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RESEARCH REGARDING THE INFLUENCE OF DOUGH PREPARATION PROCESS ON THE ACRYLAMIDE LEVEL IN BREAD

Mioara NEGOIȚĂ[1], Gabriel MUSTĂȚEA[1,2], Enuța IORGA[1], Alina ADASCĂLULUI[1], Giuseppe SPADARO[1], Nastasia BELC[1], Luminița CATANĂ[1], Andreea STAN[2]

[1]National R&D Institute for Food Bioresources, 6 Dinu Vintilă Street, 021102, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine, 59 Marasti Blvd., 011464, Bucharest, Romania
Corresponding author: mioaranegoita@yahoo.com

Abstract

The influence of the dough preparation process on the acrylamide level formed in different bread types was studied. For each preparation process – direct (PD) and indirect (PI), four types of bread: "Pan bread", "Simple bread stick", "Olive bread stick" and "Onion bread stick" were obtained.Water content, acrylamide level and chromatic parameters, CIELab (L, a*, b*) for each type of bread were measured. Acrylamide analysis was performed by GC/MS/MS-SRM, by using the internal standard method of labelled acrylamide (1,2,3-^{13}C), min. 99% purity.The results show that, acrylamide level obtained for the bread samples prepared through PI process is lower than the acrylamide level obtained for the bread samples prepared through PD process. Preparation of dough through PI process decreases the acrylamide level, in all tested samples, with 6.29% to 31.63% compared with the PD process.Regarding color parameters (L*, a*, b*), samples obtained through both PD and PI process, had similar variations. Also it was found no correlation between CIELab color parameters and the level of acrylamide.*

Key words**:** *acrylamide, bread, process, GC/MS/MS.*

INTRODUCTION

Since 1994, International Agency for Research on Cancer (IARC) classified acrylamide as potential carcinogen to humans (group 2A), and in 2001, Scientific Committee on Toxicity, Eco-toxicity and Environment demonstrated health risks and toxic properties of acrylamide such as neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity (IARC, 1994; Dybing et al., 2003; Wilson et al., 2006; Keramat et al., 2011).

In April 2002, scientists from National Food Administration in Sweden along with scientists from Stockholm University have raised fears, discovering that people consume acrylamide through their diet by eating common foods, such as bread, biscuits, chips, coffee, etc., at levels much higher than the dose allowed in drinking water (SCF, 2002). Their results were quickly confirmed by working groups consisting of experts from the World Health Organization, Food and Agriculture Organization, National Center for Food Safety and Technology (FAO/WHO, 2002).

In February 2005, The Joint FAO/WHO Expert Committee on Food Additives (JECFA) made an assessment of acrylamide in terms of food safety concluding its risks to human health and the need for efforts to be done in order to reduce the exposure to acrylamide (JECFA, 2005).

Acrylamide content in food varies from one type to another, depending on the raw material and technological process (343 mg/kg in cereals and cereal-based products, 477 mg/kg in potatoes and processed potato products, 509 mg/kg in coffee and green tea, 19 mg/kg in meat and offal, 17 mg/kg in dehydrated fruits and vegetables). In most countries, food products that contribute to acrylamide intake by dietwere: French fries (16-30%), potato chips (6-46%), coffee (13-39%), bakery products (10-30%), and

confectionery products (10-20%) (Svensson et al., 2003; Konings et al., 2003; Hamlet et al., 2005; Amrein et al., 2007; Olmez et al., 2008).

Processing conditions, such as fermentation time, baking time, and temperature as well as food matrix also influence formation and reduction of acrylamide.

This paper aim was to investigate the influence of dough preparation process (direct and indirect) on acrylamide content in several bread types.

By using direct process for dough preparation in obtaining bread processes like activation and adaptation of yeast to dough environment, yeast cell multiplication, lactic acid accumulation through lactic fermentation and flavor substances accumulation through alcoholic and acid fermentation are determined.

Obtained products were analyzed regarding CIELab chromatic characteristics in an attempt to correlate the color of products with acrylamide content.

