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Impact of ingredients added in sweet and savory biscuits on the contents of furanic compounds and 3-methylbutanal

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A R T I C L E I N F O

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A B S T R A C T

Ingredients and processing conditions influence the content of volatile compounds and heat-generated contaminants in baked products including biscuits. However, limited research has focused on the effect of specific ingredients. Hence, the present study investigated the influence of various added ingredients in sweet and savory biscuits – including dried plums and apricots, roasted pistachios and peeled almonds for sweet biscuits; and black olives (with and without brine), sunflower and sesame seeds for savory biscuits - on the contents of furan derivatives and 3-methylbutanal, analyzing the final products, raw doughs and individual ingredients by HS-SPME-GC–MS instrument. The content of furanic compounds was higher in ingredients added in sweet preparations especially in dried plums and apricots. In addition, sweet biscuits were richer in furan derivatives especially those formulated with dried plum. Therefore, careful selection of ingredients together with processing optimization are crucial to balancing both safety and sensory quality features of baked products.

1. Introduction

Biscuits are among the most widely consumed baked goods globally, appreciated for their taste, convenience and long shelf-life. According to a recent study by Motiwala et al. (2023), biscuit and cookie consumption increased by 40.9 % during the COVID-19 pandemic among the Indian population, reflecting shifts in dietary behaviour toward convenient and shelf-stable products. Globally, the biscuit market has also shown steady growth, with an estimated market size exceeding USD 100 billion and a projected compound annual growth rate (CAGR) of over 5 % from 2022 to 2028 (Mordor Intelligence; Statista). This highlights the growing importance of studying the overall quality and safety characteristics of these baked products and the ingredients used in their formulation. Biscuits are primarily made from wheat flour, sugar, fat and leavening agents, which are combined through various mixing techniques (Misra and Tiwari, 2014). The dough is then formed into shapes by sheeting, cutting and molding (Xu et al., 2020), before being baked at certain temperatures for specific durations (Nakov et al., 2018). Once baked, the biscuits undergo cooling and packaging as final steps (Xu et al., 2020). Sugar in the formulation provides sweetness, color and volume while also affecting the texture of biscuits by influencing starch gelatinization. Similarly, fat plays a crucial role in biscuit production, with the type of fat used having a significant impact on the quality of the final product (Mamat and Hill, 2018). Moreover, the baking process involves complex physical and chemical mechanisms, including heat and mass transfer, moisture exchange and biochemical reactions (Mosalam, 2021). Specifically, heat during baking affects enzymatic reactions, starch gelatinization, protein denaturation and water migration, all of which influence the quality of the final product (Mosalam, 2021). The moisture exchange between the dough and the environment, as well as the moisture content, are crucial for the baking process. The completion of the starch gelatinization and the achievement of the desired moisture content are therefore important indicators of a successful baking (Semenov et al., 2021).

The Maillard reaction is a complex series of chemical reactions between reducing sugars and amino acids that occur during the baking

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process. This non-enzymatic browning reaction plays a crucial role in determining the color, flavor and aroma of baked goods. (Al-Abbasy et al., 2024; Tamanna and Mahmood, 2015). It proceeds through the initial formation of a Schiff base, followed by Amadori rearrangement and the generation of various intermediate compounds. (Gao et al., 2023; Lee et al., 2022 These intermediates can undergo further reactions leading to the formation of flavor-active molecules, such as pyrazines and thiols, as well as heat-induced contaminants, including furanic compounds and 3-methylbutanal (Starowicz and Zieliński, 2019). The specific profile and concentration of these compounds depend on the ingredient composition, baking temperature and moisture content (Acquaticci et al., 2024; Huault et al., 2016). Furan is produced during the thermal processing of various foods due to the thermal degradation of carbohydrates and other food constituents (Javed et al., 2021). Some of these compounds are implicated in carcinogenicity, genotoxicity, and other adverse health effects (Javed et al., 2021).

In both industrial and artisanal biscuit production, a variety of ingredients are commonly used, such as dried fruits, nuts, olives, and seeds. In sweet formulations, dried plums and dried apricots are widely employed for their high sugar content, acidity and moisture, all of which can influence Maillard and caramelization reactions. Roasted pistachios and peeled almonds are used for their lipid and amino acid content, affecting aroma and flavor development through Strecker degradation and lipid oxidation pathways. In savory preparations, black olives (with and without brine) are included as examples of fermented plant-based ingredients, which can introduce organic acids, sugars and volatiles. Sunflower and sesame seeds contribute amino acids and unsaturated fats, with the potential to act as precursors in the formation of volatile compounds during baking. The addition of these ingredients may alter the compositional profile and have a technological impact on biscuit formulations, making them suitable for investigating their role in the formation of furanic compounds and 3-methylbutanal.

Particularly, furan has been classified as Group 2B by the International Agency for Research on Cancer (IARC) and defined as "possibly carcinogenic to humans" based on sufficient evidence in experimental animals (Monographs of IARC, List of Classifications). Among the other furan derivatives, 5-(hydroxymethyl) furfural (5-HMF) and furfuryl alcohol (FFA) have shown significant evidence of toxic effects. 5-HMF can be metabolized into 5-sulfoxymethylfurfural, which has been shown to react with DNA and proteins, potentially causing mutations (Petisca et al., 2013; Shen et al., 2016). Furfuryl alcohol has also been classified as Group 2B by the IARC, indicating it is possibly carcinogenic to humans (Monographs of IARC, List of Classifications). These furanic derivatives are of particular concern due to their potential health risks and the need for monitoring their levels in food products. 5-HMF and furfural (FF) are formed mainly by caramelization and Maillard reaction (Capuano and Fogliano, 2011; Lee et al., 2022). When absorbed by the body, they can be converted into metabolites that cause genotoxicity, carcinogenicity and hepatoxicity. These furanic compounds have a carbonyl group in their structures that can interact with free asparagine through the Maillard reaction and/or during digestion, leading to AA formation (Aktağ et al., 2022; de Borba et al., 2024).

Similarly, acrylamide (AA) is another heat-generated contaminant classified as probably carcinogenic to humans (Group 2A) by the International Agency for Research on Cancer (Monographs of IARC, List of Classifications).

This study investigates the effects of adding various ingredients on the concentration of some volatile organic compounds (VOCs) in the formulation in sweet and savory biscuits. The investigation comprises three primary components: (1) profiling furanic compounds (furfural, furfuryl acetate, 5-methylfurfural, 5-HMF and furfuryl alcohol) and 3-methylbutanal in the individual additional ingredients used to enrich the biscuits formulations; (2) comparing the furanic compounds and 3-methylbutanal in both dough and baked biscuits to understand how baking and ingredients influences these compounds; and (3) examining the correlation between the previously mentioned VOCs and acrylamide in the baked biscuits. This study aims to address the limited research on how specific ingredients differently affect the formation of heat produced compounds in complex food matrices like biscuits.

