Influence of *Monascus purpureus* BD-M-4 on the physicochemical properties, proteolysis and volatile compounds of surface mould-ripened cheese

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Abstract The effect of adjunct culture *Monascus purpureus* BD-M-4 on the physicochemical, proteolytic, and lipolytic properties of surface mold-ripened cheese were evaluated. During the maturation of *Monascus*-fermented cheese, the total microbiota count and the content of soluble nitrogen increased steadily, whereas the total protein content showed no significant difference. Moreover, a 17-fold increase in total free amino acids was observed in *Monascus*-fermented cheese. The use of adjunct culture *M. purpureus* BD-M-4 in the production of surface-ripened cheeses did not show a significant effect on the total fat content in the ripening period, nor did it change the lipolysis of cheese during ripening. Compared to 52 volatile compounds of the control cheese, a total of 62 compounds were detected in *M. purpureus*-fermented cheese, including 16 acids, 16 ketones, 11 alcohols, 5 aldehydes, 11 esters, and 3 unclassified compounds.

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Introduction

In most surface-ripened cheeses, primary starter cultures such as *Lactococci* spp. and *Streptococci* spp. are considered as essential parts of lactic acid accumulation. Secondary starters or adjuncts, such as *Penicillium roqueforti*, *Geotrichum candidum* and *P. camemberti*, play an important role in ripening, leading to flavor development mainly through proteolysis (Gobbetti et al., 2015). Following the primary proteolysis by residual coagulant and somatic cell proteinases, the endopeptidases and exopeptidases of these adjunct cultures increase the rate of casein hydrolysis, resulting in high levels of small peptides and free amino acids, which are precursors for many flavor and aroma compounds (Fox et al., 2017).

In the group of surface-ripened cheeses, molds have always been considered as potential cheese adjunct cultures because of their ability to ferment lactose and produce a specific texture (Chen et al., 2012). Besides, molds also play an important role in the cheese-ripening process. During the metabolism of molds, the changes in proteolysis and lipolysis lead to an improvement in texture and flavor of cheeses. Proteolysis, lipolysis, and development of volatiles are key characteristics that distinguish cheese varieties.

Monascus spp. has been used as a fermentation starter for Chinese traditional food for over 1000 years. The production of food components, natural pigments, and food supplements was proven to be effective in the management of blood cholesterol, diabetes, blood pressure, obesity, and Parkinson's disease (Hsu et al., 2014; Tseng et al., 2016).

Several reports on the production of mevinolins, γ -aminobutyric acid, and monacolin K using monocultures of *Monascus* species are presented (Vendruscolo et al., 2016). In a previous study, *M. purpureus* BD-M-4 was used as the secondary starter for producing a novel semi-hard cheese, which showed the presence of proteolytic activity against α_{s1} -casein and β -casein (Yu et al., 2016). Thus, *Monascus* spp. might have a potential application in the manufacturing of cheeses.

In the present study, we assessed the effectiveness of the *M. purpureus* BD-M-4 strain as an adjunct culture on the microbiological, physicochemical, proteolytic, and lipolytic properties of surface-ripened cheeses during 33 days ripening.

Materials and methods

Cheese manufacture

The *Monascus*-fermented cheese (MC) was manufactured in duplicate from raw whole bovine milk using a surface-ripened cheese manufacturing procedure, as described by Yu et al. (2016) with minor modifications. After molding, the cheeses were immersed into the solution containing *M. purpureus* (7.6×10^6 spores per milliliter) for 20 min (0 day). Then, the cheese blocks were transferred to ripening chambers and ripened at 25 °C for 4 days. Subsequently, the blocks were ripened at 14 °C within days 5–21 and at 4 °C within days 21–33. The control group of cheese (CC) was made through the same steps as above, except the immersion in *M. purpureus* solution. Samples from the surface of cheeses were taken at 0, 1, 5, 12, 19, 26, and 33 days of ripening for further analysis. Samples were taken in triplicate.

Physicochemical analysis of cheeses

For each part of the cheeses, the dry matter (DM), pH, and total fat and protein contents were determined. Total fat and protein were determined using the methods described previously by McCarthy et al. (2015). DM was analyzed in triplicate by drying 3.0 ± 0.3 g cheese samples at 150 °C in an MB 45 moisture analyzer (Ohaus International Trading Co., Ltd., Shanghai, China) until constant in weight. Ground cheeses (5.0 ± 0.1 g) were mixed with 45 mL deionized water; then, the mixture was stirred for 10 min, and its pH was measured using a Delta 320 pH meter (Mettler-Toledo Ltd., Shanghai, China).