MATERIALS AND METHODS

Dough preparation is one of the key steps in bakery products technological process. The quality of dough, after mixing and fermentation directly influence the quality of final products.

Two types of dough preparation processes were used: direct or mono-phase process (dough) – PD and indirect or bi-phase process (sponge – dough) – PI. For each process were obtained four types of products: "Pan bread", "Simple bread stick", "Olive bread stick" and "Onion bread stick" using the same 480 wheat flour type.

The main operations in dough preparation process were ingredient selection and scaling, mixing and fermentation.

Direct process (PD) of dough preparation consisted in mixing and kneading, in a single step, of all the raw and auxiliary materials: 2 kg flour, 300 mL sodium chloride solution (10% w/v), 400 mL of fresh yeast emulsion (15% w/v) and 590 mL of water. All components were mixed in a mixer for 10 minutes.The obtained dough was left in a fermenting room at a temperature of 30°C for 90 minutes. After fermentation time ended, corresponding ingredients were added: divided black olives and dehydrated onion, rehydrated in advance. Each type of bread was kneaded again for another 5 minutes. Dough was divided in pieces of 110 grams each for bread sticks and 600 grams for pan bread. Divided dough was properly modeled and placed in rectangular trays (20 mm x 100 mm) and were left in a steam fermenting room at 30°C and 85% relative humidity for 30 minutes (bread stick) respectively 45 minutes (pan bread). Bread types were baked in an oven with two overlapped chambers, with controlled temperature and time. "Bread sticks" were baked at 220°C for 40 minutes, while "Pan bread" was baked at 230°C for 40 minutes.

Indirect process (PI) of dough preparation consisted insponge preparation, which was then used to prepare the dough. Sponge preparation role is to obtain a favorable environment for both yeast cells propagation and fermentation for several compounds production: primarily lactic acid, which helps to improve dough characteristics and also taste and flavor of bread. Sponge was prepared using flour, water and yeast. Flour amount used was half of total amount of flour used for dough preparation. Flour: water ratio was approximately 2:1. All yeast amounts were used to obtain the sponge which was left for 2 hours at 30°C to ferment. After fermentation, remaining flour was added together with water and salt and then was mixed for about 10 minutes. Obtained dough was transferred in a fermenting room for 45 minutes at 30°C. After fermentation time was completed corresponding ingredients were added: divided black olives and dehydrated onion, rehydrated in advance. Each type of bread was kneaded again for another 5 minutes. Dough was divided, modeled and baked in the same way as in direct process.

Experimental samples obtained both through direct and indirect processes are presented in Table 1. Samples were obtained in 3 batches of 4 types prepared by 2 processes. For acrylamide analysis was used a homogenous sample from all 3 batches.

Table 1. Experimental variants for obtainingbread through direct and indirect process

Sample name	Ingredients, kg	Process type						
		Direct process (PD)			Indirect process (PI)			
		Dough preparation		Fermentation time, min.	Dough preparation			Fermentation time, min.
		Dough	Total	Dough/ Final fermentation	Sponge	Dough	Total	Sponge/ Dough/ Final fermentation
Pan bread (code 1 – PD) (code 2 – PI)	Flour	2.0	2.0	90/45	1.0	1.0	2.0	120/45/45
	Yeast	0.06	0.06		0.05	-	0.05	
	Salt	0.03	0.03		-	0.03	0.03	
	Water	1.20	1.20		0.6	0.6	1.20	
Simple bread stick (code 9 – PD) (code 10 – PI)	Flour	2.0	2.0	90/30	1.0	1.0	2.0	120/45/40
	Yeast	0.06	0.06		0.05	-	0.05	
	Salt	0.03	0.03		-	0.03	0.03	
	Water	1.20	1.20		0.6	0.6	1.20	
Olive bread stick (code 5 – PD) (code 6 – PI)	Flour	2.0	2.0		1.0	1.0	2.0	
	Yeast	0.06	0.06		0.05	-	0.05	
	Salt	0.03	0.03		-	0.03	0.03	
	Water	1.2	1.2		0.6	0.6	1.20	
	Olives	0.24	0.24		-	0.24	0.24	
Onion bread stick (code 7 – PD) (code 8 – PI)	Flour	2.0	2.0		1.0	1.0	2.0	
	Yeast	0.06	0.06		0.05	-	0.05	
	Salt	0.03	0.03		-	0.03	0.03	
	Water	1.2	1.2		0.6	0.6	1.20	
	Onion	0.24	0.24		-	0.24	0.24	

