Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.124222

Characterization of the volatile compound profile of artisanal Minas cheese from different regions

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Abstract

Artisanal Minas cheese (AMC) is a product with great economic, historical, and cultural value, and the final organoleptic characteristics of AMC can be influenced by soil and climate factors at the production site (*terroir*). This study aimed to evaluate the profile of volatile compounds in cheeses from seven certified producing regions in Minas Gerais, Brazil, to characterize the volatile compounds according to their origin. A total of 78 samples were obtained from producers registered with the health certification body (Instituto Mineiro de Agropecuária), and the health requirements, minimum ripening time, and characteristic production methods of the cheeses from each region were followed. In general, a total of 166 compounds were identified, which were divided into several chemical groups, including aldehydes (20), ketones (17), carboxylic acids (20), terpenes (6), esters (57), hydrocarbons (15), and alcohols (31). The terpenes α-pinene and caryophyllene-oxide were possible plant markers for cheeses from the Canastra and Serro regions, as these compounds were not identified in cheeses from the other regions. For cheeses from different regions, interactions between some origin-related compounds were verified, which may help establish guidelines and standards for chemical compounds at different production sites, as there are differences between the profiles of volatile compounds and the region.

Keywords: traceability; chemical characterization; denomination of controlled origin.

Practical Application: The study provides information on the difference in volatile compounds profile in artisanal Minas cheese according to the region of origin. Therefore, this profile of compounds can contribute to traceability for the denomination of controlled origin in a process for artisanal Minas cheese, improving the economic value of these products, and ensuring authenticity for consumers.

1 INTRODUCTION

Artisanal Minas cheese (AMC) is a product produced from raw milk according to the determinations of Article 1 of State Law No. 14,185 of January 31, 2002 (Minas Gerais, 2002a). AMC has great historical and cultural value, as it is associated with a centuries-old tradition that has been passed on for generations. AMC supports the survival of families and rural communities that participate in cheese making and fosters the economy of several cities and regions of Minas Gerais, the state most involved in cheese production.

Currently, AMC is produced in 10 regions that are certified and recognized by the Instituto Mineiro de Agropecuária (IMA) (Minas Gerais, 2022), as supported by studies that evaluated environmental and geographic, cultural, historical, economic, and social factors in these regions. As AMC is a handmade product and no legally defined characterization exists, AMC products in the market are not uniform, even when they originate from the same region. In addition, the final characteristics of the product are influenced by physical factors associated with where the cheese is produced (*terroir)*, and these factors include the endogenous microbiota of the milk, humidity and altitude, and type of pasture provided to the herd (Kamimura et al., 2019).

In 2002, the State Institute of Historical and Artistic Heritage of the State of Minas Gerais registered the process of producing AMC as "Intangible Heritage of the State of Minas Gerais" (Minas Gerais, 2002a, 2002b). Later, in 2008, the Institute for National Artistic and Historical Heritage (IPHAN) approved the artisanal method of producing AMC to be registered as a Brazilian Intangible Heritage, which was requested in 2006; thus, the traditional method was recognized as a national cultural heritage title (IPHAN, 2006). Recognizing the traditions and production method of AMC is of utmost importance for strengthening this product, its regions, and the families involved in the process and adding value to these cheeses. However, although the manufacturing technology for obtaining AMC is well defined, the production process varies among producers due to differences in the composition of milk and lactic fermentation culture ("pingo"). Edaphoclimatic factors in each region can affect the final composition of the regions, as these factors favor the development of different microorganism species and

Received Dec. 9, 2022.
Accepted July 24, 2023.
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consequently influence the microbiological composition that is used during production, in addition to the process of maturing cheese (Kamimura et al., 2019). The types of microorganisms present in raw milk and lactic ferment culture generally originate from *Streptococcus*, *Lactococcus*, *Staphylococcus*, and *Lactobacillus* groups and play a key role in forming compounds responsible for the aroma, flavor, and texture characteristics of cheeses in different regions (Bank, 1998; Kamimura et al., 2019; Luiz et al., 2017; Montel et al., 2014; Pereira, 2019).

Thus, considering the influence of edaphoclimatic and microbiological factors on the final characteristics of cheeses in each region, studies that evaluate volatile components can provide guidelines regarding the chemical characterization of AMCs; these results assist the inspection and certification process. In addition, these studies facilitate the process of granting the geographical indication (GI) title once the influence of the production location and its particularities (*terroir)* on the chemical, physical, and sensory parameters of the final product is determined. Compounds present in pastures can be transferred to products of animal origin and differentiate the products based on the type of animal feed and production systems; thus, these compounds can provide guidelines for the location at which milk and its derivatives are produced, a concept based on labeling products with Denomination of Controlled Origin, as performed in Europe (Bonanno et al., 2013; Engel et al., 2007).

Knowing that the final characteristics of AMC are influenced by regional factors, this study aimed to evaluate the volatile compound profile of cheeses from seven regions to identify the presence of compounds that can help characterize and identify cheeses in relation to their origin, aiding in the process of certifying the origin of cheeses.

2 MATERIALS AND METHODS

2.1 Determination of the samples

For the study, 78 samples were collected of AMCs from producers in the seven regions recognized and certified for their production between the months of May and November 2019 (Serro – n=25; Canastra – n=19; Triângulo Mineiro – n=11; Cerrado – n=10; Serra do Salitre – n=6; Araxá – n=4; and Campo das Vertentes – n=3), with the assistance of the Technical Assistance and Rural Extension Company of Minas Gerais (EMATER-MG). The aim was to collect samples from producers registered with the IMA to ensure that the cheeses met the sanitary requirements, in addition to presenting their own production characteristics and the minimum ripening time indicated for each region, which was at least 14 days for the Araxá region, 17 days for Serro, and 22 days for the other regions (Minas Gerais, 2020).

Cheese samples from the Canastra, Serro, and Triângulo Mineiro regions were obtained from regional competitions promoted by the Government of Minas Gerais through EMATER-MG and IMA, in which a quarter of cheese was collected from each enrolled producer, individually packed in nontoxic and sterile polyethylene plastic packaging, externally identified with an adhesive label, and transported in an isothermal box with ice. For the other regions, the producers were asked to collect whole cheese from their production lot on the first day corresponding to the minimum ripening time indicated for each region. The samples were packed in a specific package corresponding to each producer, collected, and sent to the laboratory in an isothermal box with ice for analysis.

The variation observed in the total number of samples collected according to the region of origin was due to the number of producers that participated in each regional competition (Canastra, Serro, and Triângulo Mineiro) and the collaboration of producers in each region who provided a cheese sample for the research (Araxá, Cerrado, Campo das Vertentes, and Serra do Salitre).

2.2 Determination of the volatile compounds

The samples collected remained intact when evaluating the volatile compounds, were individually packaged in nontoxic, sterile polyethylene bags, and were stored in a freezer at -18°C. The samples were then thawed and fractionated only when prepared for analysis.

To determine the volatile compounds, 5 g of each sample was aliquoted, and these samples included the rind and interior of the cheeses, which were obtained through previous grinding. Later, the samples were fractioned into small portions and placed in 20-mL vials. The solid-phase microextraction (SPME) technique coupled with high-resolution gas chromatography/mass spectrometry (GC/MS) was used. The samples were incubated at 60°C for 10 min, and the volatile components were extracted by SPME using divinylbenzene/carboxen/polydimethylsiloxane fiber with a film thickness of 50/30 mm for 30 min. After the extraction was finished, the fiber was introduced into the injector of the gas chromatography equipment (SHIMADZU, GC/MS-QP2010) to separate and identify the volatile compounds with an automatic injector (SHIMADZU, AOC-5000) at 250°C in splitless injection mode and with a 31-mm injector. The chromatograph was equipped with a Slb-5MS capillary column (Supelco, 30 m × 0.25 mm × 0.25 μm) with initial and final temperatures of 40 and 230 °C, respectively. Helium was used as the carrier gas with a flow rate of 2 mL/min.