2. Materials and methods

2.1. Reagents and standards

Deionized water was further purified using a Milli-Q SP Reagent Water System (Millipore, Bedford, MA, USA). Before ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) analysis, all samples were filtered with a PhenexTM RC 4 mm 0.2 μm syringeless filter. Pure standards of furfural (CAS 98–01-1), furfuryl acetate (CAS 623–17-6), 5-methylfurfural (CAS 620–02-0), 5-(hydroxymethyl) furfural (5-HMF) (CAS 67–47-0), furfuryl alcohol (CAS 90–00-0), 3-methylbutanal (CAS 590–86-3), 2-methylpentanal (CAS 123–15-9) and HPLC-grade ethanol were purchased from Sigma Aldrich (Milan, Italy). 2-Methylpentanal were used as internal standard (IS). The stock solutions of target volatile compounds were prepared by mixing 10 mg of pure standards with 10 mL of HPLC-grade ethanol (1000 $\mu g \cdot mL^{-1}$). Standard working solutions at different concentration were prepared by diluting the stock solutions.

2.2. Biscuit samples

All the ingredients used for enrichment, including dried plums, dried apricots, roasted pistachios, peeled almonds, black olives (with and without brine), sesame seeds and sunflower seeds, were purchased from local Italian markets.

The basic recipes for the biscuits were prepared according to the procedure reported by Schouten et al. (2024). In detail, wheat flour type "00" (250 g), sugar (62.5 g), pasteurized eggs (62.5 g), sunflower oil (50 g) and raising agent (sodium diphosphate and carbonates, 7.5 g) were used for the sweet products. While, the basic recipe for the savory biscuits included wheat flour type "00" (250 g), water (100 g), extra virgin olive oil (25 g), salt (5 g) and raising agent (diphosphate and carbonates, 5 g).

To study the effect of different ingredients, 10 % (*w*/w) of selected ingredients (calculated based on the total amount of all other ingredients in each basic recipe) were added to both sweet and savory biscuit formulation. For sweet biscuits, enrichment consisted of dried plums, dried apricots, roasted pistachios or peeled almonds, while the savory ones were enriched with black olives with brine, black olives without brine, sesame seeds or sunflower seeds. To prepare the dough, flour, sugar, salt and raising agent were combined in a Bimby Robot TM31 (Vorwerk, Germany) on speed 3 for 15 s. Eggs, oil and water were added and mixed on speed 4 for 1 min and 30 s. The enriched biscuits samples were made by adding the selected enrichment ingredients to the dough and mixing on speed 3 for 1 min. The almonds and pistachios were added crushed, the apricots, plums and olives were diced, and the seeds were added whole. After the dough was formed, it was kneaded by hand for 1 min to ensure a uniform texture. They were then left to rest for 20 min at around 4 °C in the refrigerator before being shaped. After the resting period, the dough was rolled out to a thickness of 3 mm using a semi-industrial dough sheeter (S-420 INOX, Gam International, Italy). The round biscuits were cut out using a plastic mold with a diameter of 8 cm.

For each recipe, six raw biscuits (weighing approximately 19.8 g each) were baked in a static electric oven preheated to 175 °C (Apollo PS1 Steamer, AEG-Electrolux, Germany) for the optimum baking time, selected based on preliminary sensorial tests and by monitoring key baking parameters (i.e., moisture and texture). The sweet control sample required 14 min to bake, while the biscuits enriched with peeled almonds and roasted pistachios took 9 min and those with dried apricots and dried plums took 13 min. For the savory biscuits, the control sample and the varieties with sunflower and sesame seeds required 20 min baking, while the biscuits with black olives, both with and without brine, took 23 min. The ingredients added to the sweet and savory base recipes differed significantly in moisture content and chemical composition, resulting in different rates of water evaporation and chemical reaction kinetics during baking and therefore different final baking times to achieve the optimum required/desired quality characteristics. Each dough recipe and baking process was repeated twice, resulting in 12 biscuits for each recipe and a total of 96 biscuits for all samples.

2.3. Furanic compounds quantification by HS-SPME-GC–MS

2.3.1. Sample preparation

The furanic compounds quantification in dough, added ingredients and baked biscuits followed previous analytical methods by Acquaticci et al. (2023) with slight modifications. Briefly, immediately after the opening, sample (respectively ingredients, dough, and baked biscuits) was frozen with liquid nitrogen and ground in small particles, and 1 g of it was placed into a 20 mL vial. Then, it was rapidly added the internal standard and immediately after the vial was sealed with a screw cap with a PTFE septum.

2.3.2. Headspace solid-phase microextraction (HS-SPME)

Furanic compounds were determined using a headspace solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS). The analysis was performed with a method previously developed by Acquaticci et al. (2023). Briefly, the fiber was conditioning for 10 min at 250 °C and then it was inserted inside the headspace of sample vial with a speed of 20 mm·s^{-1} and a penetration depth of 40 mm. The extraction was performed at 60 °C for 30 min and then the fiber was inserted into injector port at a speed of 100 mm·s^{-1} and a penetration depth of 40 mm. The desorption occurred at 250 °C for 1 min. After desorption, the fiber was conditioning at 250 °C for 10 min.

2.3.3. GC–MS analysis

The GC–MS analysis was carried out using an 8890-gas chromatograph (GC) equipped with a PAL RTC 120 autosampler and a 5977B mass spectrometer (MSD) Agilent Technologies (Santa Clara, CA, USA). The ionization was obtained by using an electron ionization source (EI). The injector temperature was set at 250 °C and the liner used was recommended for SPME injection namely Inlet liner, Ultra Inert, splitless, straight, 0.75 mm i.d. from Agilent Technologies. The gas carrier was helium at flow rate of 1 mL·min^{-1}. The separation of target molecules was established on DB-WAX capillary column (60 m, 0.25 mm i.d., 0.25 μm film thickness) with the following ramp of temperature: 35 °C held for 3 min, 35–200 °C at 5 °C/min, 200–250 °C at 15 °C/min and 250 °C held for 5 min. The run time was about 44.3 min. The transfer line was set at 250 °C and the temperature of the ionization source and the mass analyzer were set at 230 and 150 °C, respectively. The ion species selection and optimization for all the volatile compounds were carried out by injecting a standard solution (10 μg·mL^{-1}) in SCAN mode (35–450 *m/z*) and the three most abundant ions were selected for each analyte. The acquisitions were carried out in "Selected Ion Monitoring" (SIM) mode and detection was divided into time windows to enhance the sensitivity (Acquaticci et al., 2023). The most abundant ions were used for quantitation while the other to confirm the presence of the analytes. The GC–MS acquisition parameters including the selected ions, retention time (Rt) and time windows are reported in **Table S1**. Data results were managed by MSD ChemStation Software (Agilent, Version G1701DA D.01.00). The internal standard used was 2-methylpentanal (it was added in each sample at a concentration of 10 μg·mL^{-1} prior to extraction process). Moreover, the LODs and LOQs for all analyzed compounds were determined based on a signal-to-noise ratio of 3:1 and 10:1, respectively, and are reported in the Supplementary Materials (**Table S5**).

