FERNANDA COSTA PRATES

ARTISANAL MINAS CHEESE PRODUCED IN THE CAMPOS DAS VERTENTES/MG REGION: MICROBIOTA AND VOLATILE COMPOUNDS PROFILE

LAVRAS – MG
2023

FERNANDA COSTA PRATES

ARTISANAL MINAS CHEESE PRODUCED IN THE CAMPOS DAS VERTENTES/MG REGION: MICROBIOTA AND VOLATILE COMPOUNDS PROFILE

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração Ciência dos Alimentos, para a obtenção do título de Doutor.

Prof. Dr. Luis Roberto Batista
Orientador

Prof. Dr. Luiz Ronaldo de Abreu
Coorientador

LAVRAS – MG
2023

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Prates, Fernanda Costa.
Artisanal Minas cheese produced in the Campos das Vertentes/MG region : microbiota and volatile compounds profile / Fernanda Costa Prates. - 2023.
102 p. : il.

Orientador(a): Luis Roberto Batista.
Coorientador(a): Luiz Ronaldo de Abreu.
Tese (doutorado) - Universidade Federal de Lavras, 2023.
Bibliografia.

1. Artisanal Minas Cheese. 2. Volatile compounds. 3. Metagenomics. I. Batista, Luis Roberto. II. Abreu, Luiz Ronaldo de. III. Título.

FERNANDA COSTA PRATES

ARTISANAL MINAS CHEESE PRODUCED IN THE CAMPOS DAS VERTENTES/MG REGION: MICROBIOTA AND VOLATILE COMPOUNDS PROFILE

QUEIJO MINAS ARTESANAL PRODUZIDO NA REGIÃO CAMPOS DAS VERTENTES/MG: MICROBIOTA E PERFIL DE COMPOSTOS VOLÁTEIS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência dos Alimentos, área de concentração Ciência dos Alimentos, para a obtenção do título de Doutor.

APROVADA em 26 de julho de 2023.
Dra. Jaqueline de Paula Rezende - UFLA
Dr. Cleube Andrade Boari - UFVJM
Dra. Kamilla Soares de Mendonça - IFMG

Dr. Luis Roberto Batista
Orientador

Dr Luiz Ronaldo de Abreu
Coorientador

LAVRAS/ MG
2023

AGRADECIMENTOS

A Deus, por ter me permitido experienciá-lo, me revelando a verdade e ressignificando minha vida.

Ao meu orientador Luis Roberto, um grande exemplo de elegância, educação, respeito, confiança e liberdade, por todo conhecimento compartilhado.

À minha mãe, Bethinha, minha maior inspiração como profissional, pessoa e mãe.

Aos meus filhos Beatriz, João Vitor e Laura, que chegaram durante este caminho e são a razão de tudo.

Ao meu esposo Dalberth, pela parceria, apoio incondicional, incentivos diários, paciência infinita, amizade e amor.

Às minhas irmãs, Nina e Oli, e demais familiares, pelo apoio e confiança.

À minha amiga Dra. Luciana Junqueira, por compartilhar comigo a maternidade e tornar minha vida mais leve e alegre.

Aos amigos que fiz durante este tempo e tornaram o caminho menos árduo, especialmente à Priscila, Fabiana, Natasha, Míriam, Nádjla, Kátia e à IC Vitória por toda ajuda na execução das análises.

Ao coorientador Luiz Ronaldo de Abreu, pela valiosa contribuição na realização deste trabalho e à Creusa, pela solicitude em me auxiliar na realização das análises.

Aos produtores de Queijo Minas Artesanal da região Campos das Vertentes por prontamente cederem as amostras.

Às agências de fomento CAPES, Fapemig e CNPq. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001

À Universidade Federal de Lavras, ao Programa de Pós- Graduação em Ciências dos Alimentos (PPGCA) e ao Departamento de Ciências dos Alimentos, pela oportunidade de crescimento profissional e pessoal.

Muito obrigada!

RESUMO

O queijo artesanal produzido em Minas Gerais é um produto de extrema importância econômica e cultural para a região e foi reconhecido como patrimônio imaterial brasileiro. Sua fabricação ocorre em propriedades rurais, utilizando leite cru recém ordenhado, coalho, pingo e sal. A região Campos das Vertentes foi reconhecida em 2009 como produtora de Queijo Minas Artesanal (QMA) e os queijos devem ser maturados por um período mínimo de 22 dias para garantir sua segurança microbiológica. O presente trabalho foi realizado com o objetivo de revelar a microbiota e o perfil de Compostos Orgânicos Voláteis, presentes no QMA produzido na região Campos das Vertentes/MG. Foi realizado o isolamento e a identificação da microbiota utilizando metodologia dependente de cultivo, através de MALDI – TOF, e independente de cultivo, através do sequenciamento de regiões conservadas 16S e ITS do rRNA, extraído diretamente dos queijos, a composição centesimal, pH e perfil de compostos orgânicos voláteis, utilizando CG/MS, de QMA produzido por 4 queijarias certificadas localizadas na região Campos das Vertentes/MG. As amostras apresentaram umidade entre 38,7 e 44,7%, e teor de gordura e em média entre 33 e 37%, o que equivale a 57% de matéria gorda no extrato seco, classificando o queijo como gordo. O teor de proteínas variou entre 22,3 e 25,02 e o pH variou entre 5,0 e 5,5. Nas análises dependentes de cultivo, as leveduras identificadas nos queijos foram *Geotrichum candidum*, *Kluyveryomyces lactis, Diutina catenulata e* algumas espécies de candida e através da metagenômica revelou-se a predominância de *G. candidum*, *Corynebacterium variabile*, *D. catenulata, K. lactis* e *Debaryomyces prosopidis.* Não foram identificadas as espécies *Yarrowia lipolítica* e *Debaryomyces hansenii*, amplamente descritas em queijos artesanais, sugerindo que a ausência pode ser um marcador de autenticidade para os queijos produzidos nesta região. Para compostos voláteis, foi constatada maior área relativa dos ácidos acético, decanoico, hexanoico e octanoico, álcool 3-metilbutanol, cetonas 2-heptanona, 2-nonenona e 2-butanona, e ésteres etil hexanoato e etil octanoato. Espera-se que o queijo produzido nesta região possua notas aromáticas de vinagre, oleoso, rançoso, adocicado, frutado, maltado, queimado e verde. Os resultados obtidos neste trabalho ampliam o conhecimento sobre a microbiota naturalmente presente nos queijos artesanais e os principais compostos voláteis produzidos na maturação dos queijos da região Campos das Vertentes e se configuram como um importante passo para assegurar a fabricação dos queijos de casca florida, embasar novas legislações que visem regulamentar a produção desses queijos e endossar a requisição da certificação de Denominação de Origem para a região.

Palavras – chave: Microbiota. Queijo Minas Artesanal. Compostos voláteis. *Geotrichum candidum.* Metagenômica.

ABSTRACT

The artisanal cheese produced in Minas Gerais is a product of extreme economic and cultural importance for the region and has been recognized as intangible Brazilian heritage. Its manufacture takes place in rural properties, using freshly milked raw milk, rennet, endogenous yeast (pingo) and salt. The Campos das Vertentes region was recognized in 2009 as a producer of Artisanal Minas Cheese (AMC) and the cheeses must be matured for a minimum period of 22 days to ensure their microbiological safety. The present work was carried out with the objective of revealing the microbiota and the profile of Volatile Organic Compounds, present in the AMC produced in the Campos das Vertentes/MG region. The isolation and identification of the microbiota was carried out using a culture-dependent methodology, through MALDI - TOF, and culture-independent, through the sequencing of conserved 16S and ITS regions of the rRNA, extracted directly from the cheeses, the centesimal composition, pH and profile of volatile organic compounds, using GC/MS, of AMC produced by 4 certified cheese dairies located in the Campos das Vertentes/MG region. The samples showed moisture between 38.7 and 44.7%, and fat content on average between 33 and 37%, which is equivalent to 57% of fat in the dry extract, classifying the cheese as fat. The protein content varied between 22.3 and 25.02 and the pH varied between 5.0 and 5.5. In the culture-dependent analyses, the yeasts identified in the cheeses were *Geotrichum candidum, Kluyveryomyces lactis, Diutina catenulata* and some *candida* species and through metagenomics it was revealed the predominance of *G. candidum*, *Corynebacterium variabile*, *D. catenulata*, *K. lactis* and *Debaryomyces prosopidis*. The species *Yarrowia lipolitica* and *Debaryomyces hansenii*, widely described in artisanal cheeses, were not identified, suggesting that their absence may be a marker of authenticity for the cheeses produced in this region. For volatile compounds, a greater relative area was found for acetic, decanoic, hexanoic and octanoic acids, 3-methylbutanol alcohol, 2-heptanone, 2-nonenone and 2-butanone ketones, and ethyl hexanoate and ethyl octanoate esters. The cheese produced in this region is expected to have aromatic notes of vinegar, oily, rancid, sweet, fruity, malty, burnt and green. The results obtained in this work expand the knowledge about the microbiota naturally present in artisanal cheeses and the main volatile compounds produced in the maturation of cheeses from the Campos das Vertentes region and are configured as an important step to ensure the manufacture of cheeses with a flowering rind, to support new legislation aimed at regulating the production of these cheeses and endorsing the request for Denomination of Origin certification for the region.

Keywords: Microbiota. Artisanal Minas Cheese. Volatile Compounds. *Geotrichum candidum*. Metagenomics.

LIST OF FIGURES

PART ONE

PART TWO – ARTICLES

ARTICLE 1

ARTICLE 2

LIST OF TABLES

PART ONE

PART TWO – ARTICLES

ARTICLE 1

SUMMARY

PART ONE

1 INTRODUCTION

Artisanal cheese is a food manufactured in a simple way, usually using raw milk, endogenous yeast (pingo), enzymatic rennet and salt and is produced in several regions of the world, Brazil and Minas Gerais, this being called Artisanal Minas Cheese (AMC). Artisanal cheeses differ due to the particularities in the manufacturing process of each cheese factory, in addition to the influence of the region and the microbiota, which makes each AMC unique. After manufacture, the AMC is matured in maturation chambers, without controlled conditions, where the modification of its physical, chemical and sensory characteristics occurs, through the action of microbial enzymes. The way of making AMC is an intangible heritage of Brazil (IPHAN, 2008) and the cheese stands out as a product of extreme economic and cultural importance for the region.

The cheese factories producing AMC must be registered with the inspection bodies, such as the Instituto Mineiro de Agropecuária (IMA) or have the ARTE seal in order to guarantee that Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) are adopted, and that the consumption of AMC is safe, allowing its commercialization. Currently there are ten regions recognized by the IMA as producers of AMC, namely: Serro, Canastra, Cerrado, Araxá, Campo das Vertentes, Serra do Salitre, Triângulo Mineiro, Diamantina, Serras do Ibitipoca and Entre Serras da Piedade ao Caraça. The minimum maturation period of AMC is regulated (MINAS GERAIS, 2020) and in the Campo das Vertentes region is 22 days.

Microorganisms are naturally present in AMC and are transferred by the milk, the endogenous yeast and also by the environment in which it is being produced and have a fundamental role in the formation of chemical compounds that will guarantee the specific sensory characteristics, through enzymatic reactions promoted by microbial enzymes. In recent years there has been an increased interest in studying the specific characteristics of artisanal cheeses by several research groups from Brazilian Universities, including knowing the microbiota of AMC produced in different regions and their specific characteristics after maturation. For this, techniques developed to reveal the microbiota of foods, either culture-dependent or culture-independent, are used. Cultivation-dependent techniques limit the analysis only to cultivable microorganisms, but allow the isolate to be studied separately and preserved, but cannot reveal all the microbiota present, requiring the use of molecular

techniques such as DNA or rRNA sequencing to reveal all the microbiota existing in the cheeses.

Despite the progress in research, there is still little knowledge about filamentous fungi and yeasts present in AMC produced in the different regions of Minas Gerais, and it is of interest to all those involved in the production chain to know the relationship between microorganisms and the chemical and sensory characteristics of cheeses. From this knowledge it will be possible to propose improvements in all stages of product manufacture, adding value to the cheese, without ignoring the importance of ensuring its microbiological safety and stability and also to support new health legislation that authorizes and regulates its manufacture.

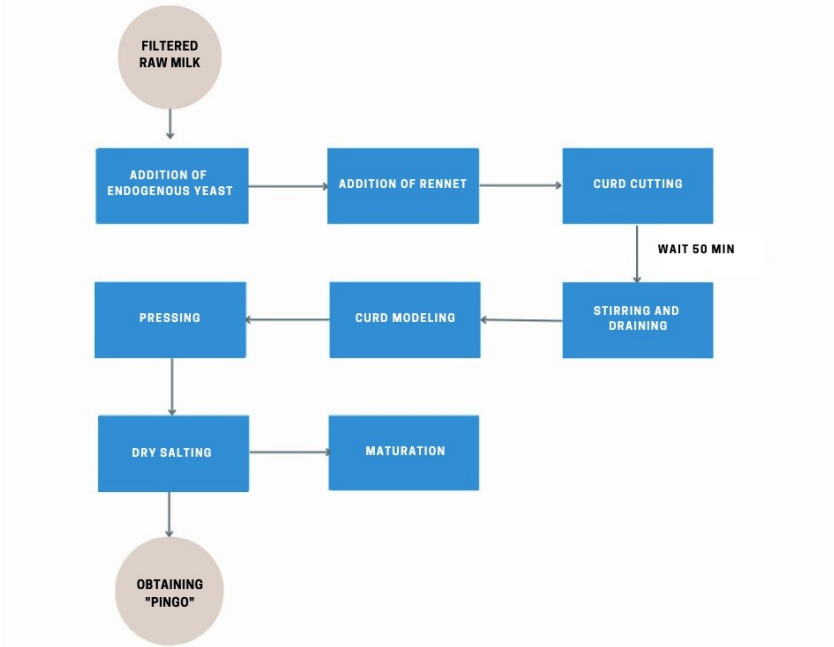
The present work was developed with the objective of revealing and describing the microbiota of AMC produced in the Campos das Vertentes/MG region by dependent and independent cultivation methods, as well as its composition and profile of Volatile Organic Compounds, expanding the knowledge about the cheeses produced in this region. In addition, this work intends to transfer the knowledge obtained to the producers of AMC of the Campos das Vertentes region, in order to encourage the production and commercialization of artisanal cheeses of Bloomy Rind, adding value to the product and also allowing the region studied to apply for the certification of Denomination of Origin, avoiding fraud and valuing the product before society, which also benefits from the greater circulation and access to a product with typical and unique sensory characteristics.

2 THEORETICAL REFERENCE

2.1 Artisanal Minas Cheese

According to Brazilian legislation, cheese is defined as the fresh or matured product obtained by partial separation of whey or milk serum, coagulated by the physical action of rennet, specific enzymes of specific bacteria, organic acids, with or without the addition of food substances and/or spices and/or condiments and matured cheese is the cheese that has undergone the necessary biochemical and physical changes and characteristics of the cheese variety (BRASIL, 2017). In the process of making artisanal minas cheese, rennet is added to the milk at room temperature and the curd is left to rest for about 50 minutes, until the dough reaches the firmness necessary for cutting. Then the dough is cut manually into cubes or grains to promote the desorption of the dough. Afterwards, the dough is placed in the molds where the desorption is completed and the dry salting is performed on the formed cheese. At the end of the manufacturing process, the dripping is collected and used in the next batch of cheese (Figure 1).

Figure 1. Artisanal Minas Cheese manufacturing flowchart.



Source: Sant’anna et al. (2019).

Once the cheese is manufactured, it is removed from the mold and sent to the maturing rooms, where it remains for the period necessary to develop its characteristics of texture, color and aroma, typical of each region where it is produced. Traditionally, the cheese is matured on wooden boards and is turned frequently (Figure 2). In addition, in some regions, the cheese may be subjected to treatments on the rind, such as the use of edible coatings (DE SOUSA LEÃO et al., 2020).

Figure 2. (A) Artisanal Minas Cheese (AMC) produced in the Campos das Vertentes region. (B) Maturation of AMC on wooden planks.

(A) (B)

Source: author.

As soon as the cheese is made, it is called "fresh", but over time, microbiological and chemical changes occur, which alter its sensory characteristics, turning them into "matured" cheeses. The first advantage of matured cheese is that it prolongs the consumption of coagulated milk through the loss of moisture. Maturing is the last stage of cheese making and its duration varies from weeks to months, depending on the type. It is during this period that chemical, biological and sensory transformations occur, mainly due to the action of lipolytic and proteolytic enzymes produced by microorganisms, which modify the physical and chemical structure of the cheese, contributing to the texture, aroma and flavor and promoting a variety of matured cheeses (JÚNIOR et al., 2014).

Most cheeses produced in Brazil go through the maturation process before consumption, such as prato, parmesan and provolone cheeses, including those produced by hand. To be considered artisanal, the cheese must be made from raw, healthy, whole milk, produced on the farm itself, with the use of whey (pingo) and have a firm consistency, its own

color and flavor, uniform mass, free of dyes and preservatives, with or without mechanical eyes (BRASIL, 2017). In addition, the cheese must be processed within 90 minutes after the beginning of milking, made with milk that has not undergone heat treatment and matured according to the period stipulated for micro-regions that have scientific research or, in its absence, for the longest period determined through scientific studies (MINAS GERAIS, 2020).

Although it is possible to draw a flowchart of artisanal cheese production, due to the similarity in the process in different regions and in different cheese factories in the same region, it is necessary to emphasize that each cheese factory has particularities, unique details of the artisanal way, which guarantee the typicality and uniqueness of each piece of artisanal cheese produced.

2.2 History of AMC production

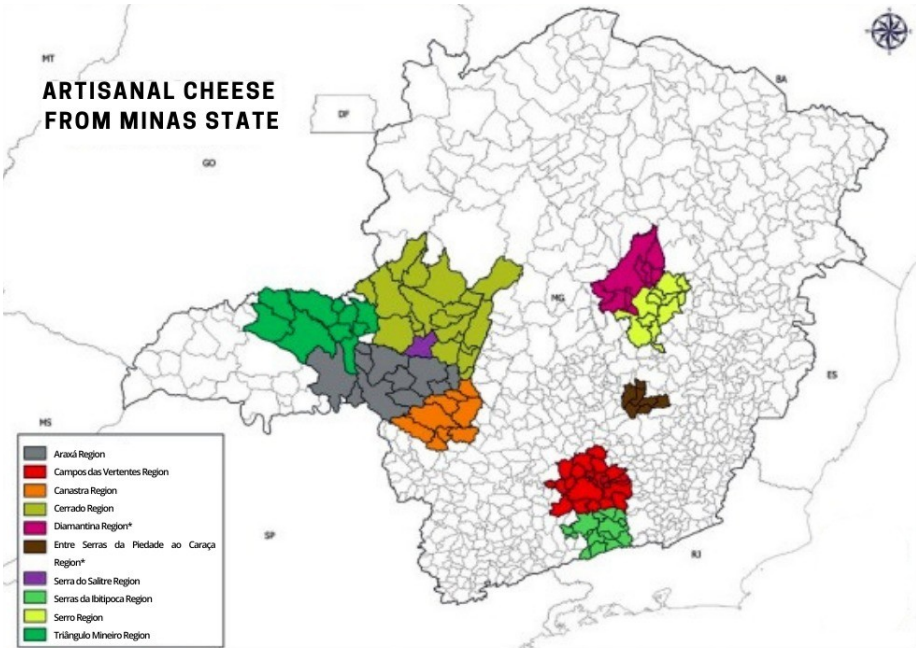
Some findings indicate that the techniques for making Artisanal Minas Cheese (AMC) in the Campos das Vertentes region were brought from Serra da Estrela by Portuguese colonizers (MENESES, 2006). However, the true origin of Artisanal Minas Cheese is the Azores archipelago in Portugal, more specifically the islands of Pico and São Jorge (NETTO, 2011). Milk and cheese production in the Campo das Vertentes region began in the colonial period, along with gold mining, between the 18th and 19th centuries. The food produced in the region was consumed by traders, prospectors and travelers and could not be perishable due to the long journeys they faced. In this context, cured cheese, known as "cheese from Minas", "cheese from Minas Gerais" or simply "Minas cheese", stood out as a transportable delicacy capable of reaching distant cities (MORENO, 2013). The way of making artisanal cheese has been present since colonial times to the present day, based on a tradition that the colonizers brought to Minas Gerais. Thus, the AMC has not lost the strength of its artisanal tradition and has not ceased to be important, culturally and economically, in its original way of doing (IPHAN, 2008).

