European Food Research and Technology (2019) 245:2393–2400
https://doi.org/10.1007/s00217-019-03357-2

ORIGINAL PAPER

Acrylamide determination in baked potatoes by HPLC–MS: effect of steam and correlation with colour indices

Chiara Bignardi[1] · Antonella Cavazza[1] · Maria Grimaldi[1] · Carmen Laganà[1] · Chiara Manzi[2] · Massimiliano Rinaldi[3] · Claudio Corradini[1]

Received: 29 October 2018 / Revised: 30 June 2019 / Accepted: 16 August 2019 / Published online: 30 August 2019

Abstract
Acrylamide is a toxic compound and a thermal marker of food, deriving from Maillard reaction. A recent European Regulation established mitigation measures and benchmark levels for its reduction in many products encouraging the use of colourimetric scales providing a statistical correlation between colour intensity and acrylamide content. This study was focused on acrylamide determination by liquid chromatography coupled to mass spectrometry in baked potato samples cooked at different time, temperature and steam conditions. Effect of steam on acrylamide formation has been evaluated. Portions of cooked product characterized by different colours were sampled to create a colour scale. Acrylamide level was correlated with colour indices based on RGB channels. Results showed that similar colours, even obtained under different cooking conditions, were characterized by similar acrylamide levels. Statistical elaboration of the data allowed to find a high correlation between the two sets of data and to build a colour gradation that could be a starting point to elaborate a suitable tool to provide information on acrylamide content from colour analysis.

Keywords Acrylamide · Cooking process · Thermal marker · Baked potatoes · Colour indices · Steam cooking

Introduction

Acrylamide is a neurotoxin and a probable human carcinogen. Studies on cell cultures and laboratory animals have highlighted that acrylamide exposure can induce genetic modification, cancer as well as neurological damages and low fertility [1]. However, toxicological and epidemiological studies in humans are still necessary to confirm the existence of a statistically significant association between acrylamide intake and risk cancer increase [2].

From a chemical point of view, acrylamide is an amide containing an electrophilic double bond and an amine group and exhibits both weakly acidic and basic properties. The carboxamide group retains electrons and activates the double bond, which reacts with nucleophilic reagents. Akalinity conditions allow the addition of amines, mercaptans, sulphides, ketones, nitroalkanes and alcohols [3].

Interest about acrylamide has increased in recent years; the main cause of its ingestion is due to specific food products such as cakes, bread and fried or baked potatoes, as well as non-starch foods such as black olives and coffee [4]. Besides, it has also a wide variety of industrial non-food uses and is also present in tobacco smoke. Therefore, some people may be exposed by skin absorption or by inhalation.

Acrylamide is formed in foods by several pathways connected to the Maillard reaction [5] during cooking processes requiring temperature greater than 120 °C such as frying, roasting and baking. Its presence has not been found in boiled or steamed foods.

Several factors influence acrylamide formation in foods: type and concentration of reagents, physico-chemical characteristics of food matrices, and process parameters for food cooking.

Reagents that lead to acrylamide formation are reducing sugars and amino acids, mostly asparagine [6]. In potatoes,

✉ Antonella Cavazza
antonella.cavazza@unipr.it

[1] Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

[2] Cucina Evolution Academy, Viale Mentana 41, 43121 Parma, Italy

[3] Dipartimento di Scienze degli Alimenti e del Farmaco, Università di Parma, Parco Area delle Scienze 47/A, 43124 Parma, Italy

its content is about 940 mg/Kg over a total amino acids of about 2400 mg/Kg (about 40% on percentage basis), while in cereal flour it accounts for 200–400 mg/Kg over 1900 mg/Kg of total amino acids (about 15% on percentage basis) [5]. Despite the high content of this amino acid, some researchers found no direct correlation between its concentration and acrylamide content in potatoes [7], whereas higher amounts of reducing sugars result in a significant acrylamide increase [8]. The recent FoodDrinkEurope Toolbox [9] also confirms that reducing sugar content in potatoes is generally a good indicator of acrylamide forming potential, together with the ratio value between asparagine and total free amino acids. As for cereal products, a direct correlation between acrylamide formation and the initial asparagine concentration has been found [10] and, therefore, asparagine, rather than sugars, can be considered the key determinant [9]. Acrylamide concentration is also strongly dependent on the type of sugar involved in the reaction, with monosaccharides generally having a greater influence [10].

