A study on the microbiological and biochemical changes in flavour compounds during ripening of Xinjiang specialty cheese

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Abstract

Xinjiang specialty cheese is produced by natural fermentation of milk. During fermentation, a complex succession of changes takes place in the milk, including pH decline, protein denaturalisation, whey discharge, and etc. The ripening process of Xinjiang specialty cheese was studied for 50 days. Microbial count showed that Lactobacillus helveticus was the dominant strain in the cheese during ripening. The contents of pH 4.6 soluble nitrogen and 12% TCA soluble nitrogen gradually increased during the ripening process of the cheese. An electrophoretogram showed that the degree of protein degradation was high after 30 days of ripening. The contents of total organic acids and free fatty acids were determined by high performance liquid chromatography (HPLC). The results showed that the contents of total organic acids and free fatty acids in the flavour compounds increased significantly (p < 0.01). Twenty-five compounds were detected by gas chromatography-mass spectrometry (GC-MS), mainly alkanes, ketones, alcohols, esters, aldehydes, and acids. The sensory evaluation scores increased with ripening, and reached the highest value after 50 days.

Keywords

Xinjiang specialty cheese, microbiological changes, biochemical changes, flavour compounds

Introduction

Xinjiang specialty cheese is one of the most important foods consumed by Kazak nomads in Xinjiang Uygur Autonomous Region in north-western China; because of its dainty nature, special flavour, low cost, and high productivity (Ma et al., 2012). Xinjiang specialty cheese is distinctive, high in nutrients, and considered a good staple food. Xinjiang specialty cheese is often a dry cheese with rough skin. The texture can be hard, semi-hard, or soft, with a sour or sweet taste (Wang et al., 2019). The sweet type is made of milk fat with high aroma, while the sour type is less fatty and fragrant, but still milky. Sour cheeses are commonly used to make local flour food, such as noodles and bread. Xinjiang specialty cheese has a high-acid, sharp milky flavour, and is thicker and darker than ordinary cheese (Azat et al., 2016). During the production process, the protein and fat contents of raw milk were enriched 10 times (Gobbetti et al., 2018).

Lactic acid is produced through sugar fermentation by lactic acid bacteria. Some of the lactic acid is transferred to the whey thus alleviating lactose intolerance. By sugar fermentation, protein allergic reaction can also be avoided (Fox et al., 2017). In addition, a portion of the protein is broken down into nutrients, such as polypeptides and amino acids, which are favourable for assimilation (Garcia et al., 2019). Xinjiang specialty cheese also contains a variety of vitamins, mainly vitamin A, followed by vitamin B and vitamin A source substances, which can protect eyesight and prevent skin diseases (Gobbetti et al., 2018). After fermentation, milk can decompose the macromolecular substances into small molecular compounds that are easily absorbed by the human body, especially as the degradation rate of protein can reach about 97% (Lee et al., 2016). The unsaturated fatty acids generated by decomposition can prevent diseases such as hypertension and cardiovascular diseases (Lee et al., 2016).

A great deal of research on cheese flavour from different kinds of cheese have been conducted (Pionnier et al., 2002; Lawlor et al., 2003; Taborda et al., 2008; Bergamaschi and Bittante, 2018; Del Toro-Gipson et al., 2020). Cheese flavour is believed to result from a balance between a number of components released by enzymic reactions (Delahunty and Piggott, 2010). The characteristics of the flavour profile of ripened cheeses are mainly affected by proteolysis of casein, and in some types, by lipolysis (Ayad et al., 2004). Some research has been carried out in this regard. However, only a few studies have focused on the reasons for the different flavours (Ballesteros et al., 2006; Ahmadi et al., 2013;
Cheese ripening is a complex microbiological and enzymatic process, characterised by the production of compounds that lend the cheese a certain aroma and texture characteristics (James et al., 2013). The potential products of amino acid metabolism, including organic acids and volatile compounds, significantly contribute to cheese flavour (Kieronczyk et al., 2001).

