Soluções Sustentáveis: Enfrentando os Desafios do Clima e da Alimentação
9º SIMPÓSIO DE SEGURANÇA ALIMENTAR
19 A 21 DE MAIO DE 2025 | BENTO GONÇALVES | RS

INFLUENCE OF BREAD BAKING CONDITIONS ON LEVELS OF ACRYLAMIDE, HMF AND STARCH DIGESTIBILITY

A. C. Lemos[1], V.S de Borba[2], P.T. Scaglioni[3], E. Babiale-Furlong[4].

1- Mycotoxins and Food Science Laboratory, School of Chemistry and Food, Federal University of Rio Grande – FURG, Avenida Itália Km 8, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil. Phone (53) 992135083 e-mail: (andressa.cunhalemos@gmail.com)
2- Laboratory for the Analysis of Organic Compounds and Metals, School of Chemistry and Food, Federal University of Rio Grande – FURG, Avenida Itália Km 8, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil. Phone: (53) 991171489 e-mail: (veronicasimoesborba@gmail.com)
3- Food Technology Laboratory, School of Chemistry and Food, Federal University of Rio Grande – FURG, Avenida Itália Km 8, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil. Phone (53) 981011132 e-mail: (priscilascaglioni@gmail.com)
4- Mycotoxins and Food Science Laboratory, School of Chemistry and Food, Federal University of Rio Grande – FURG, Avenida Itália Km 8, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil. Phone (53) 999710840 e-mail: (dqmefb@furg.br)

RESUMO – Este estudo avaliou a influência das condições de assamento nos níveis de acrilamida e HMF em pães pré-congelados. Os pães foram fermentados por 3 h a 30 °C e assados a 180 °C, 200 °C e 220 °C por 8, 10 e 12 min, além dos contaminantes, também foram avaliados parâmetros tecnológicos de volume específico e índice de escurecimento, e nutricionais (digestibilidade proteica e do amido). O pão assado a 200 °C por 10 min resultou na menor formação de acrilamida (1194 µg/kg) e o pão assado a 180 °C por 10 min na menor formação de HMF (152 µg/kg). Os pães apresentaram volume específico adequado, e a digestibilidade do amido (712,53 mg/g) e da proteína (122%) foi maior no pão assado a 200 °C por 12 min. As condições testadas resultaram em pães com características tecnológicas adequadas, mas com acrilamida em níveis superiores aos permitidos pela União Europeia.

ABSTRACT – This study evaluated the influence of baking conditions on acrylamide and HMF levels in pre-frozen breads. The breads were fermented for 3 h at 30 °C and baked at 180 °C, 200 °C and 220 °C for 8, 10 and 12 min. In addition to contaminants, technological parameters of specific volume and browning index, and nutritional parameters (protein and starch digestibility) were also evaluated. The bread baked at 200 °C for 10 min resulted in the lowest acrylamide formation

(1194 µg/kg) and the bread baked at 180 °C for 10 min in the lowest HMF formation (152 µg/kg). The breads presented adequate specific volume, and the digestibility of starch (712.53 mg/g) and protein (122%) was higher in the bread baked at 200 °C for 12 min. The conditions tested resulted in breads with adequate technological characteristics, but with acrylamide at levels higher than those permitted by the European Union.

PALAVRAS-CHAVE: Amido, Contaminantes, Panificação.
KEYWORDS: Starch; Contaminants; Breadmaking.

1. INTRODUCTION

Bread is one the world's most consumed bakery products, and consequently, it has been deeply studied Mesci; Kilic andOguz (2011). There are several types of bread, and those made from refined wheat flour, known as white bread, are the most popular ones due to their sensory aspects (Bakke; Vickers, 2007). French fresh bread usually presents an appealing brownish and crunchy crust, a pleasant roast aroma and a soft and elastic crumb texture. However, fresh bread is a product with a short shelf-life and during its storage chemical and physical alterations occur, known as staling (Carr *et al.*, 2006).

The bakery sector is growing, with a projection of U$ 810 billion by 2029, and is always looking for innovations, such as large-scale production and new products, like frozen bread (Perfecta, 2024). In this sense, frozen unfermented dough stands out as an alternative for small businesses (Intelligence, 2025). In Rio Grande do Sul state, there are industries that produce up to 100,000 frozen loaves a day and distribute them to more than 60 establishments in the city (Bevilaqua, 2019).

