Comparison of taste characteristics between koji mold-ripened cheese and Camembert cheese using an electronic tongue system

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ABSTRACT

Koji mold, classified in the Aspergillus genus, is used to produce traditional Japanese fermented foods such as miso, soy sauce, and sake. In recent years, the application of koji mold to cheese ripening has attracted attention, and cheese surface-ripened with koji mold (koji cheese) has been studied. In this study, to evaluate the taste characteristics of koji cheese, an electronic tongue system was employed to measure the taste values of cheese samples ripened using 5 strains of koji mold in comparison with commercial Camembert cheese. All koji cheese samples exhibited lower sourness and greater bitterness, astringency, saltiness, and umami richness than the Camembert cheese samples. The intensity of each taste characteristic differed depending on the koji mold strain. These results indicate that koji cheese has a different taste value than conventional mold-ripened cheese. Furthermore, the results also indicate that various taste characteristics can be achieved by selecting different koji molds.

Key words: mold ripened cheese, Aspergillus oryzae, Aspergillus sojae, electronic tongue system

INTRODUCTION

Cheese is a popular fermented food that is produced and consumed globally. Cheese production is stated to have started 8,000 years ago in the Fertile Crescent (Fox and McSweeney, 2017). Generally, cheese is prepared by adding lactic acid bacteria as a starter culture along with rennet to coagulate milk and by removing whey from casein aggregates, followed by the ripening process (Zheng et al., 2021). Many types of cheese have been uniquely developed in relation to the climate of the region. It is suggested that more than 1000 varieties of cheese have been developed, and they can be grouped by differences in moisture content (such as hard, semi-hard, and soft type cheese) and the ripening method (bacteria and/or mold-ripened cheese, wash cheese, and fresh cheese) (Sandine and Elliker, 1970).

Mold-induced ripening is a typical cheese manufacturing method. There are 2 categories of mold-ripened cheese: cheese ripened with surface mold and blue vein cheese (Galli et al., 2016). Penicillium camemberti, Penicillium roqueforti, and Geotrichum candidum are used for the production of mold-ripened cheeses. Although there are some differences depending on the strain, these molds grow well around 20–30°C, and they exhibit strong protease and lipases activities (Kalai et al., 2017). These activities are important for creating the characteristic flavor and texture of cheeses.

A variety of fermented foods are consumed in Japan, such as sake (Japanese rice wine), shochu (Japanese distilled liquor), miso (fermented soybean paste), and soy sauce. These foods are important in Washoku (Japanese foods), which was registered on the UNESCO (United Nations Educational, Scientific and Culture Organization) Intangible Cultural Heritage list as “Washoku, traditional dietary cultures of the Japanese” in 2013 (United Nations Educational, Scientific, and Cultural Organization, accessed July 28, 2022). Koji, which is essential for producing the aforementioned foods, is produced by inoculating koji molds, such as Aspergillus oryzae, Aspergillus sojae, and Aspergillus luchuensis, onto a steamed cereal followed by cultivation while adjusting the temperature and humidity. The optimum temperature of koji mold is 35–38°C, and the optimum water content is approximately 40% (Yamashita, 2021). Proteases, lipases, and amylases produced by koji mold degrade protein, triacylglycerol, and starch in cereals.

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and promote fermentation. Thus, the use of koji mold as an enzyme source has attracted attention in recent years, and its use in foods other than grains (e.g., tea, seaweed, eggs) has resulted in improvements in nutritional components, production efficiency, and flavor (Kewpie Corp.; Watanabe et al., 2008; Uchida et al., 2020).

Conversely, there is little information about cheese produced using koji mold (koji cheese). In around 1960 in Japan, an A. oryzae strain was selected for its high amino acid production in casein-based model cheese culture (Nakanishi and Tokita, 1959). This mold grew on cheese curds for short periods and degraded casein, producing a cheese with a unique flavor. This study was the first report to suggest the use of koji mold for cheese ripening. Unfortunately, this strain was lost several decades after this study.

