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INFLUENCE OF TECHNOLOGICAL FACTORS ON ACRYLAMIDE LEVEL FROM BISCUITS

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Abstract

The objective of this study was to investigate the influence of wheat flour extraction degree and baking parameters on acrylamide (AA) level in biscuits by using gas chromatography tandem mass spectrometry (GC-MS/MS). Color parameters were analyzed in an attempt to establish correlations with the investigated factors. Biscuits baked between 25 min ÷ 60 min at 200°C recorded values of AA level < LOD (4.63 µg/kg) to about 1.580 µg/kg. By baking biscuits at a temperature varying between 220°C ÷ 240°C for 20 min the AA level increased from 67.44 to 212.87 µg/kg. The highest AA level and the darkest color was obtained when using in the biscuits recipe the whole-wheat flour (F3) with an ash content of 2.37% d.m. (1580.33 µg/kg, ΔE= 35.66), followed by white flour F1 with 0.53% d.m. (387.82 µg/kg, ΔE* = 61.79), respectively F2 with 0.44% d.m. (308.38 µg/kg, ΔE* = 67.47). From the results obtained it can be said that the AA level is strongly influenced by biscuits baking parameters (temperature and baking time) and by the ash content of wheat flour used in the manufacture recipe.*

***Key words**: acrylamide, baking parameters, biscuits, GC-MS/MS, wheat flour.*

INTRODUCTION

Acrylamide (AA) is a thermal chemical process contaminant that can be formed when foods are subjected to roasting, baking, frying processes at temperatures above 120°C (Ahrné et al., 2007, FDA, 2016). High temperature and long baking time may produce pleasant taste and specific color to food, and also can lead to the development of toxic substances such as AA (2-propenamide) (Mottram et al., 2002). In April 2002 a group of researchers from Sweden have announced that people consume AA through their daily diet by eating some common foods such as bread, crackers, chips, coffee etc. at much higher levels than the allowed dose in the drinking water (Tareke et al., 2002). The highest concentrations of AA were found to be formed in fried and baked potato products, followed by cereal products, coffee, and small amounts of AA were found in dairy, meat and fish products (Becalski et al., 2003; Ahrné et al., 2007; Alves et al., 2010).

Since acrylamide is known for its neurotoxic effects and was classified by the International Agency for Research on Cancer as a carcinogen in animals and "probable carcinogenic to humans" (Group 2A) (IARC, 1994), there is a permanent concern of experts from this field to decrease the concentration of AA from food at the lowest possible level.

There are several ways for AA formation in thermally treated foods, such as the Maillard reaction, involving a number of specific amino acids, for example asparagine, and compounds with a carbonyl group, for example, glucose, fructose (Mottram et al., 2002; Friedman, 2003; Claus et al., 2008; Hedegaard et al., 2008), and the second route for the formation of AA is the degradation of glycerol to acrolein, then to acrylic acid which reacts with ammonia and leads to the development of AA (IARC, 1994; Claus et al., 2008; Hedegaard et al., 2008).

The acrylamide content of food varies depending on the raw material, formulation recipe, processing conditions, production method etc. (Claus et al., 2008; Curtis et al., 2014; Przygodzka et al., 2015). Nguyen et al. (2016) showed that the acrylamide content of baked products is influenced by the type of sugar used in the manufacture recipe. By baking biscuits at 200°C, glucose produced a higher level of acrylamide than sucrose and fructose, the concentration of asparagine being higher in these samples. The lowest concentration of acrylamide was observed for the recipe of biscuits obtained with sucrose. Besides the type and concentration of sugars, the formation of acrylamide in biscuits was influenced by the concentration of amino acids, temperature and baking time, pH, water activity, leavening agent. Food surface color is the first qualitative parameter evaluated by consumers and it is essential in product acceptance. The color can be easily considered an indication of Maillard reaction products. Food color formation is influenced by factors such as pH, water content, water activity, oxygen levels, relative humidity (Ahrné et al., 2007), but the most important are the parameters of the heat treatment, temperature and baking time.

