



Formation of acrylamide in biscuits during baking under different heat transfer conditions

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ABSTRACT

The effects of heat-transfer modes on acrylamide (AA), AA precursors, and the main quality characteristics of biscuits were investigated. Biscuits were baked in ventilated mode (V), with forced air convection, and static mode (S), based only on conduction, at 175 °C for 18, 20, 22, 24, 26 min. Both heat-transfer types promoted an increase in AA levels during baking. However, in the V mode the heat was distributed more evenly compared to the S one, causing a faster rise of temperature, as showed by recorded time-temperature profiles. As a consequence, the V biscuits reached higher AA levels compared to those baked under S conditions at 20 and 22 min. Both samples baked at 20 and 22 min in S mode were acceptable based on the results of quality parameters. Nevertheless, under the formulation and baking conditions applied, all samples showed AA levels below the reference value of Commission Regulation (EU) 2017/2158.

1. Introduction

Acrylamide (AA) has been classified as a toxic and carcinogenic compound that is formed naturally during high-temperature processes applied in foods, such as roasted coffee, baked goods, baked and fried potatoes, causing several global concerns (IARC, 1994; SNFA, 2002). In view of the risks associated with this substance, the interest in AA reduction in foods is becoming an important goal to achieve for the food legislation and industry. The latest Regulation from the European Commission (EU) 2017/2158 regulates the presence of AA in various food products (EC, 2017). Although no real legal limit is defined, this Regulation introduced benchmark levels with the aim to invite the food industries to implement strategies for reducing AA levels in foods. Moreover, to assist in the control of AA levels, Food Drink Europe has developed a Toolbox indicating possible strategies applicable at different stages of processing of different food categories (FDE, 2019).

Bakery products are one of the food categories at AA risk due to both the presence of asparagine and reducing sugars in the most commonly used ingredients, and the baking process carried out at temperatures above 120 °C. This triggers the Maillard reaction, the main route of AA formation (Mesías et al., 2016). The baking process is a complex stage in which heat and mass transfers occur simultaneously, generating a temperature gradient that influences the rates of chemical reactions taking place in the food matrix (Mesías et al., 2016, 2020).

Several studies conducted on bakery products have established that at low temperatures the formation of AA takes longer, whereas at higher temperatures the maximum level is reached in a short time (Açar & Gökmen, 2010; Ahrné et al., 2007; Amrein et al., 2004; Elmore et al., 2005; Mogol & Gökmen, 2014; Sakin-Yilmazer et al., 2013). However, the development of AA is also influenced by the mode of heat transfer, resulting in differences in the formation of temperature gradients within the product, which, consequently, influence the rate of the chemical

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reactions, including the AA formation induced by the process (Anese et al., 2008; Claus et al., 2008; Mogol & Gökmen, 2014; Sakin-Yilmazer et al., 2013). For example, a study of Claus et al. (2008) investigated the differences between the use of a multi-deck oven, characterised by heat transfer via conduction and radiation, and of a convection oven, based on forced air circulation, for bread baking. The results indicated that at 220 °C, AA levels were 60% higher in bread baked in a ventilated oven (173.9 µg/kg) than in a static oven (109.6 µg/kg), whereas at 260 °C these differences were reduced by up to 35%, probably due to the high formation rate of AA at this high temperature. The highest AA levels in bread baked in the convection oven were attributed to the forced air circulation, leading to faster and more intense drying of the bread surface crust.

As an alternative to traditional conduction or convection baking, Anese and co-authors (2008) proposed the use of radiofrequency in the final baking stage as a promising strategy to reduce the concentration of AA in leavened cakes and biscuits baked to a final moisture content of 3.5% and 3.0% respectively. Radiofrequency baking allows food to be cooked quickly and evenly. In fact, in the case of the cakes, the only use of air-circulating oven heating was responsible for a high formation of AA, reaching 400 µg/kg. In contrast, a final heating by radiofrequency on a cake partially baked by convection and with a moisture content of 6%, permitted to reduce AA levels by up to 300 µg/kg at the same final moisture content of 3.5%. Radiofrequency heating was even more effective when applied to product conventionally baked until a moisture content of 10%, reaching values of less than 200 µg/kg at a final moisture content of approximately 3.5%. Similarly, a 35% of AA reduction with a final moisture content of 3% was achieved in biscuits by combined baking methods. However, in the case of cakes, the radiofrequency heating has resulted in a darker colour on the internal portion than on the external one. In contrast, in very low moisture contents and thin bakery products, such as biscuits, the internal colour was not affected; this highlights the limitation of the radiofrequency heating for certain products with particular shape and moisture content.

Another study by Sakin-Yilmazer et al. (2013) analysed AA formation in muffins baked in forced convection and steam-assisted hybrid ovens at 145, 160, and 175 °C for different baking times. The muffin baked in a steam-assisted hybrid oven resulted in lower AA concentrations (~33 µg/kg) at all baking temperatures, compared to samples baked in a convection oven. These results have been attributed to a less drying rate of the food matrix in steam-assisted hybrid oven, confirming once again that, besides time and temperature, AA is significantly affected by the heat-transfer method. Moreover, at the same baking times for all baking temperatures, the browning index values of the muffins baked in the steam oven were significantly lower than those of samples baked in natural and convection ovens.

