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Malgorzata A. Majcher, Henryk H. Jeleń. Acrylamide formation in low-fat potato snacks and its correlation with color development. Food Additives and Contaminants, 2007, 24 (04), pp.337-342. 10.1080/02652030601185709 . hal-00577543

HAL Id: hal-00577543

<https://hal.science/hal-00577543>

Submitted on 17 Mar 2011

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Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-247.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	12-Dec-2006
Complete List of Authors:	Majcher, Malgorzata; Agricultural University of Poznań, Food Science and Nutrition Jeleń, Henryk; Agricultural University of Poznań, Food Science and Nutrition
Methods/Techniques:	Chromatography - GC/MS
Additives/Contaminants:	Acrylamide
Food Types:	Snack products

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Acrylamide formation in low-fat potato snacks and its correlation with colour development

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Abstract

In the Institute of Food Technology of Plant Origin at Agricultural University of Poznań a recipe and technological process for production of low-fat potato snacks (7% of fat), using a combination of extrusion and a roasting process was developed. Due to the character of the product obtained, the amount of acrylamide was analyzed. At the same time, the influence of temperature and time of heating on the levels of acrylamide were monitored as well as correlated with colour development and acrylamide content. The amount of acrylamide determined in low-fat potato snacks was $489 \mu\text{g kg}^{-1} \pm 26 \mu\text{g}$

kg⁻¹, which is comparable to that of French fries, crackers and cookies and almost three times less than in potato chips analyzed by other authors. At the same time it was shown that temperature and length of heating time have a significant influence on acrylamide formation, with temperature having a stronger effect than time. Measurement of the colour and the acrylamide content in low-fat potato snacks produced using different temperatures and time of roasting showed that there is a substantial correlation between lightness (L^*), redness (a^*) and yellowness (b^*) and acrylamide concentration as a function of time: $r^2 = -0.995$, $R^2 = 0.996$, $r^2 = 0.998$ respectively. Graduated increases in roasting temperatures showed correlation between lightness (L^*) or redness (a^*) and acrylamide concentration: $r^2 = -0.947$ and $r^2 = 0.968$. Yellowness (b^*) was not correlated with acrylamide content as a function of temperature.

Keywords: *low-fat potato snacks, acrylamide, colour development, gas chromatography-mass spectrometry, (GC-MS/MS)*

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Introduction

The high temperature used for the preparation of potato snacks to bring a pleasant flavor and colour can also at the same time cause the development of toxic substances such as acrylamide (2-propenamide). Since the year 2002 when a group of Swedish researchers announced the presence of acrylamide in a wide range of fried or oven-treated foods, many product types have been investigated. The highest amounts of acrylamide were found to be in fried and baked potato products followed by cereal products, whereas only low amounts of acrylamide were found in meat products (Ahn et al. 2002, Becalski et al. 2003). Since acrylamide is known to be a neurotoxin, a carcinogen in animals and a probable carcinogen to humans as defined by the International Agency for Research on Cancer (IARC, 1994) it is a concern of food technologists to decrease its amount to the lowest possible levels.

There are several hypotheses concerning formation of acrylamide in cooked foods, among which the Maillard browning reaction involving free asparagine and reducing sugars is the most likely and frequently described mechanism (Mottram et al., 2004). The second pathway considered for acrylamide formation is degradation of glycerol to acroleine and later to acrylic acid, which in reaction with ammonia can develop into acrylamide (Becalski et al. 2003). The optimal temperature for acrylamide formation has been shown to range from 140°C to 180°C (Freidman 2003, Elmore et al. 2005). The Maillard reaction is being used intentionally to develop a nice, desirable texture, flavor and colour of the food product. Several factors are known to influence the colour formation, such as pH, water content and activity, oxygen level but most important is temperature and length of heating time. Increase in temperature by 10°C speeds up a non-enzymatic browning reaction by factors of 3-4 times (Belitz et al. 2004).