The purpose of this research was to investigate the influence of the wheat flour extraction degree (expressed as ash content) and the influence of the cooking parameters (time, temperature) on the AA content of biscuits; also it were evaluated the CIELab chromatic characteristics (L^*, a^*, b^*) in order to establish the correlations between color parameters and investigated factors.

Investigation of technological factors on the AA level in biscuits, on the one hand is important for consumers to select the use of everyday products that have a lower level of AA, on the other hand it is important for producers of bakery products in order to know the factors leading to the formation of a higher level of AA in food matrices such as biscuits and similar products, and to determine optimal mitigation strategies to reduce this contaminant.

MATERIALS AND METHODS

Biscuits preparation

Wheat flour was the main raw material, which came in the highest proportion in the composition of biscuit samples, made in experimental variants. In Romania, based on the extraction degree it is defined the type of flour, which is presented as mineral content (ash), expressed in dry matter percent. The more the extraction degree is higher, the ash content of flour is higher.

In this study three types of wheat flours obtained by milling the same wheat variety were used: white flour with an ash content of 0.53% d.m. (F1), white flour with an ash content of 0.44% d.m. (F2), respectively whole-wheat flour with an ash content of 2.37% d.m. (F3).

It were made 18 experimental variants of biscuits, varying the:

- baking time (25, 30, 40, 50, 60 min) and maintaining a temperature of 200°C, using wheat flour type with a different ash content (F1, F2, F3)
- baking temperature (220°C, 230°C, 240°C) and mantaining the baking time at 20 min, using the white wheat flour (F1).

For the biscuits preparation of the 18 experimental variants were used: flour (F1, F2, F3) (750 g), margarine (250 g), sugar (250 g), eggs (4, around 200 g), baking soda ($NaHCO_3$, 10 g), vinegar (3.5 mL), and salt (NaCl, 5 g). Margarine was mixed with sugar until a frothy masses was obtained, then the eggs, baking powder dissolved in vinegar, salt and finally flour were added. Kneading was done for 10-15 min, until getting a loose dough with proper consistency, which could be modeled easily. After kneading, the dough was packed in polyethylene bags and was allowed to rest in a refrigerator at 7°C for 60 - 120 min. The dough was shaped in the biscuits form, using a shaping machine, and then biscuits were placed in trays lined with parchment paper. Biscuit trays were placed in the oven, under the experimental conditions presented below. The experimental variants were realised in three separate batches, and for analyse were used average samples from 3 batches. Measurements were performed in triplicate, and the values were presented as mean ± standard deviation.

To get the biscuit assortments in the experimental variants were used specific equipment for the manufacturing technology of bakery products, from the Pilot Experiments Plant for Cereals and Flours Processing from IBA Bucharest: Buhler laboratory automated flour mill, MLU-202 (10-12 kg wheat/h), dough mixer (Diosna, DM 08- 4/6), dough roller, shaped machine, rotary kiln oven with controlled temperature and baking time (ROTOR model) etc.

Reagents and Consumables

For the determination of AA content from biscuits were used a native AA standard, min.

99% purity, of concentration 1000 μg/mL in methanol (Ultra Scientific Analytical Solution, N. Kingstown, Rhode Island, USA), internal standard (IS) of labeled acrylamide with the carbon atoms marked (1,2,3-^{13}C), min. 99% purity (+100 ppm hydroquinone), of concentration 1000 μg/mL in methanol (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). All chemicals, solvents and reagents used to analyze the AA were of chromatographic purity and were purchased from Merck (Darmstadt, Germany), LGC Promochem GmbH (Wesel, Germany) and Scharlau (Sentmenat, Spain). The water used was obtained from a purification system, PURELAB Option- S7/15 and PURELAB Ultra Ionic system (Elga Labwater, High Wycombe, UK).

Moisture and ash content determination

Determination of wheat flour and biscuits moisture of the experimental variants was realised by the method AOAC (2000). The ash content of wheat flour was performed by gravimetric method by sample calcination at 550°C, according to method AOAC (2000).