The results of the analysis were expressed as percentages of the area of each analyte present in the cheese samples. The compounds were identified by integrating the peaks generated in the chromatogram and the database of the GCSolution *software* (NIST, 2020) as a function of the mass spectrum. The volatile compounds present in the samples were also identified by comparing between the Kovats index values, which were calculated through the retention indices obtained experimentally for each compound, and those in the literature (Adams, 2007).

2.3 Statistical analysis

The averages of data obtained from the volatile compound profile of the cheeses from each region were used to evaluate the behavior of variables in relation to the regions through principal component analysis (PCA) in the R environment (R Team, 2019) and the FactoMineR package (Lê et al., 2008).

3 RESULTS AND DISCUSSION

The profile of volatile compounds identified in the analyzed AMC samples revealed several chemical groups, including 20 aldehydes, 17 ketones, 20 carboxylic acids, 6 terpenes, 57 esters, 15 hydrocarbons, and 31 alcohols (Table 1).

Among the compounds in the aldehyde group, benzeneacetaldehyde and 6-decenal were found in larger quantities in the cheeses from the Campo das Vertentes region and in the Canastra cheeses, respectively (Table 1). The aldehydes are compounds that mainly result from the proteolytic process, in

Table 1. Profile of volatile compounds of AMC from seven regions certified for AMC production.

Compounds	Regions						
	ARA (n=4)	CAN (n=19)	CER (n=10)	SEE (n=3)	SER (n=25)	SAL (n=6)	TMI (n=11)
Aldehydes							
(E)-2-hexenal	-	0.42±0.93	-	-	0.25±0.49	0.24±0.54	-
1,3-p-Menthadien-7-al	-	0.07±0.27	0.01±0.01	0.15±0.21	0.40±1.77	0.09±0.19	-
2,4-Hexadienal	2.18±3.07	0.57±0.97	3.50±6.42	0.03±0.04	0.20±0.59	-	0.29±0.91
2,4-Octadienal	-	0.07±0.20	-	-	0.06±0.26	-	-
2,6-Nonadienal	-	-	-	-	0.02±0.04	0.04±0.08	-
2-Methyl-2-(methyldithio)propanal	-	0.01±0.06	-	-	0.51±1.41	-	0.16±0.49
2-Octenal	-	2.55±7.81	0.97±1.93	-	1.57±3.16	0.03±0.07	0.03±0.09
3-Hexenal	-	0.08±0.24	-	-	0.09±0.42	-	-
3-Nonenal	-	0.07±0.17	1.85±4.10	0.10±0.13	1.13±2.34	-	0.86±2.70
6-Decennial	-	5.08±5.15	-	-	0.87±2.68	-	0.15±0.48
benzaldehyde	-	-	-	-	0.01±0.03	0.08±0.18	0.15±0.33
Benzeneacetaldehyde	-	2.54±8.93	2.18±2.75	6.26±8.85	1.33±2.42	0.91±1.98	-
Decadal	-	0.30±0.82	-	-	0.03±0.04	-	0.05±0.16
Heptanal	-	0.15±0.43	2.90±8.65	-	0.08±0.22	-	0.47±0.70
Hexanal	-	0.57±1.13	-	1.65±2.33	0.27±0.95	0.63±1.40	-
Methional	-	0.29±0.53	2.29±4.68	0.34±0.47	0.29±0.49	-	0.14±0.31
Nonanal	-	0.10±0.17	0.04±0.09	0.13±0.17	0.06±0.15	-	-
Perilla aldehyde	-	-	-	-	0.83±3.98	0.01±0.02	-
Tridecanal	-	-	-	0.44±0.34	0.02±0.07	0.01±0.02	-
Undecanaldehyde	-	0.70±1.39	-	0.04±0.05	0.59±2.76	-	-
Alcohols							
(E,Z)-3,6-nonadien-1-ol	-	4.51±2.98	3.77±4.75	7.36±1.85	2.18±2.97	-	2.06±4.37
(Z)-3-hexenol	-	1.94±2.86	0.19±0.21	-	0.41±1.45	-	-
2,3-Butanediol	-	-	-	-	0.17±0.59	-	-
2-Hexenol	-	0.01±0.02	-	-	0.07±0.17	-	0.19±0.59
2-Nonanol	-	-	-	-	0.11±0.40	1.15±2.57	1.41±3.81
2-Pentanol	-	0.02±0.07	-	-	0.02±0.05	-	-
2-Phenylethyl alcohol	1.70±2.94	0.16±0.40	0.36±1.07	-	0.56±1.26	0.45±1.00	1.04±1.83
3-Cyclohexen-1-ol,1-methyl	-	0.91±0.03	0.26±0.12	-	0.43±0.20	-	-
4-Methylhexanol	-	-	-	-	0.07±0.23	-	-
4-Penten-1-ol	-	0.57±2.40	-	-	0.33±1.11	-	-
5-Propylpentan-5-olide	-	-	-	-	0.25±0.88	0.23±0.50	-
Benzyl alcohol	-	-	0.30±0.75	-	0.23±0.69	-	0.29±0.93
Bornyl formate	-	-	0.02±0.05	-	0.10±0.35	0.15±0.34	0.01±0.04
Butanediol	0.62±1.07	1.37±2.15	0.01±0.03	-	0.50±0.83	0.02±0.04	-
Caprylate isoamyl	-	-	0.02±0.01	-	0.25±	0.06±0.01	0.02±0.01
Carveol	6.61±1.87	0.18±0.49	0.25±0.53	-	1.05±1.76	4.57±4.41	1.02±1.61
Decanol	-	0.01±0.03	-	-	0.41±1.05	-	-
Dihydrocarveol	-	-	0.14±0.28	-	0.64±1.29	1.97±4.39	1.59±3.85
Ethylphenol	-	-	0.01±0.01	-	0.01±0.02	-	-
Fufuryl mercaptan	-	0.11±0.46	-	-	0.47±2.15	-	0.51±1.62
Guaiacol	-	-	0.15±0.36	-	0.98±3.28	-	0.02±0.06
Heptanol	-	-	-	-	0.04±0.16	-	0.11±0.21

Continue...

Table 1. Continuation.