The artisanal manufacturing process of Minas Gerais cheese has been resisting the trends and demands of production modernization, due to the attachment to tradition among generations, isolation of the producing properties and remarkable economic importance for the region, ensuring the preservation of regional products with their own characteristics and high cultural value. Despite being traditionally marketed and consumed products, artisanal cheeses did not always have the guarantee of being microbiologically safe and the cheese

factories did not comply with current legislation, forcing producers to market cheeses in a clandestine manner, without authorization from the supervisory bodies (EMATER, 2019).

An important milestone in the history of AMC production was the recognition of the Artisanal Way of Making Minas Cheese, in the regions of Serro, Serra da Canastra and Salitre, in Minas Gerais, by the National Historical and Artistic Heritage Institute as Brazilian intangible heritage, in 2008 (IPHAN, 2008). Each region recognized as a producer of AMC its own characteristic "know-how", which gives the cheese its own identity, according to the place where it is made. Currently, 10 regions are recognized as producers of AMC: Araxá, Campos das Vertentes, Canastra, Cerrado, Diamantina, Serra do Salitre, Serro, Triângulo Mineiro, Serras do Ibitipoca and Entre Serras da Piedade ao Caraça (Figure 3).

Figure 3. Mapping of Minas Artisanal Cheese producing regions.



Source: EMATER, 2019.

2.3 Legislation

The Government of Minas Gerais, aiming at the regularization and expansion of the commercialization of one of the most traditional products of the State, has been carrying out, through public agencies, such as the Secretariat of Agriculture, Livestock and Supply , the Technical Assistance and Rural Extension Company of the State of Minas Gerais and the Minas Gerais Institute of Agriculture, important actions to improve the quality of cheeses, in

addition to seeking technical-scientific support to develop and approve new legislation that ensures the quality of cheese, without preventing its commercialization (EMATER, 2019).

The first regulation that applied to artisanal cheese was the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA), approved by Decree No. 30,691 on March 19, 1952 (BRASIL, 1962). However, the first specific legislation for Artisanal Minas Cheese (AMC) is State Law No. 14.185/02, which established microbiological and physical-chemical standards for raw materials and water quality of cheese factories, in addition to requiring ideal physical structure conditions, procedures for sanitizing equipment and utensils, requirements related to the health conditions of the herd and the hygiene and health of the handlers (MINAS GERAIS, 2002). This law was an important milestone in the regulation of AMC production and aimed to guarantee the quality and food safety of cheeses, in addition to preserving the tradition and culture of artisanal production.

In 2011, State Law No. 19,492 was sanctioned, which assigned the responsibility of supervising and registering the production processes of AMC to public agencies (MINAS GERAIS, 2011). Soon after, in 2012, State Law No. 20,549 was sanctioned, repealing Law 14,185 and detailing the requirements for the production and commercialization of artisanal minas cheese, half-cured cheese, cabacinha cheese and artisanal cottage cheese (MINAS GERAIS, 2012). In 2017, Decree No. 9,013, known as the new RIISPOA, was published, responsible for establishing the hygienic-sanitary conditions that must be followed by establishments that produce products of animal origin, including artisanal cheeses. This regulation allowed artisanal cheese factories to be registered and inspected by competent bodies in a simplified manner, provided they meet specific production, storage and transportation requirements (BRASIL, 2017).

Law 20.549/12 was repealed by State Law No. 23.157/18, which recognized the production of other types of cheeses and made AMC production official as a small agroindustry (MINAS GERAIS, 2018a). The government of the state of Minas Gerais sanctioned Law No. 22,926/2018, which provides for the Certification Program for Agricultural and Agroindustrial Products - Certifica Minas. This law establishes criteria and procedures for the certification of agricultural and agroindustrial products, establishing norms and standards for their production and commercialization, with the objective of ensuring quality and safety for the population (MINAS GERAIS, 2018b).

In 2019, Decree No. 9,918 was published, which regulated the ARTE seal and determined that the agriculture and livestock agencies would be responsible for inspecting

artisanal products and granting the "ARTE Seal" (BRASIL, 2019a). In December of this year, MAPA published IN 73, which established the Technical Regulation of Good Agricultural Practices for rural producers supplying milk for the manufacture of artisanal dairy products and assigned the responsibility of assessing compliance with GAP to the states for granting the ARTE seal (BRASIL, 2019b).

In 2020, Decree 48.024 regulated Law No. 23.157, of December 18, 2018, which provides for the production and marketing of artisanal cheeses in Minas Gerais, requiring the registration of cheese factories with MAPA or SIM and the implementation of Good Agricultural and Manufacturing Practices, including animal health, physical structure of handling areas, quality of raw materials and water, hygiene and health of handlers and health records available to inspection bodies (MINAS GERAIS, 2020).

Regarding the ripening period of cheeses, the first regulation occurred in November 2000 (BRASIL, 2000), where the minimum period of 60 days was determined for cheeses produced from raw milk. This measure was necessary to ensure the microbiological safety of the cheeses, however, producers reported that during this period undesirable sensory changes occurred, developing the bitter taste, in addition to weight loss and dryness of the rind. With the advancement of studies related to the microbiological safety and stability of cheeses, Normative Instruction No. 57 (BRASIL, 2011) was approved in 2011 and, in 2017, Decree 9.013 (BRASIL, 2017) allowing the minimum maturation period to be changed if the safety and microbiological safety of the product with a shorter maturation period are proven. Therefore, the minimum maturation period of AMC was defined by Ordinances published by the IMA, which over time were updated based on scientific studies carried out on the maturation of AMC, specific in each region, being 14 days for the Araxá region, 17 days for the Serro region and 22 days for the other regions, including Campos das Vertentes (MINAS GERAIS, 2020).

On December 27, 2022, the Secretary of Agriculture, Livestock and Supply of Minas Gerais, recognized through Resolution 42, the artisanal minas cheese in the bloomy rind variety and defined as bloomy rind "visual dominance of filamentous fungi", with predominance of fungi *Galactomyces Geotrichum* (*Geotrichum candidum* or *Geotrichum silvicola*), also defining the cheese bloomy rind as "cheese obtained from raw, hygienic, whole milk, of its own production, with the use of serum ferment (pingo)" and that has "its own color and flavor, uniform mass, free of dyes and preservatives, with or without mechanical eyes and rind of rough aspect", being possible to observe the "dominance of

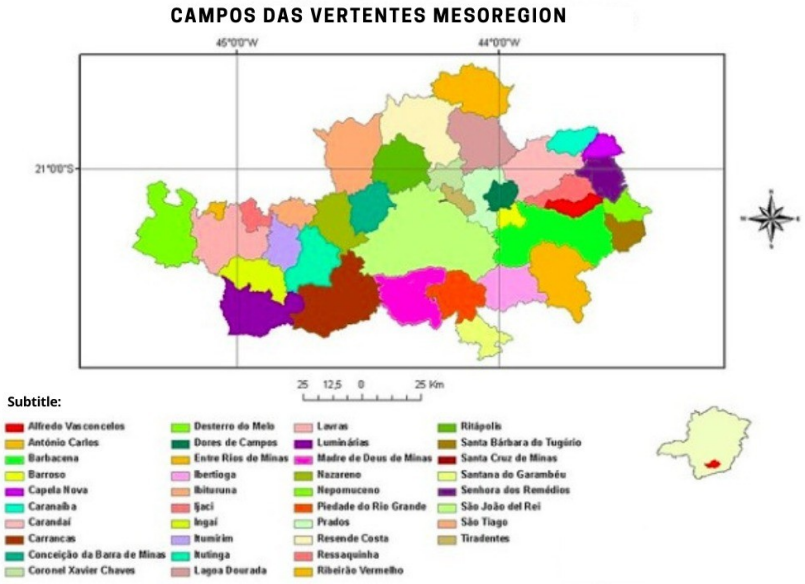
filamentous fungi of white color and rough aspect" (MINAS GERAIS, 2022). The next step, based on the resolution, is the regulation of the rules applied to the production and commercialization of this cheese, by the IMA. The publication of the Technical Regulation of Identity and Quality of AMCBR will soon allow the sanitary qualification of this cheese.

The growing adherence to these measures and the regulation of cheese production have driven the search for registration and legalization of cheese factories with the bodies responsible for health inspection and inspection services. This allows the regularization of cheese factories, expanding their borders and allowing distribution throughout the national territory, ending clandestinity in marketing.

2.4 Campos das Vertentes/MG region

The Campos das Vertentes region is located in the state of Minas Gerais, in southeastern Brazil and is divided into 3 micro-regions: Lavras, Barbacena and São João Del Rei. It is a transition region between the Brazilian central plateau and the Mantiqueira and Espinhaço mountains, and covers about 3,678 km² of area (MORENO, 2013). Among the 38 municipalities that make up the region (Figure 4), the following are authorized to produce AMC: Barroso, Carrancas, Conceição da Barra de Minas, Coronel Xavier Chaves, Lagoa Dourada, Madre de Deus de Minas, Nazareno, Prados, Piedade do Rio Grande, Resende Costa, Ritápolis, Santa Cruz de Minas, São João Del Rei, São Tiago and Tiradentes (IMA, 2009). The climate of the Campos das Vertentes region is tropical highland, with hot and rainy summers and dry and cold winters and the average annual temperature between 17.4 °C and 20.5 °C (BARUQUI et al., 2006).

Figure 4. Mapping of municipalities belonging to the Campos das Vertentes mesoregion.



Source: EMATER, 2019.

The Campos das Vertentes region was officially recognized as a AMC producer in 2009, through Ordinance No. 1,022 (MINAS GERAIS, 2009). In 2022, 5 cheese factories were registered in the Certifica Minas program, instituted by IMA after State Law 22.926/18 (MINAS GERAIS, 2018b) and coordinated by SEAPA together with EMATER, EPAMIG and IMA.

In 2019, the Association of Coffee Growers of Campo das Vertentes requested the recognition of the Indication of Origin (IP) for green coffee beans, roasted coffee beans and ground roasted coffee and obtained the certification in November 2020 by the National Institute of Industrial Property - INPI. This certification is essential to protect and value coffee products, valuing the entire region in the national market for artisanal products. The Denomination of Origin (DO) certification has not yet been requested for the AMC produced in this region, however, according to reports from producers, a producers' association is being formed with the aim of strengthening the product and making the request to the National Institute of Industrial Property (INPI, 2023).

The artisanal cheese produced in the Campos das Vertentes region has a strong flavor, soft and creamy texture, and is molded in a cylindrical shape with a diameter between 10 and 17 cm, average height of 4 cm and weight between 750-900g (CASTRO et al., 2016). Its rind has a straw-yellow color with a medium and semi-hard texture. When cut, it tends to break

into small pieces with mechanical eyes and when tasted, the predominant flavor is slightly acidic (MORENO, 2013).

In a visit to the IMA certified cheese factories located in the Campos das Vertentes region, a heterogeneity was observed in the AMC process conditions, as well as in the resources invested in the construction of the cheese factories. It was observed that all cheese factories adopted measures of good manufacturing practices, such as window screens, water chlorination, use of uniforms, disposable caps and props, and adequate sanitization of hands, environments and utensils, fundamental factors to ensure the microbiological quality of cheeses. However, the cheesemakers adopted different process conditions, such as the criteria and frequency for washing or turning the cheeses in the ripening chambers, criteria for sanitizing or replacing the wooden boards, criteria for storage and use or disposal of the drip and use or not of cloth at the time of hanging. The cheese producers were resistant to the manufacture of Artisanal Minas Cheese Bloomy Rind opting for frequent washing of the cheeses to remove the fungi grown on the surface and reported the lack of knowledge and prejudice regarding the commercialization and consumption of this type of cheese, in addition to its manufacture being prohibited by the bodies responsible for the inspection of the establishments. However, we found in some cheese factories pieces of AMC with a white coating, typical of the presence of fungi *Geotrichum candidum*, reserved for marketing "in loco", that is, products that are not labeled and distributed, but are sold to visitors, tourists who go to cheese factories in search of a product with different characteristics.

2.5 Cheese production and consumption

In 2019, the global cheese market increased by 2.3% to $114.1 billion, rising for the third consecutive year after two years of decline. The market value increased at an average annual rate of +1.1% over the period 2013-2019; the trend remained consistent, with only minor fluctuations in certain years. The pace of growth was strongest in 2017, when the market value increased by 7.1%. In the period under review, the global market reached its maximum level in 2019 (ABIQ, 2019).

In Brazil, 35.3 billion liters of milk were produced in 2021 and Minas Gerais stands out as the Brazilian state with the highest milk production, responsible for 9.6 billion liters, which represents 27.2% of national production and the largest cheese producing state in the country, on average 40% of national production (85 thousand t/year). Most of the existing agro-industries in Minas Gerais are milk producing farms, totaling 11,158 units and, among

them, more than 7,000 establishments are also dedicated to the production of artisanal cheeses. Of these, more of 3,100 agro-industries are producers of Artisanal Minas Cheese, producing an average of 21.8 thousand tons of AMC per year, which represents 65.2% of the production of artisanal cheeses of family agro-industries (EMATER, 2019).

Regarding consumption, cheeses are among the most consumed dairy products in Brazil, second only to fluid milk. According to ABIQ, Brazilians consume, on average, 5.5 kilograms of cheese per year. Compared to other countries, the highest levels of annual cheese consumption per capita in 2019 were in the Czech Republic (64 kg per person), Germany (37 kg per person) and France (25 kg per person) (ABIQ, 2019). Although there is no data on the consumption of artisanal cheeses, the general data on cheese consumption show how much the consumer market can still grow, and an alternative to stimulate the increase in cheese consumption is the valorization of regional products produced by small agro-industries, such as Artisanal Minas Cheese.

According to information provided by AMC producers in the Campos das Vertentes region who have their cheese factories registered with the IMA, the AMC consumer market is predominantly local. In addition to the points of sale distributed in the cities of the region (São João Del Rei, Tiradentes, Bichinho and Santa Cruz de Minas), there is a large number of hotels, inns and restaurants that offer these products to visitors and use them in their preparations, as it is a tourist region. In addition, many of the cheese factories are open to visits and participate in the cheese route, allowing visitors to know, try and purchase their products directly at the place of production.

2.6 Microorganisms in artisanal cheeses produced with raw milk

The action of microorganisms throughout the maturation of the cheese is essential to ensure the expected characteristics of the product. The microorganisms found in each artisanal cheese are distinct and specific to each producing region, as their growth and predominance depend on characteristics such as location, altitude, soil type, climate, breed and feeding of cattle, whose set of factors is called "terroir", in addition to the ingredients and manufacturing process (NERO et al., 2021).

Currently there are some research groups in Brazilian universities and institutions, such as EMATER, EMBRAPA and IMA, developing works aiming to reveal the microbiota of artisanal cheeses using cultivation-dependent and cultivation-independent molecular techniques, such as metagenomics (Table 1). These studies have been carried out mainly

through sequencing of the conserved 16S region, revealing the bacterial community present, highlighting lactic acid bacteria and assessing the presence of undesirable contaminants, spoilers or pathogens. In contrast, there are few studies revealing the mycobiota, i.e. the fungal community through sequencing of the ITS region, existing in Brazilian artisanal cheeses, given the importance of these microorganisms for the maturation and biological signature of Brazilian artisanal cheeses.

Lactic acid bacteria (LAB) are the most reported microorganisms (KOTHE, MOHELLIBI, RENAULT, 2022; NERO ET AL., 2021; KAMIMURA ET AL., 2020; DE FREITAS MARTINS ET AL., 2018) and studied in cheeses. Its importance for the ripening of cheeses is related to the degradation of milk casein into aromatic compounds and the transformation of residual sugars into lactic acid and CO2, favoring the formation of eyes and modifying the texture of the dough, by the action of enzymes (proteases) responsible for protein hydrolysis, they help inhibit the growth of pathogenic microorganisms such as Listeria monocytogenes and Salmonella spp (ANDRETTA et al., 2019).

The presence of yeasts in cheeses is fundamental and unavoidable and the most commonly found are *Geotrichum candidum, Kluyveromyces Lactis, Debaryomyces hansenii* and *Diutina catenulata* (ARAGÃO et al., 2021; SILVA, 2020) and its main functions are: to consume the available residual sugars, avoiding excessive acidification; to deacidify and neutralize the cheese surface, allowing the growth of fungi and bacteria sensitive to acidic environments; to assist in the production of aromas - due to its proteolytic activity, and to degrade bitter peptides (PEREIRA; SPERAT-CZAR; ROUSTEL, 2017).

G. candidum was initially classified as a fungus, then it was classified as a yeast and currently there is still a discussion about its classification as it has characteristics of both fungi and yeasts. It is of great importance in cheese ripening and contributes to the formation of characteristic flavors and can grow both on the surfaces and inside the cheeses. On surfaces, it is responsible for forming a white rind, rich in lipolytic and proteolytic enzymes, which contribute to the ripening process. Several strains of *G. candidum* are known, but phenotypic identification methods allow to classify strains of *G. candidum* isolated from cheese at the genus level due to their similarity, whereas strains can only be identified at the species level using molecular methods (BOUTROU, GUÉGUEN, 2005).

Table 1. Microbiota of Brazilian Artisanal Cheeses revealed by molecular techniques.