In recent years, research on cheese has increased. A number of differences exist between most enzyme cream cheese and Xinjiang specialty cheese. In the present work, the microbial, physical, chemical, and flavour changes of Xinjiang specialty cheese were systematically studied. The main microbial flora, fatty acid change, and volatile flavour constituent of ripened Xinjiang specialty cheese were determined. The present work also provides some important information on Xinjiang specialty cheese.

Materials and methods

Cheese production

Raw milk (cow milk) was pasteurised at 80 - 85°C for 2 - 3 s or 65°C for 30 min. After the milk has been cooled to 32°C, the starter culture containing Lactobacillus helveticus and L. lactis were added at a level of 4 g/100 g. At 37°C and pH 4.4, the curd was cut into cubes. The curd and whey mixture were left standing for a few minutes. Subsequently, pressure was applied at 0.3 MPa for 24 h, or until whey drainage stopped, or decreased to a low level. The curd was then cut into blocks and cooked (60°C). Later on, the cheese was packaged and transferred to the constant temperature and humidity cabinet maintained at 10°C and 80% RH. The cheese was selected at random when ripened for 0, 10, 20, 30, 40, and 50 days. Each sample was made up of one whole cheese. The cheese was taken to the laboratory under refrigeration, below 5°C, and analysed upon arrival.

Microbiological analysis

Colony counting

Using aseptic techniques, 25 g of each sample (two replicates) were homogenised in 225 mL of sterile peptone saline (1 g of peptone and 9 g of NaCl per litre water). After shaking at 230 rpm for 10 min with a stomacher, this suspension was serially diluted in triplicate (1:10) in peptone saline, and 1 mL dilutions were inoculated onto Plate Count Agar (PCA agar, LuQiao Company, Beijing, China) to obtain the total aerobic count, and onto de Man Rogosa Sharpe agar (MRS, Oxoid, Britain) for the determination of lactic acid bacteria. The plates were then incubated for 48 h at, 37 and 30°C, respectively. The colonies developing on the plates were counted. Escherichia Coli on MacConkey Agar (MAC, LuQiao Company, Beijing, China) was incubated at 37°C for 48 h. Yeasts on Potato Dextrose Agar (PDA agar, LuQiao Company, Beijing, China) was incubated at 28°C for 3 d.

Proteolysis analyses

Soluble nitrogen fractions

The pH4.6-soluble and 12% TCA-SN extracts of the cheeses were prepared following the method of Kuchroo and Fox (1982) with slight modification, as outlined by Hayaloglu et al. (2004). The extracts were determined by the Kjeldahl method (AOAC, 1995).

SDS-PAGE

Cheese samples were grated and homogenised with 10 mL of pH 4.6 acetate buffer solution. The mixtures were centrifuged, and the supernatants were removed. The precipitate was added to a treatment solution (4:1), which contained 50 mM of Tris-HCl sample buffer (pH 6.8), 5% β-mercaptoethanol (w/v), 2% SDS (w/v), 0.1% bromophenol blue (w/v), and distilled water. The solution was heated at 95°C for 4 min (Laemmli, 1970).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by using a consort mini gel electrophoresis unit (DYCZ, Liuyi Instrument Factory, Beijing, China). 15% separation gel and 5% stacking gel were prepared for SDS-PAGE. The gels were run at a constant voltage of 80 V for 3 - 4 h. The gels were stained with Coomassie Blue G-250 (0.1%, w/v) in a mixture of water, methanol (40%, w/v), and acetic acid (10%, w/v) for 30 min and destained in a mixture of water, methanol (10%, w/v), and acetic acid (10%, w/v).

Densitometric evaluation of electrophoretograms was performed by using a system of electrophoresis gel imaging. The positions of the casein fractions were identified based on the ratio to the marker.

Determination of flavour compounds

Assessment of organic acids

All cheese samples were taken from each refrigerated aging period at -20°C. 0.5 g of samples and 8 mL of 0.01 mol/L H$_2$SO$_4$ were placed into 10 mL volumetric flask, extracted for 30 s on a shaker, and then H$_2$SO$_4$ was added until it reached the mark. The extracted organic acid sample was centrifuged at 8,000 g for 10 min. The supernatant was filtered through a 0.45 µm membrane filter.