2.4. Acrylamide quantification by HPLC-MS/MS system

Acrylamide quantification was not performed in the present study. The authors used previously published data from Schouten et al. (2024), where acrylamide levels were determined for the same biscuit samples. The procedure involved ultrasound-assisted extraction with water, lipid removal by liquid-liquid extraction and sample concentration by freeze-drying, followed by SPE purification, as detailed in earlier work. Finally, the samples were injected into an Agilent 1290 Infinity Series UHPLC and 6420 Triple Quadrupole with electrospray (ESI) ionization source working in positive polarity and the acquisition was performed in Multiple Reaction Monitoring" (MRM) mode. This approach allowed for the consistent detection of acrylamide levels across various biscuit samples.

2.5. Statistical analysis

All the data were reported as means ± standard deviation. Significant difference among treatments was evaluated through analysis of variance (ANOVA) and Tukey's post-hoc (*p*-value <0.05). Moreover, principal component analysis (PCA) and Pearson correlation (r) were used to analyses the correlation between the evaluated VOCs and acrylamide (*p*-value <0.05). The XLSTAT (Premium, Lumivero, USA) statistical program was used.

3. Results and discussion

3.1. Furanic compounds and 3-methylbutanal: Ingredients analysis

Fig. 1 provides a detailed overview of the concentrations of furanic compounds (furfural, furfuryl acetate, 5-methylfurfural, furfuryl alcohol and total furans) and 3-methylbutanal in the additional ingredients used for sweet and savory biscuits formulations. The data underscores the significant differences between the various additional ingredients, which have an impact on the safety and sensory qualities of the final biscuit products.

3.1.1. Ingredients added in sweet biscuits

Among the sweet ingredients, dried plums exhibited the highest total concentration of furanic compounds (65.35 ± 9.84 mg/kg), largely due to the high level of furfural (58.39 ± 8.97 mg/kg). Statistical analysis confirmed that furfural content in plums was significantly higher (*p*-value <0.05) than in other ingredients (Fig. 1a). To the best of authors' knowledge, few papers reported the presence of furan derivatives in dried fruits. For instance, Fromberg et al. (2014) focused on other furan derivatives such as furan, 2-alkylfurans and 2-methylfuran in dried plum, while Batool et al. (2020) reported furfural levels in various dried fruits, including plum, with significantly lower concentrations (ranging from < LOD to 7.21 μg/kg) compared to the current study. These differences can be attributed to differences in fruit variety/agricultural practices, as well as variation in the processing methods/conditions, including drying techniques and other treatments applied by different manufacturers, which can influence the chemical composition of the final product (Batool et al., 2020). Following the dried plums, dried apricots contained furfural as the main abundant furan derivative with a concentration of 2.93 ± 0.47 mg/kg, although it occurred almost twenty-times lower than those found in plum. Similarly, Göğüş et al. (2007) identified furfural as the major volatile component in dried apricots by studying the influence of various drying techniques on volatiles formation.

Among the ingredients evaluated, dried pistachios have shown the highest concentrations of 3-methylbutanal (18.85 ± 1.09 mg/kg) and furfuryl alcohol (2.46 ± 0.05 mg/kg). The high presence of 3-methylbutanal, a branched-chain aldehyde associated with the flavor of hard and semi-hard cheese varieties, contributes to the distinct flavor profile of pistachios in enriched baked products (Afzal et al., 2017). Acena et al.