Acrylamide analysis

Biscuits acrylamide was quantified by GC-MS/MS (Negoiță et al., 2014, Negoiță and Culețu, 2016). The calibration solutions and the derivatized extracts sample were analyzed using a gas chromatograph, type TRACE GC Ultra, coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS) from Thermo Fisher Scientific (USA).

The analyses were performed in the electron impact ionization operation mode, positive (+ EI); acquisition mode: selected reaction monitoring (SRM) and ion scanning mode - Product ("Product"). A capillary column based on polyethylene-glycol, TraceGOLD™ TG-WaxMS (Thermo Fisher Scientific, USA) with a length of 30 m x 0.25 mm inner diameter and film thickness of 0.25 μm was used as a stationary phase.

The column temperature in the oven was programmed at 65°C for 1 min, and then increased with 15°C/min up to 170°C (0 min), 5°C/min up to 200°C (0 min), respectively 40°C/min up to 240°C with maintaining at this temperature for 15 minutes. The flow of the mobile phase, He, was 1.6 mL/min, transfer line temperature was 230°C. From the sample, 1 μL was injected into an injector PTV Right type, using an autosampler TriPlus AS (Thermo Fisher Scientific, USA), in split mode with a split ratio of 1:10 and the injector temperature 220°C. Under these conditions, the retention time of AA was 10.65 min ± 0.5 min AA and IS were identified by the appropriate ion fragments of ion derivatized 2-BPA (2 brompropenamida) and 2-BP ($^{13}C_3$) A.

The fragmentation of the precursor ions *m/z* 151 and 154 was carried out with argon (1 mTorr), leading to the formation of product ions (daughter) with *m/z* 70 (2-BPA) and 73 (2-BP ($^{13}C_3$) A), being used for quantification. The calculation of the AA concentration in the test samples were based on the appropriate peaks area ratios product ions with *m/z* 70 for 2-BPA and *m/z* 73 for 2-BP ($^{12}C_3$)A.

AA concentration values of the experimental variants of biscuit samples were expressed both on dry matter (% d.m.) and as μg/kg AA of biscuits.

The analytical procedure to determinate the AA level from biscuits was characterized by a high degree of sensitivity (LOD = 4.63 μg/kg, LOQ = 13.89 μg/kg) and good precision (RSD (r) ≤ 5%, RSD (R) ≤ 6%) (Negoiță and Culețu, 2016).

Color analysis

The most common way of measuring food color was introduced by Commission Internationale de L'éclairage (CIE) in 1976, in which color is described in a three-dimensional scale, the amounts of *L**, *a**, *b** (CIE Colorimetric Committee, 1974, Francis and Clydesdale, 1975).

The color analysis was performed using a HunterLab colorimeter (Universal Software V4.01 Miniscan XE Plus). Instrument calibration was done with black and white pads supplied by the manufacturer. The color of the samples was measured using illuminate D65 with an angle of view of 10°.

It were calculated CIELAB'76 color parameters: *L**, *a**, *b**. According to CIE, *L** measured the object luminance intensity on a scale from 0 to 100, where 0 represents black and 100 white, *a** represents the color position of the object on a scale from pure red and pure green, where pure green is -127, and pure red is +127, and b^* represents the position of the object on a scale color of pure blue and pure yellow, where pure blue is -127 and pure yellow is +127. For each sample, measurements were made on 10 different points, and the mean value was determined. Total color difference, ΔE^* was calculated using the following equation (1):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (1)$$

where $\Delta L^* = L^*_1 - L^*_0$; $\Delta a^* = a^*_1 - a^*_0$; $\Delta b^* = b^*_1 - b^*_0$, L^*_0, a^*_0, b^*_0 it refers to the standard color (black, where $L^*_0 = 0$, $a^*_0 = 0$, $b^*_0 = 0$), L^*_1, a^*_1, b^*_1 refers to the sample color.

Statistical analysis

AA content was expressed as mean ± standard deviation.