Organic acids were analysed using Shimadzu Chromatography (LC-2010). Operating conditions were mobile phase, methanol:NaH$_2$PO$_4$ (0.01 mol/L,
pH 2.6) = 3.97 (v/v). The column used was C18 (5 µm, 4.6 × 250 mm); the column flow rate was 0.8 mL/min at 40°C; and the injection volume was 10 µL. Diode array detector was set at 210 nm. Quantification was carried out based on external standard method.

Free fatty acids
Cheese lipids were extracted following the Rose-Gottlieb method (Ng-Kwai-Hang et al., 1998). A Shimadzu GC-C14 gas chromatograph equipped with a flame ionisation detector (FID) were used: a capillary column DM-Wax (30 m × 0.25 mm × 0.25 µm). The column temperature was kept at 50°C for 1 min, increased at 25°C/min to 200°C, increased at 3°C/min to 240°C, and then were kept for 18 min; injector temperature was 250°C; detector temperature was 280°C; injection volume was 0.1 µL; carrier gas was N₂. Calibration curves were prepared using a mixture of 37 FFA standards, purity > 99%. All determinations were performed in triplicate.

Analysis of volatile flavour compounds
SPME extraction
Briefly, 6 g of cheese was ground, then added into a 10 mL sample vial and sealed. At the first sample, SPME extraction fibre (75 µm CAR/PDMS, Supelco Company, America) needed staling for 2 h at the injection port (250°C) and latter for 0.5 h. The SPME extraction fibre was inserted into the headspace of the sample container. Ultrasonic extraction was used for 40 min at 40°C. After the extraction step, the analytes were thermally desorbed from the fibre into an injector port operating at 250°C in the splitless mode for 2 min. Each sample was analysed twice.

GC-MS analysis
Thermo Trace GC Ultra gas chromatograph and Thermo DSQII mass were used for the separation and identification of the analytes. Chromatographic separation was performed on a TR-5-MS (30 m × 0.25 mm × 0.25 µm) with the following temperature program: 33°C for 3 min, increased to 42°C at a rate of 10°C/min, increased to 140°C at a rate of 5°C/min, increased to 240°C at a rate of 18°C/min, and held for 8 min. Helium was used as the carrier gas at a constant flow of 0.8 ml/min. MS detector was programmed as follows: EI electron energy of 70 eV; ion source, transfer line temperature were set to 200 and 250°C; ionisation model was EI+; emission current was 200 µA; and mass scan range: 43–500 amu.

Each compound was identified using its mass spectral data (NIST library), and each content of the compound was calculated by area normalisation method.

Sensory evaluation
Sensory evaluation of Xinjiang specialty cheese samples during ripening was carried out at 0, 10, 20, 30, 40, and 50 days using Scoring method. The organoleptic assessment was performed by a trained sensory panel composed of ten members, consisting of five females and five males. Xinjiang specialty cheese samples were cut into small pieces (1 × 1 × 1 cm) and placed on a plate. Two whole slices from each Xinjiang characteristics cheeses were distributed simultaneously and anonymously to all panel members. Appearance (colour), texture (firmness), and flavour intensity (taste) were scored on a scale from 0 (absent) to 5 (high) (Jéssica et al., 2020).

Statistical analysis
In the present work, analyses of the samples were duplicated. All statistical calculations were performed using Origin7.5 Statistical Software. Significance was evaluated using analysis of SPSS for repeated measurements, followed by Duncan’s multiple range tests. Values of p < 0.05 were considered to be significant.

Results and discussion
Microbiological analysis
Colony counting
Cheese could be considered as an ecosystem in continuous change, in which some species are succeeded by the other, depending on the physicochemical conditions, which are modified by their own microbial metabolism. High densities of microorganisms are present in cheese throughout ripening, and they play a significant role during the ripening process (Coda et al., 2006). Among the microorganisms, interactive associations are being formed, such as that resulting from the degradation of proteins and carbohydrates by some species to produce simple substrates, and the production of vitamins as well as the growing factors which assist the growth of other species (Boddy and Wimpenny, 2010).

