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Research Article

Assessment of Acrylamide Formation in Various Iraqi Bread Types and Mitigation of Acrylamide Production by Calcium Carbonate in White Flatbread

Mustafa A. M. Alsharafani,[1] Abeer S. Alhendi,[2] Taghreed K. Sharif,[1] Abdulwahed Ahmed Hassan,[3] Ali M. Jawad,[1] Rana T. Alsallami,[2] Amir Abdulmawjood,[4] and Izhar U. H. Khan[5]

[1]*Food Safety Center, Science and Technology, Environment and Water Research Department, Ministry of Science and Technology, Baghdad, Iraq*
[2]*Quality Control Department, Grain Board of Iraq, Ministry of Trade, Altaji, Baghdad, Iraq*
[3]*Department of Veterinary Public Health (DVPH), College of Veterinary Medicine, University Mosul, Mosul, Iraq*
[4]*Institute of Food Quality and Food Safety, Bischofsholer Damm 15, University of Veterinary Medicine Hannover, Bünteweg 17, D-30559 Hannover, Germany*
[5]*Agriculture and Agri-Food Canada, Ottawa Research and Development Centre, 960 Carling Ave, Ottawa, ON K1A 0C6, Canada*

Correspondence should be addressed to Izhar U. H. Khan; izhar.khan@agr.gc.ca

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Acrylamide is a neuro- and reproductive toxicant to humans and has carcinogenic effects in animal species which naturally forms in high-carbohydrate foods at high (≤121°C) temperatures. This study assessed acrylamide formation in various Iraqi bread types, including whole-wheat flatbread, white flatbread, stone-baked bread, whole-wheat baguette, and white baguette prepared in the selected local bread factories in Baghdad, Iraq, using HPLC assay. Among several types of bread, white flatbread is the most commonly consumed bread in Iraq; therefore, the impact of calcium carbonate ($CaCO_3$), added to the flour at different concentrations: 240, 260, and 280 mg/100 g, in reducing acrylamide concentration only in white flatbread was assessed. Acrylamide was detected at various concentrations in each type of bread tested, with the highest concentration in whole-wheat flatbread ($470.2 \pm 6.7\,\mu$g/kg) followed by stone-baked bread ($418.8 \pm 6.4\,\mu$g/kg), whole-wheat baguette ($408.3 \pm 9.1\,\mu$g/kg), white flatbread ($400.9 \pm 7.4\,\mu$g/kg), and white baguette ($362 \pm 9.2\,\mu$g/kg), respectively. However, after supplementing $CaCO_3$ in white flatbread, a significant ($p < 0.05$) reduction in acrylamide (219 ± 8.5, 121.8 ± 1.7, and $115 \pm 3.4\,\mu$g/kg, for the three calcium carbonate concentrations) as compared to the control ($308.3 \pm 2.9\,\mu$g/kg) was observed without impacting the rheological properties of bread. In conclusion, the acrylamide concentration can be reduced by supplementing $CaCO_3$ in the white flatbread without compromising the rheological properties (i.e., dough strength, volume, and water absorption) and overall bread quality. The study results would help to mitigate the risk of toxic effects in humans.

1. Introduction

Acrylamide (2-propenamide) or acrylic amide is an organic, thermogenic compound mainly generated during the baking process in thermal-treated plants (e.g., potato) and cereal-grain (e.g., bread and other baked products) based food as a result of the Maillard reaction, where the Schiff base initially forms between the carbonyl group of reduced sugar and α-amino group of amino acid asparagine [1–4]. Acrylamide was first detected at relatively high levels in various fried (French fries, crisps, and roast) and baked (jacket potatoes, bread, and baked goods) food products and has been considered as a harmful indicator for humans, but it is still unclear what risk exactly acrylamide poses to human health [5–7]. Acrylamide is a precursor for cancer formation that is naturally produced in carbohydrates and protein food in conjunction with low humidity during frying, roasting, or extruding at high (≥121°C) temperature [3]. The European Food Safety Authority (EFSA) has determined that acrylamide in food may increase the risk of developing cancer in all age groups particularly young people who are highly vulnerable [6]. Heat-treated bakery products may include a wide range of chemicals including acrylamide, hydroxymethylfurfural, and derivatives that are generated during the baking process [8].

In 2007, FAO/WHO organized the first international meeting on acrylamide to develop strategies to reduce acrylamide levels and determine the maximum daily levels in food [9]. Until now, there are no regulations that specify the maximum allowable and safe level of acrylamide in food and food products. Many European countries such as Portugal, Italy, and France are concerned about reducing the level of acrylamide in cooked food [10]. Generally, bread contains less acrylamide than fried food or potato products. Reducing daily acrylamide intake is still of high priority to many countries highlighting an urgent need to reduce acrylamide in bread [3]. The starting concentration and ratio of the precursors (asparagine and free reducing sugars), the quality of flour, such as milling intensity, fermentation conditions, and thermal processing techniques (temperature, length of heating time, pH, and water content), and physical state of food additives are some of the elements that play a vital role in the final product and impact on the level of acrylamide in food [8, 11–15]. Similarly, sensory characteristics are extremely important for consumer satisfaction based on the European Parliament regulations (European Union No. 1333/2008) on food additives [16]. The U.S. Food and Drug Administration (FDA) has recommended acrylamide levels within the range of 0.3 to 0.8 μg/kg bodyweight per day (bw/day) in food [2]. Based on the highest reported level of acrylamide in bread samples (2565 μg/kg), the European Commission recommended further research on acrylamide known as a food contaminant. According to these recommendations, bread should be sampled at the point of sale or manufacture and analysis should be performed before the samples' expiry date. Furthermore, the benchmark levels of acrylamide have been set for bread and infant food (30 μg/kg) and all other food products (50 μg/kg) [17]. Acrylamide concentration in conventionally baked bread crust ranges between 85 and 230 μg/kg baked at 200–270°C for 10–20 min [11, 18]. According to the French Total Diet study, acrylamide was analyzed in 192 samples of food from mainland France chosen to be typical of the diet and processed in the same way as it would be for the population as a whole. The highest mean concentrations were recorded (724 μg/kg) in fried potato products other than potato chips (697 μg/kg) [19]. A minimum concentration of 0.17 and 0.43 mg/kg bw/day of acrylamide can have tumour-causing or neurological effects on humans or animals [5, 20]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) determined that the average acrylamide intake ranges between 0.3 and 2.0 μg/kg bw/day, with a mean of 1 μg/kg bw/day, and the average acrylamide intake for high level consumers ranges between 0.6 and 5.51 μg/kg bw/day, with a mean of 4 μg/kg bw/day. Upon calculating the margin of exposure, the JECFA found that acrylamide in food is a human health hazard that may cause genotoxicity and carcinogenicity [21].