Compounds	Regions						
	ARA (n=4)	CAN (n=19)	CER (n=10)	SEE (n=3)	SER (n=25)	SAL (n=6)	TMI (n=11)
Hexanethiol	-	0.03±0.06	-	-	0.04±0.10	-	-
Hexanol 2-ethyl	-	-	-	3.86±1.93	0.69±0.02	0.17±0.02	-
Methoxymethylbutanethiol	-	-	0.49±1.47	-	0.07±0.19	-	0.10±0.31
Methyldihydrofuranthiol	-	0.33±0.90	-	-	0.16±0.56	-	-
Methyltetrahydrofuranthiol	-	0.09±0.26	0.15±0.31	-	1.66±4.93	-	0.16±0.35
Methyl-thiophenethiol	-	-	-	-	0.23±0.75	3.60±6.95	0.66±2.08
Nonan-2-ol	2.5±1.35	0.03±0.13	0.25±0.25	-	0.33±1.42	0.16±0.27	0.72±0.45
Norfuraneol	2.60±4.49	2.05±3.83	2.00±2.59	-	1.66±2.54	2.78±6.21	2.42±3.77
Phenethyl alcohol	2.68±3.10	-	0.48±0.57	0.15±0.21	1.48±2.97	9.19±10.43	0.22±0.50
Terpenes							
Camphene	-	0.09±0.29	-	-	0.07±0.18	-	-
Caryophyllene oxide	-	0.28±0.33	-	-	0.04±0.08	-	-
Limonene	0.28±0.49	-	0.40±0.41	-	0.54±0.98	0.92±1.91	1.32±1.68
α-Picoline	-	0.04±0.12	0.13±0.38	-	0.07±0.22	-	0.03±0.08
α-Pinene	-	0.46±1.35	-	-	0.16±0.51	-	-
α-Xylene	-	-	-	-	0.16±0.52	-	-
Ketones							
1,5-Octadienone	-	0.02±0.06	0.02±0.07	0.61±0.86	0.41±1.62	0.14±0.29	0.07±0.11
1-Cyclohexen-3-one	-	0.02±0.07	-	-	0.12±0.47	-	0.06±0.18
2-Hexanone	-	0.50±0.1	0.01±0.02	-	0.05± 0.02	-	-
8-Nonen-2-one	-	-	2.84±6.60	-	0.42±0.81	-	0.29±0.55
Benzothieno[2,3-C]quinolin-6(5H)-one,2-methoxy	-	1.61±3.76	0.25±0.17	-	0.22±1.05	-	0.41±0.83
Butanedione	-	-	-	-	0.57±0.2	-	-
Decalactone delta	-	-	0.17±0.18	-	0.04±0.15	0.08±0.17	0.31±0.63
Dimethyl sulfone	-	0.31±1.32	-	-	0.10±0.23	-	-
Ethylfuranone	-	-	1.12±3.33	-	1.12±3.09	0.49±0.45	0.10±0.23
Heptanone	-	0.99±4.05	9.10±10.36	-	2.02±5.60	-	0.08±0.20
Hydroxypentanone	-	0.62±0.84	0.20±0.27	0.35±0.26	0.55±0.80	0.48±0.87	0.18±0.38
Mercaptopentanone	-	0.01±0.03	0.57±1.15	0.39±0.55	0.37±1.49	-	3.39±7.34
Methylbutanone	-	1.01±2.85	0.01±0.02	-	0.77±2.33	-	-
Nonanone	2.11±1.15	-	-	-	0.08±0.24	-	0.65±1.33
Nonyl methyl ketone	-	0.10±0.24	-	-	0.02±0.04	-	-
Octenone	-	0.02±0.07	-	0.29±0.41	0.02±0.05	-	0.07±0.10
Undecanone	-	0.02±0.06	0.08±0.08	-	0.02±0.06	0.02±0.03	0.08±0.15
Carboxylic Acids							
2-Hydroxy-3-methylbutanoic acid	-	-	1.73±2.24	-	1.34±3.47	-	3.61±11.43
4-Methylhexanoic acid	-	2.60±8.20	0.74±1.39	-	0.63±2.09	-	-
9-Octadecenoic acid (Z)	-	0.17±0.20	-	-	0.02±0.05	0.07±0.22	-
Benzoic acid	-	-	1.00±3.01	0.01	1.18±3.60	-	-
Benzoic acid hexahydro	-	0.02±0.08	-	-	0.04±0.1	0.02±0.14	-
Butanoic acid, 3-methyl	-	0.15±0.43	0.02±0.06	-	0.13±0.24	-	0.05±0.15
Butyric acid	-	0.08±0.28	0.10±0.28	-	0.63±1.71	-	2.57±6.41
Caprylic acid	-	1.12±1.61	1.08±2.21	1.75	1.32±3.29	0.01±0.13	2.33±5.05
Decanoic acid	14.98±6.55	-	2.32±4.01	-	0.07±1.47	5.89±9.40	4.53±1.75
Hexanoic acid	-	1.03±4.24	2.38±3.72	-	0.70±2.27	-	0.07±0.22
Hexanoic acid,2-methylpropyl	-	-	0.03±0.04	-	0.02±0.08	-	-
Isobutyric acid	-	1.13±3.28	0.89±2.56	0.01	1.16±2.65	-	1.57±4.98
Lauric acid	-	0.02±0.06	-	0.15	0.19±0.35	0.28±2.21	-
Methyl octanoic acid	-	-	-	-	0.14±0.02	-	-
Methylbutyric acid	-	1.26±2.70	0.10±0.22	-	-	-	-
Nonanoic acid	-	-	-	-	0.04±0.02	0.02±0.03	-

Continue...

Table 1. Continuation.

Compounds	Regions						
	ARA (n=4)	CAN (n=19)	CER (n=10)	SEE (n=3)	SER (n=25)	SAL (n=6)	TMI (n=11)
Nonanoic acid, 5-hydroxy-	-	-	-	-	0.13±0.40	-	-
Octanoic acid	1.49±2.58	0.28±0.88	0.46±1.38	4.28	0.42±1.15	-	1.53±3.09
Pelargonic acid	-	-	0.08±0.13	0.04	0.28±0.76	0.01±0.2	0.04±0.13
Propanoic acid, 2-methyl-3-methylbutyl	-	-	1.96±2.38	-	1.17±3.67	3.98±0.46	-
Esters							
(Z)-3-hexenyl butanoate	-	-	0.01±0.02	-	0.09±0.01	-	-
(Z)-3-hexenyl-2-methylbutanoate	-	0.52±0.03	0.32±0.12	1.51±0.23	0.82±1.02	0.52±0.32	1.39±1.02
2-Methylpropyl octanoate	-	-	-	-	0.01±0.04	-	-
2-Phenylethyl propanoate	-	-	-	-	0.04±0.09	-	-
4-(5,7-Dichloro-1,3-benzoxazol-2-yl)sulfanyl-methylphenyl 1,1,2,2-Tetrafluoroethyl ester	-	-	0.29±0.39	-	-	-	0.14±0.29
Acetic acid, 2-phenylethyl ester	-	0.03±0.10	-	-	0.46±2.01	-	-
Benzyl acetate	-	-	0.69±0.34	-	0.13±0.06	0.02±0.01	-
Butanoic acid, 2-ethyl-, 1,2,3-propanetriyl ester	-	0.43±1.83	2.89±5.80	-	1.02±3.13	-	2.59±8.20
Butanoic acid, ethyl ester	-	-	0.20±0.34	0.96±1.35	0.25±0.83	-	0.19±0.40
Butanoic acid, propyl ester	-	-	-	-	0.06±0.17	-	-
Butyl acetate	2.77±4.79	0.48±2.02	0.63±1.20	-	0.18±0.73	3.96±6.67	0.27±0.67
Butyl isothiocyanate	-	0.05±0.09	-	-	0.02±0.03	-	-
Butyl octanoate	-	-	-	-	0.02±0.05	-	-
Isoamyl caproate	-	-	-	-	0.02±0.06	-	0.03±0.08
Caproate propyl	-	0.36±0.67	0.03±0.08	0.25±0.34	0.77±2.58	-	-
Ethyl capronate	-	3.02±2.78	0.50±1.27	1.28±0.94	0.81±1.30	-	0.19±0.61
Carbamic acid, ethyl ester	-	1.56±4.72	0.76±0.74	-	0.20±0.53	-	-
Ethyl decanoate	-	-	-	-	0.33±1.48	-	0.06±0.15
Decanoic acid, ethyl ester	-	-	-	-	0.07±0.19	-	0.10±0.20
Diethyl succinate	-	2.15±0.20	1.13±0.30	-	1.09±0.79	0.01±0.02	-
Ethyl 3-hydroxybutanoate	-	0.47±1.42	-	-	0.27±0.64	-	-
Ethyl 3-methylbutanoate	-	0.11±0.04	-	-	0.29±0.03	-	-
Ethyl butyrate	3.30±5.70	0.07±0.24	0.27±0.33	-	0.10±0.25	0.67±1.27	0.45±0.64
Ethyl heptylate	-	-	-	-	0.02±0.01	-	-
Ethyl hydroxybutanoate	-	0.31±0.74	-	-	0.12±0.59	-	0.71±2.02
Ethyl isohexanoate	-	0.01±0.08	0.32±0.51	0.07±0.05	0.05±0.03	0.30±0.38	0.51±0.21
Ethyl mercaptopropionate	-	0.08±0.21	0.05±0.10	-	0.26±0.44	-	-
Ethyl octenoate	-	1.54±3.61	0.29±0.60	-	1.92±4.38	0.92±1.70	0.74±1.44
Ethylhydroxyhexanoate	-	0.02±0.01	-	-	-	-	-
Hexadecanoic acid, ethyl ester	-	-	0.01±0.01	-	0.30±1.40	0.03±0.07	0.04±0.09
Isobutyl hexanoate	-	-	-	0.11±0.12	0.12±0.30	-	0.02±0.05
Hexyl octanoate	1.59±1.08	-	0.54±0.26	-	0.03±0.11	-	0.27±0.26
Isoamyl n-butyrate	-	1.19±5.06	1.40±2.25	-	1.08±2.94	0.93±1.20	2.77±6.45
Isobutyl decanoate	-	-	-	-	0.01±0.03	-	-
Isobutyrate< isopropyl->	0.51±0.46	1.34±1.11	0.09±0.08	-	0.13±0.35	0.06±0.08	0.05±0.12
Isobutyrate isoamyl	0.94±1.63	-	-	-	-	-	0.14±0.29
Isopentyl 2-methylbutanoate	-	-	-	-	0.05±0.15	-	0.02±0.07
Isopropyl hexanoate	2.27±3.93	0.40±1.07	-	-	0.55±1.37	-	-
Isopropyl octanoate	-	-	2.24±6.68	-	-	-	-
Methyl benzoate	-	0.06±0.17	-	-	0.02±0.07	-	-
Methyl cyclohexanecarboxylate	-	-	-	-	-	0.01±0.03	-
Methyl laurate	-	0.64±0.23	-	0.55±0.76	0.08±0.15	-	-
Methyl nonanoate	-	1.06±0.13	0.17±0.32	-	0.17±0.03	1.77±1.00	0.07±0.23
Methyl octanoate	-	0.33±0.97	0.41±0.76	-	0.29±0.65	-	-
Methyl-2-(methylthio)acetate	-	0.19±0.76	0.68±2.00	0.07±0.09	4.32±8.30	-	4.05±8.96