The evolutions of the different microbial group count throughout the ripening of Xinjiang specialty cheese are shown in Figure 1. The total number of bacteria increased rapidly in the first ten days, but later decreased dramatically (Figure 1a). The counts of bacteria remained stable during the last ripening period, and the change trend of the total
number of bacteria was the same with lactic acid bacteria.

Lactic acid bacteria were the predominant microflora during the ripening of Xinjiang specialty cheese (Figure 1b). During the initial period of ripening, the activity of the starter culture was strong, and the metabolism increased, so the total number of lactic acid bacteria increased rapidly. However, during the middle-later period of ripening, the metabolism of lactic acid bacteria decreased. In addition, the low pH value inhibited the growth and autolysis of bacteria, which led to a decrease in the number of lactic acid bacteria.

The total number of *E. coli* decreased dramatically in the first ten days of ripening (Figure 1c), as the growth of lactic acid bacteria led to a low pH value and inhibited its growth. With the ripening of cheese, the number of *E. coli* decreased gradually. Twenty days later, it was not detected in the cheese.

The number of yeasts in the cheese increases during the whole ripening time (Figure 1d). Cheese during production will harbour natural yeasts from the environment; with a large number of lactic acid bacteria growths, sour curd cheese will have low pH, and this condition is suitable for yeast growth, and the number of yeasts increases throughout the maturity time.

**Proteolysis**

The changes in the proteolysis parameters obtained throughout the ripening of Xinjiang specialty cheese are shown in Figure 2.

Figure 2a shows the values of the pH 4.6-soluble nitrogen at the beginning and end of ripening. The content of pH 4.6-soluble nitrogen increased significantly throughout ripening (*p* < 0.05), because protein was hydrolysed to small and medium peptides by the protease. During the initial period of ripening, the content of pH 4.6-soluble nitrogen increased slightly, and then the activity of protease increased causing increased proteolysis, which led to the rise in pH values. These reasons contributed to the increase in pH 4.6-soluble nitrogen. A similar trend was observed in study on the Prato cheese (Gorostiza *et al.*, 2004; Merheb-Dini *et al.*, 2012; 2016).

12% TCA-soluble nitrogen is composed of small peptides and free amino acids. They are produced by the proteases and peptidases of microbial origin or rennet. It has been found that 12% TCA-soluble nitrogen include many volatile flavour compounds, and that their contents directly influence
Since the curd of Xinjiang specialty cheese depend on natural fermentation, no rennet was added, thus the 12% TCA-soluble nitrogen obviously came from the functions of proteases and peptidases, which were produced by the starter culture. Figure 2b shows the values of the 12% TCA-soluble nitrogen at the beginning and end of ripening. The content of 12% TCA-soluble nitrogen increased significantly throughout ripening ($p < 0.05$). In the previous period, it increased slightly; however, because of the production of small peptides and amino acids by the proteases and peptidases that were released as a result of the death of most lactic acid bacteria in the latter ripening period, the 12% TCA-soluble nitrogen then increased rapidly.

The pH 4.6-soluble nitrogen represents the length of proteolysis of a cheese sample, while the 12% TCA-soluble nitrogen indicates the depth to which proteolysis of a cheese takes place. Ripening also increased pH 4.6-soluble nitrogen and 12% TCA-soluble nitrogen, but the former was higher than the latter. During the initial period, the rate of pH 4.6 soluble nitrogen increase was faster than that of 12% TCA-soluble nitrogen, but their rates of increase were the same in the latter period. The reason for this is as follows: at the beginning of ripening, protein was decomposed into amount of peptides by the proteases. However, the ability of the proteases was decreased in the latter period, and the peptides were decomposed into small peptides and amino acids by the peptidases at the same time, i.e., the degradation of peptides were more obvious in the latter ripening period; in addition, it contributed to the improved flavour of the cheese.