The differences among sample groups were analyzed by one-way analysis of variance ANOVA followed by Tukey's test, $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Since duration and temperature at which dough is thermally treated during baking highly influence the level of acrylamide and the color of the baked product, the objective of this study was to establish the influence of these parameters on the acrylamide content and color changes of biscuits.

Characterisation of flour types

The physico-chemical properties and color parameters of wheat flour used in the experimental variants are presented in Table 1.

Influence of the baking time and the flour type on the AA formation in biscuits

In this study 15 experimental variants of biscuits were made, varying the baking time (25, 30, 40, 50, 60 min) and the wheat flour used, with different extraction degrees, expressed in ash content (F1, F2, F3). The same baking temperature, 200°C, was used for all 15 experimental variants. The experimental variants when varying the baking time are presented in Table 2.

Table 1. Physico-chemical and sensorial properties of wheat flour used in the experimental variants

Physico-chemical and sensorial properties	**Wheat type A**		
	White flour		Whole-wheat flour
	F1	F2	F3
Moisture (%)	12.60	12.71	11.99
Ash (% d.m.)	0.53	0.44	2.37
Glucides (% d.m.)	73.2	74.1	70.21
Reducing sugars (% d.m.)	0.68	0.65	1.3
Celulose (% d.m.)	0.65	0.59	1.48
Color parameters, CIELab			
L^*	88.87	88.16	74.63
a^*	0.64	0.27	2.48
b^*	8.89	7.81	8.85
ΔE^*	89.31	88.51	75.19

From the data shown in Table 3 where the AA content of the experimental samples is presented as a function of baking time it can be noticed that for the three types of flour used in the manufacture recipe of biscuits, the AA level increases as increasing the baking time, respectively when decreasing the water content of biscuits. Also AA level increases with increasing ash content of the flour, as follows:

- AA level increased from < LOQ (13.89 μg/kg) up to 387.82 μg/kg for wheat flour F1 (0.53% d.m.),
- AA level increased from < LOD (4.63 μg/kg) up to 308.38 μg/kg for wheat flour F2 (0.44% d.m.),
- AA level increased from 14.34 μg/kg up to 1580.33 μg/kg for whole-wheat flour F3 (2.37% d.m.).

Table 2. Experimental variants to obtain biscuits using three different types of flour, varying the baking time

No.	Flour type	Variant	Cooking parameters, temp. (^{0}C), time (min)	Experimental variants
1	White wheat flour (F1)	V21	200, 25	
2		V31	200, 30	
3		V41	200, 40	
4		V51	200, 50	
5		V61	200, 60	
6	White wheat flour (F2)	V22	200, 25	
7		V32	200, 30	
8		V42	200, 40	
9		V52	200, 50	
10		V62	200, 60	
11	Whole-wheat flour (F3)	V23	200, 25	
12		V33	200, 30	
13		V43	200, 40	
14		V53	200, 50	
15		V63	200, 60	

Table 3. Acrylamide and water content when varying the baking time of experimental variants

Time / min	**Parameter**[a]								
	AA (μg/kg)			AA (% d.m.)			Water content (%)		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
25	<LOD[b]	<LOD[b]	14.34[b] ± 0.03	0.84[c] ± 0.00	0.29[c] ± 0.00	1.58[c] ± 0.00	13.17[d] ± 0.06	10.21[d] ± 0.05	9.29[d] ± 0.12
30	<LOQ[b]	<LOQ[b]	25.51[b] ± 0.03	1.22[c] ± 0.00	0.44[c] ± 0.00	2.74[c] ± 0.00	11.12[d] ± 0.04	7.54 ± 0.10	7.00[d] ± 0.21
40	55.12[b] ± 0.0	45.70[b] ± 0.18	533.73[b] ± 0.02	5.89[c] ± 0.00	4.85[c] ± 0.00	54.35[c] ± 0.04	6.41[d] ± 0.08	5.73[d] ± 0.05	1.80[d] ± 0.07
50	108.87[b] ± 0.04	59.53[b] ± 0.26	1214.79[b] ± 0.02	11.38[c] ± 0.00	6.17[c] ± 0.00	123.09[c] ± 0.07	4.30[d] ± 0.04	3.60[d] ± 0.10	1.31[d] ± 0.06
60	387.82[b] ± 0.07	308.38[b] ± 0.12	1580.33[b] ± 0.03	39.46[c] ± 0.03	31.38[c] ± 0.02	159.81[c] ± 0.12	1.70 ± 0.07	1.72 ± 0.06	1.11[d] ± 0.08

