Romanian Biotechnological Letters *Vol. 21, No. 5, 2016*

ORIGINAL PAPER

Investigation regarding influence of different food ingredients addition on the acrylamide level in bread

Received for publication, June 6th, 2015
Accepted, November 13th , 2015

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Abstract

Influence of three food ingredients (nuts, olives and dehydrated onion) addition on the acrylamide level formed in different types of bread: Simple bread, Nut bread, Olive bread and Onion bread, made of 2 types of flour (white flour 480 and black flour 1250) was studied. For all bread assortments were evaluated CIELab parameters, L, a*, b*. Acrylamide (AA) analysis in bread samples was performed by gas chromatography tandem mass spectrometry (GC/MS/MS) using a gas chromatograph TRACE GC ULTRA coupled to triple quadrupole mass spectrometer, TSQ Quantum XLS (Thermo Fisher Scientific, USA).The results revealed that the lowest values of AA content (26.92 to 33 mg/kg) were found in Simple bread and the highest values (79.22 to 88.5 mg/kg) were found in Onion bread for both types of flour used. AA level doesn't correlate with the color, but with the carbohydrate content of the ingredients.*

Keywords: acrylamide, bread, CIELab parameters, GC/MS/MS

1. Introduction

Acrylamide (AA), CH_2=CH-CO-NH_2, CAS 79-0601 was discovered in food in April 2002 by the researchers from Stockholm University, Sweden. It has been shown that acrylamide is formed when foods are processed (roasted, baked) at temperatures above 120^0C, as the result of chemical reaction of specific amino acids (i.e. asparagine) and compounds with carbonyl groups (i.e. glucose, fructose, maltose). This reaction, known as the Maillard reaction, gives the food the corresponding color and flavor, and, on the other hand, leads to the formation of AA (BECALSKI *et al.* [1], MOTTRAM *et al.* [2], FRIEDMAN [3]).

In 2005, The Joint FAO/WHO Expert Committee on Food Additives made an assessment of acrylamide in terms of food safety and concluded that AA is a potentially genotoxic and carcinogenic compound with risks to human health (JECFA, [4]). AA intake through food causes damage to the nervous system and affects reproductive function, having mutagenic and carcinogenic properties in animals. Increasing the concentration of AA in laboratory animals led to lung tumor multiplicity, skin tumors, thyroid gland tumors, brain tumors, uterine tumors, etc. Neurotoxicity is the only adverse effect in humans, affecting sensory and/or motor functions (COSTA *et al*., [5], DOERGE *et al*. [6, 7], FRIEDMAN [3], LOPACHIN *et al*. [8], PAULSSON *et al*. [9], WILSON *et al*. [10]). The aim of this paper was to determine the influence of added ingredients, olives, nuts, dehydrated onion on AA content in some bread types, experimentally obtained. For all bread samples were determined chromatic parameters CIELab'76, *L**, *a**, *b**. It was intended to establish correlations between parameters and color and level of AA formed in experimentally obtained bread samples, respectively correlation between carbohydrate content of added ingredients and levels of AA.

2. Materials and methods

Raw materials and added ingredients can be considered a key factor in AA formation. Thus, the influence of some natural ingredients: olives, nuts and dehydrated onion on AA formation was studied in four bread types: *Simple bread*, *Nut bread*, *Olive bread* and *Onion bread*. Bread samples were obtained using two types of wheat flour: white type 480 and black type 1250.

Dough preparation of the experimental samples consisted in the following main operations: dosing of the raw and auxiliary materials, mixing, fermentation, division and the final fermentation of the dough. For each type of bread dough was prepared as shown in Table 1.

Table 1. Experimental types of bread obtained with added ingredients

Flour type	Bread types (code)	Components						
		Flour, kg	Yeast, kg	Salt, kg	Water, L	Olives, kg	Nuts, kg	Onion, kg
White wheat flour, type 480	Simple bread (1)	100	3	1.5	60	-	-	-
	Nut bread (2)	100	3	1.5	60	-	12	-
	Olive bread (3)	100	3	1.5	60	12	-	-
	Onion bread (4)	100	3	1.5	60	-	-	12
Black wheat flour, type 1250	Simple bread (5)	100	1.5	1.5	60	-	-	-
	Nut bread (6)	100	1.5	1.5	60	-	12	-
	Olive bread (7)	100	1.5	1.5	60	12	-	-
	Onion bread (8)	100	1.5	1.5	60	-	-	12

The amount of yeast used to obtain dough from black wheat flour was half of the amount used to obtain dough from white wheat flour, whereas acidity of black wheat flour had a double value (5.5 degrees) than that of white wheat flour (2.8 degrees). Each of the obtained dough was allowed to ferment at 30°C for 90 minutes. After fermentation was finished, corresponding ingredient was added (nuts, olives and dehydrated onion). Each type of dough has been kneaded another 5 minutes in order to incorporate the ingredients. Divided dough pieces were shaped and placed in rectangular trays in a fermenting room for 30 minutes at a temperature of 30°C and a relative humidity of 85% (Fig. 1). The bread samples were baked for 40 minutes at 220°C.