Figure 2c shows an electrophoretogram of Xinjiang specialty cheese during ripening. The breakdown patterns of α-CN and β-CN were similar in all batches of cheeses, and α-CN was hydrolysed more extensively than β-CN during ripening. During ripening, casein was decomposed into small peptides, and more bands were found in the electrophoretogram. There were significant changes in the casein during the 50 days of ripening. At day 10, the band of α-CN was darker than that of β-CN, and there were a few bands in the whole lane. This showed that hydrolysis of casein was not active, and protein remained in the form of large fragment polypeptides. From day 20 to 30, polypeptides were hydrolysed into small peptides; so, many bands could be found. From day 40 to 50, both α-CN and β-CN were hydrolysed strongly, and the small peptides increased. In addition, the greater hydrolysis of α-CN than β-CN was found to be similar with the report...
(Gorostiza et al., 2004; Merheb-Dini et al., 2012; 2016). Both α-CN and β-CN were not completely hydrolysed at the end of ripening.

**Determination of flavour compounds**

**Assessment of organic acids**

The changes in the organic acids’ parameters obtained throughout the ripening of Xinjiang specialty cheese are shown in Table 1. The organic acids contribute to the flavour and aroma of most cheese varieties. A suitable content can endow the cheese with mellow sour taste. The total organic acid content and each organic acid content of the cheese increased very significantly from day 10 to day 50 ($p < 0.01$). The total organic acid content reached 15.51 mg/g at day 50 from 7.84 mg/g at day 10.

The formation of lactic acid is essential for proper production, flavour development, normal ripening, and good storage quality of cheese. Lactic acid is the major product of sugar fermentation by lactic acid bacteria. Its content varies throughout the ripening period. In the present work, it was the most abundant organic acid found in Xinjiang specialty cheese, with a mean content of 7.99 mg/g of cheese. Some reports have shown that the content of lactic acid ranged from 1.94 to 17.4 mg/g in different cheeses. The result of the lactic acid content of Xinjiang specialty cheese in the present work falls within this range.

Malic acid increased in concentration as the cheeses were ageing. Fifty days after the cheeses were made, they had the highest concentration of malic acid, which was 2.94 mg/g. Acetic acid is another product of sugar fermentation by lactic acid bacteria or by the metabolism of citric acid, lactic acid, and amino acid. Some researchers had reported that the acetic acid content ranged from 0.13 to 2.96 mg/g in Cheddar and Provolone cheeses, as well as in other varieties. Table 1 shows that the maximum amount of acetic acid was 0.95 mg/g, which was in the range of the reports.

The content of citric acid was 0.87 mg/g after 50 days of ripening, which was the lowest amount of all organic acids detected. The reason for the low amount could be attributed to the metabolism of citric acid by lactic acid bacteria into flavour components, such as acetic acid, acetaldehyde, and diacetyl (Murtaza et al., 2017).

**Assessment of free fatty acid**

The changes in the free fatty acids’ parameters obtained throughout ripening of Xinjiang specialty cheese are shown in Table 2. The free fatty acids have important function related to the basic taste. The total content of free fatty acids increased significantly ($p < 0.01$); at 50 days, the content was 236.16 mg/100 g. The values are higher than those found in other cheeses, for example Babia-Laciana (Franco et al., 2003), but Wit et al. (2005) obtained similar values. It is known that some of the main factors that contribute to free fatty acid content in cheese are the kind and quality of milk, its heat-treatment, the lactic acid starters used, ripening and storage temperature, brine concentration, milk lipase (if raw milk is used), and lipases found in rennet. The result may be supported by these reasons: 1) The lipoprotein lipase of goat milk is distributed primarily in cream and in milk serum, and only a small part is associated with casein micelles, which are incorporated into cheese. 2) The interactions of caseins with indigenous lipase in cow milk are higher than in goat milk. In addition, some research has shown that cheese stored at 10–20°C had a higher degree of lipolysis than that stored at 5°C. The Xinjiang specialty cheese and the cheese studied by Wit et al. (2005) were made from cow milk, and stored at 10°C, but Babia-Laciana cheese was made from goat milk, and stored at 5°C. Therefore, the content of free fatty acid in Xinjiang specialty cheese was higher than that in Babia-Laciana cheese.

