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The conversion of glucosamine to deoxyfructosazine and its impact on bread quality

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ABSTRACT

Bread serves as a crucial medium for developing functional foods. Functional bread enriched with glucosamine (GlcN) can be a dietary supplement to support joint health. This study investigated the retention of GlcN in bread and the formation of deoxyfructopyrazine (DOF) under various formulations. Bread samples containing GlcN and sugar alcohols demonstrated the highest GlcN retention, accounting for 69.7 %–71.0 % of the added GlcN, and exhibited the greatest production of DOF. The moisture distribution in the dough samples and the acetal reaction of sugars with DOF within bread system led to these outcomes. Additionally, bread with these additives displayed a darker and browner color than the control sample containing GlcN alone. Furthermore, 34 distinct flavor substances were identified across all bread samples. The study provides valuable insight for developing functional food utilizing GlcN, especially for baked foods.

1. Introduction

Bread, a stable food consumed worldwide, is an excellent source of carbohydrates and protein (Hasan et al., 2024). With increasing work pressure and the rapid pace of modern life, the prevalence of suboptimal or sub-health has risen significantly (Li, Dessie, et al., 2024). As a result, there is a growing demand for healthier dietary options that not only satisfy hunger but also provide additional health benefits (Azeredo et al., 2021; Dominguez-Viera et al., 2022). Functional substances are often incorporated during bread production, which can address these demands of consumers for functional foods while maintaining its desirable qualities, such as color and flavor.

There is a growing need for functional breads that enhance joint flexibility and physical performance among functional nutritional foods. However, such products remain relatively scarce, making their development highly anticipated. Glucosamine (GlcN), a widely used dietary supplement, is known for its ability to mitigate cartilage wear and protect joints as well as its proven efficacy and minimal side effects (Figueroba et al., 2021; Jiang et al., 2018; Reginster et al., 2012). The recommended daily intake of GlcN varies by region, with a suggested amount of 1500 mg/day in the United States, Canada, and Australia and 1000–1500 mg/day in Europe (Shintani et al., 2021). Research has shown the potential of GlcN in various food applications (Shintani et al., 2021). For instance, incorporating GlcN into low-salt breakfast sausages has enhanced flavor and consumer acceptance without compromising texture (Restaino et al., 2019; Soladoye et al., 2021). In addition, studies have demonstrated that incubating GlcN with proteins extracted from shrimp by-products increased its antibacterial and antioxidant activities, highlighting its potential in functional foods (Djellouli et al., 2020). As bread is a vital staple food consumed worldwide, integrating GlcN into bread formulation presents an innovative opportunity. Bread enriched with GlcN could serve as a convenient dietary source of this beneficial supplement, offering a practical approach to supporting joint health on a broader scale.

Some studies have demonstrated that under specific conditions, GlcN undergoes self-condensation to produce deoxyfructosazine (DOF), a functional substance with notable properties such as immune enhancement. DOF has been shown to inhibit the growth of T-cell interleukin-2, thereby supporting the immune system in combating inflammation (Zhu et al., 2007). During the bread-baking process, GlcN may degrade due to the reaction, while functional substances known as DOF are simultaneously generated. Consequently, bread may contain both GlcN and DOF, offering a valuable reference for developing bread with multiple functional properties.

Sugar and sugar alcohols are often added during the bread-making process to ensure the sweetness of bread (Ding & Yang, 2021). These additives contain reactive functional groups, such as carbonyl and hydroxyl groups, which can react with GlcN and have a specific effect on

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the presence of GlcN and DOF in bread. This study investigated the change rule of DOF produced by GlcN reaction in the baked bread system and the effects of sugar or sugar alcohols on their amount. Additionally, the research examined the key quality differences among bread samples under varying conditions. The study provides an important reference for developing multifunctional bread and advancing the application of GlcN in functional baked foods.

2. Materials and methods

2.1. Materials

High-gluten flour was purchased from Beijing Guchuan Food Co. Ltd. (Beijing, China), this flour complies with GB/T 8607 in China and contains 12.2 g protein, 1.8 g fat, and 73 g carbohydrates per 100 g. Edible butter was purchased from Shanghai Gaofu Food Co., Ltd. (Shanghai, China). Edible sodium chloride was bought from the China National Salt Industry Corporation (Beijing, China). Edible fructose and edible glucose were bought from Zibo Prebiotic Edge Biotechnology Co., Ltd. (Shandong, China) and Weifang Revitalizing Coke Co., Ltd. (Shandong, China), respectively. Yeast powder was obtained from Angel Yeast Co. Ltd. (Beijing, China). Glucosamine (purity ≥99 %) was bought from Sigma-Aldrich. Xylitol (purity ≥98 %) and sorbitol (purity ≥98 %) were obtained from J&K Scientific Co., Ltd., which also supplied 2,5-DOF (≥ 95 % purity) and HPLC-grade acetonitrile. All other chemicals used were of analytical grade, and deionized water was utilized throughout the study.

2.2. Preparation of bread samples

Baked bread samples were prepared using 24.0 g high-gluten flour, 2.4 g edible sodium chloride, 3.0 g glucosamine, 2.4 g yeast powder (which was evenly mixed with 120 mL of water at approximately 40 °C), and a certain amount of sugar or sugar alcohol. The additive formulations included 21.0 g glucose (GlcN-G), 21.0 g fructose (GlcN-F), 17.73 g xylitol (GlcN-X) or 21.23 g sorbitol (GlcN-S). The molar numbers of sugar and sugar alcohol added were equivalent.

The ingredients were mixed into dough using a dough mixer (ASM-DA600, Appliance Co. of America, USA). Subsequently, 10.0 g butter was added and blended into the dough to achieve a smooth surface. The dough was placed in an oven (T4-L326F, Midea, China) and fermented at 30 °C for 1 h. Afterward, the dough was removed, kneaded, vented, and divided into three portions of equal weight. Each portion was placed in the same toast mold (7.5 cm × 7.5 cm × 7.5 cm) and fermented at 30 °C for 30 min. The toast mold was then covered, and the doughs were baked at 160 °C for 30 min. Once baked, the bread samples were removed from the mold and allowed to cool at room temperature for 2 h before testing.

2.3. Determination of GlcN content

The bread samples weighing 5.0 g were initially mixed with 40 mL of water and subjected to ultrasonic treatment with the ultrasonic instrument (KQ-600DE, Kunshan Shumei Co., Ltd., China) for 30 min. The resulting solution was centrifuged with the high-speed centrifuge (H1750R, Cence Co., Ltd., China) at 3000 rpm for 10 min to obtain the supernatant. Subsequently, 10 mL of the supernatant was mixed with 30 mL of anhydrous ethanol and refrigerated at 4 °C for 3 h. The mixture was centrifuged at 5000 rpm for 10 min to obtain the supernatant. The collected supernatant was purified using a C_{18} column, which had been pre-activated with methanol and water. The purified liquid was evaporated under rotation with the rotary evaporator (OSB-2100, EYELA U.S.A. branch office, Japan) at 70 °C until approximately 10 mL remained, and its volume was adjusted to 25 mL. Finally, the solution was diluted tenfold and filtered through a 0.22 μm PES filter membrane to obtain the test samples.