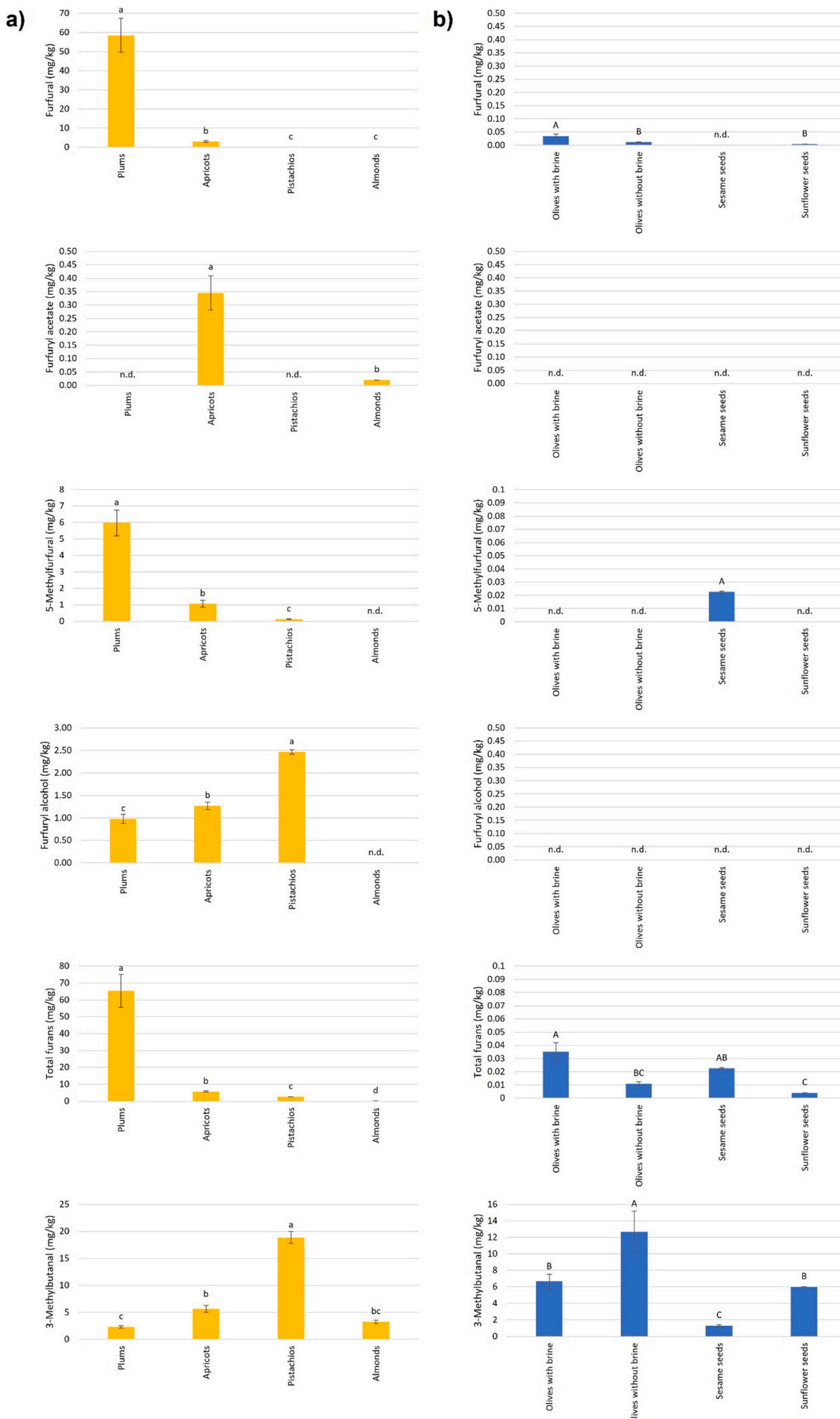

Fig. 1. Concentration of furfural, furfuryl acetate, 5-methylfurfural, furfuryl alcohol, total furans and 3-methylbutanal in the ingredients used for sweet (**a**) and savory (**b**) biscuits. Significant differences (p-value <0.05) between ingredients are indicated by different letters above the bars and n.d. means not detected because below the limit of quantification (< LOQ).

(2011) also reported 2- and 3-methylbutanal as main odorants in roasted pistachios nuts, derived via Maillard reaction, lipid degradation/oxidation and sugar degradation. Comparable levels of furfuryl alcohol (from 260.19 ± 2.19 to 933.83 ± 39.60 μg/kg) have been reported in differently hulled pistachios by Sonmezdag et al. (2019) while Mesías et al. (2024) also reported the presence of fructosyl-lysine (determined as furosine), 5-HMF and furfural in roasted almonds. Moreover, Kelebek et al. (2020) reported that the ripening stage of pistachios influences their chemical composition, with unripe pistachios being preferred for use in desserts, sauces, ice creams and chocolate, while ripe pistachios are more commonly consumed as a roasted snack. On the other hand, dried almond had lower concentrations of the evaluated furanic compounds compared to other ingredients analyzed. This aligns with other published studies (Lee et al., 2014; Xiao et al., 2014), which reported only trace (some micrograms per kilograms) of these furan derivatives. In detail, Xiao et al. (2014) reported contents of furfural and furfuryl alcohol of 0.10–0.46 mg/kg and of 1.20–5.20 μg/kg, respectively, in differently roasted almonds. While Lee et al. (2014) found variable concentration of furfural (4.9–460.0 μg/kg) and furfuryl alcohol (0.4–5.2 μg/kg) according to the roasting levels and storage times. These results indicate that furanic compounds levels in almonds are affected by multiple factors, including processing methods/conditions such as roasting, blanching and storage times (Lee et al., 2014; Pasqualone et al., 2018; Xiao et al., 2014). Finally, 3-methylbutanal was also present in dried almond (3.25 ± 0.29 mg/kg), in agreement with the levels reported in literature ranging from 0.91 to 4.27 mg/kg (Xiao et al., 2014). In line with our observations, Mesías et al. (2024) reported the formation of both acrylamide and 5-HMF in roasted almonds, highlighting how temperature and precursor availability significantly affect the formation of these thermal contaminants in nuts.

3.1.2. Ingredients added in savory biscuits

In the ingredients added to savory biscuits, just traces of furfural and 5-methylfurfural were found in table black olives (both with and without brine) and in sesame seeds. Instead, in all other cases the concentrations of the furan derivatives were below the limit of quantification (< LOQ). Differently, 3-methylbutanal was found in concentrations ranging from 1.29 ± 0.14 to 12.68 ± 2.51 mg/kg, showing levels comparable to those observed in the ingredients added in sweet biscuits. According to Fig. 1b, the highest concentration of 3-methylbutanal was measured in olives without brine (12.68 ± 2.51 mg/kg), followed by olives with brine (6.65 ± 0.84 mg/kg), sunflower seeds (6.00 ± 0.00 mg/kg) and sesame seeds (1.29 ± 0.14 mg/kg). Consistent with our findings, Berk et al. (2019) demonstrated that roasting sesame seeds significantly enhances the formation of Maillard-derived markers such as furans, supporting the role of seeds as potent precursors in thermally induced reactions. The furan derivatives and in general VOCs present in table olives with/without brine are influenced from various factors, including fruit characteristics, ripening stage and processing conditions. In particular, the fermentation steps involved in table olive processing contribute to a more complex aroma/volatiles profile (Sabatini and Marsilio, 2008). In addition the presence of low or high amount of NaCl can impact the fermentation procedure and consequently the volatilome of table olives (Alvanoudi et al., 2024). Despite this, very few studies have been conducted on volatile compounds analysis in table olive (Sabatini and Marsilio, 2008; Sansone-Land et al., 2014). For example, Sansone-Land et al. (2014) found 3-methylbutanal concentrations in black-ripe table olives from 25 to 132 μg/kg, considerably lower than the levels observed in the present study. On the other hand, to the best of authors' knowledge, this is the first study regarding the quantification of furanic compounds in table black olive, both with and without brine. Interestingly, Sánchez et al. (2022) reported that sterilization treatments applied to Californian-style black olives significantly affected their volatile profiles, another factor which can contribute to the differences found between olive with and without brine. Additionally, this research represents the first documented quantification of furan derivatives in sunflower and sesame seeds. The only relevant study in the literature that indirectly addresses this topic is the work by Guo et al. (2019), which analyzed the influence of roasting condition on the flavor profile of sunflower seeds. The study reported 3-methylbutanal concentrations ranging from 0.11 to 1.60 mg/kg and furfural levels between 0.35 and 8.18 mg/kg in sunflower seeds. The significant differences in concentration observed in the present study may be attributed to a myriad of factors, including seeds origin, pre-treatment, processing methods/conditions and other variables that influence their chemical composition.

3.2. Furanic compounds and 3-methylbutanal: Dough and biscuits analysis

The comparison on the concentrations of furanic compounds (furfural, 5-methylfurfural, furfuryl alcohol and 5-HMF) and 3-methylbutanal among biscuits and starting doughs is highlighted in the following subsections as well as among different sweet and savory biscuits.

3.2.1. Sweet dough and biscuits

Table 1 presents the contents of furanic compounds and 3-methylbutanal in the raw doughs and the resulting sweet biscuits, formulated with dried plums, dried apricots, roasted pistachios and peeled almonds.