With respect to the main fatty acids, $C_{18:1}$, $C_{16:0}$ and $C_{14:0}$ had the highest content, but they had no effect on the flavour of cheese. Regarding the individual free fatty acid, not all of them changed at the same rate. $C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$ and $C_{12:0}$ changed very significantly ($p < 0.01$); $C_{14:0}$, $C_{16:0}$ and $C_{18:1}$, and

### Table 1. The contents of organic acids in Xinjiang specialty cheese during ripening (mg/g).

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<tr>
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<th>10 days</th>
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<th>40 days</th>
<th>50 days</th>
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</thead>
<tbody>
<tr>
<td>Malic acid</td>
<td>1.48 ± 0.06a</td>
<td>2.03 ± 0.02b</td>
<td>2.14 ± 0.05b</td>
<td>2.40 ± 0.01c</td>
<td>2.94 ± 0.04d</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>6.06 ± 1.15a</td>
<td>7.51 ± 0.10b</td>
<td>7.68 ± 0.05b</td>
<td>7.97 ± 0.06b</td>
<td>10.75 ± 0.08c</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.26 ± 0.06a</td>
<td>0.36 ± 0.01b</td>
<td>0.45 ± 0.03c</td>
<td>0.83 ± 0.01d</td>
<td>0.95 ± 0.06c</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.04 ± 0.01a</td>
<td>0.10 ± 0.01b</td>
<td>0.15 ± 0.02c</td>
<td>0.65 ± 0.03d</td>
<td>0.87 ± 0.04c</td>
</tr>
<tr>
<td><strong>ΣOA</strong></td>
<td>7.84 ± 1.01a</td>
<td>10.00 ± 0.13b</td>
<td>10.41 ± 0.05b</td>
<td>11.85 ± 0.05c</td>
<td>15.51 ± 0.10d</td>
</tr>
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</table>

Means with different letters within the same row differed significantly ($p < 0.05$).
C18:3 had notable changes ($p < 0.05$); only C18:2 changed insignificantly. Wit et al. (2005) also observed that C18:2 experienced no significant change. Short chain fatty acids have an important role on the formation of special aroma. Table 3 shows that the content of short chain fatty acids changed significantly during ripening. C4:0 was the main short chain fatty acid, and this was similarly reported by Poveda and Cabezas (2006). C14:0 was the main medium chain fatty acid. The increase in short and medium chain fatty acids contributes to the digestion of the cheese in humans. The nutrition value of the cheese improved obviously with the ripening.

Assessment of volatile flavour compounds

The changes in the volatile flavour compounds’ parameters obtained throughout the ripening of Xinjiang specialty cheese are shown in Table 3. In total, 25 compounds were detected, including mainly alkanes, ketones, alcohols, esters, aldehydes, and acids.

Alkanes

Fourteen alkanes were identified. Heptane and 5-ethyl-2,2,3-trimethyl heptane had the highest value. The threshold values of alkanes are higher; they had a little effect on the flavour of cheese, but this effect is not exhibited by branched chain alkanes and olefins. The types of alkanes increased with the ripening of cheese, and the content of alkanes reached the highest values at 50 days (21.5%).

Ketones

Ketones are usually reported as one of the main fractions of volatile compounds of cheese (Gursoy et al., 2018). They have very particular odours and low perception thresholds (Sonmezdag, 2019; McSweeney and Sousa, 2000). Methylketone is related to the lipolytic activity of microflora in cheeses. The ketones played an important role in the ripening of cheese, and most of them formed flowery and fruity flavour. During the initial 20 days of ripening, no ketone was detected. Only 2-nonanone was detected at 30 days, and reached the highest value at 50 days (3.46%, w/w). The type and content of ketone in the present work were less than those reported by other researchers (Coda et al., 2006; Bertuzzi et al., 2018).

Alcohols

The content of 1-butanol increased from 0.71% at day 10 (w/w) to 1.64% at day 20, but disappeared thereafter. Regarding branch chain alcohols, 3-methyl-1-butanol is an important volatile compound in cheese, and it is produced from leucine catabolism by Strecker degradation (Katayama et al., 2017). This compound provides the pleasant aroma of fresh cheese (Bertuzzi et al., 2018; Picon et al., 2019). It was found at 50 days, and the content was 0.16%.

