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

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**Abstract**

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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.

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The amount of acrylamide determined in low-fat potato snacks was  $489 \mu\text{g kg}^{-1} \pm 26 \mu\text{g}$

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3 25 kg<sup>-1</sup>, which is comparable to that of French fries, crackers and cookies and almost three  
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5 26 times less than in potato chips analyzed by other authors. At the same time it was  
6  
7 27 shown that temperature and length of heating time have a significant influence on  
8  
9 28 acrylamide formation, with temperature having a stronger effect than time.  
10  
11 29 Measurement of the colour and the acrylamide content in low-fat potato snacks  
12  
13 30 produced using different temperatures and time of roasting showed that there is a  
14  
15 31 substantial correlation between lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) and  
16  
17 32 acrylamide concentration as a function of time:  $r^2 = -0.995$ ,  $R^2 = 0.996$ ,  $r^2 = 0.998$   
18  
19 33 respectively. Graduated increases in roasting temperatures showed correlation  
20  
21 34 between lightness ( $L^*$ ) or redness ( $a^*$ ) and acrylamide concentration:  $r^2 = -0.947$  and  $r^2$   
22  
23 35 = 0.968. Yellowness ( $b^*$ ) was not correlated with acrylamide content as a function of  
24  
25 36 temperature.  
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32 **Keywords:** *low-fat potato snacks, acrylamide, colour development, gas*  
33  
34 *chromatography-mass spectrometry, (GC-MS/MS)*  
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## 41 Introduction

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

53

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

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3 67 Potato-based products represent an important part of the daily intake of mainly the  
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5 68 adolescent population from western countries. The most well-known and most popular  
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7 69 are French fries and potato chips which, beside high fat content (30-45%), also cause  
8  
9 70 concern as the products with the highest amount of acrylamide. World interest in  
10  
11 71 healthier way of life has driven the food industry to find new ways to obtain food  
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13 72 products with lower oil content, while still retaining desirable texture and flavor. The  
14  
15 73 authors recent paper has introduced an extrusion process followed by roasting as a  
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17 74 way of producing low fat (7%) potato snacks (Majcher and Jeleń 2005).  
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22 75  
23 76 The colour of the food surface is the first quality parameter evaluated by consumers  
24  
25 77 and it is critical in the acceptance of the product. At the same time, colour can be easily  
26  
27 78 measured as an indication of other Maillard reaction products such as acrylamide. The  
28  
29 79 most popular and the easiest way of food colour measurement was introduced by the  
30  
31 80 Commission Internationale d'Eclairage (CIE) in 1976, where the colour is described in  
32  
33 81 a three-dimensional scale represented by the  $L^*a^*b^*$  values. According to CIE,  $L^*$   
34  
35 82 indicates the level of light and dark,  $a^*$  value indicates redness or greenness and  $b^*$   
36  
37 83 value indicates values of yellowness or blueness (Papadakis et al. 2000). Additionally  
38  
39 84 the newest paper by Gökmen et. al (2007) presents possibilities of employment  
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41 85 computer vision-based analysis which uses digital colour images for the estimation of  
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43 86 acrylamide content in food products.  
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49 88 The aim of this study was to (i) calculate the amount of acrylamide in low-fat potato  
50  
51 89 snacks obtained by extrusion cooking and roasting, (ii) investigate the influence of  
52  
53 90 roasting time and temperature on acrylamide formation and (iii) examine the correlation  
54  
55 91 of the colour and acrylamide concentration in low-fat potato snacks as a function of  
56  
57 92 roasting time and temperature.  
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### 93 **Materials and methods**