As expected, all baked biscuits contained higher concentrations of furanic compounds compared to their respective raw doughs since baking process promotes the formation of these molecules through various physical and chemical mechanisms (Acquaticci et al., 2024). However, some studies indicate that under prolonged high-temperature baking in a model cookie, compounds such as furfural and 5-HMF may undergo degradation or volatilization into the baking vapors, leading to their reduction in the final product (Ameur et al., 2008). In contrast to furanic compounds, 3-methylbutanal was found at lower levels in baked biscuits compare to raw doughs, with statistically significant reductions observed in formulations containing dried apricots, roasted pistachios and peeled almonds (p-value <0.05). This reduction can be attributed to the low boiling point of 3-methylbutanal (94 °C), which facilitates its evaporation during the baking process (Acquaticci et al., 2024).

The type of added ingredient influenced the final concentrations of total furanic compounds of biscuits. Biscuits formulated with dried plum (19.60 ± 1.57 mg/kg) had the higher levels followed by the ones with dried apricots (2.16 ± 0.11 mg/kg), pistachios (2.11 ± 0.22 mg/kg) and peeled almonds (1.55 ± 0.09 mg/kg). This trend aligns with the results found in the ingredients themselves, where dried plums contained the highest levels of furanic compounds, followed by dried apricots, pistachios and peeled almonds. Interestingly, all enriched biscuits, except those with dried plums, contained lower concentrations of furanic compounds than that found in the control biscuits (without additional ingredient). In biscuits formulated with dried plums and with peeled almonds the concentrations of furanic compounds were statistically significant higher and lower, respectively, than that reported in the control biscuits (p-value <0.05). This again confirm the influence of the ingredients in the biscuit formulations as the two ingredients, respectively, with the highest and lowest content of furans have affected more their levels in biscuits. Among the furanic compounds detected, furfural (11.91 ± 0.35 mg/kg) and 5-methylfurfural (3.79 ± 0.52 mg/kg) were the most abundant in dried plum biscuits. Notably, 5-HMF (2.50 ± 0.53 mg/kg) was detected only in these biscuits, likely due to its degradation into furfural and 5-methylfurfural during the later stages of baking (Ameur et al., 2008). The high moisture content (12.7 ± 0.1 %) and low pH (3.7 ± 0.0) of dried plum ingredient, as reported by Schouten et al. (2024), are known to both modulate the development of chemical reactions, possibly favoring the formation of 5-HMF and related compounds. In addition, higher content of sugar, in this case gave by dried plum, can promote the formation of these furanic compounds, as previously observed in other studies (Mesias et al., 2021).

Table 1
Contents (mg/kg) of furanic compounds and 3-methylbutanal in sweet dough and biscuits. Results are presented as average values ± standard deviation. Different letters in the same column indicate significant differences between samples (Tukey's test, $p \leq 0.05$).

Sweet samples	Furfural	5-Methylfurfural	Furfuryl alcohol	5-HMF	Total furanic compounds	3-methylbutanal
Cd	0.098 ± 0.011^{d}	0.015 ± 0.001^{b}	n.d.	n.d.	0.113 ± 0.012^{e}	6.721 ± 1.294^{abc}
Cb	0.297 ± 0.002^{d}	0.078 ± 0.004^{b}	2.421 ± 0.011^{a}	n.d.	2.795 ± 0.013^{bc}	5.987 ± 0.420^{cd}
PLd	2.513 ± 0.134^{b}	0.292 ± 0.019^{b}	n.d.	0.914 ± 0.050^{b}	3.718 ± 0.204^{b}	6.665 ± 1.279^{abc}
PLb	11.914 ± 0.348^{a}	3.791 ± 0.523^{a}	1.393 ± 0.171^{c}	2.499 ± 0.532^{a}	19.597 ± 1.574^{a}	8.260 ± 0.815^{a}
APd	0.067 ± 0.004^{d}	0.025 ± 0.002^{b}	n.d.	n.d.	0.091 ± 0.006^{e}	7.880 ± 1.602^{ab}
APb	0.818 ± 0.038^{c}	0.149 ± 0.010^{b}	1.192 ± 0.062^{d}	n.d.	2.159 ± 0.110^{cd}	5.371 ± 0.371^{cd}
PId	0.276 ± 0.042^{d}	0.071 ± 0.004^{b}	n.d.	n.d.	0.347 ± 0.047^{e}	6.090 ± 0.129^{bcd}
PIb	0.259 ± 0.020^{d}	0.015 ± 0.001^{b}	1.842 ± 0.203^{b}	n.d.	2.115 ± 0.223^{cd}	3.354 ± 0.339^{e}
ALd	0.094 ± 0.009^{d}	0.044 ± 0.006^{b}	n.d.	n.d.	0.138 ± 0.015^{e}	7.027 ± 1.023^{abc}
ALb	0.159 ± 0.028^{d}	0.055 ± 0.001^{b}	1.341 ± 0.062^{cd}	n.d.	1.554 ± 0.091^{d}	4.262 ± 0.710^{de}

Cd, control dough; **Cb**, control biscuits; **PLd**, dough formulated with dried plums; **PLb**, biscuits added with dried plums; **APd**, dough formulated with dried apricots; **APb**, biscuits added with dried apricots; **PId**, dough formulated with pistachios; **PIb**, biscuits added with pistachios; **ALd**, dough formulated with almonds; **ALb**, biscuits added with almonds. n.d. = not detected.

The concentrations of furanic compounds observed in this study are in line with those reported in literature. For example, Petisca et al. (2014) analyzed 16 commercial biscuits, finding 5-HMF and furfural concentrations ranging from 1.65 to 82.78 mg/kg and from not detectable to 10.49 mg/kg, respectively. Similarly, Acquaticci et al. (2024) found 5-methylfurfural (0.2–1.4 mg/kg) and furfuryl alcohol (0.9–3.1 mg/kg) in commercial biscuits, which are comparable to the values reported in the present study.

Similar to the furanic compounds, the highest content of 3-methylbutanal concentration was detected in dried plums biscuits (8.26 ± 0.81 mg/kg), while pistachios biscuits sample contained the lowest level (3.35 ± 0.34 mg/kg). No significant differences were observed in 3-methylbutanal concentrations among all raw dough samples (enriched vs control). However, two differences emerged in the baked samples. The first between dried plum biscuits and control and the second between pistachios sample and the control (p-value <0.05). These variations can be attributed to the added ingredients, which influence both the chemical composition and physical properties of the doughs, thereby favoring or inhibiting the formation and degradation of 3-methylbutanal.

3.2.2. *Savory dough and biscuits*

Table 2 reports the concentration of furanic compounds and 3-methylbutanal in raw doughs and the resulting savory biscuits, formulated with black olives with and without brine, sesame and sunflower seeds.

Similar to the sweet samples, all the savory biscuits contained higher contents of furanic compounds than the related raw doughs, demonstrating again their formation during the baking process. However, since furanic compounds were present only in trace amounts in the individual additional ingredients (Fig. 1), their supplementation resulted in an overall decrease in these volatiles levels in all raw doughs. At the same time, these ingredients acted as precursors that favored their formation during baking. In fact, previous studies have reported that the presence of seeds or spices could promote the formation of furfural and other related molecules. For example, the sesame seeds increased the formation of the Maillard reaction products over baking (Mesias et al., 2021).