Aldehydes

Benzaldehyde, 3-methyl-butanal, and pentanal were identified in the cheese. Benzaldehyde was the predominant aldehyde. Its final content was 8.41%. Pentanal was identified at 40 days, and a content of 0.79% was found; however, it disappeared by the end of ripening. 3-methyl-butanal was detected at 30 days, and increased with the ripening; at 50 days, its content reached 1.12%. The aldehydes were mostly related to the conversion of acids and

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<tbody>
<tr>
<td>C4:0</td>
<td>13.47 ± 0.74a</td>
<td>14.59 ± 0.45a</td>
<td>19.22 ± 1.42b</td>
<td>20.04 ± 0.33bc</td>
<td>21.42 ± 1.45c</td>
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<tr>
<td>C6:0</td>
<td>10.63 ± 0.38a</td>
<td>11.49 ± 0.19b</td>
<td>14.96 ± 0.67b</td>
<td>15.51 ± 0.23b</td>
<td>14.52 ± 1.00b</td>
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<tr>
<td>C8:0</td>
<td>9.71 ± 0.39a</td>
<td>10.32 ± 0.17b</td>
<td>12.88 ± 0.19b</td>
<td>13.23 ± 0.10b</td>
<td>19.72 ± 2.69c</td>
</tr>
<tr>
<td>C10:0</td>
<td>9.35 ± 0.45a</td>
<td>10.30 ± 0.48a</td>
<td>12.69 ± 0.10c</td>
<td>13.19 ± 0.32c</td>
<td>12.51 ± 0.75c</td>
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<tr>
<td>C12:0</td>
<td>10.36 ± 0.52a</td>
<td>10.77 ± 0.32a</td>
<td>14.02 ± 0.18b</td>
<td>14.50 ± 0.40bc</td>
<td>16.20 ± 1.96c</td>
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<tr>
<td>C14:0</td>
<td>14.29 ± 0.70a</td>
<td>14.16 ± 0.43a</td>
<td>19.62 ± 0.63a</td>
<td>21.73 ± 0.99a</td>
<td>32.53 ± 9.15b</td>
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<tr>
<td>C16:0</td>
<td>19.36 ± 1.17a</td>
<td>19.02 ± 1.01a</td>
<td>26.71 ± 0.26a</td>
<td>30.92 ± 2.87a</td>
<td>41.00 ± 9.85b</td>
</tr>
<tr>
<td>C18:1</td>
<td>21.31 ± 2.07a</td>
<td>23.51 ± 2.32a</td>
<td>30.20 ± 1.82ab</td>
<td>37.17 ± 2.14bc</td>
<td>60.22 ± 20.71c</td>
</tr>
<tr>
<td>C18:2</td>
<td>16.09 ± 4.96a</td>
<td>15.57 ± 5.45a</td>
<td>20.82 ± 6.11a</td>
<td>22.90 ± 7.31a</td>
<td>13.59 ± 1.52a</td>
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<tr>
<td>C18:3</td>
<td>14.79 ± 0.32a</td>
<td>16.06 ± 0.98a</td>
<td>21.43 ± 0.73a</td>
<td>18.67 ± 0.48b</td>
<td>14.46 ± 1.53a</td>
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<tr>
<td>∑FFA</td>
<td>139.35 ± 7.24a</td>
<td>145.79 ± 5.29a</td>
<td>192.70 ± 1.34b</td>
<td>207.86 ± 10.74bc</td>
<td>236.16 ± 37.38c</td>
</tr>
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</table>

Means with different letters within the same row differed significantly ($p < 0.05$).
<table>
<thead>
<tr>
<th>Compound</th>
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Table 3. Volatile flavour compounds in Xinjiang specialty cheese during ripening.
esters. Branch chain aldehydes, such as 3-methyl-butanal, originated from isoleucine and leucine, by Strecker degradation (McSweeney and Sousa, 2000; Katayama et al., 2017).

**Acids**

Eleven acids were identified, mainly saturated fatty acids of even number of carbon atoms from C₂ to C₁₀ as well as branch chain acids. The main ones were pentanoic acid and hexanoic acid. Acids made up the largest group of aroma compounds, ranging from 60.22 to 88.39% (w/w), and played an important role in the aroma development of aged cheeses. During the initial period of ripening, the acids were mainly formed by the lactic acid bacteria, while during the latter period of ripening, they were mainly formed by the starter culture and part of the nonstarter culture bacteria. Though it is considered that acids have little effect on the flavour of cheese, some acids were of advantage to the release of flavour compounds, such as butanoic acid and hexanoic acid.