The analysis of GlcN was performed using an ion chromatography (IC) system equipped with a pulse amperometric detector (ICS-5000+, Thermo Scientific, USA) and a Carbopac™ PA10 column (Thermo Scientific, USA, 4 × 250 mm), and the mobile phase comprised phase A (H_2O) and phase B (250 mmol/L NaOH). The elution program was as follows: 0–15 min, 80 % A and 20 % B; 15.1–20 min, 20 % A and 80 % B; and 20.1–30 min, 80 % A and 20 % B. The flow rate was maintained at 0.6 mL/min, with an injection volume of 25 μL. Standard solutions of GlcN were prepared at various concentration gradients to generate standard curves, which were subsequently used to calculate the GlcN content in different bread samples.

2.4. Determination of DOF content

The supernatant was obtained following the procedure outlined in Section 2.3 and transferred to a 50 mL volumetric flask, and the volume was adjusted to 50 mL. The solution was filtered through a 0.22 μm PES filter membrane to obtain the test samples. These samples were analyzed using an HPLC system with an ultraviolet detector (SPD20-A, Shimadzu, Japan). An amino column (Shim-pack GIST NH2, 4.6 × 250 mm, 5 μm, Shimadzu, Japan) was used to analyze the DOF contents, with the column temperature maintained at 30 °C. The injection volume was set to 5 μL. The mobile phase consisted of a 20:80 (*v*/v) isocratic mixture of water and acetonitrile, with a 0.4 mL/min flow rate. Each analysis cycle lasted 30 min, and the resulting data were processed using LabSolutions software (Version 5.98). Standard curves for 2,5-DOF were generated using a series of standard solutions with varying concentration gradients, and the 2,5-DOF content in various bread samples was calculated according to these standard curves.

2.5. Determination of moisture

Low-field nuclear magnetic resonance (LF-NMR) was used to detect the moisture distribution in dough samples made with different additives. The detection method followed the approach of Pan et al. (Pan et al., 2024) with slight modifications. LF-NMR measurements were performed using a LIME-MRI-D12 analyzer (Limecho Technology Co., Ltd., Beijing, China). Each dough sample, weighing 20.0 g, was placed into the sample cells for analysis. T2 relaxation scanning was then performed with the experimental parameters set as follows: the echo time was 300 μs, the echo count was 5000, the reduction time was 3 s, and the number of scans was 16. The T2 transverse relaxation time of the sample was obtained using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, and the data were inverted using the synchronous iterative reconstruction technique.

2.6. Determination of acetal reaction products

The method for extracting acetal reaction products from bread samples followed the same procedure as the previous DOF extraction. Acetal reaction product was detected using an Agilent 1260 liquid chromatography system coupled with an Agilent 6460 ESI-triple quadrupole mass spectrometry system (Agilent, USA). For analysis, a Shim-pack GIST NH2 amino column (Shim-pack GIST NH2, 4.6 × 250 mm, 5 μm, Shimadzu, Japan) was used with the column temperature maintained at 30 °C. The injection volume was set at 5 μL. The elution was executed using a mobile phase comprising a 20:80 (*v*/v) isocratic mixture of Solution A (water with 0.1 % v/v formic acid) and Solution B (acetonitrile with 0.1 % v/v formic acid) at a flow rate of 0.4 mL/min. Each cycle for the acetal reaction products lasted 25 min, whereas the standard DOF cycle lasted 40 min. The mass operating conditions were set as follows: sheath gas temperature, 400 °C; gas flow, 12 L/min; nebulizer pressure, 45 psi; and capillary voltage, 3850 V. Positive ion multiple reaction monitoring (MRM) and the product ion mode were used for sample analysis.

In the quantitative determination using MRM mode, the precursor ion for the acetal reaction product of sugar and DOF was $m/z = 467$, with its characteristic product ion was $m/z = 377$. The fragmentation voltage for the precursor ions was set at 135 V, and the collision energy for the product ions was 30 V. For 2,5-DOF, the precursor ion was $m/z = 305$, with the characteristic product ion was $m/z = 227$. The fragmentation voltage for these precursor ions was also set at 135 V, while the collision energy for the product ions was 15 V. The standard curve was constructed using 2,5-DOF as a reference, and the quantities of the acetal reaction products were calculated accordingly.

2.7. Color and flavor substance determination of bread

2.7.1. Color

The color (L^* - lightness, a^* - redness, and b^* - yellowness) of the bread samples was measured using a chroma meter (CR-400, Konica Minolta, Japan). The L^*, a^*, and b^* values for each group were analyzed as the average of three readings. The total color difference ΔE between the control and the samples with different additives was calculated according to the methods described by Sui (Sui, 2017), with $\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$.

2.7.2. Flavor substance

The flavor compounds were detected using Headspace-Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS, FlavourSpec®, G.A.S., Germany). The detection method was based on Ping and Zhao et al. (Ping et al., 2024; Zhao et al., 2024) with slight modifications. Each sample (2.0 g) was placed in a 20 mL headspace vial and equilibrated at 60 °C in a water bath for 15 min. Subsequently, 500 μL of headspace air was injected into the injector column without splitting and driven into the 60 °C column by nitrogen (≥99.999 %) as the carrier gas. The IMS was operated in positive ion mode with the following carrier gas flow rate program: 0–2.0 min, 2.0 mL/min; 2.1–10.0 min, a linear increase to 10.0 mL/min; 10.1–20.0 min, a linear increase to 100.0 mL/min, 20.1–40.0 min, held constant at 100.0 mL/min. Flavor compounds were identified based on retention index and IMS time databases (G.A.S).

2.8. Statistical analysis

All experiments were performed in at least three independent trials, with the results expressed as the mean ± standard deviations. Data analysis was performed using IBM SPSS Statistics 19.0 software (SPSS Inc., USA).

3. Results and discussion

3.1. Retention amount of GlcN

The recommended daily intake of GlcN is between 1000 and 1500 mg/day to play the role of joint protection better. In addition, according to survey data, people usually consume an average of 100–200 g of bread per day (Cauvain, 2016). The bread samples in this study were prepared using a common recipe, with each batch consisting of 240 g of flour and a specified proportion of sugar and other ingredients to produce 350–400 g of bread (Graça et al., 2022; Tuta Şimşek, 2022). Therefore, 3.0 g of GlcN was added to each batch of bread to make functional bread products containing an amount suitable for consumers to obtain the dietary supplement.

However, reactions involving GlcN may occur in bread baking, resulting in its reduction. Additionally, adding sugar or sugar alcohol may influence the change of GlcN. IC was used to determine the change in GlcN levels in bread samples (350–400 g) post-baking. The standard curve used for quantification was $y = 4.1923\times - 1.4951$ ($R^2 = 0.9987$). The remaining GlcN content in bread samples with different additives is presented in Fig. 1. For all the experimental samples, the amount of GlcN decreased after baking, which was consistent with previous studies

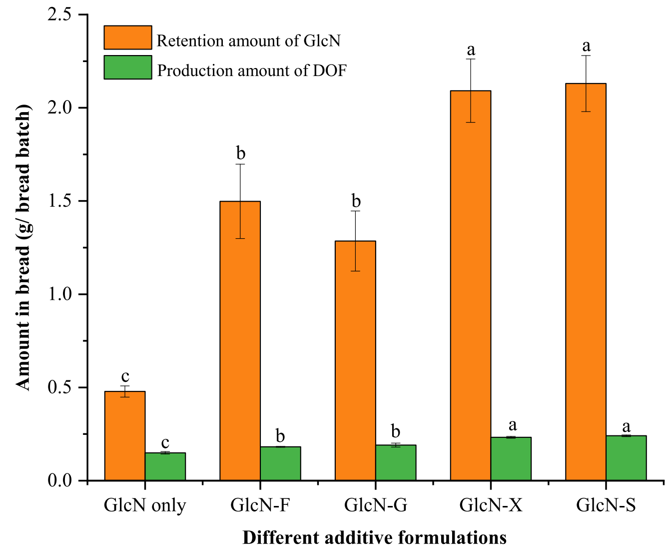

Fig. 1. The GlcN retention and DOF production ($n = 3$).
Note:
Data values with different superscript letters (a–c) in the same group differ significantly (Duncan test at $p < 0.05$).
Different abbreviations mean: GlcN only (the sample added with 3 g GlcN but no other sugars or sugar alcohols), GlcN-G (the sample added with 3 g GlcN and 21 g glucose), GlcN-F (the sample added with 3 g GlcN and 21 g fructose), GlcN-X (the sample added with 3 g GlcN and 17.73 g xylitol), GlcN-S (the sample added with 3 g GlcN and 21.23 g sorbitol).