Equipment. Bread samples were obtained by using the following equipments: oven with two chambers and controlled time and baking temperature (Mondial Forni), dough mixer (Diosna), manual divider (Vitella), molding machine (Kohler). For acrylamide analysis a gas chromatograph (Trace GC Ultra) coupled with a triple quadruple mass spectrometer (TSQ Quantum XLS) purchased from Thermo Fisher Scientific, USA 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.

Acrylamide was analyzed using an internal GC/MS/MS method (Negoita et al., 2014; Negoita et al., 2015) adapted after Pittet et al., 2004; Nemato et al., 2002; Cheng et al., 2006.

Acrylamide concentration was calculated with the following formula:

$$C = [440 \cdot C_0 \cdot (100 - U1)]/[w \cdot (100 - U_2)] \ \mu g/kg \ bread$$

where:

440 Total sample volume extract (400 µL ethyl acetate + 40 µL triethylamine), µL

C_0 Acrylamide concentration measured by GC/MS/MS fromfood matrix, mg/L

$$[C_0 = \frac{1}{b} * (\frac{A_{aaN}}{A_{aaM}} * F_{ISTD} - a)]$$

w Amount of breadcrumbs used, g

U_1 Fresh matrix humidity, %

U2 Breadcrumbs humidity, %

b Calibration curve slope

A_{aaN} Area of corresponding acrylamide signal of food matrix

A_{aaM} Area of corresponding acrylamide signal of internal standard

$\frac{A_{aaN}}{A_{aaM}}$ Ratio between analyte signal area and internal standard signal area is defined as response factor corresponding to the

analyte from food matrix

F_{ISTD} Correction factor of internal standard, defined as ratio of matrix internal standard concentration and calibration internal standard concentration

a Intercept

Color evaluation of samples was made at room temperature, using a HunterLab colorimeter and Universal Software V4.01 Miniscan XE Plus. The CIELab'76 parameters were evaluated:

L* - Color luminance: 0 – black and 100 – white

a* - red-green 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.

RESULTS AND DISCUSSIONS

Raw and auxiliary materials were analyzed regarding physico-chemical characteristics. The results are presented in Table 2.

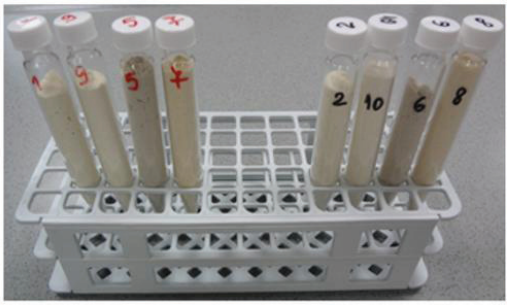
Table 2. Physico-chemical characteristics of raw and auxiliary materials

Characteristic	White flour, type 480	Sliced black olives	Dehydrated onion
Moisture, %	12.71	55.0	14.5
Ash, %	0.42	-	-
Proteins, %	10.04	2.4	10.5
Lipids, %	0.88	22.8	1.6
Carbohydrates, %	64.24	17.2	70.0
Fiber, %	0.61	-	-
Acidity, grades	2.44	-	-

All fourth bread types: "Pan bread", "Simple bread stick", "Olive bread stick", "Onion bread stick", obtained through both processes, were analyzed regarding acrylamide content and chromatic characteristics *CIELab*. Obtained samples (Figure 1) were transformed in breadcrumbs (Figure 2) by drying and grinding.