However, in recent years, natural cheese consumption has been increasing in Japan (Ministry of Agriculture Forestry and Fisheries, 2022). Furthermore, the export volume of domestically produced cheese is increasing annually, and Japanese cheese is attracting attention not only from within Japan but also from abroad. Therefore, we focused on koji cheeses as novel and original Japanese natural cheeses. Some characteristics of koji cheese that differ from those of existing mold-ripened cheeses have been revealed using modern analytical techniques. In our previous study, we measured the protease and lipase activities of cheese curds incubated with 5 strains of koji mold (Suzuki et al., 2021). The results indicated that protease and lipase activities differed among the koji strains. Furthermore, to investigate the effects of koji mold on the chemical characteristics of ripened cheeses, metabolite profiling was performed (Tomita et al., 2022; Hagi et al., 2022). The results illustrated that different koji molds have different metabolites that characterize the ripened cheese, and that the addition of sake lees increases the levels of γ-glutamyl peptides known as kokumi-active glutamyl peptides.

Previous studies have revealed that koji mold can be used for cheese ripening, and that the compositional characteristics of koji cheeses differed from those of other mold-ripened cheeses. However, the effect of koji molds on the taste of ripened cheese, which is important for cheese consumption, remains unclear.

In recent years, the electronic tongue system has been used as an objective, rapid, and accurate measurement method for the taste of foods (Phat et al., 2016; Liu et al., 2017; Hwang et al., 2020). This system has sensors that mimic the human tongue, and it can convert the tastes of foods to numerical data. Although human sensory tests are often performed to assess the taste of foods, this approach has few drawbacks. For instance, the evaluation of several food samples can be affected by the physical conditions and differences in the preferences of the panelists. It is also difficult to evaluate many samples because of the burden on the panelists (Hayashi et al., 2020; Toko, 2022). Moreover, human sensory tests are difficult to use for accurately comparing the samples from different days. However, these problems can be resolved by using an electronic tongue system. In addition, it can obtain data rapidly and safely without the risks and costs of human experiments. So, this system has been utilized in the food and drug industries. Therefore, an electronic tongue system was used in this study to evaluate the taste characteristics of koji-ripened cheeses. We aimed to develop a new Japanese cheese having different characteristics than the other mold-ripened cheeses. For this purpose, 5 surface-ripened cheeses were produced using Aspergillus strains instead of Camembert mold. In addition to the taste characterization of koji cheese, analyses of pH, moisture content, free amino acids (FAAs), and organic acids and the comparisons with Camembert cheese were performed.

**MATERIALS AND METHODS**

**Koji cheese**

Koji cheeses used in this study were produced on the same day using the same production method and materials used for preparing experimental cheeses in our previous study (Tomita et al., 2022).

The A. oryzae strains B, C, D, and K as well as A. sojae strain 2041 were obtained from Higuchi Matsu-no-se Shoten (Osaka, Japan). In the food industry, strains 2041 and K are industrially used to produce soy sauce, whereas strains B and D are used to make miso; strain C is a multipurpose mold.

Milk was obtained from Holstein cows maintained in the Institute of Livestock and Grassland Science, NARO (Tsukuba, Japan). The raw milk was stored at 4°C throughout the day. Before using this milk for cheese production, 1.5% (wt/vol) NaCl was added to 36 kg of raw milk, and the milk was then pasteurized at 75°C and quickly chilled at 30°C. While stirring gently, 360 mg of the lactic fermentation starter (CHN-11, Chr. Hansen Holdings A/S) and 0.9 g of rennet powder (Fro-mase 2200 TL Granulate; DSM Food Specialties) were added to pasteurized milk. After 1 h of incubation, the coagulated curd was cut into cubes using a curd knife. After standing for 1 h, 18 L of 1.5% (wt/vol) NaCl was added to the curd and the mixture was gently stirred for 30 min. The curd was stuffed into 36 plastic molds (φ62 mm × 90 mmH) and incubated overnight at 30°C. Green curds were inoculated with Aspergillus strains by
dipping them in a $1 \times 10^6$ /mL conidia solution. The cheeses were then ripened for 10 d at 30°C and 90% relative humidity in plastic cases with lids, allowing sufficient growth of koji molds. Experimental manufacturing was conducted in 3 independent batches.

**Sampling**

Ripe cheese samples were collected 10 d after inoculating the *Aspergillus* strains. Each sample was minced and mixed well. Additionally, to compare koji cheeses with conventional Camembert cheese, 3 different commercial Camembert cheeses were purchased in Tsukuba, Japan. The pH values of these cheeses were 5.82, 5.75, and 6.33. These commercial cheeses were also minced, mixed in the same amount, and used as comparators. The pH and moisture content were measured immediately after sampling. For other analyses, the samples were stored at −30°C until further use.