Microbiological analysis of cheese

Grated cheese (5 g) was placed into a sterile mortar with 45 mL sterile 0.9% (w/v) NaCl solution and blended for 5 min at room temperature.

Serial dilutions were made using 0.9% (w/v) NaCl solution for all further steps. The total microbiota and *M. purpureus* BD-M-4 counts were determined using plate count agar and potato dextrose agar (PDA) plate (pH 6.5) (Sincere Biochemical Technology, Shanghai, China) by incubating under aerobic conditions at 30 °C for 3 days. The microbiological analysis was conducted in duplicate for each sample at each sampling point.

Analysis of proteolysis in cheese

Nitrogen fractions and free amino acid (FAA) measurements

The water-soluble nitrogen (WSN) of the cheeses were prepared according to Kuchroo and Fox (Kuchroo and Fox, 1982), where nitrogen (N) was determined by Leclercq-Perlat et al. (2000). Results were expressed as percentages of total N. The levels of 12% (w/v) trichloroacetic acid soluble nitrogen (TCA-SN) and 5% (w/v) phosphotungstic acid-soluble cheese nitrogen (PTA-SN) were determined using the method by Leclercq-Perlat et al. (2000) and were expressed as percentages of total nitrogen.

Individual FAAs were determined in duplicate from PTA-SN filtrates. FAAs were analyzed using the method described by Jarrett et al. (1982). All analyses were carried out in duplicate.

SDS-PAGE analysis in cheese

An aliquot of each cheese sample (0.05 g) was taken at each sampling point and frozen at -20 °C. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) were carried out as described by Ong (2007). Standard α - (C6780) and β -casein (C6905) used in the electrophoresis were obtained from bovine milk (Sigma-Aldrich Co., St. Louis, MO, USA).

Free fatty acid analysis

Free fatty acid (FFA) extraction was performed on 0.3 g of grated cheese, according to the method described by De Jong and Badings (1990). The FFA extracts were aliquoted into amber glass vials and capped with PTFE/white silicone septa. The FFA extracts were derivatized as methyl esters, as outlined by Mannion et al. (2016). Fatty acid methyl ester extracts were analyzed using 7890A-5975C gas chromatograph (Agilent, Santa Clara, CA, USA), with

a CTC auto-sampler and flame ionization detector (GC-FID). For the GC-FID analysis, 1 μ L was injected into a DB-5 column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) (Agilent). Results are expressed as g/10 kg DM of cheese.

Volatile compounds analysis

Volatile compounds were extracted according to the method of Mei et al. (2015), with modification. A total of 5 g of cheese was placed in a 20-mL headspace vial, and then 8 mL of NaH_2PO_4 (25%, w/v) was added. The headspace vials were equilibrated to 40 °C for 30 min in an ultrasonic processor (THC-28; Tianhua, Jinjing, China). Then, the samples were centrifuged at 8000 rpm for 30 min, and the supernatants were obtained by using a 50/30 μ m Divinylbenzene/Carboxen/PDMS SPME fiber (Supelco, Bellefonte, PA, USA). After extraction, the sample was directly desorbed into the injection port at 260 °C. Injection was made on GC-MS (7890A-5975C; Agilent, Santa Clara, CA, USA) equipped with a DB-wax column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m). The carrier gas, helium, was at a flow of 1.0 mL/min. The temperature program method was isothermal at 40 °C for 5 min and then adjusted to 5 °C. The GC-MS transfer line temperature was at 280 °C. The MS was operated in electron impact mode with an electron impact energy of 70 eV. Data were collected at a rate of 0.7 scans/s over a range of 20–400 m/z. Compounds were identified based on mass spectra comparison, with the spectra obtained from the NIST 2011 database. All analyses were conducted in duplicate.

Statistical analysis

The data were evaluated using one-way analysis of variance (ANOVA). The significance for all analyses was determined at $p < 0.05$. A Student–Newman–Keuls test was applied to compare the mean values of the variables. Principal component analysis (PCA) was applied to the data of the free amino acids and volatile compounds for MC at different ripening times. IBM statistical product and service solutions (SPSS) statistics software version 20 (IBM Corporation, Armonk, North Castle, NY, USA) was used in the statistical analysis.