Continue...

Table 1. Continuation.

Compounds	Regions						
	ARA (n=4)	CAN (n=19)	CER (n=10)	SEE (n=3)	SER (n=25)	SAL (n=6)	TMI (n=11)
Octyl acetate	-	-	-	-	0.16±0.12	-	0.08±0.01
Pentyl butanoate	-	-	2.84±2.63	-	0.03±0.09	0.35±0.37	1.21±2.11
Pentyl methylbutyrate	-	0.03±0.10	-	-	0.01±0.04	-	-
Phenethyl2-methylpropanoate	-	0.01±0.02	-	0.10±0.14	0.03±0.09	-	0.01±0.04
Phenethyl acetate <2->	-	-	-	-	0.04±0.12	-	-
Propane, 1,2-dimethoxy	-	-	0.13±0.24	0.89±1.25	0.24±0.70	2.31±3.22	0.46±0.96
Propanoic acid, 2-methyl-, ethyl ester	-	-	-	-	0.03±0.09	-	-
Propyl butyrate	-	0.02±0.04	0.03±0.10	-	0.30±0.85	0.02±0.03	0.11±0.24
Propyl decanoate	1.97±0.74	-	0.13±0.17	-	-	0.45±0.35	0.41±0.36
Propyl propanoate	-	0.33±0.86	0.34±1.00	-	0.66±1.38	2.17±2.18	0.04±0.11
S-(2-furfuryl)-ethanethioate	-	0.02±0.05	0.04±0.01	-	0.01±0.01	-	0.17±0.03
β-Phenethyl acetate	-	-	-	-	0.34±0.92	-	0.02±0.07
Hydrocarbons							
Benzene ethenyl	-	0.04±0.15	0.18±0.55	-	0.35±0.76	0.14±0.31	-
Bicyclo[7,2,0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (Z)-(1R,9S)	-	0.02±0.07	-	-	0.01±0.02	-	-
Cyclopentane	-	0.03±0.43	-	-	0.48±1.59	0.08±0.01	-
Docosane	-	0.02±1.01	-	-	0.03±0.01	-	-
Dodecane	0.68±0.32	-	1.47±1.16	4.04±3.01	0.18±0.20	0.84±0.29	-
Eicosane	-	0.89±0.82	0.29±0.14	1.30±0.48	0.45±0.08	-	1.17±1.14
Hexadecane	-	0.02±0.11	0.01±0.02	0.09±0.12	0.31±0.20	0.01±0.02	-
Methane	-	-	-	-	0.13±0.11	-	0.02±0.01
Methyldithiofurane	-	1.42±1.01	0.68±0.54	-	0.41±0.03	1.39±1.05	0.69±0.40
Nitro-phenylethane	-	0.10±0.14	1.95±1.05	0.17±0.21	0.68±0.23	0.11±0.16	0.37±0.20
Pentadecane	4.59±2.01	0.25±0.11	-	-	0.15±0.43	-	-
Styrene	6.61±6.35	0.60±0.61	1.72±4.43	0.66±0.90	1.05±1.60	0.40±0.60	4.19±4.90
Tetradecane	-	0.52±2.21	0.19±0.60	0.03±0.04	0.73±1.96	-	-
Trans-sabinene hydrate	-	0.01±0.03	0.15±0.14	0.03±0.05	0.56±2.16	0.14±0.30	0.47±1.09
Tridecane	-	0.01±0.04	-	-	0.04±0.06	-	-

Results refer to the peak area as a percentage of the analyzed samples; ARA: Araxá; CAN: Canastra; CER: Cerrado; VER: Campo das Vertentes; SER: Serro; SAL: Serra do Salitre; TMI: Triângulo Mineiro.

which amino acids derived from the breakdown of large peptides by the enzymatic action of the coagulation agent are catabolized by proteinases and peptidases in lactic bacteria to aldehydes and other compounds, such as alcohols, amines, and carboxylic acids (McSweeney & Sousa, 2000). According to Curioni and Bosset (2002), aldehydes are transient compounds in cheese, as they are reduced to primary alcohols or oxidized to carboxylic acids. This may explain the absence of benzeneacetaldehyde and 6-decenal compounds in some regions evaluated in this study, in addition to the low percentages generally observed for the compounds in this group within other regions where these aldehydes were not found.

The Araxá region showed higher values for the alcohol group in relation to the compounds nonan-2-ol and carveol compared with that of other regions. The phenethyl alcohol compound was higher in Serra do Salitre and was not identified in Canastra cheeses. The hexanol 2-ethyl compound was found in larger quantities in cheeses from the Campo das Vertentes region, and it was not found in cheeses from other regions. Serra do Salitre differed from the other regions in contrast to methyl-thiophenethiol, which was higher. Campo das Vertentes, in turn, showed a higher percentage value compared to that in other regions with regard to the compound (E,Z)-3,6-nonadien-1-ol, which was also identified in the Canastra, Cerrado, and Serro regions but was not observed in Araxá and Serra do Salitre. The compound 5-propylpentan-5-olide was identified only in cheeses from the Serro and Serra do Salitre regions (Table 1).

The compounds in the alcohol chemical group are formed by proteolysis, as occurs for aldehydes, are derived from these groups through reduction, or are produced by lipolysis from the degradation of free fatty acids. Lipolysis, as well as secondary proteolysis, is performed by lactic bacteria enzymes, which are present in milk and/or the culture, and the action of these enzymes on the free fatty acids triggers reactions that produce secondary alcohols such as nonan-2-ol (Dransfield, 2008; McSweeney & Sousa, 2000). Thus, among the cheeses from the evaluated regions, the variation observed in the compounds from the alcohol group may mainly reflect the enzymatic activity of the lactic bacteria during the ripening process, which is influenced by the microbiological composition of the natural culture ("pingo") and/or by more favorable conditions for these enzymes to function during the ripening process (Delgado et al., 2010; Pereira, 2019).

In this study, six compounds belonging to the terpene group were identified. Compounds such as camphene, caryophyllene-oxide, and α-pinene were identified only in Canastra and Serro cheeses, which may be possible plant markers for cheeses from these regions, as well as the compound α-xylene for Serro cheeses, as it was not identified in the other regions. The cheeses from the Triângulo Mineiro showed the highest percentage of limonene, a compound that showed the highest values in the terpene group, and this was not observed in the Canastra and Campo das Vertentes regions (Table 1).

