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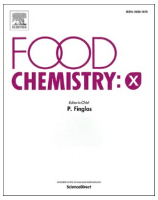

Comparison and evaluation of *L. reuteri* and *L. rhamnosus*-fermented egg yolk on the physicochemical and flavor properties of cookies

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ABSTRACT

The study aims to explore an effective approach to improve the sensory quality and consumer satisfaction of cookies in the food industry. *L. reuteri* and *L. rhamnosus* were chosen to ferment egg yolk and their effects on dough properties and physicochemical properties, flavor, texture, color, and sensory acceptability of cookies were studied. Results show that the utilization of fermented egg yolk significantly decreased baking loss and increased spread factor of cookies. GC–MS analysis indicates different Lactobacillus species enhanced cookie flavor through unique mechanisms. Texture analysis shows cookies prepared with *L. rhamnosus*-fermented egg yolk had significantly lower hardness (1807.12 g) than control cookies (2028.34 g). Sensory evaluation reveals the *L. reuteri*-fermented egg yolk significantly improved the overall acceptability of cookies by enhancing appearance, flavor, and mouthfeel scores. These findings have practical implications for food manufacturers seeking to enhance their product's quality and appeal, thereby gaining a competitive edge in the market.

1. Introduction

Cookies are ready-to-eat bakery products traditionally composed of flour, sugar, egg, and shortening (Bakare et al., 2020). Since their palatability, affordability, storability, and diverse tastes, cookies are widely consumed worldwide (Aljobair, 2022). The texture and flavor properties of cookies are significant factors influencing consumer choices (Ashwath Kumar, & Sudha, 2021; Erinc, Mert, & Tekin, 2018). Egg yolk, as an important emulsifier in cookie dough, plays a critical role in contributing to these properties (Jia et al., 2023; Ren, Ma, Xu, Lv, & Tong, 2022). Therefore, it is interesting to explore natural ways for modifying egg yolks to improve the sensory attribute of cookies.

Lactic acid fermentation is an ancient, natural, and low-cost biotechnology that has been recently applied to egg yolk and mayonnaise (Jia et al., 2023; Tian et al., 2021). Tian et al. (2021) reported that lactic acid bacteria grow well in egg yolk and 3 h of fermentation significantly improves its emulsifying properties. Meanwhile, the 3 h-fermented egg yolk enhances the flavor score of mayonnaise by altering the composition of volatile compounds, as well as improving its textural property (Jia et al., 2023). Additionally, lactic acid fermentation can also enhance the texture and flavor of dough and baked goods (Jia et al., 2022; Liu et al., 2020a; Sulieman et al., 2019). For example, Sulieman et al. (2019) added fermented Agaricus bisporus polysaccharide in composite gluten-free dough, resulting in better functional, pasting, and rheological properties of dough and the texture of biscuits. Jia et al. (2022) used fermented egg whites to prepare angel food cakes and found that 9 h-fermented egg white significantly increases the abundance of alcohol compounds in cakes, which positively affects their odor score. Accordingly, we are interested in further investigating the potential application of lactic acid-fermented egg yolk to improve the sensory properties including texture and flavor of cookies.

For lactic acid fermentation, it is worth mentioning that homofermentative and heterofermentative lactic acid bacteria have different ways of enhancing the aroma of sourdough, the former tends to generate more aldehydes and ketones, while the latter produces more ethanol and esters (Liu et al., 2020a). Our recent work investigated the impacts of 10 species of lactic acid bacteria on the flavor of egg yolk, finding that *Limosilactobacillus reuteri* (*L. reuteri*) and *Lacticaseibacillus rhamnosus* (*L. rhamnosus*) endow egg yolk with the most pleasant aroma. Since *L. reuteri* and *L. rhamnosus* are heterofermentative and facultatively heterofermentative species, respectively, they produce different small molecule metabolic during fermentation, which occurs in complex

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reaction and generate diverse volatile compounds during baking (Jia et al., 2022). Therefore, in this work, the effects of *L. reuteri* and *L. rhamnosus*-fermented egg yolk on the physicochemical properties, flavor, texture, and sensory acceptance of cookies were evaluated and compared, aiming to give some scientific basis for the application of lactic acid fermentation technology in baked foods.

2. Material and methods

2.1. Material

Hen eggs were purchased from a local market (Yangling, Shaanxi, China). Low-gluten wheat flour (9.5 % protein) and white granulated sugar were supplied by Angel Yeast Co., Ltd. (Yichang, Hubei, China). *L. reuteri* JYLB-291 and *L. rhamnosus* JYLR-005 were acquired from Shandong Zhongkejiayi Biological Engineering Co. Ltd. (Qingzhou, Shandong, China). C_4-C_{40} *n*-alkanes series was supplied by Sigma-Aldrich Co., Ltd. (St. Louis, Mo, USA).

2.2. Preparation of fermented egg yolk powder

2.2.1. Fermentation treatment

The fermentation of egg yolk was performed using the previous method (Jia et al., 2022; Tian et al., 2021). First, egg yolk was separated using a separator and rolled on a filter paper to remove egg white. After that, the egg yolk membrane was punctured to collect the egg yolk solution in a sterilized bottle. Then, *L. reuteri* JYLB-291 and *L. rhamnosus* JYLR-005 were inoculated into egg yolk solution (1 %, w/v egg yolk solution), respectively, and fermented at 37 °C for 3.5 h. The native egg yolk without fermentation was regarded as a control.

2.2.2. Spray drying condition

Native, *L. reuteri*-fermented, and *L. rhamnosus*-fermented egg yolk were diluted with equal volumes of distilled water, respectively, then were spray-dried by a laboratory-scale spray dryer (SP-1500, Shanghai Sunyitech Co., Ltd, Shanghai, China) based on previous reference (Dhal, Anis, Shaikh, Alhamidi, & Pal, 2023). The inlet air temperature and speed of fluid flow were set to 160 °C and 500 mL/h, respectively. The outlet air temperature was 63 °C.

2.3. Preparation of dough and cookie samples

Dough and cookie samples were prepared based on the previous reference with slight modifications (Yang, Guo, & Zhao, 2020). To investigate the influences of fermented egg yolk powder on the flavor of cookies, 16.0 g egg yolk powder and 14.0 g water were added to the original formulation. Due to 16.0 g egg yolk powder containing about 10 g lipid, the total lipid content in the original formulation was reduced accordingly. The modified formulation included 100.0 g low-gluten wheat flour, 20.0 g white granulated sugar, 18.0 g butter, 16.0 g native, *L. reuteri*-fermented, or *L. rhamnosus*-fermented egg yolk powder, and 28.0 g water. The white granulated sugar was ground into powdered sugar in a grinder and then dissolved in water. Low-gluten wheat flour and egg yolk powder were sieved through a 60-mesh sieve. All the ingredients were mixed by a kitchen mixer (M6, Qingdao Hauswirt Baking Electrical Appliances Co., Ltd, Qingdao, Shandong, China) at speed 2 for 1 min and speed 5 for 3 min. After gently hand rounded, the dough was left to stand at room temperature (25 °C) for 30 min. After that, the dough was laminated in pieces of 3 mm by an automatic sheeter (Deqing Baijie Electrical Appliances Co., Ltd, Huzhou, Zhejiang, China), cut with a circular die (5 cm diameter) and then baked at 190 °C for 13 min in electric oven (SEB-3Y-3S, Jiangsu SUN-MATE Food Machinery Co., Ltd, Yancheng, Jiangsu, China). Subsequently, the cookie samples were cooled to room temperature.

