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Influence of some factors on the acrylamide content in bread

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Abstract

It was studied the influence of flour type, of weight of piece of dough and of baking time on the content of acrylamide in bread assortments made within Pilot Plant. For all bread assortments were evaluated CIELab parameters, L, a*, b*.*
Separation, detection, identification and quantification of derivative compound of acrylamide, 2-bromopropenamide (2-BPA), are achieved by GC/MS/MS-SRM, by using method of the internal standard of labelled acrylamide (1,2,3-^{13}C), min. 99% purity.
In the case of experimental samples, both for samples with lower grammage as well as for samples with higher grammage, the use of white wheat flours type 480 lead to formation of a higher quantity in acrylamide (28.52 - 33 µg/kg), compared to the use of black wheat flour type 1000 (22.10 – 26.33 µg/kg) and type 1250 (19.21 - 26.92 µg/kg), respectivelly. Increasing the baking time with 10, 20 minutes, increased the acrylamide content in bread, from 16.60 µg/kg to 18.80 µg/kg, and to 21.48 µg/kg, respectively.

Keywords: acrylamide, baking time, bread, CIELab parameters, flour, GC/MS/MS

1. Introduction

Acrylamide has been classified in 1994 by the International Agency for Research on Cancer as "probably carcinogenic for humans" (Group 2A), having toxicological properties, as: neurotoxicity, genotoxicity, carcinogenity and toxicity for reproduction. Acrylamide is an α, β-unsaturated carbonyl compound (CH2=CHCONH2), with electrophilic reactivity and can react with nucleophile groups of some biological compounds generating compounds with toxic effects (IARC, [1]).

According to experiments on animals, acrylamide is rapidly absorbed mainly on gastrointestinal tract after oral administration and is transmitted to all organs and tissues. In the body, acrylamide is metabolized to glycidamide in the presence of cytochrome P450. Formation of glycidamide is considered as a critical step for genotoxic effects of acrylamide, with the potential to induce mutagene genes at chromosome level. Acrylamide and glycidamide can react with macromolecules, such as: hemoglobin, DNA, serum albumin and enzymes, forming adducts. The formation of DNA adducts is such to confirm the toxic, carcinogenes potential of acrylamide (PAULSSON *et al*. [2], EFSA Scientific Colloquium [3], FAO/WHO [4]).

Acrylamide is predominantly formed when the food are subjected to technological processes of roasting, baking, at temperatures higher than 120^{0}C, being the result of reaction between the amino group of the asparagine and a carbonyl group of a compound derived from carbohydrates (mainly, glucose, fructose and maltose). This reaction, known as Maillard reaction, gives to food, on the one hand, the corresponding color and flavor, and on the other hand, leads to the formation of this undesirable compound, acrylamide.

Foods with the highest contribution to the intake of acrylamide, vary from country to country, depending on national food habits and food preparing methods. In general, potato-based products, coffee and bakery products are the major sources of acrylamide.

Basic foods, such as bread, contain acrylamide in very small quantities, but still can contribute considerably to Romanian consumer's daily diet. Considering that in Romania the daily bread consumption for an adult weighing 80 kg is 300 g/day and given that bread has a mean acrylamide concentration of 30 µg/kg, it can be deduced theoretically that in our country only bread contributes with 14% to 37.5% at the maximum allowed dose, for foods, in daily diet estimated by FAO/WHO (0.3 – 0.8 µg acrylamide/kg body/day) (FAO/WHO [4-5]).

The aim of this paper was to determine the influence of the flour type, the weight of piece of dough and the baking time on acrylamide content in some bread assortments, experimentally obtained. Also for all bread assortments were evaluated CIELab parameters, *L*, a*, b**, using HunterLab colorimeter.

2. Materials and methods

In experiments there were used bread assortments made within the Pilot Experiments Plant for Cereals and Flours Processing of INCDBA – IBA Bucharest.

In the experiments performed there were used wheat flour with different rate of extraction: type 480, 550, 1000 and 1250.