Potato-based products represent an important part of the daily intake of mainly the adolescent population from western countries. The most well-known and most popular are French fries and potato chips which, beside high fat content (30-45%), also cause concern as the products with the highest amount of acrylamide. World interest in healthier way of life has driven the food industry to find new ways to obtain food products with lower oil content, while still retaining desirable texture and flavor. The authors recent paper has introduced an extrusion process followed by roasting as a way of producing low fat (7%) potato snacks (Majcher and Jeleń 2005).

The colour of the food surface is the first quality parameter evaluated by consumers and it is critical in the acceptance of the product. At the same time, colour can be easily measured as an indication of other Maillard reaction products such as acrylamide. The most popular and the easiest way of food colour measurement was introduced by the Commission Internationale d'Eclairage (CIE) in 1976, where the colour is described in a three-dimensional scale represented by the $L^*a^*b^*$ values. According to CIE, L^* indicates the level of light and dark, a^* value indicates redness or greenness and b^* value indicates values of yellowness or blueness (Papadakis et al. 2000). Additionally the newest paper by Gökmen et. al (2007) presents possibilities of employment computer vision-based analysis which uses digital colour images for the estimation of acrylamide content in food products.

The aim of this study was to (i) calculate the amount of acrylamide in low-fat potato snacks obtained by extrusion cooking and roasting, (ii) investigate the influence of roasting time and temperature on acrylamide formation and (iii) examine the correlation of the colour and acrylamide concentration in low-fat potato snacks as a function of roasting time and temperature.

Materials and methods

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94 **Chemicals**

95 Acrylamide (99%) and d3-acrylamide (isotopic purity 98%) were commercially available
96 from Aldrich (Poznań, Poland) and Cambridge Isotope Laboratories (Andover, MA,
97 USA), respectively; acetonitrile, potassium bromide, hydrobromic acid were obtained
98 from Sigma-Aldrich (Poznań, Poland); *n*-hexane, sodium thiosulfite, ethyl acetate,
99 saturated bromine water were from Chempur (Tarnowskie Góry, Poland); anhydrous
100 sodium sulfide was obtained from POCh (Gliwice, Poland). All chemicals were of
101 analytical grade. SEP-PAK Silica cartridges for extract purification were purchased
102 from Waters (Warsaw, Poland).

103 **Preparation of extruded potato snacks**

104 Experiments were conducted with extruded potato snack produced according a recipe
105 developed by us using a procedure described in the aforementioned paper (Majcher
106 and Jeleń 2005). After extrusion, products were cut into small pieces and heated in an
107 oven at 85°C for 15 min followed by 45 min of roasting at 130°C to develop proper
108 texture and aroma.

109 **Extraction of acrylamide**

110 The analysis was based on a previously described (Castle, 1993) method based on
111 bromination of acrylamide into 2-bromopropenamide, followed by GC/MS/MS analysis.
112 Extruded and roasted potato snacks (20 g) were ground and mixed with water (150 ml),
113 spiked with 100 µl of 100 mg l⁻¹ d₃-acrylamide internal standard, homogenized during 5
114 min and centrifuged after at 14j000 rpm for 15 min. 20 ml of the organic extract was
115 transferred to 100 ml screw-top vial and bromination reagents were added: 5 g of
116 potassium bromide; 0.2 ml of hydrobromic acid; 4 ml of saturated bromine water. The
117 entire mixture was stirred and set aside in a refrigerator at 4°C overnight. Excess
118 bromine was then neutralized by the addition of 0.7 M sodium thiosulfite solution until
119 yellow colour disappeared. The mixture was then extracted with ethyl acetate (2 x 5 ml)

using mechanical shaker for 10 min. After separation the organic layer was collected and filtered through anhydrous sodium sulfate and concentrated to ~30 μ l under a soft stream of nitrogen. To remove the last traces of ethyl acetate, 200 μ l of hexane was added and then evaporated to dryness. The residue was re-dissolved in 20% v/v ethyl acetate/hexane (250 μ l) prior to cleanup. A SPE-silica cartridge was preconditioned with 7 ml 20% ethyl acetate/hexane and the sample was applied. The cartridge was eluted with 6 ml 30% ethyl acetate/hexane and the first 2 ml was discarded. The rest of the eluate was collected in an amber vial, and finally evaporated to a volume of ~50 μ l ready for GC/MS/MS analysis.