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3 94 **Chemicals**  
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6 95 Acrylamide (99%) and d<sub>3</sub>-acrylamide (isotopic purity 98%) were commercially available  
7  
8 96 from Aldrich (Poznań, Poland) and Cambridge Isotope Laboratories (Andover, MA,  
9  
10 97 USA), respectively; acetonitrile, potassium bromide, hydrobromic acid were obtained  
11  
12 98 from Sigma-Aldrich (Poznań, Poland); *n*-hexane, sodium thiosulfite, ethyl acetate,  
13  
14 99 saturated bromine water were from Chempur (Tarnowskie Góry, Poland); anhydrous  
15  
16 100 sodium sulfide was obtained from POCh (Gliwice, Poland). All chemicals were of  
17  
18 101 analytical grade. SEP-PAK Silica cartridges for extract purification were purchased  
19  
20 102 from Waters (Warsaw, Poland).  
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24 103 **Preparation of extruded potato snacks**  
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26 104 Experiments were conducted with extruded potato snack produced according a recipe  
27  
28 105 developed by us using a procedure described in the aforementioned paper (Majcher  
29  
30 106 and Jeleń 2005). After extrusion, products were cut into small pieces and heated in an  
31  
32 107 oven at 85°C for 15 min followed by 45 min of roasting at 130°C to develop proper  
33  
34 108 texture and aroma.  
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38 109 **Extraction of acrylamide**  
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40 110 The analysis was based on a previously described (Castle, 1993) method based on  
41  
42 111 bromination of acrylamide into 2-bromopropenamide, followed by GC/MS/MS analysis.  
43  
44 112 Extruded and roasted potato snacks (20 g) were ground and mixed with water (150 ml),  
45  
46 113 spiked with 100 µl of 100 mg l<sup>-1</sup> d<sub>3</sub>-acrylamide internal standard, homogenized during 5  
47  
48 114 min and centrifuged after at 14j000 rpm for 15 min. 20 ml of the organic extract was  
49  
50 115 transferred to 100 ml screw-top vial and bromination reagents were added: 5 g of  
51  
52 116 potassium bromide; 0.2 ml of hydrobromic acid; 4 ml of saturated bromine water. The  
53  
54 117 entire mixture was stirred and set aside in a refrigerator at 4°C overnight. Excess  
55  
56 118 bromine was then neutralized by the addition of 0.7 M sodium thiosulfite solution until  
57  
58 119 yellow colour disappeared. The mixture was then extracted with ethyl acetate (2 x 5 ml)  
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3 120 using mechanical shaker for 10 min. After separation the organic layer was collected  
4  
5 121 and filtered through anhydrous sodium sulfate and concentrated to ~30  $\mu\text{l}$  under a soft  
6  
7 122 stream of nitrogen. To remove the last traces of ethyl acetate, 200  $\mu\text{l}$  of hexane was  
8  
9 123 added and then evaporated to dryness. The residue was re-dissolved in 20% v/v ethyl  
10  
11 124 acetate/hexane (250  $\mu\text{l}$ ) prior to cleanup. A SPE-silica cartridge was preconditioned  
12  
13 125 with 7 ml 20% ethyl acetate/hexane and the sample was applied. The cartridge was  
14  
15 126 eluted with 6 ml 30% ethyl acetate/hexane and the first 2 ml was discarded. The rest of  
16  
17 127 the eluate was collected in an amber vial, and finally evaporated to a volume of ~50  $\mu\text{l}$   
18  
19 128 ready for GC/MS/MS analysis.  
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### 23 129 ***Gas Chromatography/Mass Spectrometry and quantification***

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25  
26 130 The acrylamide content was determined by gas chromatography – tandem mass  
27  
28 131 spectrometry (GC/MS/MS). Brominated sample extracts were analyzed on a  
29  
30 132 Thermoquest Trace 2000 gas chromatograph coupled to Finnigan PolarisQ ion trap  
31  
32 133 mass spectrometer. The chromatograph was equipped with fused silica column type  
33  
34 134 DB 5 (25 m x 0.25 mm x 0.25  $\mu\text{m}$ ). The operating conditions of GC-MS were as  
35  
36 135 follows: helium flow 0.8 ml, initial oven temperature 65°C (1 min); then raised 15°C min<sup>-1</sup>  
37  
38 136 to 170°C; then at 5°C min<sup>-1</sup> to 200°C; finally at 40°C min<sup>-1</sup> to 280°C (5 min). The GC-  
39  
40 137 MS interface transfer line was held at 280°C. Mass spectrometer was running in ms<sup>2</sup>  
41  
42 138 mode with the following ions selected for secondary fragmentation: m/z 152 for 2-  
43  
44 139 bromopropenamide and m/z 155 form 2-bromopropenamide d<sup>9</sup>. Comparison of the  
45  
46 140 integrated abundances of the daughter ions of the unlabeled (m/z 135) and labeled  
47  
48 141 (m/z 137) compounds provided data for the quantification of the acrylamide (Schieberle  
49  
50 142 and Grosch, 1987). The response factor was calculated in a blank sample spiked with  
51  
52 143 a known amount of unlabeled and labeled acrylamide in three different ratios: 250:50;  
53  
54 144 250:250; 250:500 ppb (Sen et al. 1991). The average response factor was calculated  
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56 145 at a value close to one (1.01  $\pm$  0.02) therefore was omitted in further calculations.  
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### 146 ***Measurement of sample colour***