Producing region	Methodology	Microrganisms			Reference
		Bacteria	**Yeast**	**Filamentous fungi**	
Serra da Canastra	Metagenomics Shotgun – 16S + ITS	*Lactococcus lactis subsp. Lactis, Streptococcus spp. (S. thermophilus, S. salivarius e S. infantarius) and Corynebacterium variabile*	*Diutina catenulata, Debaryomyces hansenii and Kodamaea ohmeri*		KOTHE, MOHELLIBI, RENAULT, 2022
Serra da Canastra	Sequencing of isolates			*Fusarium solani, Geotrichum candidum, Aspergillus (A. versicolor and A. westerdijkiae), Penicillium steckii and Cladosporium*	CÉSAR ET AL., 2022
South (colonial cheese)	Metagenomics - 16S	*Acinetobacter, Bacillus, Pseudomonas, Staphylococcus, Lactobacillus, Lactococcus, Leuconostoc, Pseudoalteromonas, Psychrobacter, Streptococcus, Enterobacter, Enterococcus and Escherichia*			REMOR ET AL., 2021
Serro	Metagenomics - 16S	*Lactococcus lactis, Streptococcus (S. salivarius and S. thermophilus) and Acinetobacter johnsonii*			NERO ET AL., 2021
Serra da Canastra	Metagenomics - ITS		*Diutina catenulata, Kluyveromyces, Torulaspora and Debaryomyces*	*Geotrichum candidum, Fusarium sp., Paecilomyces sp.,Trichosporon (T. coremiforme and T. japonicum), Aspergillus sp.*	ARAGÃO ET AL., 2021

				(A. oryzae and A. ochraceus), Penicillium spp. and Cladosporium	
Serra da Canastra	Metagenomics - 16S		*Debaryomyces and Diutina catenulata*	*Trichosporon*	SILVA, 2020
Serra da Canastra	Metagenomics – ITS	*Lactococcus, Lactobacillus and Leuconostoc*			KAMIMURA ET AL., 2020
Serra do Salitre	Metagenomics - 16S	*Planococcaceae, Streptococcaceae and Leuconostocaceae*			SANT'ANNA ET AL., 2019
Northeast (Coalho and Manteiga), Central (Caipira), Southeast (Araxá, Campo das Vertentes, Cerrado, Canastra and Serro) and South (Colonial and Serrano)	Metagenomics – 16S + ITS	*Streptococcus, Leuconostoc and Lactococcus*			KAMIMURA ET AL., 2018
Pará	Sequencing of isolates	*Lactobacillus, Lactococcus, Weissella, Enterococcus, Pediococcus e Leuconostoc, Macrococcus caseolyticus, Corynebacterium variabile, Granulicatella elegans and Aerococcus sanguinicola*			DE FREITAS MARTINS ET AL., 2018

Serro, Canastra, Serra do Salitre, Araxá and Campo das Vertentes	Sequencing of isolates	*Lactobacillus, Lactococcus, Enterococcus and Weissella*	PERIN ET AL., 2017
Araxá	Sequencing of isolates	*Lactobacillus spp. (L. plantarum, L. brevis, L. rhamnosus, L. casei), Enterococcus (E. faecalis, E. rivorum), Lactococcus lactis and Pediococcus sp.*	LUIZ ET AL., 2016
Campo das Vertentes	Sequencing of isolates	*Lactococcus lactis subsp. Lactis, Lb. plantarum, Lb. rhamnosus, Lc. garvieae, and Enterococococcus (E. faecium and E. faecalis)*	CASTRO ET AL., 2016
South (Serrano cheese)	Sequencing of isolates	*Lactobacillus (L. plantarum, L. paracasei, L. rhamnosus, L. acidophilus, L. curvatus and L. fermentum), Lactococcus and Enterococcus*	DELAMARE ET. AL, 2012

In addition to bacteria and yeasts, the presence of filamentous fungi is also reported in cheeses, which are responsible for giving greater typicality to cheeses, as they act more deeply. Fungi found more frequently in artisanal cheeses are from the genera *Penicillium, Aspergillus, Cladosporium, Geotrichum, Mucor* and *Trichoderma* (ARAGÃO et al., 2021). The presence of filamentous fungi in cheeses is generally undesirable, as some fungi are producers of mycotoxins, secondary metabolites of their metabolism and are related to, in addition to acute intoxication, damage to the liver, kidneys, immune system and cancer. The presence of mycotoxins in artisanal cheeses was reported by Anelli et al. (2019) and the main toxins reported in artisanal cheeses are citrinin, penitrem A, roquefortin C, sterigmatocystin, aflatoxin and ochratoxin A. However, mycotoxins require specific conditions for their production and recent research has indicated that the presence of the fungus is not a determining factor for the presence of the toxin, as investigated and described by Martin and Cotter (2023).

2.7 Methods for the identification of microorganisms

There are several techniques to identify the microbiota present in food, which can be classified as culture-dependent or culture-independent techniques. The culture-dependent techniques are restrictive, as they analyze only microorganisms that can be cultivated in the laboratory by plating the samples in specific culture media and then incubating them at a predetermined time and temperature. Among the culture-dependent techniques, the use of "Matrix-Assisted Laser Desorption Ionization - Time of Flight" (MALDI - TOF) stands out.

This technique has been used in several areas, such as in the analysis of proteins in biomedical research and in the identification of microorganisms in clinical and food microbiology (PASTERNAK, 2012), including to identify bacteria (PYZ-ŁUKASIK et al, 2021; GANTZIAS et al., 2020; SÁNCHEZ-JUANES et al., 2020), yeasts and filamentous fungi (ARAGÃO et al., 2021; PENLAND et al., 2021; DE SOUZA et al., 2021; ANDRADE et al., 2017) present in artisanal cheeses. The main disadvantage of this technique is the need for cultivation, limiting it to a small number of microorganisms and the need to obtain an updated database, presenting a high cost, in addition to requiring a trained professional to perform the technique.

Among the culture-independent methods, we can highlight metagenomic analysis, which is an innovative technology that focuses the study of the metagenome of a given system, environment or food, through the amplification and sequencing of DNA directly from

the sample, being an alternative to traditional microbiology, as it allows to identify all microorganisms present in a sample at the genus and species level without the need to cultivate isolates in the laboratory (PUIG et al., 2018).

The sequencing technique is used to identify the presence of already known genes with reported functions or their homologues in non-cultivated microorganisms. This technique can be applied in any system where it is desired to know microorganisms and has been widely used in genetic identification and parentage tests, early diagnosis of genetic syndromes, forensic medicine, to compare DNA samples collected with possible criminal suspects, rapid diagnosis of infectious diseases and industrial quality control (COUGHLAN et al., 2015).

The oldest method for sequencing the nucleotides of the DNA molecule is the Sanger enzymatic method, developed by biochemist Fred Sanger in 1977 and used to sequence the human genome. This method involves the production of many copies of a target region of DNA and uses the enzyme DNA polymerase, specific primers that will act as primers for the enzyme, DNA nucleotides (dATP, dTTP, dCTP, dGTP) and chain terminators for the 4 nucleotides (ddATP, ddTTP, ddCTP, ddGTP) marked with different dyes. These components are placed in a flask, heated and cooled at defined temperatures successively. After the reaction the samples are denatured in the presence of formamide and analyzed on a urea polyacrylamide gel by capillary electrophoresis. In electrophoresis, short fragments move rapidly through the pores of the gel, while long fragments move more slowly, separating the DNA fragments. Each fragment that reaches the end of the tube is illuminated by a laser, allowing the dye to be detected, and from the colors of the dyes recorded one after the other on the detector, the DNA sequence can be constructed one nucleotide at a time. The recorded data consists of a series of peaks in fluorescence intensity and the DNA sequence is read from the peaks on the chromatogram (ZAHA; FERREIRA; PASSAGLIA, 2014).

This technique, while useful, is time-consuming and subject to human error. To overcome these limitations, automated DNA sequencers have been developed. These techniques use PCR (polymerase chain reaction), which involves successive cycles of DNA annealing and denaturation, with the incorporation of specific and labeled dideoxyribonucleotides. After the reaction, the sample is applied to a gel and the fragments are separated and excited by a laser emitting light at a specific wavelength. The resulting spectrum pattern is recorded and analyzed by an algorithm that generates DNA sequences with high precision and efficiency (ZAHA; FERREIRA; PASSAGLIA, 2014).

Before starting sequencing, it is necessary to extract the DNA from the sample to be sequenced, using standardized extraction methodologies, which use denaturing reagents or

commercially available ready-made extraction kits. There are several methodologies that can be used in this step and the efficiency of the extraction depends on the type of sample, the methodology used, the types of reagents chosen and, mainly, the execution of the extraction step, since DNA is highly susceptible to degradation and contamination by operator DNA (RUPPERT; KLINE; RAHMAN, 2019).

After DNA extraction and purification, it is necessary to amplify the DNA to obtain sufficient sample for sequencing, through the polymerase chain reaction (PCR). The principle of PCR is to make successive copies of DNA fragments obtained from the use of specific primers and consists of 3 steps that occur successively in a thermal cycler: denaturation (96 - 98°C) which disassembles the DNA strands, allowing them to separate into two single strands that will serve as templates for the next step; annealing (55°C - 65°C), where the reaction cools so that the primers can bind to their complementary sequences in the single-stranded template DNA; and the third step, extension (68°C - 72°C), where the reaction temperature is raised so that DNA polymerase extends the primers, synthesizing new strands of DNA. This process is repeated several times to produce enough DNA to be sequenced (OLIVEIRA et al., 2007).

After obtaining the amplified DNA, the sequencing is done in specific equipment that recognizes the nucleotide sequence of the sample analyzed and the result of the sequences obtained is analyzed by a software that compares the sequences obtained with a database and reassembles the DNA. Among the most used databases is Genbank, which has free access and is produced and maintained by the National Center for Biothecnology Information (NCBI). Using the functionality called BLAST (Basic Local Alignment Search Tool), it is possible to compare information from primary biological sequences, such as amino acid sequences, nucleotides, proteins and DNA sequences, allowing an unknown sequence to be compared with a sequence library, and to identify sequence libraries that resemble the sequences consulted (BENSON et al., 2012).

With the growing interest in knowing the complete sequence of the Genome of different organisms, besides the human being, more sophisticated techniques for DNA sequencing have been developed, automating the process and reducing high costs, called Next Generation Sequencing (NGS). Among the NGS sequencers, one of the oldest and most used are from Illumina, which use reversible didesoxifluorescent terminators to sequence the amplified DNA. There are a few options of sequencers, with different indications, which should be selected according to the user's needs. The MiSeq system is the first DNA sequencing platform that integrates clustering, amplification, sequencing and data analysis in

a single instrument. This system has been widely used due to the equipment's size (0.19 m²), speed and cost-effectiveness. Illumina continues to produce new platforms with a variety of throughputs, such as the miniSeq, NextSeq 550 Series, NextSeq 1000 and 2000 and provides alternatives and indications for each platform developed on its website.

NGS enables the direct and parallel sequencing of millions and billions of DNA molecules, as they require a small amount of sample and can be applied for sequencing of complete genomes, metagenomes, RNA-seq, non-coding RNAs, small RNAs, amplicons, tagged regions, immunoprecipitated, libraries enriched with target fragments (KAHVEJIAN; QUACKENBUSH; THOMPSON, 2008) and can be applied in different types of soil samples, water, human intestine, food, among others, making possible the analysis of complex samples, composed of different nucleic acids, being able to recover complete genomes of unknown organisms (PUIG et al., 2018).

The main challenge of metagenomics is the occurrence of false positives, when the target microorganism is no longer present in the sample but its DNA is recovered, and false negatives, when the target organism is present but the DNA cannot be recovered. Detection of the microorganisms depends on many factors, such as proper DNA isolation in the sample, extraction efficiency, barriers to analysis such as PCR inhibitors that prevent amplification, and sensitivity of analytical methods (GARLAPATI et al., 2019). False positives are a cause for concern and can occur due to contamination of the sample with environmental or human DNA, poor selection of the specific primer, or ambiguities presented in sequencing, when two microorganisms have very similar sequences (RUPPERT; KLINE; RAHMAN, 2019).

In the food sector, metagenomics has been widely used to understand the response of microorganisms in different systems, mainly due to their adaptive capacity and the interaction of the entire microbiome. In fermented foods, for example, metagenomics can be extremely useful to explore functions directly in the food matrix and understand the behaviour of yeasts in response to different process conditions and thereby optimize the production of products of interest, such as alcohol, acids and enzymes (DE FILIPPIS; PARENTE; ERCOLINI, 2017).

Metagenomics can assist in the identification of enzymes with desirable technological properties, capable of catalyzing new reactions or replacing chemically synthesized catalysts that may be difficult or expensive to produce, and able to work under a wide range of environmental conditions found in food and processing cycles, including extremes of temperature, pH, osmolarity, etc. In addition, metagenomics enables the discovery of novel bioactives produced by microorganisms, including antimicrobials of interest to the food

industry, and allows investigation of important issues such as the development of resistance of bacteria to industrial processing (COUGHLAN et al., 2015).

2.8 Volatile Organic Compounds

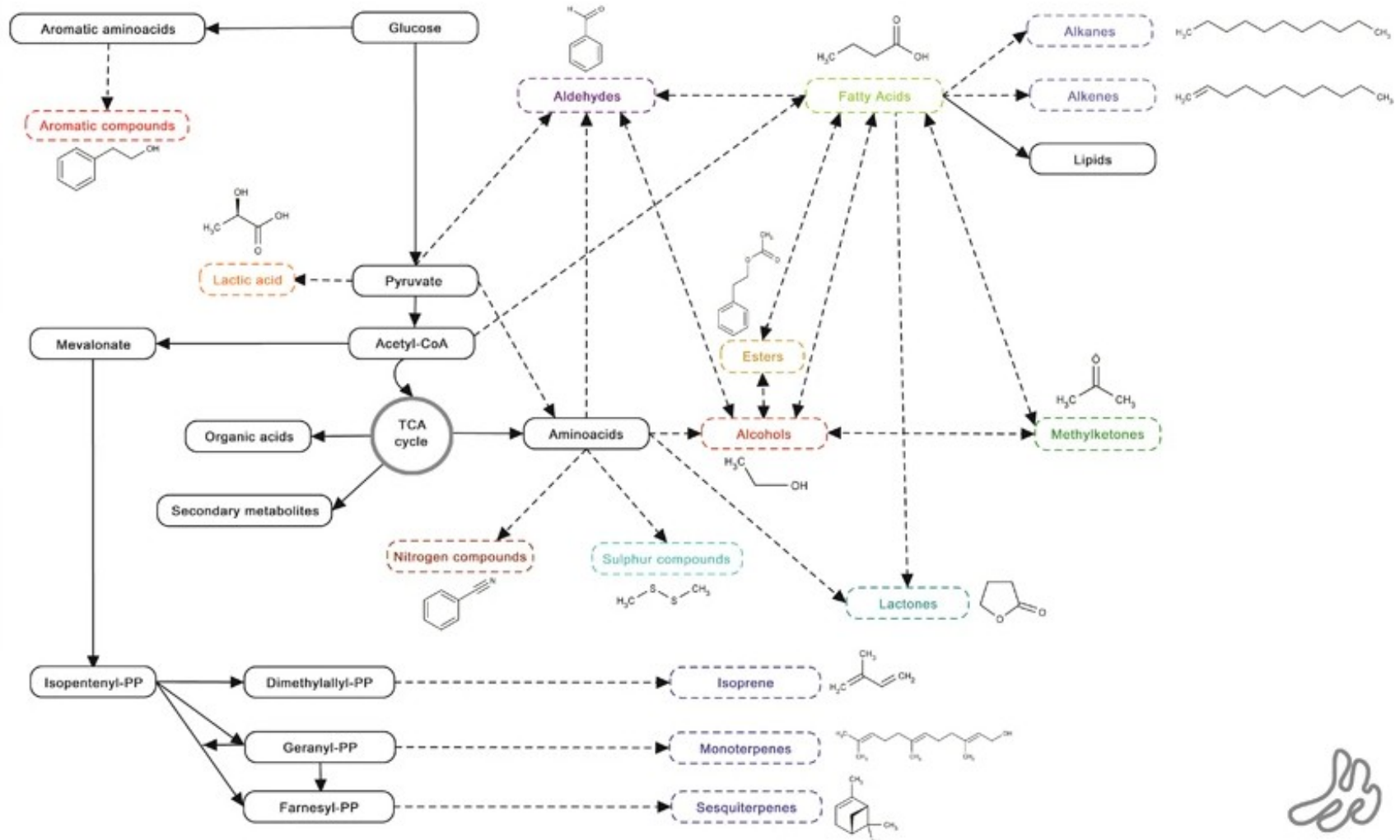
Volatile organic compounds (VOCs) are organic molecules that have a low boiling point and high vapor pressure at room temperature, i.e. they volatilize easily under normal temperature and pressure conditions. These compounds are produced by a wide variety of sources, including industrial processes, human activities, and biological processes (SCHMIDT et al., 2015).

In food, VOCs are related to flavor perception and can be produced naturally during the ripening process or formed during processing or storage, as a product of the metabolic pathways of different microorganisms (Figure 5) (FOX et al., 2017; DRAKE, DELAHUNTY, 2017; SCHMIDT et al., 2015). The volatiles responsible for the typical aroma of cheese are mainly produced by lipolytic and proteolytic pathways and by the metabolism of lactose, lactate and citrate (BERTUZZI et al., 2018). Microbial enzymes that degrade amino acids produce acids, alcohols, aldehydes, amines and various sulfur compounds, while those that break down fatty acids produce esters, methyl ketones and secondary alcohols (MCSWEENEY, 2004).

VOCs are grouped into classes, according to their function and organic form, being divided into alcohol, ester, ketone, carboxylic acids, aldehydes and aromatic hydrocarbons. The formation of VOCs during the maturation of artisanal cheeses is related to the proteolytic and lipolytic activity of enzymes produced by the microorganisms present in the cheeses (Ni et al., 2020), resulting in different volatile compounds in cheeses produced in different regions, ensuring the typicality of the cheeses (SCHMIDT et al., 2015).

Analyses of VOCs in artisanal cheeses have been carried out in several countries (Table 2), mainly relating changes in the volatile profile to the ripening time of the cheeses. Many of these studies, in addition to revealing the volatile compounds, aim to obtain the odor profile which, combined with the human sensory profile, can allow the establishment of a kind of odor signature for cheeses, which can be used, for example, to obtain Denomination of Origin certification for a specific region (GEZGINC et al., 2022).

Figure 5. Metabolic pathways of production of Volatile Organic Compounds by microorganisms.



Source: SCHIMIDT et al., 2015.

Table 2. Volatile Organic Compounds described in artisanal cheeses.

Name/Type of cheese	Region	Volatiles number	Main VOCs reported	Reference
Tulum	Türkiye	36	pentadecanoic acid, octadecanoic acid, tetradecanoic acid, octanoic acid, hexanoic acid, ethanol, 2-butanol, diacetyl, and 2-butanone	GEZGINC et al., 2022
Pecorino di Carmasciano	Irpinia/ Italy	82	hexanoic acid, butyric acid, octanoic acid, ethyl acetate, ethyl butyrate, ethyl hexanoate, 2-heptanone, 2-nonanone, 2-ethyl hexanol, benzeneethanol, and benzaldehyde	COZZOLINO et al., 2021
Artisanal Cazak	Uighur/ China	65	heptanoic acid, benzoic acid, acetic acid, 7-hydroxy-3,7-dimethyl-octanal, 2-isononenal, octanoic acid ethyl ester, 2-hydroxy-propanoic acid ethyl ester, and 3-hydroxy-2-butanone	ZHENG et al., 2021
P'elardon (with DOP)	Cevennes/ France	54	3-methylbutanoic acid, 2-phenylacetaldehyde, butanoic acids, hexanoic, pentan-2-one, heptan-2-one, hexan-2-one and nonan-2-one, and heptan-2-ol	PENLAND et al., 2021
Artisanal goat cheese	Türkiye	51	Decanoic acid, octanoic acid, hexanoic acid, 2-hexanol, 3-methylbutanol, 2-heptanone, and 3-hydroxy-2-butanone	SAY, 2021
Canastra	Serra da Canastra/ Brazil	52	acetic acid, hexanoic acid, butanoic acid, 2-butanone, acetoin, 2-butanol, ethanol, 3-methyl-1-butanol, ethyl hexanoate, acetaldehyde, and 3-methyl butanal	DE JESUS FILHO et al., 2021
Fresh mexican	Mexico	61	3-methyl-butanoic acid, ethanol, 3-methyl-1-butanol, phenylethyl alcohol, 2,3-butanedione, and 2-heptanone	REYES- DIAZ et al., 2020
Manchego (with DOP)	La Mancha/ Spain	79	dodecanoic acid, butyric acid, 2-heptanone, 2-nonanone; nonen-2-one, 3-methyl-1-butanol, heptanol, o 2-nonanol, 2-methylbutanal, 3-methylbutanal, benzaldehyde, and phenylacetaldehyde	GÓMEZ-RUIZ et al., 2020
Nanos (with DOP)	Vivapa Valley/ Slovenia	62	acetic acid, butanoic acid, hexanoic acid, octanoic acid, 1-hexanol, 2-ethyl hexanol, 2,3 heptadione, 6-methyl 5-hepten-2-one, benzaldehyde, heptanal, nonanal, and octanal	BOLTAR et al., 2015
Gokceada goat artisanal	Gokceada Island/ Türkiye	50	ethyl acetate, propyl acetate, ethyl hexanoate and ethyl octanoate, acetic acid and heptanoic acid	HAYALOGLUA et al., 2013

Idiazabal	Northern Spain	190	2-ethyl hexanoic acid, 2-nonanone, 2-heptanone, 2-nonanol, 2-heptanol, and 2-ethylhexanol	GUILLEN E ABASCAL, 2012
Lighvan	Azerbaijan/ Iran	41	butanoic acid, 3-methylbutanoic acid, hexanoic acid, octanoic acid, decanoic acid	AMINIFAR et al., 2012
Feta	Greece	25	acetic acid, butanoic acid, hexanoic acid, ethanol, 3-methylbutanol, butan-2-one, ethylbutanoate, ethylhexanoate, 3-methylbutylacetate, and 2-phenylethylacetate	KONDYLI, PAPPA,VLACHOU, 2012
Xynotyri	Naxos / Greek island	114	Acetic acid, ethanol, 3-Octen-2-ol, 2-methyl, 2-heptanol, 4-methylhexanoic acid, 2-ethylhexanoic acid, ethyl ester, 2,3-butanedione, and butanone	BONTINIS et al., 2012
Artisanal from Leiden (with DOP)	Netherlands	39	acetaldehyde, diacetyl, 2-butanone, butanal, ethanol, and alkyl moiety	GALLE et al., 2011
Darfiyeh	Lebanon	16	3-methylbutanal, 1-phenylethanol, octanol, 3-methylbutanol, 1-propanol, 2-heptanone, 2-nonanone, and 2-undecanone	SERHAN et al., 2010
Piacentinu Ennese	Province of Enna/ Italy	35	Hexanoic acid, acetic acid, butanoic acid, nonanal, (E)-nonenal, (Z)-nonenal, decanal, vanillin, ethyl butanoate, and ethyl hexanoate	HORNE et al., 2005

The efficacy of the best-known methods used to detect volatile compounds in cheese depends on the nature of the extraction polymeric material (fiber), the chromatographic column and the detector used, influencing the sensitivity and reliability of detection of specific analytes. Studies conducted on surface-ripened cheese varieties have shown that the volatiles detected were predicted based on the characteristics of the extraction techniques and the stationary phase of the column used. Furthermore, a multidisciplinary approach incorporating sensory and chemical analysis methods is fundamental for a complete assessment of cheese quality and authenticity (BERTUZZI et al., 2018).

