Journal of Food and Agriculture Research
Vol. 4, No. 2, 2024, pp. 113-130

URL: www.arfjournals.com
https://doi.org/10.47509/JFAR.2024.v04i02.01

Effect of Baking Time-temperature Combination on the Formation of Processing Toxicants and Physicochemical Properties of Biscuit

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Abstract: Acrylamide (AA) and 5-hydroxymethylfurfural (HMF) formation in baked goods is a concern due to their potential health risks. This study investigated the effects of different baking time-temperature combinations on the formation of HMF and AA and biscuits' physicochemical, sensory, and textural properties. Results revealed that increasing baking time and temperature significantly decreased biscuits' residual reducing sugar and asparagine levels. Additionally, color analysis showed increased browning intensity with higher baking temperatures, indicating the presence of melanoidins. The sensory evaluation demonstrated that overall acceptability increased with baking time and temperature, particularly at 180°C for 20 and 25 min. Finally, AA and HMF content in biscuits increased significantly with higher baking temperatures and longer durations. This study provides valuable insights for the food industry to produce healthier and safer biscuit products.

***Keywords*:** Baking, Time-Temperature Reducing sugar, Acrylamide, Hydroxymethylfurfural, Asparagine.

Received : 28 June 2024
Revised : 20 July 2024
Accepted : 26 July 2024
Published : 24 December 2024

TO CITE THIS ARTICLE:

Verma, V., Singh, Z., & Yadav, N. 2024. Effect of Baking Time-temperature Combination on the Formation of Processing Toxicants and Physicochemical Properties of Biscuit. *Journal of Food and Agriculture Research,* 4: 2, pp. 113-130. *https://doi.org/10.47509/JFAR.2024.v04i02.01*

1. Introduction

Biscuits represent a universally beloved snack enjoyed for convenience, taste, and nutritional content. However, the production of biscuits presents a dual challenge for the food industry: achieving sensory excellence and ensuring food safety. Among the various compounds formed during baking, acrylamide (AA) and 5-hydroxymethylfurfural (HMF) have emerged as significant concerns due to their potential health implications. These compounds are formed as byproducts of the Maillard reaction and caramelization processes,

particularly when foods rich in reducing sugars and asparagine are subjected to high temperatures (Mottram *et al*., 2002; Verma and Yadav, 2022a). However, health risks such as diabetes, cancer, coronary heart disease, and hypertension are associated with consuming fried foods (Milicevic *et al*., 2020). Cancer is a global health problem, with an estimated 19.3 million cases and 9.9 million deaths in 2020 and projections indicating an increase in cases up to 28.4 million by 2040 (Sung *et al*., 2021). Efforts to mitigate the presence of AA and HMF in biscuits align with evolving consumer preferences for healthier and safer food options. So, it is essential to reduce the formation of these compounds and preserve the desired physicochemical properties, sensorial attributes, and textural characteristics of biscuits (Kukurova *et al*., 2013). Previous research has shown that baking parameters, particularly time and temperature, play a crucial role in modulating the formation of AA and HMF. Understanding how these variables influence biscuits' sensory, textural, and chemical properties is essential for developing strategies to minimize the presence of harmful compounds while maintaining product quality (Lee *et al*., 2020; Mesias *et al*., 2020). In this study, we aim to investigate the influence of various baking time-temperature combinations on the formation of HMF and AA and their subsequent effects on biscuits' physicochemical, sensorial, and textural properties. By comprehensively examining these factors, we seek to contribute valuable insights to developing healthier and safer biscuit products that meet consumer demands and regulatory standards.

2. Materials and Methods

2.1. Preparation of biscuits

The biscuits recipe followed the American Association of Cereal Chemists (AACC) method: 80 g refined flour, 17.6 g deionized water, 35 g sucrose, 1.0 g sodium chloride, 1.0 g sodium bicarbonate, 20 g butter (AACC, 2000). Model biscuit recipes were modified by the replacement of flour with other flour/the replacement of salt with additives/addition of sugar alternatives.

2.2. Physical evaluation of the biscuits

The physical evaluation of the biscuits includes thickness and diameter measures according to the AACC (1995) methods. Five biscuits were placed edge to edge to determine the thickness, and the combined diameter was measured, resulting in an average value reported in centimeters. Four biscuits were aligned edge to edge for the diameter, and the average value was reported

in centimeters. The spread ratio was then calculated by dividing the diameter by the thickness.

2.3. Asparagine analysis

A spectrophotometer estimated the asparagine content in french fries (Hurst *et al*., 1995). The samples were extracted with methanol and ground in a 10 mL solution (1:4 methanol: 0.133 M Tris HCI) (pH 6.0). pH levels were adjusted to 6 for the amino acid analysis, and the isolated solution was centrifuged with an alcoholic ninhydrin solution. The absorbance of solutions were measured at 340 nm after 3 hours of incubation at 37°C using a spectrophotometer. The 1 mL of methanol-Tris and 9 mL of ethanolic ninhydrin were used for the blank.

