

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

^{1,2}Li, Y. H., ^{1,2*}Wang, J. G. and ^{1,2}Liu C. J.

¹Xinjiang Academy of Agricultural and Reclamation Science, 832000, Xinjiang, People's Republic of China

²Key Laboratory of Agro-Products Processing, Xinjiang Academy of Agricultural and Reclamation Science, 832000, Xinjiang, People's Republic of China

Article history

Received: 22 April 2020

Received in revised form:

15 July 2020

Accepted:

1 October 2020

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.

© All Rights Reserved

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 (García *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;

*Corresponding author.
Email: wjgang728@126.com

Dugat-Bony *et al.*, 2019).

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 pH 4.6-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 H₂SO₄ were placed into 10 mL volumetric flask, extracted for 30 s on a shaker, and then H₂SO₄ 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 μ m membrane filter.

Organic acids were analysed using Shimadzu Chromatography (LC-2010). Operating conditions were mobile phase, methanol:NaH₂PO₄ (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

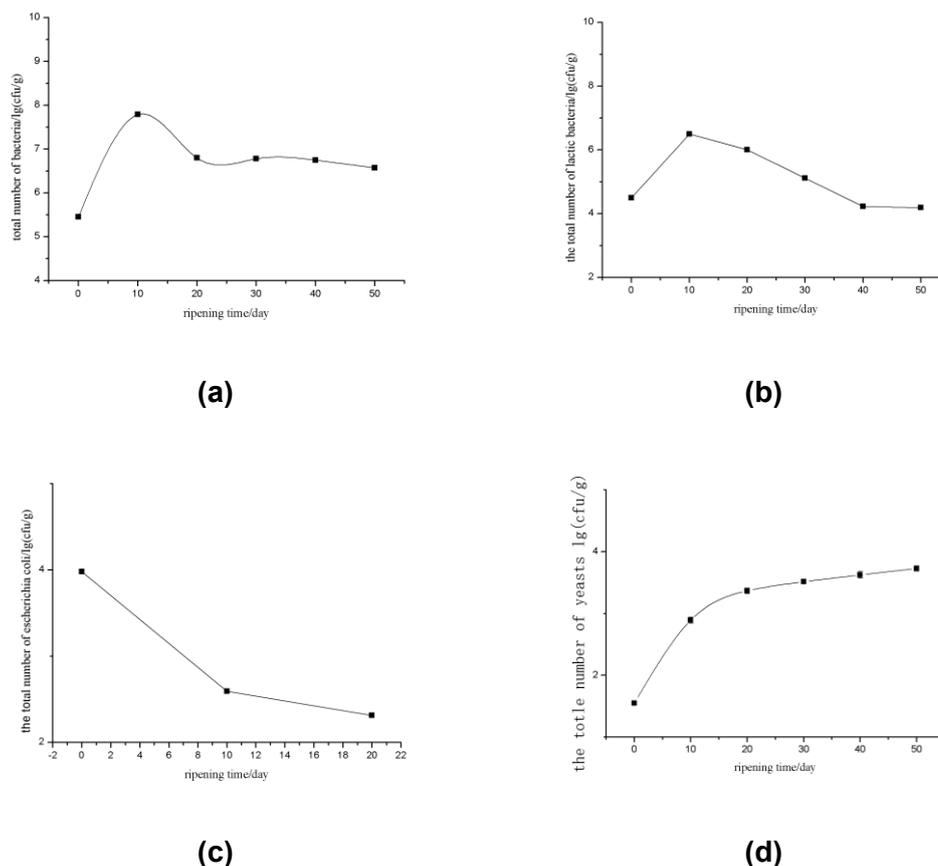


Figure 1. The change in the total number of bacteria (a), lactic acid bacteria (b), *Escherichia coli* (c), and yeasts (d) in Xinjiang specialty cheese during ripening.