A previous study has investigated the effect of calcium salts combined with carbohydrate-rich foods containing the free amino acid asparagine to prevent the formation of N-glycosyl asparagine and cut the chains of the initial stages of the Maillard reaction, which resulted in reacting amino acids with reducing sugars and consequently prevented acrylamide formation [22]. The food processing industry has come up with ways to lower the amount of acrylamide in its products without compromising food quality by modifying product formulations, preparation, and baking methods including temperature, reducing pH, and baking time to limit acrylamide development [5, 23]. Moreover, in a previously reported study, a minimal level of acrylamide was reduced in baked products by simulating 300 U/kg purified asparaginase and crude asparaginase [24].

The present study was aimed to estimate, firstly, the concentration of acrylamide in five different types of commercially baked breads collected from Baghdad bread factories, Iraq. Secondly, we investigated the effect of calcium carbonate ($CaCO_3$) on reducing acrylamide formation and concentration in the most commonly consumed white flatbread in Iraq. The findings would help authorities including risk assessment and food safety managers to improve and/or develop strategies to mitigate acrylamide formation in food.

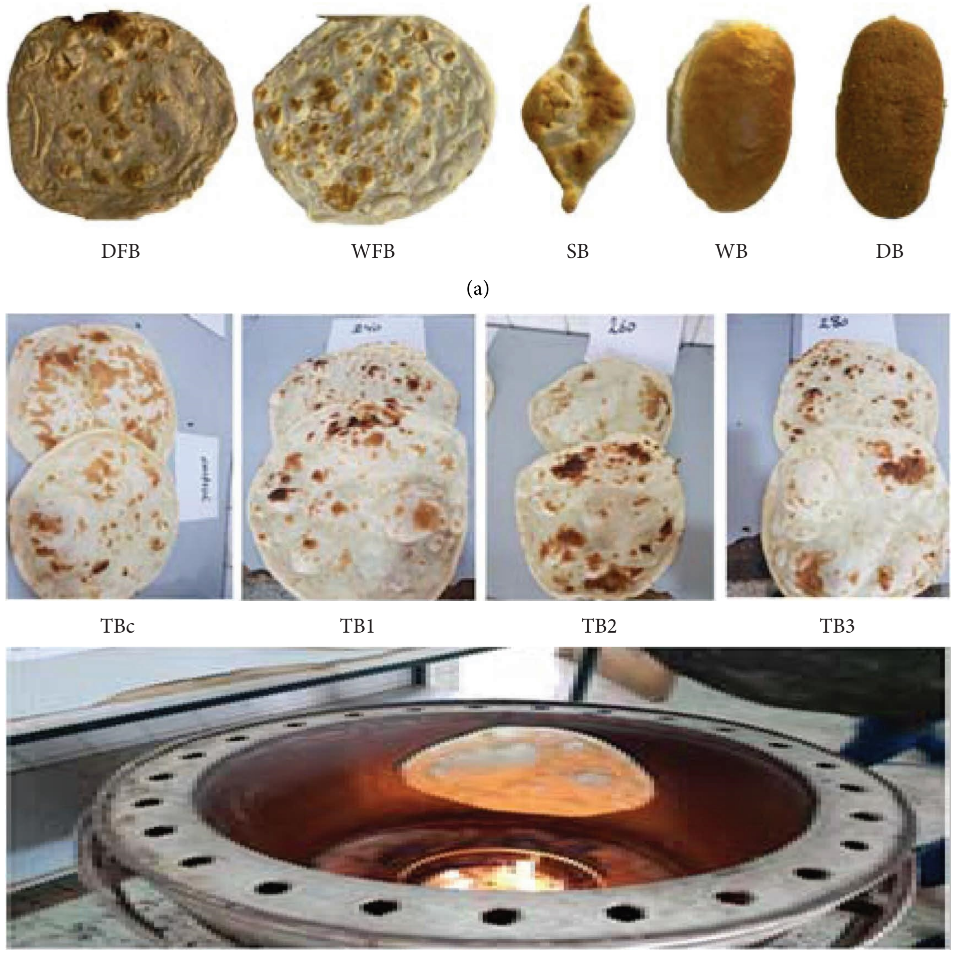
2. Materials and Methods

2.1. Bread Types and Collection. Five most commonly consumed commercial bread types including whole-wheat flatbread, white flatbread, stone-baked bread, whole-wheat baguette, and white baguette (Figure 1(a)) were prepared in different local bread factories in Baghdad.