The experimental variants were achieved by variation of the wheat flour type, expressed as rate of extraction, the weight of the piece of dough and the baking time on the acrylamide content in different bread assortments:

a) Wheat flour type and weight of piece of dough

For the experimental samples were used 3 wheat flour types: type 480, 1000 and 1250.

In Romania, based on the mineral content, it is defined *flour type,* which means mineral content (ash), expressed in percentages at dry matter, multiplied by 1000 (BORDEI [6], SR 877-1:2002 [7]).

Dough preparation of the experimental samples consisted in the following main operations: dosing of the raw materials and materials, mixing, fermentation, division and the final fermentation of the dough. There were done 6 bread assortments according to Table 1. All ingredients were mixed in a mixer of low capacity for 10 minutes. The obtained dough was fermented in a proofer at temperature 30^{0}C, for 90 minutes. Dough was divided in pieces of 100 g (bread sticks) and 460 g (bread), respectively.

Table 1. Experimental variants of the bread assortments

No.	Ingredients	Bread assortments					
		Wheat flour type 480		Wheat flour type 1000		Wheat flour type 1250	
		Bread stick (code 9)	**Bread** (code 1)	**Bread stick** (code 19)	**Bread,** (code 11)	**Bread stick** (code 23)	**Bread** (code 21)
1.	Flour (kg)	2.00	2.00	2.00	2.00	2.00	2.00
2.	Yeast (kg)	0.06	0.06	0.04	0.04	0.03	0.03
3.	Salt (kg)	0.03	0.03	0.03	0.03	0.03	0.03
4.	Water (l)	1.20	1.20	1.20	1.20	1.20	1.20

To obtain those 6 bread assortments there were used specific equipment of pilot plant: *oven with two overlapping hobs, with controlled baking temperature and time* (Mondial Forni, Mod. 4T – 40/60 UT25 + VAP), *dough mixer* (Diosna, DM 08 – 4/6). Baking parameters for the bread assortments experimentally obtained were: 235^0C, 40 minutes – for bread and 220^0C, 40 minutes – for bread sticks.
Experimental samples were done in 3 batches x 6 variants (3 flour types x 2 assortments). For the acrylamide analysis were used the average samples of those 3 batches.

b) Baking time

Bread experimental variants were done with wheat flour type 550. Dough were prepared according to the main operations specified at pct. a., with difference that after fermentation the dough was divided in pieces of 650 g weight.
It was studied the influence of the baking time on the acrylamide content in product "Pan bread", in variants:

- **V1** – baking time, t_1 = 40 minutes, baking temperature 235^0C
- **V2** – baking time, t_2 = 50 minutes, baking temperature 235^0C
- **V3** – baking time, t_3 = 60 minutes, baking temperature 235^0C

Used methods Wheat flours and bread assortments used within the experiments were physical-chemical analyzed, using the following methods:

- moisture determination, according to SR 90:2007
- lipids content determination, according to SR 91:2007
- protein content determination, according to SR EN ISO 20483:2007
- ash content determination, according to SR EN 2171:2010
- salt content determination, according to SR 91:2007
- acidity determination, according to SR 90:2007
- sugar content determination, according to SR 91:2007
- determination of crude fiber, according to SR EN ISO 6865:2002
- acrylamide determination by GC/MS/MS (internal procedure)
- color measurements (CIELab, *L**, *a** and *b**, HunterLab colorimeter)

Determination of acrylamide

Acrylamide in bread was quantified by GC/MS/MS-SRM, after derivatization, using the preparation methods, adapted by Saturo *et al*. [8], Pittet *et al*. [9], Wei-Chih *et al*. [10], with modifications. Concentration of acrylamide for each bread assortment was expressed in μg/kg and represents the arithmetic mean of the obtained results by processing at least three parallel samples. Preparation of the bread assortments in order to determine the acrylamide level was made according to the working protocol presented by NEGOITA *et al*. [11]).