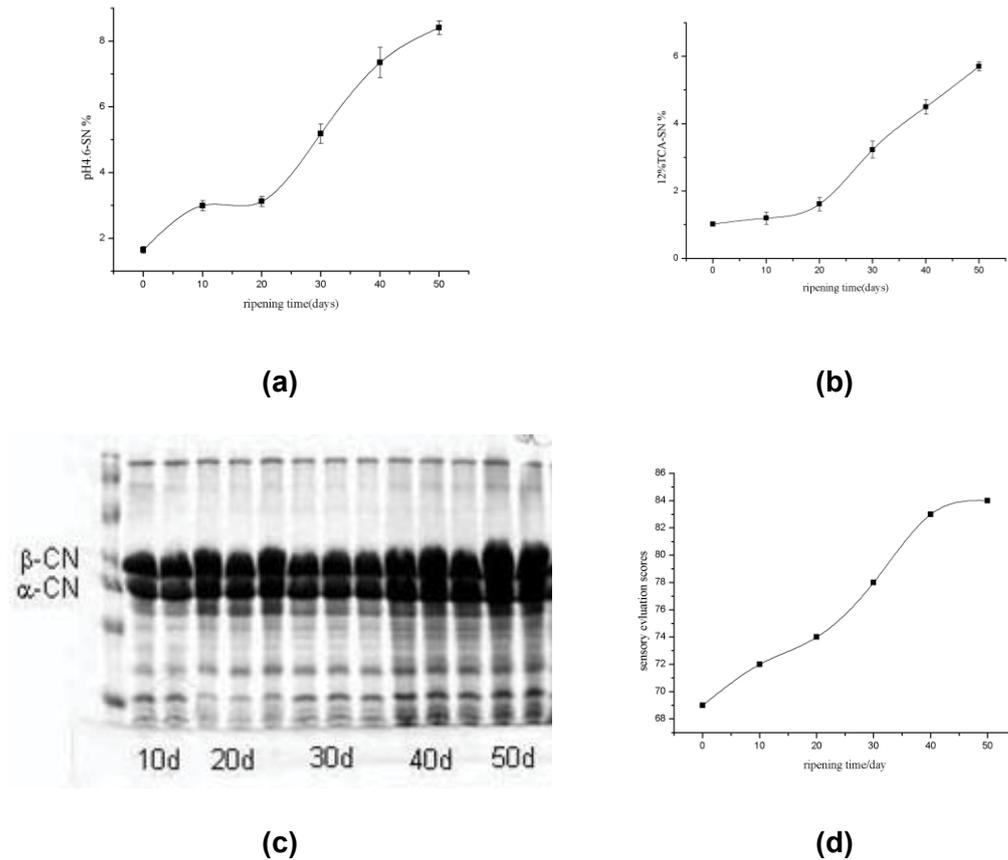


Figure 2. Changes in pH 4.6-SN (a), 12% TCA-SN (b), electrophoretogram of SDS-PAGE (c), and sensory evaluation scores (d) in Xinjiang specialty cheese during ripening.

the flavour of cheese (Whetstine *et al.*, 2007). 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 case in 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 ($p < 0.01$); $C_{14:0}$, $C_{16:0}$, $C_{18:1}$, and

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

	10 days	20 days	30 days	40 days	50 days
Malic acid	1.48 ± 6.06 ^a	2.03 ± 0.02 ^b	2.14 ± 0.05 ^b	2.40 ± 0.01 ^c	2.94 ± 0.04 ^d
Lactic acid	6.06 ± 1.15 ^a	7.51 ± 0.10 ^b	7.68 ± 0.05 ^b	7.97 ± 0.06 ^b	10.75 ± 0.08 ^c
Acetic acid	0.26 ± 0.06 ^a	0.36 ± 0.01 ^b	0.45 ± 0.03 ^c	0.83 ± 0.01 ^d	0.95 ± 0.06 ^e
Citric acid	0.04 ± 0.01 ^a	0.10 ± 0.01 ^b	0.15 ± 0.02 ^c	0.65 ± 0.03 ^d	0.87 ± 0.04 ^e
ΣOA	7.84 ± 1.01 ^a	10.00 ± 0.13 ^b	10.41 ± 0.05 ^b	11.85 ± 0.05 ^c	15.51 ± 0.10 ^d

Means with different letters within the same row differed significantly ($p < 0.05$).

Table 2. The contents of free fatty acids in Xinjiang specialty cheese during ripening (mg/100 g).