The pH of the food affects acrylamide formation because it influences the reactivity of the amine group of the amino acid involved, but also of the carbonyl group of reducing sugars. By increasing the pH, the amine group is deprotonated favoring the interaction between amino acid and sugar [11]. By decreasing the pH, the amine group is protonated and this penalizes the formation of the Schiff base (reaction intermediate in the acrylamide formation). The use of acidifying substances allows the reduction of the acrylamide content in foods [11, 12].

Water content of foods also affects the mechanisms of acrylamide formation [13, 14]. Low moisture content (less than 20% water relative to the total weight in potatoes and 10% in bakery products) generally leads to an increase in its final concentration [14, 15].

The two main process parameters that directly influence the acrylamide development are time and temperature of the heat treatment [16]. Parameters such as heat transfer mechanism, heating and cooling rate, shape and thickness of food, have an influence on concentration of acrylamide [17].

Several studies have shown that the use of certain compounds reduces acrylamide levels in foods and model systems. For example, the addition of amino acids other than asparagine in a biscuit model [18, 19], or the use of baking agents and organic acids led to a reduction in acrylamide in cracker [20], and corn chips [21]. Addition of natural antioxidants extracted from bamboo and green tea leaves could reduce acrylamide levels in foods [22]. Finally, in fried snack products, water-soluble vitamins reduce acrylamide formation [23].

The discovery of high concentrations of acrylamide in foods has raised many concerns. In 1994, acrylamide was classified as probably carcinogenic to humans by the International Agency for Research on Cancer [24]. In 2006, the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Joint Expert Committee of Food Additives (JECFA) confirmed that its presence in foods had to be considered a cause for concern [25]. In 2007, the European Commission adopted a Recommendation for monitoring levels in food [26], extended in 2010 [27] with the aim of investigating the dietary intake and identify possible strategies for reducing its formation. In 2015, after a careful risk assessment, the European Food Safety Authority published a scientific opinion [28] confirming that acrylamide can increase the risk of developing cancer and other effects for consumers in all age groups, especially children. Very recently, a European Commission Regulation [29] established mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. It also encourages the finding of colourimetric scales providing that a statistical correlation between colour intensity and measured acrylamide level can be demonstrated. Colour guides should be developed for a specific product type.

The use of colour scale is linked to the assumption that colour development during cooking process is associated to Maillard reaction progress [30]; therefore, a possible correlation between colourimetric indices and amount of acrylamide can be expected (as already proposed for fried potatoes by Pedreschi et al. [31, 32]). A relationship between the change of the redness parameter a^* and acrylamide levels in coffee, flour and potato chips has been proposed as a possible indicator by Gokmen and Senyuva [33]. On this basis, to enhance accuracy, a computer vision-based image analysis system has been proposed [34] and may represent a promising rapid method for detecting acrylamide although it still needs further improvements to become a portable device [35].

In this work, an investigation on acrylamide content in baked potatoes cooked in different conditions, involving traditional and air/steam combination ovens, was performed. An important aim was to evaluate the effect of the cooking method on acrylamide formation since no data about the effect of steam on acrylamide content in potato have been previously reported. Another purpose of this research was to explore whether baked potatoes characterized by similar colour, even obtained under different cooking conditions, present similar acrylamide content. Such finding, in perspective, would be a starting point for the development of a chromatic indicator based on the combination of the RGB indices, suitable to provide toxicity level linked to acrylamide occurrence, as auspicated by the recent European Regulation.