Results and discussion

Physicochemical and microbiological characteristics

The compositional results of surface-ripened cheeses with or without the adjunct culture *M. purpureus* BD-M-4 following 33 days of ripening are outlined in Table 1.

Regardless of the group, the dry matter increased gradually from day 0 to day 33. This increase may be attributed to the surface evaporation of water and volatile products. The pH of the curd was about 4.80 at day 0 as a result of lactose conversion to lactic acid. Due to the alkaline product accumulation through proteolysis, the pH of CC gradually increased during the whole ripening time (Leclercq-Perlat et al., 2004). The pH of MC rapidly reached up to 6.89 at day 5 and increased even further to 7.04 at day 33 due to proteolysis combined with the consumption of lactic acid by *M. purpureus*. Minor reductions in fat and protein during ripening were observed, but these reductions were not statistically significant over time ($p > 0.05$). In the initial stage of ripening (day 0 to day 5), the count of total microbiota in both MC and CC increased rapidly from 5.6×10^8 CFU/g to 1.55×10^9 CFU/g and 5.9×10^8 CFU/g to 3.7×10^9 CFU/g, respectively. After day 5, the total microbiota count of CC continuously decreased and reduced to about 5.75×10^8 CFU/g at day 33. However, the total viable microorganisms of MC indicated a steady increase from day 5 to day 19 (4.4×10^9 CFU/g to 1.0×10^{10} CFU/g) and remained at about 6.7×10^9 CFU/g until the end of ripening. MC showed a count of viable cells for *M. purpureus* BD-M-4 at 9.0×10^5 CFU/g on day 0, after inoculation. At day 1, the count of total microbiota of MC was 3.2×10^7 CFU/g and decreased to 6.1×10^6 CFU/g at day 19. These results agree with the report of Leclercq-Perlat et al. (2013), who observed a reduction in *Penicillium camemberti* mycelial concentration in Camembert cheese. This result may be attributed to the fact that the lack of fermentable carbohydrate and low temperature probably led to the decrease of total microbiota. Ong also reported that the viable counts of probiotic adjunct in Cheddar cheese decreased after 24 weeks because of low pH and lack of fermentable carbohydrate (Ong, 2007).

Proteolysis in cheeses during ripening

The assessment of proteolysis in CC and MC by determining the WSN/TN%, TCA-SN/TN%, and PTA-SN/TN% during 33 days of ripening period is depicted in Table 2. The WSN/TN% indicates primary proteolysis. During the 33-day ripening period, the amount of WSN increased progressively. CC and MC was found to have no significant differences ($p > 0.05$) at the beginning of the ripening period (0–1 day), which was in agreement with the report that rennet is principally responsible for primary proteolysis in cheeses (Alichanidis and Polychroniadou, 2008). After 5 days of ripening, significant differences of WSN were observed between MC and CC, which could be attributed to the growth of *Monascus*.

Table 1 Changes in microorganisms and physicochemical characteristics in cheeses during ripening

Parameters	Day 0	Day 1	Day 5	Day 12	Day 19	Day 26	Day 33
DM (% wt)							
Control	46.05 ± 1.78a ¹	48.05 ± 0.97b	50.44 ± 0.43c	50.84 ± 0.25c	51.50 ± 0.25c	52.39 ± 0.35c	52.79 ± 0.22c
<i>Monascus</i>	45.32 ± 0.14a	47.60 ± 1.27b	51.13 ± 0.64c	50.91 ± 0.26c	51.37 ± 0.21c	51.73 ± 0.23c	52.33 ± 1.14c
pH							
Control	4.76 ± 0.03b	4.73 ± 0.04b	4.72 ± 0.01b	4.78 ± 0.05b	4.78 ± 0.11b	4.85 ± 0.07b	5.02 ± 0.02a
<i>Monascus</i>	4.80 ± 0.02b	4.91 ± 0.01b	6.89 ± 0.10a	6.87 ± 0.01a	7.01 ± 0.01a	7.04 ± 0.08a	7.04 ± 0.18a
Fat (g 100 g ⁻¹ DM)							
Control	52.14 ± 3.36	51.25 ± 1.21	51.03 ± 2.46	50.29 ± 0.55	48.91 ± 0.59	48.98 ± 0.15	48.76 ± 3.91
<i>Monascus</i>	51.93 ± 2.07	50.42 ± 0.47	49.99 ± 0.69	48.84 ± 3.12	47.55 ± 0.82	46.62 ± 0.69	45.79 ± 1.26
Protein (g 100 g ⁻¹ DM)							
Control	37.18 ± 1.09	37.12 ± 1.12	37.14 ± 0.55	36.99 ± 0.42	36.63 ± 0.55	36.88 ± 0.28	36.87 ± 1.63
<i>Monascus</i>	37.35 ± 1.23	37.25 ± 0.88	36.89 ± 0.27	36.64 ± 1.08	36.28 ± 0.13	36.10 ± 1.25	35.19 ± 0.42
Total microflora count (log CFU g ⁻¹)							
Control	8.76 ± 0.12b	9.26 ± 0.31ab	9.57 ± 0.02a	9.26 ± 0.08ab	9.05 ± 0.21b	8.90 ± 0.08b	8.76 ± 0.01b
<i>Monascus</i>	8.74 ± 0.09b	9.18 ± 0.10ab	9.62 ± 0.22a	9.74 ± 0.39a	9.96 ± 0.27a	9.82 ± 0.01a	9.70 ± 0.39a
<i>Monascus</i> spp. (log CFU g ⁻¹)							
Control	ND	ND	ND	ND	ND	ND	ND
<i>Monascus</i>	5.90 ± 0.08b	6.46 ± 0.19a	6.73 ± 0.02a	6.75 ± 0.03a	6.79 ± 0.01a	6.69 ± 0.12a	6.49 ± 0.23a