Among the monitored furans, furfuryl alcohol (0.80–3.98 mg/kg) and furfural (0.47–0.90 mg/kg) were the most abundant, followed by traces of 5-methylfurfural (0.04–0.09 mg/kg). Conversely, 5-HMF was always under the limit of quantification in all samples. This aligns with findings from other studies, which reported that its concentration in baked products can vary widely depending on numerous factors such as differences in the composition of the cereals, recipe ingredients, and type of processing conditions (Ameur et al., 2008; Mesias et al., 2021).

The concentration of 3-methylbutanal varied depending on the type of sample. Notably, control biscuits and black olive biscuits (with brine) contained significantly lower levels of 3-methylbutanal than their respective raw doughs. On the other hand, its contents were statistically significantly higher than their raw doughs in sunflower and sesame seeds biscuits, while no statistically differences were observed for black olives without brine sample. The formation and degradation of this aldehyde is controlled by numerous factors related to cooking conditions, recipe and presence of additional ingredients. In detail, this compound is mainly formed during the Strecker reaction by deamination and decarboxylation of amino acids by dicarbonyls produced in the Maillard reaction (Van Boekel, 2006). Hence the presence of protein and certain amino acids plays a key role in regulating 3-methylbutanal formation. Additionally, the pH seems an important modulating factor, affecting both the generation and degradation of Strecker aldehydes such as 3-methylbutanal (Pasqualone et al., 2014).

All the ingredients produced an increase of furanic compounds and 3-methylbutanal levels respect to control biscuits, as reported also in

Table 2
Contents (mg/kg) of furanic compounds and 3-methylbutanal in savory dough and biscuits. Results are presented as average values ± standard deviation. Different letters in the same column indicate significant differences between samples (Tukey's test, $p \leq 0.05$).

Savory samples	Furfural	5-Methylfurfural	Furfuryl alcohol	5-HMF	Total furanic compounds	3-methylbutanal
Cd	0.602 ± 0.121^{b}	0.066 ± 0.013^{bc}	n.d.	n.d.	0.668 ± 0.134^{e}	8.674 ± 1.240^{a}
Cb	0.470 ± 0.024^{c}	0.058 ± 0.003^{cd}	0.796 ± 0.134^{d}	n.d.	1.324 ± 0.161^{d}	3.646 ± 0.235^{cd}
OBd	0.140 ± 0.013^{d}	0.028 ± 0.006^{f}	n.d.	n.d.	0.168 ± 0.018^{f}	6.537 ± 0.667^{b}
OBb	0.610 ± 0.023^{b}	0.043 ± 0.003^{def}	2.347 ± 0.095^{c}	n.d.	3.000 ± 0.120^{c}	4.756 ± 0.981^{c}
ONBd	0.187 ± 0.039^{d}	0.037 ± 0.012^{ef}	n.d.	n.d.	0.223 ± 0.051^{ef}	7.675 ± 1.192^{ab}
ONBb	0.901 ± 0.058^{a}	0.079 ± 0.006^{ab}	3.982 ± 0.342^{a}	n.d.	4.961 ± 0.406^{a}	8.101 ± 0.416^{a}
SEd	n.d.	0.043 ± 0.008^{ef}	n.d.	n.d.	0.043 ± 0.008^{f}	2.735 ± 0.035^{d}
SEb	0.832 ± 0.076^{a}	0.045 ± 0.005^{de}	3.721 ± 0.369^{ab}	n.d.	4.598 ± 0.441^{ab}	6.593 ± 0.028^{b}
SUd	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SUb	0.834 ± 0.065^{a}	0.088 ± 0.008^{a}	3.450 ± 0.284^{b}	n.d.	4.372 ± 0.357^{b}	4.790 ± 0.260^{c}

Cd, control dough; **Cb**, control biscuits; **OBd**, dough formulated with olives with brine; **OBb**, biscuits added with olives with brine; **ONBd**, dough formulated with olives without brine; **ONBb**, biscuits added with olives without brine; **SEd**, dough formulated with sesame seeds; **SEb**, biscuits added with sesame seeds; **SUd**, dough formulated with sunflower seeds; **SUb**, biscuits added with sunflower seeds. n.d. = not detected.

literature (Mesias et al., 2021; Pasqualone et al., 2014). Specifically, black olives without brine and sesame seeds biscuits had the highest concentrations of furanic compounds (4.96 ± 0.41 and 4.60 ± 0.44 mg/kg, respectively) and 3-methylbutanal (8.10 ± 0.42 and 6.59 ± 0.03 mg/kg, respectively).

3.2.3. Biscuits comparison

Regarding the comparison among the two types of preparations, sweet control biscuits had higher contents of furanic compounds and 3-methylbutanal than savory control biscuits, while their concentrations varied based on different added ingredients. The major differences between the two recipes regarded the presence of egg and sunflower oil in the sweet basic recipe and the use of salt and extra virgin olive oil in the savory basic recipe. All these ingredients can influence the formation of volatile and non-volatile compounds during baking. For example, eggs provide a certain amount of proteins and some sulphur amino acids, which can react with 5-HMF through Michael addition reactions (Hamzalıoğlu and Gökmen, 2017). Kocadağlı and Gokmen (2016) studied the impact of different salts on the formation of α-dicarbonyl compounds and furfurals in cookies during baking. They found that the concentrations of 5-HMF and furfural improved using NaCl. In addition, the type of the oil used can also impact the content of volatile compounds. Vurro et al. (2022) found that 3-methylbutanal and furfural concentrations vary depending on the type and quantity of oil used, with differences observed between sunflower oil and durum wheat oil in biscuit formulations. Additionally, sugar content may have contributed to the differences. Sweet biscuits contained added sugar and the presence of sugar in provides essential substrate for Maillard and caramelization reactions, which are key pathways for the formation of both flavor-active and potentially harmful compounds such as furan derivatives. This difference in formulation could partially explain the distinct profiles.

3.3. Correlation between 3-metylbutanal, furanic compounds and acrylamide

Note: Acrylamide concentrations used in this section were not determined within the present study but were retrieved from a previous publication by Schouten et al. (2024). This allowed the authors to explore potential associations with the thermally induced compounds analyzed herein.

Principal Component Analysis (PCA) was used to evaluate the relationships between the analyzed furanic compounds, 3-metylbutanal and acrylamide in both sweet (Fig. 2) and savory (Fig. 3) biscuits. The acrylamide concentrations previously determined by Schouten et al. (2024) in the same biscuit samples were included in the analysis (**Table S2** in supplementary materials). This to investigate the impact of additional ingredients on the formation of potentially harmful compounds (e.g., furfuryl alcohol and acrylamide) in biscuits, offering insights into their impact on overall product safety and quality.