Materials and methods

Chemical and reagents

Acrylamide standard (99%), d_3-acrylamide (500 mg/L in acetonitrile) and formic acid (≥ 98%) were purchased

from Sigma-Aldrich (Steinheim, Germany). Methanol and n-Hexan (HPLC grade) were obtained from Carlo Erba Reagents (Milan, Italy). Potassium hexacyanoferrate (Carrez I) and zinc sulphate (Carrez II) were supplied by Merck (Darmstadt, Germany). Deionized water was obtained with a Milli-Q™ system (Millipore, Bedford, MA, USA).

Samples

Potatoes belonging to variety Primura and Arizona found on the market were peeled, ground till obtaining a puree and placed on the baking tray with a thickness of about 1 cm. Cooking was carried out using "dry air" ventilated function or "25% steam" (corresponding to the degree of steam injected, expressed as percentage over total time) ventilated function. Preliminary experiments at different time (from 30 to 55 min) and temperature (from 160 to 200 °C) conditions were performed prior to select the final parameters for obtaining the samples that were 50 min at 180 °C, and 40 min at 200 °C.

From both cooking modalities, ten portions of cooked product (5 x 5 cm) characterized by different colours were sampled and analysed to measure acrylamide levels and colour parameters. All preparations were performed in triplicate.

The temperatures of the surface of the samples were measured immediately after cooking by means of an infrared thermometer (MTP Instrument, Montréal-Nord, Québec, Canada) interfaced with a personal computer and data points were collected in an Excel® worksheet as reported by Bignardi et al. [30].

Sample preparation

Acrylamide extraction procedure from baked potatoes without seasoning and oil was developed from a method present in the literature [36] with slight modifications. Briefly, cooked potatoes were treated with liquid nitrogen and ground in a blender. 1 g of sample was weighed in a 50 mL plastic tube and then internal standard solution (100 µL d_3-acrylamide 10 mg/L) followed by 0.1% (v/v) formic acid for a final volume of 10 mL were added. After stirring using vortex mixer for 1 min, extraction was carried out using a magnetic stirrer at room temperature for 10 min. Sample was centrifuged (80 Hz at 4 °C for 15 min). The obtained supernatant was transferred into a cleaned conical tube and filtered through a 0.45 µm disposable nylon filters prior to instrumental analysis. All preparations were performed in triplicate.

Colour analysis

Colourimetric parameters of potato samples were evaluated by means of image analysis: samples were scanned by means of a desktop flatbed scanner (Hewlett Packard Scanjet 8200, Palo Alto, CA, USA) at 236 pixels per cm (600 dpi of resolution; true colour—24 bit), equipped with a cold cathode lamp for reflective scanning. All images were scanned at the same conditions, during image acquisition, the scanner was held in a black box, to exclude surrounding light and external reflections. Flatbed scanner colour (R, G and B) was characterized and corrected as previously reported by N'Dri et al. [37]. Measures were performed in triplicate.

Instrumental analytical method

The instrumental analysis was performed on an Agilent 1200 liquid chromatograph coupled to a mass spectrometer, equipped with an electrospray (ESI) interface and a linear ion trap analyzer (LC/MSD Trap XCT Ultra, Agilent Technologies). LC separation was carried out on a Luna C18 column (250 mm × 2 mm, 5 µm particle size; Phenomenex Inc., CA, USA) thermostated at 30 °C using an injection volume of 10 µL and a gradient solvent elution system composed by: eluent A, water:formic acid 0.1 M at ratio 99.5:0.5, containing 0.1% methanol (v/v); eluent B: methanol 100%.

Electrospray ionization was set in positive mode (ESI +). The nebulizer gas (nitrogen, 99.9% purity) and the dry gas (nitrogen, 99,9% purity) were delivered at flow rates of 60.0 psi and 10.0 L/min, respectively. Other conditions of the interface were: ESI voltage 4.5 kV, skimmer voltage 40.0 V, dry temperature 365 °C. Masses were recorded in SIM (Selected Ion Monitoring) mode, selecting signals of molecular ions $[M+H]^+$ at m/z 72 and m/z 75 for acrylamide and d_3-acrylamide, respectively. Confirmation of acrylamide identity was performed by a comparison of migration time and mass spectrum obtained from sample and standard solution.