2.4. Total ash (AOAC, 2016)

The charring process determined the ash percent in the sample. 5 g sample was placed in a silica crucible and fired on a Bunsen burner until the fumes stopped (charring procedure), after which it was transferred to a muffle furnace until clean ash was achieved. The weight of the residue was recorded, and the percentage of ash was computed using the following formula:

$$Ash\ (\%) = (Wt.\ of\ residue) / (Wt.\ of\ sample) \times 100$$

2.5. Water activity

The water activity meter Aqualab (Decagon Device USA) at 25 °C was used to determine the water activity of crushed potato samples.

2.6. Moisture Content

Moisture percent of the sample was estimated by hot air drying method. 5 g of samples was placed in a hot air oven at 70 °C for 22 h until a consistent weight was achieved. Calculate the raw and fried samples' moisture content (%) (AOAC 2016).

2.7. Color Value

An Xrite colorimeter (Grandville, MI, USA) was used to measure the color value in the raw and fried samples (Grandville, MI, USA). The following formula can be used to determine the color differences referred to as the Euclidean distance (ΔE), where lightness (L*) varies from 0 to 100, a* (green to red), and b* (blue to yellow) both range from -120 to 120.

$$\Delta E = \sqrt{\{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\}}$$

ΔL, Δa, and Δb were the difference between L*-L, a*-a, and b*-b, respectively. The letters L*, a*, and b* stand for the color values of French fries, while the letters L, a, and b stand for the color values of raw potato strips. The CIE values of L*a*b* were used to calculate the Browning index (BI) (Mohapatra *et al*., 2010):-

$$BI = [100 \times (X - 0.31)] / (0.17)$$

Where, $$X = (a^* + 1.75L)/(5.645L + a^* - 3.012b^*)$$

2.8. HMF

The UV-spectrophotometer method measured the HMF of French fries (White, 1979). Transfer 5 g of the homogenized sample to a 50 mL volumetric flask and add 25 mL of water. Add 0.25 mL of Carrez I solution and 0.25 mL of Carrez II solution (1-2 drops of alcohol may also be added to suppress surface bubbles). The initial 10 mL was discarded after filtration. Transfer 5 mL of supernatant to each of the two test tubes, then add 5 mL of ultrapure water to the sample and 5 mL of bisulfite at 0.2 percent to the blank test tubes. Note the absorbance of the sample at 284 and 336 nm to the standard and calculate the HMF content in the formula by putting the absorbance value.

2.9. AA

High-Pressure Liquid Chromatography quantified french fries' AA content with a Diode Array Detector (HPLC-DAD). The sample preparation involved defatting with hexane 3 times, followed by AA extraction using acetone. The purification was done through syringe filters according to the modified method by Geng *et al*. (2008). The defatted mixture weighed 1 g and was centrifuged with 10 ml HPLC water in 11200g for 10 min at 10 °C. After transferring the filtrate to a new centrifuge tube, 0.5 mL Carrez I and II of each were added to the solution. Discard the first 2 ml of filtrate; the rest 8 mL was dried under a vacuum oven at 40 ºC, and add 20 mL of acetone. Vortexed the sample for 10 minutes and kept it at 40°C for about 20 minutes in an ultrasonic water bath. The solvents were filtered through a filter paper (Whatman No.1). The filtrate was evaporated under a rotary evaporator to dryness. 2 ml of mobile phase HPLC grade water: acetonitrile (90:10) was added and shaken thoroughly to dissolve the residue. The aqueous solution was purified using 0.45 µm of syringe filter paper (cellulose acetate) (Merck, Sigma Aldrich). Samples were

injected with a 50µl manual syringe into the HPLC system (Agilent) (Model no. 1260 Infinity, Agilent Technologies, USA). An isocratic elution pattern was adopted to separate the analyte, and the water and acetonitrile ratio of 90:10 was used for the mobile phase. The method comprised of reverse, Zorbax column (SB-C18, 2.1×150 mm) set at temperature 40°C with flow rate 1 mL per min, and by injecting 20 µL volume of solutions in HPLC system, a peak was detected at wavelength 210 nm. The retention time was 0.46 min, and the total run time was 15 min. The linear calibration curve of ten concentration levels were plotted with regression equation: y = mx + b where, m: 94.12764, b: 150.32727, x: Amount and y: Area. The peak area is linearly related to the AA concentration with a regression coefficient (R^2) of 0.9997. Calculated the LOD (Limit of detection) and LOQ (limit of quantification) by using the formula (3.3 × SD per S) and (10 × SD per S), respectively, SD (standard deviation of response), and S (slope of the calibration curve) (Muthaiah *et al*., 2019). The values of LOD and LOQ are 3.733 and 11.045 (ng per µL), respectively. Furthermore, the recovery % was achieved by applying known amounts of AA standard to reference samples and running in HPLC. The recovery ranged from 98 % to 110 %, with a correction value (CV) of less than 10 %. The detailed validation procedure is published in my previous study by Verma and Yadav (2022a).

2.10. Fat content

The oil content of samples was measured by weight using the soxhlet method, where petroleum ether was used as the solvent (AOAC 2016).

2.11. Reducing sugar

The dinitro-salicylic acid (DNSA) method determines the reducing sugar (Miller, 1959). The OD of the sample was noted down and plotted against the OD of standard glucose solution in the range of 1–10 ppm. The result was given as mg/100 g.