Equipment: For obtaining bread samples the following equipment were used: oven with two chambers and controlled time and baking temperature (Mondial Forni), dough mixer (Diosna), and manual divider (Vitella). For AA analysis a gas chromatograph (Trace GC Ultra) coupled to triple quadrupole mass spectrometer (TSQ Quantum XLS) purchased from Thermo Fisher Scientific was used. **Methods:** Moisture and acidity were analyzed according to SR 90:2007, lipids content and carbohydrates content were analyzed according to SR 91:2007, protein content was determined according to SR EN ISO 20483:2007, ash content was analyzed according to SR EN 2171:2010 and crude fiber content was determined according to SR EN ISO 6865:2002. AA was analyzed using a gas chromatograph (TRACE GC ULTRA) coupled with triple quadrupole mass spectrometer (TSQ Quantum XLS) from Thermo Fisher Scientific (USA) and color measurements were performed using a HunterLab colorimeter.

Figure 1. Phases of the technological process for obtaining bread with various ingredients

AA analysis in bread samples were performed in the electron impact positive ionization mode (EI^+), acquisition mode "Selected Reaction Monitoring - SRM" and ion scanning mode "Product". A capillary column (30 m x 0.25 mm, 0.25 µm thickness), TraceGOLD™ TG-WaxMS (Thermo Fisher Scientific, USA), having polyethylenglycol as stationary phase and helium with a purity of 99.9995% as mobile phase was used. Collision gas used was argon (1 mTorr). 1 µL sample was injected into an injector type Right PTV using an autosampler TriPlus AS autosampler (Thermo Fisher Scientific, USA), split mode, split ratio of 1:10 and a temperature of 220°C. AA quantification was performed by the internal standard method, using 1,2,3-^{13}C labelled AA (IS). Under these conditions, the retention time of AA and IS was 10.65 minutes ± 30 sec. AA and IS were identified by the ion fragments corresponding to the derivatized ions, 2-BPA (2-bromopropenamide) and 2-BP($^{13}C_3$)A. Collision of precursor ions with m/z 151 and 154 resulted in the formation of daughter ions m/z 70 (2-BPA) and 73 (2-BP($^{13}C_3$)A), which were used for the quantification of AA. Calculation of the AA concentration in the test sample is based on peak area ratio corresponding to daughter ions m/z 70 for 2-BPA and m/z 73 for 2-BP($^{13}C_3$)A (NEGOITA *et al.*, [11, 12]). For the analysis of AA a homogeneous sample from 3 experimental batches was used. **Color measurement** of bread samples was performed at room temperature, using a HunterLab colorimeter with Miniscan XE Plus Universal Software V4.01. Samples color was measured using D65 illuminator, measuring angle being of 10°. Samples were put in a transparent container with low reduction and placed above colorimeter opening. For each sample, were performed measurements in 10 different points and the average value was calculated. The following CIELab'76 color parameters were calculated:

L^* - color luminance: 0 is black and 100 is white; a^* - red-green color coordinate: positive values are red, negative values are green and 0 is neutral; b^* - yellow-blue coordinate: positive values are yellow, negative values are blue and 0 is neutral.

$$\Delta E^* = \sqrt{\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2} \quad ,$$

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 refers to color etalon ($L^*_0 = 0, a^*_0 = 0, b^*_0 = 0$) and L^*_1, a^*_1, b^*_1 refers to colored sample.

3. Results and discussion

Both flour types used in the experiments were analyzed in terms of physico-chemical characteristics. Obtain results are presented in Table 2.