Mogol and Gökmen (2014) evaluated the effect of baking at atmospheric pressure and under vacuum (at 500 mbar pressure) on the AA content of biscuits. While the kinetic trends of AA formation were identical for both baking methods, their content was significantly different. The mean levels detected ranged from 11 to 51 µg/kg in biscuits baked under vacuum and from 39 to 211 µg/kg in biscuits baked in conventional oven. This difference was justified by the different time-temperature profiles due to the lower boiling point of the water at low pressure (81.6 °C at 500 mbar), allowing the same final biscuit moisture to be obtained at lower baking temperatures and leading to less AA formation. Nevertheless, the development of colour was lacking in vacuum-baked biscuits compared to the conventional baked ones due to the limited convective heating that lowered the thermal load and browning reactions. To avoid this problem a solution proposed was to add brown-coloured ingredients to the recipe; where it is not possible to change the formulation of bakery products the application of this alternative baking method is still limited. There are therefore alternative baking methods studied in the literature, which however do not achieve the same final results in terms of the quality of some baked products.

The aim of this study was to evaluate the influence of two commonly

used baking methods, such as by conduction and by forced air convection, on the AA formation in biscuits. Moreover, AA precursors (glucose, fructose, asparagine) and some quality characteristics (weight loss, moisture, water activity, texture, and colour) were analysed in biscuits during baking in order to identify the optimal baking conditions to obtain the lowest AA development and at the same time acceptable quality characteristics of the final product.

2. Materials and methods

2.1. Preparation of biscuits

The biscuit dough was prepared in a standardised way following the formulation described by Canali et al. (2020). In detail, the ingredients purchased from a local market, such as wheat flour (500 g), sucrose (125 g), pasteurized eggs (125 g), butter (100 g), milk (100 mL), and a leavening agent containing sodium diphosphate and carbonates (15 g), were weighed and placed in a household mixer (mod. Bimby Robot TM31, Vorwerk, Wuppertal, Germany). All the ingredients were mixed and kneaded by setting the speed regulator to position 5 (about 3000 rpm) for 1 min and 30 s, reversing the direction of rotation after 80 s, to allow the walls of the robot's kneading container to be cleaned. The dough was then kneaded quickly by hand for about 30 s to compact it. The dough, thus prepared, was characterised by moisture content and water activity of $23.53 \pm 0.41\%$ and 0.90 ± 0.01 , respectively.

After letting it rest in a refrigerator at 4 °C for 20 min, the dough has been sheeted to a thickness of 3.0 mm by a pasta filler machine (mod. SFSI 42040050 T, GAM International, Santarcangelo di Romagna, Italy) and cut by using a stainless-steel circular mould of 6 cm diameter.

2.2. Baking

The uncooked biscuits were placed on a baking tray that was put on the middle level of a domestic electric oven (mod. Procombi Plus, AEG-Electrolux, Berlin, Germany) and baked in static mode (S), with heat transfer mainly by conduction, and in ventilated mode (V), based on forced air convection. In the forced convection condition, the hot air was constantly re-circulated inside the oven by a fan installed on the backside of the oven chamber (air speed: 0.5 m/s); the fan was turned off in the static air condition. For both baking methods, the oven was pre-heated for the time necessary to reach and maintain a constant air temperature equal to 175 °C and the baking was carried out for 18, 20, 22, 24, and 26 min. The temperature and time conditions studied were selected after preliminary trials in order to obtain biscuit samples baked at different levels. For each baking condition, 3 batches of 10 biscuits (about 180 g of raw dough per batch) were produced.

For each baking cycle, the temperature of the air inside the oven chamber and inside biscuits were recorded every 20 s using 1 mm diameter type K thermocouples (RS PRO Italy, Milan, Italy) with an accuracy of 0.1 °C, connected to the acquisition and registration system mod. 34970 (Agilent Technologies, Santa Clara, California, USA). A thermocouple was placed in the centre of the oven chamber above the baking tray and another one inside a biscuit placed in a central position of the baking tray. The temperatures were monitored continuously throughout the duration of each cooking cycle.

After baking, biscuits were removed from the oven, placed on a grid, and kept cooling at room temperature for 1 h.

A part of each raw dough sample was freeze-dried for reducing sugars, asparagine, and AA analyses.

2.3. Chemicals and reagents

AA (for molecular biology, $\geq 99\%$ (HPLC), C_3H_5NO , molecular weight 71.08 g/mol, CAS No 79-06-1), 2,3,3-d₃-AA (AA-d₃, AA-d₃) standard solution, 500 mg/mL in acetonitrile (analytical standard, CAS 122775-19-3), L-asparagine ($\geq 98\%$ (HPLC), $C_4H_8N_2O_3$, molecular

weight 132.12 g/mol, CAS No 70-47-3), *D*-(−)-fructose (≥99%, C₆H₁₂O₆, molecular weight 180.16 g/mol, CAS No 57-48-7), *D*-(+)-glucose (analytical standard, C₆H₁₂O₆, molecular weight 180.16 g/mol, CAS No 50-99-7) and sucrose (BioUltra, for molecular biology, ≥99.5% (HPLC), C₁₂H₂₂O₁₁, molecular weight 342.30 g/mol, CAS No 57-50-1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution of each analyte has been prepared by dissolving the pure powder (10 mg) into 10 mL of ultrapure water except for asparagine which has been prepared at 500 mg/L. All stock solutions were stored in a glass vial at −18 °C up to use. Daily, standard working solutions at different concentrations were prepared by adequate dilution of the stock solutions in water. For AA quantitation an internal standard (IS), i.e., AA-d₃, has been used. An exact aliquot of AA-d₃ at 5 µg/mL was combined with the standard working solutions of native AA prepared at diverse concentrations. LC-MS grade acetonitrile was supplied by Sigma-Aldrich (Milano, Italy) and HPLC-grade formic acid (99%) was obtained from Merck (Darmstadt, Germany). Ultrapure water was prepared by using a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). All other solvents and reagents were analytical grade. Two different cartridges for solid-phase extraction (SPE) were employed for sample clean-up such as Bond Elut-Accucat, 200 mg, 3 mL cartridges, from Agilent Technology (Santa Clara, CA, USA) and Oasis HLB 200 mg, 6 mL cartridges, from Waters (Milford, MA, USA). Before high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) analysis each sample was filtered by Phenex™ RC 4 mm, 0.2 µm syringeless filter, Phenomenex (Castel Maggiore, BO, Italy). Captiva PTFE 13 mm, 0.45 µm syringeless filter was bought from Agilent Technology (Santa Clara, CA, USA).

