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Correlation between acrylamide content and colour in some baked products

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Abstract: The objective of this study was to quantify the concentration of acrylamide in biscuits, bread, and cake obtained from industrial and local bakeries, classified based on their colour intensity as light, medium, or dark. The analysis was performed using gas chromatography-mass spectrometry (GC-MS). Sugar, moisture, and asparagine analyses were also carried out simultaneously. The results demonstrated that the acrylamide content in biscuits, bread, and cakes varied according to the colour (light 109.9 ± 7.95, 214.7 ± 27.40, and 128.6 ± 7.05; medium 176.3 ± 15.16, 387.0 ± 87.71, and 804.3 ± 17.16; and dark 407.6 ± 105.13, 555.8 ± 16.20, and 1 015.0 ± 83.68 $\mu g \cdot kg^{-1}$, respectively). Statistically, significant differences were observed between acrylamide content and product colour density ($P < 0.05$). It can be concluded that the acrylamide content increases as the product colour intensity rises due to the increased baking temperature at which the reaction between reducing sugars and asparagine takes place, resulting in the formation of acrylamide. The results suggest that selecting commercial bakery products based on colour may be beneficial for reducing the daily intake of acrylamide by consumers. Therefore, it is recommended to avoid dark-coloured bakery products.

Keywords: asparagine; biscuit; bread; cake; gas chromatography-mass spectrometry (GC-MS)

Acrylamide is an organic crude chemical compound used for industrial purposes (Stadler and Scholz 2004; Zhao et al. 2021). It is a white crystalline solid with a low molecular weight that is highly soluble in water (Friedman 2003; Mollakhalili-Meybodi et al. 2021). It has been classified as probably carcinogenic to humans by the International Agency for Research on Cancer due to its carcinogenic effects in rodents (Tareke et al. 2000; Mori et al. 2022). In 2002, significant levels of acrylamide were discovered in starch-based foods by the Swedish National Food Agency (SNFA) and Swedish researchers at Stockholm University (Geng et al. 2011; Sarion et al. 2021). Since acrylamide is considered unavoidable in some foods, researchers focused on the effects of food processing, which play a significant role in its formation (Deribew and Woldegiorgis 2021). Some studies suggest that food's temperature, time, and composition may have different effects on acrylamide (Gökmen et al. 2006). Nevertheless, the reaction between asparagine and carbonyl sources at temperatures above 120 °C is the most probable route to acrylamide formation during the Maillard reaction (Omar et al. 2015). Fernandes et al. (2019) reported that acrylamide formation in bakery products

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is parallel to the browning process. Under controlled laboratory conditions, a direct correlation between browning colour and acrylamide content in baked goods has been established (Mesias et al. 2021). In this way, a large part of the population is exposed to acrylamide through food (Bušová et al. 2020) because the colour of the food surface is usually the first quality parameter evaluated by consumers and is crucial for the acceptance of the product (Purlis 2010).

For this reason, it is necessary to introduce recommendations for consumers to reduce the excess of acrylamide in bakery products. Therefore, the selection matrix was based on the fact that these foods are a daily source of acrylamide due to their widespread consumption. It is important to gain more knowledge about acrylamide formation in some bakery products and find an approach to control it. In this study, the samples of different colours were selected based on the consumer's perspective, and the main goal of this study was to determine the acrylamide content of some bakery products, such as bread, biscuit, or cakes, and then estimate the relationship between acrylamide content and browning colour.

MATERIAL AND METHODS

Reagents and standards. Acrylamide (99%), reducing sugar (98%), and asparagine (98%) were obtained from Sigma Aldrich (Madrid, Spain). Hexane, hydrochloric acid, and methanol were purchased from Merck (Darmstadt, Germany). All solvents were suitable for liquid chromatography. Ultrapure water was obtained from Milli-Q Water Purification System (Millipore, USA). Isolute ENV+ (6 mL, 500 mg) and Isolute Multimode (6 mL, 500 mg) solid phase extraction (SPE) cartridges were purchased from Biotage (Uppsala, Sweden). Acrylamide standard stock solution (1 mg·mL^{-1}) was prepared with methanol. Working standard solutions were prepared by diluting the stock solution into concentrations in the range of 20–2 000 μg·kg^{-1}.

Samples collection. Totally, 27 bakery products (bread, cake, and biscuit) were randomly selected from different production batches and local bakeries in Khartoum, Sudan. Before analysis, the samples were dried at a temperature of 105 °C. The dried samples were then homogenised using a stainless-steel blender and stored in polyethylene bottles until analysis.