2.4. Dough properties

2.4.1. Moisture content

The moisture content of dough samples was evaluated using the gravimetric method according to Miśkiewicz et al. (2018). For each sample, 3.0 g of ground dough powder was dried at 105 °C in an electric blast drying oven (101-1AB, Tianjin Taisite Instrument Co., Ltd., Tianjin, China) until constant weight.

2.4.2. Rheological properties

The rheological properties of dough samples were evaluated utilizing a dynamic shear rheometer (DHR-1, TA Instruments, Waters, UK) with parallel plate geometry (20 mm diameter) according to the modified procedure of Zhang et al. (2020). The measurement was carried out at 25 °C. First, the dough sample was loaded onto the parallel plate and compressed with a gap of 1 mm. Then, the edge of the dough was covered in silicone oil to avoid moisture loss. Subsequently, the dough was left for 5 min to relax normal stresses induced during loading. G' and G'' were determined over a frequency range of 0.1–10 Hz under 1.5 % strain.

2.4.3. Texture

Based on the modified procedure of Miśkiewicz et al. (2018), dough texture was determined as an average value of five individual dough samples by a texture analyzer (TA-XTplusC, Stable Microsystems Ltd, Godalming, UK) equipped with a load cell of 5 kg and P/6 probe. 100.0 g of dough sample was loaded in the cylinder and extruded to a thickness of 25.0 mm. The pre-test speed, test speed, and post-test speed were all set to 3.0 mm/s, the distance was 12 mm, and the trigger force was 5.0 g.

2.5. Physicochemical properties of cookies

2.5.1. Baking loss

Dough and cookie weights were measured as an average value of six individual cookies (Yang et al., 2020). The baking loss was calculated following the equation (Šarić et al., 2019):

Baking loss (%) = (dough weight - cookie weight) × 100 / dough weight.

2.5.2. Moisture content

The moisture content of cookie samples was evaluated using the same method as described above (section 2.4.1).

2.5.3. Physical dimensions

The diameter and thickness were measured based on the previous method with slight modifications (Mudgil, Barak, & Khatkar, 2017). Six cookies were placed edge to edge and the total diameter was measured. After rotating each cookie to 90, the diameter of six cookies was measured again. The cookie diameter was calculated by the average value of before and after rotation. The cookie thickness was measured by calculating the average thickness of random stacking six cookies three times. Cookie volume was determined using the seed displacement method (Azaripour & Abbasi, 2020). The spread factor was calculated following the equation (Mudgil et al., 2017):

Spread factor = cookie diameter (mm) / cookie thickness (mm)

2.6. Sensory quality of cookies

2.6.1. Volatile compounds

2.6.1.1. Headspace solid-phase microextraction (HS-SPME). Volatile compounds of cookie samples were extracted by the method of HS-SPME (AOC-6000, Shimadzu, Kyoto, Japan). 2.0 g of ground cookie powder was placed into 20 mL headspace vials and balanced at 60 °C for 30 min. A 2 cm CAR/PDMS/DVB (50/30 μm) SPME fiber (Supelco, Bellefonte, PA, USA) was inserted into a sample bottle for 20 min. After that, the fiber was desorbed in the gas chromatography (GC) injector at 250 °C for 3 min.

2.6.1.2. Gas chromatography-mass spectrometry (GC–MS). The extracted volatile compounds were separated and identified by GC–MS (GCMS-QP2010 Ultra, Shimadzu, Kyoto, Japan). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min through the DB-1MS column (60 m × 0.25 mm × 0.25 μm; Agilent Technologies, Santa Clara, CA, USA). The oven temperature program was: 50 °C for 1.00 min, and then raised to 250 °C at 4 °C/min and maintained for 10.50 min. Mass spectra were obtained using the electron impact ionization (EI) mode with 70 eV of ionization energy; in full scan mode, the scanning range was m/z 35–500. The injector, ion source, and interface temperatures were 250 °C, 230 °C, and 230 °C, separately.

2.6.1.3. Qualitative analysis. Volatile compounds were identified by comparing the mass spectra fragments with those in the NIST MS library, and retention indices calculated by the C_4-C_{40} *n*-alkanes series with those in the NIST MS library. The compounds with a similar index (SI) greater than 85 are considered as effective volatile components.

2.6.2. Texture

Based on the modified method of Torra et al. (2021), the texture of the cookies was detected as an average value of five individual cookies using a texture analyzer (TA-XTplusC, Stable Microsystems Ltd, Godalming, UK) equipped with a 5 kg load cell, a 3-point bending test holder and a probe (HDP/3PB). The distance between the two supporting beams was adjusted to 28 mm. The pre-test speed, test speed, and post-test speed were all set to 2.0 mm/s, the probe travel distance was 5 mm, and the trigger force was 10.0 g.

2.6.3. Color

The facial color of cookies was measured using a Colorimeter (Ci7600, X-rite color technology Co., Ltd, Shanghai, China). First, the colorimeter was calibrated by a white and black standard plate, respectively. The facial color was determined at 3 points of five individual cookies. Results were presented as CIE L^*, a^*, and b^* values. The total color difference (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{(L^* - L_0{}^*)^2 + (a^* - a_0{}^*)^2 + (b^* - b_0{}^*)^2}$$

Here, $L_0{}^*$, $a_0{}^*$, and $b_0{}^*$ were the lightness, green-redness, and blue-yellowness of control cookies, and L^*, a^*, and b^* were those parameters of cookies prepared with fermented egg yolk.

ΔE is interpreted as follows: $\Delta E < 1$, the differences of color are not obvious to human eyes; $1 < \Delta E < 3$, the differences of color are not appreciated by human eyes; and $\Delta E > 3$, the differences of color are obvious to human eyes (Jia et al., 2022).

2.6.4. Sensory evaluation

Cookie samples were evaluated by 36 semi-trained panelists (ranging from 22 to 36 years of age) from the College of Food Science and Engineering, Northwest A&F University, China, who had experience in sensory evaluation. The cookies tested were safe for consumption. All participants acknowledged informed consent before this study, and the rights and privacy of each participant were ensured. They were able to withdraw from the sensory evaluation at any time without giving a reason. All cookie samples were given randomly codes and simultaneously to each panelist. Panelists evaluated the acceptability of cookie samples based on the following indicators (appearance, flavor, texture, mouth feel, and overall acceptability) using a 9-hedonic scale from 1 (extremely dislike) to 9 (extremely like) (Bhat et al., 2018).

2.7. Experimental design and statistical analysis

Except for the baking loss, physical dimensions, texture, color, and sensory evaluation, other experiments were carried out in triplicate. Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests with a 95 % confidence interval ($p < 0.05$) using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). All the results were shown as the mean ± SD. Bar and line graphs were drawn by GraphPad Prism software (GraphPad Prism 6.0, GraphPad Software Inc., San Diego, CA, USA). The principal component analysis (PCA) was performed using the Genescloud tools (https://www.genescloud.cn). The hierarchical cluster analysis (HCA) was carried out by the OmicStudio tools (https://www.omicstudio.cn/tool).