	10 days	20 days	30 days	40 days	50 days
C _{4:0}	13.47 ± 0.74 ^a	14.59 ± 0.45 ^a	19.22 ± 1.42 ^b	20.04 ± 0.33 ^{bc}	21.42 ± 1.45 ^c
C _{6:0}	10.63 ± 0.38 ^a	11.49 ± 0.19 ^a	14.96 ± 0.67 ^b	15.51 ± 0.23 ^b	14.52 ± 1.00 ^b
C _{8:0}	9.71 ± 0.39 ^a	10.32 ± 0.17 ^a	12.88 ± 0.19 ^b	13.23 ± 0.10 ^b	19.72 ± 2.69 ^c
C _{10:0}	9.35 ± 0.45 ^a	10.30 ± 0.48 ^b	12.69 ± 0.10 ^c	13.19 ± 0.32 ^c	12.51 ± 0.75 ^c
C _{12:0}	10.36 ± 0.52 ^a	10.77 ± 0.32 ^a	14.02 ± 0.18 ^b	14.50 ± 0.40 ^{bc}	16.20 ± 1.96 ^c
C _{14:0}	14.29 ± 0.70 ^a	14.16 ± 0.43 ^a	19.62 ± 0.63 ^a	21.73 ± 0.99 ^a	32.53 ± 9.15 ^b
C _{16:0}	19.36 ± 1.17 ^a	19.02 ± 1.01 ^a	26.71 ± 0.26 ^a	30.92 ± 2.87 ^a	41.00 ± 9.85 ^b
C _{18:1}	21.31 ± 2.07 ^a	23.51 ± 2.32 ^a	30.20 ± 1.82 ^{ab}	37.17 ± 2.14 ^{bc}	60.22 ± 20.71 ^c
C _{18:2}	16.09 ± 4.96 ^a	15.57 ± 5.45 ^a	20.82 ± 6.11 ^a	22.90 ± 7.31 ^a	13.59 ± 1.52 ^a
C _{18:3}	14.79 ± 0.32 ^a	16.06 ± 0.98 ^a	21.43 ± 0.73 ^c	18.67 ± 0.48 ^b	14.46 ± 1.53 ^a
ΣFFA	139.35 ± 7.24 ^a	145.79 ± 5.29 ^a	192.70 ± 1.34 ^b	207.86 ± 10.74 ^{bc}	236.16 ± 37.38 ^c

Means with different letters within the same row differed significantly ($p < 0.05$).

C_{18:3} had notable changes ($p < 0.05$); only C_{18:2} changed insignificantly. Wit *et al.* (2005) also observed that C_{18:2} 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. C_{4:0} was the main short chain fatty acid, and this was similarly reported by Poveda and Cabezas (2006). C_{14:0} 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-butanol, 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-butanol 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

Table 3. Volatile flavour compounds in Xinjiang specialty cheese during ripening.