3.3.1. Sweet biscuits

The PCA biplot for sweet biscuits (Fig. 2) indicates that the two principal components (F1 and F2) together account for 96.39 % of the total variance, with the F1 contributing for 72.40 % and F2 for 23.99 %.

It is noteworthy that the control sweet biscuits and the pistachio-

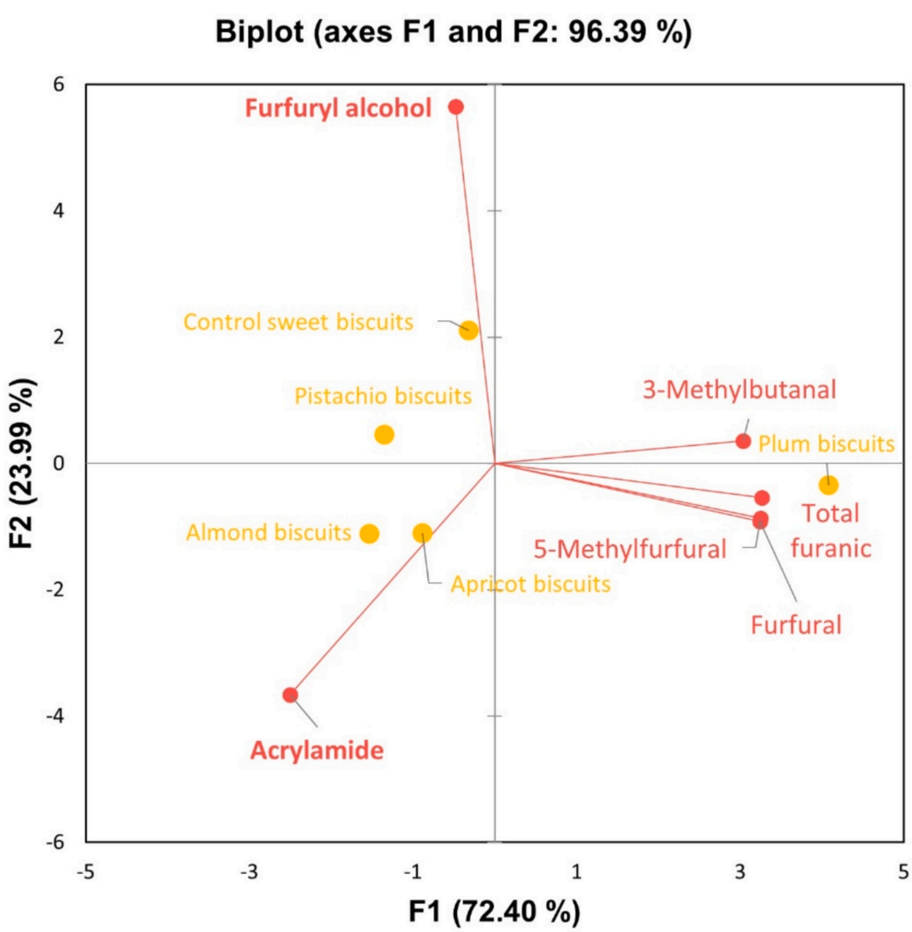

Fig. 2. Biplot from Principal Component Analysis (PCA) of sweet biscuits illustrating the relationships between various chemical compounds associated with thermal processing, including acrylamide, 3-methylbutanal and furanic compounds.

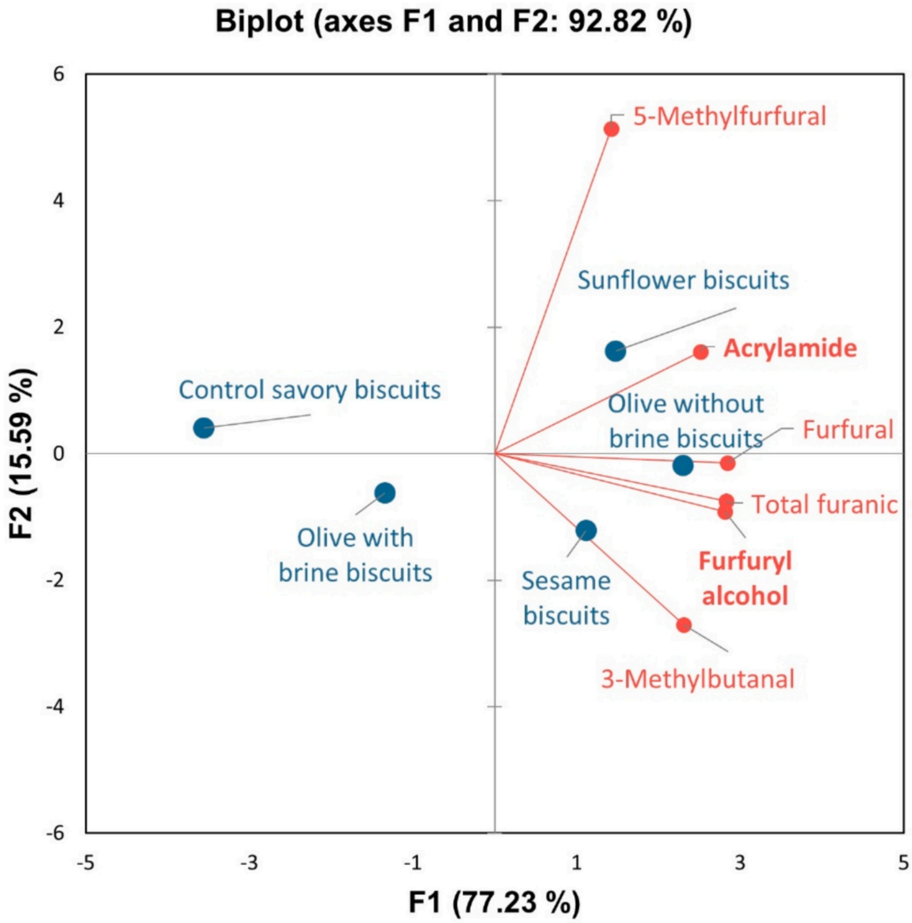

Fig. 3. Biplot from Principal Component Analysis (PCA) of savory biscuits illustrating the relationships between various chemical compounds associated with thermal processing, including acrylamide, 3-methylbutanal and furanic compounds.

enriched ones occupy the negative quadrant of F1 showing minimal association with furfural, 5-methylfurfural and 3-methylbutanal. However, their placement in the positive F2 quadrant indicates a significant association with furfuryl alcohol. In contrast, apricot and almond biscuits are in the negative F1 and F2 quadrants, indicating that these samples have lower levels of furanic compounds and 3-methylbutanal. Despite this, these biscuits exhibited high acrylamide concentrations. The formation of acrylamide in these biscuits can be attributed to the thermal decomposition of intermediate products of the Maillard reaction, such as 5-HMF and furfural, especially at a processing temperature around 180 °C (Abedi et al., 2023; Aktağ et al., 2022; de Borba et al., 2024). In addition, lipid oxidation products such as acrolein and acrylic acid can also contribute to acrylamide formation when they interact with nitrogen-containing compounds, a reaction that is particularly pronounced in fatty foods (Abedi et al., 2023; de Borba et al., 2024). These pathways of acrylamide formation may be less pronounced in control biscuits (without additional ingredients) and in those made with dried plums and pistachios.