3. Results and discussion

3.1. Dough properties

3.1.1. Moisture content

The moisture content of dough samples is a vital factor in determining their rheological properties (Dhal et al., 2023) and the rate of Maillard reaction during baking (Morell, López-García, Hernando, & Quiles, 2023). Typically, the moisture content of dough ranges between 20 % and 25 % (Dhal et al., 2023). As presents in Fig. 1(a), the moisture content of control dough samples was 22.44 %, being consistent with values reported by Dhal et al. (2023). Fermented egg yolk did not significantly affect the moisture content of dough samples, remaining at around 22.56 %. This finding aligns with Schouten et al. (2023), reporting that dough samples with the same recipes have similar moisture content.

3.1.2. Rheological properties

The rheological properties of dough samples have a significant impact on their workability and baking performance, ultimately affecting the quality of cookies (Demirkesen, 2016; Yang et al., 2020). The elastic and viscous properties were used to determine the rheological behavior of dough, which were expressed in terms of storage modulus (G′) and loss modulus (G″), respectively (Jiang et al., 2020). The former represents the solid-like behavior, and the latter reflects the fluid-like behavior of dough samples (Jiang et al., 2020). As depicts in Fig. 2, under a constant strain of 0.1 %, G′ values were consistently higher than G″ values over the frequency range in all dough samples, indicating solid-like elastic behavior. The G′ and G″ values of all dough samples increased gradually with the frequency, which is consistent with the results obtained by Yang et al. (2020) and Zhang et al. (2020). Compared to control dough samples, those prepared with *L. reuter* and *L. rhamnosus*-fermented egg yolk showed a gradual decrease in G′ and G″ values. This suggests a reduction in elastic and viscous properties of dough samples, which would contribute to a softer texture of cookies. The primary reason is that egg yolk proteins undergo hydrolysis after fermentation by lactic acid bacteria, which may hinder the formation of gluten networks and reduce the elasticity of dough samples (Yang et al., 2020).

3.1.3. Texture

The texture of the dough was evaluated by determining hardness and stickness, and the obtained results are presented in Table 1. It is observed that there was no significant difference in the hardness among the dough samples. As reported by Sai Manohar and Haridas Rao (1999), the moisture content of dough samples significantly influences their hardness. The unchanged hardness level of dough samples can be attributed to their similar moisture content. However, the stickiness of the dough significantly reduced from 57.32 to 29.86 g.*sec* upon the substitution of native egg yolk with *L. reuter*-fermented egg yolk. As mentioned above, the utilization of fermented egg yolk may weaken the

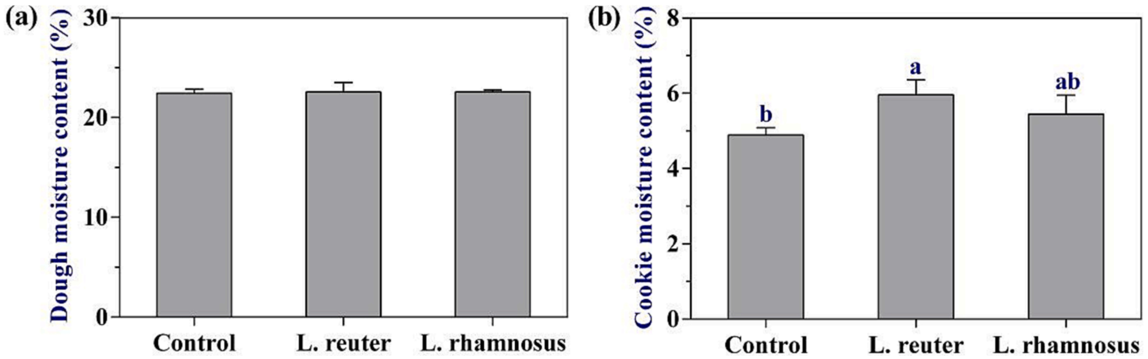

Fig. 1. The moisture content of dough samples (a) and cookie samples (b) prepared with native, *L. reuteri*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively. Different letters (a-b) indicated significant differences ($p < 0.05$).

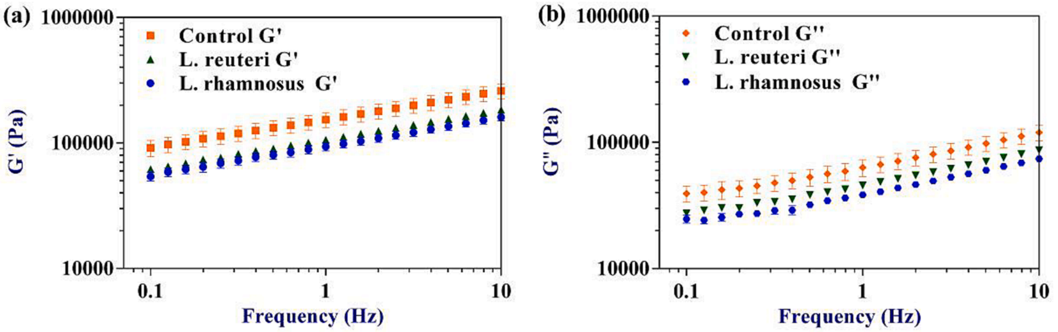

Fig. 2. Rheological properties of cookie samples prepared with native, *L. reuteri*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively. (a) Storage modulus (G′) of cookie samples; (b) Loss modulus (G″) of cookie samples.

Table 1
Textural characteristics of dough samples prepared with native, *L. reuter*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively.

	Control	*L. reuter*	*L. rhamnosus*
Hardness (g)	410.07 ± 28.44	399.33 ± 17.36	415.49 ± 41.59
Stickness (g.*sec*)	−57.32 ± 6.44^{a}	−29.86 ± 7.74^{b}	−52.07 ± 27.81^{ab}

Different letters (a-b) within the row indicated significant differences ($p < 0.05$).

gluten network formation in dough samples, thereby leading to a more sticky structure.

3.2. *Physicochemical properties of cookies*

3.2.1. *Baking loss*

Loss of moisture during baking, also known as baking loss, impacts the texture and staling properties of baked goods (Jia et al., 2022; Mohibbullah et al., 2023). As shows in Table 2, control cookies had the highest baking loss at 19.51 %. When replacing native egg yolk with fermented egg yolk, the baking loss of cookies was significantly reduced to 18.72 % and 18.56 %. This finding is consistent with the above result of rheological properties of dough samples (Fig. 2). The lower elastic and viscous properties of dough samples prepared with fermented egg yolk may cause an increase in air bubbles rising to the surface and result in fewer channels in the dough, thereby decreasing moisture loss. Another possible reason is that the presence of protein hydrolysates in fermented egg yolk samples facilitates the binding of water molecules through ion–dipole and dipole–dipole bonds. The decreased baking loss of dough samples can potentially increase the moisture content of cookies and thus affect their quality.

Table 2
Weight of dough samples prepared with native, *L. reuter*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively. Weight, baking loss, physical dimensions, textural characteristics, color parameters, and sensory characteristics of cookie samples prepared with native, *L. reuter*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively.

