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INFLUENCE OF MANUFACTURING RECIPE AND BAKING CONDITIONS ON ACRYLAMIDE CONTENT IN BREAD

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Abstract

The aim of the study was to assess and correlate the acrylamide content, determined by GC-MS/MS, and colour parameters of bread samples prepared using different wheat flours, dough preparation process and baking conditions (230, 240°C/40, 50 min). Our results showed that, regardless of the preparation process and baking conditions, the acrylamide content in bread varied under the effect of flour type. Refined wheat flour bread showed lower acrylamide levels (6.42-12.02 µg/kg) compared to whole-wheat flour bread (9.57-33.04 µg/kg). Acrylamide formation varied in response to dough preparation, ranging between 6.42-15.80 µg/kg for indirect process (IP), and 10.22-33.04 µg/kg for direct process (DP). With respect to the influence of baking conditions, a 10°C increase in baking temperature elevated the acrylamide content by up to 2.87-fold. Similarly, a 10 min extension of baking time resulted in higher acrylamide content (up to 1.30-fold). Moreover, strong negative correlations were observed between the acrylamide content and the L colour parameter of breads, with r= -0.7522 ÷ -0.8428 for DP, and -0.9796 ÷ -0.9724 for IP. Our findings revealed the lowest acrylamide content achieved in bread prepared using wheat flour, indirect process, and lower baking time and temperature.*

***Key words**: acrylamide, bread, flour type, manufacturing recipe, sourdough.*

INTRODUCTION

Bread is known to be one of the most consumed food products, being a basic food resource all over the world. Flour, water, yeast and salt are the basic ingredients in the manufacturing recipe of this food product. For breadmaking the following steps are followed: dough mixing, dough fermentation and baking.

During baking, the Maillard reaction also known as non-enzymatic browning reaction takes place between asparagine and reducing sugars, such as glucose and fructose. This reaction contributes to obtaining the organoleptic characteristics of food products but it is also responsible for acrylamide formation. Acrylamide is a chemical contaminant, formed in starchy products under conditions employing temperatures above 120°C and low moisture (EFSA, 2015). This compound was classified by the International Agency for Research for Cancer (IARC, 1994) as a “probable human carcinogen”.

Bread usually contains low levels of acrylamide (Andačić et al., 2020; Mihai et al., 2020). However, given its high consumption, bread is an important contributor to acrylamide dietary intake. As such, acrylamide mitigation in bread has received increased consideration in both industrial and domestic processing.

In this context, reports involving acrylamide content mitigation studies on bread samples included factors such as: flour selection (Capuano et al., 2009; Przygodzka et al., 2015), asparaginase addition (Capuano et al., 2009; Mohan Kumar et al., 2014; Ghorbani et al., 2019), amino acids addition (Capuano et al., 2009), using sourdough fermentation (Codină et al., 2021; Zhou et al., 2022), baking parameters modulation (Onacik-Gür et al., 2022), baking to a lighter colour endpoint of final product (Abdulazeez et al., 2023; Gunduz, 2023), antioxidants addition such as green tea extract (Onacik-Gür et al., 2022). The content can be reduced by recipe reformulation or by modifying the baking conditions.

Sourdough utilization to obtain bakery products is an old technique that can improve the physical, nutritional and sensorial characteristics of bread, also prolonging the product shelf life (Codină et al., 2021; Susman et al., 2021; Kezer, 2022).

This technique started to be reused lately due to the high interest for traditional products. Sourdough is obtained my mixing cereal flour and water which are fermented through lactic acid bacteria and yeasts (Lutter, Jõudu and Andreson, 2023). Sourdough confers to bread a special flavour as a result of volatile and non-volatile compounds formed during fermentation (Warburton et al., 2022). Through fermentation, the acidity increases as organic acids are formed, while the pH decreases, this mechanism being related to the acrylamide reduction in bread (Zhou et al., 2022). Also, during this fermentation process, the protein content is reduced, asparagine being consumed by yeast fermentation (Wang et al., 2017; Zhou et al., 2022), while sucrose, is transformed to glucose and fructose due to the invertase enzyme present in yeast (Nachi et al., 2018).

