat_{(\text{intermediate precision})}$ (Equation 6) was calculated. Values of $HorRat_{(\text{intermediate precision})}$ lower or equal to 2 indicate appropriate intermediate precision.

$$Si_{(j,k)} = \sqrt{\frac{1}{2.t} \cdot \sum_{j=1}^{t} (y_{j1} - y_{j2})^2} \quad (4)$$

$$CV\ (\%)_{(intermediate\ precision)} = \frac{s_{i\ x\ 100}}{\bar{x}} \quad (5)$$

$$HorRat_{(intermediate\ precision)} = \frac{CV\ (\%)_{(intermediate\ precision)}}{44\%} \quad (6)$$

Where,
Si = standard deviation of intermediate precision
$CV\ (\%)_{(\text{intermediate precision})}$ = coefficient of variation of intermediate precision
$\dot{x}$ = average of the concentrations measured for the repetitions
t = total of samples tested;
y_{j1} = determination of variation 1;
y_{j2} = determination of variation 2;
n = total of tests run per sample.
Values of $HorRat_{(\text{intermediate precision})}$ lower or equal to 2 indicate appropriate intermediate precision.

Robustness

To check the robustness of the method, two equal samples were analyzed five times in similar columns of the same manufacturer (A e B) and in different batches. The homogeneity of the variances encountered in the tests of column A and B through the test F (SNEDECOR; COCHRAN, 1989) (Equation 7) was checked. Statistic F was calculated and compared with the critical value F.

Confirming the homoscedasticity, the average of the concentrations was compared through test T (Equation 8). To calculate test T, the average of the differences (d) (Equation 9) and the variance of the differences (s^2) (Equation 10) were determined.

$$F_{calculado} = \frac{s_1^2}{s_2^2} \quad (7)$$

Where,

s_1^2 and s_2^2 are the variances of each sample with the higher variance in the numerator

$$t_{calculado} = \frac{\bar{d}}{\sqrt{s^2/n}} \quad (8)$$

$$\bar{d} = \frac{\sum_{i=1}^{n}(x_{i_1} - x_{i_2})}{n} \quad (9)$$

$$s^2 = \sum_{i}^{n} \frac{(x_{i1} - x_{i2})^2}{n-1} \quad (10)$$

Being,

x_{i_1} = concentration of the i-th replicate of the sample tested in the normal conditions of the methodology,

x_{i_2} = concentration of the i-th replicate of the sample tested for variation,

s^2 = variance of the differences.

Statistical Analysis

The data were analyzed for frequency and data cross-checking using the Microsoft Office Excel 2016. The electronic spreadsheet developed by Bazílio and collaborators (2012) was used to check the adjustment of analytical curves to the linear model.

For the intra-lab validation, the statistical methods adopted for the treatment of outliers in the assessment of linearity as the tests to check the normality and homogeneity of the variances of the data, the autocorrelation of the residues, as well as the tests for regression analysis and deviation of linearity, were mentioned throughout the presentation of the results.

ETHICS COMMITTEE

Because human beings are not involved in this part of the research, the Ethics Committee approval was not necessary.

RESULTS AND DISCUSSION

ANALYSIS OF THE SAMPLES

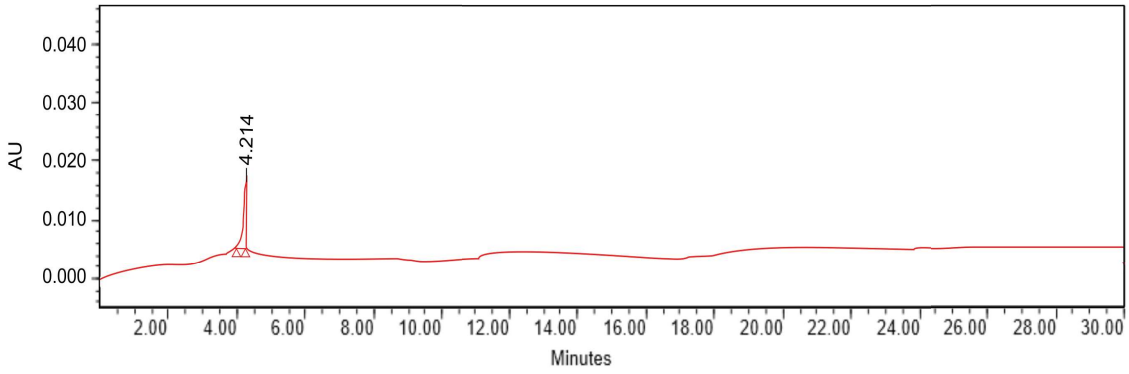
The analysis performed with straw potatoes showed good results in regard to the separation of acrylamide after various changes of the chromatographic conditions. The standard of acrylamide was injected always in the same conditions when samples were analyzed to show the guarantee of confirmation of acrylamide identity. The parameter used to determine the concentration of acrylamide present in each sample was the height of the peak, since the peaks of acrylamide are very low and the base line might interfere in the area of the peaks (Figure 4 and 5).