(Dhungel et al., 2018; Hong & Betti, 2016). Bread containing only GlcN retained 0.48 g, corresponding to 15.9 % of the initially added GlcN, which was significantly lower than other groups ($P < 0.01$). The results indicate that adding sugar or sugar alcohol can mitigate the loss of GlcN during baking. Bread samples with fructose or glucose retained 1.29–1.50 g of GlcN, equivalent to 42.9–49.9 % of the added amount. However, there was no significant difference in retention amount of GlcN between bread samples with fructose and glucose. Meanwhile, there was also no significant difference in retention amount of GlcN between the bread samples with xylitol and sorbitol. But bread samples with sugar alcohol retained more GlcN than bread samples with sugar, which retained the highest GlcN levels. Bread samples with sugar alcohol retained 2.09–2.13 g GlcN, accounting for 69.7–71.0 % of the initial GlcN, and the formulation may serve as effective dietary supplement for GlcN.

3.2. Production amount of DOF

Since GlcN would self-condense to form the functional substance DOF under heating conditions, which was associated with the decrease in GlcN (Jia et al., 2017; Zhu et al., 2007). The production of DOF was analyzed. HPLC was utilized to quantify the DOF content in bread samples of each batch after baking. The standard curve was determined as $y = 22{,}285\times - 353.27$ ($R^2 = 0.9993$) and is shown in Fig. 1.

No DOF was produced in the control sample without GlcN. The DOF production in bread samples containing only GlcN was 0.14 g, which differed significantly from the other groups ($P < 0.01$). The DOF production in samples with reducing sugar ranged from 0.18 to 0.19 g for the GlcN-F and GlcN-G groups. In contrast, samples with sugar alcohol, GlcN-X, and GlcN-S exhibited higher DOF production amounts of 0.23–0.24 g.

These results indicate that reducing sugar and sugar alcohol enhances DOF production, with sugar alcohols showing a more pronounced effect. However, no significant difference in DOF production was observed between samples containing fructose and glucose or those containing xylitol and sorbitol.

The study considered two factors in investigating the differences in DOF production. First, the moisture distribution within the reaction system likely plays an important role because the moisture distribution can affect the chemical reaction (Chaplin, 2006). Second, other reactions may influence DOF production. Therefore, further analyses were conducted to explore the moisture distribution in bread doughs with different additives and to identify other reactions affecting DOF formation.

3.3. Moisture status in dough samples

LF-NMR has been used to study the moisture existence status in the food according to the T2 relaxation time graph. The graph includes three peaks: T21 (0–1 ms), T22 (1–100 ms), and T23 (100–1000 ms), representing bound water, immobilized water, and free water, respectively. A longer T2 relaxation time indicates greater water mobility (Li et al., 2012).

The proportion of different types of moisture in dough samples was analyzed and presented in Fig. 2. The results showed that the proportion of T21 bound water in the sample dough containing only GlcN was 2.5 %, a value significantly lower than those in other groups ($P < 0.01$). Dough samples containing sugar or sugar alcohol additive exhibited higher T21 bound water proportions, ranging from 5.0 % to 7.4 %. This increase might be attributed to the poly-hydroxyl nature of fructose, glucose, xylitol, and sorbitol, which are present in greater amounts than GlcN in the bread system. These hydroxyl groups bind water molecules via hydrogen bonding, increasing the bound water proportion in the dough (Ball, 2008; Chaplin, 2006). Moreover, sugar and sugar alcohols exhibit stronger water-binding capacities than gluten and starch, hindering the interaction of water molecules with gluten and starch. Thus, it relatively increased the immobility of this part of water (Huang, Guo, Wang, Ding, & Cui, 2016; Peng, Li, Ding, & Yang, 2017). It was shown by an increase in the proportion of T21.

The bound water in the GlcN-F dough sample was 5.10 % of T2 moisture, and that of the GlcN-G dough sample was 5.16 %, with no significant difference between them. In addition, GlcN-X exhibited a bound water proportion at 6.81 %, and GlcN-S showed the highest bound water proportion at 7.36 %. Among these, the dough sample containing sorbitol had the largest bound water proportion, attributable to the higher number of hydroxyl groups per molecule in sorbitol, which enhances its water-binding ability (Ding & Yang, 2021; Mantzari et al., 2010). For samples with different additives, the Pearson correlation analysis was carried out for the proportion of dough T21 and the production amount of DOF in bread. The result showed a significant positive correlation between them ($r = 0.97$, 95 % BCaCI [0.94, 0.99], $P < 0.05$). It was because the self-condensation of two molecules of GlcN can form one molecule of DOF, losing two molecules of H_2O (Henry et al., 2012; Hrynets et al., 2016; Rohovec et al., 2001). The reaction was be promoted through reducing the existence of free water, due to these additives' stronger bonding ability with water molecules.

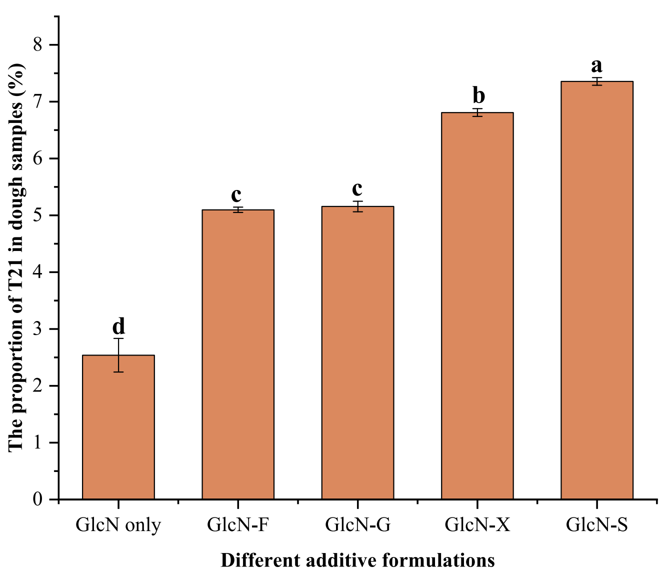

Fig. 2. Moisture difference of difference dough samples (n = 3).
Note:
Data values with different superscript letters (a–c) differ significantly (Duncan test at $p < 0.05$).
Different abbreviations mean: GlcN only (the sample added with 3 g GlcN but no other sugars or sugar alcohols), GlcN-G (the sample added with 3 g GlcN and 21 g glucose), GlcN-F (the sample added with 3 g GlcN and 21 g fructose), GlcN-X (the sample added with 3 g GlcN and 17.73 g xylitol), GlcN-S (the sample added with 3 g GlcN and 21.23 g sorbitol).
T21 corresponds to the moisture existence status of bound water in the samples.