Agilent ChemStation B.01.03 and LC–MSD Trap 6.0 Build 458.0 softwares were used to control the instrument and for data processing.

Method validation

To validate the analytical method employed for acrylamide quantification, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy (precision and trueness) were calculated according to Eurachem guidelines [38]. Boiled potatoes were used as blank matrix after excluding the presence of acrylamide.

LOD and LOQ values were calculated from the matrix calibration curve as 3 *s*/slope and 10 *s*/slope, respectively, where *s* is the standard deviation of the blank signal

obtained from ten independent blank measurements. Linear range was investigated starting from LOQ value to 12,500 ng/g (6 concentration levels).

After variance homogeneity assessment ($p > 0.05$) and testing significance of the intercept ($p < 0.05$ at 95% confidence level), linearity was also mathematically verified by applying the Mandel fitting test ($p > 0.01$ at the 99% confidence level). Quantitative analyses were performed using the calibration curve built in matrix by internal standard method. The matrix-matched calibration curve was obtained by analysing the matrix extracts fortified with a mixture of the five standards at six concentration levels in duplicate and treated applying the entire analytical procedure. The same amount of d_3-acrylamide, as internal standard, was added in each solution to reach the final concentration of 100 ng/mL. The analytical signal considered was the value of peak area/peak area of internal standard (A/AIS).

Accuracy was assessed in terms of precision and trueness. Precision was assessed as RSD % in terms of intra-day repeatability and intermediate precision (inter-day repeatability) of migration times and peak areas. In detail, the intra-day repeatability was evaluated by performing three independent extractions of blank matrix fortified with acrylamide standard solutions at 1000 ng/g concentration level, and three injections for each sample in the same day. The inter-day repeatability was calculated on 5 days by performing five independent extractions of the blank matrix fortified with acrylamide standard solution of 1000 ng/g concentration level, and three injections per day for each sample.

Trueness was evaluated in terms of recovery as a ratio of determined and added standard content on matrix at two concentration levels (LOQ and intermediate calibration level) by analysing in triplicate blank matrix samples fortified before extraction.

Percentage of recovery was then calculated with the formula:

$$\text{Recovery } (\%) = \left[(C1 - C2)/C3\right] \times 100$$

where C1 is the concentration determined in fortified sample, C2 is the concentration determined in unfortified sample (in this case, C2 = 0 since blank matrix did not contain acrylamide) and C3 is the concentration of fortification.

Statistical analysis

The coefficients of the regression model for each standard were calculated using the R-based chemometric software developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemometric Society. Acrylamide values were correlated with R, G and B parameters obtained in the colourimetric scale.

Results and discussion

Validation in matrix

Method validation for quantitative purposes was conducted according to Eurachem guidelines, as reported in "Materials and methods". A wide range of linearity has been established to cover the concentrations found in the investigated samples. The considered range was chosen on the basis of preliminary experiments, accordingly to the appearance of a sensible darkening of the product, and including concentration values corresponding to the benchmark levels established by the European Regulation [29].

Obtained data for the set parameters are presented in Table 1, reporting values of LOD and LOQ and calibration curve equation, characterized by a satisfactory R^2, higher than 0.98. An excellent value of trueness (99%) was also achieved, and good precision in terms of peak area and retention time repeatability (intra- and inter-day) has been assessed.

Samples analysis

Cooking was performed in domestic ovens by selecting different time/temperature combinations. Preliminary tests were performed to set up the parameters values to obtain a wide range of potato colours, from lightest to darkest, comprehending also undercooked and almost burnt products, to reproduce the possible colourations that consumers can obtain. The best condition to adopt was found to be 50 min at 180 °C and 40 min at 200 °C. Different cooked product portions (squares of size 5 × 5 cm) were sampled from different areas and subsequently analysed for acrylamide quantification. In detail, darkest portions were sampled by the back area of the tray, whereas the lighters were sampled by the front area.