2.12. Texture (hardness)

The texture of French fries was measured using a stable micro system TA-XT2i texture analyzer (Texture Technology Corp, UK) with a small three-point bend rig probe. The pre-test speed of 1.0 mm/s, the test speed of 1.0 mm/s, and the post-test speed of 10 mm/s, a distance of 20 mm, and 5 kg of load cell were used to measure the hardness of French fries. The hardness value was expressed in Newtons (N) as the mean peak compression force. The fracturability was

measured by the average number of first positive peaks on the texture analyzer graph during product compression.

2.13. Sensory evaluation

The semi-trained panel scored the french fries using a 9-point hedonic scale (1=extremely dislike, 9=extremely like). The samples were judged on their color, flavor, texture, and overall acceptability (Rangana, 2005).

2.14. Crude fiber (AOAC, 2005)

To estimate crude fiber, 2.5 g of a defatted sample was placed in a gooch crucible before being transferred to a fiber tech unit (FibraPlus, Pelican/FES08). 150 ml of sulphuric (1.25 % w/v) was added and effluxed at 450°C for 90 min. The sample was washed twice with 150 ml of a 1.25 % sodium hydroxide solution and refluxed for 60 min at 450°C. The remnants were washed with water for 30 min to remove alkali, then dried at 130±2° C for 1 hour, cooled in a desiccator, and weighed. The capsules were placed in a muffled furnace for ashing at 550°C for 2 hours in the preweighed crucible. Desiccators were used to cool crucibles to room temperature gradually. The change in residue weight after combustion was reported as crude fiber.

Calculation

$$\textit{Crude fiber}\ (\%) = W3\ (W1 \times C) - (W5 - W4 - D) / (W2) \times 100$$

In this formula: W1 = weight of empty capsules with lids that have been pre-dried, W2 = weight of sample, W3 = weight of capsule containing residue after extraction, W4 = weight of an empty crucible that has been pre-dried, W5 = weight of the crucible with the ash, C = weight of blank capsule after extraction/weight of blank capsule at the beginning, D = weight of ash obtained from the blank capsule.

2.15. Amino acids

Total free amino acids were measured using the Ninhydrin method. Ninhydrin solution dissolved stannous chloride (0.16g) in 100 mL citrate buffer (pH5.0) and 4 g ninhydrin in 100 mL methyl cellosolve. To 0.1 mL hydrolysate, 1 mL. Ninhydrin and 2 mL water were added and then heated in boiling water for 10 min till the blue color developed. Then, 5 mL diluent (water+n-propanol) was added, and after 15 min, absorbance was measured at 570 nm. The standard curve of Leucine amino acid at Img/mL was used to calculate total free amino acid content (Sadasivum and Manickam, 2009).

2.16. Microstructure

The microstructure of french fries was examined to identify the effect of various treatments on the crust of french fries, which can explain its hardness through observing the structure of pores of the crust region by using a Scanning Electron Microscope (SEM, model-JSM-7800F, JEOL Ltd, Japan. The fine powder of dried and defatted samples was suspended on a microscopic slide using double-sided adhesives carbon tape, and a 25 nm thick graphite layer was deposited on the sample using a vacuum evaporator (Jeol-Model-JEE420). At an accelerating voltage of 10kV or 15kV, images of a sample were captured at 300× magnification by an electron probe microanalyzer (EPMA, model-JXA8100, JEOL). It consists of four spectrometers and eight spectroscopic crystals. The Department of Mineralogy and Petrology, University of Allahabad, supported this study (Li *et al*., 2020).

2.17. Data analysis

The statistical analysis was done by a factorial design approach, incorporating variables such as baking time and temperature. Data analysis encompassed ANOVA, coefficient regression, and correlation assessments via Minitab software (Version 21, Minitab, LLC), with significance determined by the Tukey test. The values were expressed as means ± standard deviations of triplicates (n=3).

3. Result and Discussion

3.1. Effects of baking conditions on residual asparagine and residual reducing sugar in biscuits

The levels of asparagine and reducing sugar found in baked biscuits of different time-temperature combinations are presented in Table 1. Both reducing sugar and asparagine are the primary precursors of AA formation. Hence, these precursors were found to be utilized in AA formation, and their levels were reported to be in lesser quantity in baked products than in raw ingredients (Elmore *et al.*, 2005). The amount of reduced sugar and free asparagine contents in biscuits under various baking conditions are shown in Figures 3a and 3b. The amount of reduced sugar in the biscuit significantly decreased as baking time and temperature increased, whereas asparagine content significantly decreased with an increase in baking temperature (Table 1). The stability of asparagine and its content is affected by heating time and temperature in foods,

Table 1: Physicochemical analysis of biscuits at different baking time-temperature combinations