3.4. Analysis of the acetal reaction product

In the bread system, the generated DOF contains multiple ortho-hydroxyl groups. Fructose and glucose contain carbonyl groups that may undergo an acetal reaction, influencing the amount of DOF (BeMiller, 2019). The acetal reaction product of sugar with DOF was monitored using HPLC-MS/MS.

The MS^2 analysis was conducted in positive ion mode to identify the structure of the reaction product. The m/z of precursor ion $[M + H]^+$ was at 467, and the m/z of fragment ions were at 449, 431, 377, 305, and 287, respectively. According to these fragmentation ions, the fracture rule of the acetal reaction product can be derived, as shown in the Fig. 3. Glucose was used as an example, with fructose following a similar fragmentation pattern.

The acetal reaction occurred between the carbonyl group of the reducing sugar and the two hydroxyl groups of the DOF, and a molecule of water was lost at the same time. The precursor ion ($m/z = 467$) and the corresponding fragments were detected in product ion mode. The precursor ion contained multiple hydroxyl groups, which were easily shed together with hydrogen from adjacent carbon atoms, losing a molecule of water. This dehydration produced product ion (1) $m/z = 449$ and product ion (2) $m/z = 431$ after losing one or two water molecules, respectively. In the product ion (1), the carbonyl group formed by dehydration could form a conjugate structure with pyrazine ring, which was stable. Fragmentation at site A of the precursor ion generated (3) ion ($m/z = 377$), corresponding to the loss of a glyceraldehyde molecule. The (4) ion ($m/z = 305$) was formed when the chemical bond of precursor ion was broken at site B, losing a molecule of glucose residue. Further dehydration of (4) ion resulted in product ion (5) with $m/z = 287$. According to these fragmentation rules, it was confirmed there was the acetal compound in the bread system. Additionally, the content of the compound was quantitatively analyzed.

MRM mode was used to calibrate and qualitatively detect the acetal reaction product, with its content measured using 2,5-DOF. The ion pair ($m/z = 467/377$) was selected to quantitative analysis for the acetal product, the ion pair ($m/z = 305/227$) was selected to quantitative analysis for 2,5-DOF, and 2,5-DOF was used as a reference for quantifying the acetal product. It could be obtained that in group GlcN-F, the content of acetal reaction product of sugar and DOF in bread sample was 32.98 ± 3.93 mg, whereas, it was 36.37 ± 4.79 mg in group GlcN-G. These values were measured using 2,5-DOF. The results indicate that the GlcN-G group produced more acetal products than the GlcN-F group. This difference was attributed to the greater reactivity of the aldehyde group in glucose during the acetal reaction compared to the ketone group in fructose (Asatkar & Basak, 2023).

Through qualitative and quantitative analysis, it was observed that the acetal reaction between sugar and DOF led to decreased DOF content in bread samples. In contrast, sugar alcohols, which contain only hydroxyl groups without carbonyl groups, did not react with the hydroxyl

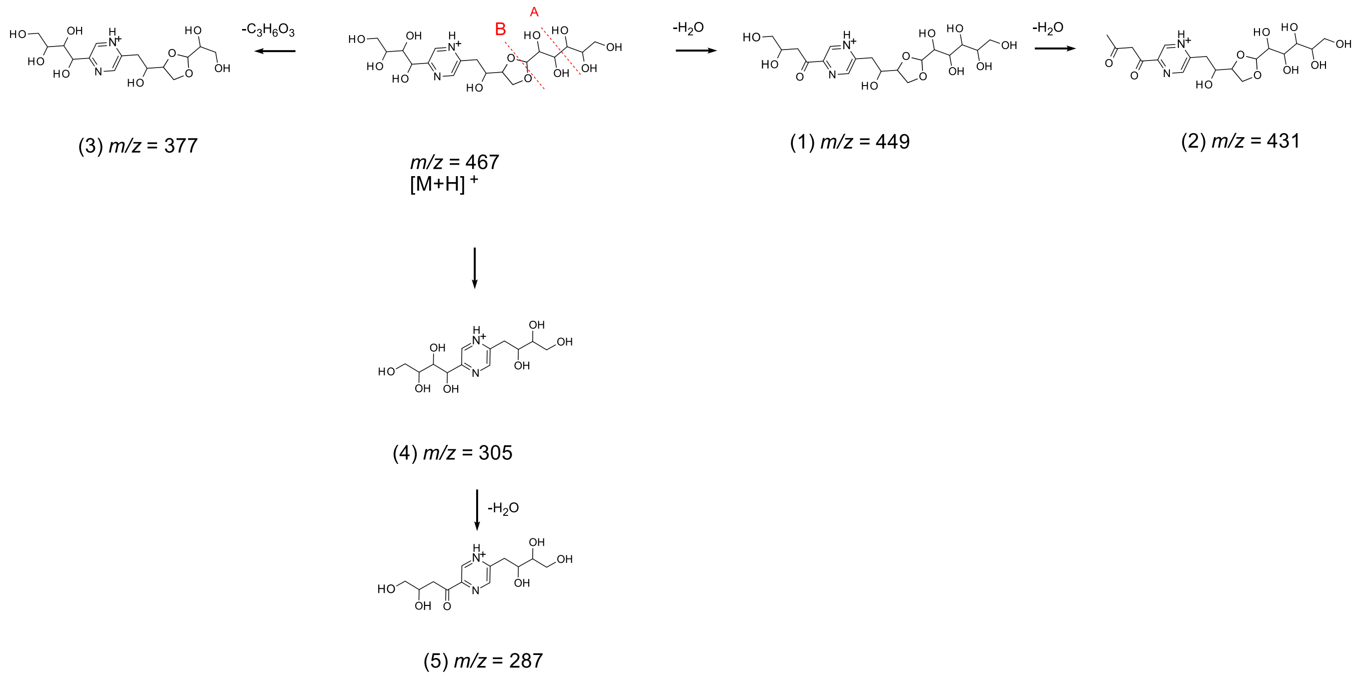

Fig. 3. Fracture path of the acetal reaction product.
Note: The chemical bonds broke at sites of "A" and "B".

group of DOF, thereby reducing the consumption of DOF. Consequently, both the acetal reaction and the moisture difference resulted in lower DOF content in bread samples containing sugar than in those containing sugar alcohol.

3.5. *Color and flavor substance of bread samples*

The color and flavor of bread are critical factors influencing its quality and serve as key consumer preference indicators. Hence, it is essential to evaluate whether adding GlcN to the bread system impacts