2.4. Reducing sugars, sucrose, asparagine and acrylamide analysis by HPLC-MS/MS system

The quantification of reducing sugars (fructose and glucose), sucrose, asparagine, and AA has been performed by following previous analytical methods (Fernandes et al., 2019; Schouten et al., 2021) with some adjustments especially in the extraction and purification steps. The instrument employed for the current project was an Agilent 1290 Infinity series and a Triple Quadrupole 6420 from Agilent Technology (Santa Clara, CA, USA) equipped with an electrospray ionization (ESI) source operating in positive ionization mode. The separation of target compounds was achieved on Kinetex HILIC analytical column (100 mm × 4.6 mm i.d., particle size 2.6 µm) from Phenomenex (Torrance, CA, USA) preceded by a KrudKatcher ULTRA HPLC In-Line Filter (2.0 µm Depth Filter × 0.004 in I.D.). The mobile phase was composed of water (A) and acetonitrile (B) both with 0.1% of formic acid and the separation has been obtained at 0.8 mL/min in gradient elution mode. The composition of the mobile phase varied as follows: 0–2.5 min, isocratic condition, 85% B; 2.5–3.5 min, 85–70% B; 3.5–5.5 min, isocratic condition, 70% B; 5.5–6.5 min, 70–60% B; 6.5–10 min, isocratic condition, 60% B; 10–12 min, 60–85% B; 12–20 min, isocratic condition, 85% B. The injection volume was 2 µL, the temperature of the drying gas in the ionization source was kept at 350 °C, the gas flow was 12 L/min, the nebulizer pressure was 45 psi and the capillary voltage was set at 4000 V. The acquisition was performed in “Multiple Reaction Monitoring”

mode and the most abundant transitions were used for quantification while the other for confirming the analyte. Table 1 reports the HPLC-MS/MS acquisition parameters for each target compounds such as precursor ion, product ion, fragmentor, collision energy, and polarity. For AA quantification the response factor was measured by calculating the ratio between the area of native AA and AA-d₃. Limit of detection (LOD) and limit of quantification (LOQ) have been calculated by injecting gradually lower concentrations of standard mixtures and the concentration that determined a signal-to-noise (SNR) ratio of 3 was assigned to LOD while that generated a SNR of 10 was assigned to LOQ. The present method was characterised by good sensitivity, which agrees with those reported in the literature (Fernandes et al., 2019; Schouten et al., 2021; Wenzl et al., 2006). LOQ for glucose, fructose, sucrose, asparagine, and AA were 50, 50, 10, 5, and 5 µg/kg, respectively.

The extraction process was performed by using ultrasound-assisted extraction (UAE) with water. Briefly, 1 g of ground biscuits was fortified with AA-d₃ (0.5 mL at 500 ng/mL), and 9.5 mL of water was added into it. Different attempts were performed to optimize the solvent to sample ratio to obtain the best extraction efficiency especially for AA (data not shown). Then, the sample was agitated with a vortex mixer for 30 s and sonicated for 1 h at room temperature with an ultrasonic bath (FALC, Treviglio, Italy) at a frequency of 59 kHz. After centrifugation at 5000 rpm for 10 min, the sample was filtered with filter paper and the lipids were removed by a defatting process with hexane (four times with a total volume of 35 mL). Afterward, the sample was filtered with a 0.45 µm filter and for the analysis of glucose, fructose, and asparagine, it was diluted 1:40 in the mobile phase, filtered with 0.2 µm pore membrane filter and injected into HPLC-MS/MS system. For the analysis of sucrose, an aliquot of sample was diluted 1:50000 in the mobile phase and injected into HPLC-MS/MS instrument.

For the AA quantitation, a purification step composed of a double SPE has been carried out. With the aim to increase the content of AA in the final sample solution, several volumes (1.5, 3, 4, and 6 mL) of the sample have been individually loaded in the first cartridge but keeping then the same elution volume. The best results in terms of signal-to-noise ratio (SNR) of the quantitative transition of AA (1.5 mL, SNR = 8; 3 mL, SNR = 18; 4 mL, SNR = 26; 6 mL, SNR = 27) have been generated from 4 mL to 6 mL. More than 4 mL did not generate an advantageous enhancement of AA signal therefore 4 mL has been selected for the current study. The first employed cartridge was the Oasis HLB. This was first conditioned with 3.5 mL of methanol and then with 3.5 mL of water. 4 mL of sample were loaded onto cartridge followed by 0.5 mL of water. The sample was allowed to pass completely through the sorbent material. For AA elution, 1.5 mL of water was added onto the cartridge and the eluent was collected in a 3 mL glass vial. Before conditioning the second SPE column, a mark was placed on the outside of the cartridge at a height equivalent to 1 mL of liquid above the sorbent bed. The Bond Elut-Accucat column was conditioned with 2.5 mL of methanol followed by 2.5 mL of water. The solvents used for conditioning were discarded. The eluent collected from the first cartridge was added to the Bond Elut-Accucat cartridge. The sample was allowed to eluate from the column up to the mark previously placed on the outside; the eluent was then collected into a 6 mL glass vial. Lastly, the samples were filtered with a 0.2 µm filter and injected into HPLC-MS/MS.