[a]The results are expressed as mean ± standard deviation (n = 3); [b,c,d] data followed by different superscript letters within each column indicates a highly significant difference among each parameter according to Tukey's test ($p < 0.001$) ([b] for AA level expressed in μg/kg, [c] for AA level expressed in % d.m., [d] for water content)

When varying the baking time of the experimental variants of biscuits, lower concentrations of AA were formed in biscuit samples obtained with white flour (F1, F2), compared with the biscuits samples obtained from whole-wheat flour (F3). Extraction degree of flour, expressed in ash content, is an important factor affecting the acrylamide level in the final product (Claus et al., 2008). Also, the acylamide content of bakery products is influenced by the asparagine content of wheat flour used, products made from whole-wheat flour with an ash content higher than white flour producing a higher level of acrylamide in the equivalent model system (Claus et al., 2008; Przygodzka et al., 2015; FDA, 2016). In a study realized by Przygodzka et al. (2015) it was shown that the baking conditions and the wheat flour used influence the acrylamide formation in white and dark breads. By increasing the baking temperature from 200°C to 240°C and decreasing the baking time from 35 min to 30 min, for both white bread and whole-wheat dark bread, the crust of bread baked at higher temperature produced a higher AA content. The AA content was higher in the bread crust than in bread sliced.

The results are in agreement with the findings of Capuano et al. (2009) who investigated the effect of flour type (wheat, rye, and whole-wheat flour), also the process conditions on a bread crisp model system and it was determine that the acrylamide amount was higher in the samples containing rye flour (301.0 μg/kg) and whole-wheat flours (291.0 μg/kg), than the ones with wheat flours (262.0 μg/kg). It can be concluded that these results can be due to a higher content of free asparagine and ash content of rye and whole-wheat flours.

As acrylamide forms in foods through the Maillard reaction, the content of reducing sugars influence the level of acrylamide in the food system. The AA content of experimental variants of biscuits obtained in this study was in direct ratio with the content of reducing sugars presented in the flour used. A higher level of AA was obtained for the biscuits made with the flour F3 which presented the highest content of reducing sugars (1.3% d.m.), followed by the flour F1 (0.68% d.m.), respectively F2 (0.65% d.m.) (table 3). This result is probably due to the fact that sucrose began to hydrolyse, resulting a higher level of reducing sugars which produce asparagine degradation and acrylamide formation (Van Der Fels-Klerx et al., 2014).

Correlation between AA level and biscuits baking time

To establish a correlation between the AA level and the biscuits baking time (25 min, 30 min, 40 min, 50 min, 60 min) it were drawn the regression lines for each type of flour which led to the line equations and regression coefficient values. For each type of flour it were obtained positive linear correlation between the acrylamide level and the baking time:

- y = 9.825x – 288.85, R = 0.8876, for wheat flour F1 (0.53% d.m.),
- y = 7.637x – 229.04, R = 0.8550, for wheat flour F2 (0.44% d.m.),
- y = 48.678x – 132.00, R = 0.9883, for whole-wheat flour F3 (2.37% d.m.).

By using in the biscuits formulation recipe of a type of flour with a higher ash content (F3), a higher level of AA was observed compared to the biscuit samples obtained by using a flour with a lower ash content (F1, F2). Applying a higher baking time for biscuits, provide a higher level of AA in all cases of flour used.

The correlation between the AA level and the baking time of the biscuit is higher as the ash content of the wheat flour is higher.