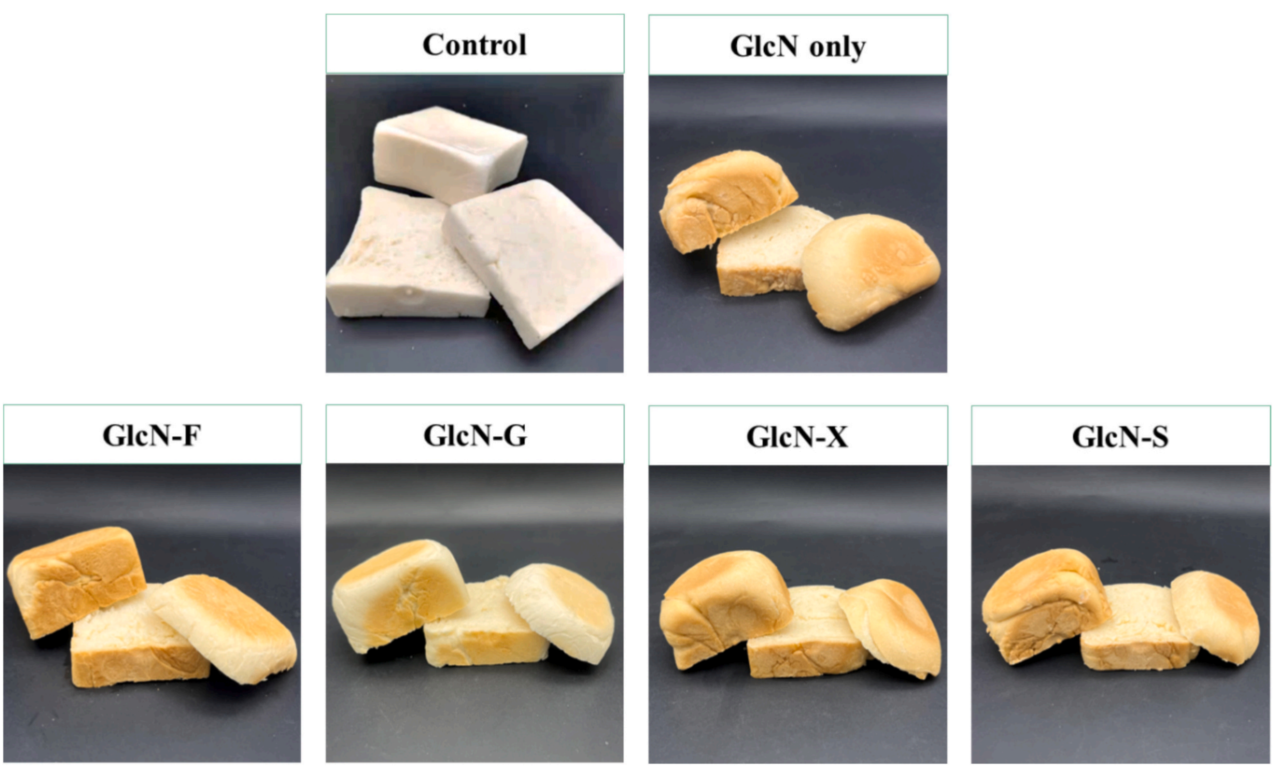

Fig. 4. Appearance of bread samples.
Note: Different abbreviations mean: Control (the sample without GlcN or sugars or sugar alcohols), GlcN only (the sample added with 3 g GlcN but no other sugars or sugar alcohols), GlcN-G (the sample added with 3 g GlcN and 21 g glucose), GlcN-F (the sample added with 3 g GlcN and 21 g fructose), GlcN-X (the sample added with 3 g GlcN and 17.73 g xylitol), GlcN-S (the sample added with 3 g GlcN and 21.23 g sorbitol).

these two key qualities (Dong et al., 2024).

3.5.1. Chromatic aberration and appearance of bread samples

The color of bread, especially the crust and internal color, is an important indicator of bread quality and significantly influences consumer preferences (Castro et al., 2017; Chen et al., 2022).

When different additives were used in bread, their appearance were shown as Fig. 4. Noticeable color differences were observed in the crust between bread samples containing additives and the control sample without additives. Chromatic aberration analysis was conducted on both the bread's outer surface and interior portion, with the results presented in Table 1.

When the values of $\Delta E > 3$ were observed, it indicated that this color difference was noticeable to the human eye (Sozer et al., 2014). For the crust of the sample bread, the value of $\Delta E > 3$ was observed in the samples with added GlcN, reducing sugar or sugar alcohol, compared to the control bread. However, for the internal of the bread, $\Delta E < 3$ was recorded across samples with additives and the control bread, aligning with the bread's visual appearance. These results suggest that the additives had a more pronounced effect on the crust than internal color. This difference might be attributed to the crust's greater exposure to air than the inside portion, which facilitates water evaporation and creates more suitable water activity conditions for the Maillard reaction during the baking (Sun et al., 2024). Additionally, the crust of the bread containing additives appeared darker and browner than the control bread, and the measured values of chromatic aberration were also in accordance with this phenomenon. The L^* values of additive-containing bread samples were significantly lower than those of the control bread, while the a^* and b^* values were higher than those of the control bread. These differences might be due to the addition of GlcN which further promoted the Maillard reaction during bread baking process (Purlis, 2010), which is consistent with the previous works (Viturat et al., 2023; H. Yang et al., 2020). However, no significant difference in L^*, a^*, and b^* values was detected among the samples with different additive formulations. In summary, these findings demonstrated that adding GlcN, sugars, or sugar alcohols contributes to enhancing the crust color of bread with limited impact on the internal crumb color.

3.5.2. Flavor substance of bread samples

To evaluate whether the flavor characteristics of bread were altered by using GlcN, six samples, including a control bread sample, were analyzed using GC-IMS to identify volatile flavor substances. Across these six samples, a total of 34 volatile flavor substances were detected, comprising seven alcohols, six aldehydes, seven ketones, three acids, four esters, one furan, and six pyrazines. Twelve substances were detected to exist in both monomeric and dimeric forms, marked as "M" and "D" respectively. The fingerprint shown in Fig. 5 was employed to showcase pertinent details about the compounds in the samples. In this graph, rows corresponded to the selected signal peaks from individual samples, while columns represented the signal peaks of the same compound across all samples (Zhang, Wang, et al., 2024). The intensity of each substance's concentration is depicted by the hue of the signal peaks (Zhang, Guo, et al., 2024). Brighter colors have higher concentrations of volatile components (Li et al., 2024). The results reveal that bread samples with different formulations showed minimal variation in overall flavor substances, indicating that the addition of GlcN, sugar, and sugar alcohol did not have a remarkable effect on the flavor characteristics of the bread. However, bread samples containing sugar exhibited a higher production of flavor substances, especially ketones. This increase was likely due to the higher degree of Maillard reaction between reducing sugar and amino acids or proteins in the bread system compared to the sugar alcohol (Conceição et al., 2024; Han et al., 2023; Liu et al., 2024; F. Yang et al., 2025; Zhou et al., 2024).

4. Conclusion

GlcN was incorporated into the bread-baking process to create functional bread to support joint health. The retention amount of GlcN and the production amount of DOF under different formulations were investigated. The bread samples with sugar alcohol had the highest retention of GlcN, with 69.72 %–71.00 % of the added GlcN, effectively supplementing GlcN than the other samples. A portion of the lost GlcN was converted into DOF. Meanwhile, the bread samples with sugar alcohol yielded the highest DOF production. The mechanism of the above phenomenon was explained via the water distribution in the dough samples and the acetal reaction of sugar with DOF in the bread system. In addition, bread samples with GlcN additives exhibited a darker appearance compared to the samples without GlcN. As for flavor substances, bread samples with sugar produced more ketones, and there was no significant difference among the other groups. The amounts of GlcN and DOF functional substances were simultaneously analyzed, which provides a valuable reference for the development of foods with multiple functions, especially for baking bread.

CRediT authorship contribution statement

Ruiqi Sun: Writing – original draft, Investigation, Formal analysis, Data curation. **Mengdi Niu:** Project administration, Investigation. **Hao Liu:** Resources. **Jun Wang:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial

Table 1
Chromatic aberration of bread samples (n = 3).