Table 2. Physico-chemical characteristics of wheat flour

Physico-chemical characteristics	Flour	
	White flour type 480	**Black flour type 1250**
Moisture, %	12.71	11.10
Ash, % d.m*.	0.48	1.27
Proteins, % d.m.	11.5	13.70
Lipids, % d.m.	1.01	2.09
Carbohydrates, % d.m.	73.6	70.61
Reducing sugars, % d.m.	0.7	1.3
Fiber, % d.m.	0.7	1.23
Acidity, degrees	2.8	5.5
Color	Yellowish-white	Yellowish-white with faint gray color, containing particles of bran
Smell	Pleasant, specific healthy flour, without the smell of mold, musty or other foreign smell	
Taste	Specifically, slightly sweet, nor sour nor bitter, without squealing in mastication	

*d.m. – dry matter

The ingredients used to differentiate the samples were analyzed regarding moisture and the content of proteins, lipids and total carbohydrates. The results are presented in Table 3.

Table 3. The physico-chemical characteristics of used ingredients

No.	Characteristics	Ingredients		
		Olives	**Nuts**	**Onion**
1.	Moisture, %	55	3.9	14.5
2.	Total carbohydrates, %	17.2	16.2	70
3.	Proteins, %	2.4	14.3	10.5
4.	Lipids, %	22.8	64.3	1.6

All experimental samples (Fig. 2) were analyzed as follows: CIELab (L^*, a^*, b^*) - hromatic parameters for bread crumb obtained after drying and grinding of bread samples; Water content of bread samples; Water content of bread crumbs obtained after drying and grinding of bread samples; AA content of bread samples.

CIELab color parameters (L^*, a^*, b^*) for each bread type obtained from white/black flour vary in the same way (Fig. 3 and 4). For both wheat flour types used brightness (L^*) varies in the same way; the highest value was obtained for “Simple bread” while the lowest value was obtained for “Olive bread”.

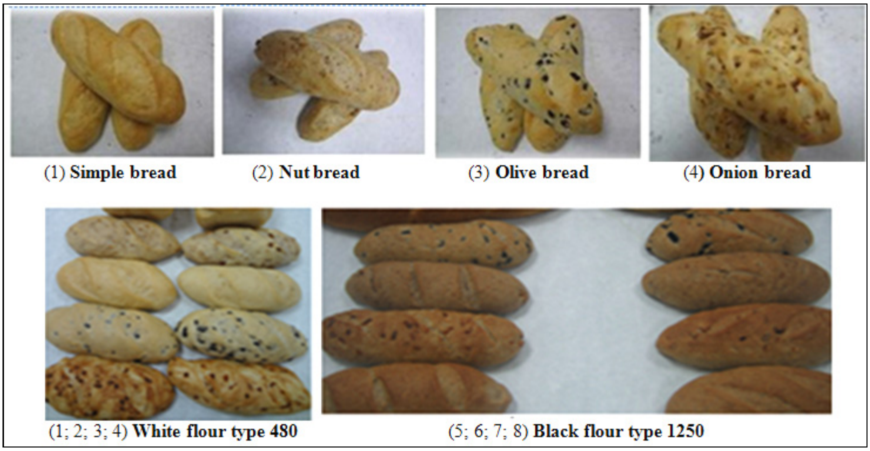

Figure 2. Bread types with different ingredients

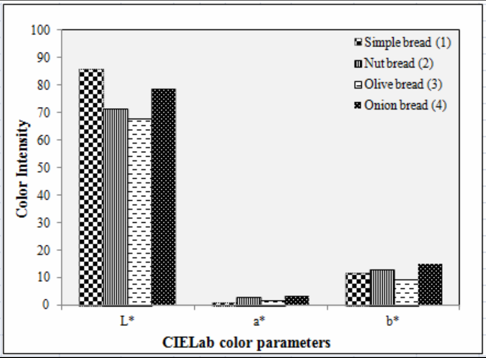

Figure 3. CIELab color parameters of bread types obtained from white flour (480 type)

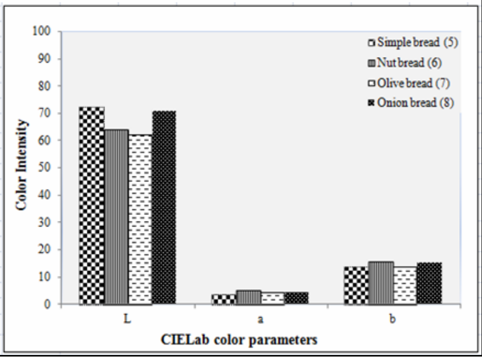

Figure 4. CIELab color parameters of bread types obtained from black flour (1250 type)

Plotting in Figure 5, the total color difference, *ΔE** of the samples of the experimental variations, it shows that it varies in the same manner for both types of wheat flour used.