Gas Chromatography/Mass Spectrometry and quantification

The acrylamide content was determined by gas chromatography – tandem mass spectrometry (GC/MS/MS). Brominated sample extracts were analyzed on a Thermoquest Trace 2000 gas chromatograph coupled to Finnigan PolarisQ ion trap mass spectrometer. The chromatograph was equipped with fused silica column type DB 5 (25 m x 0.25 mm x 0.25 μ m). The operating conditions of GC-MS were as follows: helium flow 0.8 ml, initial oven temperature 65°C (1 min); then raised 15°C min⁻¹ to 170°C; then at 5°C min⁻¹ to 200°C; finally at 40°C min⁻¹ to 280°C (5 min). The GC-MS interface transfer line was held at 280°C. Mass spectrometer was running in ms² mode with the following ions selected for secondary fragmentation: m/z 152 for 2-bromopropenamide and m/z 155 form 2-bromopropenamide d³. Comparison of the integrated abundances of the daughter ions of the unlabeled (m/z 135) and labeled (m/z 137) compounds provided data for the quantification of the acrylamide (Schieberle and Grosch, 1987). The response factor was calculated in a blank sample spiked with a known amount of unlabeled and labeled acrylamide in three different ratios: 250:50; 250:250; 250:500 ppb (Sen et al. 1991). The average response factor was calculated at a value close to one (1.01 $\pm$ 0.02) therefore was omitted in further calculations.

Measurement of sample colour

Colour was measured with a Spectro-Pen (Dr Lange, Poland) in the L^* , a^* , b^* modes, which provide uniform colour differentiation in relation to visual differences. L^* , a^* and b^* are chromaticity coordinates, where L^* = the lightness of the colour, positive a^* = red colour, negative a^* = green, positive b^* = yellow and negative b^* = blue. Because the surface of the potato snacks are not uniform in a colour and shape, colour measurements were performed after milling of 10 g of the sample, where the optical probe was placed over in a central position. Results are reported as an average of 3 measurements.

Results and discussion

Influence of time and temperature on acrylamide formation

Production of low-fat potato snacks consisted of two major technological processes:- extrusion cooking and roasting. As has been demonstrated in the aforementioned paper (Majcher and Jeleń 2005) the roasting process is a crucial phase for developing Maillard reaction flavor products. Therefore, the authors chose to leave the extrusion parameters stable and consider only the influence of varying temperatures and roasting times. Ten potato snacks samples were obtained. Five were obtained using a constant time of 45 minutes, but varying the roasting temperatures: 130; 140; 150; 160; 170°C. Five were obtained using a constant temperature of 130°C, but varying the roasting times: 45; 55; 65; 75 and 85 min. For each product the acrylamide content was calculated in triplicates and presented as a mean value with standard deviation. The results are presented in Figure 1.

[Insert Figure 1 about here]

The average amount of acrylamide formed in low-fat potato snacks using our original recipe when the roasting process is performed at 130°C for 45 min was estimated at 489 $\mu\text{g kg}^{-1} \pm 26 \mu\text{g kg}^{-1}$. This amount is comparable to acrylamide content in products

172 such as biscuits, crackers ($\sim 423 \mu\text{g kg}^{-1}$) or French fries ($\sim 537 \mu\text{g kg}^{-1}$) but almost
173 three times less than in potato chips ($\sim 1312 \mu\text{g kg}^{-1}$) (WHO 2002).