Category	The crust of bread				The internal of bread			
	L^*	a^*	b^*	ΔE	L^*	a^*	b^*	ΔE
control	79.00 ± 2.59 ^{a}	−0.41 ± 0.49 ^{d}	23.54 ± 3.17 ^{d}		83.15 ± 1.03 ^{c}	−1.24 ± 0.09 ^{a}	16.82 ± 0.53 ^{c}	
GlcN only	60.95 ± 0.41 ^{c}	9.66 ± 0.16 ^{b}	33.43 ± 1.09 ^{c}	22.91	83.31 ± 0.49 ^{c}	−1.80 ± 0.08 $^{b\,c}$	17.96 ± 1.39 $^{b\,c}$	1.28
GlcN-F	62.94 ± 0.10 $^{b\,c}$	10.20 ± 0.15 ^{b}	33.75 ± 1.20 ^{c}	21.79	81.99 ± 0.90 ^{c}	−1.51 ± 0.04 $^{a\,b\,c}$	16.89 ± 1.81 ^{c}	1.19
GlcN-G	57.43 ± 0.44 ^{d}	11.86 ± 0.31 ^{a}	33.68 ± 1.45 ^{c}	26.81	82.97 ± 0.43 ^{c}	−1.72 ± 0.20 $^{b\,c}$	19.18 ± 0.26 ^{b}	2.42
GlcN-X	64.42 ± 1.04 $^{b\,c}$	8.76 ± 0.72 ^{c}	32.44 ± 0.56 ^{c}	19.39	82.75 ± 0.66 ^{c}	−1.87 ± 0.04 ^{c}	17.43 ± 0.96 $^{b\,c}$	0.96
GlcN-S	60.78 ± 1.03 ^{c}	11.43 ± 0.70 ^{a}	33.53 ± 0.04 ^{c}	23.92	82.41 ± 1.41 ^{c}	−1.45 ± 0.42 $^{a\,b\,c}$	17.65 ± 0.61 $^{b\,c}$	1.13

Note:
Data values with different superscript letters (a–d) in the same column differ significantly (Duncan test at $p < 0.05$).
Different abbreviations mean: Control (the sample without GlcN or sugars or sugar alcohols), GlcN only (the sample added with 3 g GlcN but no other sugars or sugar alcohols), GlcN-G (the sample added with 3 g GlcN and 21 g glucose), GlcN-F (the sample added with 3 g GlcN and 21 g fructose), GlcN-X (the sample added with 3 g GlcN and 17.73 g xylitol), GlcN-S (the sample added with 3 g GlcN and 21.23 g sorbitol).
ΔE *is* the total color difference and $\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$.

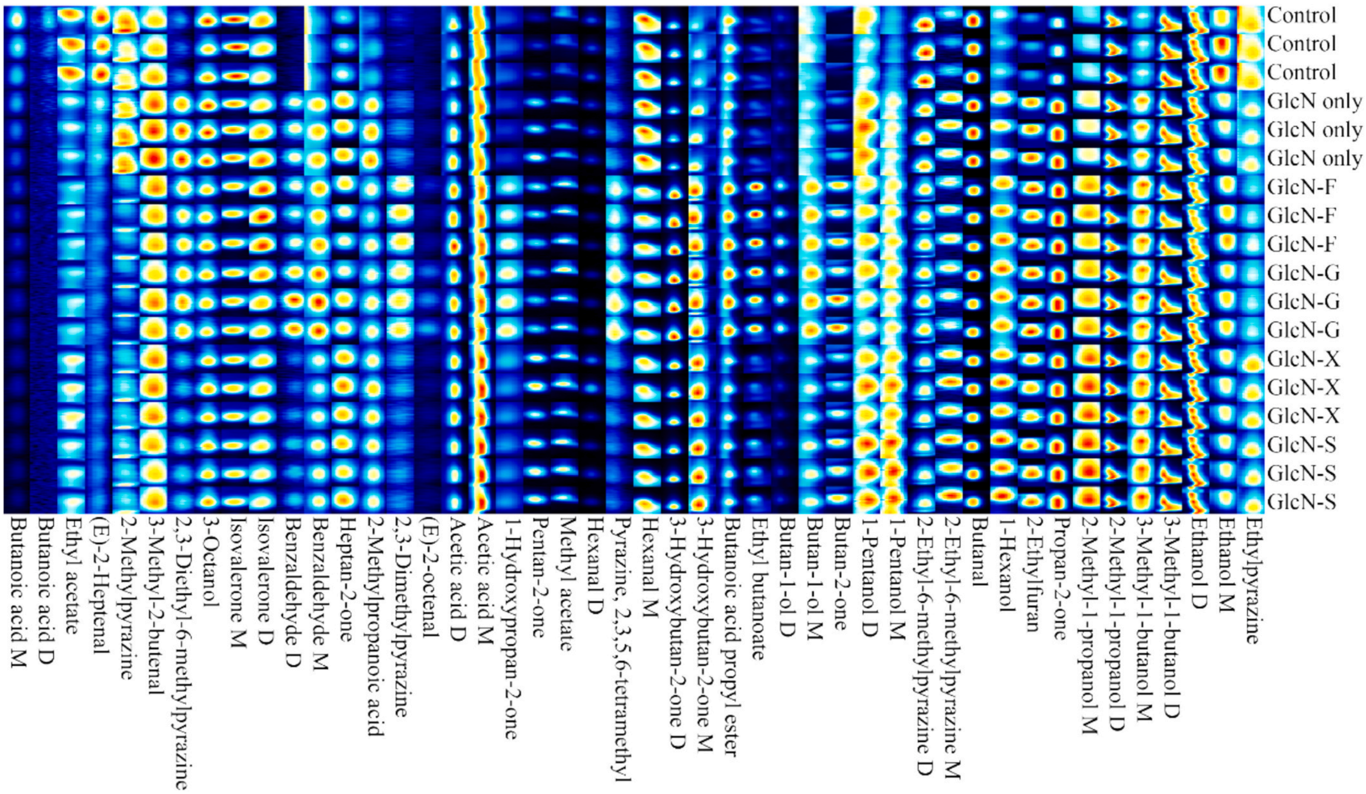

Fig. 5. Flavor substance of bread samples (n = 3).
Note: Different abbreviations mean: Control (the sample without GlcN or sugars or sugar alcohols), GlcN only (the sample added with 3 g GlcN but no other sugars or sugar alcohols), GlcN-G (the sample added with 3 g GlcN and 21 g glucose), GlcN-F (the sample added with 3 g GlcN and 21 g fructose), GlcN-X (the sample added with 3 g GlcN and 17.73 g xylitol), GlcN-S (the sample added with 3 g GlcN and 21.23 g sorbitol).

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

Asatkar, A. K., & Basak, R. K. (2023). Chapter 2 - carbohydrate: Introduction and fundamentals. *Handbook of Biomolecules.*. https://doi.org/10.1016/B978-0-323-91684-4.00020-7

Azeredo, H. M. C., Tonon, R. V., & McClements, D. J. (2021). Designing healthier foods: Reducing the content or digestibility of key nutrients. *Trends in Food Science & Technology, 118*, 459–470. https://doi.org/10.1016/j.tifs.2021.10.023

Ball, P. (2008). Water as an active constituent in cell biology. *Chemical Reviews, 108*(1), 74–108. https://doi.org/10.1021/cr068037a

BeMiller, J. N. (2019). Carbohydrate reactions. In *Carbohydrate chemistry for food scientists* (pp. 25–48). Elsevier. https://doi.org/10.1016/B978-0-12-812069-9.00002-9.