ND not detected

¹Values are expressed as mean ± SD. Different letters as superscripts in the same row indicate that the data differ significantly ($p > 0.05$)

A similar trend was found in the amount of TCA-SN. No significant difference was found ($p > 0.05$) in the level of TCA-SN between CC and MC up to 5 days of ripening. The majority of proteolysis took place during the initial 19 days of ripening; further ripening (19–33 days) contributed to only 10% rise in TCA-SN. After day 5 a large extent of proteolysis due to the growth of *Monascus* can be observed, and more products of the primary proteolysis become available as substrates for subsequent proteolysis by peptidases.

Considering that PTA is a discriminating protein precipitant, which only precipitates the peptides to greater than 600 Da (Ong, 2007), PTA-SN is produced primarily by the action of microbial peptidases. PTA-SN was higher in MC than in CC after day 12, but the observed differences were not statistically significant ($p > 0.05$).

The SDS-PAGE profiles of CC and MC ripened for 0, 1, 5, 12, 26, and 33 days are presented in Fig. 1A, B. Electrophoretograms showed two major bands, namely, α s-CN and β -CN with molecular weights the same as in a previous report (Van Hekken et al., 2004). Qualitative differences between the SDS-PAGE profiles of MC were evident after 5 days. Besides these two fractions, the SDS-PAGE profile also contained many low molecular weight peptides

(LMWP) in the range of 10–15 kDa. The observed differences in the two major bands and LMWP, as the products of proteolysis, could well be attributed to the peptidase enzyme systems of the adjunct cultures. This finding was in agreement with those reported by Folkertsma et al. (1996) and Ong and Shah (2009).

Total free amino acids also represent the extent of secondary proteolysis. Table 2 shows that the concentration of total FAA was similar at the initial stage of ripening ($p > 0.05$). The total FAA levels of MC increased from 2.81 to 13.03 mg/mL from day 5 to 26, following a similar dynamic in the PTA-SN index. In this case, the concentration of WSN and TCA-SN became significantly high, which may be attributed to the secondary proteolysis by the action of the proteinases secreted by *Monascus*. This result agreed with the research of O'Brien et al. (2017).

As shown in Fig. 1C, D, the principal FFAs prevalent in both cheese groups were Glu, Val, Tyr, Pro, Leu, and Lys. Similar to the total FAAs, the concentrations of individual FAAs increased during ripening. The MC group had about 10 times higher levels of individual FAAs than the CC group, indicating a faster development of proteolysis in MC than CC. Changes in the profiles and concentration of FAAs due to peptidolysis may impact the regulation of