One purpose of this research was to build a colourimetric scale and to explore whether the achieved colour intensity was dependent on the acrylamide content of the product.

Table 1 Method validation results obtained for mashed baked potatoes analysis

Standard	LOD; LOQ (µg/kg)	Linearity range (µg/kg)	Calibration curve	R^2	Trueness (%)	Intra-day rep (area, RT)	Inter-day rep (area, RT)
Acrylamide	225; 600	600–12,500	$y = 0.001x$	0.981	99 ± 1	3.1%, 0.8%	4.1%, 1.4%

Besides, colourimetric scales from samples cooked in the two ovens were compared to explore if steam presence (and therefore cooking conditions) could affect acrylamide formation.

In particular, ten different colour gradations belonging to the set of samples obtained by dry air ventilation cooking (see Fig. 1a) and ten belonging to the cooking at 25% steam function were chosen (see Fig. 1b) and analysed.

Table 2 shows the quantities of acrylamide found that were ranging between not detectable values (below LOD) and almost 10 mg per kilogram of product. Samples are enumerated in ascending order from the lightest to the darkest one (as ordered in Fig. 1). It can be seen that the amounts reported for samples cooked under dry air conditions are always lower with respect to those processed in the presence of steam. Besides, the last value recorded accounts for about 10 times the first one in dry air cooking, whereas it reaches values about 20 times higher than the first one in steam cooked samples.

The existence of a correlation between colour intensity and acrylamide content might be evidenced and, in particular, acrylamide amount is found to increase in a very steep way when the colour turns from clear brown to dark. About the same point, and in particular between sample n. 6 and 7 for dry air, and between n. 5 and 6 for steam cooking, the acrylamide concentration corresponds to the benchmark levels established by the European Regulation (fixed to 750 ng/g for potato-based products). All the following samples, in fact, were characterized by a definite dark colouration that can be easily recognized using the colourimetric scale.

About the higher amounts of acrylamide found in samples cooked at higher moisture level, our finding does not support a previous work on baked bread obtained under different steam occurrence [39], showing that steam cooking reduced acrylamide level in the product. Romani et al. [40] also reported that the decrease in the moisture content led to a corresponding increase of the activation energy for acrylamide formation. However, a study from Ciesarova et al. [41] described that an increase in moisture enhanced acrylamide formation in model system based on both potato and wheat starch. Besides, the different heat transfer of the moisture and the dry air on the surface of the product may play a crucial role. It can be assumed that, in the presence of moisture, the relative humidity inside the oven chamber may cause steam condensing, able to transfer heat more rapidly. Consequently, the higher levels of acrylamide found in steam-cooked potatoes (together with a greater browning) could depend on the higher temperature reached on the surface of the food due to a more effective heat transfer of steam with respect to dry air. This hypothesis was confirmed by the data of higher surface temperatures recorded for steam–air cooked potatoes. In general, air–steam function gave a difference ranging from 8 to 13 °C compared to air ventilated ones for the same cooking conditions. For instance, temperature values recorded on the central part of the baking tray, when oven was set at 180 °C, were 137 ± 2 °C and 148 ± 2 °C in dry and steam conditions, respectively.

Colour analyses performed on the same samples, on the basis of the colour channels Red, Green and Blue, showed

Table 2 Acrylamide levels in mashed baked potatoes employing the two oven functions

Sample	Acrylamide content (µg/kg)	
	Dry air	25% steam
1	n. d	n. d
2	n. d	n. d
3	n. d	n. d
4	n. d	389 ± 10
5	269 ± 19	671 ± 48
6	269 ± 70	860 ± 10
7	990 ± 30	1069 ± 53
8	972 ± 38	1346 ± 17
9	1077 ± 71	7562 ± 21
10	2993 ± 49	9550 ± 55