Observing the findings in the literature, it is impossible to trace a common pattern among the various artisanal cheeses produced in different regions around the world. The profile of volatile compounds is multiple and diverse, as it is dependent on the microbiota present in the cheese, influenced by intrinsic and extrinsic factors, such as type of milk, region, soil, climate, breed and feeding of animals, as well as the conditions of the manufacturing process.

2.9 Geographical indication and authenticity verification methods for artisanal cheeses

Food fraud is an intentional action aimed at deceiving consumers and obtaining economic advantages from the marketing of food, including artisanal cheeses (CARDIN et al., 2022). Among the actions taken to combat cheese fraud, in addition to increased labeling requirements, is the obtaining of geographical identification (GI) certifications. In Brazil, these registrations are divided into indication of Procedence (IP), a certification that indicates that a product has a reputation, characteristic or specific quality associated with its geographical origin and denomination of origin (DO), which assumes that the qualities or characteristics of a particular geographical area, including natural and human factors, exclusively or essentially influence a product or service, typifying it (INPI, 2023).

The process of obtaining a GI certificate is regulated by Law No. 9.279/1996 (BRASIL, 1996) and, in order to obtain certification from the National Institute of Industrial Property, producers need to follow a series of established criteria, which may include factors such as production methods, varieties of ingredients used, cultural or historical traditions. This certification can confer a differential of quality, authenticity and added value to the producer and protects consumers, ensuring that they are purchasing genuine and authentic products, thus avoiding fraud and imitations. Among Brazilian artisanal cheeses, 6 have GI certification,

5 of which are IP: colonial and colonial cheese with green pepper produced in the German rural community Colônia Witmarsunm, AMC do Serro, AMC da Serra da Canastra, AMC do Cerrado and Marajó cheese and 1 DO certificate, belonging to the Serrano artisanal cheese produced in Campos de Cima da Serra, in the States of Rio Grande do Sul and Santa Catarina (INPI, 2023).

To verify the occurrence of cheese fraud, there are efficient food authentication methods, such as stable isotope ratio mass spectrometry, inductively coupled plasma, infrared spectroscopy, nuclear magnetic resonance, gas chromatography-based fatty acid profile analysis, gas chromatography volatile compound analysis and DNA-based methods such as metagenomics (CARDIN et al., 2022). Although these methods help to verify the authenticity of cheeses, they all have some advantages and disadvantages, and it is not possible to choose the most efficient method for this purpose.

Andrade et al. (2022) determined the mineral composition of Brazilian artisanal cheese and classified cheese types using chemometric techniques. Samples of cheeses produced in the Northeast (Coalho and Manteiga), Midwest (Caipira), Southeast (Araxá, Campo das Vertentes, Cerrado, Serra da Canastra and Serro) and South (Colonial and Serrano) were evaluated and the results obtained indicated that this analysis is a good method to ensure the authenticity of artisanal cheeses. In a study with Emmental cheese with DO, Pillonel et al. (2003) were able to discriminate the country of origin of the cheeses, differentiating Polish, French and Swiss Emmental cheeses, through the concentrations of volatile compounds by principal component analysis, validating this analysis as an efficient method of verifying authenticity. Authenticity methods based on the revelation of the microbiota through sequencing techniques have also been shown to be suitable methods for authentication in artisanal cheeses with Designation of Origin (CARDIN et al., 2022) and new authentication methods should be developed in order to support the achievement of this certification.

3 FINAL CONSIDERATIONS

The results obtained in this work expand the knowledge about the microbiota naturally present in artisanal cheeses produced in the Campos das Vertentes region and are an important step to support new legislation aimed at regulating the production of these cheeses. In addition, this study can be used to endorse the request for the Denomination of Origin certification, with the INPI, guaranteeing greater added value to the cheeses, benefiting the producer and also the consumer, by encouraging the consumption of this product with unique and typical characteristics.

The results exposed in this work in article format will be presented to the producers who provided the evaluated samples, through a banner (APPENDIX B), highlighting the main microorganisms present and their action during the maturation of the cheeses, thus allowing the improvement of good agricultural and manufacturing practices procedures, aiming to avoid the growth of undesirable microorganisms, in addition to encouraging the production of bloomy rind cheeses. This knowledge guarantees the producer the necessary security to maintain the manufacture of this type of cheese and the incentive to consumption, through the dissemination of information, since G. candidum is recognized as a bioprotector and the commercialization of AMCBR is being regulated.

In addition, the microorganisms isolated and identified from the cheeses and the environment (*terroir*) were deposited in the Microorganisms Culture Collection of the Food Science Department of the Federal University of Lavras (CCDCA - UFLA), thus expanding the currently existing collection and making the isolates available for new research, such as monitoring the modification of the native microbiota over time, complete DNA sequencing of species essential for maintaining cheese quality and performing other molecular analyzes, such as the mechanisms of gene expression of *G. candidum* gene expression mechanisms.

REFERENCES

AMINIFAR, Mehrnaz et al. Investigation on proteolysis and formation of volatile compounds of Lighvan cheese during ripening. **Journal of Food Science and Technology**, v. 51, p. 2454-2462, 2014.

ANDRADE, Rafaela Pereira et al. Yeasts from Canastra cheese production process: Isolation and evaluation of their potential for cheese whey fermentation. **Food Research International**, v. 91, p. 72-79, 2017.

ANDRADE, Barbara M. et al. Chemometric classification of Brazilian artisanal cheeses from different regions according to major and trace elements by ICP-OES. J**ournal of Food Composition and Analysis**, v. 109, p. 104519, 2022.

ANDRETTA, M. et al. Microbial safety status of Serro artisanal cheese produced in Brazil. **Journal of dairy science**, v. 102, n. 12, p. 10790-10798, 2019.

ANELLI, Pamela et al. Fungal mycobiota and mycotoxin risk for traditional artisanal Italian cave cheese. **Food Microbiology,** v. 78, p. 62-72, 2019.

ARAGÃO, Michele et al. Fungal community and physicochemical profiles of ripened cheeses from the Canastra of Minas Gerais, Brazil. **Journal of Food Science and Technology**, v. 59, n. 12, p. 4685-4694, 2022.

ASSOCIAÇÃO BRASILEIRA DAS INDÚSTRIAS DE QUEIJO. ABIQ. Disponível em: <https://www.abiq.com.br/index.asp>. Acesso em: 01 mar. 2023.

BARUQUI, Alfredo Melhemet al et al. Levantamento de reconhecimento de média intensidade dos solos da Zona Campos das Vertentes-MG. 2006. Disponível em: <https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/856021/1/bpd962006levantcamposvertentes.pdf>. Acesso em: 30 jun. 2022.

BERTUZZI, Andrea S. et al. 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**, v. 17, n. 2, p. 371-390, 2018.

BENSON, Dennis A. et al. GenBank. **Nucleic acids research,** v. 41, n. D1, p. 36-42, 2012.

BOLTAR, I. et al. Volatile compounds in Nanos cheese: their formation during ripening and sesonal variation. **Journal of Food Science and Technology**, v. 52, p. 608-623, 2015.

BONTINIS, Th G. et al. Study of proteolysis, lipolysis and volatile profile of a traditional Greek goat cheese (Xinotyri) during ripening. **Small Ruminant Research**, v. 105, n. 1-3, p. 193-201, 2012.

BOUTROU, Rachel; GUÉGUEN, Micheline. Interests in Geotrichum candidum for cheese technology. **International journal of food microbiology**, v. 102, n. 1, p. 1-20, 2005.

BRASIL. Decreto n° 9.013 de 29 de março de 2017. Regulamenta a lei n° 1.283, de 18 de deembro de 1950, e a lei n° 7.889, de 23 de novembro de 1989, que dispõem sobre a inspeção industrial e sanitária de produtos de origem animal. Disponível em: <https://www.planalto.gov.br/ccivil_03/_ato2015-2018/2017/decreto/d9013.htm> Acesso em: 18 jan. 2022.

BRASIL. Decreto nº 1.255, de 25 de junho de 1962. Altera o Decreto nº 30.691, de 29 de março de 1952, que aprovou o Regulamento da Inspeção Industrial e Sanitária de Produtos de Origem Animal. Diário Oficial, Rio de Janeiro, 1962. Disponível em: <http://www.planalto.gov.br/ccivil_03/Decreto/Historicos/DCM/dcm1255impressao. Htm> Acesso em 17 jul. 2022.

BRASIL. Decreto nº 9.918, de 18 de julho de 2019. Regulamenta o art. 10-A da Lei nº 1.283, de 18 de dezembro de 1950, que dispõe sobre o processo de fiscalização de produtos alimentícios de origem animal produzidos de forma artesanal. 2019a. Disponível em: <https://presrepublica.jusbrasil.com.br/legislacao/868766972/decreto-9918-19> Acesso em: 18 ago. 2022.

BRASIL. Instrução Normativa do MAPA no 57, de 15 de dezembro de 2011. Diário Oficial da União, Brasília, 2011. Disponível em: <http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=consultarLegislacaoFederal> Acesso em: 15 fev. 2023.

BRASIL. Instrução Normativa n° 73 de dezembro de 2019. Regulamento para enquadramento de produtores fornecedores de leite para fabricação de produtos lácteos artesanais. 2019b. Disponível em; <https://www.gov.br/agricultura/pt-br/assuntos/producao-animal/selo-arte-selo-queijo-artesanal/legislacao> Acesso em: 30 jun. 2022.

BRASIL. Lei Nº 13.680, de 14 de junho de 2018. Altera a Lei nº 1.283, de 18 de dezembro de 1950, para dispor sobre o processo de fiscalização de produtos alimentícios de origem animal produzidos de forma artesanal. Diário Oficial da União, Brasília, DF, 2018. Disponível em: http://www.planalto.gov.br/ccivil_03/_ato2015-2018/2018/Lei/L13680.htm>. Acesso em 13 jan. 2022.

BRASIL. Ministério da Agricultura. Instrução Normativa no. 30, de 7 de agosto de 2013. Diário Oficial da União: seção 1, Brasília, DF. ano 150, no. 152, p. 19, 8 ago. 2013. Disponível em: <http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=08/08/2013&jornal=1&pagina=19&totalArquivos=88>. Acesso em: 14 jan. 2023.

BRASIL. Portaria n° 146, de 07 de março de 1996. Aprova Regulamentos de Identidade de Produtos Lácteos. 1996. Disponível em: <https://www.defesa.agricultura.sp.gov.br/legislacoes/portaria-mapa-146-de-07-03-1996,669.html> Acesso e: 13 jan. 2022.

BRASIL. Resolução n° 7 de 28 de novembro de 2000. Oficializa os Critérios de Funcionamento e de Controle da Produção de Queijarias, para seu Relacionamento junto ao Serviço de Inspeção Federal. 2000. Disponível em: <http://www.cidasc.sc.gov.br/inspecao/files/2012/08/RESOLU%C3%87%C3%83O-07_00_funcionamento-queijarias.pdf> Acesso em: 10 jan. 2022.

CARDIN, Marco et al. Authenticity and Typicity of Traditional Cheeses: A Review on Geographical Origin Authentication Methods. **Foods**, v. 11, n. 21, p. 3379, 2022.

CASTRO, R. D. et al. Lactic acid microbiota identification in water, raw milk, endogenous starter culture, and fresh Minas artisanal cheese from the Campo das Vertentes region of Brazil during the dry and rainy seasons. **Journal of Dairy Science,** v. 99, n. 8, p. 6086-6096, 2016.

CÉSAR, Isabel Cristina da Rocha et al. Filamentous Fungi from Artisanal Cheeses Produced in Canastra Region, Brazil. **SSRN,** 2022. Disponível em: <https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4142335> Acesso em 13 jan. 2023.

COUGHLAN, Laura et al. Biotechnological applications of functional metagenomics in the food and pharmaceutical industries. **Frontiers in microbiology,** v. 6, p. 672, 2015

COZZOLINO, Rosaria et al. SPME GC-MS monitoring of volatile organic compounds to assess typicity of Pecorino di Carmasciano ewe-milk cheese. **International Journal of Dairy Technology**, v. 74, n. 2, p. 383-392, 2021.

DE FILIPPIS, F.; PARENTE, E,; ERCOLINI, D. Metagenomics insights into food fermentations. **Microbial biotechnology**, v. 10, n. 1, p. 91-102, 2017.

DE FREITAS MARTINS, Mayra Carla et al. Bacterial diversity of artisanal cheese from the Amazonian region of Brazil during the dry and rainy seasons. **Food Research International,** v. 108, p. 295-300, 2018.

DE JESUS FILHO, Milton et al. Key aroma compounds of Canastra cheese: HS-SPME optimization assisted by olfactometry and chemometrics. **Food Research International**, v. 150, p. 110788, 2021.

DE SOUSA LEÃO, Gabriel et al. Influência da aplicação de revestimento comestível em queijo minas artesanal durante o período de maturação. **Holos**, v. 2, p. 1-20, 2020.

DE SOUZA, T. P. et al. Mycobiota of Minas artisanal cheese: Safety and quality. **International Dairy Journal**, v. 120, p. 105085, 2021.

DELAMARE, Ana Paula Longaray et al. Microbiological, physico-Chemical and sensorial characteristics of serrano, an artisanal brazilian cheese. **Food and Nutrition Sciences**, v. 3, n. 8, p. 1068, 2012.

DRAKE, Mary A.; DELAHUNTY, Conor M. Sensory character of cheese and its evaluation. In: **Cheese**. Academic Press, 2017. p. 517-545.

EMATER - Empresa de Assistência Técnica e Extensão Rural do Estado de Minas Gerais. Programa queijo Minas artesanal. 2019. Disponível em: <http://www.emater.mg.gov.br/portal.cgi?flagweb=site_tpl_queijo&id=3301>. Acesso em: 19 fev. 2022.

FOX, Patrick F. et al. Biochemistry of cheese ripening. **Fundamentals of cheese science**, p. 391-442, 2017.

GALLE, Sara A. et al. Typicality and geographical origin markers of protected origin cheese from the Netherlands revealed by PTR-MS. **Journal of Agricultural and Food Chemistry**, v. 59, n. 6, p. 2554-2563, 2011.

GANTZIAS, Charalampos et al. MALDI-TOF MS profiling of non-starter lactic acid bacteria from artisanal cheeses of the Greek island of Naxos. **International journal of food microbiology**, v. 323, p. 108586, 2020.

GARLAPATI, Deviram et al. A review on the applications and recent advances in environmental DNA (eDNA) metagenomics. **Reviews in Environmental Science and Bio/Technology**, v. 18, p. 389-411, 2019.

GEZGINC, Yekta et al. Metagenomics and volatile profile of Turkish artisanal Tulum cheese microbiota. **Food Bioscience**, v. 45, p. 101497, 2022.

GÓMEZ-RUIZ, Jose Ángel et al. Relationships between volatile compounds and odour in Manchego cheese: comparison between artisanal and industrial cheeses at different ripening times. **Le Lait**, v. 82, n. 5, p. 613-628, 2002.

GUILLÉN, María D.; ABASCAL, Begoña. Nature and distribution of the volatile components in the different regions of an artisanal ripened sheep cheese. **Journal of dairy research**, v. 79, n. 1, p. 102-109, 2012.

HAYALOGLU, A. A. et al. Characterizing volatile compounds and proteolysis in Gokceada artisanal goat cheese. **Small Ruminant Research**, v. 113, n. 1, p. 187-194, 2013.

HORNE, J. et al. Differences in volatiles, and chemical, microbial and sensory characteristics between artisanal and industrial Piacentinu Ennese cheeses. **International Dairy Journal**, v. 15, n. 6-9, p. 605-617, 2005.

IMA - Instituto Mineiro de Agropecuária. **Queijo Minas Artesanal**. 2009. Diponível em: <http://www.ima.mg.gov.br/queijo-minas-artesanal> Acesso em: 13 jan. 2023.

INPI – Instituto Nacional de Propriedade Industrial. **Indicações Geográficas**. Disponível em; <https://www.gov.br/inpi/pt-br/servicos/indicacoes-geograficas> Acesso em: 10 mai. 2023.

IPHAN - Instituto do Patrimônio Histórico e Artístico Nacional. **Registro dos Queijos Artesanais de Minas, a ser inscrito no Livro dos Saberes**. 2008. Disponível em: http://portal.iphan.gov.br/. Acesso em: 05 de jan de 2023.

JÚNIOR, Luiz Carlos Gonçalves Costa et al. Maturação do Queijo Minas Artesanal da Microrregião Campo das Vertentes e os efeitos dos períodos seco e chuvoso. **Revista do Instituto de Laticínios Cândido Tostes**, v. 69, n. 2, p. 111-120, 2014.

KAHVEJIAN, A.; QUACKENBUSH, J.; THOMPSON, J. F. What would you do if you could sequence everything?. **Nature biotechnology**, v. 26, n. 10, p. 1125-1133, 2008.

KAMIMURA, Bruna A. et al. Amplicon sequencing reveals the bacterial diversity in milk, dairy premises and Serra da Canastra artisanal cheeses produced by three different farms. **Food microbiology**, v. 89, p. 103453, 2020.

KAMIMURA, Bruna A. et al. Brazilian artisanal cheeses: an overview of their characteristics, main types and regulatory aspects. **Comprehensive Reviews in Food Science and Food Safety**, v. 18, n. 5, p. 1636-1657, 2018.