	Control	*L. reuter*	*L. rhamnosus*
Dough weight (g)	7.24 ± 0.30	7.48 ± 0.29	7.34 ± 0.29
Cookie weight (g)	5.83 ± 0.24	6.08 ± 0.23	5.98 ± 0.23
Baking loss (%)	19.51 ± 0.65^{a}	18.72 ± 0.55^{b}	18.56 ± 0.52^{b}
Cookie physical dimensions			
Thickness (mm)	3.81 ± 0.04^{a}	3.70 ± 0.04^{b}	3.81 ± 0.02^{a}
Diameter (cm)	4.48 ± 0.00	4.46 ± 0.03	4.46 ± 0.01
Volume (mL)	5.36 ± 0.56	5.72 ± 0.10	5.28 ± 0.54
Spread factor	11.76 ± 0.12^{b}	12.06 ± 0.09^{a}	11.71 ± 0.05^{b}
Cookie textural characteristics			
Hardness (g)	2028.34 ± 137.19^{a}	1963.84 ± 108.29^{ab}	1807.12 ± 135.46^{b}
Fracturability (mm)	0.50 ± 0.04	0.55 ± 0.10	0.50 ± 0.05
Cookie color parameters			
L^*	76.17 ± 0.94^{b}	77.72 ± 0.40^{a}	78.43 ± 0.12^{a}
a^*	3.19 ± 0.04	2.98 ± 0.30	3.27 ± 0.41
b^*	32.21 ± 0.68	31.51 ± 1.23	31.25 ± 0.48
ΔE	–	2.46	1.71
Cookie sensory characteristics			
Appearance	6.69 ± 2.01^{b}	7.47 ± 1.56^{a}	7.50 ± 1.13^{a}
Flavor	6.86 ± 1.25^{b}	7.47 ± 1.00^{a}	7.19 ± 1.35^{ab}
Texture	6.31 ± 2.11	6.89 ± 1.75	6.08 ± 1.89
Mouth feel	5.72 ± 1.61^{b}	6.58 ± 1.79^{a}	6.25 ± 1.61^{ab}
Overall acceptability	6.64 ± 1.33^{b}	7.25 ± 1.08^{a}	7.22 ± 1.22^{a}

Different letters (a-b) within the same row indicated significant differences ($p < 0.05$).

3.2.2. *Moisture content*

The moisture content of cookies is a crucial factor that affects their texture (Dhal et al., 2023). It can be seen from Fig. 1(b) that compared with control cookies (4.89 %), the cookies prepared with *L. rhamnosus*-fermented egg yolk had a significantly higher moisture content (5.96 %). This intriguing result can be attributed to the significantly lower baking loss (Table 2) and higher water absorption capacity of *L. rhamnosus*-fermented egg yolk (Supplemental Table S1), which reduce the water evaporation during baking (Azaripour & Abbasi, 2020; Miśkiewicz et al., 2018). Recent studies have also established that the better water absorption capacity of dough ingredients contributes to an increase in the moisture content of cookies (Azaripour & Abbasi, 2020; Miśkiewicz et al., 2018). Within a specific range, the higher moisture content is associated with a softer cookie texture in a certain range (Azaripour & Abbasi, 2020). Further, no significant change in moisture content is observed between cookies made with native and *L. reuter*-fermented egg yolk, which is consistent with dough samples.

3.2.3. *Physical dimensions*

The physical dimensions of cookies were evaluated based on their thickness, diameter, volume, and spread factor. According to Table 2, there were no significant differences in diameter and volume among cookie samples, which ranged from 4.46 to 4.48 mm and 5.28 to 5.72 mL, respectively. The thickness of cookies decreased significantly from 3.81 to 3.70 mm when using *L. reuter*-fermented egg yolk instead of native egg yolk, leading to an increased spread factor. This can be attributed to the dough prepared with *L. reuter*-fermented egg yolk having lower elasticity and viscosity (Table 2), causing it easier to flow after molding (Demirkesen, 2016; Jribi, Sahagún, Belorio, Debbabi, & Gomez, 2020). A higher spread factor is generally associated with better cookie quality (Dhal et al., 2023). On the other hand, the control cookies had a lower spread factor and a more compact structure, which would contribute to a harder texture (Guerra-Oliveira, Belorio, & Gómez, 2021; Jribi et al., 2020).

3.3. *Sensory quality of cookies*

The flavor, texture, and color are the important quality parameter that affects consumer acceptance of cookies (Demirkesen, 2016; Jribi et al., 2020). Thus, in this work, the sensory quality of cookie samples was evaluated in terms of their volatile compounds, texture, color, and sensory acceptability.

3.3.1. *Volatile compounds*

During the fermentation of egg yolk, lactic acid bacteria secrete specific enzymes that hydrolyze carbohydrates, fats, and proteins into fatty acids, glucose, amino acids, and other small molecule metabolites (Jia et al., 2022; Jiang et al., 2020; Tian et al., 2021). During the baking process, these small molecules act as flavor precursors and are involved in various chemical reactions like Maillard reaction, caramelization, lipid oxidation, and Strecker degradation which produce various volatile compounds that directly affect the final flavor of the bakery foods (Jia et al., 2022). To investigate the effects of fermented egg yolk on the flavor of cookies, HS-SPME-GC–MS was performed to identify the volatile compounds. PCA was used to analyze the credibility of the identification results and differences of volatile compounds among three cookie samples. As presents in Fig. 3(a), the variance contribution rate of PC1 and PC2 was 97.3 % and 1.2 %, respectively, accounting for 98.5 % of the information of the original data cumulatively. This suggests that PC1 and PC2 could reflect the overall information of the samples well. PCA analysis shows that the samples in the same group clustered together, indicating that the experiments possessed high reliability and repeatability. Different groups were well separated, indicating that different Lactobacillus species had varying effects on the flavor profile of cookies. Consistent with PCA results, HCA (Fig. 3b) confirms that cookie samples could be divided into three clusters, corresponding to the cookies prepared with *L. rhamnosus*-fermented, *L. reuter*-fermented, and native egg yolk respectively. These findings suggest that the fermented egg yolk changed the flavor profile of cookies, and *L. rhamnosus*-fermented egg yolk showed the largest effect.

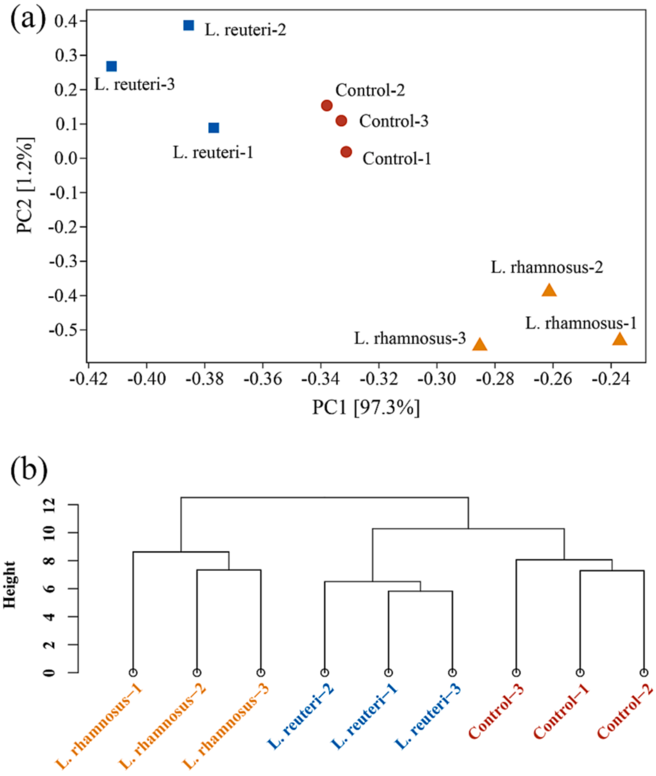

Fig. 3. Principal component analysis (a) and hierarchical cluster analysis (b) of volatile compounds in cookie samples prepared with native, *L. reuteri*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively.