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3 147 Colour was measured with a Spectro-Pen (Dr Lange, Poland) in the  $L^*$ ,  $a^*$ ,  $b^*$  modes,  
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5 148 which provide uniform colour differentiation in relation to visual differences.  $L^*$ ,  $a^*$  and  
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7 149  $b^*$  are chromaticity coordinates, where  $L^*$  = the lightness of the colour, positive  $a^*$  = red  
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9 150 colour, negative  $a^*$  = green, positive  $b^*$  = yellow and negative  $b^*$  = blue. Because the  
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11 151 surface of the potato snacks are not uniform in a colour and shape, colour  
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13 152 measurements were performed after milling of 10 g of the sample, where the optical  
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15 153 probe was placed over in a central position. Results are reported as an average of 3  
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17 154 measurements.

## 21 155 **Results and discussion**

### 24 156 ***Influence of time and temperature on acrylamide formation***

26 157 Production of low-fat potato snacks consisted of two major technological processes:-  
27  
28 158 extrusion cooking and roasting. As has been demonstrated in the aforementioned  
29  
30 159 paper (Majcher and Jeleń 2005) the roasting process is a crucial phase for developing  
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32 160 Maillard reaction flavor products. Therefore, the authors chose to leave the extrusion  
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34 161 parameters stable and consider only the influence of varying temperatures and roasting  
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36 162 times. Ten potato snacks samples were obtained. Five were obtained using a constant  
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38 163 time of 45 minutes, but varying the roasting temperatures: 130; 140; 150; 160; 170°C.  
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40 164 Five were obtained using a constant temperature of 130°C, but varying the roasting  
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42 165 times: 45; 55; 65; 75 and 85 min. For each product the acrylamide content was  
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44 166 calculated in triplicates and presented as a mean value with standard deviation. The  
45  
46 167 results are presented in Figure 1.

51 168 [Insert Figure 1 about here]

53 169 The average amount of acrylamide formed in low-fat potato snacks using our original  
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55 170 recipe when the roasting process is performed at 130°C for 45 min was estimated at  
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57 171 489  $\mu\text{g kg}^{-1} \pm 26 \mu\text{g kg}^{-1}$ . This amount is comparable to acrylamide content in products

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3 172 such as biscuits, crackers ( $\sim 423 \mu\text{g kg}^{-1}$ ) or French fries ( $\sim 537 \mu\text{g kg}^{-1}$ ) but almost  
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5 173 three times less than in potato chips ( $\sim 1312 \mu\text{g kg}^{-1}$ ) (WHO 2002).  
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10 175 Changes in roasting conditions showed that the amount of acrylamide is highly  
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12 176 influenced by both temperature or heating time, however an increase in temperature  
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15 177 had a stronger effect than an increase in time. Ten minutes of additional roasting time  
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17 178 beyond 45 min doubled the amount of acrylamide, whereas  $10^\circ\text{C}$  increase in  
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19 179 temperature from  $130^\circ\text{C}$  to  $140^\circ\text{C}$  increased the acrylamide content more than five  
20  
21 180 times. The longest time used for the roasting (85 min) caused a lower amount of  
22  
23 181 acrylamide formation in low-fat potato snacks ( $2010 \mu\text{g kg}^{-1} \pm 64.5 \mu\text{g kg}^{-1}$ ) than the  
24  
25 182 smallest raise of temperature from  $130^\circ\text{C}$  to  $140^\circ\text{C}$  ( $2767 \mu\text{g kg}^{-1} \pm 93.9 \mu\text{g kg}^{-1}$ ). This  
26  
27 183 work indicates that to have a lower acrylamide content in potato based products using  
28  
29 184 a longer time at a lower heating temperature is more satisfactory than using a higher  
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31 185 temperature. Ehling and Shibamoto in 2005 obtained similar results by performing  
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33 186 experiments on model systems using a different ratio of asparagine and D-glucose and  
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35 187 found the acrylamide content to be greater using higher temperatures rather than  
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37 188 longer heating times.  
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44 190 Further analysis of the results showed that the amount of acrylamide continued to  
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46 191 increase during entire experiment with variable time as well as increasing temperature  
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48 192 but only up to  $160^\circ\text{C}$ , at which point any further increase did not change significantly  
49  
50 193 the acrylamide formation. Compared to fried regular potato chips containing a high  
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52 194 amount of fat, described by Pedereshi at al (2005), the average acrylamide content  
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54 195 increased  $\sim 58$  times as the frying temperature increased from  $120^\circ\text{C}$  to  $180^\circ\text{C}$ . Their  
55  
56 196 results gave no indication that acrylamide formation might slow significantly at some  
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58 197 point although the temperature continued to rise. This is opposite to the findings of the  
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60 198 authors, which indicate acrylamide formation recessed at  $160^\circ\text{C}$  At that moment either