Compound	0 day		10 days		20 days		30 days		40 days		50 days		
	Relative percentage content%	Compound	Relative percentage content%	Compound	Relative percentage content%	Compound	Relative percentage content%	Compound	Relative percentage content%	Compound	Relative percentage content%	Compound	
<i>Alkanes</i>		<i>Alkanes</i>		<i>Alkanes</i>		<i>Alkanes</i>		<i>Alkanes</i>		<i>Alkanes</i>		<i>Alkanes</i>	
Toluene	0.90	Heptane	0.28	Toluene	4.03	Heptane	2.01	Toluene	2.01	Toluene	1.75	Ethylene oxide	3.06
Heptane	0.15	Toluene	1.26	Heptane	6.45	Toluene	2.46	Heptane	2.46	Heptane	1.18	Heptane	3.37
Dodecane	0.46	2,6,7-trimethyl-Decane	1.49	2,6,7-trimethyl-Decane	1.73	2,6,7-trimethyl-pentamethyl-Heptane	1.18	4-ethyl-2,2,6,6-tetramethyl-Heptane	1.18	4-ethyl-2,2,6,6-tetramethyl-Heptane	0.71	Toluene	3.04
Pentadecane	0.78	Dodecane	0.58	5-ethyl-2,2,3-trimethyl-Heptane	2.54	Decane, 2,6,7-trimethyl-	2.19	5-ethyl-2,2,3-trimethyl-Heptane	2.19	5-ethyl-2,2,3-trimethyl-Heptane	2.41	2,2,4,6,6-pentamethyl-Heptane,	1.17
Hexadecane	0.50	Pentadecane	0.43	Dodecane	0.89	5-ethyl-2,2,3-trimethyl-Heptane	2.38	2,3,4-trimethyl-Decane	2.38	2,3,4-trimethyl-Decane	1.14	Decane, 2,6,7-trimethyl-	2.96
<i>Aldehydes</i>		Hexadecane	0.43	Pentadecane	1.05	2,3,6-trimethyl-Decane	1.17	Dodecane	1.17	Dodecane	0.96	5-ethyl-2,2,3-trimethyl-Heptane,	3.40
Benzaldehyde	5.85	<i>Aldehydes</i>		<i>Aldehydes</i>		Dodecane	0.71	Pentadecane	0.71	Pentadecane	1.05	Decane, 2,3,6-trimethyl-	1.68
<i>Acids</i>		Benzaldehyde	3.70	Benzaldehyde	11.45	Pentadecane	0.79	Hexadecane	0.79	Hexadecane	0.79	Dimethyldecane	0.19
Oxalic acid	1.47	<i>Alcohols</i>		<i>Alcohols</i>		Hexadecane	0.51	<i>Aldehydes</i>	0.51	<i>Aldehydes</i>		Dodecane	0.90
Acetic acid	23.92	1-Butanol	0.71	1-Butanol	1.64	<i>Aldehydes</i>		Pentanal	0.79	Pentadecane	1.10	Pentadecane	1.00
Propanoic acid	0.07	<i>Acids</i>		<i>Acids</i>		Butanal, 3-methyl-	0.70	Butanal, 3-methyl-	0.70	Hexadecane	8.37	Hexadecane	0.74
Butanoic acid	0.08	Oxalic acid	2.36	Oxalic acid	9.28	Benzaldehyde	8.08	Benzaldehyde	8.08	<i>Aldehydes</i>		Butanal, 3-methyl-	1.12
Pentanoic acid	42.84	Acetic acid	24.24	Acetic acid	0.83	<i>Acids</i>		<i>Acids</i>		Oxalic acid	1.82	Benzaldehyde	8.41
Hexanoic acid	0.43	Propanoic acid	0.03	Propanoic acid	0.26	Oxalic acid	3.42	Oxalic acid	3.42	Oxalic acid	0.38	Benzaldehyde	
Octanoic Acid	19.58	Butanoic acid	0.07	Trimethylpentanoic acid	2.05	Acetic acid	1.12	Acetic acid	1.12	Acetic acid	0.46	<i>Alcohols</i>	
<i>Esters</i>		Pentanoic acid	48.68	Butanoic acid	1.57	Propanoic acid	0.05	Propanoic acid	0.05	Propanoic acid	0.98	3-Methyl-1-butanol	0.16
propyl butanoic	2.97	Hexanoic acid	10.53	Pentanoic acid	16.51	L-(+)-Tartaric acid	1.50	L-(+)-Tartaric acid	1.50	L-(+)-Tartaric acid	0.14	Oxalic acid	2.42
		Octanoic Acid	1.49	Hexanoic acid	0.23	Butanoic acid	0.44	Butanoic acid	0.44	Butanoic acid, 2-methyl-	1.65	Acetic acid	0.96
		<i>Esters</i>		Octanoic Acid	30.17	Pentanoic acid	37.59	Pentanoic acid	37.59	Pentanoic acid	40.75	Propanoic acid	0.07
		methyl acetic propyl butanoic	0.04	n-Decanoic acid	2.00	Hexanoic acid	21.86	Hexanoic acid	21.86	Hexanoic acid	0.07	Butanoic acid	0.51
			1.80	<i>Esters</i>		Octanoic Acid	5.79	Octanoic Acid	5.79	Octanoic Acid	13.98	Pentanoic acid	19.34
		butyl butanoic	1.91	3-Buten-1-ol, 3-methyl	1.03	<i>Ketones</i>		<i>Ketones</i>		Octanoic Acid	2.14	Hexanoic acid	0.03
		ethyl hexanoic	0.79	ethyl hexanoic	5.34	2-Nonanone	2.39	2-Nonanone	2.39	<i>Ketones</i>	2.14	Octanoic Acid	36.64
				ethyl octanoic	0.96	butyl butanoic ethyl hexanoic	2.50	<i>Esters</i>	2.50	n-Decanoic acid	8.94	n-Decanoic acid	3.51
							1.18	propyl butanoic ethyl hexanoic	1.18	2-Nonanone	8.43	<i>Ketones</i>	3.46
										ethyl octanoic	1.60	<i>Esters</i>	
										Nonalactone	0.26	Nonalactone	0.26