Table 3 presents a total of 60 volatile compounds identified in cookies, including 12 aldehydes, 7 alcohols, 7 acids, 8 alkanes, 7 ketones, 1 amine, 2 alkenes, 3 aromatic compounds, 4 heterocyclic compounds, 4 lactones, 1 terpenoid, 2 esters, and 2 sulfur compounds. The odor of each compound was also described. Among all the compounds, aldehydes were the most prominent, constituting approximately 10.56 %-13.02 %. This is consistent with the findings of Pasqualone et al. (2019) who reported that aldehydes are also the primary volatile compounds in cookies made with flour, sucrose, and olive oil. Due to their pleasant aroma and low odor thresholds (Gemert, 2011), aldehydes were an important contributor to the flavor of cookies, providing an obvious fatty odor. In particular, hexanal was the most abundant aldehyde in control cookies and was also detected in heated or spray-dried egg yolk (Liu, Liu, Wang, & Jin, 2020b; Rannou et al., 2013; Ren et al., 2022). Its relative abundance remained unchanged despite replacing native egg yolk with fermented egg yolk. Acetaldehyde, octanal, and nonanal are associated with pleasant fat, flower, citrus, and lemon aromas, and were also detected in heated egg yolk (Liu et al., 2020b). Their relative abundances in cookies prepared with *L. reuter*-fermented egg yolk were significantly higher than that in control cookies. Meanwhile, nonanal abundance was also significantly increased in cookies prepared with *L. rhamnosus*-fermented egg yolk compared to control cookies. However, isobutyraldehyde and isovaleraldehyde, which have pleasant cocoa and almond aromas, were significantly lower in cookies prepared with *L. rhamnosus*-fermented egg yolk than in control cookies. On the other hand, some aldehydes are associated with off-flavors, such as 2-methylbutyraldehyde and benzaldehyde, which have unpleasant almond odors. Their relative abundances decreased significantly in cookies made with *L. rhamnosus*-

Table 3
Main volatile compounds of cookie samples prepared with native, *L. reuter*-fermented, and *L. rhamnosus*-fermented egg yolk, respectively.

Class	Compound	CAS No.	RIa [1]	RIb [2]	Identification Methods [3]	Area (%) [4]			Odor description
						Control	*L. reuter*	*L. rhamnosus*	
Aldehydes									
	Acetaldehyde	75-07-0	459	408	MS	0.58 ± 0.22	1.04 ± 0.10*	0.97 ± 0.13	Flower, green apple [5]
	Isobutyraldehyde	78-84-2	514	543	MS, RI	0.85 ± 0.01	0.77 ± 0.07	0.64 ± 0.01*	Cocoa, burnt [5]
	Isovaleraldehyde	590-86-3	591	643	MS	2.32 ± 0.23	2.13 ± 0.14	0.79 ± 0.09*	Fat, almond [6]
	2-Methylbutyraldehyde	96-17-3	603	643	MS, RI	0.63 ± 0.08	0.60 ± 0.03	0.32 ± 0.04*	Cocoa, almond [5]
	Valeraldehyde	110-62-3	641	707	MS	1.56 ± 0.14	1.28 ± 0.08*	0.97 ± 0.02*	Pungent, bitter [5]
	Hexanal	66-25-1	765	806	MS, RI	3.48 ± 0.14	3.68 ± 0.16	3.36 ± 0.25	Fat, apple [5]
	Benzaldehyde	100-52-7	926	982	MS	0.55 ± 0.06	0.41 ± 0.08	0.38 ± 0.05*	Burnt sugar, bitter almond [5]
	Octanal	124-13-0	976	1005	MS, RI	-	0.37 ± 0.17	-	Fat, citrus [5]
	Phenylacetaldehyde	122-78-1	1006	1081	MS	0.45 ± 0.12	0.32 ± 0.10	0.27 ± 0.04	Pungent, nut [5]
	2-Octenal	2548-87-0	1029	1013	MS, RI	0.07 ± 0.02	0.07 ± 0.02	0.11 ± 0.03	Fat, grass [5]
	Nonanal	124-19-6	1080	1104	MS, RI	1.91 ± 0.12	2.20 ± 0.12*	2.59 ± 0.34*	Fat, lemon [5]
	Decanal	112-31-2	1182	1204	MS, RI	0.14 ± 0.00	0.14 ± 0.03	0.15 ± 0.04	Flower, tallow [5]
	∑Aldehydes					12.53 ± 0.53	13.02 ± 0.23	10.56 ± 0.70*	
Alcohols									
	Ethanol	64-17-5	473	463	MS, RI	6.23 ± 0.44	8.05 ± 0.67*	4.94 ± 0.74	Pungent, vinous [6]
	1-Methoxy-2-propanol	107-98-2	620	657	MS, RI	0.25 ± 0.05	0.11 ± 0.01*	0.17 ± 0.06	Mild, ethereal [6]
	1-Pentanol	71-41-0	735	761	MS, RI	0.50 ± 0.04	0.43 ± 0.03	0.41 ± 0.03*	Pungent, fruit [5]
	1-Hexanol	111-27-3	843	860	MS, RI	0.22 ± 0.01	0.23 ± 0.01	0.28 ± 0.03*	Flower, banana [5]
	1-Octen-3-ol	3391-86-4	958	969	MS, RI	0.58 ± 0.03	0.58 ± 0.03	0.66 ± 0.06	Fat, cucumber [5]
	3-Octen-2-ol	76649-14-4	987	987	MS, RI	0.12 ± 0.03	0.10 ± 0.01	0.14 ± 0.02	Savory [5]
	1-Octanol	111-87-5	1050	1059	MS, RI	0.22 ± 0.07	0.26 ± 0.04	0.36 ± 0.05*	Fat, flower [5]
	∑Alcohols					8.12 ± 0.33	9.75 ± 0.72*	6.95 ± 0.90	
Acids									
	Acetic acid	64-19-7	545	576	MS, RI	4.82 ± 0.20	4.56 ± 0.19	3.40 ± 0.10*	Pungent, sour [5]
	Butyric acid	107-92-6	750	775	MS, RI	0.45 ± 0.02	0.42 ± 0.01	0.45 ± 0.07	Cheese, sour [5]
	Isovaleric acid	503-74-2	812	811	MS, RI	0.06 ± 0.01	0.06 ± 0.01	-	Cheese, pungent [5]
	Hexanoic acid	142-62-1	954	974	MS, RI	0.89 ± 0.20	0.73 ± 0.05	1.15 ± 0.02	Cheese, sour [5]
	Octanoic acid	124-07-2	1146	1173	MS, RI	0.19 ± 0.07	0.12 ± 0.02	0.25 ± 0.03	Cheese, grass [5]
	2-Ethylbutyric acid	88-09-5	1195	910	MS	0.22 ± 0.03	0.22 ± 0.09	0.33 ± 0.02*	Fruit [5]
	Nonanoic acid	112-05-0	1243	1272	MS, RI	0.24 ± 0.05	0.24 ± 0.01	-	Fat, sour [5]
	∑Acids					6.87 ± 0.38	6.36 ± 0.19	5.57 ± 0.21*	
Alkanes									
	Pentane	109-66-0	494	518	MS, RI	1.23 ± 0.07	0.78 ± 0.26*	0.53 ± 0.07*	Petroleum-like [6]
	2,4-Dimethylpentane	108-08-7	556	589	MS, RI	4.34 ± 0.36	5.46 ± 0.50*	3.14 ± 0.25*	nf
	Octane	111-65-9	792	816	MS, RI	0.31 ± 0.04	0.21 ± 0.02*	0.17 ± 0.02*	Gasoline-like [6]
	2,2,4,6,6-Pentamethylheptane	13475-82-6	993	981	MS, RI	0.11 ± 0.01	0.11 ± 0.02	0.16 ± 0.00*	nf
	3-Methylundecane	1002-43-3	1169	1150	MS, RI	-	0.10 ± 0.02*	-	nf