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3 199 one of acrylamide substratum (i.e. asparagine or reducing sugars) had been all  
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5 200 exploited or acrylamide might take part in further Maillard reactions. Ehling and  
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7 201 Shibamoto (2005) who experimented on model systems of asparagine and glucose  
8  
9 202 also noted an increase of acrylamide formation with a rise in temperature but only up to  
10  
11 203 a certain point, after which the level of acrylamide started to decrease. Their results  
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13 204 showed that heating above 160°C for a period of 45min reduced acrylamide level as it  
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15 205 started to decompose. Although it could be considered as a way to decrease  
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17 206 acrylamide content in foods, products obtained with such a high temperature or with  
18  
19 207 such a long time of heating are generally unacceptable to the consumer.  
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22  
23 208 ***Correlation between colour and acrylamide content in low-fat potato snacks in a***  
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25 209 ***function of roasting time and temperature***  
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27  
28 210 In a second experiment, the effect of colour development and acrylamide formation  
29  
30 211 were studied. Samples roasted at 5 different temperatures (130, 140, 150, 160 and  
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32 212 170°C) and 5 different times ( 45, 55, 65, 75 and 85 min) were measured for the  
33  
34 213 intensity of lightness ( $L^*$ ), redness ( $a^*$ ) and blueness ( $b^*$ ) and correlated with  
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36 214 acrylamide content at  $p < 0.005$ . Each colour measurement was performed in triplicates  
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38 215 and presented as a mean value with calculated standard deviation.  
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44 217 Measurements of  $L^*$  value showed that with increasing time of roasting, lightness of the  
45  
46 218 sample was decreasing as well as with temperature increase but only up to 160°C.  
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48 219 Higher temperatures did not change significantly the level of lightness. On the contrary  
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50 220 acrylamide content was increasing during time extension as well as with temperature  
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52 221 increase. However, by increasing the temperature from 160°C to 180°C the acrylamide  
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54 222 content did not rise. Calculated correlation coefficient equal to  $r^2 = -0.995$  for time  
55  
56 223 variable and  $r^2 = -0.947$  for temperature change proved that there is a high reverse  
57  
58 224 linear correlation between lightness of the product ( $L^*$ ) and acrylamide content (A) as a  
59  
60 225 function of time (t):  $\uparrow T \rightarrow \downarrow L^* \uparrow A$  and temperature (T):  $\uparrow t \rightarrow \downarrow L^* \uparrow A$  (Figure 2).