2.2. Ingredients and Preparation of Breads

2.2.1. Whole-Wheat Flatbread. The whole-wheat flatbread was prepared using wheat flour mixed with water, yeast (0.6%), and table salt (1.5%). The fresh mixture was kneaded using a commercial kneading machine to produce consistent dough and left for 60 min at 25°C for fermentation. Finally, the dough was divided into small (50 g) pieces, then shaped as pizza, and baked in a traditional mud (clay free) oven. The dough was baked for 10 min at 270°C as previously described [25].

2.2.2. White Flatbread. The flour was produced at a 72% extraction rate of wheat to make white flatbread. The flour was mixed with water, yeast (*Saccharomyces cerevisiae*, 0.6%), and table salt (1.5%). The mixture was processed and baked as the procedure described above using traditional mud oven [25, 26]. As compared to the whole-wheat flour, the white flour is rich in carbohydrates and low in fiber, protein, minerals, and vitamins.



(b)

Figure 1: (a) Five bread types: DFB (wheat flatbread); WFB (white flatbread); SB (stone-baked bread); WB (white baguette); and DB (wheat baguette), used in the study. (b) White flatbread supplemented with different concentrations ($TB_1 = 240$, $TB_2 = 260$, and $TB_3 = 280$ mg/100 g flour) of $CaCO_3$ and TB_c used as control.

2.2.3. Stone-Baked Bread. The stone-baked bread is recognized for its distinct flavor and soft texture as compared to other types of bread. The dough was prepared with wheat flour (72%), water (60 g), yeast (*Saccharomyces cerevisiae*, 1%), table salt (1%), sugar (0.5%), and oil (1.5%) at 85–90% relative humidity. The fresh mixture was kneaded to produce consistent dough and fermented at 30°C for 60 min. The dough was divided into 100 g pieces, with final proofing for about 10 min prior to the bread formation. The dough was molded into a rhombus about the size of a hand. The bread was manually flattened and shaped narrow from the two edges and wide in the middle. After the final proofing, baking was done horizontally above a hot plate of brick oven at 225 ± 5°C [27, 28].

2.2.4. Whole-Wheat Baguette. The whole-wheat baguette was prepared with wheat flour mixed with water, yeast (*Saccharomyces cerevisiae*, 0.9%), table salt (1%), and ascorbic acid (0.01%). The mixture was kneaded mechanically until the dough bowl walls were clean from dough pieces. Then, the dough was incubated for fermentation at 25°C for 20 min as previously described [29]. The fermented dough was gently divided and pre-proofed to activate the yeast in the dough for 10 min. The dough was made manually by making a very thin layer sheet and folded. The final proofing time was about 60 min prior to baking in an electric oven at 270°C for 10 min.

2.2.5. White Baguette. The white baguette bread was prepared with white refined wheat flour, yeast (*Saccharomyces cerevisiae*, 0.9%), table salt (1%), and ascorbic acid (0.01%). The mixture was kneaded mechanically until the dough bowl walls were clean from dough pieces. Then, the dough was processed and baked as described above.

2.3. Preparation of White Flatbread Supplemented with $CaCO_3$. Since consumption of white flatbread is over 80% in the area, therefore, we used white flatbread for this assay. The white flatbread was prepared in the lab as described in the preceding section, and the white flour was supplemented with $CaCO_3$ using three concentrations: 240 (TB_1), 260

(TB_2), and 280 (TB_3) mg/100 g flour. A sample (TB_c) without $CaCO_3$ was prepared and used as a control (Figure 1(b)). The supplemented levels for wheat flour were within the lower (235 mg/100 g) and the upper (390 mg/100 g) limits of the British Standard for $CaCO_3$ Baking Flour fortification [30]. For statistical analysis, a total of 12 samples including three samples of each treated and control (untreated) bread were processed and analyzed.

The white flatbread was prepared with white flour mixed with water depending on farinograph water absorption, yeast (*Saccharomyces cerevisiae*, 0.6%), and table salt (1.5%). The dough was incubated for the fermentation process for 60 min at 27°C. The fresh dough was divided into pieces (165 ± 5 g) and rested for about 10 min prior to the bread formation. The dough was shaped flat and circular (diameter: ~30 cm) and baked vertically in a gas oven for 5 min.

2.4. Rheological Properties of Bread Supplemented with $CaCO_3$. Rheological properties including water absorption, volume, stability, development time, mixing tolerance index, and farinograph quality parameters of dough were determined by the falling number test according to AACC 56-81B method and International Association for Cereal Science and Technology (115/1) standards [31–33]. In brief, 300 g of control and treated wheat flour were placed in the mixer of the farinograph device, and the flour temperature was adjusted to 30°C for 1 min prior to the test. The standard curve was developed at 500 BU by adding water and controlled to generate a curve. Falling number was determined by placing 7 g of flour at 14% moisture content inside a tube, and 25 mL of distilled water was added. The tubes were shaken before placing them in the boiling water bath of the falling number device. The tubes were shaken again before dropping, and the required time(s) for the tubes was measured and recorded.