Table 1
HPLC-MS/MS acquisition parameters (MRM mode) adopted for the quantification of acrylamide, asparagine, and sugars.

Compound	Precursor Ion (<i>m/z</i>)	Product Ion (<i>m/z</i>)	Fragmentor (V)	Collision Energy (V)	Retention Time (min)	Polarity
Acrylamide	72	55	45	8	1.52	Positive
Acrylamide-d ₃	75	58	45	8	1.52	Positive
Fructose	203	203	80	0	1.96	Positive
Glucose	203	203	80	0	2.18	Positive
Sucrose	365	365	130	0	2.72	Positive
Asparagine	133	74 ^a 98	64	16 16	5.17	Positive

^a This product ion was used for quantitative analysis; the rest for confirmatory analysis.

The same procedure has been adopted for the extraction of the analytes from the freeze-dried dough.

2.5. Physicochemical analysis

2.5.1. Weight loss

Weight loss (%) of biscuits was determined as the percentage of weight variation among 10 biscuits before and after each baking cycle.

2.5.2. Moisture

Moisture (%) of raw dough and baked biscuits was determined by gravimetric method following the AOAC method (2000). For each sample about 3 g of product, exactly weighed, was placed in an oven at 105 °C until constant weight. Three replicates were performed for each baking batch per sample.

2.5.3. Water activity

Water activity (a_w) of raw dough and baked biscuits was determined on ground samples using a dew point hygrometer AQUALAB (mod. Meter 4 TE, Pullman, Washington, USA) at 25 °C. Three replicates were performed for each baking batch per sample.

2.5.4. Colour

The colour of biscuits surfaces was measured using a tristimulus spectrophotometer Colorflex (HunterLab, Sunset Hills Road Reston, Virginia, USA). Colour was determined using the CIE $L^*a^*b^*$ scale and illuminant D65.

From numerical values of the measured parameters, hue angle (h°) and browning index (BI) were calculated by the following equations (McGuire, 1992; Sakin-Yilmazer et al., 2013):

$$h^\circ = \left(\left(\tan^{-1} \frac{b^*}{a^*} \right) / 2\pi \right) \times 360$$

$$BI = \frac{[(X - 0.31) \cdot 100]}{0.17}, \text{ where } X = \frac{a^* + 1.79 \cdot L^*}{5.645 \cdot L^* + a^* - 3.012 \cdot b^*}$$

The colour measurements were carried out on the two surfaces of 5 biscuits for each baking batch per sample.

2.5.5. Texture

The texture measurement on biscuit samples was performed at room temperature using a Texture Analyser mod. TA-HDi500 (Stable Micro System, Surrey, UK) equipped with an HDP 3-point bending rig and 25 kg load cell. The distance of the two beams was 30 mm and the other settings were: pre-test speed of 5.00 mm/s, test speed of 1.00 mm/s, post-test speed of 10.00 mm/s, and distance of 5 mm. The downward movement was advanced till the biscuit was broken. The acquired parameters were expressed as hardness, calculated by means of maximum force values; index of crispness, calculated by using the linear distance between the first and the last peaks registered (Tylewicz et al., 2019), and fracturability, expressed as one/breakpoint distance between the origin of curve till the point where the biscuit breaks (Romani et al., 2012). Force vs distance curves were obtained from 10 biscuits for each baking batch per sample.

2.6. Statistical analysis

The results were reported as the mean value \pm standard deviation. Significant differences between data were calculated by unidirectional analysis of variance (ANOVA) followed by Tukey's post-hoc comparison test, with a significance level of $P < 0.05$. The relationship between the average values of AA, the content of precursors, and the physicochemical characteristics of the biscuits baked under different conditions were evaluated with Pearson correlation coefficient (r). An r -value between $0.60 \leq r \leq 1.00$ indicates a positive linear correlation, $-1.00 \leq$

$r \leq -0.60$ indicates a negative linear correlation, and $-0.60 < r < 0.60$ indicates no correlation, with a significance level $P < 0.05$. The statistical package STATISTICA 8.0 software (StatSoft, Tulsa, UK) was used.

3. Results and discussion

The air temperatures measured inside the oven and those of biscuits during the baking process at 175 °C for 18 (a), 20 (b), 22 (c), 24 (d), and 26 (e) min are shown in Fig. 1. Every thermal profile represents the average value of triplicate baking cycles, confirming a high standardisation of the baking process.