The aim of this study was to investigate the influence of processing conditions such as wheat flour (refined and whole-wheat), dough preparation method (direct and indirect processes), and baking parameters (temperature, time) on the acrylamide content and colour parameters of bread samples. Moreover, we evaluated the corresponding relationships between the resulting acrylamide content and colour parameters.

MATERIALS AND METHODS

Chemicals and solvents

A standard of native acrylamide of min. 97.7% purity, purchased from Dr. Ehrenstorfer (LGC Group, North Charleston, USA), and an internal standard of acrylamide of ≥ 98% purity, with labelled carbon atoms (1,2,3-13C), containing 100 ppm hydroquinone, purchased from Cambridge Isotope Laboratories (Andover, MA, USA), were used for acrylamide determination.

All chemicals, solvents and reagents used in experiments were of chromatographic purity. Solvents used (n-hexane, ethyl acetate, and methanol) in experiments were picograde for residue analysis and were purchased from LGC Promochem GmbH (Wesel, Germany).

For the solid-phase extraction (SPE), two cartridges from Biotage (Upsala, Sweden) were used: Isolute Multimode (1.000 mg, 6 ml), and Isolute ENV+ (500 mg, 6 ml).

Ingredients

For bread formulation, the main ingredient comprised 2 kg of either refined wheat flour (type 650) or whole-wheat flour (type 2200). The following raw materials were also used: *Saccharomyces cerevisiae* yeast (0.05/0.06 kg), salt (0.03 kg), and water (1.2 l).

Bread preparation

The breads assessed in the present study were prepared using two types of flour (wheat flour and whole-wheat flour), and two fermentation processes, direct and indirect.

Bread prepared through DP (natural fermentation)

Through the natural fermentation, without sourdough, all raw materials were mixed and kneaded in one step.

The dough preparation included the following main operations: dosing of raw materials, kneading and fermentation. When preparing the dough, a larger amount of yeast (0.06 kg) was used compared to sourdough fermentation, and the duration of the dough preparation cycle was shorter. Flour was mixed for 10 min in a small capacity blender (Diosna, DM 08 - 4/6) with 300 ml of 10% sodium chloride solution (w/v), 300 ml of 15% fresh yeast emulsion (w/v) and 600 ml tap water. Subsequently, the resulting dough was subjected to fermentation (Gima Forni fermenter, Mod. Forno Rotor 60-80 Elett, 50 kW) for 90 min at 30°C. The dough was then divided into pieces of 600 g, which were properly shaped, placed in rectangular trays (20 x 100 mm) and left for final proofing (steam proofers) at 30°C and 85% relative humidity for 45 min. The loaves of bread were baked in a rotary kiln oven with controlled temperature and baking time (Mondial Forni, Italy), at either 230°C or 240°C, for 40 min or 50 min.

Bread prepared through IP (sourdough fermentation)

In the case of IP, the sourdough was prepared prior to obtaining the dough. The scope of using sourdough fermentation is to ensure a proper environment for yeast cells multiplication, which will in turn loosen the dough during fermentation, as well as to achieve the formation of fermentation compounds, especially lactic acid; this will improve the properties of the dough and further contribute to the sensorial properties of the bread. Sourdough was prepared from flour, water and yeast. To obtain the sourdough, 50% of the total quantity of flour was used for the preparation of the dough. The ratio of the amount of flour to water was about 2:1. The amount of yeast used to obtain the sourdough was 100% of the total amount of yeast used (0.05 kg). The resulting sourdough was left to ferment for 2 h at 30°C. After fermentation, the remaining flour, water and salt were added to the sourdough and kneaded for about 10 min. The obtained dough underwent fermentation for 45 min at 30°C. Similar to the case of DP, the dough followed the same steps throughout preparation and baking (230°C/ 240°C; 40 min/ 50 min).