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 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

The present work was financially supported by the National Natural Science Foundation of China (grants no.: 31760451).

References

Ahmadi, E., Mortazavian, A. M. and Mohammadi, R. 2013. The effect of heat inactivating process on biochemical, microbiological and sensory

- characteristics of Iranian drink based on fermented milk (Doogh). *Journal Food Processing Technology* 4: article no. 271.
- Association of Official Analytical Chemists (AOAC). 1990. *Official methods of analysis of AOAC international*. 15th ed. United States: AOAC.
- Association of Official Analytical Chemists (AOAC). 1995. *Official methods of analysis of AOAC international*. 16th ed. United States: AOAC.
- Ayad, E. H. E., Awad, S., Attar, A. E., Jong, C. D. and El-Soda, M. 2004. Characterisation of Egyptian Ras cheese. 2. Flavour formation. *Food Chemistry* 86(4): 553-561.
- Azat, R., Liu, Y., Li, W., Kayir, A., Lin, D. B., Zhou, W. W. and Zheng, X. D. 2016. Probiotic properties of lactic acid bacteria isolated from traditionally fermented Xinjiang cheese. *Journal of Zhejiang University Science B* 17(8): 597-609.
- Ballesteros, C., Poveda, J. M., González-Viñas, M. A. and Cabezas, L. 2006. Microbiological, biochemical and sensory characteristics of artisanal and industrial Manchego cheeses. *Food Control* 17(4): 249-255.
- Bergamaschi, M. and Bittante, G. 2018. From milk to cheese: evolution of flavor fingerprint of milk, cream, curd, whey, ricotta, scotta, and ripened cheese obtained during summer Alpine pasture. *Journal of Dairy Science* 101(5): 3918-3934.
- Bertuzzi, A. S., McSweeney, P. L. H., Rea, M. C. and Kilcawley, K. N. 2018. Detection of volatile compounds of cheese and their contribution to the flavor profile of surface-ripened cheese. *Comprehensive Reviews in Food Science and Food Safety* 17(2): 1-20.
- Bezerra, T. K. A., Arcanjo, N. M. O., Araújo, A. R. R., Queiroz, A. L. M., Oliveira, M. E. G., Gomes, A. M. P. and Madruga, M. S. 2017. Volatile profile in goat coalho cheese supplemented with probiotic lactic acid bacteria. *LWT - Food Science and Technology* 76: 209-215.
- Boddy, L. and Wimpenny, J. W. T. 2010. Ecological concepts in food microbiology. *Journal of Applied Microbiology* 73(21): 23-38.
- Coda, R., Brechany, E., De Angelis, M., Candia, S., De Cagno, R. and Di Gobbetti, M. 2006. Comparison of the compositional, microbiological, biochemical, and volatile profile characteristics of nine Italian ewes' milk cheeses. *Journal of Dairy Science* 89(11): 4126-4143.
- 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.
- Delahunty, C. M. and Piggott, J. R. 2010. Current methods to evaluate contribution and interactions of components to flavour of solid foods using hard cheese as an example. *International Journal of Food Science Technology* 30(5): 555-570.
- Dugat-Bony, E., Bonnarne, P., Fraud, S., Castelotte, J. and Helinck, S. 2019. Effect of sodium chloride reduction or partial substitution with potassium chloride on the microbiological, biochemical and sensory characteristics of semi-hard and soft cheeses. *Food Research International* 125: article ID 108643.
- Fox, P. F., Guinee, T. P., Cogan, T. M. and McSweeney P. L. H. 2017. *Fundamentals of cheese science*. 2nd ed. New York: Springer.
- Franco, I., Prieto, B., Bernardo, A., Prieto, J. G. and Carballo, J. 2003. Biochemical changes throughout the ripening of a traditional Spanish goat cheese variety (Babia-Laciana). *International Dairy Journal* 13(2): 221-230.
- García, C., Rendueles, M. and Díaz, M. 2019. Liquid-phase food fermentations with microbial consortia involving lactic acid bacteria: a review. *Food Research International* 119: 207-220.
- Gobbetti, M., Neviani, E. and Fox, P. 2018. *The cheeses of Italy: science and technology*. 1st ed. United States: Springer.
- Gorostiza, A., Cichoscki, A. J., Valduga, A. T., Valduga, E., Bernardo, A. and Fresno, J. M. 2004. Changes in soluble nitrogenous compounds, caseins and free amino acids during ripening of artisanal prato cheese; a Brazilian semi-hard cows variety. *Food Chemistry* 85(3): 407-414.
- Gursoy, O., Ahmet, K., Özge, G., Firuze, E. and Kocatrürk, K. 2018. Physicochemistry, microbiology, fatty acids composition and volatile profile of traditional Söğle tulum (goat's skin bag) cheese. *Anais da Academia Brasileira de Ciências* 90(4): 3661-3674.