KONDYLI, E.; PAPPA, E. C.; VLACHOU, A. M. Effect of package type on the composition and volatile compounds of Feta cheese. **Small Ruminant Research**, v. 108, n. 1-3, p. 95-101, 2012.

KOTHE, Caroline Isabel; MOHELLIBI, Nacer; RENAULT, Pierre. Revealing the microbial heritage of traditional Brazilian cheeses through metagenomics. **Food Research International**, v. 157, p. 111265, 2022.

LUIZ, L. M. P. et al. Isolation and identification of lactic acid bacteria from Brazilian Minas artisanal cheese. **CyTA-Journal of Food**, v. 15, n. 1, p. 125-128, 2016.

MARTIN, José Guilherme Prado; COTTER, Paul D. Filamentous fungi in artisanal cheeses: A problem to be avoided or a market opportunity?. **Heliyon**, v. 9, p. 15110, 2023.

MCSWEENEY, Paul LH. Biochemistry of cheese ripening: Introduction and overview. In: **Cheese**. Academic Press, 2017. p. 379-387.

MCSWEENEY, Paul LH. Biochemistry of cheese ripening. **International journal of dairy technology,** v. 57, n. 2-3, p. 127-144, 2004.

MENESES, José Newton Coelho de. Queijo artesanal de Minas. **Patrimônio Cultural do Brasil**, 2006.

MINAS GERAIS. Decreto n° 48.024 de 19 de agosto de 2020. Regulamenta a Lei nº 23.157, de 18 de dezembro de 2018, que dispõe sobre a produção e a comercialização dos queijos artesanais de Minas Gerais. 2020. Disponível em:<https://www.legisweb.com.br/legislacao/?id=400191> Acesso em: 18 jan. 2022.

MINAS GERAIS. Lei estadual n° 19.492, de 13 de janeiro de 2011. Altera dispositivos da lei nº 14.185, de 31 de janeiro de 2002, que dispõe sobre o processo de produção do queijo minas artesanal e dá outras providências. 2011. Disponível em: <https://www.almg.gov.br/legislacao-mineira/LEI/19492/2011/;PORTAL_SESSIONID=27A0E21B4F830519A5766360FEEF651E.worker2> Acesso em: 22 jan. 2022.

MINAS GERAIS. Lei estadual n° 20.549, de 18 de dezembro de 2012. Dispõe sobre a produção e a comercialização dos queijos artesanais de Minas Gerais. 2012. Disponível em:

<https://www.almg.gov.br/consulte/ legislacao/completa/completa.html?
tipo=LEI&num=20549&comp=&ano=2012>. Acesso em: 17 jan. 2022.

MINAS GERAIS. Lei estadual n° 22.926 de 12 de janeiro de 2018. Dispõe sobre o Programa de Certificação de Produtos Agropecuários e Agroindustriais Certifica Minas e dá outras providências. 2018b. Disponível em:
<https://www.mg.gov.br/sites/default/files/servicos/arquivos/2018/
lei_n_22.926_de_12012018.pdf> Acesso em: 19 jan. 2022.

MINAS GERAIS. Lei estadual n° 23.157, de 18 de dezembro de 2018. Dispõe sobre a produção e a comercialização dos queijos artesanais de Minas Gerais. 2018a. Disponível em:
<https://www.almg.gov.br/consulte/legislacao/completa/completa.htmltipo=LEI&num=23157
&comp=&ano=2018>. Acesso em: 17 jan. 2022

MINAS GERAIS. Lei n° 14.185, de 31 de janeiro de 2002. Dispõe sobre o processo de produção do queijo minas artesanal e dá outras providências. 2002. Disponível em:
<https://www.almg.gov.br/consulte/ legislacao/completa/completa.html?
ano=2002&num=14185&tipo=LEI>. Acesso em: 15 jan. 2022.

MINAS GERAIS. Portaria IMA n° 1.969, de 26 de março de 2020. Dispõe sobre a produção de Queijo Minas Artesanal - QMA em queijarias e entrepostos localizados dentro de microrregiões definidas e para as demais regiões do Estado, caracterizadas ou não como produtora de Queijo Minas Artesanal – QMA. 2020. Disponível em:
<http://ima.mg.gov.br/index.php?
preview=1&option=com_dropfiles&format=&task=frontfile.
download&catid=1706&id=17923&Itemid=1000000000000>. Acesso em: 18 fev. 2022.

MINAS GERAIS. Portaria SEAPA nº 1.022, de 03 de novembro de 2009. Identifica a Microrregião do Campo das Vertentes. Disponível em:<http://ima.mg.gov.br/index.php?
preview=1&option=com_dropfiles&format=&task=frontfile.download&catid=1576&id=1655
8&Itemid=1000000000000>. Acesso em: 10 jan. 2022.

MINAS GERAIS. Resolução SEAPA N° 42, de 27 de dezembro 2022. Reconhece e define sobre Queijo Minas Artesanal de Casca Florida (QMACF). Disponível em:
<http://jornal.iof.mg.gov.br/xmlui/handle/123456789/276763 >. Acesso em 31 jan. 2023.

MORENO, V. J. Caracterização física e físico-química do queijo Minas artesanal da microrregião Campo das Vertentes. 2013. **Tese de Doutorado**. Dissertação de mestrado, Universidade Federal de Juiz de Fora. Programa de Pós-Graduação em ciênca e Tecnologia do Leite e Derivados.

NERO, Luís Augusto et al. Lactic microbiota of the minas artisanal cheese produced in the serro region, Minas Gerais, Brazil. **LWT**, v. 148, p. 111698, 2021.

NETTO, Marcos Mergarejo. A geografia do queijo minas artesanal. **Tese de doutorado**, 2011.

NI, Qianlin et al. The volatile organic compound profile of ripened cheese is influenced by crude protein shortage and conjugated linoleic acid supplementation in the cow's diet. **Journal of dairy science**, v. 103, n. 2, p. 1377-1390, 2020.

OLIVEIRA, MC de S. et al. Fundamentos teóricos-práticos e protocolos de extração e de amplificação de DNA por meio da técnica de reação em cadeia de polimerase. 2007. Disponível em: <https://www.alice.cnptia.embrapa.br/alice/bitstream/doc/48295/1/LivroProtMolecular.pdf> Acesso em: 30 mar. 2021

PASTERNAK, Jacyr. New methods of microbiological identification using MALDI-TOF. **Einstein (Sao Paulo)**, v. 10, p. 118-119, 2012.

PENLAND, Marine et al. Linking Pélardon artisanal goat cheese microbial communities to aroma compounds during cheese-making and ripening. International **Journal of Food Microbiology**, v. 345, p. 109130, 2021.

PEREIRA, Débora; SPERAT-CZAR, Arnaud; ROUSTEL, Sébastian. **Guia de Cura de Queijos**. 2° ed. Rio Doce – MG, 2017.

PERIN, Luana Martins et al. Bacterial ecology of artisanal Minas cheeses assessed by culture-dependent and-independent methods. **Food microbiology**, v. 65, p. 160-169, 2017.

PILLONEL, L. et al. Analytical methods for the determination of the geographic origin of Emmental cheese: volatile compounds by GC/MS-FID and electronic nose. **European Food Research and Technology,** v. 216, p. 179-183, 2003.

PUIG, M. S. et al. Metagenómica aplicada a la industria alimentaria. T**ecnoalimen: tecnología alimentaria y packaging**, n. 23, p. 24-26, 2018.

PYZ-ŁUKASIK, Renata et al. Occurrence of Listeria monocytogenes in artisanal cheeses from Poland and its identification by MALDI-TOF MS. **Pathogens**, v. 10, n. 6, p. 632, 2021.

REMOR, Ariane et al. Investigação metagenômica em queijos tipo coloniais, produzidos com leite pasteurizado e comercializados no estado do Rio Grande do Sul, Brasil. **Veterinária e Zootecnia**, v. 28, p. 1-9, 2021.

REYES-DÍAZ, Ricardo et al. Volatile and sensory evaluation of Mexican Fresco cheese as affected by specific wild Lactococcus lactis strains. **Journal of Dairy Science**, v. 103, n. 1, p. 242-253, 2020.

RUPPERT, K. M.; KLINE, R. J.; RAHMAN, M. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. **Global Ecology and Conservation**, v. 17, p. e00547, 2019.

SÁNCHEZ-JUANES, Fernando et al. Identification of species and subspecies of lactic acid bacteria present in spanish cheeses type “Torta” by MALDI-TOF MS and pheS gene analyses. **Microorganisms**, v. 8, n. 2, p. 301, 2020.

SANT'ANNA, Felipe Machado et al. Microbial shifts in Minas artisanal cheeses from the Serra do Salitre region of Minas Gerais, Brazil throughout ripening time. **Food microbiology**, v. 82, p. 349-362, 2019.

SAY, Dilek. Physicochemical composition, nitrogen fraction and volatile profiles of goat cheese made with artisanal liquid coagulant. **Journal of Food Science and Technology**, v. 59, n. 6, p. 2469-2478, 2022.

SCHMIDT, Ruth et al. Volatile affairs in microbial interactions. **The ISME journal**, v. 9, n. 11, p. 2329-2335, 2015.

SERHAN, Mireille et al. Changes in proteolysis and volatile fraction during ripening of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. **Small Ruminant Research**, v. 90, n. 1-3, p. 75-82, 2010.

SILVA, João Marcos Maia. Micobiota core de queijos de leite cru produzidos na região da Serra da Canastra. **Dissertação.** 2020.

ZAHA, Arnaldo; FERREIRA, Henrique Bunselmeyer; PASSAGLIA, Luciane MP. **Biologia Molecular Básica-5**. Artmed Editora, 2014.

ZHENG, Xiaochun et al. Dynamic changes in bacterial microbiota succession and flavour development during milk fermentation of Kazak artisanal cheese. **International Dairy Journal,** v. 113, p. 104878, 2021.

PART TWO – ARTICLES

ARTICLE 1 - MYCOBIOTA AND PROFILE OF VOLATILE ORGANIC COMPOUNDS IN ARTISANAL MINAS CHEESE PRODUCED IN THE CAMPOS DAS VERTENTES REGION/MG

Formatted according to African Journal of Food Science guidelines
ISSN:1996:0794
(preliminary version)

Fernanda Costa Prates[1], Vitória Luisa Soares[2], Fabiana Reinis Franca Passamani[3], Michelle de Oliveira Paiva Aragão[4] and Luis Roberto Batista[5]*

Department of Food Science/ Federal University of Lavras – Minas Gerais/ Brazil

[1]fernanda.prates@estudante.ufla.br +5532991583784

[2]vitoria.soares@estudante.ufla.br +5535998171796

[3]fabireinis@gmail.com +5535988694908

[4]micheleoaragao@gmail.com +553599820413

[5]*Corresponding author: luisrb@ufla.br +5535984769559

ABSTRACT

The artisanal cheese produced in Minas Gerais/Brazil is a product of extreme economic and cultural importance to the region and was recognized as Brazilian intangible heritage. Its production takes place on rural properties, using raw milk, rennet, "pingo" (endogenous ferment), and salt. After production, the cheese undergoes maturation, a stage where chemical reactions catalyzed by microbial enzymes occur, ensuring its typicality and uniqueness. Centesimal composition analysis, identification of fungi using culture-dependent methodology, and profile of volatile organic compounds were performed on cheese samples produced by four certified cheese dairies located in the Campos das Vertentes region, Minas Gerais. The analyzed samples presented moisture content 38.7% and 44.7% and fat content between 33% and 37%, equivalent to 57% fat in the dry matter, classifying it as a fatty cheese. The predominant fungi in the cheeses produced in this region were *Geotrichum candidum, Kluyveromyces lactis, Diutina catenulata*, and some species of *Candida*. Regarding volatile compounds, a higher relative area was observed for acetic acid, decanoic acid, hexanoic acid, octanoic acid, 3-methylbutanol alcohol, 2-heptanone ketone, 2-nonenone ketone and ethyl hexanoate ester. It is expected that the cheese produced in this region presents aromatic notes of vinegar, oily, rancid, sweet, fruity, burnt, and green.

Keywords: artisanal cheese, volatile compounds, mycobiota, *Geotrichum candidum*.

1. Introduction

Artisanal Minas Cheese is produced using freshly-milked raw milk, rennet, endogenous ferment, and salt. The manufacturing process is straightforward and involves adding enzymatic rennet to the milk, allowing the curd to rest, cutting the curds, adding the endogenous ferment, molding, and manual salting. The specific ferment used in artisanal Minas cheese, known as "pingo," refers to the whey collected during the draining stage of the cheeses produced in the previous batch (ANDRADE et al., 2017). In raw milk, over 400 different species of lactic acid bacteria, yeasts, and fungi have been identified. This diversity decreases within the cheeses, where only a few species of lactic acid bacteria become dominant. However, on the surface of the cheeses, a wide diversity of microbial species is reported, primarily due to significant variations in the dynamics of these same species in each specific cheese and the *terroir* of artisanal cheeses. This is due to the transfer of microorganisms present in the environment, surfaces, equipment, and aging chambers of cheese factories to the cheeses (MONTEL et al., 2014). Pingo is also rich in microorganisms, which, combined with the microorganisms present in raw milk, contribute to the cheese's maturation process, resulting in physical, chemical, biological, and sensory changes that ensure the distinctiveness and uniqueness of the cheeses (PENLAND et al., 2021).

Artisanal Minas Cheese has been recognized as an intangible heritage of Brazil (IPHAN, 2008), and currently, 10 production regions are officially recognized by the Instituto Mineiro de Agropecuária (IMA), including the Campos das Vertentes region, which was officially recognized in 2009 through Ordinance No. 1.022 (MINAS GERAIS, 2009). Artisanal cheeses produced in this region must be aged for a minimum of 22 days (MINAS GERAIS, 2020) and have a straw-yellow color with a medium to semi-hard texture. When cut, it tends to crumble into small pieces with mechanical holes, has a pronounced cheese odor, and a slightly acidic flavor predominates when tasted (CASTRO et al., 2016).

There is still limited knowledge about the composition, microbiota, and profile of volatile compounds in artisanal cheeses produced in this region. It is of interest to all stakeholders in the production chain to understand the relationship between the present microorganisms and the intrinsic characteristics of the cheeses. With this knowledge, it will be possible to propose improvements in all stages of the manufacturing process to enhance the value of the cheese while ensuring its microbiological safety and stability. Additionally, this knowledge can serve as a basis for new sanitary regulations that authorize and regulate its production. Several techniques can reveal the microbiota in food; however, culture-dependent techniques limit the analysis to cultivable microorganisms but allow for the isolation and separate study of the identified strains. Among the techniques that rely on microbial culture, the use of Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) stands out. This technique is widely used in various fields, such as protein analysis in biomedical research and the identification of microorganisms in clinical and food microbiology (PASTERNAK, 2012). It has been used to identify bacteria (PYZ-ŁUKASIK et al., 2021; GANTZIAS et al., 2020; SÁNCHEZ-JUANES et al., 2020), yeasts, and filamentous fungi (ARAGÃO et al., 2021; PENLAND et al., 2021; DE SOUZA et al., 2021; ANDRADE et al., 2017) present in artisanal cheeses.

During the maturation process of artisanal cheeses, the microorganisms present produce volatile organic compounds through various metabolic pathways. These compounds are associated with flavor perception (MCSWEENEY ET AL., 2017; DRAKE, DELAHUNTY, 2017; SCHMIDT et al., 2015) and contribute to the typical aroma of the cheese. They are primarily produced through lipolytic and proteolytic pathways, as well as lactose, lactate, and citrate metabolism (BERTUZZI et al., 2018). Microbial enzymes that degrade amino acids produce acids, alcohols, aldehydes, amines, and various sulfur compounds, while those that break down fatty acids produce esters, methyl ketones, and secondary alcohols (NI et al., 2020), resulting in different volatile compounds in cheeses produced in different regions, thus ensuring cheese typicity (SCHMIDT et al., 2015).

The most commonly used technique for extracting and identifying volatile compounds in cheese is solid-phase microextraction (SPME) coupled with gas chromatography. This technique integrates sample extraction, concentration, and introduction into a single device, eliminating the need for organic solvents (TETER et al., 2020). The main advantages of this technique are reduced sample requirements, automated sample preparation and chromatographic analysis, simplicity, high selectivity, and fast results. However, the effectiveness of the method depends on various factors, including the nature of the polymer material used in the extraction (fiber), the selected chromatographic column, the type of detector used, and the pre-established analysis parameters, such as extraction time and temperature, sample equilibrium in the vial, ionic strength, pH, agitation, sample quantity, and dilution (BERTUZZI ET AL., 2018; ARCARI ET AL., 2017).

The mapping of volatile compounds in cheeses has been used as a method to verify the authenticity of artisanal cheeses, preventing the occurrence of food fraud, which are intentional actions to deceive consumers and obtain economic advantages with the commercialization of the products (CARDIN et al., 2022). This knowledge can also serve as a basis for obtaining Geographical Indication certificates. In Brazil, the process of obtaining the IG certificate is regulated by Law nº 9.279/1996 (BRASIL, 1996) and to obtain the certificate from the National Institute of Industrial Property (INPI), the producer needs to follow a series of requirements, which can include factors such as production methods, varieties of ingredients used, cultural or historical traditions. This certification can provide a quality differential and greater added value for the producer and protect the consumer, guaranteeing the acquisition of genuine and authentic products, avoiding fraud and imitations.

The profile of volatile compounds in artisanal cheeses was conducted in various countries (GEZGINC et al., 2022; COZZOLINO et al., 2021; ZHENG et al., 2021; PENLAND et al., 2021; DE JESUS FILHO et al., 2021; REYES-DIAZ et al., 2020; GÓMEZ-RUIZ et al., 2020; BOLTAR et al., 2015), primarily focusing on the relationship between changes in volatile profiles and cheese maturation time. Many of these studies aim not only to identify the volatile compounds but also to obtain the olfactory profile, which, combined with human sensory perception, may establish a sort of olfactory signature for cheeses. This can be utilized, for example, to obtain Denomination of Origin (DO) certification for a specific region (GEZGINC et al., 2022).

Therefore, this study aims to contribute to the expansion of knowledge regarding the composition of artisanal cheeses produced in the Campo das Vertentes region, Minas Gerais. Additionally, it seeks to reveal the presence of the microbial community in these cheeses and

correlates its presence with the production of volatile compounds. This research will facilitate the attainment of Protected Designation of Origin/Geographical Indication titles once the influence of the production location and its specific characteristics on the microbiological and sensory parameters of artisanal cheeses has been proven.

2. Methodology

2.1. Sampling

Environmental and artisanal Minas Cheese (AMC) samples were collected from 4 certified cheese dairies located in the Campo das Vertentes microregion in Minas Gerais, Brazil, with a maturation period of 22 to 25 days (Table 1) The samples, identified as CV1, CV2, CV3, and CV4, were packed and stored in sterile containers and transported to the Mycology and Mycotoxins Laboratory, Department of Food Science, Federal University of Lavras, where they were analyzed and stored in an ultrafreezer at -80°C for subsequent compositional and volatile organic compound analyses.

Table 1. Geographical coordinates of the cheese factories.

Sample	Latitude (S)	Longitude (W)	Altitude (m)
CV1	21°02’20”	44°11’39”	961
CV2	21°05’08”	44°14’26”	890
CV3	21°07’21”	44°10’43”	917
CV4	21°04’33”	44°14’44”	943

2.2. Centesimal Composition and pH Analysis

The following parameters were evaluated in triplicate for centesimal composition analysis, according to official methodologies: Moisture content (AOAC 926.08), Ash content (AOAC 935.42), Nitrogen compounds using the Kjeldahl method (AOAC 2001.14), Fat content (BRITISH STANDARDS INSTITUTION, 1989), and Protein content determined by multiplying the percentage of total nitrogen (TN) by the correction factor 6.38 (BRASIL, 2006). pH measurements were determined by placing the pH electrode (Portamess 913, Knick, Berlin, Germany) directly into the grated cheese mass.