Sample preparation for acrylamide and colour determination

Subsequent to drying (3 h; 50°C), samples were finely ground (B-400, BÜCHI Labortechnik AG, Switzerland), transferred in centrifuge tubes, and stored at -21°C until analysis.

Acrylamide determination by GC-MS/MS

Preparation of stock, working and calibration solutions for acrylamide determination were realized as previously described by Negoiță et al. (2022).

For the acrylamide analysis, the method presented by Negoiță et al. (2022) with modification was used. Briefly, 1.5 g of homogenized sample was weighted into 50 ml centrifuge tubes, to which 440 µl internal standard solution (1 mg/l) and 30 ml water were added. Samples were vortexed for 30 min at ambient temperature and then centrifuged at 5°C, 10.000 x g for 15 min (5804R Eppendorf centrifuge with cooling, Eppendorf, Germany). The aqueous extract was collected (10 ml) and cleaned-up using the HyperSep Universal Vacuum Solid Phase Extraction Manifold (Thermo Fisher Scientific, USA). The Isolute Multimode SPE cartridge was conditioned with methanol (3 ml) and water (12 ml). The acrylamide extract was loaded onto the cartridge and the resulting eluate was collected. The Isolute ENV$^+$ cartridge was conditioned with methanol (5 ml) and water (5 ml), followed by the extract loading. The cartridge was rinsed with 4 ml water which was discarded with the eluate. A volume of 5 ml of 60% MeOH in water was loaded on the cartridge and the acrylamide extract was collected.

The resulting extract was derivatized with bromine compounds and was subsequently extracted and concentrated as described by Negoiță et al. (2022). The final extract was injected on GC-MS/MS (Thermo Fisher Scientific, USA).

The method was validated internally based on the following parameters: linearity, linearity range, limit of detection (LOD), limit of quantification (LOQ), selectivity, precision, accuracy, recovery and measurements uncertainty, as described by Negoiţă & Culeţu (2016). Two calibration curves were plotted, with both correlation coefficients above 0.998, within the ranges of 0.05–0.5 mg/L, and 0.4–3 mg/L, respectively. The linearity domain was achieved within the range of 6.20- 116.34 µg/kg, accompanied by a correlation coefficient (R^2) higher than 0.998. The LOD and LOQ were set at 2.06 and 6.20 µg/kg, respectively. To verify the selectivity, the SRM detection mode and internal standard method were used. The method's accuracy was expressed as relative standard deviation (RSD) under repeatability (0.82-3.57%) and reproducibility (4.54-12.94%) conditions. The recovery of the method was ≤ 110%. The method uncertainty was set at ± 17.5% of concentration. To demonstrate the method's precision and accuracy, the laboratory participated in a proficiency test launched by Food Analysis Performance Assessment Scheme (FAPAS) (Sand Hutton, UK) on cereal-based products (biscuits- PT 30114) and obtained a z-score of -0.3. The validated parameters fulfilled the criteria set by European Commission (2017/2158).

Colour determination

For colour analysis a Konica Minolta colorimeter (Universal Software V4.01 Miniscan XE Plus) was used. The CIELAB´76 colour parameters were assessed on ground bread. Measurements were realized in 10 different areas of the ground bread samples, followed by the calculation of the colour parameters (L^*, a^* and b^*), expressed as mean ± standard deviation (SD).

Statistical analysis

The analyses were performed in duplicate, all data are expressed as mean ± standard deviation (SD). Results were submitted to Minitab statistical software version 20. One-way analysis of variance (ANOVA) was performed to examine the statistical differences between samples, followed by Tukey's test using a significance level of $p < 0.05$. Pearson correlation was used to assess the relationship between acrylamide content and colour parameters of the tested bread samples.