In order to confirm if the peak encountered was actually of acrylamide, the time of retention obtained with the injection of the standard in the same run was considered. In addition, the samples

enriched with acrylamide were also injected, to obtain the elevation of the peak. With the enrichment, there was always an elevation of only the peak corresponding to acrylamide.

The concentration of acrylamide present in straw potato was determined from the use of the validated analytical curve. The sample was diluted until a chromatographic peak was obtained with a concentration within the calibrated analytical curve. Based on this result, the concentration of acrylamide present in the intact sample of straw potato, i.e., before the dilution, was calculated.

FIGURE 4 – CHROMATOGRAM OBTAINED FROM THE STANDARD OF ACRYLAMIDE.



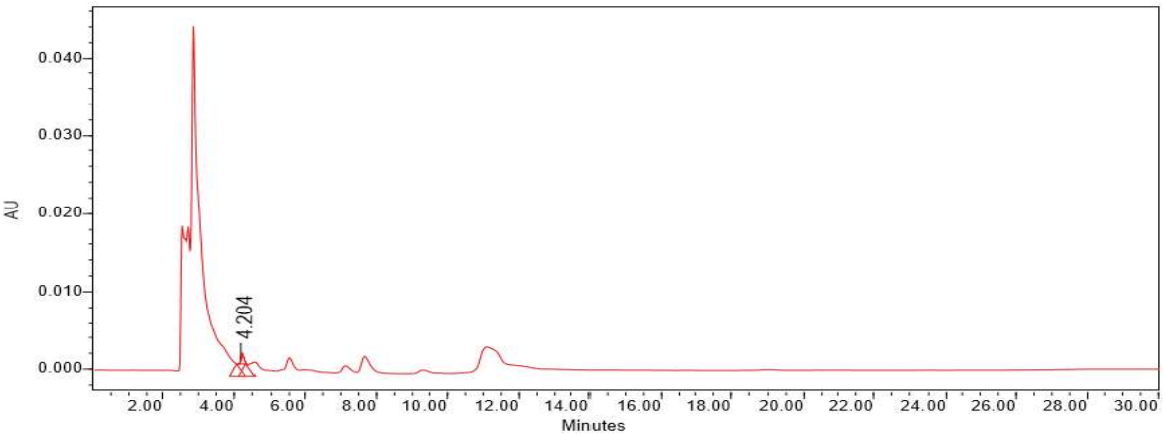
Chromatographic conditions: column C18 (25 cm x 4 mm), mobile phase: water/acetonitrile (95:5, v/v); flow: 1.0 $mLmin^{-1}$; length of the wave: 200 nm; volume of injection: 150 µL.

Three different brands of straw potato purchased in a market in Tijuca neighborhood were analyzed. The brands coded as A, B and C presented an average concentration of acrylamide of 427.42 μgkg^{-1}, the brands coded as D, E and F presented an average concentration of 306.56 μgkg^{-1} and the sample coded as G, H and I presented an average concentration of 134.27 μgkg^{-1}.

The average concentration of acrylamide in straw potato was 289.42 μgkg^{-1}.

In Figure 5, the chromatogram obtained for the determination of the content of acrylamide in straw potato F is displayed.