Terpenes are volatile compounds derived from the metabolism of plants available in pastures, mainly dicotyledons, and have been used as plant markers to trace the origin of products with protected designation of origin (PDO) or GI; this is because the type of food ingested by an animal can be identified through the presence of terpenes in animal products (Kalac, 2011). In addition, Bosset et al. (1994) compared *Gruyère* cheeses produced with milk from cows raised in highland fields with those with milk from cows raised in lowland fields, and it was observed that the products from highland fields exhibited a higher concentration and variety of terpenes. This information is an important parameter when evaluating the geographic origin of cheeses.

The Canastra region presents altitude variations of up to 1,485 m (Kamimura et al., 2019), which is the highest among the seven regions evaluated in this study, which may help explain the presence of camphene, caryophyllene-oxide, and α-pinene in cheeses from this region; these compounds were absent in the others cheeses except for Serro. The presence of these compounds in Serro's cheeses may be related to a greater biodiversity of native pastures, as well as a greater presence of dicotyledons in the pastures, as the average altitude of this region (900 m) is lower than that of Canastra and other regions, such as Araxá (1,135 m), Cerrado (975 m), and Serra do Salitre (1,200 m). This can also explain the higher percentage of limonene compounds observed in cheeses from Triângulo Mineiro (900 m). In contrast, the cheeses from Campo das Vertentes did not contain the terpenic compounds identified in this study, and this absence may be related to the composition of the pasture, in addition to the lower average altitude of this region, which ranges from 400 to 1,300 m (Kamimura et al., 2019).

The ketone compounds that occurred more often in this study were 8-nonen-2-one and nonanone. Compound 8-nonen-2-one was found in larger quantities in cheeses from the Cerrado region, while nonanone, in turn, presented a higher percentage in the Araxá region. However, the butanedione compound was observed only in cheeses from the Serro region (Table 1). Ketones are compounds derived from the decarboxylation of free fatty acids during the process of lipolysis, which is caused by lactic bacteria, as mentioned above. The amount of ketonic compounds in cheeses depends mainly on the concentration of fatty acids available in the cheese matrix (McSweeney & Sousa, 2000), and the greater presence of 8-nonen-2-one and nonanone compounds in cheeses from the Cerrado and Araxá regions, respectively, suggests that the products from these regions contained a higher concentration of fatty acids in relation to that of the others. When comparing the aromatic compounds of *Ragusano* cheese from cows fed on a pasture and cows fed with mixed rations, Carpino et al. (2004) observed that the cheese from animals fed on native pasture exhibited higher concentrations of fatty acids and, consequently, ketone compounds.

Among the carboxylic acids identified in this study, decanoic acid was notable, as these compounds exhibited the highest percentage in cheeses from the Araxá region and were not observed in Canastra and Campo das Vertentes. In addition to decanoic acid, the carboxylic acids hexanoic acid, 2-hydroxy-3-methylbutanoic acid, and octanoic acid presented the highest percentages in these cheeses. Hexanoic acid presented the highest percentage in the Cerrado cheeses, 2-hydroxy-3-methylbutanoic acid was more prevalent in Triângulo Mineiro and Cerrado cheeses, and octanoic acid was identified in a higher percentage in Campo das Vertentes cheeses. When present in high concentrations, the compounds in the carboxylic acid group mentioned above can be associated with the rancid taste in animal products, such as meat and cheese; in addition, when present in desirable amounts, these compounds are responsible for the characteristic aroma of some cheeses, such as provolone and *Roquefort* (Costa et al., 2009; Donadel, 2013; Perry, 2004). The nonanoic acid 5-hydroxy compound was identified only in Serro cheeses. The presence of fatty acids in cheeses is mainly due to the lipolysis of triglycerides, which occurs by the action of lipolytic enzymes in lactic bacteria present in the culture and milk, and their concentrations are directly related to the fat content and its composition in the milk (McSweeney & Sousa, 2000; Pereira, 2019).

Regarding the ester grouping, the main compounds observed were capronate ethyl, isoamyl n-butyrate, pentyl butanoate, and carbamic acid ethyl ester (Table 1). The capronate ethyl compound showed a higher percentage in the Canastra cheeses compared to the cheeses from the other regions. The compound isoamyl n-butyrate showed a higher percentage value in the Triângulo Mineiro and was not identified in cheeses from the Araxá and Campo das Vertentes regions. The pentyl butanoate compound presented the highest value for the Cerrado cheeses and was not identified in the Araxá, Canastra, and Campo das Vertentes cheeses. The Canastra and Cerrado cheeses showed a higher percentage of carbamic acid ethyl ester compared with that of the other regions, in which only the Serro cheeses contained the same compound. Furthermore, 2-phenylethyl propanoate and butanoic acid propyl ester were identified only in the Serro cheeses, as well as ethylhydroxyhexanoate for the Canastra cheeses and isopropyl octanoate for those from the Cerrado.

Esters are the most identified volatile compounds in cheeses and are formed by esterification reactions between medium- and short-chain fatty acids and primary and secondary alcohols from glycolysis and proteolysis processes, which occur during ripening. In this process, the availability of alcohols is a limiting factor for ester production (Marilley & Casey, 2004; McSweeney & Sousa, 2000). The higher percentages of compounds in the ester chemical group in cheeses from the Canastra, Triângulo Mineiro, and Cerrado regions can be associated with the lower presence or absence of some compounds in the alcohol group observed in this study for cheeses from the same regions. This is because these compounds participate in the esterification process, reflecting a higher concentration of esters and a lower concentration of alcohols in cheeses from these regions. Delgado et al. (2010) analyzed the profile of volatile compounds in Spanish cheeses made with raw milk and observed a considerable increase in ester group compounds throughout ripening, which was associated with a decrease in alcohol and carboxylic acid group compounds. This was also observed for cheeses from Canastra, Triângulo Mineiro, and Cerrado in this study, indicating that a more advanced ripening process occurs in these regions.

Regarding hydrocarbons, the compounds styrene and dodecane were identified in higher percentages in the cheeses from the regions evaluated. Styrene presented the highest values in cheeses from Araxá and Triângulo Mineiro regions. The dodecane compound was identified in higher concentrations in Campo das Vertentes cheeses but was not identified in Canastra and Triângulo Mineiro cheeses. Furthermore, it was possible to observe that the compound methane was only identified in the Serro region and the nitro-phenylethane was identified in all regions evaluated except Araxá. Hydrocarbons are precursors for the formation of other volatile compounds and generally do not exhibit a considerable influence on the formation of aroma in cheeses, which originate from animal feed or products of lipid autooxidation (Bontinis et al., 2012).