RESULTS AND DISCUSSIONS

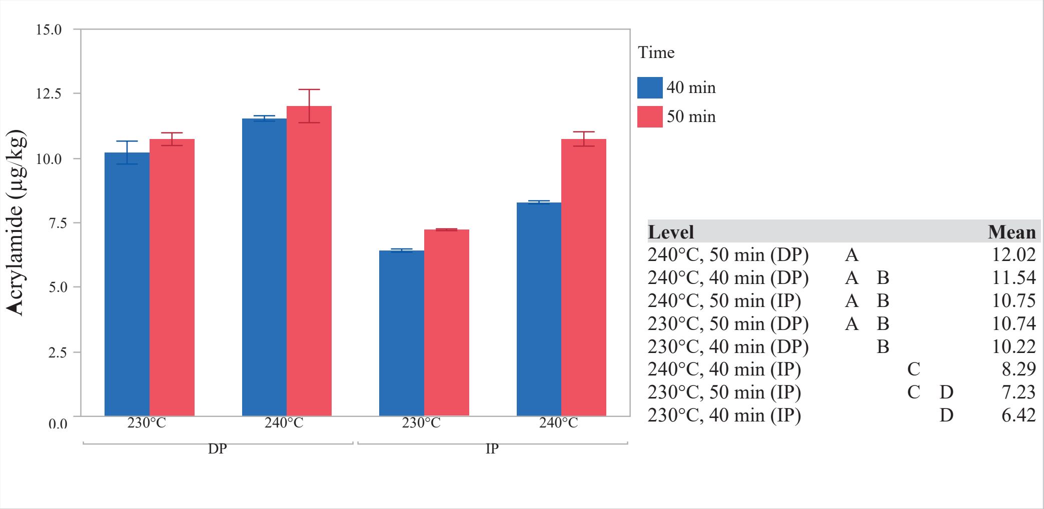
Influence of manufacturing recipe on the acrylamide content of bread

The main raw materials used in the bread manufacturing recipe were flour, water, salt and yeast. For this study, bread samples were prepared using either refined wheat flour and whole-wheat flour. The later in abundant in vitamins, minerals, and dietary fibers, and represents a rich source of antioxidants, carotenoids, flavonoids and phenolic acids (Ma et al., 2021; Sun et al., 2023). Recently it was shown that a higher intake of whole-wheat flour in consumed products helps reduce type 2 diabetes, cardiovascular, intestinal or chronic diseases (Tebben et al., 2018; Adams et al., 2020; Gomez et al., 2020; Hu et al., 2020; Reynolds et al., 2020). However, products made with this kind of flour are darker, have a harder texture, bitter taste compared with the products made using refined flour (Gómez et al., 2020).

When investigating the effect of manufacturing recipe on the acrylamide content of bread samples, our results showed that bread formulated with whole-wheat flour had a higher content than that of bread made with refined wheat flour (Figures 1 and 2). Similarly, in the study realized by Capuano et al. (2009) in a bread crisp model system, it was shown that whole-wheat flour produces more acrylamide than wheat flour.

The acrylamide content of bread samples made with wheat flour and DP ranged between 10.22-12.02 µg/kg (Figure 1), while using the whole-wheat flour and the same process, the content ranged between 11.01-33.04 µg/kg (Figure 2).

In the case of bread obtained using wheat flour through sourdough fermentation (IP), the acrylamide content varied between 6.42-10.75 µg/kg (Figure 1). By employing the same process for the whole-wheat flour, the acrylamide content registered higher levels, ranging between 9.57-15.80 µg/kg (figure 2).



Level					Mean
240°C, 50 min (DP)	A				12.02
240°C, 40 min (DP)	A	B			11.54
240°C, 50 min (IP)	A	B			10.75
230°C, 50 min (DP)	A	B			10.74
230°C, 40 min (DP)		B			10.22
240°C, 40 min (IP)			C		8.29
230°C, 50 min (IP)			C	D	7.23
230°C, 40 min (IP)				D	6.42