- Hayaloglu, A. A., Guven, M., Fox, P. F., Hannon, J. A. and McSweeney, P. L. H. 2004. Proteolysis in Turkish white-brined cheese made with defined strains of *Lactococcus*. *International Dairy Journal* 14(7): 599-610.
- 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.
- James, S., Jeffery, B. and Jan, K. 2013. Perspectives on the contribution of lactic acid bacteria to cheese flavor development. *Current Opinion in Biotechnology* 24(2): 135-141.
- Jéssica, F. R., Bruna, A. M., Jonas, G. S., Gustavo, A. L., Lorena, O. C., Erick, A. E., ... and Adriano, G. C. 2020. Sorting task as a tool to elucidate the sensory patterns of artisanal cheeses. *Journal of Sensory Studies* 35(3): article ID e12562.
- Judacewski, P., Los, P. R., Lima, L. S., Alberti, A. and Nogueira, A. 2019. Perceptions of Brazilian consumers regarding white mould surface-ripened cheese using free word association. *International Journal of Dairy Technology* 72(4): 585-590.
- Katayama, E., Kimori, Y. and Uyeda, T. Q. P. 2007. Further characterization of the configuration of actin-attached myosin-crossbridges during *in vitro* sliding (molecular motors, oral presentations). *Seibutsu Butsuri* 47: article ID S143.
- Kieronczyk, A., Skeie, S., Olsen, K. and Langsrud, T. 2001. Metabolism of amino acids by resting cells of non-starter lactobacilli in relation to flavour development in cheese. *International Dairy Journal* 11(4): 217-224.
- Kuchroo, C. N. and Fox, P. F. 1982. Soluble nitrogen in Cheddar cheese: comparison of extraction procedures. *Milchwissenschaft* 37: article ID 331.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259): 680-685.
- Lawlor, J. B., Delahunty, C. M., Sheehan, J. and Wilkinson, M. G. 2003. Relationships between sensory attributes and the volatile compounds, non-volatile and gross compositional constituents of six blue-type cheeses. *International Dairy Journal* 13(6): 481-494.
- Lee, J., Joung, J., Choi, Y. S., Kim, Y. and Oh, N. S. 2016. Characterisation of microbial diversity and chemical properties of Cheddar cheese prepared from heat-treated milk. *International Dairy Journal* 63: 92-98.
- Ma, Y., Lu, S., Li, K. X. and Li, B. K. 2012. Studies on the non-volatile flavor compounds of Xinjiang characteristics cheese during ripening period. *Food and Fermentation Industries* 38(12): 161-165.
- McSweeney, P. L. and Sousa, M. J. 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. *Le Lait* 80(3): 293-324.
- Merheb-Dini, C., Chaves, K. S., Gomes, E., Silva, R. D. and Gigante, M. L. 2016. Coalho cheese made with protease from *Thermomucor indiciae-seudaticae* N31: technological potential of the new coagulant for the production of high-cooked cheese. *Journal of Food Science* 81(1-3): 563-568.
- Merheb-Dini, C., Garcia, G. A. C., Penna, A. L. B., Gomes, E. and da Silva, R. 2012. Use of a new milk-clotting protease from *Thermomucor indiciae-seudaticae* N31 as coagulant and changes during ripening of Prato cheese. *Food Chemistry* 130(4): 859-865.
- Murtaza, M. A., Huma, N., Shabbir, M. A., Murtaza, M. S. and Anees-Ur-Rehman, M. 2017. Survival of micro-organisms and organic acid profile of probiotic Cheddar cheese from buffalo milk during accelerated ripening. *International Journal of Dairy Technology* 70(4): 562-571.
- Ng-Kwai-Hang, K. F., Moxley, J. E. and Van De Voort, F. R. 1988. Factors affecting differences in milk fat test obtained by Babcock, Rose-Gottlieb, and infrared methods and in protein test from infrared milk analysis. *Journal of Dairy Science* 71(2): 290-298.
- Picon, A., López-Pérez, O., Torres, E., Garde, S. and Nuñez, M. 2019. Contribution of autochthonous lactic acid bacteria to the typical flavour of raw goat milk cheeses. *International Journal of Food Microbiology* 299(6): 8-22.
- Pionnier, E., Engel, E., Salles, C. and Le Quéré, J. L. 2002. Interactions between non-volatile water-soluble molecules and aroma compounds in Camembert cheese. *Food Chemistry* 76(1): 13-20.
- Poveda, J. M. and Cabezas, L. 2006. Free fatty acid composition of regionally-produced Spanish goat cheese and relationship with sensory characteristics. *Food chemistry* 95(2): 307-311.
- 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.
- Sonmezdag, A. S. 2019. Characterization of aroma and aroma-active composition of Gaziantep cheese by solvent-assisted flavor evaporation (SAFE) and aroma extract dilution analysis (AEDA). *Journal of Food Processing and Preservation* 43(6): article ID e13840.
- Taborda, G., Gómez-Ruiz, J. A., Martínez-Castro, I., Amigo, L., Ramos, M. and Molina, E. 2008. Taste and flavor of artisan and industrial