In addition, wet gluten and gluten index of flour supplemented with and without $CaCO_3$ were measured according to American Association of Cereal Chemists (AACC, 38-12 method) as shown in equations (1) and (2) for calculating total wet gluten and gluten index:

$$\text{wet gluten, \% (14\% moisture basis)} = \frac{\text{total wet gluten (g)} \times 860}{100 - \text{\% sample moisture}}, \tag{1}$$

$$\text{gluten index} = \frac{\text{wet gluten remaining on sieve (g)}}{\text{total wet gluten (g)}} \times 100. \tag{2}$$

2.5. Extraction and Quantification of Acrylamide. Acrylamide concentration was determined by dehydrating 100 g of each bread at 105 ± 2°C for 4 hours using laboratory drying oven Memmert BM700 (Büchenbach, Germany). The sample including crust and pulp was ground using the Electric Grain Grinder Mill (Cgoldenwall-HC-300Y, China) and sifted using a 0.5 mm sieve. In the second step, Carrez I solution was prepared using 21.9 g of Zn acetate ($Zn(CH_3COO)_2 2H_2O$) and three gram of glacial acetic acid and dissolved in 100 mL distilled water. The second Carrez II solution was prepared with 10.6 g of potassium hexacyanoferrate ($K_4[Fe(CN)_6]3H_2O$) dissolved in 100 mL distilled water. The third step consisted of extraction of acrylamide from bread samples using 1000 mg of dried bread mixed with 5 mL of Carrez I solution followed by 5 mL of Carrez II solution. The final mixture was filtered using paper filter No. 1 (Whatman, Germany). The purification procedure was repeated when the filtrate was not clear using 10 mL of Carrez I and II solutions [34]. For statistical analysis, each bread type was tested in triplicate.

2.6. Determination of Acrylamide Concentration. A standard curve was developed using a standard solution of acrylamide (99%) (Merck, Germany) prepared by dissolving 10 mg of acrylamide in 10 mL distilled water (HPLC grade) using a tenfold serial dilution (1000 mg/L). The analytical HPLC system (Sykam, Eresing, Germany), compatible with the fluorescence spectrophotometer detector (RF20) (excitation: 280 nm and emission: 360 nm wavelengths), was used according to the manufacturer's instruction. The separation of the sample substances was carried out at 37°C using a separation column (300 mm × 3.9 mm) including 10 μm Bonda pack particles. The flow rate of mobile phase was 0.9 mL/min. The mobile phase was composed of acetonitrile and distilled water at a 70 : 30 ratio. The time required for the separation process was 25 min, and the injection volume was 100 μL. The acrylamide concentration peaked at 4.4 min where the retention time was estimated using the following equation:

$$\text{Concentration Sample} = \left(\text{Conc. Standard} \times \frac{\text{Area Sample}}{\text{Area standard}}\right) \cdot \left(\frac{\text{Dilution factor}}{\text{Weight sample}}\right). \tag{3}$$

2.7. Statistical Analyses. For statistical analysis, each sample was processed in triplicate. The data analysis was performed by one-way ANOVA using Tukey's multiple comparison test (GraphPad Prism ver. 8.0). p values <0.05 were interpreted as statistically significant.

3. Results

The types of bread used in the present study showed different properties in the dough based on various factors including the base material, fermentation time, proofing time for dough to rest before baking, oven type, and baking time as well as temperature that substantially contributed to determine the characteristics of each type of bread.

Overall, each type of bread investigated in the present study showed variable concentrations of acrylamide. The analytical HPLC system results revealed that acrylamide was detectable in the white flatbread, whole-wheat flatbread, stone-baked bread, whole-wheat baguette, and white baguette samples at the concentration of 400.9 ± 7.4, 470.2 ± 6.7, 418.8 ± 6.4, 408.3 ± 9.1, and $362 \pm 9.2\,\mu$g/kg. The acrylamide concentration in the whole-wheat flatbread was significantly ($p < 0.05$) higher than other types of bread (Figure 2).

On the other hand, the effect of $CaCO_3$ on acrylamide formation and baking properties showed no significant effect on wet gluten and gluten index (Table 1). The α-amylase activity which coincided with falling number tests of TB_1, TB_2, and TB_3 supplemented with $CaCO_3$ was significantly ($p < 0.05$) higher than α-amylase activity for TB_c control sample. Water absorption characterization of farinograph test of samples supplemented with $CaCO_3$ exhibited 63.8, 63.9, and 63.9% for TB_1, TB_2, and TB_3 with no significant ($p > 0.05$) difference as compared to TB_c (64.1%) (Table 2). On the other hand, the stability time of TB_1 and TB_2 (6.3 min) significantly ($p < 0.05$) increased than TB_3 and TB_c (5.8 min) samples. Moreover, the development time (6.5 min) of TB_c sample was significantly longer as compared to TB_3 (5.0 min) followed by TB_1 and TB_2 (5.7 and 6.1 min), respectively. Among treated samples, TB_2 development time significantly ($p < 0.05$) increased as compared to TB_3 and TB_1. When comparing the mixing tolerance index BU values between treated and control samples, TB_c control sample had significantly ($p < 0.05$) high (45.3) value, while TB_3 sample had significantly lower value (33.9) than TB_1 (35.2) and TB_2 (36.9). Overall, Q number was significantly higher in treated than control sample; however, Q number for TB_1 and TB_2 was 92.9, significantly ($p < 0.05$) higher as compared to TB_3 and TB_c (91 and 87.9) samples (Table 2).

The acrylamide concentration (219 ± 8.5, 121.8 ± 1.7, and $115 \pm 3.4\,\mu$g/kg) in TB_1, TB_2, and TB_3 supplemented with $CaCO_3$ samples was significantly ($p < 0.05$) lower as compared to TB_c ($308.3 \pm 2.9\,\mu$g/kg) sample as illustrated in Figure 3. A coefficient correlation ($R^2 = 0.9971$ and $p < 0.05$) analysis between acrylamide and $CaCO_3$ showed a positive correlation with calcium concentration compared to the TB_c control sample. The results showed an acrylamide concentration reduction with the increase of $CaCO_3$ amount added to the flour where no significant ($p > 0.05$) difference between TB_2 and TB_3 in acrylamide concentration was recorded. However, when $CaCO_3$ was added at the concentration of 280 mg/100 g flour in TB_3 sample, the formation of acrylamide concentration was significantly ($p < 0.05$) lower than TB_c control sample.