During baking, the starch gelatinizes, and the proteins are denatured when the internal temperature reaches 60-80 °C, and the dough becomes a digestible product. In addition, the Maillard reaction (MR) and caramelization occur. MR occurs between a free sugar and an amino acid at temperatures > 120 °C, resulting in changes in coloration through the formation of malanoidins, aromatic compounds and contaminants such as acrylamide, a probable carcinogen (Iarc, 1994). Caramelization is the dehydration of sugars, and is of great importance in the food industry, being used as an indicator of food quality during heat treatment (Lemos *et al.*, 2021; Ramírez-Jiménez; Guerra-Hernández; García-Villanova, 2000). Europe Union set the maximum level of acrylamide in bread of 500 µg/kg (Jecfa, 2011) while for HMF there is no recommendation in bread. In Brazil, there is no recommendation. The study aimed to evaluate the influence of bread baking conditions on the levels of acrylamide and HMF.

2. MATERIALS AND METHOD

2.1 Breadmaking experiments

For the baking experiments, bread doughs frozen before fermentation were used, purchased from an industry in the city of Rio Grande/RS. The bread samples were placed in a fermentation chamber for 3 hours at 30 °C and then baked at 180 °C, 200 °C and 220 °C at three different levels (8, 10 and 12 min).

2.2 Technological Aspects

After baking, color analysis was performed on the crumb using a Minolta® model CR400 colorimeter. The analysis was based on the L (lightness)*, and the Browning Index (BI) was calculated (BI=100-L). To determine the specific volume, homogeneous pieces of bread were cut, and their mass was measured. The piece was then placed in a measuring cylinder filled with millet seeds. The difference in the level of the seeds indicated the volume occupied by the bread, and the ratio between volume and mass corresponded to the specific volume.

2.3 Processing Contaminant Determination

The extraction method was done with methanol and cleaning whit Carrez I and Carrez II. Chromatographic separation of process contaminant was performed by an HPLC-PDA (HPLC; Shimadzu, Kyoto, Japan) (Lemos *et al.*, 2024).

2.4 Starch and Protein Digestibility

Digestible and resistant starch was determined according to the protocol proposed by Walter; Silva and Emanuelli (2005). Protein digestibility was calculated by adapting the protocol proposed by Bredariol; Carvalho and Vanin (2020). The bread baked for 10 min at 200 °C was considered the control (100% protein digestibility).

2.5 Statistical Analysis

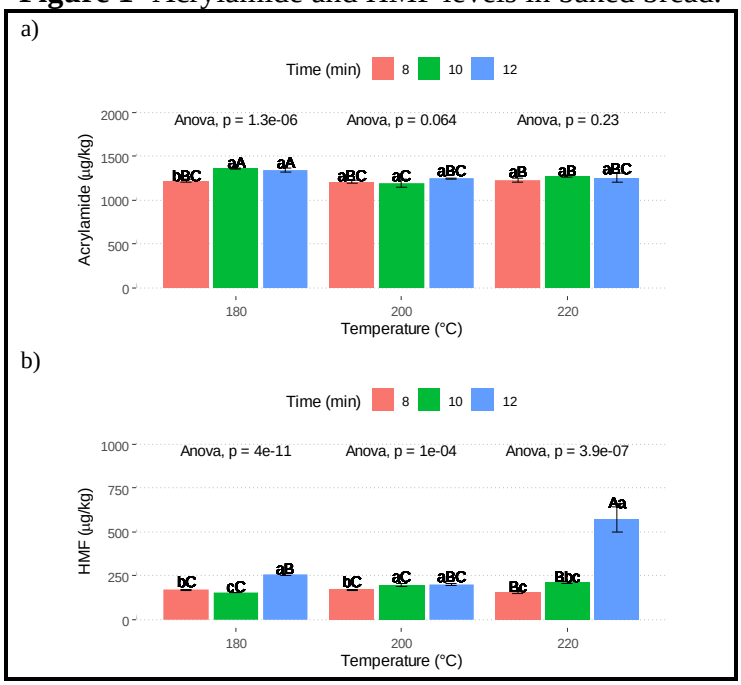
All experiments were carried out in triplicate and expressed as mean ± standard deviation. One-way ANOVA was applied to compare different times at the same temperature (α=0.05) and two-way ANOVA to compare all experiments (α=0.05).