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3 226 [Insert Figure 2 about here]  
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8 228 The second colour value of low-fat potato snacks, when measured at different  
9 229 temperatures and roasting times, demonstrated an increase of redness ( $a^*$ ) either with  
10 230 temperature or time change. However, an increase in the red colour intensity was  
11 231 much more constant with time prolongation whereas change in temperature resulted in  
12 232 substantial increases at the beginning of the heating but only till it reached 150°C, after  
13 233 which it remained without further difference. The analogous situation with acrylamide  
14 234 concentration effected again with high correlation coefficients between  $a^*$  value and  
15 235 acrylamide content:  $r^2 = 0.996$  for changes in a roasting time ( $\uparrow t \rightarrow \uparrow a^* \uparrow A$ ) and  $r^2 =$   
16 236  $0.968$  for roasting temperature ( $\uparrow T \rightarrow \uparrow a^* \uparrow A$ ) (Figure 3).  
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28 237 [Insert Figure 3 about here]  
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34 239 The third value of colour measurement - yellowness  $b^*$ , was increasing constantly  
35 240 when applying longer heating time and it was highly correlated with acrylamide  
36 241 concentration in a product with correlation coefficient calculated as  $r^2 = 0.998$ . On the  
37 242 contrary, application of higher roasting temperatures gave an increase of  $b^*$  values only  
38 243 at the beginning of heating at 140°C; after that the intensity of yellow colour started to  
39 244 decrease in opposition to the acrylamide content,  $r^2 = 0.097$  (Figure 4). Consequently  
40 245 measurement of yellowness was not considered to be a good indicator of acrylamide  
41 246 content in this type of products.  
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51 247 [Insert Figure 4 about here]  
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54 248 In a recent paper Gökmen et al. (2006) showed that in potato chips the values of  $L^*$   
55 249 and  $b^*$  decreased with temperature and time studied whereas the value of  $a^*$  increased  
56 250 to certain point and then, depending on temperature, either started to decrease or  
57 251 stayed at the same level. In this study only redness of the product and acrylamide  
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3 252 content was found to be correlated, however the same authors concluded that the  
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5 253 complexity of Maillard reaction in which acrylamide is considered to be formed as a  
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7 254 intermediate could not be explained by a simple linear regression model but rather  
8  
9 255 required explanation be a more complex quotation involving rate constants determined  
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11 256 experimentally for each product. In another paper, Pedrechi et al. (2005) reported that  
12  
13 257  $L^*$  and  $b^*$  did not show significant changes during frying of potato chips, however a  
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15 258 positive linear correlation was found between acrylamide concentration and redness of  
16  
17 259 the product at temperatures of 120, 150 and 180°C. Senyuva and Gökmen in their  
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19 260 study on instant, ground and laboratory roasted coffee found out that change in the  $a^*$   
20  
21 261 colour value was found to show a good correlation with the change in acrylamide, data  
22  
23 262 was fitted to a non-linear logarithmic function for the estimation of acrylamide level in  
24  