Tempera-ture	*Time*	*Residual Reducing (mg/100g)*	*Residual aspargine (mg/100g)*	*Amino acid (g/100g)*	*Water Activity*	*Fat (%)*	*Crude fiber (g/100g)*	*Ash (%)*	***HMF (mg/kg)***	***AA (µg/kg)***
160	**20**	1.9±0.1 ^{aA}	9.±0.2 ^{aA}	5±0.2 ^{aA}	0.4 ^{bA}	14.2±0.6 ^{aA}	1.6±0.6 ^{aA}	1.1±0.1 ^{aA}	49.5±2.2 ^{aA}	830.3±4.7 ^{aA}
160	**25**	1.8±0.1 ^{bB}	9.6±0.7 ^{aA}	5±0.1 ^{aA}	0.3 ^{bB}	13.9±0.2 ^{aA}	1.5±0.2 ^{aA}	1.3±0.2 ^{aA}	62.5±2.3 ^{aB}	1190±8 ^{aB}
170	**20**	1.7±0.2 ^{cA}	8.5±1.3 ^{aA}	4.9±0.2 ^{aA}	0.4 ^{aA}	13.9±0.2 ^{aA}	1.5±0.2 ^{aA}	1.2±0.2 ^{aA}	122±26.3 ^{bA}	1229±8.2 ^{bA}
170	**25**	1.4±0.1 ^{aB}	8±2.4 ^{aA}	5±0.1 ^{aA}	0.3 ^{aB}	13.8±0.1 ^{aA}	1.5±0.1 ^{aA}	1.1±0.1 ^{aA}	141±17.6 ^{bB}	2095±11.4 ^{bB}
180	**20**	1.5±0.1 ^{bA}	5.1±0.5 ^{bA}	5±0.1 ^{aA}	0.2 ^{cA}	13.9±0.1 ^{aA}	1.7±0.1 ^{aA}	1.1±0.1 ^{aA}	190.7±6.1 ^{cA}	3231±9 ^{cA}
180	**25**	1.3±0.1 ^{cB}	4.6±0.5 ^{bA}	5±0.2 ^{aA}	0.2 ^{cA}	13.9±0.1 ^{aA}	1.9±0.1 ^{aA}	1.2±0.1 ^{aA}	267.3±7.6 ^{cB}	4654.3±22.5 ^{cB}

All values shown are means±SD of three replicate and superscript (A-B) in a column, indicating a significant difference ($p<0.05$) between baking time and superscript (a-c) in the same column, indicate a significant difference ($p<0.05$) between baking temperature

has been reported by several researchers (Nguyen *et al.*, 2017; Weiss *et al.*, 2018). The levels of asparagine and reducing sugar, the essential AA precursors in bakery products, detected in the biscuit samples during baking closely match those reported in prior research on biscuits prepared under different baking conditions (Nguyen *et al.*, 2016). These findings provide valuable insights for food manufacturers to optimize their baking processes, thereby reducing the formation of harmful compounds and elevating the quality of their products.

3.2. *Effects of baking conditions on color value of biscuits*

The color development, a visual indicator of the Maillard reaction, is a crucial aspect of baking. The surface of the biscuits turned brownish after the baking process (Fig 1), indicating the presence of melanoidins in samples (Izydorczyk, 2005). Our study demonstrates that an increase in both baking time and temperature resulted in a significant increase ($P<0.05$) of a* and BI value in biscuits (Figure 2b, d). In contrast, ΔE was significantly increased with baking time (Figure 2e). The color difference increased with the increase in baking temperature due to the increased redness (a*) value (Table 2). However, the L* value of the biscuit was significantly ($P<0.05$) decreased as the baking time temperature increased (Figure 2a). The color development of french fries also depended on moisture loss, oil retention, Maillard reaction, frying time temperature, and the amount of asparagine-reducing sugars (Krokida *et al.*, 2001). The b* values of biscuits showed no significant difference ($p>0.05$) with an increase in baking time temperature (Figure 2b).

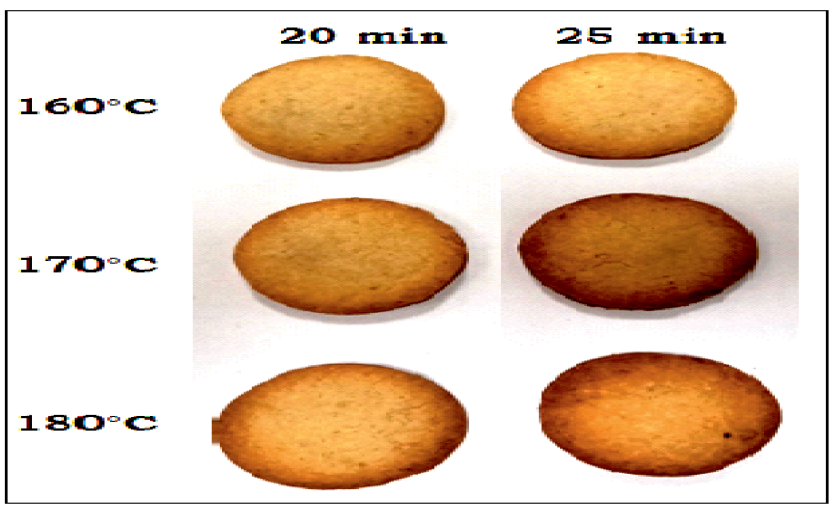


Figure 1: Photograph of biscuit baking at different time-temperature combinations