3. RESULTS AND DISCUSSION

Figure 1 shows the levels of acrylamide (a) and HMF (b) in samples baked at different temperatures and times. For acrylamide, levels ranged from 1194 µg/kg for bread baked at 200 °C

for 10 min to 1364 µg/kg for bread baked at 180 °C for 10 min. For HMF, the levels ranged from 150 µg/kg for bread baked at 180 °C for 10 min to 572 µg/kg for bread baked at 220 °C for 10 min. No sample was below the level recommended by European Union (500 µg/kg) (Jecfa, 2011).

Figure 1- Acrylamide and HMF levels in baked bread.



Different small letters: Statistical differences ($p<0.05$) among the baking times at the same temperature. Different uppercase: Statistical differences ($p<0.05$) among all tests.

For acrylamide, for 180 °C, there was no difference between baking bread for 10 or 12 min, while for HMF there was 40% more HMF baking for 12 min. For 200 °C, there was no difference in acrylamide formation among different times of baking, while for HMF, longer baking times lead to more HMF formation. For 220 °C, 12 min of baking results in 2x more HMF than in 8 min and 10 min. The formation of acrylamide was almost 6x higher than of HMF, a fact observed by Lemos *et al.* (2023) e Lemos *et al.* (2024) in bread produced in lab scale baked for different times and for collected bread of Rio Grande/RS, respectively.

Table 1 shows the technological of bread and digestibility of starch and protein. For protein, bread baked at 200 °C for 12 min was the more digestible, while those baked at 220 °C was the less digestible. For starch, bread baked for 12 min was those with more digestible starch, and there was more resistant starch in sample baked at 220 °C. For Browning Index (BI), the longer the baking time, the greater the browning, regardless of the temperature. This indicates that the oven may have heated more than indicated (sample 220 °C, 12 min), resulting in one of greater browning, lower specific volume and greater formation of HMF (Figure 1b), comparing only samples baked at 220 °C.

According to Bredariol; Carvalho and Vanin (2020), the higher the baking temperature and time, the higher the crust color change, and protein digestibility may also be favored, however it does not follow a linear correlation. The specific volume of bread ranged from 2.61 mL/g to 5.92 mL/g, results similar to reported by Bredariol; Spatti and Vanin (2019).

Table 1-Bread characteristics according to temperature and baking times.

Temperature (°C)	Time (min)	Protein Digestibility (%)	Digestible Starch (mg/g)	Resistant Starch (mg/g)	BI	Specific Volum (mL/g)
180	8	96±4^{bc}	545.24±58.42^{cde}	57.47±4.41^{de}	16.88±0.90^{e}	2.61±0.64^{d}
	10	100±8^{b}	573.75±12.65^{de}	121.07±1.69^{b}	18.05±0.53^{de}	3.09±0.22^{d}
	12	93±2^{bc}	914.38± 86.81^{a}	99.82±14.05^{c}	32.94±6.83^{c}	4.32±0.18^{b}
200	8	96±4^{bc}	661.13±26.47^{bc}	66.11±6.24^{d}	45.80±3.71^{ab}	3.26±0.27^{cd}
	10	100<0^{b}	578.41±32.87^{cd}	46.53±3.13^{e}	46.62±3.06^{a}	3.97±0.16^{bc}
	12	122±2^{a}	712.53±66.08^{b}	43.90±4.78^{e}	50.58±2.90^{a}	2.71±0.12^{d}
220	8	65±8^{d}	427.20±11.29^{ef}	117.20±2.22^{b}	28.40±6.53^{cd}	4.57±0.14^{b}
	10	62±5^{d}	407.03±27.41^{f}	64.01±3.94^{d}	36.40±3.77^{bc}	5.92±0.26^{a}
	12	86±1^{c}	573.46±60.77^{cd}	167.24±2.29^{a}	44.18±8.08^{ab}	3.20±0.26^{cd}

Different small letters: Statistical differences ($p<0.05$) among different baking conditions. BI=Browning Index.

4. CONCLUSION

Frozen bread dough sold in the city of Rio Grande/RS, subjected to different baking conditions, showed 2x levels of acrylamide than those established by the EU. Despite this, all the technological parameters evaluated were in line with the literature and the digestibility of starch and protein showed that baking was effective.