Conversely, the biscuits containing dried plums exhibit a strong correlation with furfural, 5-metylfurfural, total furanic compounds, and 3-methylbutanal, as indicated by their positive association with F1 components. The inclusion of dried plums in the biscuit formulations may enhance the formation of these thermally induced compounds while simultaneously reducing acrylamide levels. This reduced acrylamide concentration can be attributed to the high moisture content (12.7 %) and low pH (6.8) of the plum-enriched biscuits, factors that exhibited significant negative and positive correlations with acrylamide concentrations, respectively (Schouten et al., 2024). Moreover, the antioxidant activity of dried plums may further contribute to the lower acrylamide levels in this biscuit type. The antioxidant compounds present in the biscuit matrix may interact with acrylamide precursors, thereby inhibiting the formation of this toxic compound (AL-Ansi et al., 2019; Schouten et al., 2024). This interplay between moisture, pH, and antioxidant activity not only affects acrylamide formation but may also positively influence the formation of furanic compounds during the baking process of biscuits.

The findings from the Pearson correlation matrix (**Table S3** in supplementary materials) further confirm the relationships among the evaluated furanic compounds, 3-methylbutanal and acrylamide in the sweet biscuit samples. A strong positive correlation was identified between furfural and 5-methylfurfural (r = 1.000), both significantly contributing to the total furanic content (r = 0.998). This suggests that these compounds likely share similar formation pathways during baking, possibly via Maillard reaction or caramelization. Additionally, 3-methylbutanal exhibited strong positive correlations with furfural (r = 0.851) and 5-methylfurfural (r = 0.852), indicating that it is co-formed alongside these furanic derivatives under comparable conditions. In contrast, acrylamide demonstrated significant negative correlations with furfural (r = −0.647), 5-methylfurfural (r = −0.649), total furanic compounds (r = −0.692) and 3-methylbutanal (r = −0.711). This suggests that the studied ingredients that favor the formation of furanic compounds do not concurrently promote acrylamide formation in the sweet biscuits, likely due to distinct thermal degradation mechanisms. However, further investigation is needed to clarify the specific factors influencing these complex interactions.

3.3.2. Savory biscuits

The PCA biplot for savory biscuits (Fig. 3) shows that the two principal components (F1 and F2) explain 92.82 % of the total variance, with F1 contributing for 77.23 % and F2 for 15.59 %.

Contrary to sweet biscuits, the savory biscuits enriched with various ingredients exhibit strong associations between furanic compounds, 3-methylbutanal and acrylamide. Biscuits enriched with sunflower seeds, sesame seeds and black olives without brine are in the positive F1 quadrant, indicating a positive correlation with furanic compounds, 3-methylbutanal and acrylamide. This suggests that these ingredients contribute significantly to the formation of these compounds, probably due to their specific compositional characteristics, such as fats, sugars and proteins, which can promote several reaction pathways, including the Maillard reaction, caramelization and lipid oxidation during baking (de Borba et al., 2024).

In contrast, the biscuits made with black olives in brine as well as the control savory biscuits, are in the negative F1 quadrant, indicating a much lower content of these thermally induced compounds. The lower levels of furanic compounds and acrylamide in biscuits with black olive in brine can be attributed to both the lower concentration of furanic compounds and 3-methylbutanal in this ingredient (Fig. 1) and the significantly higher moisture content. A higher moisture content can reduce the formation of acrylamide by slowing down the Maillard reaction during baking (Schouten et al., 2024).

These results are also confirmed by the Pearson correlation matrix (**Table S4** in supplementary materials), which shows a strong positive correlation between acrylamide and the total furanic compounds ($r = 0.871$), especially furfural ($r = 0.866$) and furfuryl alcohol ($r = 0.867$). This indicates that the formation of furfural and furanic compounds also increases with increasing acrylamide content in most enriched-savory biscuits tested, suggesting a common mechanism or precursor pathways. An exception is the use of black olives with brine, where the higher moisture content and unique composition limit the formation of acrylamide and furanic compounds in biscuits. Thus, while most savory ingredients contribute to the formation of these heat-induced compounds to a comparable degree, black olives with brine appear to mitigate their development.

Although the present study does not propose a universal model for predicting the health impact of biscuits, it provides ingredient-specific insights under standardized processing conditions that can support formulation improvements. Given the variability in raw material composition, particularly in industrial settings, further research should address ingredient-level acrylamide content and conduct targeted exposure assessments. These steps would be essential to establish robust mitigation strategies that align with current regulatory guidelines and safety thresholds for heat-induced food contaminants.

4. Conclusions

Although the clear understanding of complex mechanisms underlying the formation of thermally induced volatile compounds remains an ongoing challenge, the present paper provides valuable insights into how the levels of furan derivatives and 3-methylbutanal vary with different added ingredients in biscuit formulations. This study is the first to explore how commonly used ingredients in sweet and savory biscuit formulations influence the presence of some important aromas and/or potential harmful thermal compounds. Despite the added ingredients, sweet preparations resulted richer in furan derivatives and the conditions which favored the formation of these compounds were not appropriate for acrylamide development. On the other hand, in savory biscuits the formation of both furan derivatives and acrylamide seemed to be related to the similar mechanisms or precursor pathways.

Therefore, careful consideration of ingredient composition and processing conditions becomes fundamental to optimize both safety and sensory attributes of baked goods. This balance ensures that the final product is not only flavorful but also safe for consumption, meeting consumer expectations and regulatory standards. In addition, the differences observed between sweet and savory biscuits underscore the importance of ingredient-specific tailored strategies to control the formation of thermal contaminants, while preserving desirable organoleptic/quality properties.

CRediT authorship contribution statement

Agnese Santanatoglia: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maria Alessia Schouten:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation. **Simone Angeloni:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Giovanni Caprioli:** Writing – review & editing, Supervision, Resources, Project administration. **Sauro Vittori:** Visualization, Supervision, Project administration, Funding acquisition. **Santina Romani:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Data curation.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2025.145776.

Data availability

Data will be made available on request.

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