**Esters**

Esters are common important constituents of the volatile fraction of cheese. Different esters have been reported, such as methyl, ethyl, propyl, and butyl esters, as a product of the reaction of free fatty acid with ethanol, methanol, propanol, and butanol in different cheese varieties (Hong et al., 2018). Ester formation is correlated with the growth of lactic acid bacteria (Bezerra et al., 2017; Thierry et al., 2017). They contribute in a synergistic way to the fruity aroma of cheese since they have a low perception threshold concentration, which is 10-fold lower than their alcohol precursors (Preininger and Grosch, 1994). In Xinjiang specialty cheese, nine esters were identified. Ester concentration represented 1.87% of the total volatile compound concentration during cheese ripening. Ethyl octanoic was the predominant ester.

**Sensory evaluation**

The sensory value of the cheese increased with ripening (Figure 2d). Temperature and relative air humidity may interfere in both microbial growth and sensory characteristics (Vale et al., 2018). The sensorial dimension included the categories of texture, flavour/taste, aroma, and appearance. The appearance of the cheese is white or light yellow, which attracts consumers. The cheese was described as having a soft, creamy texture, with a mild flavour, ammonia/milk aroma, and a velvety appearance. These characteristics were highly cited, suggesting the importance of these attributes because consumers expect standardised manufacturing and products that always have the same characteristics (Judacewski et al., 2019; Jéssica et al., 2020).

The lactic acid bacteria contributed to the fermentation of lactose, and many enzymes were released after their death, which could accelerate proteolysis and lipolysis. This led to the production of the flavour compounds of the cheese. During the initial period of ripening, the growth of lactic acid bacteria was vigorous. The flavour compounds could only be made by citrate metabolism, but with the autolysis of lactic acid bacteria, a large number of peptidases and lipases were released. They decomposed the protein and fats to flavour compounds, which formed the better flavour of the cheese.

**Conclusions**

The total bacteria had a similar trend to lactic acid bacteria. Lactic acid bacteria were the predominant flora during ripening of Xinjiang specialty cheese. Escherichia coli disappeared completely, and fungi was below 200 CFU/g. Both pH4.6-SN and 12% TCA-SN changed significantly during the latter period of ripening. The content of pH4.6-SN was higher than that of 12% TCA-SN during the whole ripening period. Protein gradually hydrolysed with the ripening of cheese, but had a higher degree at 30 days. The contents of total organic acids and the individual organic acids experienced significant changes. Lactic acid was the dominant organic acid. The total content of free fatty acids increased; short-chain fatty acids and semi-chain fatty acids also increased and were beneficial to digestion. The volatile compounds included mainly alkanes, ketones, alcohols, esters, aldehydes, and organic acids. They were characterised by a decrease in acid compounds and an increase in alkanes. Nutritional value was at the highest value after 50 days of Xinjiang specialty cheese.

**Acknowledgement**

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**References**

characteristics of Iranian drink based on fermented milk (Doogh). Journal Food Processing Technology 4: article no. 271.


Del Toro-Gipson, R. S., Rizzo, P. V., Hanson, D. J. and Drake, M. A. 2020. Sensory characterization of specific wood smoke aromas and their contributions to smoked cheddar cheese flavor. Journal of Sensory Studies 35(3): article ID e12564.


Hong, Q., Liu, X. M., Hang, F., Zhao, J. X., Zhang, H. and Chen, W. 2018. Screening of adjunct cultures and their application in ester formation...
in Camembert-type cheese. Food Microbiology 70(8): 33-41.


Preininger, M. and Grosch, W. 1994. Evaluation of key odorants of the neutral volatiles of Emmentaler cheese by the calculation of odour activity values. LWT - Food Science and Technology 27(3): 237-244.


Manchego cheese as influenced by the water-soluble extract compounds. European Food Research and Technology 227(2): 323-330.