2.3. Isolation and Characterization of the Mycobiota

The isolation of filamentous fungi and yeasts from cheese samples was carried out using the standardized culture media Dicloran Rose Bengal Chloramphenicol (DRBC) and Yeast Extract Peptone Glucose (1% Yeast Extract, 2% Bacteriological Peptone, 2% Glucose, and 1.5% Agar) through the serial dilution technique. 25g of cheese previously collected from equidistant points on the sides, one central point from the base, and one from the surface were weighed and homogenized in 225 mL of 0.1% peptone water using a Stomacher (490 strokes/min for 2 min). Serial dilutions were performed, and surface plating was carried out up to a dilution of 10^{-6}.

For the analysis of air samples from the cheese production and maturation rooms, sedimentation technique was employed for 15 minutes. Swabs moistened in peptone saline solution were used to collect samples from wooden planks. A square acetate mold, measuring 10 cm on each side, totaling 100 cm², was utilized. The DRBC plates were incubated at 25°C in a BOD incubator for 5 to 7 days to evaluate and isolate filamentous fungi, while the YEPG plates were incubated at 28°C for 48 hours for better visualization of yeasts. After the incubation period, colony-forming units (CFU/g) were counted, and morphological characterization of the colonies was performed.

Following the determination of the microorganism populations present in the environment, wooden planks, and cheese, morphological characterization (colony color, mycelium color, reverse color, presence of streaks and pigments on the reverse, shape, size, surface, edges, and profile) of the colonies grown in culture media was conducted to differentiate the morphotypes present. Filamentous fungi were isolated on Malt Extract Agar (MA), and yeasts were isolated on YEPG medium. For macroscopic and microscopic observation, the activated fungi were cultured on malt agar (MA) and subsequently transferred to culture media at standardized temperatures according to identification manuals for the observed genus. The Klich Manual (2002) was used for species belonging to the *Aspergillus* genus, and the Pitt Manual (2000) was used for the *Penicillium* genus.

The yeasts were activated on malt agar (MA) and then subcultured onto YEPG agar medium, where they were grown for 18 hours at 28°C. A loopful of the yeast mass was transferred to an Eppendorf tube containing 6 µL of 25% formic acid solution and vortexed for 1 min. Next, 1 µL of this suspension was transferred to the stainless-steel plate of the equipment. When the samples were nearly dry, 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA, Fluka; Buchs, Switzerland) matrix solution saturated in an organic solvent was added and gently mixed. The mixture was air-dried at room temperature.

The analyses were performed using a MALDI-TOF system in triplicate to assess the quality and reproducibility of the spectra. Finally, the mass spectra were processed using MALDI Biotyper software package 3.0 (Bruker Daltonics, Bremen, Germany) for microbial identification. Scores above 2.00 were considered for result evaluation.

2.4. Profile of Volatile Organic Compounds (VOC’s)

For the qualitative analysis of volatile organic compounds present in artisanal cheese samples, the solid-phase microextraction coupled with high-resolution gas chromatography/mass spectrometry (SPME-GC/MS) method was used. The methodology was developed and tested specifically for cured cheeses by the Central Analysis and Chemical Prospecting (CAPQ) of the Department of Chemistry at the Federal University of Lavras.

To prepare the samples, 30 g of each analyzed sample was ground with liquid nitrogen, fractionated (5 g), and transferred to a 20 mL vial. The samples were heated at 60°C for 10 minutes, and then the fiber was exposed in the headspace for 30 minutes to adsorb the volatile compounds. Solid-phase microextraction was performed using a DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fiber with a film thickness of 50/30 µm. The selected fiber provides greater chromatographic qualitative (number of compounds) and quantitative information (peak area of the chromatogram) (TAVARIA et al., 2004).

After extraction, the fiber was inserted into the injector of the chromatograph (SHIMADZU-CG/MS-QP2010) for separation and identification of the adsorbed volatile compounds from the samples. The molecules were desorbed in the injector (SHIMADZU-AOC5000) at 250°C for 5 minutes in Splitless mode. The equipment was equipped with a fused silica capillary column (Slb-5MS, Supelco, 30 m x 0.25 mm x 0.25 μm) with an initial temperature of 40°C for 10 minutes, and then ramped to 230°C at a rate of 5°C/min, with a total runtime of 53 minutes. Helium gas was used as the carrier gas at a flow rate of 2.0 mL/min.

The identification of compounds was performed by integrating the obtained peaks and comparing them with the mass spectra in the NIST database (GC/MS Solution Library, 2020) based on the mass spectrum. The results were expressed as the percentage of the area of each analyte present in the cheese samples. The volatile compounds present in the samples were also identified by comparing the Kovats index values calculated from the experimentally obtained retention indices for each compound with those in the literature.

2.5. Statistical Analysis

Descriptive analysis was performed for the centesimal composition and pH analyses, and the results were expressed as mean and standard deviation using the Sisvar software, version 5.6 (FERREIRA, 2015). The means of yeast and filamentous fungi populations, along with standard deviation and relative frequency graphs of microorganisms, were performed using LibreOffice Calc. Principal Component Analysis (PCA) was conducted for the analysis of volatile organic compounds using Sensomaker software (PINHEIRO; NUNES, 2013).

3. Results and Discussion

3.1. Centesimal Composition and pH Analyses

In the evaluated samples, a moisture content ranging from 38.7% to 44.7% was observed, classifying this cheese as a medium-moisture cheese (referred to as semi-hard cheese). The average fat content ranged from 33% to 37%, equivalent to 57% of fat in the dry matter, categorizing it as a fatty cheese (Table 2) (BRASIL, 1996).

Castro et al. (2016) evaluated the characteristics of AMC in natura produced in the Campos das Vertentes region and found moisture content ranging from 49% to 56% and fat content ranging from 28% to 29%. Protein values ranged from 22.3 to 25.02%, while Castro et al. (2016) reported values between 17.5% and 19.8%. The differences in the results of moisture, protein and fat in relation to our study can be attributed to the lack of maturation period in the mentioned research. Júnior et al. (2014) evaluated the pH behavior over 30 days of maturation of AMC produced in the Campos das Vertentes region and observed a slight increase in this parameter in the dry and rainy seasons. This occurs due to the metabolization of lactic acid previously produced by bacteria by yeast. The values found by these authors ranged from 5.0 to 5.5, similar to what was verified in the present study.

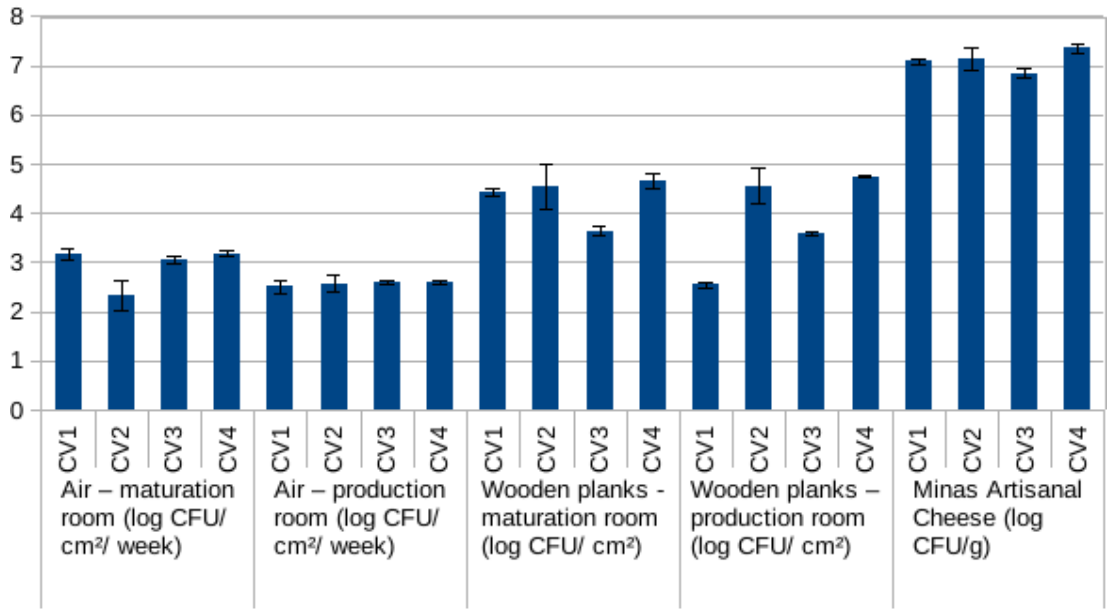
Table 2. Average values of moisture, protein, fat, mineral residue, and pH of Artisanal Minas Cheese produced in the Campos das Vertentes region, Minas Gerais.

Sample	Analysis	Mean + Standard Deviation	Coefficient of variation (%)
CV1	Moisture	38,70 ± 4,40	11,50
	Protein	25,02 ± 0,97	3,89
	Fat	34,00± 3,70	11,00
	Mineral waste	4,50 ± 0,50	10,10
	pH	5,48 ± 0,10	1,75
CV2	Moisture	42,88 ± 4,39	10,25
	Protein	23,40 ± 1,32	5,66
	Fat	33,00 ± 3,83	11,60
	Mineral waste	3,30 ± 0,40	12,62
	pH	5,45 ± 0,06	1,06
CV3	Moisture	41,62 ± 6,51	15,64
	Protein	23,00 ± 1,03	4,48
	Fat	37,00 ± 5,29	14,30
	Mineral waste	3,35 ± 0,31	9,28
	pH	5,32 ± 0,15	2,82
CV4	Moisture	44,70 ± 4,63	10,37
	Protein	22,35 ± 1,44	6,43
	Fat	31,75 ± 2,22	6,98
	Mineral waste	3,77 ± 0,77	20,47
	pH	4,98 ± 0,05	1,07

3.2. Mycobiota in AMC and the environment

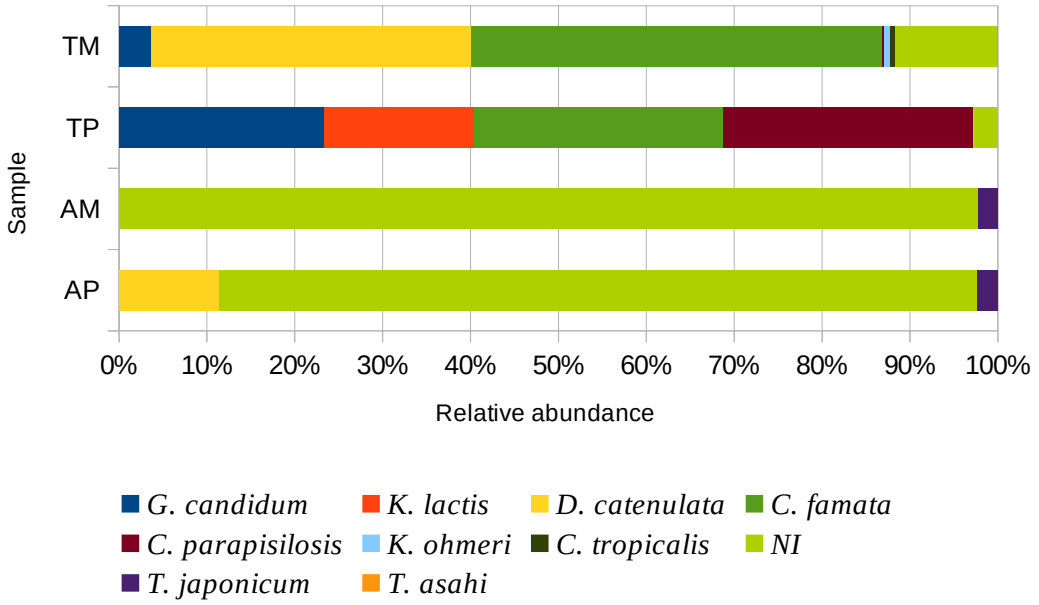
After isolation of fungi and yeasts present in the cheese and the environment using specific culture media (DRBC and YEPG), a high population was observed in all evaluated cheese samples (Figure 1), averaging 6 to 7 log CFU/g and ambient air samples between 3 and 4 log CFU/cm²/week and wooden board samples in the manufacturing and maturation sectors between 4 and 5 log CFU/cm².

Figure 1. Populations means of fungi and yeasts in the environment and in artisanal cheeses.



To facilitate the observation of the results, the cheese factories were grouped by environment, and the identification of yeasts present in the ambient air and on the wooden planks of the production room and maturation rooms was performed using MALDI-TOF, as represented in Figure 2.

Figure 2. Relative abundance of filamentous fungi and yeasts in cheese-making environments.



Legend: TM - Wooden planks from maturation rooms; TP - Wooden planks from production rooms; AM - Ambient air from maturation rooms; AP - Ambient air from production rooms; NI: Unidentified isolated.

In the samples collected in the environment, the number of yeast species identified was higher than the wooden tables collected in the maturation chambers. In the air samples,

there was a predominance of filamentous fungi (not identified), and the species of yeasts found were not also present in the wooden tables. In artisan cheesecakes, it was possible to visually observe the differences in the microbiota (Figure 3), since the cheesecakes CV1 and CV3 presented a white cover on the surface, framing the cheesecake as flowery shell (MINAS GERAIS, 2022). In our complaints, in addition to the identification of yeasts, two filamentous fungi were identified based on macroscopic and microscopic morphological characteristics (Figure 4).

Figure 3. Artisanal Minas Cheese samples collected from the complaints of the Campos das Vertentes/MG region.

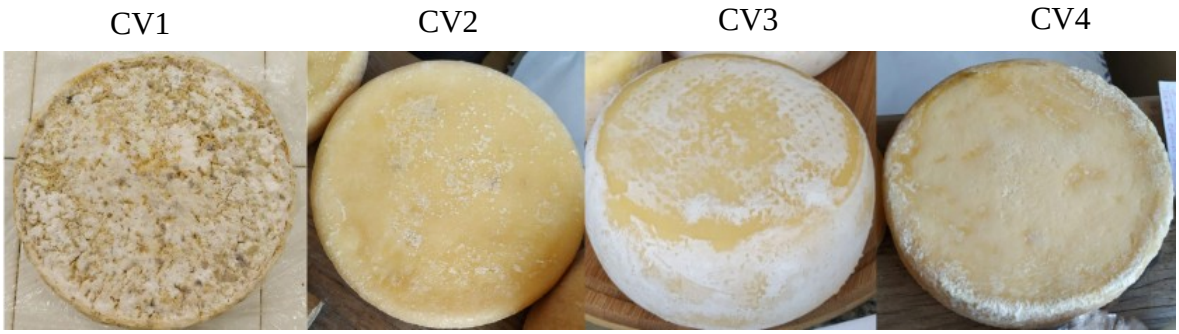
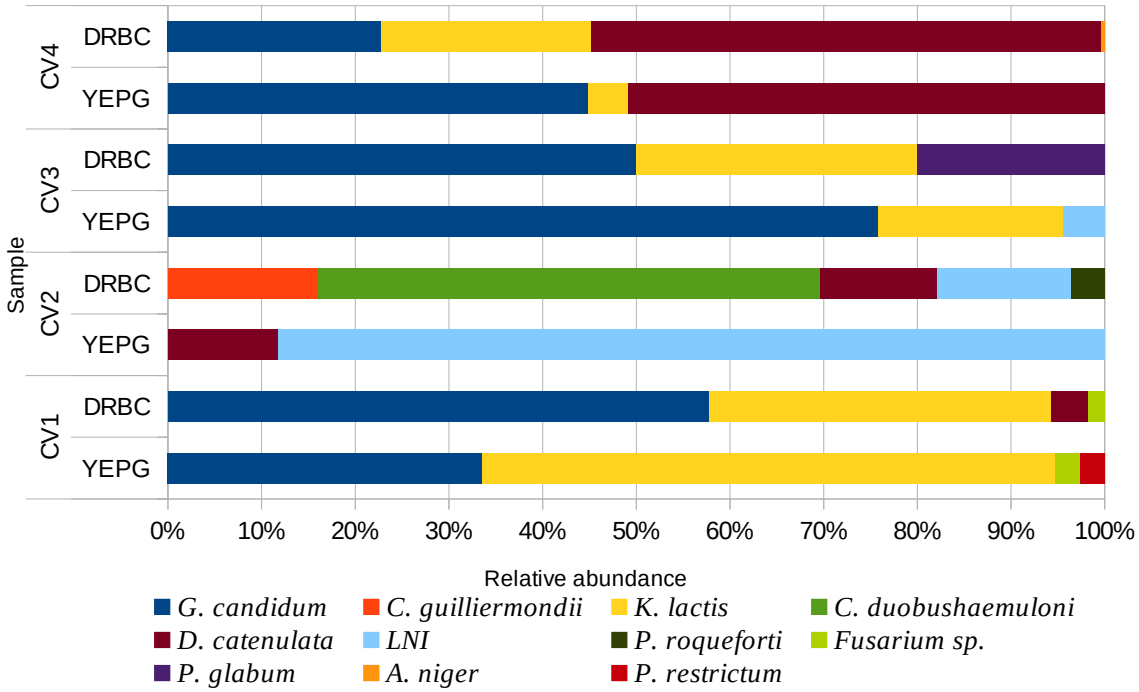


Figure 4. Relative abundance of filamentous fungi and yeast occurrence in Artisanal Minas Cheese produced in the Campos das Vertentes region, Minas Gerais.



Legend: LNI = Unidentified Isolated Yeasts.

Among the isolated and identified yeasts in artisanal cheeses, the presence of *G. candidum* stands out, except in the CV2 cheese factory where the species was not identified.

G. candidum was also identified on the wooden planks, but it was not detected in the air samples from the environments. The presence of *G. candidum* in artisanal cheeses produced in Brazil has been described by several authors (ARAGÃO et al., 2021; DE SOUZA et al., 2021), and this fungus has proven to be important in cheese ripening, contributing to the development of the characteristic flavor (ARAGÃO et al., 2021) and acting as a bioprotective agent (BOUTROU, GUÉGUEN, 2005), inhibiting the growth of spoilage microorganisms such as *Mucor spp., Aspergillus ochraceus, and pathogens Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, and Bacillus cereus* (MARIANI et al., 2007; DIEULEVEUX, V. et al., 1998). *G. candidum* is capable of alkalizing the medium through the metabolism of lactic acid, which allows its survival and growth during cheese ripening, coexisting with the predominant LAB in cheese, and is responsible for the production of various volatile organic compounds such as aldehydes and carboxylic acids through deamination reactions, influencing the sensory perception of the cheese (ŠIPOŠOVÁ et al., 2021).

These specific characteristics of *G. candidum* and the extensive discussion on the benefits of its presence in artisanal cheeses from Minas Gerais, ensuring their microbiological safety, have been instrumental in regulating its production and authorizing its commercialization through Resolution 42 of the Secretary of State for Agriculture, Livestock and Supply/ Minas Gerais, which defines the “casca florida” variety of Minas artisanal cheese as having a "visual dominance of filamentous fungi, predominantly *Galactomyces Geotrichum (Geotrichum candidum* or *Geotrichum silvicola*)" (MINAS GERAIS, 2022). However, there is still much to be discussed and defined regarding the chemical and sensory characteristics (such as the perception of odor, due to the presence of specific volatile compounds) and the physical aspects (such as appearance) that differentiate cheese with a flower rind and cheese of washed bark, in addition to verifying the presence of *G. candidum*.