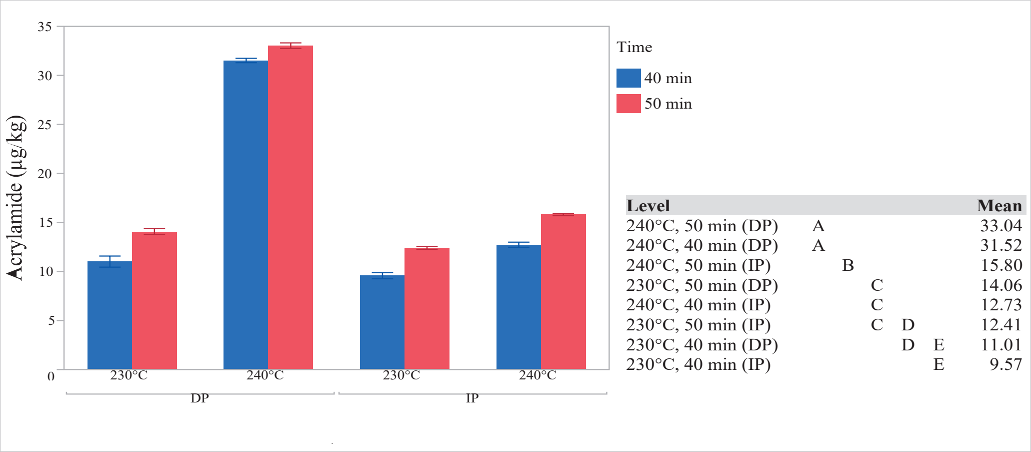
Figure 1. Changes of acrylamide content in wheat flour bread obtained by different dough preparation processes (DP and IP), under different baking conditions (230/240°C; 40/50 min). Means not connected by same letter are significantly different

By contrast to the case of natural sourdough fermentation, the acrylamide content in bread samples prepared through the IP decreased significantly irrespective of the flour type. Similar results were reported by Wang et al. (2017), Nachi et al. (2018) and Codină et al. (2021) who determined a lower acrylamide content in bread formulated with leaven/sourdough compared with the one unleavened.

Zhou et al. (2022) evaluated the effect of some *Lactobacilli* and *Saccharomyces cerevisiae* strains on sourdough in comparison with the natural fermentation of sourdough, rendering viable the utilization of these microorganisms as they enabled a considerable decrease in acrylamide content by 24.38-58.83%. During fermentation, the pH of bread samples decreased, and the acidity increased, resulting a reduction of acrylamide content.

The acrylamide content of all bread samples evaluated in the present study are lower than the ones reported by Roszko et al. (2019) of 3.6-163 µg/kg for soft bread, by Dessev et al. (2020) of 4-77 µg/kg in bread, and by Basaran et al. (2022) of 60.7-130 µg/kg, respectively.

Most importantly, the samples evaluated herein did not exceed the benchmark level of 50 µg/kg regulated by the European Commission (2017/2158) when considering wheat-based bread.



Level						Mean
240°C, 50 min (DP)	A					33.04
240°C, 40 min (DP)	A					31.52
240°C, 50 min (IP)		B				15.80
230°C, 50 min (DP)			C			14.06
240°C, 40 min (IP)			C			12.73
230°C, 50 min (IP)			C	D		12.41
230°C, 40 min (DP)				D	E	11.01
230°C, 40 min (IP)					E	9.57

Figure 2. Changes of acrylamide content in whole-wheat flour bread obtained by different dough preparation processes (DP and IP), under different baking conditions (230/240°C; 40/50 min). Means not connected by same letter are significantly different

Influence of baking conditions on the acrylamide content of bread

In the present study, we employed 4 different baking conditions comprising combinations of 230°C and 240°C with 40 min and 50 min, in order to investigate the exerted effect on the acrylamide content achieved in the formulated bread.

In this context, our findings revealed that the baking parameters influenced the acrylamide content in bread. As such, the lowest acrylamide content was measured in bread baked at the lowest temperature and shortest baking time, respectively (230°C, 40 min).