174

175 Changes in roasting conditions showed that the amount of acrylamide is highly
176 influenced by both temperature or heating time, however an increase in temperature
177 had a stronger effect than an increase in time. Ten minutes of additional roasting time
178 beyond 45 min doubled the amount of acrylamide, whereas 10°C increase in
179 temperature from 130°C to 140°C increased the acrylamide content more than five
180 times. The longest time used for the roasting (85 min) caused a lower amount of
181 acrylamide formation in low-fat potato snacks ($2010 \mu\text{g kg}^{-1} \pm 64.5 \mu\text{g kg}^{-1}$) than the
182 smallest raise of temperature from 130°C to 140°C ($2767 \mu\text{g kg}^{-1} \pm 93.9 \mu\text{g kg}^{-1}$). This
183 work indicates that to have a lower acrylamide content in potato based products using
184 a longer time at a lower heating temperature is more satisfactory than using a higher
185 temperature. Ehling and Shibamoto in 2005 obtained similar results by performing
186 experiments on model systems using a different ratio of asparagine and D-glucose and
187 found the acrylamide content to be greater using higher temperatures rather than
188 longer heating times.

189

190 Further analysis of the results showed that the amount of acrylamide continued to
191 increase during entire experiment with variable time as well as increasing temperature
192 but only up to 160°C , at which point any further increase did not change significantly
193 the acrylamide formation. Compared to fried regular potato chips containing a high
194 amount of fat, described by Pedereshi et al (2005), the average acrylamide content
195 increased ~ 58 times as the frying temperature increased from 120°C to 180°C . Their
196 results gave no indication that acrylamide formation might slow significantly at some
197 point although the temperature continued to rise. This is opposite to the findings of the
198 authors, which indicate acrylamide formation recessed at 160°C . At that moment either

one of acrylamide substratum (i.e. asparagine or reducing sugars) had been all exploited or acrylamide might take part in further Maillard reactions. Ehling and Shibamoto (2005) who experimented on model systems of asparagine and glucose also noted an increase of acrylamide formation with a rise in temperature but only up to a certain point, after which the level of acrylamide started to decrease. Their results showed that heating above 160°C for a period of 45min reduced acrylamide level as it started to decompose. Although it could be considered as a way to decrease acrylamide content in foods, products obtained with such a high temperature or with such a long time of heating are generally unacceptable to the consumer.

Correlation between colour and acrylamide content in low-fat potato snacks in a function of roasting time and temperature

In a second experiment, the effect of colour development and acrylamide formation were studied. Samples roasted at 5 different temperatures (130, 140, 150, 160 and 170°C) and 5 different times (45, 55, 65, 75 and 85 min) were measured for the intensity of lightness (L^*), redness (a^*) and blueness (b^*) and correlated with acrylamide content at $p < 0.005$. Each colour measurement was performed in triplicates and presented as a mean value with calculated standard deviation.

Measurements of L^* value showed that with increasing time of roasting, lightness of the sample was decreasing as well as with temperature increase but only up to 160°C. Higher temperatures did not change significantly the level of lightness. On the contrary acrylamide content was increasing during time extension as well as with temperature increase. However, by increasing the temperature from 160°C to 180°C the acrylamide content did not rise. Calculated correlation coefficient equal to $r^2 = -0.995$ for time variable and $r^2 = -0.947$ for temperature change proved that there is a high reverse linear correlation between lightness of the product (L^*) and acrylamide content (A) as a function of time (t): $\uparrow T \rightarrow \downarrow L^* \uparrow A$ and temperature (T): $\uparrow t \rightarrow \downarrow L^* \uparrow A$ (Figure 2).

226 [Insert Figure 2 about here]

227

228 The second colour value of low-fat potato snacks, when measured at different
229 temperatures and roasting times, demonstrated an increase of redness (a^*) either with
230 temperature or time change. However, an increase in the red colour intensity was
231 much more constant with time prolongation whereas change in temperature resulted in
232 substantial increases at the beginning of the heating but only till it reached 150°C, after
233 which it remained without further difference. The analogous situation with acrylamide
234 concentration effected again with high correlation coefficients between a^* value and
235 acrylamide content: $r^2 = 0.996$ for changes in a roasting time ($\uparrow t \rightarrow \uparrow a^* \uparrow A$) and $r^2 =$
236 0.968 for roasting temperature ($\uparrow T \rightarrow \uparrow a^* \uparrow A$) (Figure 3).