At the beginning of the baking process and in each cycle, the air temperature inside the oven decreased rapidly and markedly from 175 °C to about 115 °C due to the opening of the oven to insert the raw biscuits. The air inside the oven reached the initial temperature of 175 °C differently when set up at static and ventilated heat transfer modes. In detail, with the ventilated mode the oven reached the set temperature in the first 2 min, more quickly than with the static mode that required about 10 min (Fig. 1). This is because in a static mode the heat comes from the electrical resistances located both at the top and bottom of the oven chamber (natural convection), while in the ventilated mode the hot air is distributed through a fan by forced convection, so the chamber heats up faster and more evenly (Marcotte, 2007; Sakin-Yilmazer et al., 2009; Walker, 2016). In fact, it has been established that the fan application in the oven compared to no fan application resulted in a higher heat transfer coefficient ($W/m^2 \cdot K$), one of the main parameters of the baking process time and efficiency together with the resulting product quality (Sakin-Yilmazer et al., 2009). In both static and ventilated modes, it is necessary to consider that heat is also distributed across the contact area between products and the baking tray as well as transferred by radiation from the hot oven's inner walls (Cevoli et al., 2020; Sakin-Yilmazer et al., 2009). The total radiative heat transfer coefficient is rather limited and independent of the type of heat transfer mode, as it is mainly determined by the oven wall material and the temperature, whereas the total convective heat transfer coefficient is more than twice as high under ventilated conditions as under static conditions without the fan (Sakin-Yilmazer et al., 2009).

As expected, the differences in temperature measured inside the oven set up at the two different baking modes influenced the thermal profiles of the biscuit during baking. The inside of the biscuit baked in ventilated mode reached the temperature of 100 °C slightly faster (about 2 min earlier) compared to the biscuit baked in static mode. Generally, the time-temperature behaviour of food baked in the oven can be divided into three different phases: the first phase is characterised by a slight increase in temperature, followed by a faster increase up to 100 °C, which represents the second phase; finally, in the third phase the temperature of 100 °C is kept constant due to the evaporation of the water inside the matrix (Manley & Clark, 2011). In our case, only after 26 min of baking, in both modes, the biscuits exceeded the temperature of 100 °C, indicating a start of over-baking (Fig. 1e).

The differences in the time-temperature profiles of S and V biscuit samples, during the first minutes of baking, influenced the formation of AA and the levels of its precursors in the biscuits at the considered baking times.

In Fig. 2, AA levels of biscuit samples during baking in ventilated and static modes for different times are shown. In detail, AA was not detected in the raw dough; after the first 18 min of baking the AA levels found in S and V samples were respectively of $130.0 \pm 3.0 \mu g/kg$ and $153.1 \pm 14.5 \mu g/kg$ without significant differences. At longer baking times, the AA levels increased, reaching the highest values at 26 min of about $275 \mu g/kg$ for both biscuit samples. The increase in AA formation in biscuits with the increase of baking time is confirmed by several literature studies (Amrein et al., 2004; Cheng et al., 2014; Courel et al., 2009; Fernandes et al., 2019; Mogol & Gökmen, 2014; Žilić et al., 2020). In a recent study, Žilić et al. (2020) baked biscuits at 180 °C for 7, 10 and 13 min in a ventilated oven confirming that the formation of AA is

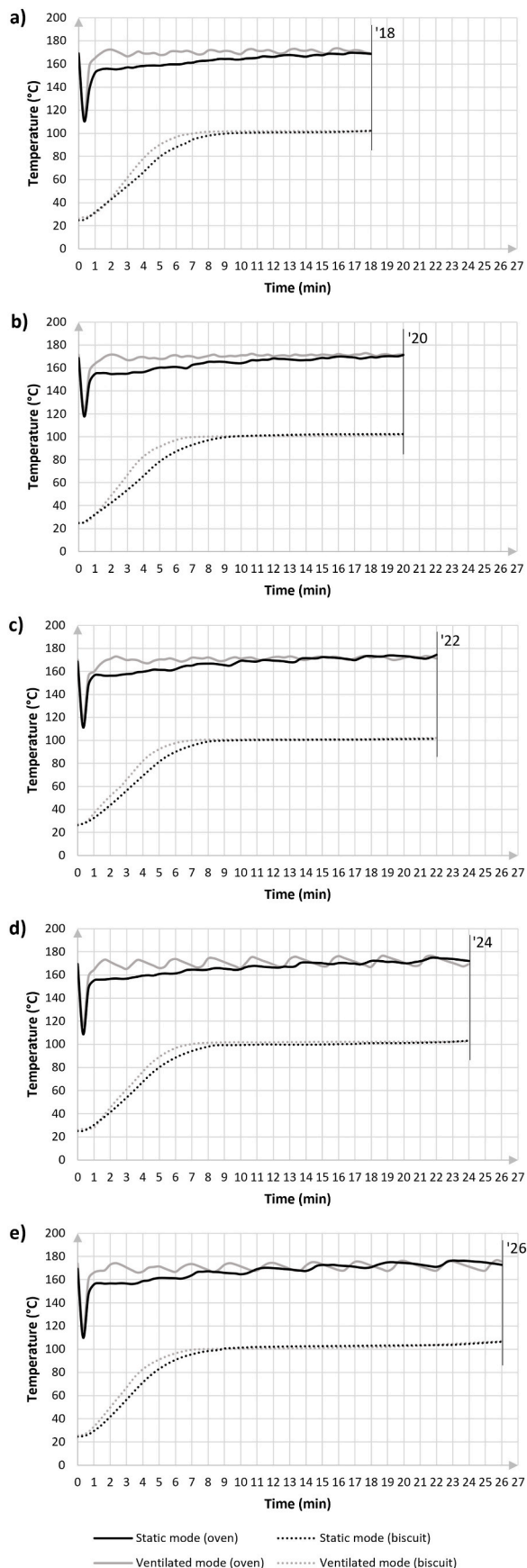


Fig. 1. Thermal profiles of the oven chamber and of the inner part of biscuit samples during baking in ventilated (grey lines) and static (black lines) modes at 175 °C for different times: 18 (a), 20 (b), 22 (c), 24 (d) and 26 (e) min.