In addition, the results showed that by increasing the baking temperature with 10°C (from 230 to 240°C) for both flour types irrespective of the baking time (40/50 min), an increase in acrylamide levels was generated (Figures 1 and 2). This increase ranged between 11.9-13.0% for bread made from wheat flour using DP of dough preparation, and much higher for bread obtained using IP of dough preparation ranging between 29.1-48.7%. For bread formulated with refined wheat flour, the acrylamide content was higher for bread baked at 240°C for 50 min (12.02 µg/kg), compared with that of bread baked at 230°C for 50 min (10.74 µg/kg). Similarly, in the case of whole-wheat flour, a 10°C increase in the baking temperature resulted in a considerably high increase in acrylamide formation when DP was used (135.0-188.3%), whereas a much lower elevation was registered for IP, ranging between 27.4-33.0%.

The maximum level of acrylamide (33.04 µg/kg) was achieved for whole-wheat bread baked at 240°C for 50 min using the DP, 2.35-fold higher than the one of the breads baked at 230°C for the same time (14.06 µg/kg).

Similarly, the increase in baking time by 10 min (from 40 to 50 min) generated higher acrylamide formation. For bread made from wheat flour baked at 230/240°C using the DP of dough preparation, the increase of acrylamide content ranged between 4.2-5.2%, while for the IP of dough preparation it ranged between 12.6-29.7%. Comparably, for bread made from whole-wheat flour baked at 230/240°C using DP, the increase in acrylamide levels ranged between 4.8-27.7%, whereas the IP preparation registered an increase in acrylamide content in the range of 24.1-29.6%. Overall, our findings showed that by increasing the baking temperature, a higher increase of acrylamide level was caused compared to the increase in baking time.

The impact of the baking parameters was investigated in several studies. Surdyk et al. (2004) reported the proportion of crust in the dry breads increased with temperature and time of baking, from 30% (170°C/17 min) to 45% (270°C/32 min). By increasing the baking parameters, the content of acrylamide in the crust increased. When bread was baked at 290°C for 25 min, the acrylamide content of dry crust was very high (1800 µg/kg).

In the study conducted by Haase et al. (2003) it was shown that flour milling intensity as well as baking temperature have a high impact on acrylamide content in bread. Additionally, Claus et al. (2018) reported that acrylamide in wheat bread increased linearly with baking parameters. Breads baked at a lower temperature and a longer baking time (200°C/70 min) compared to those presented in our study, had an acrylamide content of 124.1 mg/kg. When bread was baked at similar conditions like the ones from our study (240°C/50 min), the acrylamide content was 92.4 mg/kg, much higher than the one obtained in our study.

Colour parameters of bread samples

Colour is an important parameter that can be correlated with the acrylamide content of food products. When food products have a lighter colour, a lower acrylamide content is found in product (Figure 3).

Food and Drug Administration (FDA, 2022) recommended to modify the baking parameters and to cook cereal-based products to a lighter colour endpoint in order to reduce acrylamide content in this category of products.

Figure 3. Bread samples obtained in experiments

The colour parameters (*L**, *a**, and *b**) of bread samples were calculated and results are presented in Table 1.

The more intense the baking treatment was, the more evident the Maillard reaction occurred. Hence, while the darkest colour was observed in bread baked at the highest temperature (240°C) and time (50 min), the lightest colour was noted for bread baked at the lowest settings (230°C; 40 min). It can be said that by increasing the baking temperature, bread lightness decreased. Also, by increasing the baking time, the bread samples became darker.

In comparison with the *L** parameter, the *a** parameter showed overall lower values for refined wheat bread than those of whole-wheat bread, whereas the *b** parameter reflected the opposite outcome.

No significant patterns were observed among the two colour parameters, with regards to the effect of baking conditions or fermentation process.