Correlation between the AA level and the water content of biscuits

At baking time variation of the experimental samples of biscuits were obtained negative linear correlations between the AA level and biscuits moisture for each type of flour used:

- y = –2.8225x + 32.393, R = 0.8311, for wheat flour F1 (0.53% d.m.),
- y = –3.1228x + 26.668, R = 0.7992, for wheat flour F2 (0.44% d.m.),
- y = –16.398x + 135.04, R = 0.8636, for whole-wheat flour F3 (2.37% d.m.).

When increasing the baking time of biscuits, it was produced the decrease of water content of biscuits and thus increased the AA level for all type of flour used. During baking, moisture loss progresses (Della Valle et al., 2012, Walker et al., 2012) and acrylamide formation enhance (Claus et al., 2008).

Same results were obtaind by Acar and Gokmen (2009), who showed that the temperature and water content of baked products influence the acrylamide formation, higher levels of acrylamide being obtained at higher temperatures. Mulla et al. (2011) found that there is a correlation between the AA formation and water content of extruded snacks. An increase in the moisture content of the raw material produced a decreased of the AA level.

The correlation between the AA level and the water content of biscuits is higher as the ash content of the wheat flour is higher.

Correlation between the AA level and color parameters of biscuit samples

The effect of color development and increasment of AA level in biscuits obtained at 5 different baking time, 25, 30, 40, 50, 60 min (200°C) and 3 types of wheat flour with different ash content was studied. Color is an important food atribute and can be an indicator of acrylamide formation during processing of food products (Lu and Zheng, 2012). The formation of color during baking is due to the Maillard reaction and caramelization of sugars (Purlis, 2010).

All experimental variants were analyzed in terms of CIELab chromatic characteristics represented by the color difference ΔE^*. Lu and Zheng (2012) evaluated the AA content in biscuits by analysing the color information using the principal component analysis (PCA) and least-squares support vector machine (LS-SVM) combined with fractal color. It was found that L^*, a^*, b^* have the best performance to identify the correlation between the AA content and color changes of biscuits comparing with the prediction performance of fractal color.

In Figure 1 it is represented the AA level variation and color differences (*ΔE**) of the biscuits obtained in the experiment. It is shown that as the AA content increases, the color difference decreases.

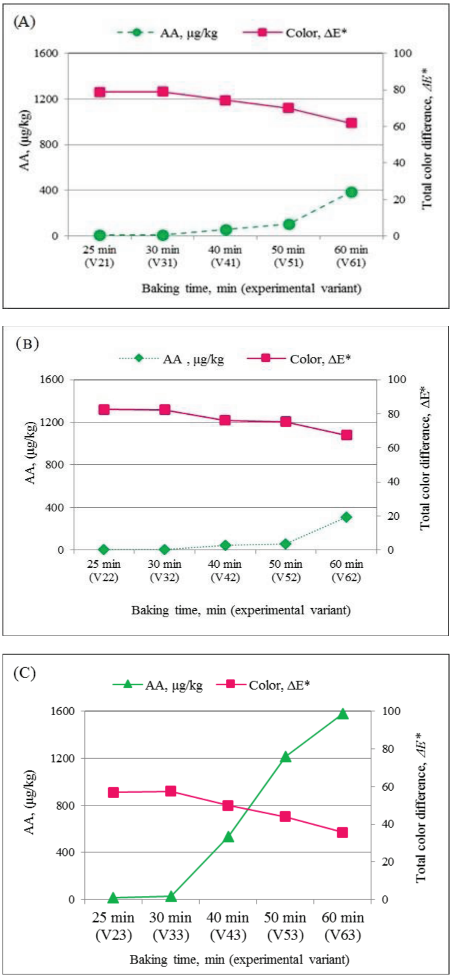

Figure 1. Variation of AA level and total color difference *ΔE** of biscuits obtained with flour F1 (A), flour F2 (B), and flour F3 (C) when varying the baking time

In order to establish the correlation between the AA level and total color difference (*ΔE**) of the experimental variants, the regression lines were plotted and the straight lines equations and the regression coefficients values between these parameters were determined.