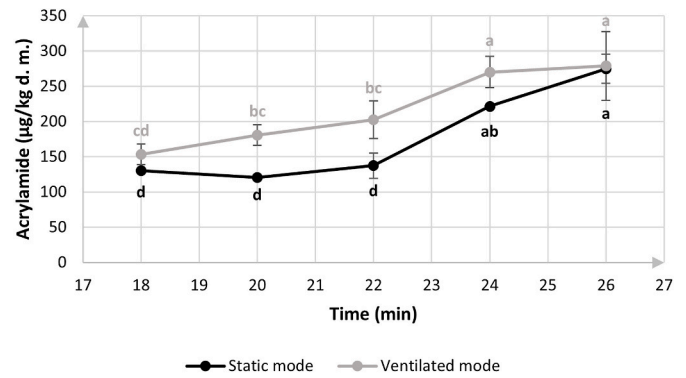


Fig. 2. Acrylamide levels expressed on dry matter (d.m.) basis of biscuit samples during baking in ventilated (grey line) and static (black line) modes at 175 °C for different times. Different letters indicate significant differences among samples at $P < 0.05$ level.

proportional to the baking time; the authors found AA levels of 72.3 µg/kg and 861.7 µg/kg after 13 min of baking in biscuits formulated with refined wheat flour and hullless oat flour, respectively.

In this study, the major differences in AA values between S and V biscuits were found after 20 and 22 min of baking; the samples baked with the ventilated mode showed significantly higher AA values compared to those baked with static mode (Fig. 2). The general higher AA values found in V biscuit samples can be related to the fact that in a ventilated mode the heat during baking is distributed faster and more evenly, thus favouring greater dehydration of the product and consequently accelerating the reactions responsible for the AA formation.

The levels of asparagine, glucose, fructose, and sucrose found in raw dough and in differently baked biscuits samples are reported in Table 2. All the AA precursors in both S and V biscuit samples decreased compared to the raw dough, indicating their involvement in the Maillard reaction and thus AA formation. The content of asparagine, the main AA precursor in bakery products, found in the biscuit samples during baking agrees with those reported in the previous literature for biscuits prepared at different baking conditions (Nguyen et al., 2016; Wenzl et al., 2006). The concentrations of asparagine appeared to rise after 20 min of baking in both S and V biscuit samples, reaching concentrations of 42.16 ± 2.41 mg/kg and 38.71 ± 1.71 mg/kg, respectively. This increase could be the result of the thermal hydrolysis of the proteins in the flour, as reported by Žilić et al. (2020). In the subsequent baking times, there was a reduction of asparagine levels that was 64.8% and 56.9% respectively for S and V samples baked for 26 min. Thus, it can be hypothesized that the observed variation in asparagine concentration was due to both protein hydrolysis and its participation in chemical reactions leading to its partial degradation, including the Maillard reaction. The effect of heating time on the stability of asparagine and its content in foods has been studied by several researchers (Curtis et al., 2014; Hamlet et al., 2008; Nguyen et al., 2017; Weiss et al., 2018; Žilić et al., 2020). This amino acid turns out to be the limiting precursor in baked products since its concentration is lower compared to the reducing sugars one, 60.11 ± 1.09 mg/kg versus 89.62 ± 3.11 mg/kg, respectively. To confirm this, the results of Pearson's analysis showed a strong correlation between asparagine content and AA value as indicated by a significant r -value of -0.90 .

No relevant differences in the content of reducing sugars were found between the two differently baked biscuit samples, except at 26 min for glucose and 24 and 26 min for fructose. However, these differences were not reflected in the AA content, which did not correlate with r -values of -0.54 and -0.24 for glucose and fructose respectively. Similar to asparagine, glucose and fructose follow a non-linear trend, probably due to simultaneous reduction in the Maillard reaction and formation through the hydrolysis of sucrose that occur during long baking (Amrein et al., 2004; Hamzaloğlu & Gökmen, 2020; Van Der Fels-Klerx et al.,

Table 2

Levels of asparagine, glucose, fructose and sucrose expressed on dry matter basis in the raw dough and in biscuits baked in static (S) and ventilated (V) modes for different times.

Baking time (min)	Asparagine (mg/kg)	Glucose (mg/kg)	Fructose (mg/kg)	Sucrose (g/kg)
Raw				
0	60.11 ± 1.09 ^a	34.32 ± 2.11 ^a	55.30 ± 4.30 ^a	209.29 ± 16.563 ^a
S				
18	29.09 ± 0.62 ^c	16.11 ± 2.09 ^{cde}	27.89 ± 3.95 ^{ef}	154.30 ± 19.93 ^b
20	42.16 ± 2.41 ^b	18.57 ± 0.21 ^{bcd}	48.34 ± 3.71 ^{abc}	156.86 ± 13.01 ^b
22	28.31 ± 1.15 ^c	16.45 ± 3.90 ^{cde}	45.24 ± 4.14 ^{bcd}	184.08 ± 8.63 ^{ab}
24	20.40 ± 1.39 ^e	15.01 ± 1.74 ^{de}	26.58 ± 3.65 ^f	162.55 ± 17.07 ^{ab}
26	14.85 ± 0.69 ^f	11.37 ± 1.19 ^e	36.49 ± 4.21 ^{cdef}	181.55 ± 8.49 ^{ab}
V				
18	25.92 ± 1.36 ^{cd}	14.84 ± 0.10 ^{de}	32.97 ± 5.89 ^{def}	142.32 ± 18.43 ^b
20	38.71 ± 1.72 ^b	23.78 ± 1.58 ^{bc}	48.57 ± 4.95 ^{abc}	181.58 ± 16.28 ^{ab}
22	20.88 ± 3.46 ^{de}	20.30 ± 3.50 ^{bcd}	36.89 ± 1.08 ^{def}	164.97 ± 22.26 ^{ab}
24	21.40 ± 1.29 ^{de}	16.44 ± 1.11 ^{cde}	40.51 ± 4.18 ^{cd}	182.75 ± 19.65 ^{ab}
26	16.70 ± 2.77 ^{ef}	24.64 ± 6.12 ^b	55.83 ± 7.26 ^a	173.51 ± 1.27 ^{ab}

Values in the same column followed by different letters differ significantly at a $P < 0.05$ level.