25 263 coffee. Studies on another potato product such as French fries (Taubert et al. 2004)  
26  
27 264 showed correlation of the colour development and acrylamide content only when a  
28  
29 265 small surface of the product was studied but no correlation was observed for large  
30  
31 266 surface material. Results for low-fat potato snacks reported in this paper were more  
32  
33 267 similar to those obtained by Gökmen et al. (2006) who studied wheat flour, where the  
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35 268 intensity of lightness decreased with time at all temperatures considered, and in most  
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37 269 cases the intensity of redness  $a^*$  and yellowness  $b^*$  increased which was correlated  
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39 270 with acrylamide content. Most of the papers (Pedreschi et al. 2005, Gökmen et al. 2006,  
40  
41 271 Mustafa et al. 2005) proved that the best correlation, and at the same time the best  
42  
43 272 prediction of acrylamide level, is the intensity of the red colour. This paper  
44  
45 273 demonstrated that there is also a high correlation with lightness  $L^*$ , which proves again  
46  
47 274 that in darker products higher amounts of acrylamide are formed. Ehling and  
48  
49 275 Shibamoto (2005) demonstrated a correlation of browning colour and acrylamide  
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51 276 formation, however their method of colour measurement was based on spectroscopy  
52  
53 277 analysis and involved much more time in sample preparation and measurement.  
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55 278 Similar results were obtained by Pedreschi et al. (2006) who studied ways of reduction  
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57 279 of acrylamide content in fried potato strips and at the same time correlated it with  
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3 280 colour development. They noted a significant correlation between colour represented  
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5 281 by  $L^*$  and  $a^*$  values and acrylamide content.  
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10 283 Mustafa et al. (2005), during studies on rye crisp bread, observed a correlation  
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12 284 between acrylamide content and colour expressed with  $a^*$  and  $b^*$  values in the time  
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14  
15 285 and temperature experiment but found no correlation when acrylamide precursors were  
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17 286 added. They concluded that asparagine is contributing to the pathway leading to  
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19 287 acrylamide but its effect on colour formation is limited.  
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24  
25 289 The results presented here show that acrylamide is formed also in low-fat potato  
26  
27 290 snacks, and may suggest that the lower fat content in such products does not influence  
28  
29 291 acrylamide formation. Temperature is the most important factor for acrylamide  
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31 292 formation in low-fat potato snacks. At the same time it has been proven that  
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33 293 measurement of colour expressed as value of lightness,  $L^*$ , and redness,  $a^*$ , could be  
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35 294 a good estimator of acrylamide content in food products.  
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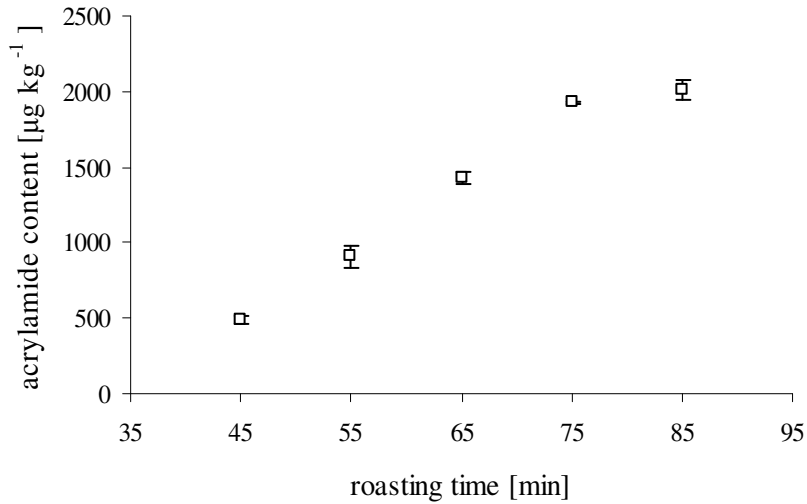
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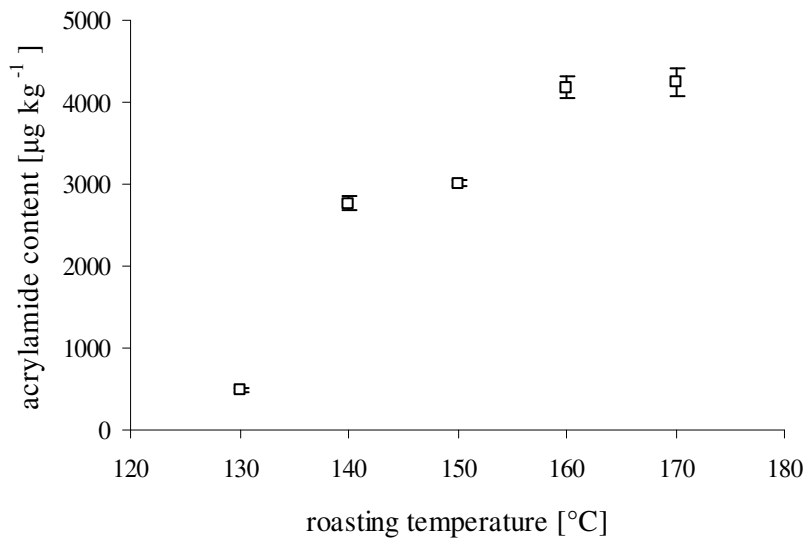