**pH and moisture measurements**

pH was determined using a pH meter. Five grams of each minced cheese sample were homogenized with 5 mL of Milli-Q water, followed by centrifugation to obtain supernatant for measurement.

Water content was measured using SMART6 Moisture and Solids Analyzer (CEM, USA) according to the manufacturer’s instructions. The experiments were conducted in duplicate.

**Organic acid measurement**

A 2.5-g sample of cheese was transferred to a 50 mL disposable centrifuge tube. Distilled water (22.5 mL) was added to the tube, and the cheese was homogenized for 1 min. The homogenate was incubated for 30 min at 50°C with shaking. The extracted sample was centrifuged at 20,000 $\times g$ for 10 min, and then the supernatant was filtered through a Vivaspin 500 ultrafiltration unit with a MWCO of 10 kDa (Sartorius AG, Göttingen, Germany). The filtered sample (10 μL) was injected into an HPLC system for organic acid analysis as described previously (Hagi et al., 2019).

**FAA measurement**

FAAs in the cheeses were extracted using a previously reported method (Asahina et al., 2020) with some modifications. Specifically, 2.5 g of each cheese was homogenized in 5 mL of 30% trichloroacetic acid solution. The suspension was centrifuged at 20,700 $\times g$ for 10 min at 4°C. One milliliter of the aqueous phase was appropriately diluted with 0.05 N HCl and filtered with a 0.45-μm filter. The samples were examined using an amino acid analyzer (L-8900, Hitachi Ltd., Tokyo, Japan). Standards were prepared using amino acid mixture standard solutions Type AN-II and Type B (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan).

**Electronic tongue measurements**

To assess the different taste attributes of the cheeses, electronic tongue measurements were performed using TS-5000Z (Intelligent Sensor Technology Inc.). The TS-5000Z system comprises sensor arrays (Ag/AgCl reference electrode and lipid/membrane electrodes), an autosampler, an electronic data collection unit, and a software program for interpreting the chemometric data. Five lipid/membrane electrodes were used to measure the taste values: sourness (CA0), saltiness (CT0), bitterness (C00), astringency (AE1), and umami (AAE). All sensors were preconditioned in a standard solution for 24 h, and a sensor check was performed before every measurement. To assure system stability, the taste values for each sample was measured 4 times using all available sensors, and the data obtained in the first run was deleted. A reference solution containing 30-mM KCl and 0.3-mM tartaric acid was prepared. The following steps were used to measure the potential of the sample solution: first, the potential of the reference solution was measured (Vr), followed by measuring the potential of the sample solution (Vs). The sensor arrays were washed lightly with the reference solution, followed by dipping the sensors again in the reference solution to obtain the potential Vr'. After the sensor array measurements, the sensor signals were converted into “taste values” using a data conversion tool (Intelligent Sensor Technology Inc.). There are 2 types of output measured by this system, i.e., a “relative value” as a first taste and “a change in the membrane potential caused by adsorption (CPA)” as an aftertaste, as described in a previous report (Toko, 2022). The relative value and CPA were calculated as follows: Vs − Vr and Vr' − Vr, respectively. The measurement parameters included “umami,” “saltiness,” “sourness,” “bitterness,” and “astringency” for the first taste, and “umami richness,” “aftertaste of bitterness,” and “aftertaste of astringency” for the aftertaste, as described by Toko (1996).

To prepare each minced cheese sample, 20 ± 0.01 g was added to 80 mL of Milli-Q water heated to 40°C and homogenized for 1 min. After centrifugation at 7035 $\times g$ for 10 min at 10°C, the aqueous phase was collected. For all taste measurements, except for “umami” the supernatant filtrated through a cell strainer (pluriStrainer® 200 μm, pluriSelect Life Science UG & Co.
Statistical analysis

pH, water content, organic acid and FAA levels, and taste values were analyzed by Tukey’s multiple comparisons test ($P < 0.05$). These data were also analyzed by performing Pearson correlation analysis. Principal component analysis (PCA) was conducted to evaluate the differences in taste values among samples. Statistical analysis was performed using the R software ver. 4.2.0.