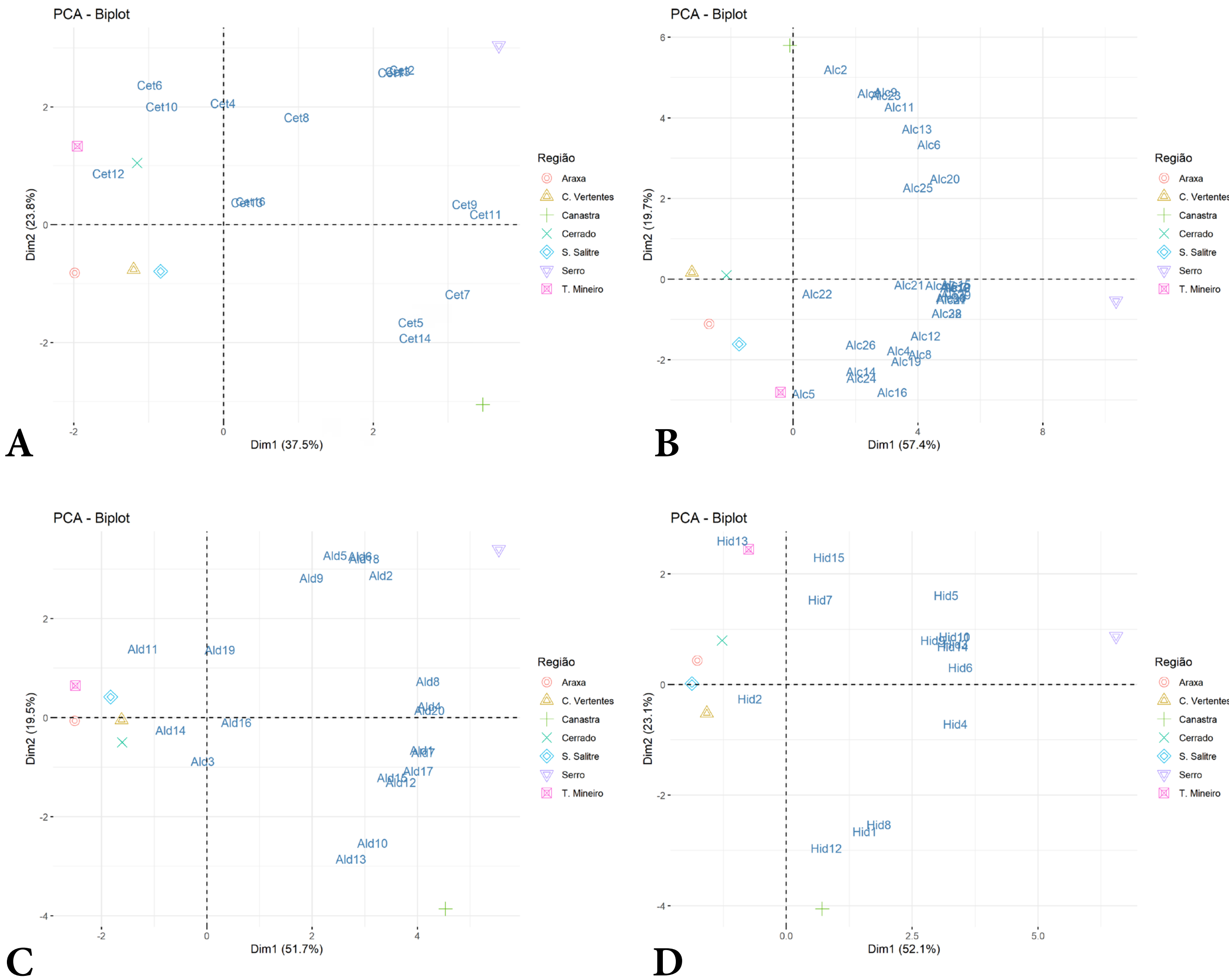
PCA for the group of ketone compounds is shown in Figure 1A. The Serro region differed from the others with respect to the compounds hydroxypentanone (Cet9) and methylbutanone (Cet11) in the Dim1 axis in the positive quadrant and the compound 1-cyclohexen-3-one (Cet2), which corresponded to the greatest influence in the Dim2 axis and the positive quadrant. The Serro region was notable for exhibiting the highest levels of these compounds (Table 1). The cheeses from the Triângulo Mineiro and Cerrado regions behaved similarly in relation to the nonanone compound (Cet12). The cheeses from Araxá, Campo das Vertentes, and Serra do Salitre were similar in relation to the negative quadrant of the Dim2 axis and showed no specific relationship with any of the ketone compounds identified in this study. The Canastra cheeses showed a relationship with the compounds 2-hexanone (Cet14), benzothieno[2,3-C]quinolin-6(5H)-one,2-methoxy (Cet5), and dimethyl-sulfone (Cet7), which were positively related to the Dim1 axis in relation to the higher values of these compounds (Table 1).

Regarding alcohols (Figure 1B), it was possible to observe that the cheeses from the Serro region behaved differently from the other regions because they contained higher quantities of the compounds 2-3-butanediol (Alc3), 2-pentanol (Alc7), 4-methylhexanol (Alc10), decanol (Alc15), and guaiacol (Alc18) compared with that of the other regions. The Canastra region was also different from the other regions and was mainly influenced by the amount of compound Z-(3)-hexenol (Alc2) (Figure 1B and Table 1). The Campo das Vertentes, Araxá, Cerrado, and Serra do Salitre regions showed similar behavior when the negative axis of Dim1 was evaluated, which may be due to the low contents of compounds that influenced the same axis. The cheeses from the Triângulo Mineiro region, in turn, showed a higher relation with the compound 2-nonanol (Alc5). The greater similarity of the cheeses from Araxá and Serra do Salitre can be justified because these cheeses presented higher levels of carveol and phenethyl alcohol compounds compared with that of the others, in addition to the absence of the compound (E,Z)-3,6-nonadien-1-ol in both (Table 1).

The cheeses from the Araxá, Campo das Vertentes, Triângulo Mineiro, Serra do Salitre, and Cerrado regions showed similar behavior in relation to the aldehyde group (Figure 1C). The similarity between these regions can be explained by the low percentage or absence of compounds from this chemical group observed in the cheeses of the regions evaluated in this study. The reduced incidence of aldehydes in mature cheeses occurs because these compounds are quickly reduced to alcohols or oxidized to carboxylic acids (Curioni & Bosset, 2002). The cheeses from the Serro and Canastra regions behaved differently when the Dim2 axis was evaluated, and this behavior was influenced expressively by the compounds 2-6-nonadienal (Ald5), 2-methyl-2-methyldithiopropanal (Ald6), and perilla-aldehyde (Ald18), which were more related to the Serro region, and the compounds 6-decenal (Ald10) and decadienal (Ald13), which related to the Canastra region.

The cheese samples from the Cerrado, Araxá, Serra do Salitre, and Campo das Vertentes regions presented similar hydrocarbon compositions, mainly in relation to the negative axis of Dim1, which is related to the dodecane compound (Hid2) (Figure 1D). Among these regions, the cheeses from Campo das Vertentes showed a higher relation with the dodecane compound (Hid2) due to the presence of a higher percentage (Table 1). The cheeses from the Canastra and Serro regions also behaved divergently in relation to the hydrocarbon chemical groups, as well as to the aldehydes group. The Canastra cheeses showed a higher relation with the compound styrene (Hid12) in the negative quadrant of Dim2, while the Serro cheeses were influenced by the Compounds pentadecane (Hid3), tetradecane (Hid4), tridecane (Hid6), cyclopentane (Hid10), and eicosane (Hid11) in the positive quadrant of Dim1. The cheeses from the Triângulo Mineiro region were also different from the others and were strongly influenced by the compound docosane (Hid13). The variation in the behavior of the regions in relation to hydrocarbons may be mainly a reflection of the variability and concentration of compounds in this group that are available in pastures, in addition to the process of lipid autooxidation (Bontinis et al., 2012).

Regarding the terpene profile in the cheese samples from the different regions, the terpenes limonene (Ter3) and α-pinene (Ter6) explained why the regions were positioned in the negative quadrant of Dim2, which showed a greater relation with the cheeses from Serra do Salitre, Cerrado and Triângulo Mineiro (Figure 2A). A divergent behavior was observed among compounds present in cheeses from the Serro and Canastra regions in contrast to the others. In Serro, notably, the cheeses were a function of the compounds α-picoline (Ter4), α-xylene (Ter5), limonene (Ter3), and α-pinene (Ter6), while for the Canastra region, the difference was a function of the compound caryophyllene-oxide (Ter2). The cheeses from Araxá and Campo das Vertentes were not influenced by any of the terpenic compounds, as these compounds were not identified in the cheeses from these