Determination of acrylamide content. Preparation of samples for determination of acrylamide content was done according to Karasek et al. 2009, and the extraction of acrylamide was conducted with some modifications. An amount of 2 g of the homogenised sample was taken and mixed with 40 mL of ultrapure water in 50 falcon tubes. The mixture was shaken with a vortex shaker (IKA® Vortex 3 Shaker; Sigma-Aldrich, St. Louis, USA) for 15–30 s. Then it was centrifuged in a cooled centrifuge (10 °C, 2 683 g, 20 min) after 60 min on a horizontal shaker (WiseShake SHR-1D; SciLab, Seoul, South Korea) set to the maximum sample-extractant agitation speed (e.g. 100 rpm). The precipitate was discarded after centrifugation, and the supernatant was passed through a preconditioned Isolute multimode cartridge. The eluate was collected and then placed on a preconditioned Isolute ENV+ cartridge. Acrylamide was eluted with 3 mL of 60% methanol in water. The eluant was evaporated to dryness under a gentle nitrogen flow at 40 °C. The remaining residue was dissolved in 1 mL of methanol. Then the samples were transferred to vials, and 10 μL portions of each sample were passed through a HP 5989 gas chromatography coupled with a mass-selective detector (Hewlett-Packard, USA) operating in selected ion monitoring mode with positive electron impact (EI) ionisation and spitless. Analyses were run under the following conditions: reagent gas: helium, flow rate: 0.9 mL·min^{-1}, column: HP-5MS capillary fused silica (J & W Scientific, Agilent, USA), oven 60 °C (2 min), 10 °C (1 min) to 200 °C (5 min) (total run time: 21 min). The observed ions were m/z 70 and 149, and the retention time for acrylamide was 6.6 min.

Reducing sugar content. Samples were prepared to determine sugar content according to the method published by Claus et al. (2008) with minor modifications. After grinding the samples,10 g of the sample was weighed into a 250 mL Erlenmeyer flask with 100 mL of 60% ethanol, homogenised with an Ultra-Turrax T25 (Janke & Kunkel, Germany), and sonicated for 20 min in a water bath at 70 °C. The sample was centrifuged for 5 min at 671 g, the liquid layer was filtered through a 0.45 μm membrane filter, and 10 μL was then injected into the high performance liquid chromatography (Agilent HPLC series 1100; Agilent, USA) is equipped with a refractive index detector (RID-10A; Agilent, USA) used for sample sugar analysis. An Agilent Zorbax carbohydrate analysis column (150 mm × 4.6 mm, 5 μm) was used. HPLC conditions were set as follows: the mobile phase consisted of 80% acetone and 20% water, pumped at a flow rate of 1.4 mL·min^{-1} under isocratic conditions. injection volume, 20 μL, and the column temperature were set to 25 °C. The amounts of the different sugars (glucose and fructose) were identified using external standards.

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Asparagine content. Sample preparation for asparagine determination was carried out according to the method described previously by Hippe (1988). After weighing, 1 g of the dried sample was shaken for at least 2 min with 10 mL dichloromethane and 36 mL water. After adding 4 mL 0.1N HCL, the extract was centrifuged (2 683 g, 15 min), and the aqueous phase was removed. The remaining sediment and dichloromethane phase was extracted again with 36 mL water, centrifuged after adding HCL and combined the two aqueous phases, the last 10 µL being injected into the amino analysis. Amino acid analyser S433 (Sykam, Fürstenfeldbruck, Germany) with a column filled with the cation-exchange resin LCA K07/Li (150 mm × 4.6 mm, 7 µm) was used for the separation. After separation, postcolumn derivatisation with ninhydrin was applied, and reaction products were detected photometrically (440, 570 nm). The flow rates for the mobile phase and reagent solution were 0.45 and 0.25 mL·min^{-1}, respectively. The injection volume was 100 µL.

Moisture content. To determine the moisture content, a 2 g sample was placed in a predried dish and subjected to drying in an oven at 105 °C for approximately 6 h. After drying, the covered sample was placed in a desiccator and cooled to room temperature before being weighed again. The weight loss was calculated as a percentage of the sample weight and expressed as moisture content (AOAC 2000).

Statistical analysis. The data were statistically analysed using the SPSS program (version 22.0). The difference between the data was evaluated using One-Way ANOVA and the post hoc test (Tukey) method.