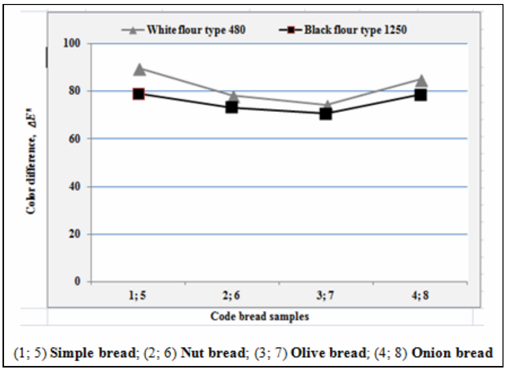

Figure 5. Color *ΔE** variation of bread types obtained from white and black flour

Samples of bread simple (1; 5) and with the addition of onion (4; 8), conducted both white wheat flour type 480 and flour black type 1250 showed the biggest differences in color, *ΔE** being the lighter compared to samples of bread with added nuts (2; 6) or olive (3; 7), which showed smaller differences in color, being darker. This is also shown visually by the color of bread crust, finely ground (Fig. 6).

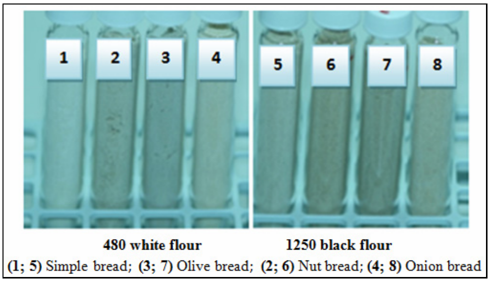

Figure 6. Bread crumbs color variation obtained by drying and grinding of bread samples

Representing graphically in Figures 7 and 8, the level of AA and the carbohydrate content of the natural ingredients added according to experimental variations, it shows that, for the two types of wheat flour used, they vary in the same way increased. The *lowest values* of AA levels were obtained for simple varieties, *Simple bread*, code 5 (26.92 µg/kg) of wheat flour black, type 1250, respectively, white wheat flour type 480, code 1 (33 µg/kg). The *highest values* of AA levels were obtained for varieties with added onions, *Onion bread*, code 4 (88.50 µg/kg) of wheat flour type 480, respectively, black wheat flour, type 1250, code 8 (79.22 µg/kg).

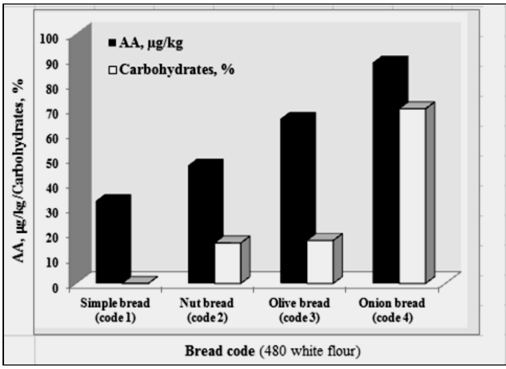

Figure 7. Level AA of experimental variants and carbohydrate content of the ingredients used

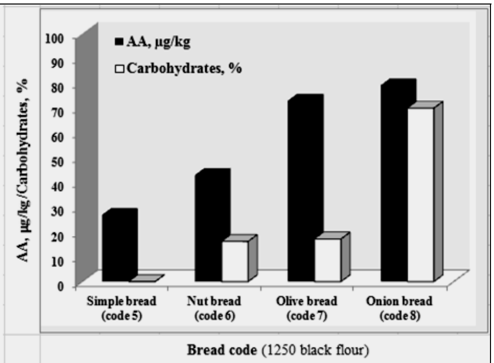

Figure 8. Level AA of experimental variants and carbohydrate content of the ingredients used

Plotting the level of AA to the difference in total color, *ΔE**, of the assortment of bread obtained in the experimental variations (Fig. 9 and 10), that the level of AA based on the carbohydrate content of the ingredients used in the manufacture varieties of bread (Fig. 11 and 12), for type 480 wheat flour and flour type 1250, we determined the line equations and correlation coefficient values of these sizes.