- Manchego cheese as influenced by the water-soluble extract compounds. *European Food Research and Technology* 227(2): 323-330.
- Thierry, A., Collins, Y. F., Abeijón Mukdsi, M. C., Mcsweeney, P. L. H., Wilkinson, M. G. and Spinnler, H. E. 2017. Lipolysis and metabolism of fatty acids in cheese. In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. and Everett, D. W. (eds). *Cheese - Chemistry, Physics and Microbiology* (4th ed), p. 423-444. United States: Academic Press.
- Vale, R. C., Rodrigues, M. P. J. and Martins, J. M. 2018. Influence of the type of ferment in the physicochemical characteristics of cheese Minas artisanal do Serro - Minas Gerais, matured in controlled conditions. *Journal of Candido Tostes Dairy Institute* 73(2): 82-90.
- Wang, C. Y., Li, Y. H., Li, Y. B., Li, B. K., Wang, T. B. and Shi, X. B. 2019. Microbial diversity and gene function analysis in traditional hand-made cheese from pastoral areas of Yili in Xinjiang. *Food Science* 40(24): 110-118.
- Whetstone, M. E. C., Luck, P. J., Drake, M. A., Foegeding, E. A., Gerard, P. D. and Barbano, D. M. 2007. Characterization of flavor and texture development within large (291 kg) blocks of cheddar cheese. *Journal of Dairy Science* 90(7): 3091-3109.
- Wit, M. D., Osthoff, G., Viljoen, B. C. and Hugo, A. 2005. A comparative study of lipolysis and proteolysis in Cheddar cheese and yeast-inoculated Cheddar cheeses during ripening. *Enzyme and Microbial Technology* 37(6): 606-616.