(*continued on next page*)

Table 3 (*continued*)

Class	Compound	CAS No.	RIa [1]	RIb [2]	Identification Methods [3]	Area (%) [4]			Odor description
						Control	*L. reuter*	*L. rhamnosus*	
	Tetradecane	629-59-4	1397	1413	MS, RI	0.05 ± 0.01	-	-	nf
	Cyclooctacosane	297-24-5	1788	3357	MS	0.10 ± 0.02	0.14 ± 0.02*	0.14 ± 0.03	nf
	Heptadecane	629-78-7	1796	1711	MS	0.18 ± 0.26	-	-	Fuel-like [6]
	∑Alkanes					6.33 ± 0.45	6.80 ± 0.50	4.14 ± 0.35*	
Ketones									
	Acetone	67-64-1	482	455	MS, RI	1.44 ± 0.88	1.88 ± 0.09	1.33 ± 0.05	Pungent [5]
	2-Heptanone	110-43-0	861	853	MS, RI	1.73 ± 0.13	1.65 ± 0.37	1.22 ± 0.10*	Blue cheese, fruit [5]
	2,5-Octanedione	3214-41-3	955	1088	MS	-	0.24 ± 0.03*	-	nf
	2-Octanone	111-13-7	965	952	MS, RI	-	-	0.07 ± 0.03	Fat, fragrant [5]
	2-Nonanone	821-55-6	1067	1052	MS, RI	0.73 ± 0.08	0.72 ± 0.05	0.66 ± 0.05	Fragrant, fruit [5]
	2-Undecanone	112-12-9	1271	1251	MS, RI	0.24 ± 0.01	0.29 ± 0.02*	0.27 ± 0.03	Fresh, orange [5]
	2-Tridecanone	593-08-8	1474	1449	MS, RI	0.08 ± 0.01	0.09 ± 0.02	0.09 ± 0.01	Savory [5]
	∑Ketones					4.21 ± 0.83	4.89 ± 0.35	3.65 ± 0.19	
Amine									
	Triethylamine	121-44-8	654	667	MS, RI	0.99 ± 0.47	1.12 ± 0.77	0.32 ± 0.15	Ammonia, fishy [6]
Alkenes									
	Vinyl acetate	108-05-4	528	576	MS, RI	0.44 ± 0.03	0.39 ± 0.01*	0.25 ± 0.04*	Sweet, fruit [6]
	6,6-Dimethylfulvene	2175-91-9	851	824	MS, RI	0.45 ± 0.05	0.40 ± 0.03	0.42 ± 0.05	nf
	∑Alkenes					0.89 ± 0.40	0.79 ± 0.73	0.68 ± 0.23*	
Aromatic compounds									
	Benzene	71-43-2	613	680	MS	0.32 ± 0.04	0.29 ± 0.01	0.21 ± 0.05*	Aromatic, gasoline-like [6]
	Toluene	108-88-3	743	794	MS	0.20 ± 0.01	0.13 ± 0.02*	0.13 ± 0.01*	Aromatic, pungent [6]
	2,6-Bis(1,1-dimethylethyl)-4-(1-oxopropyl)phenol	14035-34-8	1621	2003	MS	0.03 ± 0.02	-	-	nf
	∑Aromatic compounds					0.55 ± 0.05	0.42 ± 0.02*	0.33 ± 0.06*	
Heterocyclic Compounds									
	2,5-Dimethylpyrazine	123-32-0	881	894	MS, RI	-	0.09 ± 0.09	-	Cocoa, roasted nut [5]
	2-Pentylfuran	3777-69-3	975	1040	MS	0.46 ± 0.23	0.18 ± 0.05	0.56 ± 0.07	Butter, flower [5]
	2,3-Dihydro-3,5-dihydroxy-6-methyl-4 h-pyran-4-one	28564-83-2	1110	1269	MS	0.17 ± 0.05	0.22 ± 0.04	-	Caramelized [7]
	6-Hexyltetrahydro-2H-pyran-2-one	710-04-3	1497	1503	MS, RI	0.05 ± 0.01	0.07 ± 0.02	0.09 ± 0.02*	Peach [5]
	∑Heterocyclic Compounds					0.68 ± 0.27	0.56 ± 0.14	0.65 ± 0.07	
Lactones									
	delta-Hexalactone	823-22-3	1039	1006	MS, RI	0.07 ± 0.02	0.13 ± 0.01*	0.12 ± 0.04	Spice [5]
	delta-Octalactone	698-76-0	1237	1205	MS, RI	0.08 ± 0.03	0.08 ± 0.02	0.10 ± 0.05	Coconut, peach [5]
	delta-Decalactone	705-86-2	1452	1404	MS, RI	0.10 ± 0.02	0.13 ± 0.01	0.16 ± 0.06	Coconut [5]
	delta-Dodecalactone	713-95-1	1666	1602	MS	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	Fruit [5]
	∑Lactones					0.28 ± 0.01	0.38 ± 0.01*	0.44 ± 0.15	
Terpenoid									
	D-Limonene	5989-27-5	1021	1018	MS, RI	0.11 ± 0.02	0.11 ± 0.02	0.21 ± 0.03*	Citrus, mint [5]
Esters									
	Diisobutyl phthalate	84-69-5	1829	1908	MS	0.03 ± 0.02	0.04 ± 0.01	0.05 ± 0.02	Slight ester [6]
	Dibutyl phthalate	84-74-2	1920	2037	MS	0.08 ± 0.05	0.03 ± 0.00	0.06 ± 0.03	Slight aromatic [6]

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Table 3 (*continued*)

Class	Compound	CAS No.	RIa [1]	RIb [2]	Identification Methods [3]	Area (%) [4]			Odor description
						Control	*L. reuter*	*L. rhamnosus*	
	∑Esters					0.11 ± 0.05	0.07 ± 0.02	0.11 ± 0.04	
Sulfur Compounds									
	Methanethiol	74-93-1	466	0	MS	0.65 ± 0.13	0.36 ± 0.02	0.38 ± 0.05*	Garlic, sulfur [5]
	Dimethyl sulfone	67-71-0	846	727	MS	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	nf
	∑Sulfur Compounds					0.71 ± 0.14	0.42 ± 0.02	0.44 ± 0.07*	

*$p < 0.05$, compared with control cookies. nf: not found; -: below detection limit.