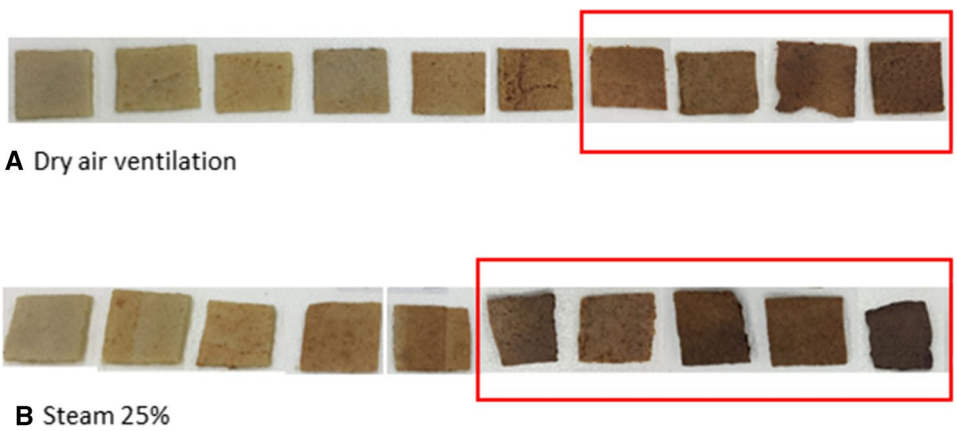

Fig. 1 Colours obtained on potato samples cooked in ovens by dry air circulation function (**a**) and 25% steam (**b**). Red squares indicate values of acrylamide higher than 750 µg/kg (benchmark levels by European Commission Regulation [29])

that generally an acrylamide increase was accompanied by a decrease of RGB values, thus leading to a darker colour development (Table 3). This confirms that acrylamide formation is directly linked to the pathways of reactions leading to brown products.

Correlations coefficients between each channel colour (R, G and B values) and acrylamide content were calculated using simple mathematical functions and are reported in Table 4. Values between 0.82 and 0.99 were obtained, showing high correlation for all the colours considered, particularly for the G channel, and for both types of cooking conditions. Good values, higher than 0.817, were achieved even when all samples were combined together and treated as a unique set, despite the different thermal treatment to which they were subjected.

Thus, the observed colour gradation can be considered as a marker able to provide information on the acrylamide level in the food product, regardless of the cooking conditions.

Moreover, to validate the procedure and to test the robustness of the finding, all data obtained by the three colour channels belonging to the 20 samples, notwithstanding the thermal treatment, were processed and correlated with acrylamide content by means of multiple regression. It could be seen that values from the two sets of samples when combined together resulted to be ordered according to colour intensity and acrylamide content. A degree of correlation with a coefficient R^2 equal to 0.844 was achieved using the following equation:

$$\text{acrylamide}\left(\mu g/_g\right) = 15.97 - 0.211 * R + 0.028 * G + 0.134 * B \quad (1)$$

This function links the intensity of the three channel colours combined together and involves all the 20 samples that were submitted to different cooking conditions demonstrating that, despite the intensity and the modality of the thermal treatment, the final colour of the product is connected to acrylamide amount.

To validate the equation, three additional samples, belonging to a different potato variety (Arizona var.), were baked under the same conditions, to reproduce colours already obtained, and verify the accuracy of the fittings of the new points. An average error of $16 \pm 3\%$ was obtained in the accuracy.

The findings of this work can represent a starting point to elaborate a colourimetric indicator possibly suitable to predict acrylamide content by a simple colour analysis providing the RGB values. It may represent a rapid method for acrylamide control, and a useful and extremely practical tool for industrial, commercial and domestic use, allowing to monitor the level of toxicity of a food product without the need of time-consuming, expensive and requiring high expertise and instrumental analyses.