RESULTS AND DISCUSSION

For acrylamide analysis, the linearity was evaluated in the range of 20–2 000 µg·L^{-1} between acrylamide concentration and peak area by injection of 10 µL. Linearity with an R^2 value of 0.99 was observed for acrylamide calibration. The limit of detection (LOD = 5 µg·L^{-1}) and limit of quantification (LOQ = 16 µg·L^{-1}) were estimated by the baseline noise method when the signal-to-noise (S/N) ratio was 3 and 10, respectively. The range and mean value of acrylamide concentration of light, medium, and dark bakery products are presented in Table 1. The results showed that acrylamide was detected in all samples. The minimum concentration of acrylamide was found in light biscuits (109.9 ± 7.95 µg·kg^{-1}), and the maximum concentration in dark cakes (1 015.0 ± 83.68 µg·kg^{-1}).

This investigation examined colour differences affecting acrylamide content in bakery products. The content of acrylamide increased with increasing colour from light to dark brown. The amount of acrylamide measured in the bread samples increased with increasing colour, reaching an apparent maximum of 555.8 ± 16.20 µg·kg^{-1} in the dark colour and then 214.7 ± 27.40 µg·kg^{-1} in the light and 387.0 ± 87.71 µg·kg^{-1} in the medium colour. The mean and median acrylamide values of the cake were 128.6 ± 7.05, 804.3 ± 17.16, and 1 015.0 ± 83.68 µg·kg^{-1} for light, medium and dark colour, respectively. In the biscuit samples, we obtained the same results, acrylamide varied between 109.9 ± 7.95, 176.3 ± 15.16, and 407.6 ± 105.13 µg·kg^{-1} for light, medium and dark colour, respectively (Table 1). The results indicate that there were significant differences between the mean value of acrylamide in the light, medium and dark samples for bread, biscuits, and cakes ($P < 0.05$). As shown in the images in Figure 1, the browning of bread, biscuits, and cakes associated with the increase in acrylamide content is clear. According to this result, there is a correlation between the amount of acrylamide content and the colour of bread, biscuits, and cakes. This

Table 1. Acrylamide contents of some bakery products (µg·kg^{-1}, n = 3)

Sample type	Average acrylamide content*	Range
Light bread	214.7 ± 27.40	189.3–243.7
Medium bread	387.0 ± 87.71	298.8–474.1
Dark bread	555.8 ± 16.20	378.9–699.0
Light biscuit	109.9 ± 7.95	101.9–117.8
Medium biscuit	176.3 ± 15.16	161.7–192.0
Dark biscuit	407.6 ± 105.13	294.9–503.0
Light cake	128.6 ± 7.05	120.5–133.0
Medium cake	804.3 ± 17.16	786.0–820.0
Dark cake	1 015.0 ± 83.68	923.4–1 087.6

* mean ± standard deviation (SD)

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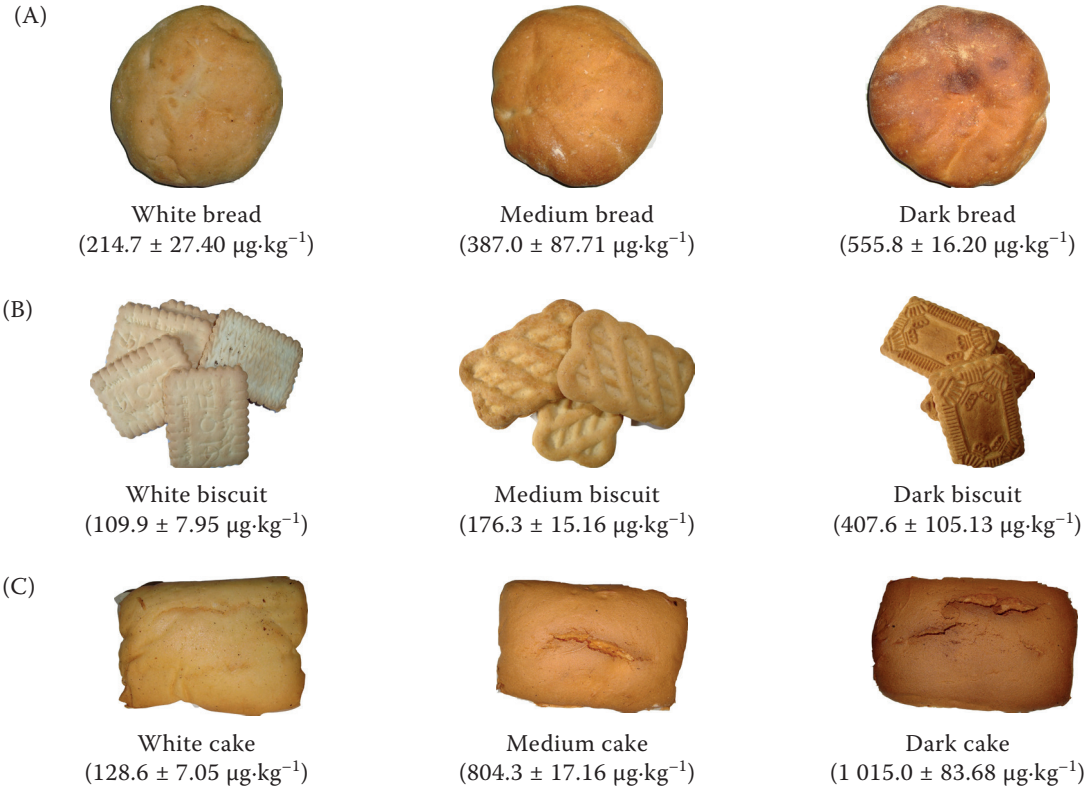