Table 1. Colour parameters of bread samples

Experimental variant			Colour parameters, mean ± sd		
			*L**	*a**	*b**
Wheat flour	DP	230°C, 40 min	76.44 ± 0.03^{a}	3.06 ± 0.03^{d}	17.43 ± 0.02^{d}
		230°C, 50 min	74.55 ± 0.05^{b}	3.95 ± 0.02^{a}	18.95 ± 0.04^{c}
		240°C, 40 min	74.41 ± 0.01^{c}	3.84 ± 0.01^{b}	19.37 ± 0.02^{a}
		240°C, 50 min	73.99 ± 0.19^{d}	3.69 ± 0.03^{c}	19.19 ± 0.04^{b}
	IP	230°C, 40 min	77.33 ± 0.04^{a}	3.14 ± 0.02^{c}	17.71 ± 0.02^{c}
		230°C, 50 min	77.21 ± 0.04^{b}	3.21 ± 0.01^{b}	18.25 ± 0.03^{b}
		240°C, 40 min	76.78 ± 0.05^{c}	2.96 ± 0.03^{d}	17.38 ± 0.10^{d}
		240°C, 50 min	75.41 ± 0.06^{d}	3.26 ± 0.03^{a}	18.53 ± 0.04^{a}
Whole-wheat flour	DP	230°C, 40 min	70.87 ± 0.01^{a}	4.28 ± 0.02^{b}	14.83 ± 0.03^{a}
		230°C, 50 min	68.27 ± 0.05^{b}	4.11 ± 0.02^{d}	12.58 ± 0.02^{d}
		240°C, 40 min	67.78 ± 0.04^{c}	4.21 ± 0.03^{c}	12.81 ± 0.08^{c}
		240°C, 50 min	66.16 ± 0.09^{d}	4.94 ± 0.04^{a}	14.33 ± 0.12^{b}
	IP	230°C, 40 min	65.89 ± 0.03^{a}	5.15 ± 0.01^{b}	14.99 ± 0.03^{a}
		230°C, 50 min	65.36 ± 0.04^{b}	5.16 ± 0.02^{b}	13.90 ± 0.05^{b}
		240°C, 40 min	65.12 ± 0.03^{c}	5.11 ± 0.03^{c}	13.46 ± 0.05^{c}
		240°C, 50 min	63.92 ± 0.06^{d}	5.27 ± 0.04^{a}	13.94 ± 0.09^{b}

*L** (lightness, 0= black to 100= white), *a** (redness, +*a**= redness, –*a**= greenness), and *b** (yellowness, +*b**= blueness, -*b**= yellowness). Values followed by different letters in the same column for each manufacturing recipe are significantly different (p< 0.05)

Variation of colour parameters based on the acrylamide content of bread samples

The changes in the *L** colour parameter in relation to the acrylamide content of bread samples is presented in Figure 4 and Figure 5.

By increasing the temperature and baking time, the acrylamide content increased, while the colour parameter *L** decreased irrespective of the flour type or baking process.

Correspondingly, the extent to which the acrylamide content and *L** colour parameter change concomitantly at the evaluated rate of temperature change, reveals high linear relationships between the two parameters.

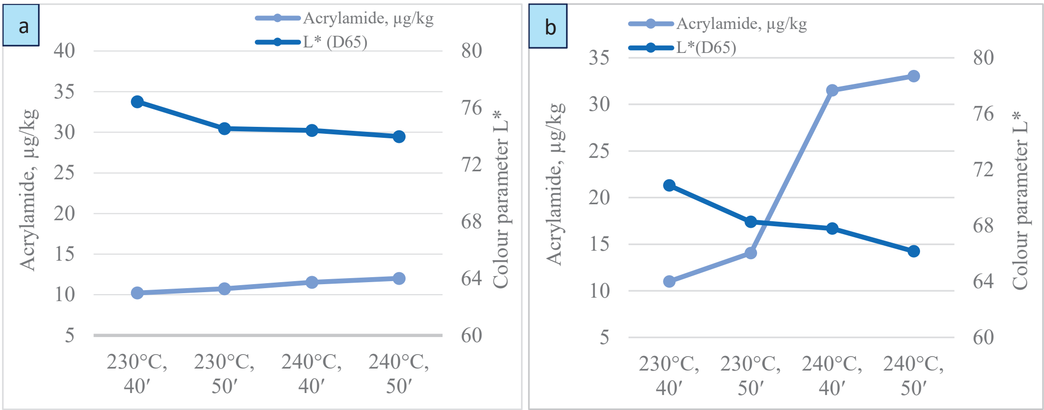


Figure 4. Changes of the colour parameter *L** based on the acrylamide content of bread made with wheat flour (a) and whole-wheat flour (b) through the direct process and baked under different conditions