Table 4 Values of correlation between colour indices and acrylamide content

	Equation	Coefficients	R^2
Dry air			
R	$\text{acrylamide} = a + b \cdot R$	$a = 3113.9$; $b = 19.9$	0.938
G	$\text{acrylamide} = a + b/G$	$a = 1434.7$; $b = 1.9*10^5$	0.971
B	$\text{acrylamide} = a \cdot e^{-B/b}$	$a = -9.274*10^6$; $b = 1.2*10^5$	0.988
25% steam			
R	$\text{acrylamide} = a + b/R$	$a = 4.6*10^3$; $b = 6.4*10^5$	0.891
G	$\text{acrylamide} = a + b/G$	$a = 2.6*10^3$; $b = 3.2*10^5$	0.921
B	$\text{acrylamide} = a \cdot e^{-B/b}$	$a = 6.7*10^4$; $b = 13.1$	0.875
All samples			
R	$\text{acrylamide} = a + b/R^2$	$a = 1209.8$; $b = 2.8*10^6$	0.817
G	$\text{acrylamide} = a + b/G^2$	$a = -759.1$; $b = 1.2*10^6$	0.911
B	$\text{acrylamide} = a \cdot e^{-B/b}$	$a = 8.5*104$; $b = 12.2$	0.904

Table 3 Results of colour analyses performed on samples cooked with dry air ventilation and 25% steam

Sample #	Dry air			25% Steam		
	R	G	B	R	G	B
1	129.32 ± 5.18	116.86 ± 5.02	88.50 ± 5.03	149.71 ± 5.43	133.10 ± 5.26	99.62 ± 5.29
2	136.71 ± 7.08	117.91 ± 7.57	77.89 ± 7.76	150.54 ± 5.42	128.71 ± 7.31	85.30 ± 9.65
3	144.53 ± 5.13	121.78 ± 5.72	76.78 ± 6.12	156.13 ± 7.10	125.11 ± 8.82	76.63 ± 8.93
4	136.04 ± 7.64	121.77 ± 8.00	88.57 ± 8.29	139.32 ± 8.02	105.14 ± 8.32	64.40 ± 7.51
5	139.27 ± 8.94	111.44 ± 9.67	71.12 ± 9.61	128.93 ± 9.38	93.45 ± 10.24	58.18 ± 8.91
6	126.01 ± 15.60	92.23 ± 16.17	56.82 ± 13.54	105.16 ± 11.81	78.64 ± 12.13	54.34 ± 11.35
7	120.87 ± 12.43	84.11 ± 12.41	53.86 ± 11.34	92.33 ± 14.43	70.27 ± 13.70	51.00 ± 11.53
8	109.46 ± 9.24	81.20 ± 9.43	52.69 ± 8.96	94.04 ± 9.04	66.26 ± 8.75	43.42 ± 8.08
9	95.15 ± 13.55	64.95 ± 12.22	43.51 ± 9.40	76.39 ± 10.86	53.83 ± 9.45	37.53 ± 8.37
10	88.92 ± 10.27	61.77 ± 10.25	43.42 ± 9.68	67.13 ± 8.61	52.06 ± 8.44	42.95 ± 8.09

Conclusions

From this study on baked potatoes, it has been revealed that the presence of steam (25%) during cooking involves an increase of acrylamide levels in the product compared with traditional cooking. This has been attributed to the greater heat transfer of steam with respect to the dry air, which usually results in a higher temperature on the food product surface.

The existence of a correlation between the acrylamide content and the colour of the product has been confirmed, regardless of the time/temperature/steam combination during the cooking process. Therefore, a possible development of this work could be the elaboration of a colourimetric scale, on the basis of the results achieved, to be proposed as an easy and useful tool to estimate the level of acrylamide in baked potatoes, as suggested by the recent European Regulation. Further studies on different potato-based preparation and formulations and other products containing acrylamide are encouraged and would be of great interest to set up a rapid way to monitor and assess consumer's safety.

In perspective, to minimize acrylamide formation, it would be also interesting to develop this research by combining together the choice of an optimized cooking procedure with previously explored strategies, already reported in the literature (such as the selection of cultivars with low amounts of reducing sugars or asparagine, the optimization of the harvest time, of storage conditions, and technological processes).

Acknowledgements The authors are thankful to Dr. Maria Mattera for her precious collaboration in the research coordination.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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