FIGURE 5 – CHROMATOGRAM OBTAINED FROM THE SAMPLE OF STRAW POTATO F.



Chromatographic conditions: column C18 (25 cm x 4 mm), mobile phase: water/acetonitrile (95:5, v/v); flow: 1.0 $mLmin^{-1}$; wave length: 200 nm; volume of injection: 150 µL.

Based on the analysis conducted in straw potatoes, we observed a fair separation of the peaks and a good extraction of acrylamide for the analysis. Both the extraction conditions (weight of the sample and volume of methanol used) and the chromatographic conditions (chromatograph, column, flow, volume of injection and UV wave-length) were modified several times in order to optimize the results obtained.

The amount of acrylamide present in the different brands of straw potato suggests that different processes of fabrication may lead to products with reduced content of this contaminant.

The cheapest brand of straw potato presented a content of acrylamide of 427.42 μgkg^{-1}, while the most expensive brand presented a content of 134.27 μgkg^{-1} and the most popular brand presented a content of 306.56 μgkg^{-1}. These results may indicate that producing facilities with more control can produce potatoes with less acrylamide. Therefore, it seems to be possible to develop and implement initiatives that clarify and encourage the industries to manufacture products with less acrylamide content.

Simple procedures may result in mitigation of acrylamide as, for example, the choice of varieties of potatoes with less sugar. In addition the use of the enzyme asparaginase, farming in specific soils, more careful storage control, frying process not generating excessive heat, and blanching the potatoes may also be implemented. Certainly, some of these procedures may generate relevant changes in the sensorial profile of the food, which would discourage the industries to use them. However, if there existed a recommendation of maximum content of acrylamide allowed for each type of food, the manufacturers would most likely endeavor to match their production to the legislation requirements. At the same time, the consumer would be exposed to a healthier product.

When we compare the results obtained in this paper with other studies worldwide, we concluded that the presence of acrylamide in straw potatoes varies widely from country to country. In Australia, researchers evaluated the presence of acrylamide in potato chips and cookies and found a variation of 25 to 1270 μgkg^{-1} of this contaminant in these foods (CROFT et al, 2004).In Belgian, the analysis of breads, cookies and potato chips encountered the presence of acrylamide between 100 and 1210 μgkg^{-1} of the sample (AFSCA, 2002).

In Turkey, researchers, found a variation between 20 to 3789 μgkg^{-1} of acrylamide in samples of cookies and potato chips (SENUYVA & GÖKMEN, 2005b).

According to the assessment performed by FAO/WHO in 2002, the content of acrylamide in potato chips varied from 1312 to 2287 μgkg^{-1} and in fried potatoes, from 537 to 3500 μgkg^{-1}. For this evaluation, 38 and 39 studies from different countries were analyzed, respectively. In 2005, the JECFA compiled 874 studies to investigate the presence of acrylamide in potato chips and concluded that the contents varied between 752 and 4080 μgkg^{-1}. In fried potatoes, JECFA encountered in 1097 studies reporting a variation from 334 to 5312 μgkg^{-1}. Until 2005, there were no Latin American studies that reported to WHO the content of acrylamide in food consumed by the population of these countries. In 2007, ARISSETO and collaborators analyzed and reported the presence of acrylamide in food consumed in Brazil, identifying a variation from 144 to 2528 μgkg^{-1} in samples of potato (fried, chips and straw). The content of acrylamide verified in the present paper (134.27 to 427.42 μgkg^{-1}) are in line with the results of the other studies.

INTRA-LAB VALIDATION

The results obtained from the procedures mentioned and related to the validation of the method are presented in the following items.