**Figure 1.** Influence of roasting time (temperature even = 130°C) (A) and temperature (time even = 45 min) (B) on acrylamide formation during preparation of low-fat potato snacks.

(A)

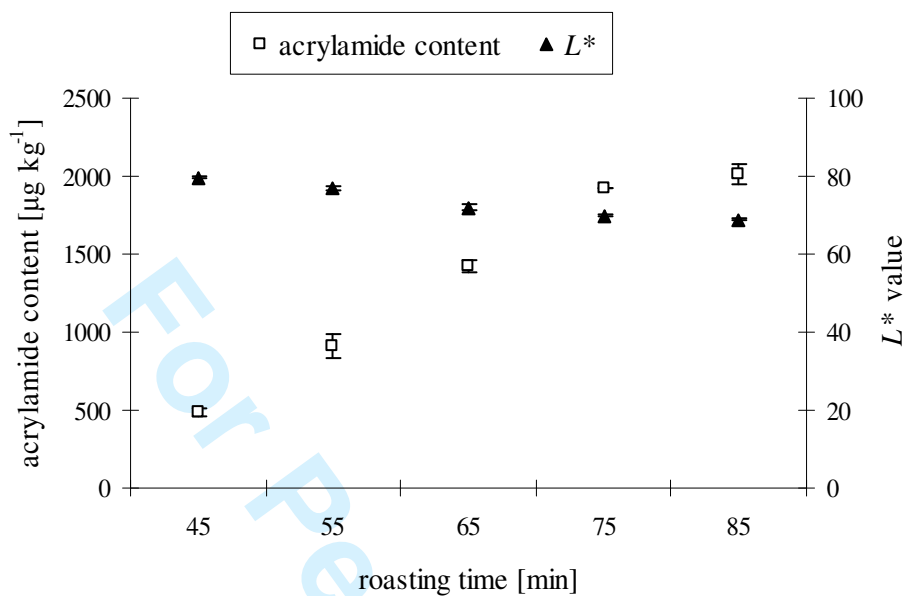


(B)

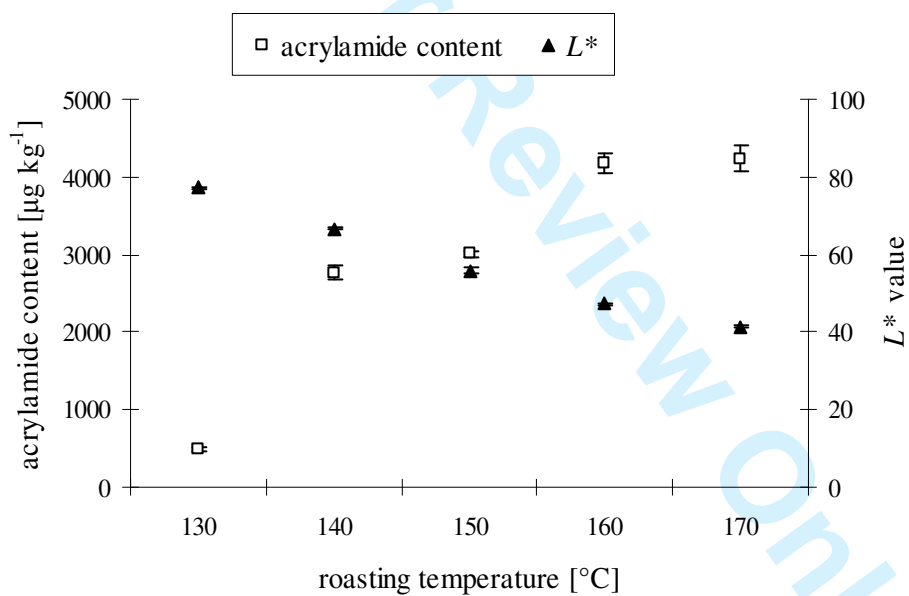


**Figure 2.** Acrylamide content and color lightness  $L^*$  in a function of roasting time (A) and temperature (B).

(A)

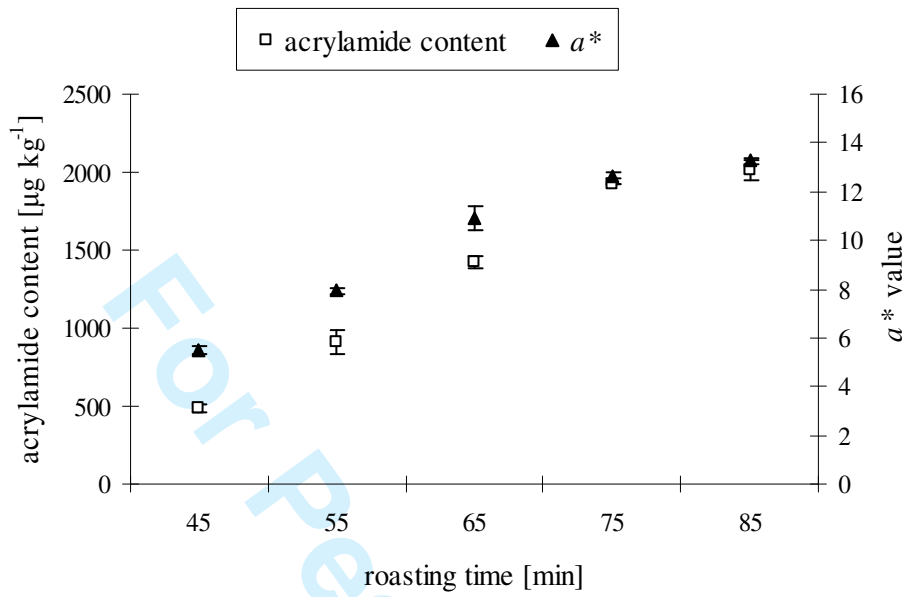


(B)