Color measurements of bread samples Measurement of color parameters of dried bread crumb was performed at room temperature, with HunterLab colorimeter, using programme Universal Software V4.01 Miniscan XE Plus, with recording of the CIELab parameters (the Commission Internationale de l'Eclairage - CIE), *L**, *a** and *b**. In this color space, value *L** represents Lightness (0 is black, and 100 is white); value *a** represents red-green coordinate (a positive a* value indicates redness and a negative a* value indicates greenness); value *b** represents yellow-blue coordinate (a positive b* value indicates yellowness and a negative b* value indicates blueness). Total color difference for two samples (control and standard), ΔE^* was calculated with equation: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. For each sample there were made measurements in 10 different points, then being achieved their average. Dried bread crumb was obtained by fine milling of bread samples, dried in etuve for 120 minutes at temperature 90°C.

3. Results and discussions

a) *Wheat flour type and weight of piece of dough*

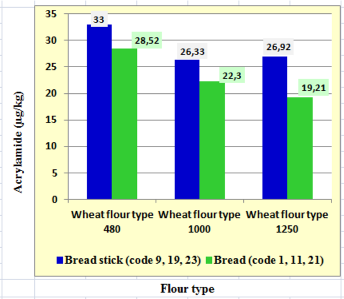
Physical-chemical characteristics of the wheat flours used to make the experimental samples are presented in the table 2.

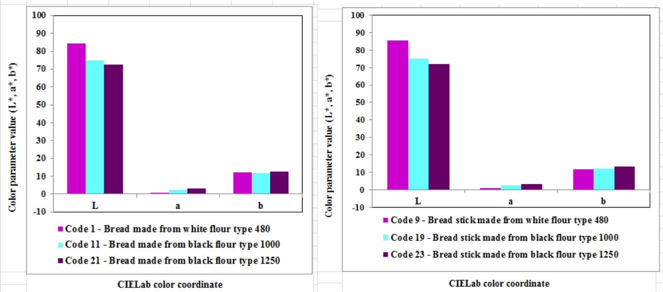
Table 2. Physical-chemical characteristics of wheat flours

Physical-chemical characteristics	Flour type		
	White flour	Black flour	
	480	1000	1250
Moisture (%)	12.71	11.30	11.10
Ash (% d.m.)	0.48	1.08	1.27
Protein (% d.m.)	11.5	13.06	13.70
Lipids (% d.m.)	1.01	1.97	2.09
Sugars (% d.m.)	73.6	71.78	70.61
Reducing sugars (% d.m.)	0.7	1.12	1.3
Cellulose (% d.m.)	0.7	0.81	1.23
Acidity (degrees)	2.8	5	5.5
Color	Yellowish-white	Yellowish-white with faint gray tint and visible traces of bran	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		

Plotting in fig. 1 the concentration of acrylamide in bread assortments depending of the used flour type and the weight of piece of dough, it is noted that both for "Bread" assortments and "Bread sticks" assortments made from white flour type 480, the level of acrylamide formed is higher than about 1.3 times (28.52 µg/kg for bread and 33 µg/kg for bread sticks, respectively) compared to bread assortments obtained from black flour type 1000 (22.10 µg/kg for bread and 26.33 µg/kg for bread sticks, respectively) and 1.2 – 1.5 times higher compared to bread assortments made from black flour type 1250 (19.21 µg/kg for bread and 26.92 µg/kg for bread sticks, respectively).

In terms of weight of divided dough, baking of pieces of dough with lower weight, "Bread sticks" - 100 g, than baking of pieces of dough with higher weight, "Bread" - 460 g, lead to a higher acrylamide level. Baking temperature of "Bread sticks" assortments was lower with 15^0C than "Bread" assortments, and the baking time was the same, 40 minutes.





(a) **(b)**

Figure 1. Variation of acrylamide level of the assortments of bread

Figure 2. CIELab parameters of the assortments of bread

From the assortments colour point of view (Fig. 2) made with different wheat flours, both "Bread stick" assortments and "Bread" assortments, made with wheat flour type 480, recorded higher values of luminance (code 9 - L^* = 85.68; code 1 - L^* = 84.52), lighter versus the values recorded of samples done from wheat flour type 1000 (code 19 - L^* = 75.13; code 11 - L^* = 75.03), and wheat flour type 1250 (code 21 - L^* = 72.46, code 23 - L^* = 71.97), respectively.