Working Range

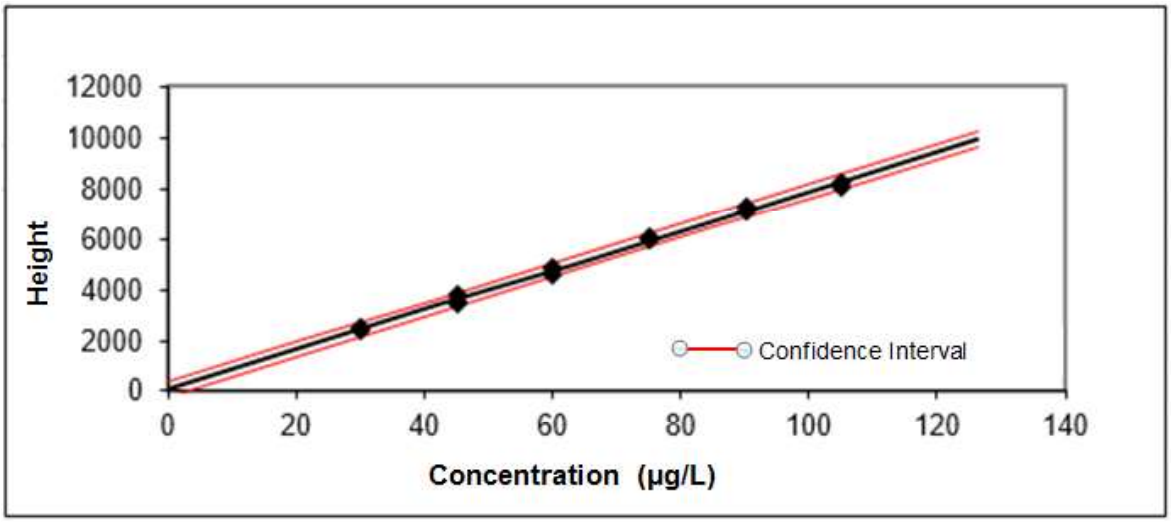
The working range for acrylamide was based on previous studies which demonstrate the amount of this contaminant in innumerous foods. As the Directive number 10/2011 of the European Commission, which determines that acrylamide must be undetectable per a method with detection limit of until 10 μgkg^{-1}.

The working range was within 30 to 105 μgL^{-1} and the amount of acrylamide present in straw potatoes was considered appropriate.

Linearity

The evaluation of the linearity and the design of the analytical curve (Figure 6) were performed through the method of ordinary least squares (OLS). The value of the outlier residues were analyzed previously by the method Jacknife. At each exclusion, the tests were performed again. The assumptions for the OLS are: normality tests, independence (non-autocorrelation of residuals) and homogeneity of the variances of the residuals, analysis of the variance for regression and deviation of the linearity of the residuals. These tests were performed for acrylamide.

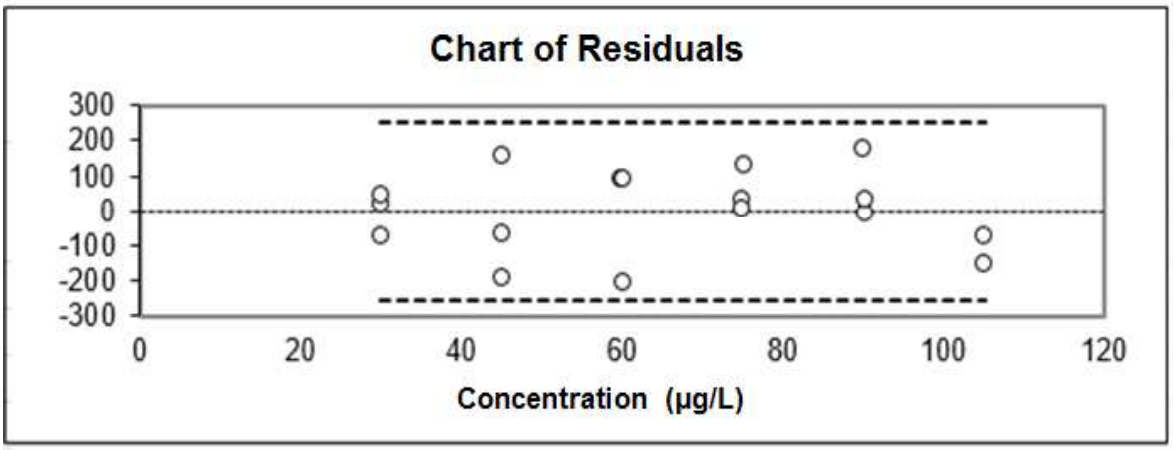
FIGURE 6 – FINAL ANALYTICAL CURVE BY METHOD HPLC FOR ACRYLAMIDE



Treatment of outliers

After performing the standard residuals Jacknife tests, it was observed that discrepant values did not overpass the limit of 22.2 % for rejected data established by Horwitz (1995), with only 1 exception (Figure 7).