RESULTS

Koji cheese manufacturing

Initially, cheese curds were salted by immersing them in 20% NaCl solution, but this method resulted in slow mold growth because of the high NaCl concentration on the surface and uneven mold growth because of surface irregularities. Therefore, in this study, NaCl was pre-added to the milk to ensure a uniform NaCl concentration throughout the curd. All Aspergillus strains were observed to grow throughout the cheese curd and develop conidia on the 10th day of ripening. B, D, and 2041 were white; C was light pink; and K was dark green (Figure 1). The observed difference in the appearance of experimental cheese and conventional Camembert cheese is an advantage of using novel cheese, and this could lead to the development of a variety of koji cheeses. After the 10 d of ripening period, the curd became softer, the cheese became deformed, and the rind was easily detached. The ripening time of koji cheese was shorter than that of the conventional Camembert cheese, which usually lasts for a few weeks. The higher ripening temperature of the koji cheeses in this study would have affected their rate of ripening.

pH and moisture analyses

The pH of cheese ripened by koji mold was higher than that of the comparator cheese (Table 1). The pH values of cheeses B, C, and D were 7.08 ± 0.37, 6.91 ± 0.35, and 7.18 ± 0.23, respectively, which were significantly higher than that of Camembert cheese (pH 6.01 ± 0.03).

All koji cheeses had significantly lower water content than comparator cheese, whereas no significant difference was noted among koji cheeses (Table 1). Given that the optimum water content of koji mold is approximately 40% (Yamashita, 2021), it was considered that the water content of cheese curds produced in this study was suitable for koji mold growth.

Organic acid analyses

Lactic acid was the major organic acid in all cheeses (Table 1). The lactic acid content of all koji cheeses was higher than that of the comparator cheese. The lactic acid content differed among the koji strains; specifically, cheese D had significantly lower lactic acid content than other koji cheeses, and cheese B also tended to have lower lactic acid content. The malic acid content was significantly higher in 2041 cheese than in other cheeses. No differences were noted for the levels of other organic acids. Citric acid, isovaleric acid, and valeric acid were not detectable in any sample. Trace amounts of propionic acid and isobutyric acid were detected in cheeses B, C, and D. Conversely, butyric acid was detected in trace amounts in B, D, K, and 2041 cheeses.

FAA and ammonia analyses

The FAA content of all koji cheeses was much higher than that of commercial Camembert cheese (Table 2). In particular, cheese 2041 displayed the highest levels of all FAAs, excluding Ile and Pro among the cheeses. Glu was the most prevalent FAA in all koji cheeses, followed by Lys. The ammonia content was also significantly higher in koji cheeses than in the comparator samples. Cheese B had the lowest ammonia content among the koji cheeses, but its ammonia content was 4.5-fold higher than that of the comparator. Additionally, pH had a stronger correlation with ammonia content ($R = 0.478$) than lactic acid content ($R = −0.032$) in the Pearson correlation analysis.

Taste value measurements

As one of the characteristics of electronic tongue measurements, the relationship between the concentration and the sensor responses is the same for human sensation, which follows the Weber-Fechner law (Wu et al., 2020). Following this law, on the electronic tongue system, one unit is defined as the concentration difference of 1.2 fold taste substance that humans can distinguish the minimum difference. (Toko, 2022).

Large taste differences between cheeses were measured for sourness, bitterness, astringency, saltiness, and umami richness (Table 3). Notably, high umami richness and low sourness values were found in all koji cheeses compared with commercial cheese. In addition, cheeses K and 2041 displayed high levels of saltiness and bitterness, respectively.
The sourness and umami richness exhibited a strong correlation with the pH of cheese in the Pearson correlation study ($R = -0.907$ and $0.887$, respectively). The moisture content was negatively correlated with bitterness, astringency, saltiness, umami, aftertaste from astringency, and umami richness ($R = -0.588$, $-0.769$, $-0.414$, $-0.900$, $-0.340$, $-0.603$, respectively). Except acetic acid, there was a weak association between sourness and organic acids ($R = 0.700$). Positive correlations were also observed between the intensity of umami and Glu, Asp, and total FAA levels ($R = 0.606$, $0.525$, and $0.729$, respectively). On the other hand, the intensity of umami richness had low correlations with these components ($R = 0.105$, $-0.037$, and $0.237$, respectively). Although the amino acids reported to elicit bitterness (Val, Cys, Met, Ile, Leu, Phe, Lys, His and Arg) had strong positive correlations with bitterness of the cheese ($0.546 < R < 0.898$), they showed negative correlations with aftertaste bitterness (Kawai et al., 2012).