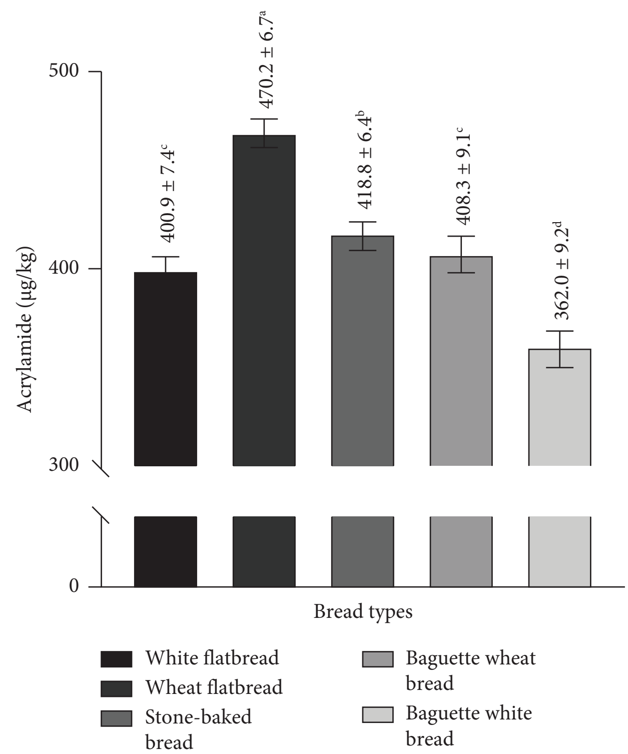


Figure 2: Concentration of acrylamide (μg/kg) in five types of bread using the HPLC method. The values above each bar (labelled as a, b, c, and d) are means, and bars represent standard deviation ($\pm$SD) indicating significant differences ($p < 0.05$).

4. Discussion

Wheat grain protein quantity has a major role in determining the quality of bread due to its function, which is responsible for affecting water absorption capacity, viscosity, elasticity, and dough consistency [35]. The gelatinization of starch and homogenization of dough could be determined by α-amylase activity by falling number [36].

Previous research found that the quantity of acrylamide in wheat flour (165.53 μg/kg) was significantly higher than the concentration (28.03 μg/kg) in white wheat flour bread [37]. This could be due to high asparagine, a main factor for acrylamide formation, in white than wheat flour [37]. The European Program on Heat-Generated Food Toxicants (HEATOX) project reported that the presence of free amino acid asparagine in wheat bran is one of the variables that contribute to an increased concentration of acrylamide in baked goods such as high-bran flour bread, which has a higher level of acrylamide when compared to bread made with low-bran flour [38]. Similarly, Taeymans et al. [39] reported that bran may increase acrylamide concentration in biscuits made from wheat flour. Our results show similar results where high

Table 1: Chemical properties of white flatbread supplemented with various concentrations of $CaCO_3$ (TB_1, TB_2, and TB_3) and control (TB_c) samples.

Attributes	Treatments ($CaCO_3$ mg/100 g flour)			
	TB_c (0)	TB_1 (240)	TB_2 (260)	TB_3 (280)
Wet gluten %	*33.5 ± 1.8**	33.3 ± 0.1	31.8 ± 1.4	33.2 ± 0.2
Gluten index %	92.1 ± 1.5	96.5 ± 1.1	95.1 ± 5.1	95.3 ± 1.0
Falling number	531 ± 28	422 ± 12	466 ± 28	445 ± 51

*Mean values. **Standard deviation values.

Table 2: Characterization of white flatbread supplemented with different concentrations of $CaCO_3$ (TB_1, TB_2, and TB_3) and control (TB_c) samples using the farinograph test.

Attributes	Treatments ($CaCO_3$ mg/100 g flour)			
	TB_c (0)	TB_1 (240)	TB_2 (260)	TB_3 (280)
Water absorption (%)	*64.1 ± 0.17**	63.8 ± 0.25	63.9 ± 0.25	63.9 ± 0.12
Stability (min)	5.8 ± 0.05	6.3 ± 0.1	6.3 ± 0.1	5.8 ± 0.05
Development time (min)	6.5 ± 0.1	5.7 ± 0.05	6.1 ± 0.05	5.0 ± 0.02
Mixing tolerance index BU	45.3 ± 0.35	35.2 ± 0.20	36.9 ± 0.13	33.9 ± 0.05
Q number	87.9 ± 0.35[a]	92.9 ± 0.20[b]	92.9 ± 0.11[b]	91 ± 0.15[a]

*Mean values. **Standard deviation values. a & b indicate significant difference.

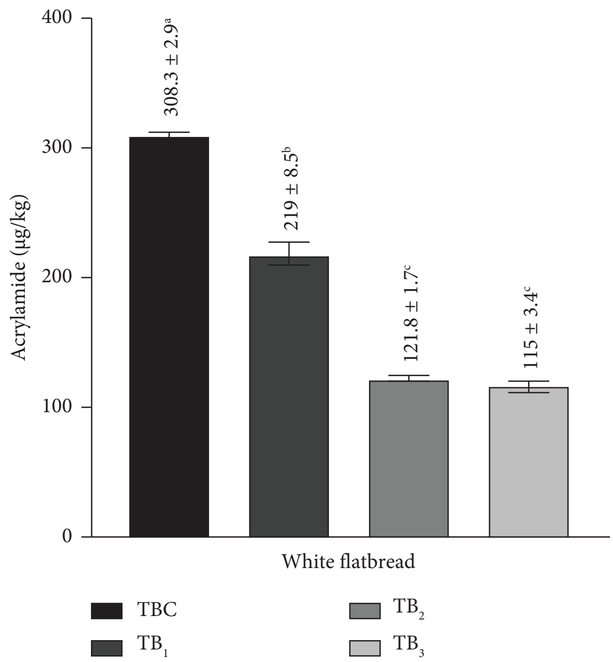


Figure 3: Acrylamide concentration in white flatbread using the HPLC method. The values above each bar (labelled as a, b, and c) are means, and bars represent standard deviation (±SD) indicating significant differences ($p < 0.05$) among samples with and without $CaCO_3$.