Castro, W., Oblitas, J., Chuquizuta, T., & Avila-George, H. (2017). Application of image analysis to optimization of the bread-making process based on the acceptability of the crust color. *Journal of Cereal Science, 74*, 194–199. https://doi.org/10.1016/j.jcs.2017.02.002

Cauvain, S. P. (2016). Bread: Breadmaking processes. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 478–483). Academic Press. https://doi.org/10.1016/B978-0-12-384947-2.00087-8.

Chaplin, M. (2006). Do we underestimate the importance of water in cell biology? *Nature Reviews Molecular Cell Biology, 7*(11), 861–866. https://doi.org/10.1038/nrm2021

Chen, W., Wu, X., Liu, Z., Liu, Y., Liu, Q., Pointer, M. R., … Khanh, T. Q. (2022). The impact of illuminance level, correlated colour temperature and viewing background on the purchase intention for bread and cakes. *Food Quality and Preference, 98*, Article 104537. https://doi.org/10.1016/j.foodqual.2022.104537

Conceição, L. D. S., Almeida, B. S. D., Souza, S. F. D., Martinez, V. O., Matos, M. F. R. D., Andrade, L. L., … Pinto Matos, L. C. (2024). Critical conditions for the formation of Maillard reaction products (MRP) in bread: An integrative review. *Journal of Cereal Science, 118*, Article 103985. https://doi.org/10.1016/j.jcs.2024.103985

Dhungel, P., Hrynets, Y., & Betti, M. (2018). Sous-vide nonenzymatic Browning of glucosamine at different temperatures. *Journal of Agricultural and Food Chemistry, 66*(17), 4521–4530. https://doi.org/10.1021/acs.jafc.8b01265

Ding, S., & Yang, J. (2021). The effects of sugar alcohols on rheological properties, functionalities, and texture in baked products – A review. *Trends in Food Science & Technology, 111*, 670–679. https://doi.org/10.1016/j.tifs.2021.03.009

Djellouli, M., López-Caballero, M. E., Arancibia, M. Y., Karam, N., & Martínez-Alvarez, O. (2020). Antioxidant and antimicrobial enhancement by reaction of protein hydrolysates derived from shrimp by-products with glucosamine. *Waste and Biomass Valorization, 11*(6), 2491–2505. https://doi.org/10.1007/s12649-019-00607-y

Dominguez-Viera, M. E., Van Den Berg, M., Donovan, J., Perez-Luna, M. E., Ospina-Rojas, D., & Handgraaf, M. (2022). Demand for healthier and higher-priced processed foods in low-income communities: Experimental evidence from Mexico City. *Food Quality and Preference, 95*, Article 104362. https://doi.org/10.1016/j.foodqual.2021.104362

Dong, Y., Chidar, E., & Karboune, S. (2024). Investigation of in situ and ex situ mode of lactic acid bacteria incorporation and the effect on dough extensibility, bread texture and flavor quality during shelf-life. *Food Chemistry: X, 24*, Article 101857. https://doi.org/10.1016/j.fochx.2024.101857

Figueroba, S. R., Moreira, J. C., Amorim, K. S., Cunha, L. D. L. L., Morais, T. M. L., Ferreira, L. E. N., & Groppo, F. C. (2021). Effect of glucosamine sulphate on the temporomandibular joint of ovariectomised rats. *British Journal of Oral and Maxillofacial Surgery, 59*(2), 202–208. https://doi.org/10.1016/j.bjoms.2020.08.078

Graça, C., Edelmann, M., Raymundo, A., Sousa, I., Coda, R., Sontag-Strohm, T., & Huang, X. (2022). Yoghurt as a starter in sourdough fermentation to improve the technological and functional properties of sourdough-wheat bread. *Journal of Functional Foods, 88*, Article 104877. https://doi.org/10.1016/j.jff.2021.104877

Han, Y., You, M., Wang, S., Yuan, Q., Gao, P., Zhong, W., Yin, J., Hu, C., He, D., & Wang, X. (2023). Enzymatic methods for the preparation of fragrant rapeseed oil: Effect of reducing sugars on flavor production using the Maillard reaction. *LWT, 189*, Article 115497. https://doi.org/10.1016/j.lwt.2023.115497

Hasan, M. M., Islam, M. R., Haque, A. R., Kabir, M. R., & Hasan, S. M. K. (2024). Fortification of bread with mango peel and pulp as a source of bioactive compounds: A comparison with plain bread. *Food Chemistry Advances, 5*, Article 100783. https://doi.org/10.1016/j.focha.2024.100783

Henry, N., Delépée, R., Seigneuret, J.-M., & Agrofoglio, L. A. (2012). Synthesis of water-compatible imprinted polymers of in situ produced fructosazine and 2,5-deoxyfructosazine. *Talanta, 99*, 816–823. https://doi.org/10.1016/j.talanta.2012.07.035

Hong, P. K., & Betti, M. (2016). Non-enzymatic browning reaction of glucosamine at mild conditions: Relationship between colour formation, radical scavenging activity and α-dicarbonyl compounds production. *Food Chemistry, 212*, 234–243. https://doi.org/10.1016/j.foodchem.2016.05.170

Hrynets, Y., Bhattacherjee, A., Ndagijimana, M., Hincapie Martinez, D. J., & Betti, M. (2016). Iron (Fe^{2+})-catalyzed glucosamine Browning at 50 °C: Identification and quantification of major flavor compounds for antibacterial activity. *Journal of Agricultural and Food Chemistry, 64*(16), 3266–3275. https://doi.org/10.1021/acs.jafc.6b00761

Huang, G., Guo, Q., Wang, C., Ding, H. H., & Cui, S. W. (2016). Fenugreek fibre in bread: Effects on dough development and bread quality. *LWT - Food Science and Technology, 71*, 274–280. https://doi.org/10.1016/j.lwt.2016.03.040

Jia, L., Liu, X., Qiao, Y., Pedersen, C. M., Zhang, Z., Ge, H., … Wang, Y. (2017). Mechanism of the self-condensation of GlcNH2: Insights from in situ NMR spectroscopy and DFT study. *Applied Catalysis B: Environmental, 202*, 420–429. https://doi.org/10.1016/j.apcatb.2016.09.058

Jiang, Z., Li, Z., Zhang, W., Yang, Y., Han, B., Liu, W., & Peng, Y. (2018). Dietary natural N-acetyl-d-glucosamine prevents bone loss in Ovariectomized rat model of postmenopausal osteoporosis. *Molecules, 23*(9), 2302. https://doi.org/10.3390/molecules23092302

Li, J., Kang, J., Wang, L., Li, Z., Wang, R., Chen, Z. X., & Hou, G. G. (2012). Effect of Water Migration between Arabinoxylans and Gluten on Baking Quality of Whole Wheat Bread Detected by Magnetic Resonance Imaging (MRI). *J. Agric. Food Chem.*

Li, Q., Zhang, C., Liu, W., Li, B., Chen, S., Wang, H., Li, Y., & Li, J. (2024). Characterization and exploration of dynamic variation of volatile compounds in vine tea during processing by GC-IMS and HS-SPME/GC–MS combined with machine learning algorithm. *Food Chemistry, 460*, Article 140580. https://doi.org/10.1016/j.foodchem.2024.140580

Li, X., Dessie, Y., Mwanyika-Sando, M., Assefa, N., Millogo, O., Manu, A., … Tang, K. (2024). Co-occurrence of and factors associated with health risk behaviors among adolescents: A multi-center study in sub-Saharan Africa,China, and India. *eClinicalMedicine, 70*, Article 102525. https://doi.org/10.1016/j.eclinm.2024.102525