Pan bread (code 1 and 2) | **Simple bread stick** (code 9 and 10) | **Olive bread stick** (code 5 and 6) | **Onion bread stick** (code 7 and 8)

Figure 1. Experimental samples



1 - Pan bread **(PD)**
9 - Simple bread stick **(PD)**
5 - Olive bread stick **(PD)**
7 - Onion bread stick **(PD)**
2 - Pan bread **(PI)**
10 - Simple bread stick **(PI)**
6 - Olive bread stick **(PI)**
8 - Onion bread stick **(PI)**

Figure 2. Breadcrumbs of experimental bread samples

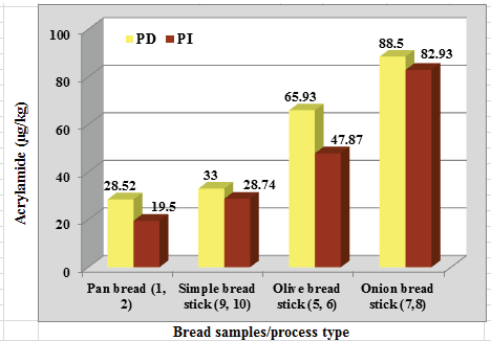


Figure 3. Acrylamide content in bread samples obtained by direct and indirect process

In Figure 3 are presented the results for acrylamide analysis in obtained bread samples.
Obtained results are according to other international researches (Fridriksson et al., 2004; CAC/RCP, 2009) which showed that a long time for dough fermentation induce a lower acrylamide level. This can be explained by the fact that both asparagine content and carbohydrates, main precursors in forming acrylamide, are consumed during fermentation. Asparagine is the source for nitrogen nutrition of yeasts while carbohydrates represents carbon source for yeasts growth and development.
Both samples obtained by direct (PD) and indirect process (PI) showed the same variations of the *CIELab* color parameters (Figure 4).

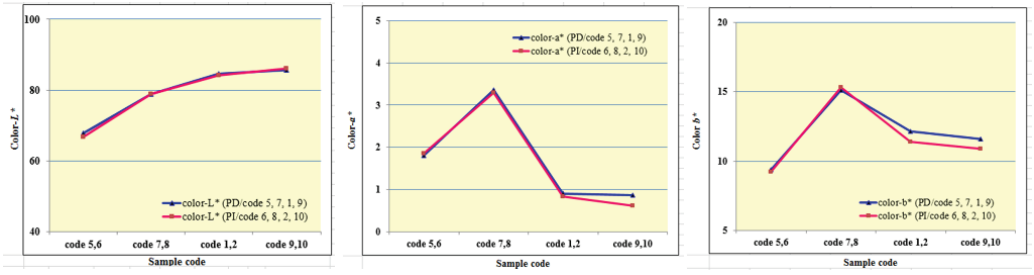


Figure 4. CIELab color parameters of bread types obtained by direct (PD) and indirect process (PI)

"Simple bread stick"(code 9, 10) and "Pan bread"(code 1, 2) samples, obtained by both processes, had the lowest acrylamide content (19.5 and 33 µg/kg) and the highest luminance values (84-86). In the case of "Olive bread stick", obtained by both PD and PI, acrylamide level do not correlate with their color. By evaluation of acrylamide level of bread samples obtained both direct (PD) and indirect process (PI) (Figure 3) with *CIELab* color parameters (Figure 4) it was found no correlation between those two.

CONCLUSIONS

Obtained results revealed that a longer fermentation of the dough, determines lower levels of acrylamide concentration. Thus, in the 4 experimental breads obtained by indirect process (PI) the acrylamide content was with 6.29% to 31.63% lower compared to samples obtained by direct process (PD).
Regarding color parameters, *L*, a*, b**, bread samples obtained through both direct and indirect process had the same variations.
There is no correlation between the acrylamide level of the achieved samples and their *CIELab* color parameters.″Simple bread

stick", "Pan bread" and "Onion bread stick" samples had highest luminosity values, while "Olive bread"samples had the lowest luminosity value, due to the main ingredient: olives.