PCA was conducted to evaluate the differences in taste between commercial Camembert cheese and each

<table>
<thead>
<tr>
<th>Cheeses</th>
<th>pH</th>
<th>Moisture (%)</th>
<th>Malic acid (μmol/g)</th>
<th>Succinic acid (μmol/g)</th>
<th>Lactic acid (μmol/g)</th>
<th>Formic acid (μmol/g)</th>
<th>Acetic acid (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>7.08 ± 0.37&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>44.82 ± 1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.40 ± 2.26</td>
<td>76.51 ± 23.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.67 ± 0.81</td>
<td>8.14 ± 4.02</td>
</tr>
<tr>
<td>C</td>
<td>6.91 ± 0.35&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>46.08 ± 0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 ± 0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90 ± 2.35</td>
<td>146.70 ± 37.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.45 ± 1.48</td>
<td>10.21 ± 12.48</td>
</tr>
<tr>
<td>D</td>
<td>7.18 ± 0.23&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>47.05 ± 2.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.88 ± 3.03</td>
<td>66.10 ± 29.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.52 ± 1.96</td>
<td>12.36 ± 5.01</td>
</tr>
<tr>
<td>K</td>
<td>6.50 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>44.80 ± 3.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.17</td>
<td>137.50 ± 11.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.72 ± 0.27</td>
<td>3.78 ± 2.26</td>
</tr>
<tr>
<td>2041</td>
<td>6.52 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>44.86 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 2.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.59 ± 0.73</td>
<td>124.03 ± 12.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.19 ± 0.81</td>
<td>1.49 ± 1.12</td>
</tr>
<tr>
<td>Comparator</td>
<td>6.01 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.67 ± 0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 ± 0.05</td>
<td>37.19 ± 1.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.67 ± 0.17</td>
<td>1.36 ± 0.28</td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard deviation (SD) of three trials each with triplicate analysis. Different superscripts indicate significant differences ($P < 0.05$). ND = Not Detected.

**Table 1.** The pH, moisture content, and organic acid content of koji and commercial Camembert cheeses

**Figure 1.** Appearance of cheese ripened with koji mold for 10 d.
koji cheese (Figure 2). The 2 principal components accounted for 83.0% of the total variance (PC1, 46.7%; PC2, 36.3%).

PCA revealed that differences in the strain more strongly affected cheese taste values than differences in manufacturing dates. In addition, cheeses made using strains B and D had negative values for PC1, whereas cheeses K and 2041 had positive values. Cheese C displayed the most similar taste value as commercial Camembert cheese.

DISCUSSION

The electronic tongue analysis revealed that koji-ripened cheeses exhibit unique taste characteristics compared with commercial Camembert cheese. The intensity of each taste characteristic also differed among the koji cheeses. In producing mold-ripened cheese, the presence of molds gives cheeses a characteristic appearance and taste (Spinnler, 2017). Similarly, in the case of koji, strain differences are believed to affect the taste of the product.

The pH values varied among the koji cheeses and had a negative correlation with sourness, which could affect the taste value of these cheeses. In addition, the moisture of koji cheeses was significantly lower than that of conventional Camembert cheese. Since the data obtained using the electronic tongue are dependent on the concentration of the taste substance, the moisture content of cheese also contributed to the difference in the intensity of the taste values between the koji and comparator cheese.

In line with a previous study (Suzuki et al., 2021), only low concentrations of butyric acid were observed in koji cheese, and this could be a distinguishing characteristic of koji cheese. Aspergillus sp. is lipolytic, and the lipase activity of molds grown on cheese curds has been confirmed previously (Suzuki et al., 2021). Two hypotheses were proposed to explain the low level of butyrate observed in koji cheeses: 1) low butyrate production and 2) quick metabolism of butyrate. Although the mechanism of butyrate production and its metabolism cannot be clarified in this study, we believe that the difference in butyrate content between koji and Camembert cheeses is an interesting finding.