Liu, S., Sun, H., Nagassa, M., He, X., Pei, H., Gao, L., Li, X., & He, S. (2024). Enhancing bread anti-staling with glucose-derived Maillard reaction products: In-depth analysis of starches, gluten networks, and moisture status. *Food Chemistry, 455*, Article 139760. https://doi.org/10.1016/j.foodchem.2024.139760

Mantzari, G., Raphaelides, S. N., & Exarhopoulos, S. (2010). Effect of sorbitol addition on the physicochemical characteristics of starch–fatty acid systems. *Carbohydrate Polymers, 79*(1), 154–163. https://doi.org/10.1016/j.carbpol.2009.07.043

Pan, C., Shi, S., Yang, X., Xiang, H., Wang, D., Zhao, Y., & Ouyang, Q. (2024). Effect of water migration on changes of quality and volatile compounds in frozen *Penaeus monodon*. *Food Chemistry, 457*, Article 140425. https://doi.org/10.1016/j.foodchem.2024.140425

Peng, B., Li, Y., Ding, S., & Yang, J. (2017). Characterization of textural, rheological, thermal, microstructural, and water mobility in wheat flour dough and bread affected by trehalose. *Food Chemistry, 233*, 369–377. https://doi.org/10.1016/j.foodchem.2017.04.108

Ping, C., Liu, Y., Bi, J., Cai, X., Li, X., & Qiao, M. (2024). Identification of characteristic flavor quality of ceramic-pot sealed meat after reheating based on HS-GC-IMS, bionic sensory combined chemometrics. *Food Chemistry: X, 23*, Article 101640. https://doi.org/10.1016/j.fochx.2024.101640

Purlis, E. (2010). Browning development in bakery products – A review. *Journal of Food Engineering, 99*(3), 239–249. https://doi.org/10.1016/j.jfoodeng.2010.03.008

Reginster, J.-Y., Neuprez, A., Lecart, M.-P., Sarlet, N., & Bruyere, O. (2012). Role of glucosamine in the treatment for osteoarthritis. *Rheumatology International, 32*(10), 2959–2967. https://doi.org/10.1007/s00296-012-2416-2

Restaino, O. F., Finamore, R., Stellavato, A., Diana, P., Bedini, E., Trifuoggi, M., … Schiraldi, C. (2019). European chondroitin sulfate and glucosamine food supplements: A systematic quality and quantity assessment compared to pharmaceuticals. *Carbohydrate Polymers, 222*, Article 114984. https://doi.org/10.1016/j.carbpol.2019.114984

Rohovec, J., Kotek, J., Peters, J. A., & Maschmeyer, T. (2001). A clean conversion of D-glucosamine hydrochloride to a pyrazine in the presence of Phenylboronate or borate. *European Journal of Organic Chemistry, 2001*(20), 3899–3901. https://doi.org/10.1002/1099-0690(200110)2001:20<3899: AID-EJOC3899>3.0.CO;2-G

Shintani, H., Ashida, H., & Shintani, T. (2021). Shifting the focus of d-glucosamine from a dietary supplement for knee osteoarthritis to a potential anti-aging drug. *Human Nutrition & Metabolism, 26*, Article 200134. https://doi.org/10.1016/j.hnm.2021.200134

Soladoye, O. P., Pietrasik, Z., Hrynets, Y., & Betti, M. (2021). The effect of glucosamine and glucosamine caramel on quality and consumer acceptability of regular and reduced salt breakfast sausages. *Meat Science, 172*, Article 108310. https://doi.org/10.1016/j.meatsci.2020.108310

Sozer, N., Cicerelli, L., Heiniö, R.-L., & Poutanen, K. (2014). Effect of wheat bran addition on in vitro starch digestibility, physico-mechanical and sensory properties of biscuits. *Journal of Cereal Science, 60*(1), 105–113. https://doi.org/10.1016/j.jcs.2014.01.022

Sui, X. (2017). Changes in the color, chemical stability and antioxidant capacity of thermally treated anthocyanin aqueous solution over storage. In X. Sui (Ed.), *Impact of food processing on anthocyanins* (pp. 49–65). Springer. https://doi.org/10.1007/978-981-10-2612-6_5.

Sun, R., Zheng, J., Niu, M., & Wang, J. (2024). Study on the effects of different ammonium salts on baked bread. *Heliyon, 10*(17), Article e37397. https://doi.org/10.1016/j.heliyon.2024.e37397

Tuta Şimşek, S. (2022). Vacuum modification of partial-baked wheat bread: Evaluation of the physicochemical, microstructural properties and acrylamide content. *Journal of Cereal Science, 105*, Article 103467. https://doi.org/10.1016/j.jcs.2022.103467

Viturat, S., Thongngam, M., Lumdubwong, N., Zhou, W., & Klinkesorn, U. (2023). Ultrasound-assisted formation of chitosan-glucose Maillard reaction products to fabricate nanoparticles with enhanced antioxidant activity. *Ultrasonics Sonochemistry, 97*, Article 106466. https://doi.org/10.1016/j.ultsonch.2023.106466

Yang, F., Chen, E., Fu, A., Liu, Y., & Bi, S. (2025). Formation of key aroma compounds in Agrocybe aegerita during hot air drying: Amino acids and reducing sugars identified as flavor precursors. *Food Chemistry, 465*, Article 141975. https://doi.org/10.1016/j.foodchem.2024.141975

Yang, H., Zhang, Y., Zhou, F., Guo, J., Tang, J., Han, Y., … Fu, C. (2020). Preparation, bioactivities and applications in food industry of chitosan-based Maillard products: A review. *Molecules, 26*(1), 166. https://doi.org/10.3390/molecules26010166

Zhang, M., Guo, D., Wu, G., Han, P., Shi, Y., Zheng, T., He, X., Zhao, E., Zhang, H., & Li, X. (2024). Analysis of volatile compound metabolic profiles during the fermentation of filler tobacco leaves through integrated E-nose, GC–MS, GC-IMS, and sensory evaluation. *Journal of Chromatography A, 1737*, Article 465472. https://doi.org/10.1016/j.chroma.2024.465472

Zhang, Q., Wang, Y., Meng, F., Wang, B., & Wang, Y. (2024). Comparative analysis of the volatile flavor compounds of Monascus-fermented cheese with different ripening periods by SPME-GC-MS, SPME-GC×GC-MS, and HS-GC-IMS. *Food Bioscience, 62*, Article 105045. https://doi.org/10.1016/j.fbio.2024.105045

Zhao, Y., He, W., Zhan, P., Geng, J., Wang, P., & Tian, H. (2024). A comprehensive analysis of aroma quality and perception mechanism in ginger-infused stewed beef using instrumental analysis, sensory evaluation and molecular docking. *Food Chemistry, 460*, Article 140435. https://doi.org/10.1016/j.foodchem.2024.140435

Zhou, Z.-X., Chen, Y.-J., Sheng, M.-M., Cui, F.-J., Chen, C., Shi, J.-C., Shu, X.-Q., & Chen, Z.-W. (2024). Improving flavor of strong fragrant rapeseed oils by supplementing commercial peptides and sugars. *Food Chemistry: X, 24*, Article 101985. https://doi.org/10.1016/j.fochx.2024.101985

Zhu, A., Huang, J.-B., Clark, A., Romero, R., & Petty, H. R. (2007). 2,5-Deoxyfructosazine, a d-glucosamine derivative, inhibits T-cell interleukin-2 production better than d-glucosamine. *Carbohydrate Research, 342*(18), 2745–2749. https://doi.org/10.1016/j.carres.2007.08.025