FIGURE 7 – EXPLORATORY CHART OF THE RESIDUALS OF THE ANALYTICAL REGRESSION CURVE OF ACRYLAMIDE



Test of normality of residuals

The analysis of the data used to elaborate the analytical curve showed that the data follow the normal distribution through the correlation coefficient of Ryan-Joiner (R_{eq}). The coefficient calculated (Table 2) was higher than the established critical value of 0.94 (α = 0.05), there is no justification to reject the hypothesis null that the data follow the normal distribution (SOUZA, 2007).

TABLE 2 – NORMALITY OF RESIDUALS

R_{eq}	0.98
R_{crit} (α = 0.05)	0.94

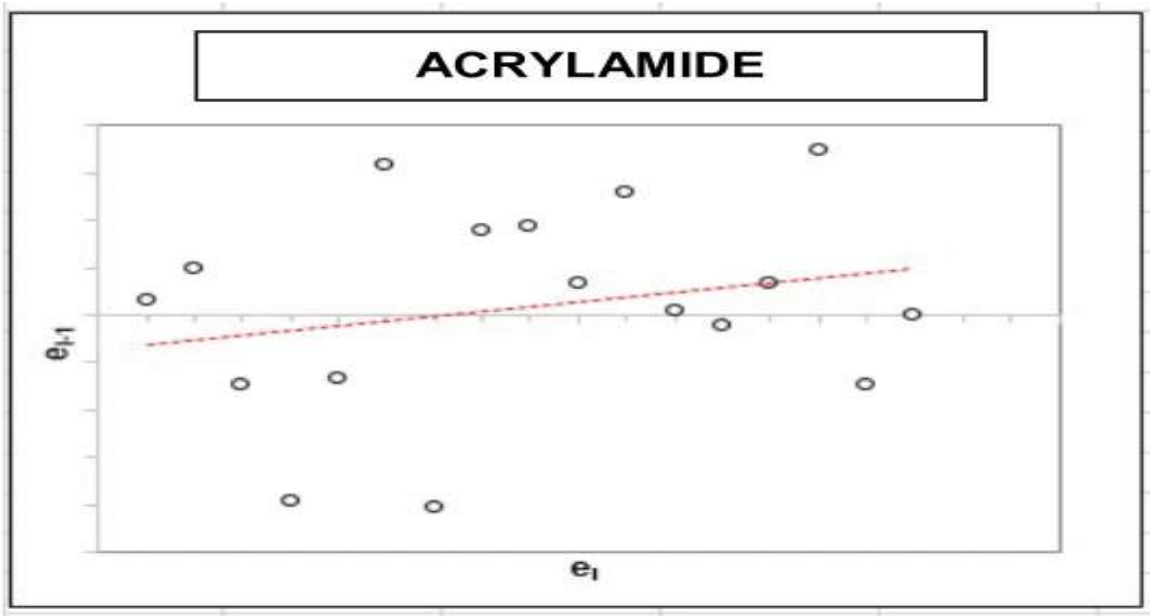
Test of homogeneity of variance of the residuals

The homogeneity of the variance of the residuals was assessed through the test of Levene adapted by Brown-Forsyte (SOUZA, 2007). The tabulated value of t (α = 0.05) is 2.14 and the value of t_L calculated was i -0.813, i.e, less than the tabulated t. So, the hypothesis null that the variances of the residuals of the regression are homogenous was accepted across the variances.

Test of non-correlation of residuals

The non-correlation or independence of the residuals of the regression was evaluated through the test of Durbin-Watson (SOUZA, 2007). The statistical value of the test is the value of *d*, inferior or superior. The value of *d* calculated was 2.04, above the critical values for d_L and d_U, respectively, 1.11 and 1.38 (α = 0.05), the hypothesis null was not rejected and it was confirmed the independence of the regression of the residuals. Figure 8 shows graphically the independence of the residuals of the regression.