The Aspergillus strain-ripened cheeses exhibited a small difference in “umami,” but their “umami richness” was stronger than that of Camembert cheese. Monosodium glutamate, disodium 5′-inosinate, and disodium 5′-guanylate are responsible for umami taste (Drake et al., 2007). In this study, there were positive correlations between the intensity of umami and Glu, Asp, and total FAA levels, and a low correlation between the intensity of umami richness and these components. Therefore, it was considered that other components were related to the intensity of umami richness. Recently, it was reported that mouthfulness and continuity are caused by γ-glutamyl peptides (Toelstede et al., 2009; Yang et al., 2021). Previous research indicated that the levels of these peptides were elevated when cheeses were ripened by koji (Hagi et al., 2022). The results of electronic tongue analysis revealed that koji-ripened cheeses exhibit unique taste characteristics compared with commercial Camembert cheese. The intensity of each taste characteristic also differed among the koji cheeses. In producing mold-ripened cheese, the presence of molds gives cheeses a characteristic appearance and taste (Spinnler, 2017). Similarly, in the case of koji, strain differences are believed to affect the taste of the product.

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Table 2. Amino acids and related compounds in the experimental cheeses

<table>
<thead>
<tr>
<th>Compounds</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>K</th>
<th>2041</th>
<th>Comparator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>0.41 ± 0.29ab</td>
<td>1.26 ± 0.56abc</td>
<td>0.54 ± 0.51abc</td>
<td>1.10 ± 0.12abc</td>
<td>2.39 ± 0.41c</td>
<td>0.13 ± 0.01a</td>
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<tr>
<td>Thr</td>
<td>0.17 ± 0.08ab</td>
<td>0.59 ± 0.31abc</td>
<td>0.19 ± 0.10abc</td>
<td>0.49 ± 0.06abc</td>
<td>0.89 ± 0.26a</td>
<td>0.05 ± 0.002a</td>
</tr>
<tr>
<td>Ser</td>
<td>0.12 ± 0.02a</td>
<td>0.55 ± 0.43abc</td>
<td>0.13 ± 0.02a</td>
<td>0.63 ± 0.10a</td>
<td>1.46 ± 0.36a</td>
<td>0.05 ± 0.002a</td>
</tr>
<tr>
<td>Glu</td>
<td>2.23 ± 1.27abc</td>
<td>5.07 ± 0.62abc</td>
<td>2.83 ± 1.79abc</td>
<td>3.95 ± 0.81abc</td>
<td>9.05 ± 2.05abc</td>
<td>0.31 ± 0.02a</td>
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<tr>
<td>Gly</td>
<td>0.24 ± 0.08ab</td>
<td>0.60 ± 0.09abc</td>
<td>0.23 ± 0.05abc</td>
<td>0.44 ± 0.07abc</td>
<td>0.92 ± 0.23abc</td>
<td>0.03 ± 0.002a</td>
</tr>
<tr>
<td>Ala</td>
<td>0.44 ± 0.17abc</td>
<td>0.84 ± 0.13abc</td>
<td>0.70 ± 0.44abc</td>
<td>0.56 ± 0.14abc</td>
<td>1.39 ± 0.29abc</td>
<td>0.11 ± 0.005a</td>
</tr>
<tr>
<td>Cit</td>
<td>0.46 ± 0.17abc</td>
<td>0.58 ± 0.14abc</td>
<td>0.49 ± 0.17abc</td>
<td>0.64 ± 0.20abc</td>
<td>0.89 ± 0.09abc</td>
<td>0.04 ± 0.001a</td>
</tr>
<tr>
<td>Val</td>
<td>0.93 ± 0.34abc</td>
<td>1.92 ± 0.35abc</td>
<td>1.21 ± 0.42abc</td>
<td>1.20 ± 0.25abc</td>
<td>1.72 ± 0.63abc</td>
<td>0.12 ± 0.004a</td>
</tr>
<tr>
<td>Met</td>
<td>0.43 ± 0.14abc</td>
<td>0.97 ± 0.13abc</td>
<td>0.55 ± 0.12abc</td>
<td>0.66 ± 0.11abc</td>
<td>1.06 ± 0.29abc</td>
<td>0.03 ± 0.002a</td>
</tr>
<tr>
<td>Ile</td>
<td>0.57 ± 0.21abc</td>
<td>1.25 ± 0.27abc</td>
<td>0.74 ± 0.27abc</td>
<td>0.94 ± 0.18abc</td>