acrylamide concentration was recorded in whole-wheat flatbread which explains that it contains a high percentage of asparagine than other types of tested bread. Also, based on the results of this study, the addition of sodium salts to the dough mixture caused strengthening of ionic bond that significantly reduced dough's ability to absorb large amount of water. Sodium salts contribute to the solubility of $CaCO_3$ that prevents a series of Maillard reaction and leads to form acrylamide [40]. Both calcium and magnesium chloride salts impact dough rheological characteristics in baked products by shortening development time and boosting water absorption capacity [41]. Previous study has investigated the effect of crude and partially purified pole bean enzymes on acrylamide concentration in bread where level was reduced by 39.7 and 42%, without changes in the final loaf properties measured by volume, weight, and texture [24]. Similarly, rosemary powder, citric acid, and food salt, separately or in combination, were used at various concentrations on potato fries that reduced acrylamide. The addition of these compounds was successful in reducing the quantity of acrylamide produced by carbohydrate-rich foods that were subjected to high temperatures [42].

Also, a comparison between samples with and without $CaCO_3$ addition has shown no discernible variations in rheological properties including water absorption, dough development duration, or dough stability (Table 2). When bread was supplemented with $CaCO_3$ derived from chicken eggshells, water absorption increased from 63.60 to 67.527%, dough development time increased from 7.38 to 11.37 min, and dough stability increased from 13.79 to 17.37 min which could potentially have an effect on increasing the parameters [43]. On the other hand, our study results are in concordance with previous study where calcium salts were supplemented to reduce acrylamide production in baked products. $CaCl_2$ and calcium lactate (Puracal) have shown a lower concentration of acrylamide in cookies. The results showed that 1% addition of calcium lactate reduced acrylamide concentration from 128 ± 10 to 24 ± 4 ng/g in cookies. Although a slight change in the color was observed, it did not affect the thickness and diameter or the sensory properties of cookies [1]. Also, the effect of adding calcium salts on the reduction of acrylamide in food containing wheat flour was investigated and results showed that positive ions can reduce dough pH, which reduced acrylamide formation after heat treatment. An addition of 0.04 M concentration of $CaCl_2$ or NaCl reduced acrylamide by 36 and 23%, respectively, while acrylamide reduction has not been observed when $CaCO_3$ was added [22]. On the other hand, calcium hydroxide supplemented in maize flour (1.5 g/100 g) reduced acrylamide concentration to 52% in tortilla chips [44]. However, tortillas prepared from maize flour were easy to absorb liquid after adding 1% calcium carbonate and difficult to break after adding calcium hydroxide [45].

Our investigation found the highest acrylamide concentrations in whole-wheat flatbread and stone-baked bread (as shown in Figure 2), suggesting that they have a significant amount of bran, a rich source of free amino acids, a primary precursor of acrylamide during baking process, which may contribute to acrylamide synthesis. The results are in concordance with the previous study where the measured mean concentration of acrylamide was mostly produced in the outer crust layer (>99%) of commercial bread [18]. Similarly, high concentration of acrylamide in the crust was also reported in flatbread [13, 37]. According to US FDA, acrylamide levels may vary depending on the bread type, e.g., grain bread has the highest concentration (59 μg/kg) followed by whole-wheat bread (between 25 and 45 μg/kg) and rye bread (31 μg/kg). According to an EFSA monitoring report, acrylamide had mean 30 μg/kg and maximum 425 μg/kg concentration in bread [6]. The acrylamide concentration in the breads may also be altered by the thickness of the crust, e.g., baguette bread had a lower level of acrylamide than flatbread and stone-baked bread [7]. Interestingly, Trabzon, rye, and oat bread crusts contain 420, 526, and 718 μg/kg of acrylamide, respectively. However, no acrylamide was detected in any of the tested bread crumbs [15]. Since bread is a poor heat conductor, the heat from the oven air is transferred to the bread, resulting in the formation of a temperature and water profile in the bread. At the completion of the baking process, the surface temperature of the loaf increases to 230–250°C, while the inside temperature may reach 100°C or slightly higher temperature. Also, acrylamide production occurs at >120°C [22].

Based on our data results, the acrylamide concentration in treated and control (TB_c, TB_1, TB_2, and TB_3) white flatbread was lower than other commercial breads (Figure 3). These results suggest that the white wheat flour, after removing bran, is more acceptable for baking since it may reduce the amount of asparagine amino acid which is a contributing factor in the formation of acrylamide. Similar results were also observed by Sadd et al. [23] where the group found that eliminating ammonium salts as biscuit enhancer, prolonging the fermentation period of the dough using yeast, lowering the pH, and adding $CaCO_3$ or calcium chloride enhanced acrylamide reduction in the dough. This suggests that mixing wheat flour with other grains such as barley or oats instead of flour with bran may help in reducing acrylamide formation. Further studies are warranted to determine the effect of baking conditions on degree and concentration of acrylamide production.