FIGURE 8 – CHART OF DURBIN-WATSON OF THE ANALYTICAL CURVE (EI X EI-1) – ACRYLAMIDE



Analysis of the variance of the residuals of the regression and deviation of linearity

The linear regression and the adjustment to the model were confirmed through the test ANOVA. The data are presented in Table 3.

TABLE 3 - RESULTS OF THE ANALYSIS OF VARIANCE IN REGARD TO THE SIGNIFICANCE OF THE REGRESSION AND NON-SIGNIFICANCE OF THE DEVIATION OF LINEARITY (A = 0.05) FOR THE ANALYTICAL CURVE OF ACRYLAMIDE

Source	*D.F.*[1]	*SS*[2]	*AS*[3]	*F*	*P*[4]
Regression	1	6.26E+07	6.26E+07	4.42E+03	5.97E-20
Residuals	15	2.12E+05	1.42E+04		
Adjustment	4	5.27E+04	1.32E+04	9.08E-01	4.92E-01
Pure error	11	1.60E+05	1.45E+04		
Total	16	6.28E+07			

[1]Degrees of freedom; [2]Sum of squares; [3]Average square; [4]Value of p for the statistic F

From the ANOVA, the regression of the analytical curve related to acrylamide was significant ($p < 0.001$) and the adjustment to the linear model or the non-deviation of the linearity was non-significant ($p > 0.05$) observed through the statistical values of the calculated *p*.

Eventually, the linearity of the method was proved in the working range 30 and 105 μgL^{-1} and the following regression equation was obtained: $y = 77.7x + 97.2$. Where *y* is the height of the peak of acrylamide and *x* the concentration of the analyte.

The criteria required by the method of OLS are shown in Table 4.

TABLE 4 – SUMMARY OF THE ANALYZES OF THE LINEARITY HYPOTHESIS BY THE METHOD OF OLS

Test of normality (a = 0.05)1º
Follows the Normal - *Req* > *Rcrit*
Homogeneity of variance
There is homoscedasticity - *p > 0.05 2º*
Autocorrelation of residuals (a = 0.05)3º
There is no autocorrelation - *d > dU*
Regression and Test of Deviation of Linearity 4º
The regression is significant - *p < 0.001* There is no deviation of linearity - *p > 0.05*

Repeatability

Based on the criteria established from the equation of Horwitz and Albert (2006), the values of *HorRat* encountered (Table 5) indicate that the method present appropriate repeatability.

TABLE 5 – EVALUATION OF REPEATABILITY

Repeatability (HORWITZ and ALBERT, 2006)		
Fried potato	Value calculated $HorRat_{repe}$	Criteria of acceptability
Straw-type	0.2152	≤ 2.0

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The analytical curve elaborated for acrylamide (Figure 6) was used for the determination of the limits (Table 6).

TABLE 6 - LIMITS AND DETECTION AND QUANTIFICATION OF THE ANALYTIC CURVES FOR THE ANALYSIS OF ACRYLAMIDE

LOD	3.96 μgL^{-1}
LOQ	11.74 μgL^{-1}

Matrix Effect

The results obtained from the test of matrix effect indicate that the amount of acrylamide measured in samples of fried potatoes type straw (concentration in the aliquot of analysis of 75 μgL^{-1}) is practically half of the amount of acrylamide measured in a standard of acrylamide with concentration of 75 μgL^{-1}. Reminding that the parameter used for concentration measure is the height of the peak, the average height encountered in the standard of acrylamide at a concentration of 75 μgL^{-1} was 6359. On its turn, the average height in straw type potato was 3196.

It is important to highlight that if the result of the matrix effect is taken into account, the amount of acrylamide present in these samples can be two-fold of the encountered.

Intermediate Precision

Based on the criteria established from the value of *HorRat,* we encountered 0.0144, indicating that the method presents intermediate precision appropriate in relation to the columns tested, since the criteria of acceptability is *HorRat* ≤ 2.0.

Robustness

Through test F, it was verified that there is homoscedasticity of the variances, since the F calculated2.54, lower than the F critical of 6.39. Based on the positiv resultto homoscedasticity among the variances, test *t* was performed, obtaining a *t* calculated in 1.42, lower than the critical *t* 2.132.Thus, the methodology is robust.