<td>1.19 ± 0.46abc</td>
<td>0.05 ± 0.002a</td>
</tr>
<tr>
<td>Leu</td>
<td>1.20 ± 0.41abc</td>
<td>2.66 ± 0.52abc</td>
<td>1.50 ± 0.61abc</td>
<td>1.74 ± 0.31abc</td>
<td>2.01 ± 0.56abc</td>
<td>0.20 ± 0.01abc</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.69 ± 0.19abc</td>
<td>1.44 ± 0.08abc</td>
<td>0.78 ± 0.31abc</td>
<td>1.28 ± 0.32abc</td>
<td>1.73 ± 0.50abc</td>
<td>0.10 ± 0.004a</td>
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<tr>
<td>Phe</td>
<td>0.82 ± 0.09ab</td>
<td>1.60 ± 0.05abc</td>
<td>1.00 ± 0.05abc</td>
<td>1.26 ± 0.27abc</td>
<td>1.70 ± 0.39abc</td>
<td>0.18 ± 0.005a</td>
</tr>
<tr>
<td>Ammonia</td>
<td>1.94 ± 0.14abc</td>
<td>2.07 ± 0.23abc</td>
<td>2.26 ± 0.26abc</td>
<td>2.32 ± 0.14abc</td>
<td>2.79 ± 0.25abc</td>
<td>0.43 ± 0.03abc</td>
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<tr>
<td>Orn</td>
<td>0.21 ± 0.05ab</td>
<td>0.37 ± 0.07abc</td>
<td>0.32 ± 0.15abc</td>
<td>0.40 ± 0.15abc</td>
<td>0.50 ± 0.008abc</td>
<td>0.10 ± 0.004a</td>
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<tr>
<td>Lys</td>
<td>2.03 ± 0.38abc</td>
<td>2.80 ± 0.83abc</td>
<td>2.56 ± 0.68abc</td>
<td>2.83 ± 0.84abc</td>
<td>4.13 ± 1.03abc</td>
<td>0.15 ± 0.009abc</td>
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<tr>
<td>His</td>
<td>0.38 ± 0.06abc</td>
<td>0.92 ± 0.27abc</td>
<td>0.42 ± 0.10abc</td>
<td>0.65 ± 0.10abc</td>
<td>0.97 ± 0.17abc</td>
<td>0.08 ± 0.003abc</td>
</tr>
<tr>
<td>Arg</td>
<td>0.11 ± 0.01abc</td>
<td>0.44 ± 0.17abc</td>
<td>0.11 ± 0.01abc</td>
<td>0.42 ± 0.02abc</td>
<td>0.85 ± 0.30abc</td>
<td>0.06 ± 0.002abc</td>
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<tr>
<td>Pro</td>
<td>0.68 ± 0.28abc</td>
<td>1.04 ± 0.52abc</td>
<td>0.91 ± 0.48abc</td>
<td>0.40 ± 0.05abc</td>
<td>0.57 ± 0.08abc</td>
<td>0.10 ± 0.02abc</td>
</tr>
</tbody>
</table>

Data are represented as mean ± standard deviation (SD) of three trials each with triplicate analysis. Different superscripts indicate significant differences (P < 0.05).
tongue analysis indicated that koji cheeses tend to have a more persistent umami than comparator cheese, and this characteristic is related to γ-glutamyl peptides produced by koji in cheeses.

Koji cheese exhibited less sourness than the comparator cheese. Related to this, the pH of koji cheeses was higher than that of the comparator cheese. As previously mentioned, the increased pH of cheese curd is related to ammonia production by the mold and the consumption of lactic acid (Karahadian and Lindsay, 1987). The lactic acid level was low in B and D cheeses and high in C, K, and 2041 cheeses. These results were in line with the higher pH values of B and D cheeses than of C, K, and 2041 cheeses. However, the pH was the lowest for comparator cheese samples despite having their lower lactic acid content relative to koji cheeses. Conversely, high ammonia production was observed in all the 5 koji cheeses. Ammonia accumulation might have contributed to the increased pH and week sourness ($R = 0.478$). In our previous report, koji strains exhibited similar or higher protease activity on the cheese surface as the Penicillium species used for Camembert cheese production, and ammonia levels displayed a strong positive correlation with protease activity (Suzuki et al., 2021).