Kluyveromyces lactis was isolated from the production area wooden planks and cheeses, except in the CV2 sample. *K. lactis* is often found in cheeses and dairy products due to its ability to utilize lactose as a substrate and its fast growth rate (LECLERCQ - PERLAT, CORRIEU, & SPINNLER, 2004). *K. lactis* is capable of producing acetaldehyde, ethanol, branched-chain aldehydes, alcohols, and acetic acid esters, which contribute to fruity and acidic notes in artisanal cheeses (ATANASSOVA et al., 2016). Zheng et al. (2018) also isolated this yeast in artisanal cheese produced in China and described its ability to produce volatile compounds important for flavor development in artisanal cheese.

Diutina Catenulata was the predominant yeast on the surfaces of the maturation rooms. This yeast was also isolated from the cheeses, except in the CV3 sample. The presence of *D. catenulata* has been reported in artisanal cheeses produced in the Serra da Canastra region (KOTHE, MOHELLIBI, RENAULT, 2022; ARAGÃO et al., 2021; ANDRADE et al., 2017). In the cheeses produced in the CV2 cheese factory, where *G. candidum* and *K. lactis* were not found, the yeasts present, in addition to *D. catenulata,* were *Candida duobushaemuloni,* and *Candida guilliermondii*. There were no findings in the literature regarding the presence of *C. duobushaemuloni* and *C. guilliermondii* species in Brazilian artisanal cheeses.

Candida parapsilosis, Candida famata, Candida tropicalis, Trichosporum japonicum, Trichosporum asahi and *Kodamaea ohmeri* were present in the environment but they were not

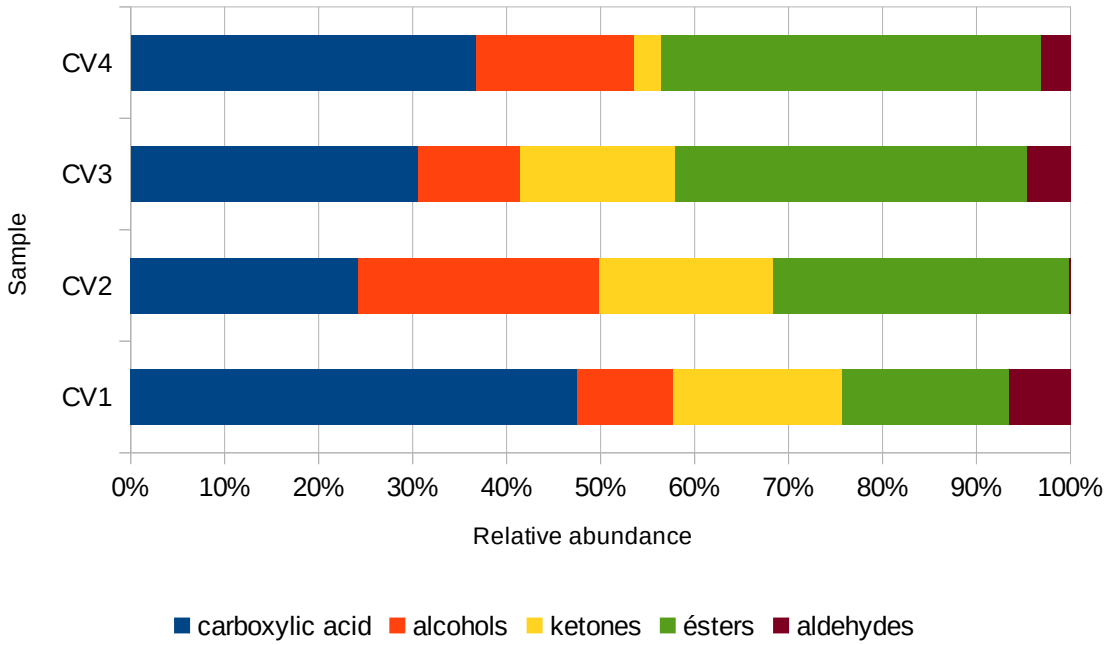
identified in the artisanal cheeses. DE SOUZA et al. (2021) assessed the mycobiota of dairy environments in the Serro region and also found the yeast *T. japonicum* in the maturation rooms. Borelli et al. (2018) reported *K. ohmeri* as one of the most frequent yeasts in Canastra cheese. The first report of *K. ohmeri* in artisanal cheeses was in cheese produced in the Serra da Canastra region (BORELLI et al., 2006). *K. ohmeri* has also been reported in AMC produced in the Serro region, where it showed low lipolytic activity and β-galactosidase production (CARDOSO et al., 2015).

The species of filamentous fungi cultivated in the media used showed lower counts, averaging two logs, compared to the yeasts. This is due to the shorter generation time of yeasts, which quickly consume the substrate and inhibit the growth of filamentous fungi. Among the filamentous fungi found in the cheeses were species of *Aspergillus niger* in the CV4 sample, *Fusarium* sp and *Penicillium restrictum* in the CV1 sample, *Penicillium roqueforti* in the CV2 sample, and *Penicillium glabrum* in the CV3 sample. Fungi belonging to the genera *Fusarium*, *A. niger*, *P. roqueforti*, and *P. glabrum* were also identified in cheese produced in the Serro region. *A. niger* is associated with the production of mycotoxins. However, the presence of the fungus is not the only indicator of mycotoxin production or pathogenicity, as several factors are required for their production, such as high population, relative humidity, and temperature of the medium (DE SOUZA et al., 2021).

3.3. Profile of Volatile Organic Compounds

Volatile organic compounds (VOCs) are released during chewing and are related to the perception of flavor through the oronasal cavity, influencing the sensory evaluation and acceptance of artisanal cheeses (BEZERRA et al., 2017). In artisanal cheeses produced in the Campos das Vertentes region of Minas Gerais, Brazil, a total of 106 different volatile compounds were identified, including 14 carboxylic acids, 19 alcohols, 6 aldehydes, 11 ketones, and 56 esters. Carboxylic acids and esters were the predominant chemical classes in the analyzed samples (Figure 5).

Figure 5. Relative abundance of different functional groups of volatile organic compounds found in cheeses produced in the Campos das Vertentes region, Minas Gerais.



The relative area (%), functional group, aromatic descriptors attributed to the compounds found in the artisanal cheese, and microorganisms producing are listed in Table 3.

Table 3. Mean values of relative area (%), odor description of volatile organic compounds found and microorganisms produtores in artisanal cheeses produced in the Campos das Vertentes region, Minas Gerais.

				Sample (relative area (%))**					
Functional group	**Compound**	**Flavor***	**TR**	**CV1**	**CV2**	**CV3**	**CV4**	**Mean**	**Producing microorganism**
Carboxylic acid (14)	hexanoic acid	caprylic, sweaty, sweaty dirty socks, vegetable oil	1047	10,07	3,35	8,51	12,48	8,60±3,86	*K. lactis*[6], *G. candidum*[4]
	decanoic acid	rancid, fat	1378	9,01	9,90	10,68	3,96	8,39±3,03	
	octanoic acid	caprilic, oily	1202	15,01	4,13	6,41	0,28	6,46±6,24	*G. candidum*[4]
	acetic acid	acetic, acidic, vinegar	ND	8,45	3,42	4,08	5,20	5,00±2,23	*K. lactis*[1,6]
	benzoic acid	urine	1192	0,00	2,53	1,75	6,82	2,77±2,90	
	propanedioic acid (malonic acid)		ND	0,00	0,00	0,00	4,15	1,04±2,08	
	benzeneacetic acid	honey, sweet	1257	3,93	0,00	0,16	0,00	1,02±1,94	
	2- hydroxypropanoic acid (lactic ácido)	acidic	920	0,17	0,00	0,00	2,77	0,74±1,36	
	2- methyl butyric acid	cheese, sweat	898	0,48	0,44	0,15	0,93	0,50±0,32	
	3- methyl butanoic acid	sweat, acid, rancid	895	0,00	0,30	0,47	0,00	0,19±0,23	*K. lactis*[6]
	nonanoic acid	green, fat	1284	0,00	0,12	0,05	0,19	0,09±0,08	*K. lactis*[1]
	propanoic acid	pungent, rancid, soy	ND	0,24	0,00	0,00	0,00	0,06±0,12	*K. lactis*[6]
	heptanoic acid		1086	0,15	0,00	0,00	0,00	0,04±0,07	
	4-methyl hexanoic acid		1080	0,00	0,00	0,15	0,00	0,04±0,07	
Alcohol (19)	3- methylbutanol	whiskey, malt, burnt	ND	2,82	2,00	1,86	4,98	2,91±1,44	*K. lactis*[1,5,6], *G. candidum*[7]
	2,7- dimethyl-4,5-octandiol		1043	0,00	11,47	0,00	0,00	2,87±5,73	

	2- phenylethanol	phenol, spice	1115	3,30	2,26	4,35	0,61	2,63±1,59	*K. lactis*[1,6]
	2- heptanol	arcticbramble, melon, mushroom	907	1,50	0,65	1,32	3,37	1,71±1,16	*G. candidum*[4]
	2,3- butanediol	fruit, onion	801	0,63	1,65	1,82	0,30	1,10±0,75	*K. lactis*[1]
	2- ethyl hexanol	rose, green	1033	1,47	2,63	0,00	0,00	1,02±1,28	
	2- propanol	alcohol, pungent	985	0,00	0,00	0,00	4,03	1,01±2,01	*K. lactis*[5], *C. guilliermondii*[5]
	2,6- dimethyl-4-heptanol		951	0,00	3,52	0,00	0,00	0,88±1,76	
	2,6- dimethyl-1,3-dioxanol		806	0,00	0,00	0,00	2,47	0,62±1,23	
	2- pentanol	alcoholic, ethery, fruity, nutty, raspberry	ND	0,49	0,73	0,46	0,52	0,55±0,12	
	2- nonanol	coconut	1103	0,00	0,38	0,82	0,00	0,30±0,39	*K. lactis*[1], *G. candidum*[4]
	3,4- hexadienol		898	0,00	0,00	0,02	0,43	0,11±0,21	
	2,2,4- trimethyl pentanediol (tmpd)		1159	0,00	0,23	0,00	0,00	0,06±0,11	
	1,2,3- cyclopentanetriol		1105	0,00	0,10	0,00	0,00	0,02±0,05	
	3 – methylthiopropanol	sweet, potato	985	0,00	0,10	0,00	0,00	0,02±0,05	
	2,3- dimethylbutoxi – 2 butanol		1154	0,00	0,00	0,08	0,00	0,02±0,04	
	2,2- dimethyl- 1-butanol	whiskey, malt, burnt	1092	0,00	0,00	0,00	0,07	0,02±0,04	
	1,3- dimethylbutoxi – 2 butanol		1154	0,00	0,00	0,06	0,00	0,02±0,03	
	2- methyl hexanol	sweat	1071	0,00	0,01	0,00	0,00	0,01±0,01	
Aldehyde (6)	phenylethanal	hawthorne, honey, sweet	1045	6,36	0,00	4,20	0,00	2,64±3,17	*K. lactis*[5], *C. guilliermondii*[5]
	nonanal	aldehyde, citrus, fatty, floral,	1105	0,22	0,00	0,00	2,80	0,75±1,37	

		green, soapy,							
	benzaldehyde	honey, sweet	963	0,00	0,00	0,40	0,00	0,10±0,20	
	heptanal	fat, citrus, rancid	1145	0,00	0,04	0,00	0,17	0,05±0,08	
	methylbutanal	apple, burnt, caramel, cheese, cherry, cocoa, malt, green, sickly	ND	0,00	0,12	0,05	0,00	0,04±0,06	*K. lactis*[1], *G. candidum*[4]
	benzeneacetaldehyde	fresh, fruity, fatty	1045	0,00	0,00	0,00	0,13	0,03±0,07	*G. candidum*[8]
Ketone (11)	2-heptanone	blue cheese, fruity, musty, peardrops, soapy	893	7,50	3,78	4,80	1,53	4,4±2,48	*K. lactis*[1,6], *G. candidum*[3]
	2 -butanone	buttery, milky	ND	5,55	2,65	4,56	0,81	3,39±2,1	*K. lactis*[5], *C. guilliermondii*[5]
	2-nonanone	blue cheese, fatty, fruity, green, ketone, musty	1091	3,49	4,17	5,21	0,35	3,31±2,10	*K. lactis*[1,5], *G. candidum*[3], *C. guilliermondii*[5]
	2-pentanone	acetone, sweet fruity	ND	0,71	6,13	0,44	0,05	1,83±2,88	*K. lactis*[1,5], *G. candidum*[3], *C. guilliermondii*[5]
	2-nonenone	pungent, mushroom	1083	0,53	0,12	0,70	0,00	0,34±0,33	
	2,3-heptanedione		945	0,00	1,15	0,00	0,00	0,29±0,58	
	2-tridecanone	fruity, green, rancid, Tallow	1293	0,09	0,28	0,70	0,08	0,29±0,29	
	4 – aminoacetophenone		962	0,00	0,19	0,00	0,00	0,05±0,09	
	ethyl phenyl ketone		1284	0,00	0,00	0,04	0,10	0,03±0,05	
	2-octanone	earthy, ethereal, ketone, mushroom,	991	0,00	0,07	0,06	0,00	0,03±0,04	
	2-decanone	fruity, musty	1292	0,12	0,00	0,00	0,00	0,03±0,06	
Éster (56)	ethyl octanoate	apple, fruity,	1196	11,48	13,33	5,29	7,31	9,35±3,70	*K. lactis*[5,6], *C.*

	sweet						*guilliermondii*[5], *G. candidum*[8]
ethyl hexanoate	aniseed, apple, fruity, green, sweet, unripe	999	2,77	2,43	7,54	7,37	5,03±2,81	*K. ohmeri*[2], *K. lactis*[5,6], *G. candidum*[8]
isobutyl propionate		1196	0,00	0,00	0,00	8,77	2,19±4,39	*K lactis*[6]
isobutyl octanoate		1145	0,00	0,05	0,04	7,19	1,82±3,58	
ethyl decanoate	apple, caprylic, fruity, solvent	1394	0,20	0,94	1,26	3,56	1,49±1,45	*K. lactis*[6], *K. ohmeri*[2], *G. candidum*[8]
ethyl pentanoate		1054	0,00	0,00	4,71	0,00	1,18±2,36	*G. candidum*[7]
isoamyl hexanoate		1250	0,17	0,67	2,56	0,77	1,04±1,05	
phenethyl ethanoate		1256	0,50	1,88	0,00	1,27	0,91±0,83	*K. lactis*[6]
pentyl isobutanoate		1058	0,33	0,94	1,52	0,54	0,83±0,52	
isoamyl ethanoate	banana, ester, solvent, Sweet	883	0,18	2,53	0,20	0,32	0,81±1,15	*K. lactis*[1]
propyl 3-acetylpropanoate		1038	0,00	2,98	0,00	0,00	0,74±1,49	
ethyl butanoate	apple, butter, fruity, papaya, perfumy, Sweet	806	0,67	0,18	1,76	0,00	0,65±0,79	*K. lactis*[5]
isobutyl hexanoate		1131	0,00	0,23	1,94	0,41	0,64±0,88	
propyl ethanoate	fruity	ND	0,17	1,54	0,19	0,59	0,62±0,64	*K. lactis*[1,5,6]
propyl hexanoate	fruity, floral	1094	0,00	0,23	1,76	0,04	0,51±0,84	
methyl 2-ethylbutanoate		848	0,00	1,35	0,18	0,00	0,38±0,65	
ethyl 3-methylbutanoate	fruity	860	0,00	0,54	0,65	0,00	0,30±0,35	*G. candidum*[8]
2-pentyl 2-methylpropanoate		1025	0,00	0,23	0,93	0,00	0,29±0,44	
ethyl propanoate		ND	1,00	0,14	0,00	0,00	0,28±0,48	

	ethyl nonanoate	sickly sweet	1295	0,00	0,00	0,00	1,01	0,25±0,51 *K. ohmeri*[2]
	propyl octanoate		1291	0,00	0,00	0,24	0,35	0,15±0,18
	isoamyl propanoate		974	0,00	0,06	0,43	0,00	0,12±0,21
	ethyl dodecanoate	caprylic, ester	1591	0,00	0,00	0,14	0,33	0,12±0,16 *K. ohmeri*[2]
	2-methylbutyl butanoate		1060	0,07	0,14	0,25	0,00	0,12±0,11
	isobutyl isobutanoate		942	0,00	0,06	0,37	0,00	0,11±0,18 *G. candidum*[8]
	isopentyl 3-methylbutanoate		1106	0,00	0,00	0,43	0,00	0,11±0,22
	delta decalactone	coconut	1591	0,00	0,20	0,00	0,21	0,10±0,12
	ethyl 2,4-hexadienoate		1103	0,00	0,00	0,40	0,00	0,10±0,20
	isopentyl butanoate		1060	0,00	0,05	0,23	0,11	0,10±0,10
	isobutyl butanoate		942	0,00	0,00	0,38	0,00	0,09±0,19 *K. lactis*[6]
	methyl 2 – hydroxycaproate		952	0,16	0,00	0,18	0,00	0,09±0,10
	ethyl isobutanoate		1102	0,00	0,00	0,30	0,00	0,07±0,15
	delta octalactone	Lactone, sweet	1287	0,07	0,08	0,11	0,00	0,07±0,05
	ethyl heptanoate	sweet, fruity, fruit peel	1097	0,00	0,00	0,00	0,25	0,06±0,12
	isobutyl isopentanoate		1008	0,00	0,00	0,25	0,00	0,06±0,12
	propyl butanoate		899	0,00	0,00	0,24	0,00	0,06±0,12
	isopropyl pentanoate		898	0,00	0,00	0,23	0,00	0,06±0,12
	phenylethyl propionate		1352	0,00	0,23	0,00	0,00	0,06±0,11
	ethyl hexadecanoate		1591	0,00	0,13	0,05	0,00	0,04±0,06
	2-phenylethyl hexanoate		1256	0,00	0,00	0,15	0,00	0,04±0,07
	isopropyl octanoate		1231	0,00	0,00	0,15	0,00	0,02±0,07
	pentyl hexanoate		1095	0,00	0,06	0,00	0,07	0,03±0,04
	2-heptanol butanoate		1210	0,00	0,00	0,11	0,00	0,03±0,06
	butyl octanoate	fruity	1325	0,00	0,00	0,11	0,00	0,03±0,05

	isopropyl isobutanoate		1142	0,00	0,09	0,00	0,00	0,02±0,04	
	propyl decanoate		1488	0,00	0,00	0,08	0,00	0,02±0,04	
	isopropyl decanoate		1427	0,00	0,00	0,07	0,00	0,02±0,04	
	acetoin butanoate		1068	0,00	0,00	0,07	0,00	0,02±0,03	
	isopropyl butanoate		846	0,00	0,00	0,06	0,00	0,02±0,03	
	isobutyl decanoate		1521	0,00	0,00	0,05	0,00	0,01±0,02	
	isobutyl 2-methylpropanoate		943	0,00	0,05	0,00	0,00	0,01±0,02	
	2-ethylhexyl butanoate		1320	0,00	0,04	0,00	0,00	0,01±0,02	
	3- methylpropyl ethanoate		1124	0,00	0,04	0,00	0,00	0,01±0,02	*K. lactis*[5], *G. candidum*[7]
	isopentyl pentanoate	fruity, perfumy, solvent	1107	0,00	0,03	0,00	0,00	0,01±0,02	
	2-methylbutyl decanoate		1600	0,00	0,00	0,03	0,00	0,01±0,01	
	butyl butanoate		997	0,00	0,00	0,03	0,00	0,01±0,01	

*Odor description found in the literature in the Flavornet database.. **Determination based on the percentage of each component relative to the peak area.
Legend: TR: Retention rate; ND: Below the lower nominal hydrocarbon. [1]PADILLA et al., 2014; [2]GRAHAM et al., 2011; [3]MOLLIMARD et al., 1997; [4]JOLLIVET et al., 1994; [5]ATANASSOVA et al., 2016; [6]CENTENO; LORENZO; CARBALLO, 2022; [7]MDAINI et al., 2006; [8]CHEN et al., 2022.