From the point of view of weight of divided dough (Fig. 2a), "Bread" assortments shown color parameters almost similar to those of "Bread stick" assortments (Fig. 2b).

According to the Code of Practice for the Reduction of Acrylamide in Foods (CAC/RCP 67-2009, [12]), it forms a level of acrylamide higher at cereal-based products, when there are used flours with higher extraction degree (higher ash content). In the case of the bread assortments experimentally obtained, when using black flour type 1250 (ash content 1.27% d.m.), so a higher extraction degree, the acrylamide level was lower compared to the assortments of bread obtained from white wheat flour type 480 (ash content 0.48% d.m.).

The data obtained are not consistent with the data given in the literature, according to which products obtained from flours with lower extraction rate (which have a lower content of asparagine than flours with high extraction rate) have a lower acrylamide content.

Taking into account that the flours used in experiments are not from the same cultivar of wheat and between the asparagine content of native wheat cultivar from which is the flour used for obtaining the analysed products and the acrylamide content of the obtained products, can intervene also the different quantity of compressed yeast used in experiments: 60 g yeast/kg flour type 480, 40 g/kg flour type 1000 and 30 g/kg flour type 1250. Compressed yeast used in experiments represented yeast cells + nutrient medium by which we can have a direct contribution of asparagine to experiment, given that the majority of culture media used for the growth of yeast use this ingredient, with ammonium salts as nitrogen source. Using of different amounts of yeast on a culture medium initially not evaluated in terms of asparagine content, makes impossible any resonable assessment of the initial amount of asparagine in flour, based on the acrylamide content in the final product.

In this respect, research will continue, on the one hand, by achievement of bread assortments made with wheat flours, originating from the same wheat cultivar, by analysis of flours in terms of asparagine content, and, on the other hand, by using of equal amounts of compressed yeast for all used types of flours.

a) *Baking time*

Bread samples (Fig. 3) obtained in those 3 experimental variants were analysed from point of view of water content, formed acrylamide level and CIELab parameters.

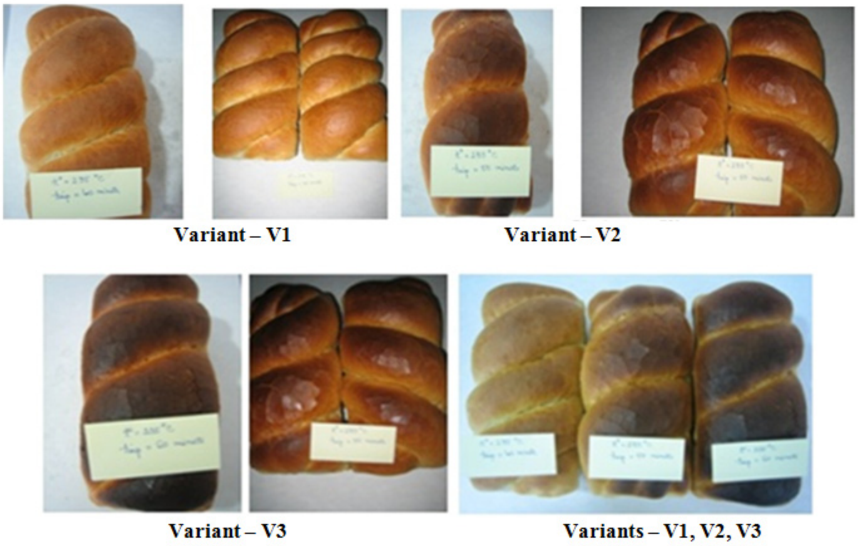
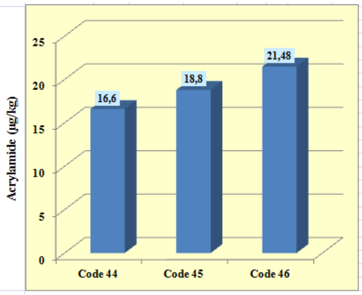