[1] RIa: calculated by formula.

[2] RIb: obtained from NIST database.

[3] MS: the MS fragments matching with the result of searching NIST MS library; RI: RIa matched with RIb.

[4] Each volatile compound was expressed as relative percentage of the GC peak area (%) on the total peak areas.

[5-7] The corresponding references showing odour description; [5] Flavor & Extract Manufacturers Association (FEMA) (2022); [6] Kim et al. (2023); [7] Pico, Bernal, & Gómez (2015).

fermented egg yolk compared to control cookies. In addition, valeraldehyde, which involves pungent and bitter odors, was significantly lower in cookies prepared with fermented egg yolk than in control cookies.

Alcohols were another dominant compound in the cookies, with ethanol being the most abundant among them. When the native egg yolk was replaced with *L. reuter*-fermented egg yolk, the ethanol abundance in cookies increased significantly from 6.23 % to 8.05 %. Conversely, 1-methoxy-2-propanol was significantly lower in cookies prepared with *L. reuter*-fermented egg yolk than in control cookies. It is worth noting that both ethanol and 1-methoxy-2-propanol have relatively high odor thresholds (Gemert, 2011) and their changed relative abundance has little effect on the flavor of cookies. Other alcohols, such as 1-pentanol, 1-hexanol, and 1-octanol, possess low odor thresholds (Gemert, 2011), and their abundance was changed when replacing native egg yolk with *L. rhamnosus*-fermented egg yolk. Specifically, the abundance of 1-hexanol (flower and banana aromas) and 1-octanol (fat and flower aromas) was increased significantly, while that of 1-pentanol (pungent and fruit) was decreased significantly. Overall, the total alcohol abundance in cookies prepared with *L. reuter*-fermented egg yolk (9.75 %) was significantly higher than that in control cookies (8.12 %) (Table 1), which would have a positive effect on the sensory analysis of cookies (Quílez et al., 2006).

Most acids are characterized by pungent and sour odors that have high thresholds for odor detection (Gemert, 2011). These smells typically have a negligible impact on the overall flavor of cookies. However, isovaleric acid has a low odor threshold (Gemert, 2011) and is commonly associated with unpleasant cheese and pungent smells. It has been detected in cookies made with native or *L. reuter*-fermented egg yolk. Interestingly, in cookies made with *L. rhamnosus*-fermented egg yolk, the level of isovaleric acid was below the detection limit. This would potentially help to reduce the unpleasant odors in cookies. Alkanes are generally described as having petroleum, gasoline, and fuel-like odor. In cookies prepared with *L. rhamnosus*-fermented egg yolk, the overall abundance of alkanes was significantly reduced in comparison to control cookies. However, given their high odor thresholds (Gemert, 2011; Guan, Liu, Li, Wang, & Zhang, 2022), this reduction may have minimal effect on the flavor of cookies.

Ketones are generated from the Maillard reaction and lipid oxidation (Jia et al., 2022). They have an intense aroma even at low concentrations (Chen, Lu, Yu, Chen, & Tian, 2019) and are associated with fruity and fragrant aromas. Interestingly, cookies made with *L. reuter*-fermented egg yolk showed a significant increase in the relative abundance of 2,5-octanedione and 2-undecanone, giving rise to fresh and orange aromas. In contrast, the use of *L. rhamnosus*-fermented egg yolk in cookies was found to significantly decrease the abundance of 2-heptanone, which imparts blue cheese and fruit aromas, while increasing the abundance of 2-octanone, contributing to pleasant fat and fragrant scents. Triethylamine is an amine compound that typically has distinct ammonia and fishy odor, and was also detected in heated duck egg gels (Ren, Ma, Lv, Tong, & Guo, 2021). When substituted for native egg yolk with fermented egg yolk, the relative abundance of triethylamine in cookie samples did not change significantly. However, it is worth mentioning that cookies made with *L. rhamnosus*-fermented egg yolk had a lower abundance of triethylamine than in control cookies, although this difference was not statistically significant. This indicates that lactic acid-fermented egg yolk had the potential to reduce the fishy odor of cookies.

Alkenes are known to have low odor thresholds (Gemert, 2011), with flower and fruit aromas. Only two alkenes were detected in cookies, of which vinyl acetate showed a significant reduction from 0.44 % to 0.39 % and 0.25 % in cookies when native egg yolk was replaced with *L. reuter*-fermented and *L. rhamnosus*-fermented egg yolk, respectively. Moreover, the total abundance of alkenes in cookies prepared with *L. rhamnosus*-fermented egg yolk was significantly lower than in the control cookies. Aromatic compounds are commonly characterized by their aromatic, gasoline-like, and pungent odor. When cookies were made using fermented egg yolk, the total abundance of these compounds was significantly lower compared to control cookies. Among the aromatic compounds, benzene possesses a high odor threshold (Gemert, 2011) and a weaker effect on odor, while toluene has a relatively lower odor threshold (Gemert, 2011), which would contribute to the reduction of aromatic and pungent flavors in cookies.

Pyrazine and furanic compounds are heterocyclic compounds that are produced through the Maillard reaction, providing a typical roasted aroma for baked foods (Jia et al., 2022). In cookies made with *L. reuter*-fermented egg yolk, 2,5-Dimethylpyrazine was generated, giving cocoa and roasted nut aromas. On the other hand, when cookies were prepared using *L. rhamnosus*-fermented egg yolk, the abundance of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (caramelized) decreased significantly, while 6-hexyltetrahydro-2H-pyran-2-one (peach) increased.

Lactones, terpenoids, esters, and sulfur compounds were also detected in three different types of cookies. Among these compounds, esters are responsible for providing a slight ester and aromatic scent to the cookies, and their abundance remained unchanged between cookies prepared with native and fermented egg yolk. The total abundance of lactones, specifically delta-hexalactone, was significantly higher in cookies prepared using *L. reuter*-fermented egg yolk than in control cookies, offering them a spice aroma. In comparison, cookies prepared with *L. rhamnosus*-fermented egg yolk showed a significant increase in D-limonene and a decrease in methanethiol. Both of these compounds have low odor thresholds (Gemert, 2011) and are also present in heated or spray-dried egg yolk (Liu et al., 2020b; Rannou et al., 2013). ᴅ-limonene imparts pleasant citrus and mint aromas to the cookies, while methanethiol has unpleasant garlic and sulfur odors.