2014). However, for both samples S and V, under our baking conditions, the sucrose content was slightly reduced non-significantly after 26 min of baking compared to the quantified content in the raw biscuit dough (Table 2) and was not correlated with the AA content of the baked biscuit samples ($r = -0.21$). The low sucrose hydrolysis under the usual baking conditions applied for biscuits has been reported in previous studies (Gökmen et al., 2007; Graf et al., 2006). Probably, for this reason, many studies have suggested that the use of sucrose (non-reducing sugar) in the formulation of bakery products is an effective way to significantly control the AA content (Gökmen et al., 2007; Graf et al., 2006; Nguyen et al., 2016).

The fact that in a ventilated mode the heat during baking is distributed faster and more evenly causing a higher AA formation, was confirmed by the evolution of the main physicochemical characteristics of biscuit samples, whose data are reported in Table 3. The baking

process promoted the dehydration of the biscuits; the moisture and the water activity for both biscuit samples progressively decreased. In particular, moisture and water activity data of S samples baked for 20 and 22 min were significantly higher than those of V ones. Several studies have reported that low moisture and water activity trigger the formation of AA, whereas, in systems containing residual water, the formation of AA is reduced because evaporation reduces the effective temperature, even in putatively dry areas of the product such as the external portions (Bräthen & Knutsen, 2005; Esposito et al., 2020; Matthäus et al., 2004). The strong relationship between pathways formation and these influencing factors was confirmed by the correlation of AA content results with weight loss ($r = 0.84$), moisture ($r = -0.85$), and a_w ($r = -0.93$). With regard to the weight loss after baking, significant differences between S and V samples were only seen after 24 and 26 min of baking when the samples baked in a ventilated mode showed a higher

Table 3

Physicochemical characteristics of biscuits baked in static (S) and ventilated (V) modes for different times.

Baking time (min)	Weight loss (%)	Moisture (%)	Water activity (a_w)	Hue angle (h°) ^a	Browning index (BI) ^a	Hue angle (h°) ^b	Browning index (BI) ^b	Hardness (N)	Crispness (linear distance)	Fracturability (1/mm)
S										
0	–	23.56 ± 0.37 ^a	0.91 ± 0.004 ^a	82.87 ± 0.03 ^a	74.64 ± 0.21 ^g	82.87 ± 0.03 ^a	74.64 ± 0.21 ^d	–	–	–
18	17.25 ± 0.04 ^f	7.77 ± 0.06 ^b	0.58 ± 0.00 ^b	76.26 ± 0.43 ^b	76.76 ± 2.93 ^f	69.64 ± 0.23 ^b	114.56 ± 5.69 ^c	44.70 ± 2.71 ^f	57.31 ± 1.52 ^g	0.39 ± 0.01 ^e
20	19.16 ± 0.05 ^e	5.89 ± 0.23 ^c	0.47 ± 0.01 ^c	74.79 ± 0.59 ^c	83.50 ± 2.63 ^e	69.27 ± 0.26 ^c	114.23 ± 5.38 ^c	45.84 ± 2.16 ^f	78.62 ± 2.54 ^e	0.56 ± 0.02 ^d
22	19.74 ± 0.16 ^d	4.72 ± 0.23 ^e	0.39 ± 0.01 ^e	71.88 ± 0.29 ^d	89.29 ± 2.61 ^d	67.98 ± 0.03 ^c	120.46 ± 5.14 ^{ab}	63.87 ± 6.14 ^c	104.85 ± 6.77 ^d	0.60 ± 0.02 ^c
24	20.87 ± 0.06 ^c	3.64 ± 0.17 ^g	0.28 ± 0.01 ^g	70.85 ± 0.03 ^{ef}	90.91 ± 2.30 ^{cd}	67.18 ± 0.22 ^f	120.58 ± 2.39 ^{ab}	62.60 ± 1.40 ^c	125.09 ± 2.09 ^c	0.70 ± 0.01 ^b
26	21.29 ± 0.08 ^{bc}	2.85 ± 0.03 ^h	0.21 ± 0.00 ^h	70.09 ± 0.11 ^g	94.95 ± 2.46 ^{ab}	67.16 ± 0.16 ^{fg}	124.25 ± 4.41 ^a	71.34 ± 2.47 ^b	169.65 ± 3.91 ^a	0.74 ± 0.01 ^a
V										
0	–	23.50 ± 0.50 ^a	0.90 ± 0.01 ^a	82.89 ± 0.01 ^a	74.62 ± 0.06 ^g	82.88 ± 0.01 ^a	74.62 ± 0.06 ^d	–	–	–
18	17.55 ± 0.21 ^f	7.55 ± 0.02 ^b	0.59 ± 0.01 ^b	74.18 ± 0.19 ^c	78.97 ± 2.39 ^f	68.49 ± 0.22 ^d	114.18 ± 2.55 ^c	51.41 ± 0.50 ^e	67.85 ± 0.69 ^f	0.42 ± 0.03 ^e
20	19.25 ± 0.13 ^e	5.40 ± 0.27 ^d	0.44 ± 0.02 ^d	71.06 ± 0.14 ^{de}	91.66 ± 2.88 ^{cd}	67.80 ± 0.19 ^e	117.46 ± 2.44 ^{bc}	56.42 ± 0.60 ^d	104.24 ± 2.47 ^d	0.53 ± 0.01 ^d
22	19.77 ± 0.12 ^d	4.29 ± 0.41 ^f	0.36 ± 0.05 ^f	70.28 ± 0.18 ^{fg}	91.83 ± 2.70 ^{cd}	67.22 ± 0.10 ^f	118.89 ± 2.82 ^{ab}	66.20 ± 1.21 ^c	123.41 ± 0.97 ^c	0.63 ± 0.02 ^c
24	21.65 ± 0.29 ^b	3.53 ± 0.25 ^g	0.28 ± 0.03 ^g	69.99 ± 0.14 ^g	94.06 ± 1.63 ^{ab}	67.26 ± 0.22 ^{fg}	119.60 ± 2.71 ^{ab}	69.80 ± 2.55 ^{bc}	147.46 ± 2.10 ^b	0.65 ± 0.04 ^{cb}
26	22.33 ± 0.10 ^a	2.95 ± 0.14 ^h	0.26 ± 0.02 ^h	69.64 ± 0.12 ^g	96.86 ± 1.89 ^a	66.78 ± 0.13 ^g	119.49 ± 2.98 ^{ab}	83.63 ± 3.46 ^a	168.96 ± 5.48 ^a	0.71 ± 0.02 ^a