5. Conclusion

In this study, we report on analyzing the concentration of acrylamide in five types of bread. Overall, acrylamide was detected in all tested breads at a high concentration ranging from 362 to 470 μg/kg. Of five tested bread types, acrylamide was detected at the highest concentration in whole-wheat flatbread followed by stone-baked bread, whole-wheat baguette, and white flatbread. White baguette had a significantly low concentration (362 μg/kg). In addition, $CaCO_3$ supplemented, at various concentrations (240, 260, and 280 mg/100 g), in white flatbread, most commonly used in Iraq, had significantly impacted on reducing acrylamide concentration as compared to the control sample. Interestingly, among three concentrations of $CaCO_3$, 280 mg/100 gm had shown the lowest (115 μg/kg) acrylamide production as compared to the control (308 μg/kg) sample without impacting rheological properties. Overall, the strategy applied for the reduction of acrylamide in white flatbread is promising and can be applied to other types of bakery products. However, further research is necessarily required to investigate its impact on quality, palatability, and shelf life.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (i) High acrylamide concentration was detected in whole-wheat flatbread as compared to four other bread types. (ii) Calcium carbonate reduces acrylamide production in bread. (iii) Calcium carbonate did not impact on rheological properties and quality of white flatbread.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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References

[1] Ö. Ç. Açar, M. Pollio, R. Di Monaco, V. Fogliano, and V. Gökmen, "Effect of calcium on acrylamide level and sensory properties of cookies," *Food and Bioprocess Technology*, vol. 5, pp. 519–526, 2012.

[2] FDA, *Survey Data on Acrylamide in Food: Individual Food Products*, FDA: Silver Spring, Silver Spring, MD, USA, 2004.

[3] D. R. Lineback, J. R. Coughlin, and R. H. Stadler, "Acrylamide in Foods: a review of the science and future considerations," *Annual Review of Food Science and Technology*, vol. 3, pp. 15–35, 2012.

[4] H. Streekstra and A. Livingston, "Acrylamide in bread and baked products," in *Breadmaking*, pp. 289–321, Elsevier, Amsterdam, Netherlands, 2020.

[5] T. Y. Curtis and N. G. Halford, "Reducing the acrylamide-forming potential of wheat," *Food and Energy Security*, vol. 5, pp. 153–164, 2016.

[6] EFSA, "Scientific opinion on peste des petits ruminants," *EFSA Journal*, vol. 13, p. 3985, 2015.

[7] FDA, *Chemical Contaminants in Food, Acrylamide*, U.S. Food and Drug Administration (FDA), Silver Spring, MD, USA, 2021.

[8] K. Miśkiewicz, E. Nebesny, J. Rosicka-Kaczmarek, D. Zyźelewicz, and G. Budryn, "The effects of baking conditions on acrylamide ˙ content in shortcrust cookies with added freeze-dried aqueous rosemary extract," *Journal of Food Science and Technology*, vol. 55, pp. 4184–4196, 2018.

[9] FAO and WHO, *Health Implications of Acrylamide in Food: Report of a Joint FAO/WHO Consultation*, WHO Headquarters, Geneva, Switzerland, 2002.

[10] C. Sarion, G. G. Codină, and A. Dabija, "Acrylamide in Bakery Products: a review on health risks, legal regulations and strategies to reduce its formation," *International Journal of Environmental Research and Public Health*, vol. 18, p. 4332, 2021.

[11] L. Ahrné, C.-G. Andersson, P. Floberg, J. Rosén, and H. Lingnert, "Effect of crust temperature and water content on acrylamide formation during baking of white bread: steam and falling temperature baking," *LWT-Food Science and Technology*, vol. 40, pp. 1708–1715, 2007.

[12] A. Gerssena, T. Boveea, L. van Ginkela, M. van Ierselb, and R. Hoogenbooma, "Food and feed safety: cases and approaches to identify the responsible toxins and toxicants," *Food Control*, vol. 98, pp. 9–18, 2019.

[13] J. Keramat, A. LeBail, C. Prost, and M. Jafari, "Acrylamide in baking products: a review article," *Food and Bioprocess Technology*, vol. 4, pp. 530–543, 2011.

[14] P. Rydberg, S. Eriksson, E. Tareke, P. Karlsson, L. Ehrenberg, and M. Törnqvist, "Factors that influence the acrylamide content of heated foods," in *Chemistry and Safety of Acrylamide in Food. Advances in Experimental Medicine and Biology*, M. Friedman and D. Mottram, Eds., vol. 561, Boston, MA, USA, Springer, 2005.

[15] H. Z. Şenyuva and V. Gökmen, "Survey of acrylamide in Turkish foods by an in-house validated LC-MS method," *Food Additives and Contaminants*, vol. 22, pp. 204–209, 2005.

[16] EU, "Regulation (EC) No. 1333/2008 of the European Parliament and of the council of 16 December 2008 on food additives," 2008, http://data.europa.eu/eli/reg/2008/1333/oj.

[17] EFSA, "Results on acrylamide levels in food from monitoring years 2007–2009 and exposure assessment," *EFSA Journal*, vol. 9, pp. 1–48, 2011.

[18] N. Surdyk, J. Rosén, R. Andersson, and P. Åman, "Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread," *Journal of Agricultural and Food Chemistry*, vol. 52, pp. 2047–2051, 2004.

[19] V. Sirot, F. Hommet, A. Tarda, and J. C. Leblan, "Dietary acrylamide exposure of the French population: results of the second French total diet study," *Food and Chemical Toxicology*, vol. 50, no. 3–4, pp. 889–894, 2012.

[20] W. Parzefall, "Minireview on the toxicity of dietary acrylamide," *Food and Chemical Toxicology*, vol. 46, pp. 1360–1364, 2008.

[21] FAO and WHO, *Proposed Draft Code of Practice for the Reduction of Acrylamide in Food*, WHO, Geneva, Switzerland, 2007.

[22] R. A. Levine and S. M. Ryan, "Determining the effect of calcium cations on acrylamide formation in cooked wheat products using a model system," *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 6823–6829, 2009.