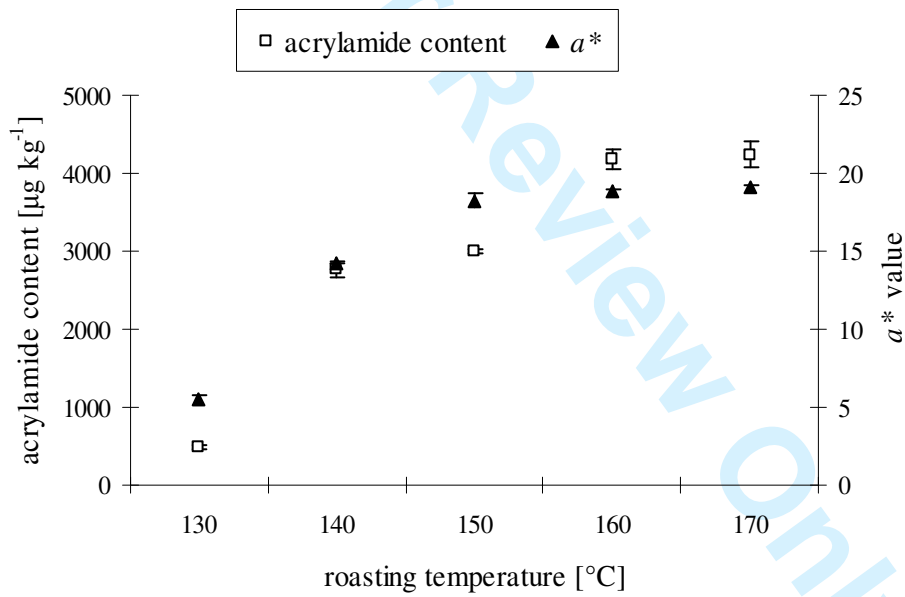


**Figure 3.** Acrylamide content and color redness  $a^*$  in a function of roasting time (A) and temperature (B).

(A)

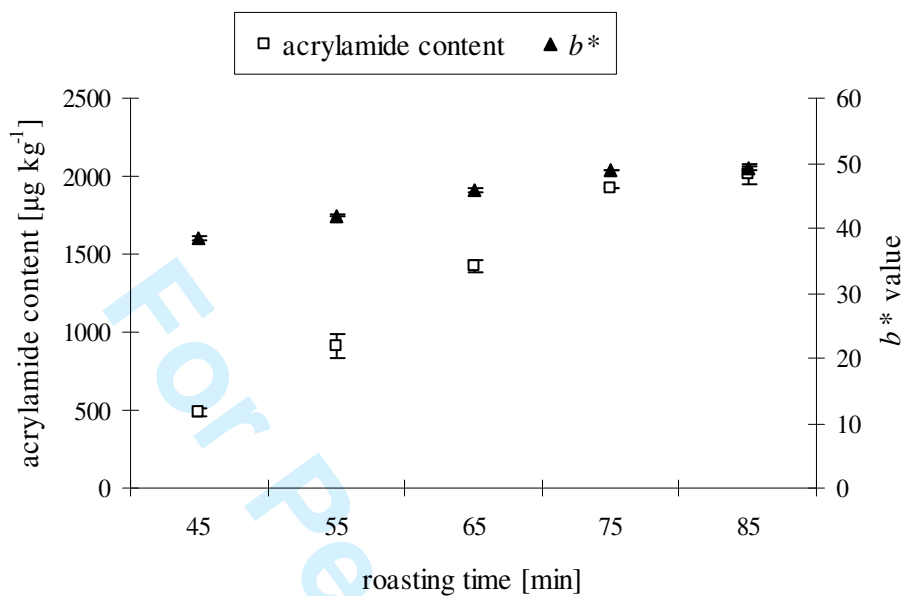


(B)



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

(A)



(B)