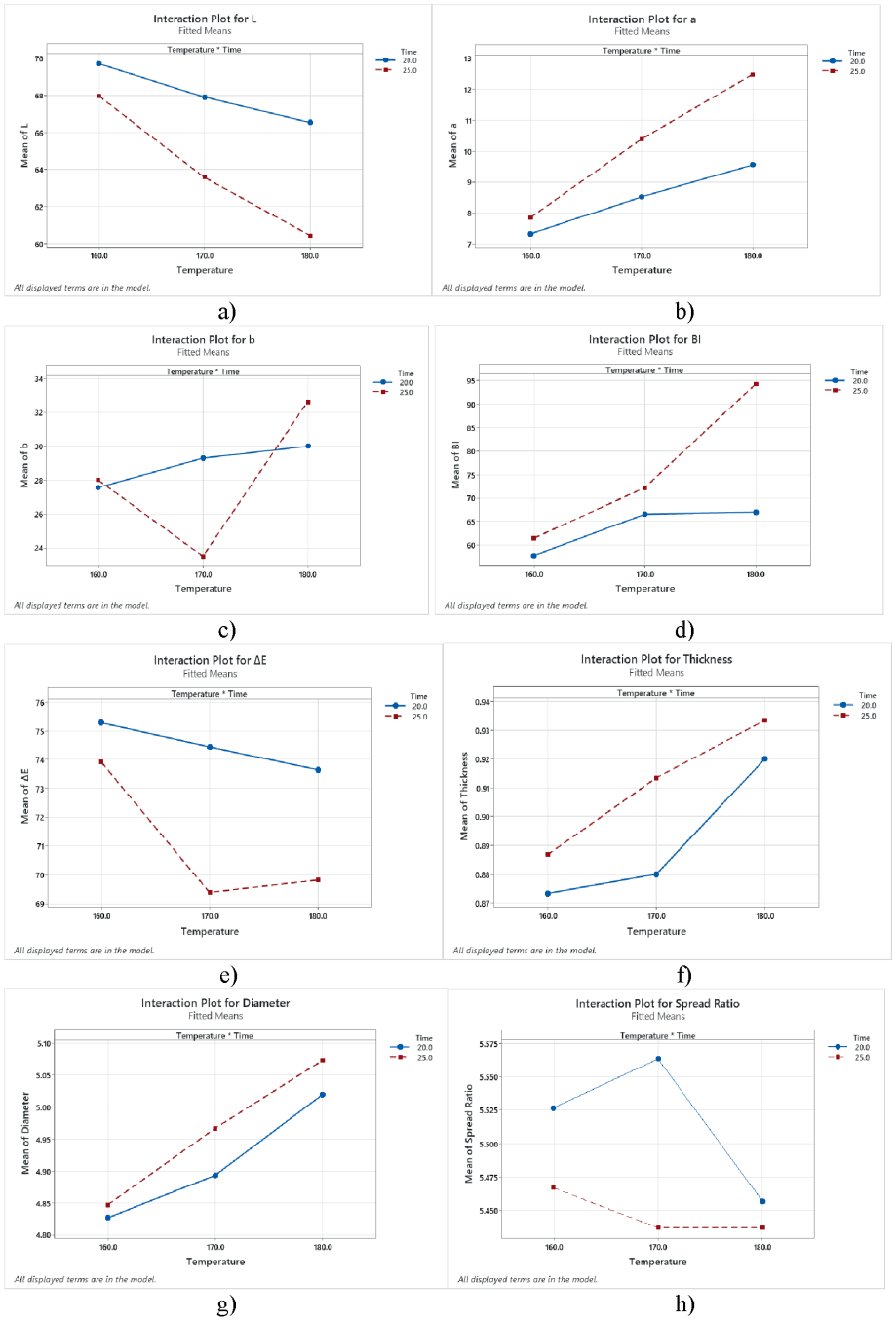


Figure 2: Effect of baking time-temperature on physical properties of biscuits a) L*(lightness) b) a*(redness) c) b*(yellowness) d) BI e) ΔE f) Thickness g) Diameter h) Spread ratio

3.3. *Effects of baking conditions on physicochemical properties of biscuits*

The results indicated that both the thickness and diameter of the biscuit were significantly ($p<0.05$) increased with baking time and temperature (Figure 2f, g). Moreover, the spread ratio decreased as baking time and temperature increased (Figure 2h). The difference in fat and crude fiber between time and temperature was insignificant (Figure 3e and f). The total ash and total free amino acids also showed a non-significant difference between time and temperature (Figure 3g and c). Water activity was measured to determine water availability in the biscuit after baking (Figure 3d). The baking process allows water migration to vapor from the inner side of the biscuits, creating a crispy skin. The water activity in the biscuit significantly ($p<0.05$) decreased as baking time and temperature increased (Table 1). Low moisture and water activity initiated the formation of AA. In contrast, the formation of AA is reduced when a system has residual water because evaporation decreases the effective temperature, even in dry areas of the product, such as the outer layer (Brathen & Knutsen, 2005; Esposito *et al.*, 2020).