[23] P. A. Sadd, C. G. Hamlet, and L. Liang, "Effectiveness of methods for reducing acrylamide in bakery products," *Journal of Agricultural and Food Chemistry*, vol. 56, pp. 6154–6161, 2008.

[24] H. N. Al Zobaidy, K. A. Shakir, S. Abdulhussain, and G. M. Strasburg, "Occurrence of Acrylamide in selected Iraqi bakery products and mitigation by partially purified L-Asparaginase," *Journal of the College of Basic Education*, vol. 22, pp. 1–10, 2016.

[25] A. S. Alhendi, D. Z. Al-Shalchi, R. T. Alsallami, and Z. S. Alobaidy, "Effect of flour extraction rate on the sensory evaluation of Iraqi flatbread," *Current Nutrition and Food Science*, vol. 17, pp. 204–209, 2021.

[26] N. Roohani, R. Hurrell, R. Wegmueller, and R. Schulin, "Zinc and phytic acid in major foods consumed by a rural and a suburban population in central Iran," *Journal of Food Composition and Analysis*, vol. 28, pp. 8–15, 2012.

[27] B. Y. Al-Abdullah, "The effect of some of the ingredients of the mixture and the process of manufacturing in the quality of bread and the characteristics of conservation," MS.c. Thesis, Agriculture Science, Faculty of Agriculture, Basra University, Basra, Iraq, 1986.

[28] M. L. Khaleel, A. M. Sharoba, A. I. El-Desouky, and M. H. Mahmoud, "Use of some hydrocolloids to improve the quality of Iraqi bread (Simon) product," in *Proceedings of the 4th International Conference on Biotechnology Applications in Agriculture (ICBAA)*, pp. 299–314, Benha University, Moshtohor and Hurghada, Egypt, January 2018.

[29] P. Baardseth, K. Kvaal, P. Lea, M. R. Ellekjær, and E. M. Færgestad, "The effects of bread making process and wheat quality on French baguettes," *Journal of Cereal Science*, vol. 32, pp. 73–87, 2000.

[30] British Food Standard Agency, "The bread and flour regulations (UK statutory instruments No. 1998 No. 141)," 2008, https://www.legislation.gov.uk/uksi/1998/141/contents/made.

[31] AACC, *Approved Methods of the American Association of Cereal Chemists*, 11th edition, 2010.
[32] AACC, *Approved Methods of the American Association of Cereal Chemists*, The American Association of Cereal Chemists, St. Paul, MN, USA, 10th edition, 2000.
[33] ICC, *Standard No: 115/1 Method For Using the Brabender Farinograph*, International Association for Cereal Science and Technology, Vienna, Austria, 1972.
[34] Z. Geng, R. Jiang, and M. Chen, "Determination of acrylamide in starch-based foods by ion-exclusion liquid chromatography," *Journal of Food Composition and Analysis*, vol. 21, pp. 178–182, 2008.
[35] H. Wieser, "Chemistry of gluten proteins," *Food Microbiology*, vol. 24, pp. 115–119, 2007.
[36] H. Perten, "Application of the falling number method for evaluating alpha-amylase activity," *Cereal Chemistry*, vol. 41, pp. 127–140, 1964.
[37] M. Negoita, I. Enuta, A. Alina et al., "Analysis and evaluation of the acrylamide levels in some bread assortments on the Romanian market by GC-MS/MS," *Journal of Environmental Engineering*, vol. 5, pp. 180–189, 2016.
[38] K.-E. Hellenas, L. Abramsson-Zetterberg, and K. Skog, "The HEATOX project," *AOAC International*, vol. 88, pp. 242–245, 2005.
[39] D. Taeymans, J. Wood, P. Ashby et al., "A Review of Acrylamide: an industry perspective on research, analysis, formation, and control," *Critical Reviews in Food Science and Nutrition*, vol. 44, pp. 323–347, 2004.
[40] B. Coto, C. Martos, J. L. Peña, R. Rodríguez, and G. Pastor, "Effects in the solubility of $CaCO_3$: experimental study and model description," *Fluid Phase Equilibria*, vol. 324, pp. 1–7, 2012.
[41] M. N. Bassett, T. Pérez-Palacios, I. Cipriano et al., "Development of bread with NaCl reduction and calcium fortification: study of its quality characteristics: bread NaCl reduction and Ca fortification," *Journal of Food Quality*, vol. 37, pp. 107–116, 2014.
[42] I. H. A. Al-Anbari, A. T. Al-Musawi, M. T. H. Al-Ani, and I. O. C. AlKaraquly, "Effect of addition of various proportions of rosemary ppwder, citric acid and table salt in reducing the ratios of acrylamide in potato fries," *Plant Archives*, vol. 19, pp. 1223–1229, 2019.
[43] F. A. Khan, K. Ameer, M. A. Qaiser et al., "Development and analysis of bread fortified with calcium extracted from chicken eggshells of Pakistani market," *Food Science and Technology*, vol. 41, pp. 14–20, 2021.
[44] R. Salazar, G. Arámbula-Villa, G. Luna-Bárcenas, J. D. Figueroa-Cárdenas, E. Azuara, and P. A. Vázquez-Landaverde, "Effect of added calcium hydroxide during corn nixtamalization on acrylamide content in tortilla chips," *LWT--Food Science and Technology*, vol. 56, pp. 87–92, 2014.
[45] M. Otsuka, H. Moritaka, H. Fukuba, S. Kimura, and M. Ishihara, "Effect of calcium carbonate and calcium hydroxide on physical properties of tortilla," *Journal of the Japanses Society for Food Science and Technology*, vol. 48, pp. 759–767, 2001.