237 [Insert Figure 3 about here]

238

239 The third value of colour measurement - yellowness b^* , was increasing constantly
240 when applying longer heating time and it was highly correlated with acrylamide
241 concentration in a product with correlation coefficient calculated as $r^2 = 0.998$. On the
242 contrary, application of higher roasting temperatures gave an increase of b^* values only
243 at the beginning of heating at 140°C; after that the intensity of yellow colour started to
244 decrease in opposition to the acrylamide content, $r^2 = 0.097$ (Figure 4). Consequently
245 measurement of yellowness was not considered to be a good indicator of acrylamide
246 content in this type of products.

247 [Insert Figure 4 about here]

248 In a recent paper Gökmen et al. (2006) showed that in potato chips the values of L^*
249 and b^* decreased with temperature and time studied whereas the value of a^* increased
250 to certain point and then, depending on temperature, either started to decrease or
251 stayed at the same level. In this study only redness of the product and acrylamide

content was found to be correlated, however the same authors concluded that the complexity of Maillard reaction in which acrylamide is considered to be formed as an intermediate could not be explained by a simple linear regression model but rather required explanation be a more complex quotation involving rate constants determined experimentally for each product. In another paper, Pedrechi et al. (2005) reported that L^* and b^* did not show significant changes during frying of potato chips, however a positive linear correlation was found between acrylamide concentration and redness of the product at temperatures of 120, 150 and 180°C. Senyuva and Gökmen in their study on instant, ground and laboratory roasted coffee found out that change in the a^* colour value was found to show a good correlation with the change in acrylamide, data was fitted to a non-linear logarithmic function for the estimation of acrylamide level in coffee. Studies on another potato product such as French fries (Taubert et al. 2004) showed correlation of the colour development and acrylamide content only when a small surface of the product was studied but no correlation was observed for large surface material. Results for low-fat potato snacks reported in this paper were more similar to those obtained by Gökmen et al. (2006) who studied wheat flour, where the intensity of lightness decreased with time at all temperatures considered, and in most cases the intensity of redness a^* and yellowness b^* increased which was correlated with acrylamide content. Most of the papers (Pedreschi et al. 2005, Gökmen et al. 2006, Mustafa et al. 2005) proved that the best correlation, and at the same time the best prediction of acrylamide level, is the intensity of the red colour. This paper demonstrated that there is also a high correlation with lightness L^* , which proves again that in darker products higher amounts of acrylamide are formed. Ehling and Shibamoto (2005) demonstrated a correlation of browning colour and acrylamide formation, however their method of colour measurement was based on spectroscopy analysis and involved much more time in sample preparation and measurement. Similar results were obtained by Pedreschi et al. (2006) who studied ways of reduction of acrylamide content in fried potato strips and at the same time correlated it with

colour development. They noted a significant correlation between colour represented by L^* and a^* values and acrylamide content.

Mustafa et al. (2005), during studies on rye crisp bread, observed a correlation between acrylamide content and colour expressed with a^* and b^* values in the time and temperature experiment but found no correlation when acrylamide precursors were added. They concluded that asparagine is contributing to the pathway leading to acrylamide but its effect on colour formation is limited.

The results presented here show that acrylamide is formed also in low-fat potato snacks, and may suggest that the lower fat content in such products does not influence acrylamide formation. Temperature is the most important factor for acrylamide formation in low-fat potato snacks. At the same time it has been proven that measurement of colour expressed as value of lightness, L^* , and redness, a^* , could be a good estimator of acrylamide content in food products.

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Figure 1. Influence of roasting time (temperature even = 130°C) (A) and temperature (time even = 45 min) (B) on acrylamide formation during preparation of low-fat potato snacks.

(A)

(B)

Figure 2. Acrylamide content and color lightness L^* in a function of roasting time (A) and temperature (B).

(A)

(B)

Figure 3. Acrylamide content and color redness a^* in a function of roasting time (A) and temperature (B).

(A)

(B)

Figure 4. Acrylamide content and color yellowness b^* in a function of roasting time (A) and temperature (B).

(A)

(B)