Table 2 Changes in proteolysis indices of cheeses during ripening

Time (days)	CC	MC
WSN/TN%		
0	8.23 ± 1.05 ¹	8.25 ± 0.25C
1	8.05 ± 0.25	8.15 ± 0.25C
5	8.15 ± 0.80a	21.65 ± 0.95bB
12	10.95 ± 0.75a	29.55 ± 0.90bAB
19	12.52 ± 0.25a	35.75 ± 3.33bA
26	12.55 ± 1.40a	41.75 ± 9.25bA
33	12.98 ± 0.25a	42.35 ± 3.25bA
TCA-SN/SN%		
0	3.65 ± 1.05A	4.80 ± 0.62A
1	4.94 ± 0.35A	5.21 ± 0.35A
5	8.35 ± 1.05aB	14.76 ± 0.45bB
12	8.53 ± 0.53aB	21.85 ± 0.93bC
19	8.95 ± 0.45aB	25.75 ± 1.32bC
26	9.75 ± 0.45aB	26.02 ± 1.32bC
33	9.65 ± 0.45aB	25.96 ± 3.96bC
PTA-SN/SN%		
0	1.55 ± 0.72A	0.78 ± 0.35A
1	1.65 ± 0.40A	2.35 ± 0.43B
5	4.55 ± 1.25B	4.17 ± 0.75C
12	4.73 ± 0.42aB	9.23 ± 1.14bD
19	6.40 ± 0.85B	9.35 ± 1.54D
26	6.35 ± 1.05B	9.52 ± 2.60D
33	6.42 ± 1.05B	9.55 ± 1.05D
Total FAA (mg g⁻¹ DM)		
0	0.53 ± 0.04A	0.69 ± 0.05A
1	0.65 ± 0.04A	0.91 ± 0.53A
5	0.75 ± 0.05aAB	2.81 ± 0.51bA
12	0.85 ± 0.02aAB	6.45 ± 1.91bAB
19	0.90 ± 0.09aAB	11.93 ± 3.50bBC
26	0.99 ± 0.30aAB	13.03 ± 1.76bC
33	1.14 ± 0.06aB	13.30 ± 1.64bC

TN total nitrogen, SN soluble nitrogen

¹Values are expressed as mean ± SD. Different lowercase letters in the same row indicate significant statistical differences ($p > 0.05$); different capital letters in the same column indicate significant statistical differences ($p > 0.05$)

specific enzyme synthesis involved in their catabolism. Apparently, ripening with *Monascus* led to a remarkable increase in total FAA and individual levels. Similar results were reported by Yu et al. (2016), who evaluated the effect of *Monascus* cultures on the extent of proteolysis in surface-ripened cheeses. PCA was applied to the individual FAA data in different ripening processes. The distribution of the scores on the first two PCs is shown in Fig. 2. Most individual amino acids were located in the positive end of PC#1, whereas arginine was located in the negative area of

Fig. 1 SDS-PAGE pattern of the water-insoluble fraction and concentrations of individual free amino acids of cheeses during the 33-day ripening. (A) CC and (B) MC; lines 1–6 represent WSN obtained from cheeses at the ripening period of 0, 1, 5, 12, 26, 29, and 33 days, respectively; (C) CC and (D) MC: individual free amino acid level of the cheeses with the mean value and SD (error bar) at different sampling points

PC#1, which was related to the reduction during the ripening process. Cysteine and lysine were near the axis

Fig. 2 Principal components analysis loading plots of free amino acids for MC

origin of PC#1 and located on the positive area of PC#2, indicating that two amino acids reached maximum levels during ripening and then decreased.

The concentrations of free fatty acids

Lipolysis is an important biochemical event that occurs during cheese ripening and is a major pathway for flavor generation. The concentrations of FFAs in the cheese samples at different stages of ripening are shown in Table 3.

The principal FFA in the two groups of cheeses at the highest ripening times were C16:0, C18:0, and C18:1, a trend consistent with their relatively high molar percentage in milk fat triacylglycerols (Atasoy and Türkoğlu, 2008). The mean fat contents of CC and MC decreased from 52.14% and 51.93% (day 0) to 48.76% and 45.79% (day 35), respectively (Table 1). The total concentration of FFAs increased with the reducing fat content ($p < 0.05$). However, no significant difference was found in the individual FAA contents, except for octadecenoic acid (C18:1) ($p < 0.05$) in CC (Table 3). A similar trend was observed in MC. These results indicated that lipolysis was not affected by the growth of *Monascus* even though a slight, though non-significant, increase in FFA of MC was found ($p > 0.05$). The increase of total FFA mainly reflected the degradation of triacylglycerols by lipases and esterases from the starter culture (Mccarthy et al., 2017).