Figure 1. Photographs of (A) bread, (B) biscuit, and (C) cake of various colours with the mean value of acrylamide concentration

result is consistent with Surdyk et al.'s (2004) finding, which reported a highly significant correlation between acrylamide and colour content. It should be noted that the heating process was the most important factor in the correlation between colour and acrylamide (Gökmen et al. 2006). Amrein et al. (2004) demonstrated this by finding a significant relationship between colour and acrylamide content in foods baked at 180 °C.

The current results support the hypothesis that the raw materials, particularly free asparagine and reducing sugars such as glucose in cereal flours, are the most important factors influencing acrylamide formation (Mustatea et al. 2015). In the present study, the mean free asparagine concentration changes with the colour and acrylamide level, a high level in the light-coloured product (2.73, 1.00, and 8.73 µmol·L^{-1}), a low level in the dark (0.33, ND, and 3.13 µmol·L^{-1}) and in the medium-coloured product (2.46, 0.001, and 4.27 µmol·L^{-1}) were found in bread, biscuit, and cake, respectively (Figure 2).

The increase in acrylamide content with the increase in colour could be attributed to the sugar content be-

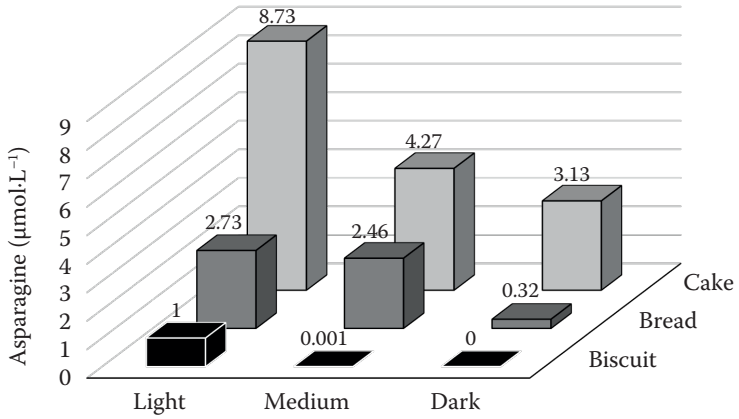

Figure 2. Free asparagine levels in bakery products

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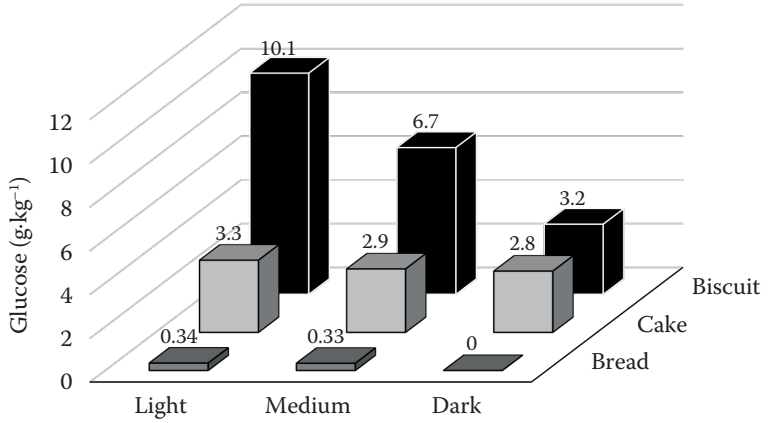

Figure 3. Glucose level in bakery products

cause sugars and the type of sugar are the main variables affecting colour formation in bakery products (Purlis 2010). Furthermore, it is now widely accepted that acrylamide is primarily formed by the reaction of asparagine with reducing sugars, mainly glucose, which is the most common reducing sugar in plants and has the greatest impact on acrylamide formation (Zhang et al. 2006). This study showed that glucose content was higher in light-coloured samples than in medium and dark-coloured samples, suggesting that some glucose plays a role in the increased acrylamide formation in dark-coloured bread, biscuit, and cake samples (Figure 3). Alvarez-Morezuelas et al. (2021) reported that the glucose concentration in potato chips was most strongly correlated with acrylamide formation. This study supports the current finding. In previous reports, fructose was found to contribute to the formation of higher amounts of acrylamide (Ciesarová et al. 2006). In contrast, in the present study, fructose results did not show a consistent direction with increased darkness (Figure 4).