In the results of PCA, koji cheeses were clearly separated from the comparator cheese. Among the Aspergillus strain-ripened cheeses, C cheese was relatively similar to Camembert cheese. In our previous report, strain C exhibited low lipase activity and low volatile short-chain fatty acid production compared with 4 other koji molds, P. candidum, and P. roqueforti on the surface of cheese (Suzuki et al., 2021). Furthermore, it has been reported that methyl ketones such as 2-nonanone and 2-heptanone hardly accumulated even when cheese was ripened for 30 d by strain C (Tomita et al., 2022). The methyl ketones are known as flavor components of blue mold cheeses (Patton, 1950) that are formed by the metabolism of ketoacyl-CoA, an intermediate product of the fatty acid β-oxidation cycle (Yan et al., 2020). These reports and the results of the present study suggest that cheese ripened with strain C has a similar taste as conventional Camembert cheese and a milder aroma than other koji cheeses.

Conversely, in PCA, the plots of B and D were similar, and those of K and 2041 were also similar. The utilization purpose was shared in each group (B and D are used for miso production, whereas K and 2041 are used for soy source production). Class separation of the 5 koji strains based on the volatile profile corresponded to their dedicated applications (Tomita et al., 2022). These results suggest that each strain has its own characteristic taste, and that the use of the strain, such as miso and soy sauce production, also affects the taste of the final cheese product. Koji strains have been

<table>
<thead>
<tr>
<th>Cheeses</th>
<th>Sourness</th>
<th>Astringency</th>
<th>Saltiness</th>
<th>Astringency</th>
<th>Umami</th>
<th>Astringency from umami</th>
<th>Umami richness</th>
<th>Aftertastes from bitterness</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>−8.16 ± 1.38ab</td>
<td>−0.05 ± 0.04ab</td>
<td>0.10 ± 0.04b</td>
<td>0.00 ± 0.10ab</td>
<td>5.87 ± 1.09c</td>
<td>11.20 ± 3.26c</td>
<td>0.93 ± 0.20a</td>
<td>11.20 ± 3.26bc</td>
</tr>
<tr>
<td>C</td>
<td>−4.54 ± 1.93c</td>
<td>0.39 ± 0.15b</td>
<td>0.77 ± 0.18b</td>
<td>0.36 ± 0.16b</td>
<td>0.36 ± 0.16b</td>
<td>0.00 ± 0.10b</td>
<td>0.00 ± 0.10b</td>
<td>5.87 ± 4.09a</td>
</tr>
<tr>
<td>D</td>
<td>−10.42 ± 0.71a</td>
<td>−0.43 ± 0.16b</td>
<td>1.65 ± 0.28b</td>
<td>0.36 ± 0.16b</td>
<td>−0.43 ± 0.16b</td>
<td>0.36 ± 0.16b</td>
<td>0.36 ± 0.16b</td>
<td>14.38 ± 1.69c</td>
</tr>
<tr>
<td>K</td>
<td>−5.01 ± 0.46c</td>
<td>7.61 ± 0.79c</td>
<td>2.59 ± 0.18c</td>
<td>0.36 ± 0.16b</td>
<td>−0.43 ± 0.16b</td>
<td>0.36 ± 0.16b</td>
<td>0.36 ± 0.16b</td>
<td>6.54 ± 0.77a</td>
</tr>
<tr>
<td>2041</td>
<td>−4.81 ± 1.12c</td>
<td>12.44 ± 0.58d</td>
<td>3.78 ± 0.22e</td>
<td>2.78 ± 0.96a</td>
<td>12.44 ± 0.58d</td>
<td>0.36 ± 0.16b</td>
<td>0.36 ± 0.16b</td>
<td>8.03 ± 2.74ab</td>
</tr>
<tr>
<td>Comparator</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
selected over a long period according to their growth on each of the grains used to make koji and the activities of enzymes such as lipases, proteases, and amylases. Although we were unable to elucidate the cause within this study, it is likely that these differences explain the different characteristics of cheeses ripened with various koji molds.

The findings highlight the possibility that surface-ripened cheeses can be produced using various koji molds, which could contribute to the development of new original cheeses in Japan.

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REFERENCES