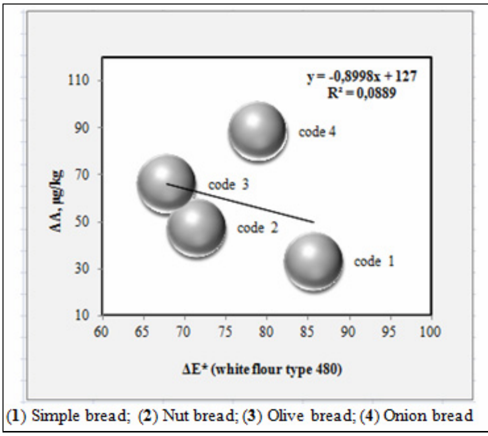

Figure 9. The correlation between the level of AA and *ΔE** color (white flour type 480)

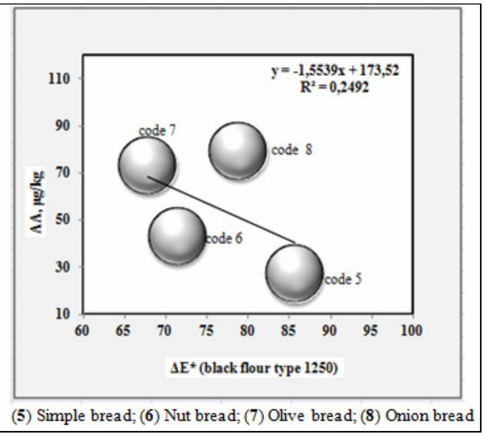

Figure 10. The correlation between the level of AA and *ΔE** color (white flour type 1250)

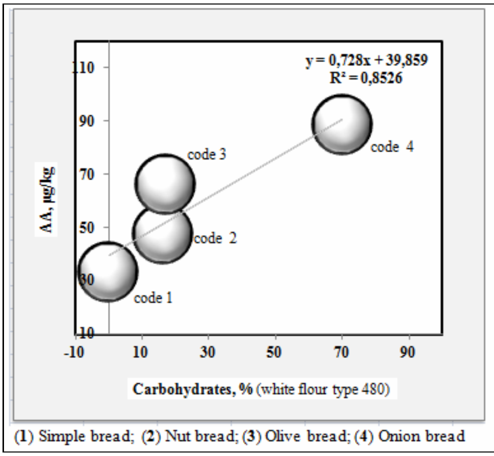

Figure 11. The correlation between the level of AA and carbohydrate content (white flour type 480)

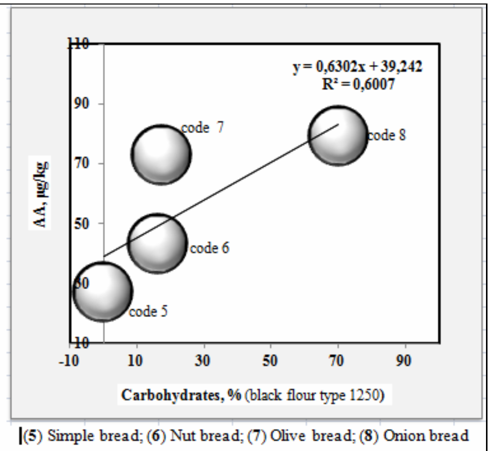

Figure 12. The correlation between the level of AA and carbohydrate content (white flour type 1250)

According to the results, the level of AA varieties of bread does not correlate with the total color difference, *ΔE**, of the correlation factors obtained, R, is less than 0.90. Added ingredient in breads may determine, on the one hand, changing color samples, because pigments in its constitution, on the other hand, may increase the level of AA, due to the intake of carbohydrates that brings in the recipe manufacturing. All tested bread samples were realized in the same processing conditions. The highest AA content was obtained for bread sample with onion, the ingredient having the highest carbohydrates content. For all samples the acrylamide content was correlated with the carbohydrates level, R = 0,9234 for white flour and R = 0,7750 for black flour and not with their color (R = 0,2981 for white flour and R = 0,4992 for black flour).

In terms of EU Recommendation 647 from 8 November 2013 (EC, [13]), bread samples with onion, obtained from white flour had a higher acrylamide level (88.5 µg/kg) than 80 µg/kg value, recommended for this type of food matrix.

4. Conclusions

The results showed lower values of acrylamide content for both types of *Simple bread* samples while the highest values of acrylamide content were obtained for *Onion bread* samples.

Acrylamide levels increase of about 1.43 to 2.7 times in bread samples containing added ingredients (onion, olives, and nuts) which can be explained by the carbohydrate content of these ingredients, considered being the main precursor in the acrylamide formation. Acrylamide level was correlated with the carbohydrate content of these ingredients and doesn't correlates with the final product color.

This paper represents a valuable effort in acrylamide content assessment for a category of products with a significant contribution in the consumer's diet.

5. Acknowledgements

This study was supported by the Ministry of National Education – State Authority for Scientific Research, Technological Development and Innovation, by Nucleu Programme PN 12 48, contract 48 N/2012.

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