It has been observed in this study that the moisture content decreased as the colour and acrylamide content increased (Figure 5). Water content appears to be a more important factor affecting acrylamide yield. Additionally, it significantly influences the chemical pathway and molecular mobility of chemical constituents, indirectly contributing to acrylamide formation (Ciesarová et al. 2006). A preview study was conducted on a model biscuit system to evaluate acrylamide formation in relation to moisture content. No acrylamide was detected at a 10% moisture level. Still, at a 2% and 6% moisture level, acrylamide ranged from 165 to 363 μg·kg^{-1} (Taeymans et al. 2004). These results are similar to the findings of our study.

The data obtained in the study showed that acrylamide was high in bread, biscuit, and cakes. The European Commission (2017) recommended a value of 50 and 350 μg per kg as the benchmark level for wheat-based bread and biscuits. Among these analysed, biscuits and bread had acrylamide content above the benchmark level of European Food Safety Authority (EFSA).

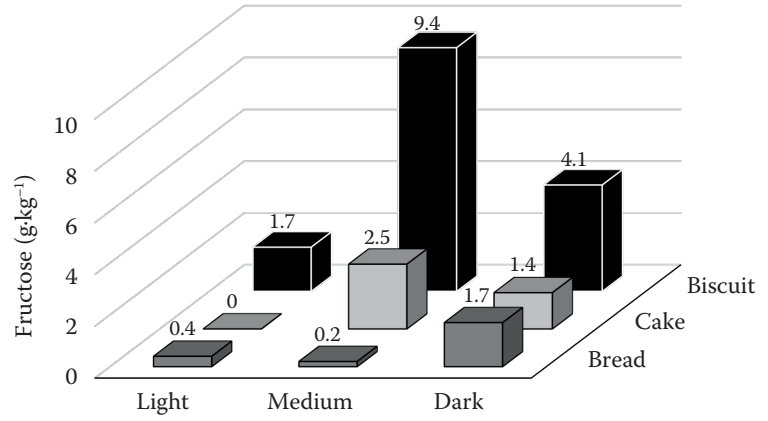

Figure 4. Fructose levels in bakery products

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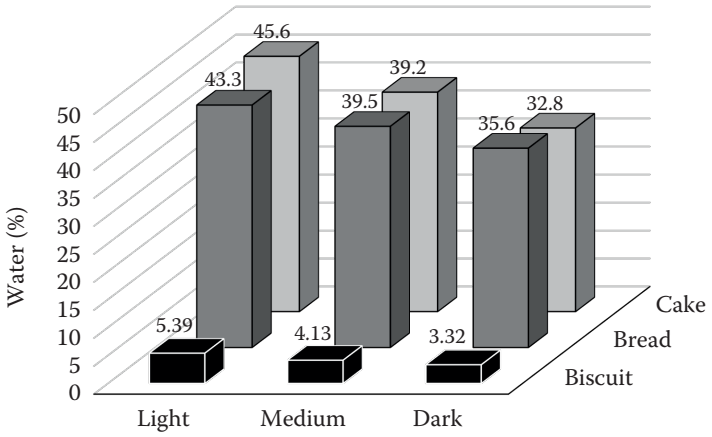

Figure 5. Water levels in bakery products

CONCLUSION

This study investigated acrylamide formation in some bakery products of different colour classes (bread, biscuit, and cake). It was apparent that acrylamide formed in this type of bakery products, and the acrylamide content increased with colour. The highest concentrations of this contaminant were found in the cake of various colours: 1 015.0 $\mu g \cdot kg^{-1}$ for dark, 804.3 $\mu g \cdot kg^{-1}$ for medium. The findings of this study confirmed that acrylamide levels in baked foods (bread and biscuits) are typically higher than benchmark levels (BL) established by the European Commission regulation (2017). This difference is due to the main compound in this study, which causes acrylamide formation. Like sugar reduction, asparagine, the main cause of the colour in bakery products, is also an essential factor in forming acrylamide. At the same time, the process of technology, such as temperature and time, clearly affects the level of hazardous compounds in bakery products. The results suggest that colour-based selection for these commercial bakery products could be beneficial in reducing the daily intake of acrylamide levels by consumers.

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