A negative linear correlation was obtained between the AA level and the total color difference (*ΔE**) of the biscuit samples tested, the straight lines equations and correlation coefficients being as follows:

- $y = -21.272x + 1660.8$, R = 0.9596, for wheat flour F1 (0.53% d.m.),
- $y = -19.069x + 1547.7$, R = 0.9264, for wheat flour F2 (0.44% d.m.),
- $y = -75.809x + y = 4375.1$, R = 0.9888, for whole-wheat flour F3 (2.37% d.m.).

In all cases of flours used, when baking time increases, the total color difference, *ΔE**, decreases. The highest values of AA level and the lowest values of total color difference were recorded when using F3 flour type (2.37% d.m.), with the highest ash content obtaining the highest correlation between these parameters (R = 0.9888). The correlation is higher as the ash content of the flour used is higher.

Influence of the baking temperature on the AA formation in biscuits

In addition to the baking time, temperature is an important factor in the formation and reduction of AA in biscuits. It were realised 4 experimental variants (Table 4), to determine the baking temperature influence on the AA content formed in biscuits. It has been shown that, by keeping constant the baking time (20 min) and temperatures ranging from 220°C to 230°C, respectively 240°C, the AA level from biscuits increased approximately 3-fold (Figure 2).

Same results were obtained by Acar and Gokmen (2009) who showed that the temperature and moisture of baked products influence the acrylamide formation, higher levels of acrylamide being obtained at higher temperatures.

From Figure 3 it can be observed that the AA level increased as the baking temperature increases and the water content of biscuits decreases. The results are in accordance with the findings of Mulla et al. (2011) who showed that the AA formation is influenced mostly by the baking temperature and product moisture for extruded snacked. An increase of temperature determine a larger amount of AA.

In 2016, US Food and Drug Administration (FDA) issued a guidance to provide information regarding the reduction of AA levels in certain foods. For bakery products it is recommended to bake the products at lower temperatures and longer times.

The baking temperature should be increased in the first stage of the baking process and decreased in the last stage of baking.

Table 4. Experimental variants of biscuits obtained with flour F1 when varying the baking temperature

No.	Flour type	Variant	Baking parameters temp. (°C), time (min)	Experimental variants
1.	White wheat flour (F1)	V200_20	200, 20	
2.		V220_20	220, 20	
3.		V230_20	230, 20	
4.		V240_20	240, 20	200 °C 220 °C 230 °C 240 °C

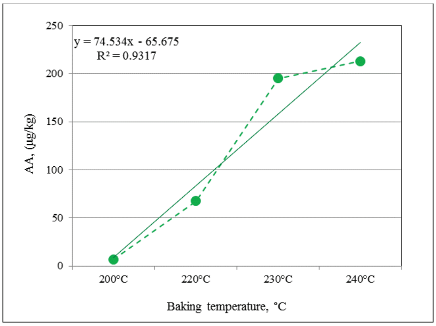

Figure 2. Variation and correlation between the AA level of biscuits and baking temperature

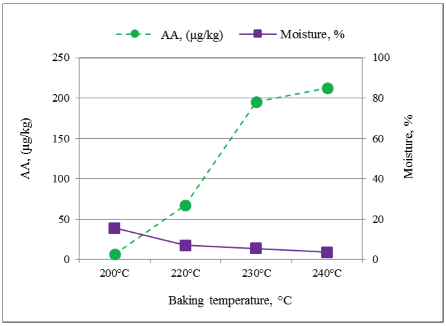

Figure 3. Variation of AA level and biscuits moisture of the experimental variants

Correlation between the AA level and the biscuits baking temperature

To establish the relationship between the AA content and the baking temperature of biscuits (200°C, 220°C, 230°C, 240°C) the regression line for the experimental variants was assign and the equation and regression coefficient was determined (figure 2).

From Figure 2 it can be noticed that there is a positive linear correlation between the AA level and baking temperature represented by a correlation coefficient of R = 0.9652. The AA content increased with baking temperature increase.