Volatile compounds analysis

The volatile compounds identified via GC–MS are listed in Tables S1 and S2 (area units, AU, $\times 10^5$). A total of 52 volatile compounds were identified in CC, 43 of which were identified on day 0 (83%). However, out of the 61 volatile compounds that was detected in MC, only 35 were detected in the cheeses on day 0 (57%). Generally, most compounds increased as ripening progressed, particularly esters and alcohols, except acetic acid, ethanol, and acetone that clearly decreased from day 0. Among them, dodecanoic acid, n-decanoic acid, benzeneacetaldehyde, and acetoin reached maximum levels during ripening and then decreased. These results were consistent with the research of hard cooked cheeses by Buchin et al. (2017) and Emmental cheeses by Thierry et al. (1999).

The levels of all carboxylic acids were increased when *Monascus* was added, except for the acetic acid. Acetic acid seemed to get consumed during ripening due to the growth of *Monascus*. Compared to CC, the cheeses with *Monascus* as adjunct contained more short and intermediate chain carboxylic acids, which were considered to be key contributors to the flavor profile due to low aroma thresholds (Mei et al., 2015). Among the acids, 3-methylbutanoic acid and hexanoic acid were the main acids found in cheeses. A previous research found that high levels of hexanoic acids contribute to the volatile flavor and have been identified as the main odorant in different cheese types (Urgeghe et al., 2012). A total of 16 ketones were found and are mentioned in Table S2. Ketones were the second most important group of aromatic compounds in the cheese after carboxylic acids, accounting for 13.78% of the total aromatic compounds. Most of the ketones in MC in this study were methyl ketones, which were produced from fatty acids via oxidative degradation. The main ketones in MC were 2-pentanone, 2-heptanone, and 2-nonanone, which were responsible for the characteristic aroma of blue-veined cheeses (Murtaza et al., 2014). Four aldehydic compounds were detected in the extracts, namely, 3-methylbutanal, nonanal, benzaldehyde, and benzene acetaldehyde. Compared to CC, the levels of 3-methylbutanal and benzene acetaldehyde in MC have a significant increase at day 33. In both hard and soft cheeses, 3-methylbutanal is the key aroma compound with fruity and nutty notes (Afzal et al., 2017). At the end of ripening, 3-methylbutanol was also the most abundant alcohol in MC, but it was not detected in CC. It was a key aroma compound for nutty and has long been recognized as an important flavor compound in Emmental cheese (Spanier et al., 2007). Further proteolysis proceeded due to the growth of *Monascus*; thus, more leucine was available as substrates for the subsequent reactions. The biosynthesis of 3-methylbutanal and 3-methyl-1-butanol from leucine