In this study, aldehydes, alcohols, acids, alkanes, and ketones were the major components contributing to the cookie flavor. These compounds accounted for 38.06 % of the total abundance of volatile compounds and contributed to the fat, cheese, flower, fruit, fragrant, and pungent aromas of cookies. It is observed that different species of Lactobacillus used to ferment egg yolk had varying effects on the flavor profile of cookies. Specifically, cookies made with *L. reuteri*-fermented egg yolk exhibited a significant increase in abundance of pleasant volatile compounds such as acetaldehyde, octanal, nonanal, 2,5-octanedione, 2-undecanone, 2,5-dimethylpyrazine, and delta-Hexalactone, while reducing the abundance of some unpleasant volatile compounds like valeraldehyde and toluene. As a result, these cookies had more flower, green apple, fat, citrus, lemon, fresh, orange, cocoa, roasted nut, and spice aromas and less pungent, bitter, and aromatic odor. Similarly, *L. rhamnosus*-fermented egg yolk also had a significant effect on the flavor of cookies, increasing the abundance of pleasant volatile compounds such as nonanal, 1-hexanol, 1-octanol, 2-octanone, 6-hexyltetrahydro-2H-pyran-2-one, ᴅ-limonene, and decreasing the abundance of 2-methyl butyraldehyde, valeraldehyde, benzaldehyde, isovaleric acid, toluene, methanethiol. This resulted in cookies with more fat, lemon, flower, banana, fragrant, peach, citrus, and mint aromas and less cocoa, almond, pungent, bitter, burnt sugar, cheese, aromatic, garlic, and sulfur odor. However, the abundance of some pleasant volatile compounds, including isobutyraldehyde (cocoa and burnt), isovaleraldehyde (fat and almond), 1-pentanol (pungent and fruit), 2-heptanone (blue cheese and fruit), and vinyl acetate (sweet and fruit), was relatively lower in cookies made with *L. rhamnosus*-fermented egg yolk. Overall, the fermentation of egg yolk effectively improved the flavor profile of cookies by producing more pleasant aromas and reducing some unpleasant odors. Further research is needed to explore the potential use of mixed lactic acid bacteria to reduce the abundance of unpleasant volatile compounds while preserving the pleasant aroma of cookies.

3.3.2. *Texture*

Texture is an important quality parameter that affects consumer acceptance of cookies (Jribi et al., 2020). Hardness and fracturability were measured to evaluate the texture of cookies and are presented in Table 2. Hardness refers to the maximum force required to break a cookie, and a higher hardness is generally considered an undesirable characteristic of cookies (Schouten et al., 2023). Compared to the control cookies (2028.34 g), cookies prepared using *L. rhamnosus*-fermented egg yolk showed a significantly lower hardness (1807.12 g). One of the primary reasons is that the hydrolysis of egg yolk proteins during fermentation attenuates the formation of gluten networks in dough samples, resulting in a softer texture of cookies (Chauhan, Saxena, & Singh, 2015; Yang et al., 2020). Additionally, the cookies prepared with *L. rhamnosus*-fermented egg yolk had higher moisture content than control cookies (Fig. 1) although not significant, which also contributed to a softer texture to some extent (Azaripour & Abbasi, 2020). Fracturability is defined as the distance between the origin and the point where the biscuit breaks. A moderate enhancement of fracturability is associated with pleasant organoleptic properties (Schouten et al., 2023). However, in this work, the fracturability between cookies prepared with native and fermented egg yolk was not significantly different. Overall, the cookies prepared with *L. rhamnosus*-fermented egg yolk had a significantly softer texture than control cookies, which can positively affect their acceptability by consumers.

3.3.3. *Color*

The color of cookies is a crucial appearance attribute that can significantly impact consumers' preferences and overall acceptability (Demirkesen, 2016). Two primary factors influence the color of cookies: the initial color of raw material; and the dextrinization of starch, Maillard reaction, and caramelization during baking (Demirkesen, 2016). The ΔE between control cookies and those made with fermented egg yolk was measured. Table 2 shows that compared with control cookies, the L^* value of cookies containing fermented egg yolk was significantly increased. It was reported that lysine plays an important role in Maillard reactions and a higher content of lysine decreases the lightness value of cookies (Sahagún & Gómez, 2018). Meanwhile, lysine is essential for the growth of Lactobacillus, and our recent research has discovered that lysine content decreases in egg whites during 3–12 h of lactic acid fermentation (Jiang et al., 2020). Additionally, Supplemental Table S2 shows that the *L. reuteri*-fermented egg yolk powder had a significantly higher lightness than native egg yolk powder. Therefore, the lighter color of cookies made with fermented egg yolk can be attributed to the lower lysine content and the lighter color of the fermented egg yolk powder. However, as indicates by the ΔE value, the color differences between control cookies and cookies prepared with *L. reuteri* or *L. rhamnosus*-fermented egg yolk were not appreciated by human eyes, with values of 2.46 and 1.71, respectively.

3.3.4. *Sensory evaluation*

The appearance, flavor, texture, mouth feel, and overall acceptability of cookies were evaluated using sensory evaluation. As presents in Table 2, the appearance of cookies made with fermented egg yolk better than that of control cookies due to their smooth and flat surface. Furthermore, cookies prepared using *L. reuter*-fermented egg yolk received significantly higher flavor scores compared to control cookies. These cookies possessed more pleasant aromas such as flower, green apple, fat, citrus, lemon, fresh, orange, cocoa, roasted nut, and spice aromas and less pungent and bitter odors as indicates in Table 3. Nevertheless, no significant difference was observed in the flavor scores between cookies prepared with native and *L. rhamnosus*-fermented egg yolk. This can be explained by the fact that although the use of *L. rhamnosus*-fermented egg yolk increased some pleasant aromas and decreased some unpleasant odors in cookies, certain pleasant aromas were also decreased. In addition, cookies prepared with *L. reuter*-fermented egg yolk also involved significantly higher mouth feel scores than control cookies due to their significantly higher spread factor and slightly softer texture (Table 2). However, the texture scores among cookie samples were not significantly different in this study, which is because the changes in texture were not able to be detected by sensory evaluators. Overall, the cookies made with fermented egg yolk, particularly *L. reuter*-fermented egg yolk, were preferred by consumers due to their higher appearance, flavor, and mouth feel scores.

4. Conclusion

In this study, native, *L. reuteri*-fermented, or *L. rhamnosus*-fermented egg yolk were utilized to prepare dough samples, respectively, which were then baked into cookies. The dough properties and the physicochemical, flavor, texture, color properties, and sensory acceptability of cookies were investigated. Results indicate that compared to the native egg yolk, (1) the fermented egg yolk significantly decreased the viscosity and elastic properties of dough samples, reduced the baking loss of cookies, and promoted their flavor via altering the composition of volatile compounds; (2) the *L. reuter*-fermented egg yolk significantly reduced the moisture content and stickiness of dough samples and increased the spread factor of cookies; (3) and the *L. rhamnosus*-fermented egg yolk significantly reduced the hardness of cookies. Despite these changes, the utilization of fermented egg yolk did not cause any perceptible fracturability or color changes in cookie samples. Sensory analysis reveals that cookies with *L. reuter*-fermented egg yolk received the highest scores for appearance, flavor, mouth feel, and overall acceptability. Overall, this work indicates that lactic acid fermented egg yolk can be used as a key ingredient in cookie production to improve their sensory quality and consumer satisfaction. This study offers valuable insights into the use of lactic acid fermentation in baking and contributes to the development of new and innovative products. Further research will focus on the optimal combination of lactic acid bacteria to improve the flavor and nutritional value of baked goods.

Ethical statement

The appropriate protocols for protecting the rights and privacy of all participants were utilized during the sensory evaluation, e.g. no coercion to participate, full disclosure of study requirements and risks, written or verbal consent of participants, no release of participant data without their knowledge, ability to withdraw from the study at any time.

CRediT authorship contribution statement

Jie Jia: Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Xiaofan Deng:** Data curation, Formal analysis, Investigation. **Xin Jia:** Formal analysis, Validation. **Chunfeng Guo:** Data curation, Methodology. **Xuebo Liu:** Resources. **Yuanjing Liu:** Conceptualization, Methodology, Supervision. **Xiang Duan:** .

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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