Correlation between color parameters and the AA level of biscuit samples when varying the baking temperature

The effect of color development and formation of AA in biscuits obtained at 4 different baking temperatures, 200°C, 220°C, 230°C, 240°C (20 min), with wheat flour with an ash content of 0.53% d.m. (F1) was studied.

Increasing the baking temperature lead to visible changes in both the biscuits crust color, viewed in the CIELab color parameters, L^*, a^*, b^*, and the level of AA formed. It is known that the crust color of products depend on the technological parameters, also the manufacture recipe and a higher level of acrylamide is located in the crust of bakery products (Ahrné et al., 2007; Claus et al., 2008; Della Valle et al., 2012; Przygodzka et al., 2015).

The variation of AA level and total color difference, ΔE^*, of biscuit assortments of the experimental variants obtained, represented in Figure 4, highlights that, as the AA content increases, the total color difference decreases.

To establish the relationship between the AA content and the total color difference, ΔE^*, the regression line for the experimental variants was assign (Figure 5) and the equation and regression coefficient were determined. It was revealed that there is a negative linear correlation between the AA level and the total color difference, represented by a correlation coefficient of R = 0.9989.

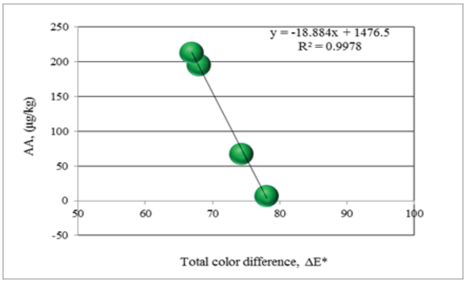

Figure 4. Variation of AA level and total color difference *ΔE** of biscuits experimental variants

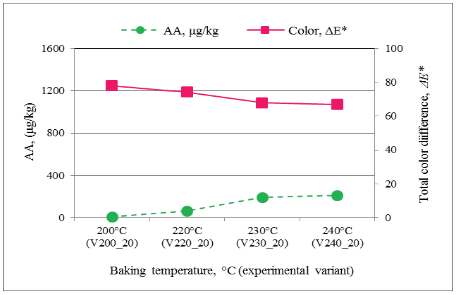

Figure 5. Correlation between AA level and total color difference ΔE*of biscuits when varying the baking temperature

As shown in figure 4 and 5, the browning ratio is an important indicator of acrylamide concentration in biscuits, being influenced by the baking parameters.

Similar results were obtained by Gökmen et al. (2008) who shown that the surface browning and acrylamide formation in cookies followed the same kinetic pattern during baking at different temperatures (200 and 220°C) and times up to 25 min.

CONCLUSIONS

Investigating the influence of technological factors on the AA level of biscuits and similar products is important for manufacturers of bakery products in order to know the factors that lead to the formation of a high level of AA and establish the best ways to reduce this processing contaminant.

The use of white flours with a low extraction degree and a low ash content in order to obtain biscuits, caused a decrease of AA level, in regard to the use of whole-wheat flour with a high extraction degree, respectively high ash content, resulted from the same variety of wheat.

Changing baking conditions showed that the AA level is strongly influenced by biscuits baking time and temperature. With the results obtained, it was found that the AA level is dependent of processing conditions (temperature and baking time), and increases with the increase of these parameters.

By measuring color parameters, *L**, *a**, *b**, of biscuits made of wheat flours, with different extraction degrees, it was shown that there is a linear correlation between the AA concentration and total color difference *ΔE**.

By increasing the time and baking temperature, the AA level increases and it were obtained darker products, producing low levels of total color difference ΔE*.

In the case of biscuits obtained from flour with a high extraction degree, and high ash content, it was formed the highest AA level and the lowest values of the total color difference (dark colors of biscuits). For biscuits obtained from flours with a low extraction degree, respectively low ash content, it was formed the lowest level of AA and the highest values of the color difference (bright colors of biscuits).

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