Table 3 Levels of individual and total free fatty acids in cheeses

FFA (g 10 kg ⁻¹ DM)	Day 0	Day 1	Day 5	Day 12	Day 19	Day 26	Day 33
CC							
C6-0	19.33 ± 3.73	20.97 ± 3.30	16.20 ± 2.04	19.69 ± 1.68	15.28 ± 2.11	21.01 ± 3.08	20.39 ± 1.29
C8-0	16.81 ± 2.09	18.55 ± 1.75	15.98 ± 1.49	17.71 ± 0.69	15.74 ± 1.04	18 ± 1.00	18.60 ± 1.69
C10-0	34.65 ± 0.13	36.24 ± 1.46	33.46 ± 2.04	34.56 ± 0.63	32.75 ± 1.73	34.31 ± 0.14	34.62 ± 0.78
C10-1	3.77 ± 0.31	3.98 ± 0.20	3.38 ± 0.37	3.92 ± 0.06	3.52 ± 0.01	3.96 ± 0.36	3.69 ± 0.39
C12-0	45.7 ± 4.68	45.88 ± 0.52	43.68 ± 2.02	44.41 ± 0.01	42.89 ± 1.77	43.59 ± 0.45	43.85 ± 0.40
C12-1	1.81 ± 0.14	1.72 ± 0.04	1.61 ± 0.11	1.76 ± 0.03	1.74 ± 0.03	1.77 ± 0.07	1.74 ± 0.04
C14-0	8.73 ± 0.32	9.07 ± 0.24	8.50 ± 0.64	9.11 ± 0.53	9.19 ± 0.27	8.84 ± 0.24	9.02 ± 0.18
C16-0	266.35 ± 14.04	272.94 ± 3.83	269.07 ± 12.38	257.79 ± 15.33	262.2 ± 15.70	267.42 ± 3.29	266.94 ± 2.42
C16-1	25.67 ± 1.13	25.58 ± 1.90	24.55 ± 1.93	25.68 ± 0.40	26.36 ± 1.27	26.74 ± 2.21	26.89 ± 0.03
C18-0	154.35 ± 11.28	165.39 ± 1.54	166.88 ± 9.26	169.29 ± 3.96	165.88 ± 9.00	167.21 ± 3.92	165.73 ± 4.16
C18-1	233.32 ± 21.59a ¹	300.13 ± 3.97b	448.63 ± 9.39c	487.29 ± 5.32 cd	505.43 ± 40.5 cd	510.52 ± 15.71 cd	526.97 ± 25.94d
C18-2	47.57 ± 2.89	48.01 ± 1.33	50.91 ± 2.37	52.96 ± 2.12	51.19 ± 2.28	52.68 ± 1.71	52.63 ± 0.30
Total	703.71 ± 50.79a	783.07 ± 3.95a	915.96 ± 16.00b	968.05 ± 64.11b	953.09 ± 20.62b	988.84 ± 20.77b	1005.31 ± 32.50b
MC							
C6-0	17.07 ± 2.14	21.35 ± 2.64	18.22 ± 1.85	21.24 ± 3.91	21.46 ± 1.06	19.24 ± 1.51	20.69 ± 3.14
C8-0	15.82 ± 0.62	18.63 ± 0.85	16.87 ± 1.29	17.79 ± 2.00	18.26 ± 0.64	16.63 ± 1.11	18.53 ± 1.43
C10-0	33.92 ± 2.42	35.69 ± 1.03	34.41 ± 1.32	33.88 ± 2.59	34.65 ± 1.09	32.83 ± 2.06	33.02 ± 1.62
C10-1	3.30 ± 0.10	4.01 ± 0.06	3.69 ± 0.31	3.87 ± 0.51	4.03 ± 0.13	3.71 ± 0.35	3.76 ± 0.78
C12-0	43.52 ± 3.06	44.93 ± 1.07	44.4 ± 1.22	43.17 ± 2.45	44.09 ± 1.64	42.5 ± 2.51	44.74 ± 0.57
C12-1	1.46 ± 0.04	1.78 ± 0.10	1.67 ± 0.17	1.71 ± 0.12	1.77 ± 0.06	1.73 ± 0.16	1.79 ± 0.08
C14-0	7.98 ± 0.09	8.31 ± 0.38	9.46 ± 0.19	8.76 ± 0.11	9.22 ± 0.15	9.10 ± 0.81	9.17 ± 0.06
C16-0	289.68 ± 28.36	262.51 ± 1.17	276.52 ± 0.58	263.95 ± 7.50	271.42 ± 13.04	274.91 ± 26.16	276.18 ± 12.68
C16-1	23.15 ± 1.62	25.15 ± 0.87	25.81 ± 0.38	24.74 ± 1.33	25.83 ± 1.11	26.93 ± 0.86	26.51 ± 1.18
C18-0	157.68 ± 3.79	162.02 ± 4.66	165.36 ± 7.85	172.83 ± 9.77	174.71 ± 1.74	167.82 ± 0.01	168.26 ± 0.98
C18-1	237.01 ± 4.45a	407.54 ± 40.28b	503.44 ± 18.42c	513.33 ± 26.85c	509.96 ± 4.19c	544.49 ± 18.35c	538.98 ± 16.5c
C18-2	44.57 ± 1.61	48.51 ± 4.56	55.73 ± 2.51	52.13 ± 2.35	55.01 ± 3.38	53.64 ± 1.71	54.82 ± 0.93
Total	717.49 ± 32.33a	878.41 ± 37.63b	990.22 ± 8.60c	984.56 ± 3.98c	995.69 ± 18.12c	1025.7 ± 53.89c	1028.16 ± 23.88c

¹Values are expressed as mean ± SD. Different letters as superscripts in the same row indicate that the data differ significantly ($p > 0.05$)

Fig. 3 Principal component analysis loading plots of the volatile compounds for MC

positive axis of PC#2, such as 9-decenoic acid, undecanoic acid, heptanoic acid, butanoic acid ethyl ester, 3-methyl butanal, and 2,3-butanediol. On the contrary, other volatile compounds of day 1 and 12 grouped on this axis, containing acetoin, 2-dodecanone, benzene acetaldehyde, 3-methyl-1-butanol, were correlated with the negative end of PC#2.

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