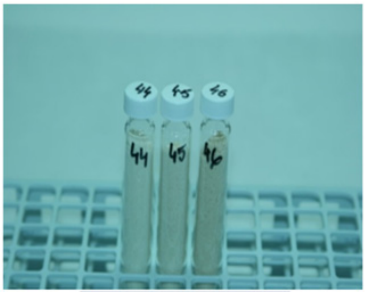
Figure 3. Bread assortments of experimental variants

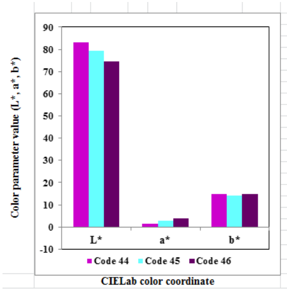
It has been shown that, at baking of "Pan bread" product, by maintaining a constantly baking temperature (235^{0}C) and varying the baking time, 40 minutes, 50 minutes, and 60 minutes, it was obtained an increase of acrylamide content in bread (Fig. 4).
Baking conditions resulted in distinct changes of crust color (Fig. 3) and, consequently, of the end product obtained by drying and milling (Fig. 5).
Changes of product color in those 3 experimental variants can be observed from measurements of parameters L^*, a^*, b^*. The results obtained are shown in figure 6.
Calculating the difference in the color of bread assortments, code 45 (time 50 minutes) and code 46 (time 60 minutes), to sample code 44 (time 40 minutes) considered standard, it obtained:

- ΔE^*_{ab} **= 4.06** (sample code 45 with sample code 44)
  - $\Delta L^* < 0$, sample code 45 is darker than standard (code 44)
  - $\Delta a^* > 0$, sample code 45 is redder than standard (code 44)
  - $\Delta b^* < 0$, sample code 45 is bluer than standard (code 44)
- ΔE^*_{ab} **= 8.79** (comparing sample code 46 with sample code 44)
  - $\Delta L^* < 0$, sample code 46 is darker than standard (code 44)
  - $\Delta a^* > 0$, sample code 46 is redder than standard (code 44)
  - $\Delta b^* > 0$, sample code 46 is yellower than standard (code 44)

The greatest difference in colour compared to the standard assortment is obtained from the sample code 46, wherein was the longest the baking time (60 minutes).
Comparing the acrylamide level formed in those three variants (Fig. 4), with the color parameter, L^* (Fig. 6), it can say that a lower acrylamide level (16.60 µg/kg) and lower baking time (40 minutes) results in a lighter color, hence a higher luminance, L^* = 83.05 (code 44), and a high acrylamide level (21.48 µg/kg), longer baking time (60 minutes) results in a darker color, so a lower value of luminance, L^* = 74.53 (code 46).







Code 44 - Pan bread, 235°C, 40 min., Code 45 - Pan bread, 235°C, 50 min., Code 46 - Pan bread, 235°C, 60 min.

Figure 4. Influence of baking time on the acrylamide level in bread

Figure 5. Dried bread crumb obtained in experimental variants

Figure 6. CIELab parameters in experimental variants

4. Conclusions

This paper is, despite some innaccuracies, a valuable effort to assess the acrylamide content in a product category with a significant contribution in the daily diet of the Romanian consumers.

In the case of bread assortments obtained experimentally, from different types of wheat flours, the acrylamide level formed in bread obtained from wheat flour type 480 was higher by 48.46% than those of bread obtained from wheat flour type 1000 and by 29.04% than those of bread obtained from wheat flour type 1250.

In the case of the bread assortments with lower grammage it obtain a higher acrylamide level (33 – 26.92 µg/kg) in comparison with bread assortments with higher grammage (28.52 - 19.21 µg/kg), in all cases of the used flours.

Achievement of bread assortments with the same manufacturing recipe, the same baking temperature but with increasing of baking time with 10 minutes, and 20 minutes, respectively, lead to an increasing of the acrylamide level of 1.1 times, and 1.3 times, respectively.

The research will continue, on the one hand, by making breads obtained of wheat flours originating from the same wheat cultivar and, on the other hand, by analyzing of flour in terms of the content of asparagine.

The results showed that the type of flour is not always the main factor in the formation of acrylamide in bread, demonstrating that in the formation of acrylamide are involved also other chemical compounds.

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