5. ACKNOWGEMENT

The authors would like to thank CAPES and FAPERGS for their grants.

REFERENCES

BAKKE, A.; VICKERS, Z. Consumer liking of refined and whole wheat breads. **Journal of Food Science and Technology**, 72, n. 7, p. S473-S480, 2007.

BEVILAQUA, J. **Com fabricação de 100 mil pães congelados por dia, padaria de Caxias quer dobrar produção até metade de 2020**. Pioneiro, 2019. Disponível em: https://gauchazh.clicrbs.com.br/pioneiro/economia/noticia/2019/10/com-fabricacao-de-100-mil-paes-congelados-por-dia-padaria-de-caxias-quer-dobrar-producao-ate-metade-de-2020-11883103.html. Acesso em: 06/02/2025.

BREDARIOL, P.; CARVALHO, R. A.; VANIN, F. M. The effect of baking conditions on protein digestibility, mineral and oxalate content of wheat breads. **Food Chemistry**, 332, p. 127399, Dec 1 2020.

BREDARIOL, P.; SPATTI, M.; VANIN, F. M. Different baking conditions may produce breads with similar physical qualities but unique starch gelatinization behaviour. **LWT**, 111, p. 737-743, 2019/08/01/ 2019.

CARR, L. G.; RODAS, M. A. B.; DELLA TORRE, J. C. M.; TADINI, C. C. Physical, textural and sensory characteristics of 7-day frozen part-baked French bread. **LWT**, 39, n. 5, p. 540-547, 2006/06/01/ 2006.

IARC. IRAC Monographs on the Evaluation of Carcinogenic Risk for Chemicals to Humans. CANCER, I. A. f. R. o. 1994.

INTELLIGENCE, M. **Tamanho do mercado de padaria congelada e análise de ações – Tendências e previsões de crescimento (2024 – 2029)** 2025. Disponível em: https://www.mordorintelligence.com/pt/industry-reports/frozen-bakery-market. Acesso em: 06/02/2025.

JECFA. **Safety evaluation of certain contaminants in food: prepared by the Seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)**. World Health Organization, 2011. 9241660635.

LEMOS, A. C.; BORBA, V. S.; CERQUEIRA, M. B. R.; PEREIRA, A. M.; SCAGLIONI, P. T.; BADIALE-FURLONG, E. White and wholewheat bread consumption and the risk of exposure to acrylamide and 5-hydroxymethylfurfural. **Food Chemistry**, 460, n. Pt 3, p. 140662, Dec 1 2024.

LEMOS, A. C.; BORBA, V. S.; SCAGLIONI, P. T.; BADIALE-FURLONG, E. Contaminantes formados durante o processamento de alimentos. *In*: **Avanços em Ciência e Tecnologia de Alimentos**, 2021. v. 1, cap. 9.

LEMOS, A. C.; BORBA, V. S. D.; BURKERT, J. F. D. M.; SCAGLIONI, P. T.; BADIALE-FURLONG, E. Role of white bread matrix components and processing parameters on 5-hydroxymethylfurfural (HMF) and acrylamide formation. **Food Control**, 145, 2023.

MESCI, B.; KILIC, D.; OGUZ, A. Dietary Breads and Impact on Postprandial Parameters. *In*: **Flour and Breads and their Fortification in Health and Disease Prevention**, 2011. p. 429-435.

PERFECTA. **Tendências para o Mercado de Panificação em 2025**. Inovações e Desafios para uma Produção de Alta Escala., 2024. Disponível em: https://perfecta.itwfeg.com.br/perfecta/noticia/tendencias-para-o-mercado-de-panificacao-em-2025. Acesso em: 06/02/2025.

RAMÍREZ-JIMÉNEZ, A.; GUERRA-HERNÁNDEZ, E.; GARCÍA-VILLANOVA, B. Browning indicators in bread. **Journal of Agricultural and Food Chemistry**, 48, n. 9, p. 4176-4181, Sep 2000.

WALTER, M.; SILVA, L. P. D.; EMANUELLI, T. Amido resistente: características físico-químicas, propriedades fisiológicas e metodologias de quantificação. **Ciência rural**, 35, p. 974-980, 2005.