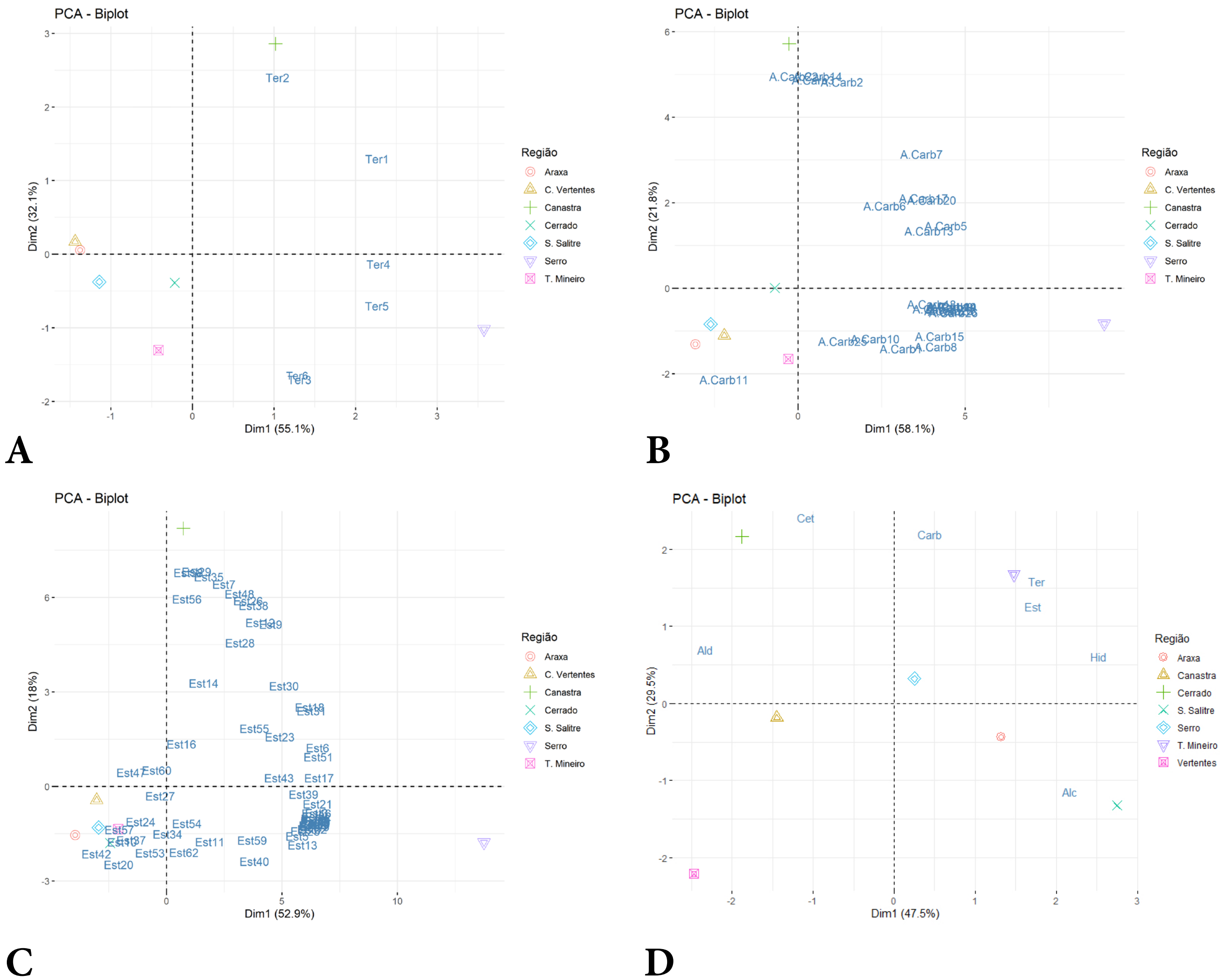


(A) Cet1: 1-5-octadienone; Cet2: 1-cyclohexen-3-one; Cet3: undecanone; Cet4: 8-nonen-2-one; Cet5: benzothieno-2-3-cquinolin-65 h-one-2-methoxy; Cet6: butanedione; Cet7: dimethyl-sulfone; Cet8: heptanone; Cet9: hydroxypentanone; Cet10: mercaptopentanone; Cet11: methylbutanone; Cet12: nonanone; Cet13: octenone; Cet14: 2-hexanone; Cet15: ethylfuranone; Cet16: nonyl-methyl-ketone; Cet17: decalactone delta. (B) Alc1: E-Z-3-6-nonadien-1-ol; Alc2: Z-(3)-hexenol; Alc3: 2-3-butanediol; Alc4: 2-hexenol; Alc5: 2-nonanol; Alc6: 2,3-butanediol; Alc7: 2-pentanol; Alc8: 2-phenylethyl-alcohol; Alc9: 3-cyclohexen-1-ol-1-methyl; Alc10: 4-methylhexanol; Alc11: 4-penten-1-ol; Alc12: benzyl-alcohol; Alc13: butanediol; Alc14: carveol; Alc15: decanol; Alc16: dihydrocarveol; Alc17: ethylphenol; Alc18: guaiacol; Alc19: heptanol; Alc20: hexanethiol; Alc21: hexanol 2-ethyl; Alc22: methoxymethylbutanethiol; Alc23: methyldihydrofuranthiol; Alc24: nonan-2-ol; Alc25: norfuraneol; Alc26: phenethyl-alcohol; Alc27: 5-propylpenthan-5-olide; Alc28: bornyl-formate; Alc29: caprylate-isoamyl; Alc30: fufuryl-mercaptan; Alc31: methyltetrahydrofuranthiol. (C) Ald1: E-2-hexenal; Ald2: 1-3-p-menthadien-7-al; Ald3: 2-4-hexadienal; Ald4: 2-4-octadienal; Ald5: 2-6-nonadienal; Ald6: 2-methyl-2-methyldithiopropanal; Ald7: 2-octenal; Ald8: 3-hexenal; Ald9: 3-nonenal; Ald10: 6-decenal; Ald11: benzaldehyde; Ald12: benzeneacetaldehyde; Ald13: decadienal; Ald14: heptanal; Ald15: hexanal; Ald16: methional; Ald17: nonanal; Ald18: perilla-aldehyde; Ald19: tridecanal; Ald20: undecanaldehyde. (D) Hid1: bicyclo-7-2-0undec-4-ene-4-11-11-trimethyl-8-methylene--Z-1R-9S; Hid2: dodecane; Hid3: pentadecane; Hid4: tetradecane; Hid5: trans-sabinene-hydrate; Hid6: tridecane; Hid7: hexadecane; Hid8: methane; Hid9: methyldithiofurane; Hid10: cyclopentane; Hid11: eicosane; Hid12: styrene; Hid13: docosane; Hid14: benzene-hethenyl; Hid15: nitro-phenylethane.

Figure 1. Principal component analysis of (A) ketone, (B) alcohol, (C) aldehyde, and (D) hydrocarbon groups referring to the volatile compound profile of artisanal Minas cheese from seven regions certified for artisanal Minas cheese production.

regions (Table 1). This behavior can be justified by the lower average altitudes in these regions in relation to the others, especially in the Campo das Vertentes region, which directly influences the variety and concentration of terpenes in the pasture.

Regarding carboxylic acids (Figure 2B), the cheeses from the Serra do Salitre, Araxá, Campo das Vertentes, and Triângulo Mineiro regions showed similar behavior that was related to higher concentrations of decanoic acid (A. Carb11) (Table 1). The cheeses from the Serro region showed a high relation with the compounds benzoic acid (A. Carb4), butanoic acid 3-methyl (A. Carb9), nonanoic acid (A. Carb12), hexanoic acid 2-methylpropyl (A. Carb16), and hexanoic acid (A. Carb19), while the Canastra region cheeses exhibited a characteristic influenced by the compounds lauric acid (A. Carb18), 9-octadecenoic acid-(Z) (A. Carb3), pelargonic acid (A. Carb14), and 4-methylhexanoic acid (A. Carb2), which resulted in a high value on the positive axis of Dim2 and a negative value on the Dim1 axis.