Very strong negative correlations were observed for both DP (r = -0.7522; p = 0.0313) and IP (r = -0.9796; p< 0.0001) when using refined wheat flour. Similarly, in the case of whole-wheat flour, we found very strong negative correlations between the acrylamide content and the *L** colour parameter for both DP (r = -0.8428; p = 0.0086) and IP (r = -0.9724; p< 0.0001). By contrast, no significant correlations were determined between the acrylamide content and the *a** and *b** colour parameters of the evaluated bread samples.

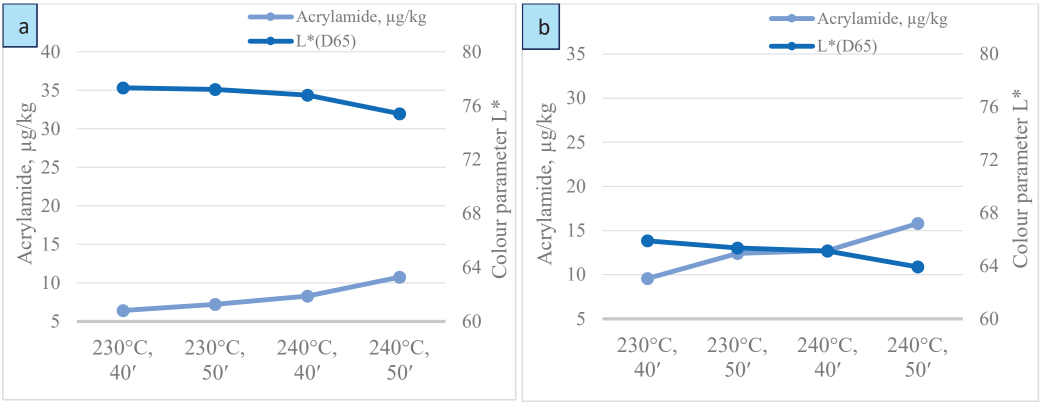


Figure 5. Changes of the colour parameter *L** based on the acrylamide content of bread made with wheat flour (a) and whole-wheat flour (b) through the indirect process and baked under different conditions

Regardless of the baking process, darker colours and higher acrylamide contents were determined for bread formulated with whole-wheat flours. Also, in the study realized by Abdulazeez et al. (2023) it was shown that the acrylamide content in cereal-based products is correlated with the colour intensity of the product, a lighter colour meaning a lower acrylamide content.

CONCLUSIONS

The acrylamide content of bread sample was influenced by the wheat flour used in the recipe, the fermentation type and the baking conditions. Even though whole-wheat flour is a rich source of bioactive compounds having beneficial effects for human health, when bread was formulated with this flour, the acrylamide content was higher.

When using the sourdough fermentation, the acrylamide content decreased compared with the DP.

The baking parameters exerted a significant influence on the acrylamide content measured in all bread samples. By increasing the temperature with 10°C, while maintaining the same baking time of 40/50 min, the acrylamide content was 1.13-2.87-fold/1.12-2.35-fold higher. By extending the baking time by 10 min and maintaining the same baking temperature of 230°C/240°C, the acrylamide content was 1.05-1.30-fold/1.05-1.30-fold higher. The highest acrylamide content was determined for the bread baked at the highest temperature (240°C), and the longest time (50 min) using the direct process of fermentation. The acrylamide content of bread formulated in this study didn't exceed the benchmark level set by EC 2017/2158.

The acrylamide content of bread was strongly correlated with the colour parameter *L**, lighter products having a lower acrylamide content.

In this context, it is important to prevent dark-colour products.

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