Taking together, the method proposed in this study to determine the acrylamide in straw potato was validated and may be used to obtain reliable results for this matrix.

CONCLUSIONS

In this study, the presence of acrylamide in three different brands of straw potatoes were analyzed and the analytic method validated was deemed appropriate and accurate in its intent to identify and quantify the presence of the contaminant in fried potatoes type straw. The method validated has been shown robust and can be applied to obtain reliable results.

The experimental results demonstrated that the levels of acrylamide in straw potato varied between 134.27 to 427.42 μgkg^{-1} and comply with data reported by other countries.

Considering studies of other countries, each one estimates the presence of acrylamide in a specific food that is chosen as representative of the regular consumption of the target population. In the study herein, straw potato was chosen because it is widely consumed by the Brazilian population and because its market is increasing due to its practicality, since it does not need to be home-fried and are off-the-shelf. More than often, this type of potato is utilized as a side dish.

It is expected that the information generated with this paper may contribute for risk assessments worldwide, in addition to allowing a better understanding of the distribution of acrylamide in food of different countries.

The effective evaluation of the acrylamide content encountered in different food consumed by the Brazilian population tends to generate data that contribute to strengthen public policies, which could interfere and determine in how the production of processed food is conducted to eventually reduce the content of this contaminant.

RESUMO

DESENVOLVIMENTO DO MÉTODO ANALÍTICO PARA DETERMINAR A ACRILAMIDA EM BATATA DE PALHA: VALIDAÇÃO E DETERMINAÇÃO

A acrilamida não está naturalmente presente nos alimentos, sendo um dos mecanismos de sua formação em alimentos baseado na reação de Maillard, entre aminoácidos, em especial a asparagina e açúcares redutores, quando submetidos a altas temperaturas. Alimentos ricos nestes dois precursores são derivados, principalmente, de produtos de origem vegetal como as batatas e cereais. A formação de acrilamida é observada na cocção, fritura, tostagem ou no processo de assar alimentos ricos em carboidratos com temperaturas superiores a 120°C e tende a aumentar com o tempo de cozimento e com a elevação da temperatura. Segundo a *International Agency for research on Cancer* (IARC) a acrilamida é classificada como uma substância provavelmente carcinogênica em humanos (grupo 2A) e, além disso, pode ser tóxica ao sistema nervoso e reprodutivo de homens e animais em determinadas doses. A Organização Mundial da Saúde (OMS) estabeleceu um valor de ingestão diária de acrilamida tolerável de 12 μgkg^{-1} de peso corporal (PC) / dia. Inúmeros estudos ao redor do mundo têm facilitado o surgimento de pesquisas mais específicas, onde é possível se dedicar a um alimento e avaliar a exposição à acrilamida em um determinado contexto. O objetivo deste estudo foi validar o método analítico por cromatografia líquida de alta eficiência (CLAE) para avaliação e quantificação da presença de acrilamida em batatas fritas do tipo palha comercializadas no município do Rio de Janeiro. O método analítico utilizado para detecção e quantificação da acrilamida nas amostras de batata palha foi cromatografia líquida de alta eficiência com detecção por ultravioleta. A validação do método contou com os testes de linearidade, efeito matriz, repetibilidade, precisão intermediária, determinação do limite de quantificação e do limite de detecção. O teor de acrilamida encontrado nas batatas palhas variou de 134,27 μgkg^{-1} a 427,42 μgkg^{-1}. A avaliação efetiva dos teores de acrilamida encontrados em diferentes alimentos consumidos pela população brasileira, geraram dados que podem contribuir para que políticas públicas sejam fortalecidas e possuam embasamento para interferir e determinar que a fabricação de alimentos industrializados seja realizada de maneira a diminuir o teor deste contaminante.

PALAVRAS CHAVE: REAÇÃO DE MAILLARD; CROMATOGRAFIA LÍQUIDA DE ALTA EFICIÊNCIA; ANÁLISE DE ALIMENTOS; SUBSTÂNCIA CARCINOGÊNICA

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