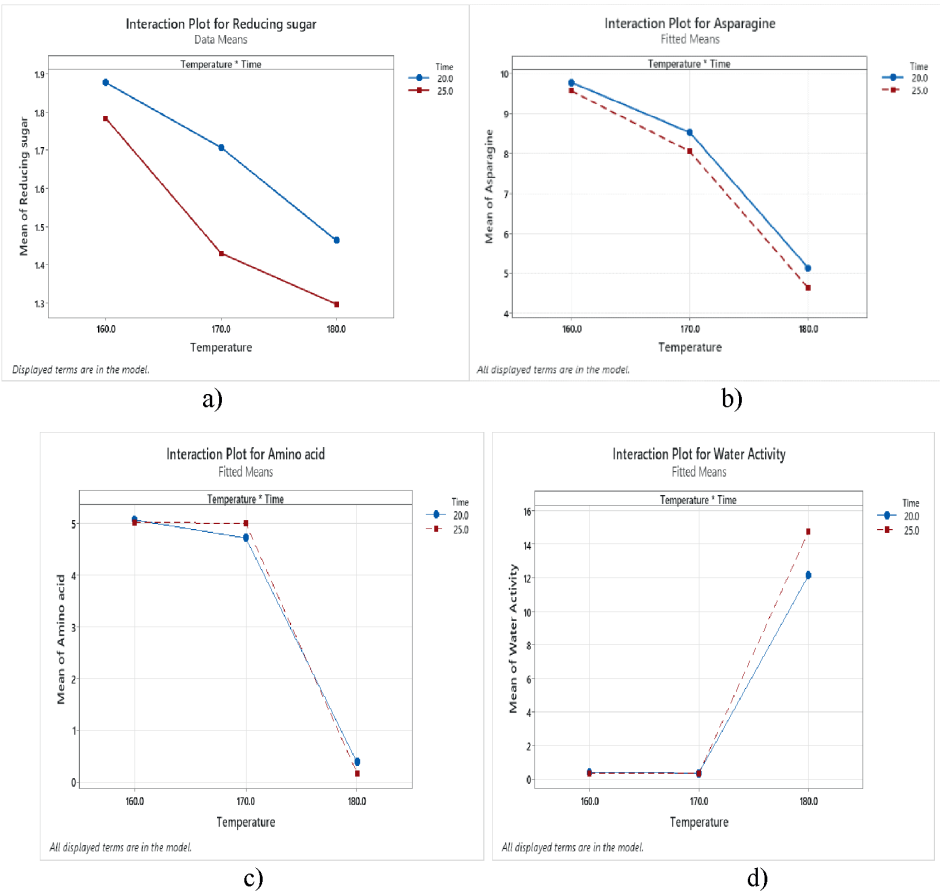
Table 2: Physical properties of biscuits at different baking time-temperature combinations

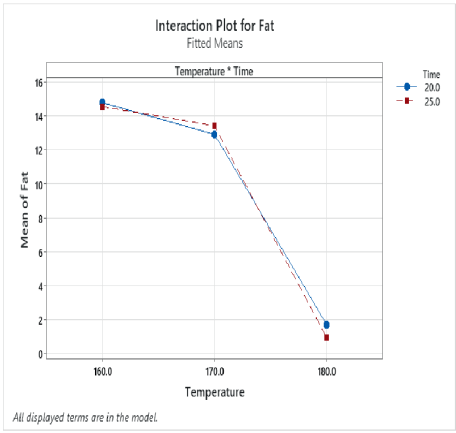
Temperature	Time	L*	a*	b*	BI	ΔE	Thickness (cm)	Diameter (cm)	Spread Ratio
160	20	69.7±0.9 ^{aA}	7.3±0.3 ^{aA}	27.6±0.5 ^{aA}	57.8±0.1 ^{aA}	75.3±0.8 ^{aA}	0.9±0.1 ^{bA}	4.8±0.1 ^{aA}	5.5±0.1 ^{aA}
160	**25**	68±1.4 ^{aB}	7.8±0.5 ^{aB}	28.0±0.6 ^{aA}	61.4±0.6 ^{aB}	73.9±1 ^{aB}	0.9±0.1 ^{bB}	4.8±0.1 ^{Bb}	5.5±0.1 ^{aA}
170	20	67.9±1.7 ^{abA}	8.5±1.1 ^{abA}	29.3±1.3 ^{aA}	66.6±1.3 ^{abA}	74.4±1 ^{aA}	0.9±0.1 ^{bA}	4.9±0.1 ^{cA}	5.6±0.2 ^{aA}
170	**25**	63.6±4.5 ^{abB}	10.4±1.7 ^{abB}	23.5±2.2 ^{aA}	72.2±5.7 ^{abB}	69.4±3.2 ^{aB}	0.9±0.1 ^{bB}	5.0±0.1 ^{Ab}	5.4±0.1 ^{aA}
180	20	66.5±2.9 ^{bA}	9.6±1.7 ^{bA}	30±1.4 ^{aA}	67±6.9 ^{bA}	73.6±2.1 ^{aA}	0.9±0.1 ^{aA}	5.0±0.1 ^{bA}	5.5±0.1 ^{aA}
180	**25**	60.4±5.1 ^{bB}	12.5±1.2 ^{bA}	32.6±0.4 ^{aA}	94.2±5.9 ^{bB}	69.8±4.4 ^{aB}	0.9±0.1 ^{aB}	5.1±0.1 ^{cB}	5.4±0.2 ^{aA}

All values shown are means±SD of three replicate and superscript (A-B) in a column, indicating a significant difference ($p<0.05$) between baking time and superscript (a-c) in the same column, indicating a significant difference ($p<0.05$) between baking temperature.