(A) Ter1: camphene; Ter2: caryophyllene-oxide; Ter3: limonene; Ter4: α-picoline; Ter5: α-xylene; Ter6: α-pinene. (B) Carb1: 2-hydroxy-3-methylbutanoic acid; A. Carb2: 4-methylhexanoic acid; A. Carb3: 9-octadecenoic acid (Z); A. Carb4: benzoic acid; A. Carb5: benzoic acid hexahydro; A. Carb6: methyl octanoic acid; A. Carb7: methylbutyric acid; A. Carb8: nonanoic acid, 5-hydroxy; A. Carb9: butanoic acid 3-methyl; A. Carb10: butyric acid; A. Carb11: decanoic acid; A. Carb12: nonanoic acid; A. Carb13: caprylic acid; A. Carb14: pelargonic acid; A. Carb15: octanoic acid; A. Carb16: hexanoic acid, 2-methylpropyl; A. Carb17: propanoic acid, 2-methyl-3-methylbutyl; A. Carb18: lauric acid; A. Carb19: hexanoic acid; A. Carb20: isobutyric acid. (C) Est1: 2-methylpropyl-octanoate; Est2: 2-phenylethyl-propanoate; Est3: acetic acid-2-phenylethyl-ester; Est4: butyl-octanoate; Est5: caproate-isoamyl; Est6: caproate-propyl; Est7: capronate-ethyl; Est8: decanoate-ethyl; Est9: ethyl-3-hydroxybutanoate; Est10: ethyl-butyrate; Est11: butanoic-acid-2-ethyl-1-2-3-propanetriyl; Est12: butanoic acid, ethyl ester; Est13: butanoic acid, propyl ester; Est14: butyric acid, propyl ester; Est15: butanoic acid, ethyl ester; Est16: butyric acid, propyl ester butanoic acid, propyl ester; Est14: ethyl-hydroxybutanoate; Est15: ethylhydroxyhexanoate; Est16: ethyl-isohexanoate; Est17: ethyl mercaptopropionate; Est18: ethyl octenoate; Est19: hexanoate isobutyl; Est20: hexyl octanoate; Est21: isobutyl decanoate; Est22: isopentyl 2-methylbutanoate; Est23: isopropyl hexanoate; Est24: isopropyl octanoate; Est25: methyl-2-(methylthio)acetate; Est26: methyl benzoate; Est27: carbamic acid, ethyl ester; Est28: hexadecanoic acid, ethyl ester; Est29: methyl laurate; Est30: methyl octanoate; Est31: (Z)-3-hexenyl butanoate; Est32: hexyl octanoate; Est33: (Z)-3-hexenyl-2-methylbutanoate; Est34: 4-(5,7-dichloro-1,3-benzoxazol-2-yl)sulfanyl-methylphenyl 1,1,2,2-tetrafluoroethyl ester; Est35: pentyl methylbutyrate; Est36: benzyl acetate; Est37: pentyl-butanoate; Est38: decanoic acid, ethyl ester; Est39: phenetyl-2-methylpropanoate; Est40: diethyl succinate; Est41: propanoic acid, 2-methyl ethyl ester; Est42: propyl decanoate; Est43: propyl propanoate; Est44: ethyl 3-methylbutanoate; Est45: β-phenethyl acetate; Est46: ethyl heptylate; Est47: butyl acetate; Est48: butyl isothiocyanate; Est49: methyl cyclohexanecarboxylate; Est50: methyl nonanoate; Est51: isopropyl hexanoate; Est52: isobutyrate isopropyl; Est53: octyl acetate; Est54: S-(2-furfuryl)-ethanethioate; Est55: isoamyl-N-butyrate; Est56: propyl butyrate; Est57: isobutyrate-isoamyl. (D) Ald: aldehyde; Cet: ketone; Carb: carboxylic acid; Ter: terpene; Est: ester; Hid: hydrocarbon; Alc: alcohol.

Figure 2. Principal component analysis of (A) terpenes, (B) carboxylic acids, (C) esters, and (D) chemical groups referring to the profile of volatile compounds in artisanal Minas cheese from seven regions certified for its production.

The ester grouping showed the highest number of identified volatile compounds (Figure 2C), corresponding to the 70.9% variation observed in the cheese samples from the different regions with respect to these compounds. The compounds ethyl butyrate (Est10), hexyl octanoate (Est20), propyl decanoate (Est42), and isobutyrate isoamyl (Est57) influenced the Campo das Vertentes, Araxá, Serra do Salitre, Triângulo Mineiro, and Cerrado regions, which presented similar behavior among themselves. The similarity of these regions is also related to the compounds isopropyl-octanoate (Est24) and pentyl-butanoate (Est37). The ethyl-butyrate, hexyl-octanoate, propyl-decanoate, and pentyl-butanoate esters were identified in the cheeses from most regions studied, while the isopropyl-octanoate compound was found only in the Cerrado region, and isobutyrate-isoamyl was identified in the cheeses from Araxá and Triângulo Mineiro. The cheeses from the Serro and Canastra regions showed different behavior from each other and from the cheeses from the other regions. The behavior of the Serro cheeses was related to the compounds caproate-propyl (Est6), ethyl-mercaptopropionate (Est17), isobutyl-decanoate (Est21), hexyl octanoate (Est32), and isopropyl hexanoate (Est51), which most explained the positive quadrant of the Dim1 axis, indicating that the Serro cheeses contained higher contents of these compounds. The behavior of the cheeses from the Canastra region, in turn, was related to the compounds methyl-laurate (Est29), pentyl-methylbutyrate (Est35), and isobutyrate-isopropyl (Est52), which showed greater influence on the behavior of the regions in relation to the positive axis of Dim2; thus, it is possible to infer that in Canastra, a higher percentage of these compounds were observed.

Figure 2D shows the PCA for the regions and chemical groups of volatile compounds identified in this study, which explained 77% of the variation in the regions' behavior. It was possible to observe that the regions exhibited divergence with respect to the groups of volatile compounds, as their positions in the PCA plot were dispersed. The characteristics of cheese from the Serra do Salitre and Araxá regions were strongly influenced by the alcohol group (Alc), unlike the Cerrado region, which, in turn, was more influenced by the aldehyde group (Ald) and ketonic compounds (Cet). The samples from the Triângulo Mineiro and Serro regions were similar and related to the groups of carboxylic acids (Carb), hydrocarbons (Hid), terpenes (Ter), and esters (Est), a behavior opposite to the Campo das Vertentes region, in which the cheese samples from the Triângulo Mineiro region were more related to the terpene (Ter) and ester (Est) groups (Figure 2D). It was also possible to observe that the Canastra, Serro, and Araxá regions showed similar behavior regarding the Dim2 axis, which was mainly influenced by ketone (Cet) and carboxylic acid (Carb) groups, and inverse behavior in relation to the Dim1 axis, which was associated with hydrocarbon (Hid) and alcohol (Alc) groups.

In general, the terpenes camphene, caryophyllene-oxide, and α-pinene were possible plant markers for cheeses from the Canastra and Serro regions, as well as the terpene α-xylene for Serro cheeses. Identifying some volatile compounds in cheeses from only one evaluated region, such as butanedione, nonanoic acid 5-hydroxy, 2-phenylethyl propanoate, and butanoic acid propyl ester for cheeses from the Serro region, can provide guidelines regarding the patterns of chemical compounds related to different production regions.

Although these differences in the profile of volatile compounds have been observed in relation to the cheeses from the regions evaluated in this study, further research should be conducted to evaluate the association of other aspects, such as ripening time and the microbiological composition of the natural culture ("pingo"), to better elucidate how the profile of volatile compounds manifests itself within and between each region certified for the production of AMC.

4 CONCLUSION

The volatile compound profiles and region of AMC production exhibited differences, with similar behavior for cheeses from Triângulo Mineiro, Cerrado, Serra do Salitre, Araxá, and Campo das Vertentes to Canastra and Serro.

Cheeses from the Canastra and Serro regions presented different characteristics regarding compounds in each group of volatile compounds identified that differ them from others. Specifically, terpenes camphene, caryophyllene oxide, and α-pinene were shown to be possible volatile compounds for cheeses from the Canastra and Serro regions, as well as the terpene α-xylene for Serro cheeses.

The identification of some volatile compounds in cheeses from only one of the evaluated regions, such as butanedione, 5-hydroxy nonanoic acid, 2-phenylethyl propanoate, and butanoic acid propyl ester for cheeses from the Serro region, for example, may provide guidelines on patterns of chemical compounds related to different production regions.

ACKNOWLEDGMENTS

The authors thank the team of the Technical Assistance and Rural Extension Company of Minas Gerais—EMATER-MG for the support in sample collection and contact with producers.

FUNDING

Minas Gerais Research Foundation (FAPEMIG) (Process APQ-00648-21).

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