Values in the same column followed by different letters differ significantly at a $P < 0.05$ level.

^a Top surface of the biscuits.

^b Bottom surface of the biscuits.

value, due to the greater evaporation of water under forced air convection conditions.

During baking, there was also a significant change in the colour of the surface of the biscuits. As expected, as the level of AA increased, the colour of the biscuits became progressively more brownish prolonging the baking times on the top/bottom surfaces according to significant r correlation values of $-0.92/-0.82$ and $0.88/0.81$ for hue angle and browning index, respectively. In general, for all samples, the bottom surface of the biscuits was characterised by a higher browning index and lower hue angle value, indicating a more intense colouring than the top surface. The top surface of samples baked in ventilated mode showed lower hue angle and higher browning index values thus a redder and browner colour than S samples (Table 3). No significant differences in top surface colour between both samples were found after 26 min of baking. Regarding the colour of the bottom surface of the biscuit, no peculiar differences were found between ventilated and static samples except for the hue angle in the samples baked for 18, 20, and 22 min showing a slightly redder colouration for the biscuits baked in a ventilated oven. This is attributed to the fact that in both modes the heat to the below part was transmitted mainly from the baking tray.

The texture is another important quality parameter for biscuits and in general bakery products. Together with the formation of AA, hardness ($r = 0.93$), crispness ($r = 0.97$), and fracturability ($r = 0.90$) also increased progressively in all samples as the baking time increased; this positive correlation is attributed to the increase in the degree of baking of the biscuit and the lowering of the moisture content. Also in this case, the S biscuits samples showed hardness and crispness values lower than V samples at all baking times, except for crispness at 26 min, in which values were not statistically different. In the ventilated mode the flow of hot air circulating throughout the oven contributes to accelerate the crust formation on the surface (Palazoglu et al., 2015). On the other hand, the fracturability increased with prolonging baking times but did not show significant differences between samples baked with the two different modes.

4. Conclusions

The results obtained in this study confirmed that in the baking process carried out in an oven set up in ventilated mode the heat is distributed in a more homogeneous way causing a faster temperature rise in the product, which consequently dehydrates and bakes faster than in static mode. This has been clearly demonstrated both by the recorded time-temperature profiles and the results of the baking parameters (weight loss, moisture, a_w , texture, and colour) measured on biscuits baked for different times. Moreover, the heat transferred by convection in the ventilated mode promoted a higher formation of AA in the biscuits compared to those baked under static conditions, mainly after 20 and 22 min of baking. On the basis of the overall quality parameter results and the lowest AA levels reached, it can be concluded that the best baking conditions were in the static baking mode at 175°C for 20 and 22 min. However, considering the formulation and the baking conditions applied in this study, all biscuit samples including the over-cooked ones, showed concentrations of AA lower compared to the reference value reported in Commission Regulation (EU) 2017/2158, which for "Biscuits and wafers" is of $350\text{ }\mu\text{g/kg}$.

Even if this study confirmed that generally baked biscuits can be considered a low/medium risky product category in terms of AA content, a deeper understanding and characterization of the presence of this toxic and carcinogenic compound in this kind of heat-processed product, as a function of the applied baking technology, is still needed and necessary.

CRedit authorship contribution statement

Maria Alessia Schouten: Writing – original draft, Investigation, Formal analysis. **Silvia Tappi:** Investigation, Writing – review & editing.

Virginia Glicerina: Investigation, Methodology, Writing – review & editing. **Pietro Rocculi:** Writing – review & editing, Resources. **Simone Angeloni:** Investigation, Methodology, Writing – review & editing. **Manuela Cortese:** Investigation, Writing – review & editing. **Giovanni Caprioli:** Investigation, Writing – review & editing. **Sauro Vittori:** Writing – review & editing, Supervision. **Santina Romani:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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