The sensory attribute of biscuits at all baking treatments was determined using a hedonic test, providing a direct connection to the consumer experience. The panelists, who played a crucial role in this study, were presented with blinded samples and asked to assess their preferences based on the perceived attributes, including taste, texture, appearance, color, and overall acceptability

(Table 3). The overall acceptability of biscuits significantly increased (P<0.05) as the baking time temperature increased, indicating a clear preference for certain baking conditions. Interestingly, baking at 180°C for 20 and 25 min shows higher overall acceptability than others (Figure 3i). The browning enhanced the appearance and taste of food products (Table 2). However, baking at high temperatures caused an extensive browning (Maillard reaction and caramelization) in biscuits due to the Maillard reaction. The baking process allows water migration from biscuits, creating a crisp surface. The hardness (fructurability) of fried products changed because of simultaneous heat and mass transfer during baking. These changes played a crucial role in fat absorption and the formation of textural structure. The maximum hardness was observed at 180°C for 25 min due to high moisture loss at high temperatures and long baking duration (Figure 3h). A similar observation was reported by Schouten *et al.* (2022) in biscuits.




Interaction Plot for Fat
Fitted Means
Temperature * Time
Time
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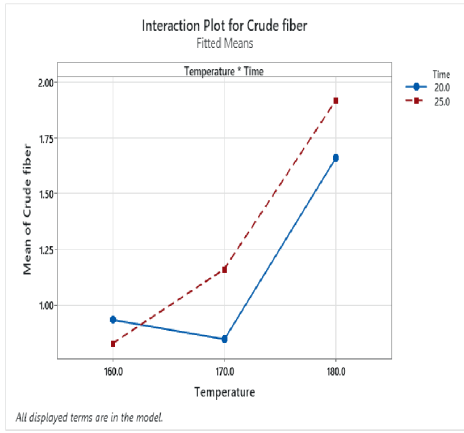
25.0
Mean of Fat
Temperature
160.0
170.0
180.0
All displayed terms are in the model.

e)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Fitted Means
Temperature * Time
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25.0
Mean of Crude fiber
Temperature
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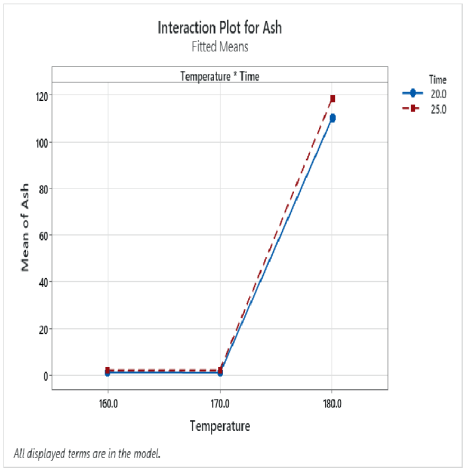
170.0
180.0
All displayed terms are in the model.

f)


Interaction Plot for Ash
Fitted Means
Temperature * Time
Time
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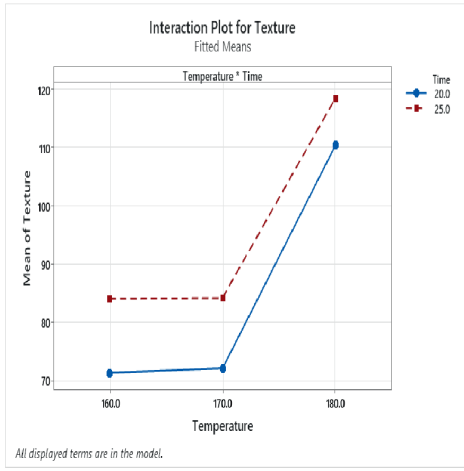
25.0
Mean of Ash
Temperature
160.0
170.0
180.0
All displayed terms are in the model.

g)


Interaction Plot for Texture
Fitted Means
Temperature * Time
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25.0
Mean of Texture
Temperature
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170.0
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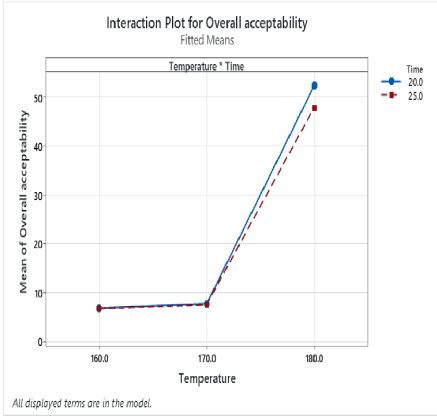
All displayed terms are in the model.

h)