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REFERENCES

Amrein T. M., Andres L., Escher F., Amado R., 2007. Occurrence of acrylamide in selected foods and mitigation options. Food Additives and Contaminants, 24, 13–25.

Cheng W. C., Hsiao S. W., Chou S. S., Sun-Hwang L., Lu T. J., Yeh A. I., 2006. Determination of Acrylamide in Chinese Foods by GC-Ion Trap MS Using 2-Bromopropenamide and 2-Bromopropenamide-^{13}C3,Journal of Food and Drug Analysis, 14(2), 207-214.

Dybing E., Sanner T., 2003. Risk assessment of acrylamide in foods. Toxicological Sciences, 75, 7-15.

Fredriksson H., Tallving J., Rosen J., Aman P., 2004. Fermentation reduces free asparagine in dough and acrylamide content in bread. Cereal Chemistry, 81(5), 650–653.

Hamlet C. G., Baxter D. E., Sadd P. A., Slaiding I., Liang L., Muller R., 2005. Exploiting process factors to reduce acrylamide in cereal-based foods. Report prepared on behalf of the U.K. Food Standards Agency, RHM Technology, High Wycombe, UK.

Keramat J., LeBail A., Prost C., Jafari M., 2011. Acrylamide in Baking Products: A Review Article, Food Bioprocess Technol., 4, 530–543.

Konings E.J.M., Baars A. J., Van Klaveren J. D., Spanjer M. C., Rensen P. M., Hiemstra M., Van Kooij J. A., Peters P. W. J., 2003. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food and Chemical Toxicology, 41, 1569–1579.

Negoita M., Catana M., Iorga E., Catana L., Adascalului A., Belc N., 2014. Determination of Acrylamide in Bread by Gas Chromatography – Tandem Mass Spectrometry, Romanian Biotechnological Letters, 19(4), 9561-9568.

Negoita M., Adascalului A., Iorga E., Catana L., Catana M., Belc, N., 2015. Internal validation of the method for determination of acrylamide in bread by gas chromatography tandem mass spectrometry, Revista de Chimie, 66(4), in press.

Nemato S., Takatsuki S., Sasaki K., Maitani T., 2002. Determination of Acrylamide in Foods by GC/MS Using ^{13}C-labeled Acrylamide as an Internal Standard, Journal of the Food Hygienic Society of Japan, 43(6).

Ölmez H., Tuncay F., Özcan N., Demirel S., 2008. A survey of acrylamide levels in foods from the Turkish market. Journal of Food Composition and Analysis, 21, 564–568.

Pittet A., Perisset A., Oberson J.M., 2004. Trace level determination of acrylamide in cereal-based foods by gas chromatography–mass spectrometry. Journal of Chromatography A, 1035, 123–130.

Svensson K., Abramsson L., Becker W., Glynn A., Hellenäs K.E., Lind Y., Rosén J., 2003. Dietary intake of acrylamide in Sweden. Food and Chemical Toxicology, 41, 1581-1586.

Wilson K. M, Mucci L. A., Cho E., Hunter D. J., Chen W. Y., Willet W. C., 2006. Dietary acrylamide intake and risk of premenopausal breast cancer. American Journal of Epidemiology, 169: 954-961.

***CAC/RCP 67-2009. (Code of Practice for the reduction of acrylamide in foods).

***FAO/WHO. Consultation on the Health Implications of Acrylamide in Food Geneva, 25-27 June (2002).

***IARC (International Agency for Research on Cancer), Lyon, France, 1994. Some industrial chemicals. IARC Monographs on the Evaluation of Carcinogenic Risk for Chemicals to Humans, vol. 60, p. 435.

***JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2005. Sixty-fifth meeting, Geneva.

*** SCF (Scientific Committee on Food), 2002. Opinion of the Scientific Committee on Food on new findings regarding the presence of acrylamide in food.