Interaction Plot for Overall acceptability
Fitted Means
Temperature * Time
Time
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25.0
Mean of Overall acceptability
Temperature
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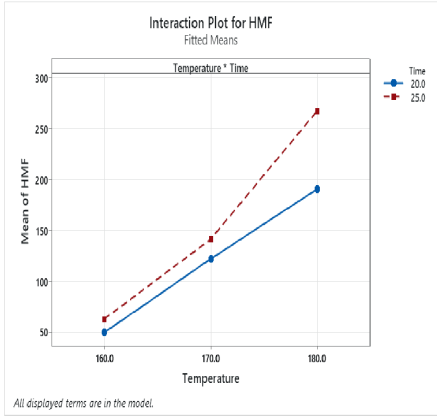
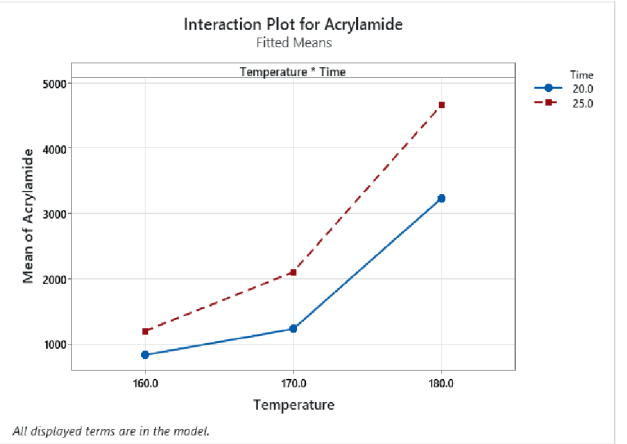
170.0
180.0
All displayed terms are in the model.

i)


Interaction Plot for HMF
Fitted Means
Temperature * Time
Time
20.0
25.0
Mean of HMF
Temperature
160.0
170.0
180.0
All displayed terms are in the model.

j)



k)

Figure 3: Effect of baking time-temperature on physicochemical analysis of biscuits a) Reducing sugar b) Asparagine c) Total free amino acids d) Water activity e) Fat f) Crude fiber g) Ash h) Texture i) overall acceptability j) HMF k) AA

Table 3: Sensory evaluation and hardness of biscuit at different baking time temperature combinations

Temperature	*Time*	***Taste***	***Texture***	***Appearance***	***Color***	***Overall acceptability***	***Fructurability (N)***
160	**20**	5.9±0.1 ^{bA}	7±0.2 ^{aA}	7.4±0.1 ^{aA}	6.7±0.1 ^{aA}	6.8±0.7 ^{aA}	71.3±0.1 ^{aA}
160	**25**	8±0.2 ^{bB}	7.3±1 ^{aA}	6.4±0.1 ^{aB}	7.1±0.1 ^{aA}	6.7±0.6 ^{aA}	84±0.2 ^{aB}
170	**20**	6.6±0.3 ^{abA}	7.8±0.4 ^{aA}	8.4±0.2 ^{bA}	7.8±0.2 ^{bA}	7.7±0.2 ^{bA}	72.1±0.1 ^{aA}
170	**25**	8±0.2 ^{abB}	7.2±0.2 ^{aA}	6.9±0.2 ^{bB}	7.6±1 ^{bA}	7.4±0.1 ^{bA}	84.1±0.3 ^{aB}
180	**20**	8.4±0.2 ^{aA}	7.5±0.6 ^{aA}	8.6±0.1 ^{cA}	7.9±0.1 ^{bA}	7.9±0.3 ^{bA}	110.3±1.2 ^{bA}
180	**25**	7.9±0.1 ^{aB}	7±0.6 ^{aA}	8.1±0.2 ^{cB}	8.1±0.1 ^{bA}	7.7±0.2 ^{bA}	118.3±3.1 ^{bB}

All values shown are means±SD of ten replicate and superscript (A-B) in a column, indicating a significant difference ($p<0.05$) between baking time and superscript (a-c) in the same column, indicating a significant difference ($p<0.05$) between baking temperature.

3.4. Effects of baking conditions on the formation HMF and AA of biscuits

The results demonstrate a significant increase in biscuits' AA and HMF content as the baking time and temperature are elevated (Table 1). Notably, the highest levels of HMF and AA were recorded at 180°C for 25 min, while the lowest levels were observed at 160°C for 20 min (Figure 4.26j and k). This observation aligns with previous reports by researchers such as Amrein *et al*. (2004) and Cheng *et al*. (2014), supporting that baking at lower temperatures curtails the rapid accumulation of HMF and AA in baked products. Furthermore, the

study of biscuits baked at 180°C for 7, 10, and 13 minutes in a ventilated oven confirmed a proportional increase in AA formation with extended baking times. Specifically, after 13 min of baking, the AA levels in biscuits formulated with refined wheat and oat flour were 72.3 µg/kg and 861.7 µg/kg, respectively. It was observed that under consistent baking temperatures, there was a marked acceleration in AA formation with prolonged baking times. Similarly, when the baking time was held constant, an elevation in AA formation was noted with increased temperatures.

Results showed that lower baking time temperature significantly decreased the formation of AA and HMF (Table 1). The sensory score of the combination (180°C for 20 min) was the highest, but the AA and HMF levels in this combination exceeded the benchmark level of the European Commission. The benchmark level of AA in biscuits is 350 µg/kg, and HMF in honey is 40 mg/kg. Hence, this combination was used in further studies to minimize HMF and AA content.

4. Conclusion

The relationship between baking time, temperature, and the quality and safety of biscuits is complex. They achieved the desired sensory qualities of biscuits while minimizing health risks and carefully balancing baking conditions. By understanding and optimizing these factors, manufacturers can produce biscuits that are not only delicious and high quality but also safer for consumption. The study revealed that the formation of AA and HMF in biscuits increased as baking time and temperature increased. Further research and technological advancements continue to provide new methods for reducing AA levels in baked goods, ensuring consumer health and satisfaction.

Competing Interests

This is to certify that all authors approve the manuscript and that there are no financial or other conflicts of interest.

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