Among the carboxylic acids found, a higher relative area percentage was observed for hexanoic, decanoic, octanoic and acetic acids. The occurrence of these acids in artisanal cheeses has been described by several authors, including studies from Turkey (GEZGINC et al., 2021; SAY, 2021), Italy (COZZOLINO et al., 2021), China (ZHENG et al., 2021), Slovenia (BOLTAR et al., 2015), artisanal ewe cheese produced in Northern Spain (SANTAMARINA-GARCÍA et al., 2023) and Brazil, specifically in the Serra da Canastra region (DE JESUS FILHO et al., 2021), where a methodology for evaluating VOCs in cheeses was optimized, and compounds were assessed at different stages of maturation. It is important to note that the high concentration of carboxylic acids is associated with the appearance of rancid flavor in fermented cheeses. However, when present in small quantities, they contribute to the characteristic aroma of certain cheeses (SOBRAL et al., 2017). Acetic acid is usually a product of acetic fermentation by bacteria and is sensorially undesirable due to its vinegar-like taste (IANNI et al., 2020).

Not all volatile compounds contribute to cheese aroma, mainly due to the human olfactory detection threshold (STAROWICZ, 2021). Generally, esters such as ethyl butanoate and ethyl hexanoate and acids have a greater impact on the active odor in artisanal cheeses, increasing during maturation (SANTAMARINA-GARCÍA et al., 2023). In this same study, butanoic, hexanoic and octanoic acids, also predominant in cheeses produced in the Campos das Vertentes region, were shown to be active in the formation of the odor of artisanal Spanish cheese. Volatile acids are aromatic compounds and have been reported to have the greatest impact on odor in various cheeses, such as cheddar cheese (WANG et al., 2021).

The aldehyde functional group was the one that presented smaller relative areas and less diversity of compounds (6), but none of them was present in all the evaluated samples. Aldehydes are primarily compounds resulting from proteolysis, where amino acids derived from the breakdown of large peptides by enzymatic action are catabolized by bacterial enzymes (MCSWEENEY; SOUSA, 2000). They are transient compounds in cheese, as during maturation, they are reduced to primary alcohols or oxidized to carboxylic acids (CURIONI E BOSSET, 2002), which explains the low representation of this group in this study. Aldehydes are related to unpleasant aromatic notes when they exceed certain thresholds and nonanal, also reported in the present work, is the most common odorant, important in the formation of the aroma of mozzarella cheese (NATRELLA et al., 2020) and cheddar (WANG et al. ., 2021).

Similar to aldehydes, compounds classified as alcohols are generally products of proteolysis caused by microbial enzymes or originating from the degradation of free fatty acids in the process of lipolysis (MCSWEENEY; SOUSA, 2000). Thus, the variation observed in the compounds belonging to the alcohol group among the evaluated cheesemaking facilities can primarily be attributed to the enzymatic activity of lactic acid bacteria during the maturation process, influenced by the microbiological composition of the endogenous ferment and more favorable conditions for the action of these enzymes during maturation (DELGADO et al., 2010). A total of 19 alcohols were found, among which the presence in all samples and the higher relative area of 2-phenylethanol, 2-heptanol, 2-pentanol, and 3-methylbutanol are noteworthy.

The average relative area of the ketone group found in this study was similar among the samples, except for cheeses produced in the CV4 region. Among the ketones found, a

higher relative area was observed for 3-hydroxy-2-butanone, 2-heptanone, 2-nonenone, and 2-pentanone. 2-heptanone and 2-nonenone were predominant in artisanal cheeses such as Pecorino di Carmasciano (COZZOLINO et al., 2021), Manchego (GÓMEZ-RUIZ et al., 2020), Idiazabal (GUILLEN E ABASCAL, 2012), and Darfyeh (SERHAN et al., 2010). Ketones are compounds resulting from the decarboxylation of free fatty acids caused by the reactions of lipolytic enzymes primarily produced by microorganisms. The quantity of ketonic compounds in cheeses mainly depends on the concentration of available fatty acids in the cheese matrix (MCSWEENEY; SOUSA, 2000). Ketones also effectively contribute to the formation of cheese aroma, due to their low detection threshold (CURIONI AND BOSSET, 2002) and the ketones 2-Heptanone, 2-Nonanone and 2-Butanone, predominant in the evaluated cheeses, were described as of high effective impact on the odor of artisanal ewe cheeses matured for up to 120 days (SANTAMARINA-GARCÍA et al., 2023), indicating notes of fruity, sweet and buttery odor.

Esters are the result of the esterification of medium and short chain fatty acids or through an alcohol deprotonation mechanism (MCSWEENEY; SOUSA, 2000). In the present work, the greatest diversity of volatile compounds among the chemical classes found was that of esters, highlighting the highest % of relative area and presence in all samples of ethyl octanoate, ethyl hexanoate, ethyl butanoate, ethyl decanoate and in lower concentration , isoamyl ethanoate and isoamyl hexanoate. The occurrence of esters in volatile compounds causes a reduction in cheese bitterness, caused by high concentrations of amines (MATERA et al., 2018). Ethyl hexanoate and ethyl octanoate were reported to be important in artisanal goat cheeses manufactured in Gokceada Island in Turkey (HAYALOGLUA et al., 2013). Matera et al. (2018) evaluated the profile of volatile compounds in artisanal rennet and similarly to the findings in this work, the compounds 3-methylbutanol, 2-heptanone, 2-nonanone and ethyl hexanoate were predominant. The ethyl octanoate and ethyl hexanoate esters were shown to be active in the formation of odor in artisanal goat cheese (SANTAMARINA-GARCÍA et al., 2023) and in Kurut cheese produced in China (WANG et al., 2020) and Swiss cheese (TAYLOR et al., 2013).

The profile of volatile compounds of artisanal cheeses is related to its microbiota, as volatile compounds are products of the primary or secondary metabolism of microorganisms (SCHIMIDT et al., 2015). Several authors have investigated the production of volatile compounds under different conditions by microbial species (CHEN et al., 2022; CENTENO, LORENZO and CARBALLO, 2022; ATANASSOVA et al., 2016; PADILLA et al., 2014; GRAHAM et al., 2011; MDAINI et al., 2006; MOLLIMARD et al., 1997; JOLLIVET et al., 1994) and the relationship between the presence of the isolates identified in the AMC produced in the Campos das Vertentes region and the volatile compounds they are capable of producing is described in Table 3. Some volatile compounds found in cheese (acetic acids, 2-methylbutanoic acids, propyl acetate esters, isoamyl ethanoate, 2-pentanone, 2-heptanone, 2-nonanone, 3-methylbutanal, 3-methyl-1-butanol, 2 ,3 – butanediol, 2-nonanol and phenylethyl alcohol) may be products of the metabolism of the yeast *Kluyveromyces lactis*, found in the environment of dairies (Figure 2) and in artisanal cheeses (Figure 4) produced in the Campos das Vertentes region, as reported by Padilla et al. (2014) when evaluating the volatile production capacity of yeasts *D. hansenii, K. lactis* and *K. marxianus*. Decanoate, dodecanoate, hexanoate and nonanoate ethyl esters have been reported as metabolism

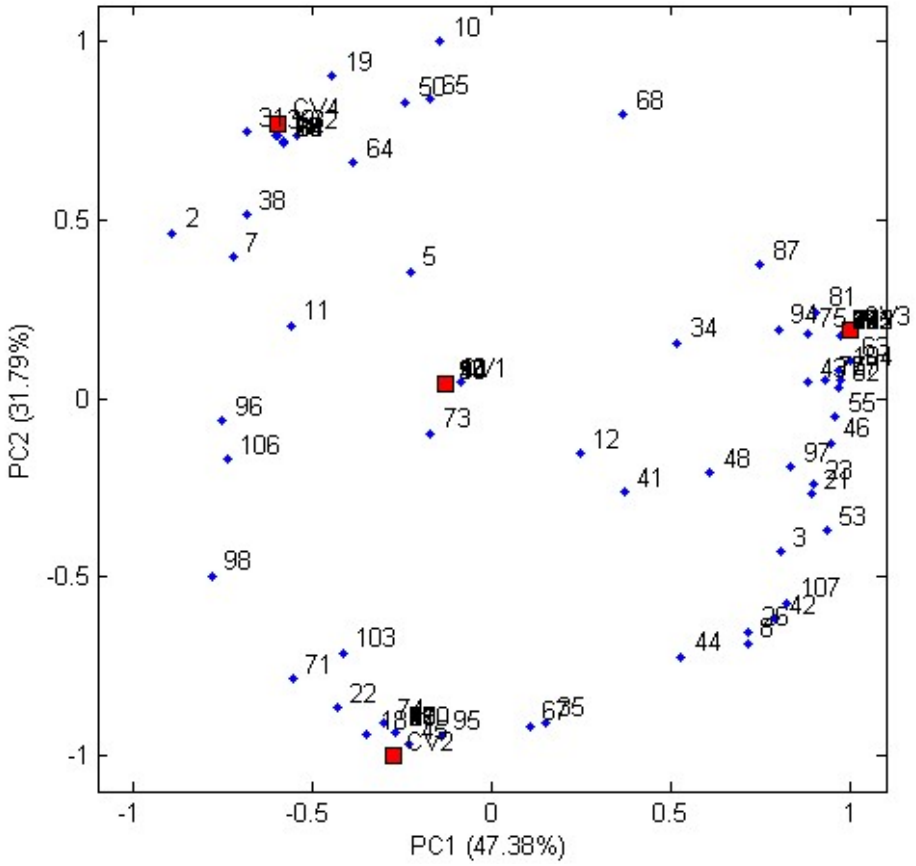
products in *Kodamaea ohmeri* (GRAHAM et al., 2011). Studies were also found that confirm that *G. candidum* is capable of producing a wide variety of ketones, such as 2-nonanone, 2-heptanone and 2-pentanone (MOLIMARD et al., 1997), in addition to 2-heptanol; 2-Nonanol; butanoic acid; hexanoic acid; Octanoic acid and 3-methylbutanal (JOLLIVET et al., 1994), all volatile compounds found in the evaluated artisanal cheeses.

It can also be observed that, in the CV2 sample, where the presence of *G. candidum* and *K. lactis* and *Diutina catenulata* and some *candida* species predominated, volatile compounds were found that were not found in the other analyzed samples, such as 2,7-dimethyl-4,5-octandiol, with high concentration (11.47%), 2,6 - dimethyl-4-heptanol, 2,2,4-trimethyl pentanediol (tmpd), 1,2,3- cyclopentanetriol, 3 – methylthiopropanol, 2,3-heptanedione, 4 – aminoacetophenone, isopropyl isobutanoate, phenylethyl propionate, 2-ethylhexyl butanoate and 3-methylpropyl ethanoate. It is suggested that new studies be carried out to expand the knowledge about microbial metabolism, related to the formation of volatile compounds, especially filamentous fungi and yeasts, so that this information can help in the establishment of odorous and microbiological signatures for artisanal cheeses.

The analysis of principal components (APC) for volatile compounds was performed in order to improve the observation of data for the evaluated cheese factories (Figure 6). The first two components explained 79.17% of the total variability of the organic compounds, with 47.38% in the first dimension and 31.79% in the second dimension. This analysis grouped the predominant volatile compounds in each sample and allowed the observation of correlations between the compounds and the samples. In sample CV1, there was the lowest diversity of volatile compounds, with a highlight on 2-pentyl-2-methylpropanoate, 2-heptanal butanoate, and benzoic acid. In sample CV2, the correlated compounds were 2-pentanol, methyl ethylbutanoate, and isoamyl ethanoate, in sample CV3, ethyl heptanoate, isobutyl 1-methyl propanoate, and ethyl hexylbutanoate, and in sample CV4, 2-heptanol, ethyl decanoate, and 3-methylbutanoic acid.

The evaluated samples showed different positions on the PCA graph, demonstrating the significant variability of volatile compounds and emphasizing the existing differences between the cheeses, despite having a similar manufacturing process and being located in the same region. This can be attributed to the artisanal character of cheese production, which guarantees the uniqueness of each cheesemaker's product due to the lack of standardization in processes and, mainly, to the differences in the microbiota present in each cheese. In a study with PDO Emmental cheese, Pillonel et al. (2003) managed to discriminate the country of origin of the cheeses, differentiating the Polish, French and Swiss Emmental cheeses, through the concentrations of butan-2-one, 3-hydroxybutanone, butan-2-ol and octene by principal component analysis (PCA) and the presence or absence of 3-methylbut-2-en-1-ol alcohol and other compounds, validating this analysis as an efficient authenticity verification method.

Figure 6. Principal Components Analysis of Volatile Organic Compounds in Artisanal Minas Cheese produced in Campos das Vertentes/MG.



Legend: 1: 2-hydroxypropanoic acid (lactic ácido); 2: 2-methyl butyric acid; 3: 3-methyl butanoic acid (valeric acid); 4: 4-methyl hexanoic acid; 5: acetic acid; 6: benzeneacetic acid; 7: benzoic acid; 8: decanoic acid; 9: heptanoic acid; 10: hexanoic acid; 11: nonanoic acid; 12: octanoic acid; 13: propanedioic acid (malonic acid); 14: propanoic acid; 15: 1,2,3-cyclopentanetriol; 16: 1,3-dimethylbutoxi-2 butanol; 17: 2,2,4-trimethyl pentanediol (tmpd); 18: 2-ethyl hexanol; 19: 2-heptanol; 20: 2-methyl hexanol; 21: 2-nonanol; 22: 2-pentanol; 23: 2-phenyiethanol; 24: 2-propanol; 25: 2,2-dimethyl-1-butanol; 26: 2,3-butanediol; 27: 2,3-dimethylbutoxi-2-butanol; 28: 2,6-dimethyl-1,3-dioxanol; 29: 2,6-dimethyl-4-heptanol; 30: 2,7-dimethyl-4,5-octandiol; 31: 3-methylbutanol; 32: 3,4-hexadienol; 33: methionol; 34: phenylethanal; 35: methylbutanal; 36: benzaldehyde; 37: benzeneacetaldehyde; 38: heptanal; 39: nonanal; 40: 2-decanone; 41: 2-heptanone; 42: 2-nonanone; 43: 2-nonenone; 44: 2-octanone; 45: 2-pentanone; 46: 2-tridecanone; 47: 2,3-heptanedione; 48: 3-hydroxy-2-butanone; 49: 4-aminoacetophenone; 50: ethyl phenyl ketone; 51: 2-ethylhexyl butanoate; 52: 2-heptanol butanoate; 53: 2-methylbutyl butanoate; 54: 2-methylbutyl decanoate; 55: 2-pentyl 2-methylpropanoate; 56: 2-phenylethyl hexanoate; 57: 3-methylpropyl acetate; 58: acetoin butanoate; 59: butyl butanoate; 60: butyl octanoate; 61: ethyl 2,4-hexadienoate; 62: ethyl 3-methylbutanoate; 63: ethyl butanoate; 64: ethyl decanoate; 65: ethyl dodecanoate; 66: ethyl heptanoate; 67: ethyl hexadecanoate; 68: ethyl hexanoate; 69: ethyl isobutanoate; 70: ethyl nonanoate; 71: ethyl octanoate; 72: ethyl pentanoate; 73: ethyl propanoate; 74: isoamyl ethanoate; 75: isoamyl hexanoate; 76: isoamyl hexanoate; 77: isoamyl propanoate; 78: isobutyl 2-methylpropanoate; 79: isobutyl butanoate; 80: isobutyl decanoate; 81: isobutyl hexanoate; 82: isobutyl isobutanoate; 83: isobutyl isopentanoate; 84: isobutyl octanoate; 85: isobutyl propionate; 86: isopentyl 3-methylbutanoate; 87: isopentyl butanoate; 88: isopentyl pentanoate; 89: isopropyl butanoate; 90: isopropyl decanoate; 91: isopropyl isobutanoate; 92: isopropyl octanoate; 93: isopropyl pentanoate; 94: methyl 2-hydroxycaproate; 95: methyl 2-ethylbutanoate; 96: pentyl hexanoate; 97: pentyl isobutanoate; 98: phenethyl acetate; 99: phenylethyl propionate; 100: propyl 3-acetylpropanoate; 101: propyl butanoate; 102: propyl

decanoate; 103: propyl ethanoate; 104: propyl hexanoate; 105: propyl octanoate; 106: delta decalactone; 107: delta octalactone.

In short, the averages of the compounds found in all studied samples showed a greater relative area of acetic, decanoic, hexanoic and octanoic acids, 3-methylbutanol, 2-heptanone, 2-nonenone and 2-butanone, and ethyl hexanoate and ethyl octanoate . Considering the aroma descriptors, it is expected that the cheese produced in this region presents aromatic notes of vinegar, oily, rancid, sweet, fruity, malted, burnt and green. However, these inferences are not enough to describe the aroma of the cheese, being necessary to continue this study, determining the active odor index, which takes into account the human detection threshold and the aromatic profile using human descriptors, thus allowing to develop a signature olfactory. for artisanal cheeses produced in the Campos das Vertentes region, in order to contribute to obtaining Protected Designation of Origin or Indication of Source seals and to prevent the occurrence of fraud, enhancing the value of the product and benefiting both the producer and the consumer.

4. Conclusions

Artisanal cheeses produced in the Campos das Vertentes region, Minas Gerais, Brazil, with a maturation period of 22 to 25 days, can be classified as semi-hard, fatty cheeses, with an average protein content of 24% and pH between 5.0 and 5.5. The mycobiota present in the environment and in artisanal cheeses is predominantly composed of *Geotrichum candidum*, *Kluyveromyces lactis*, *Diutina catenulata* and several species of *Candida*. In cheeses where *G. candidum* and *K. lactis* were not found, *C. duobushaemuloni* and *C. guilliermond* predominated. The volatile compounds described for cheeses produced in this region include carboxylic acids (acetic, decanoic, hexanoic and octanoic), alcohol (3-methylbutanol), ketones (2-heptanone, 2-nonenone and 2-butanone) and esters (ethyl hexanoate and ethyl octanoate), which contribute to the aromatic notes of vinegar, oil, rancidity, sweetness, fruity, malty, burnt and green. However, more studies are needed to verify the compounds with active odor in cheese, considering the human detection thresholds and also to correlate the production of volatiles with the cheese microbiota. In addition, sensory analysis by human descriptors is necessary to develop an odor profile for cheeses, contributing to obtaining geographical indication or designation of origin seals.

Disclosure of conflict of interest

The authors declare that they have no known competing financial, political or personal interests.

Acknowledgments

This study was partly financied by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES). The authors also thank the Conselho Nacional de

Desenvolvimento Científico e Tecnológico do Brasil (CNPq) and Fundação de Amparo a Pesquisa de MG (FAPEMIG) for their financial support.

References

Andrade, R. P., Melo, C. N., Genisheva, Z., Schwan, R. F., & Duarte, W. F. (2017). Yeasts from Canastra cheese production process: Isolation and evaluation of their potential for cheese whey fermentation. Food Research International, 91, 72-79. https://doi.org/10.1016/j.foodres.2016.11.032

AOAC. Official methods of analysis of the Association Analytical Chemists, 2006. 18.ed. Gaithersburg, Maryland.

Aragão, M. O., Evangelista, S. R., Passamani, F. R. F., Guimarães, J. P. M., Abreu, L. R., & Batista, L. R. (2022). Fungal community and physicochemical profiles of ripened cheeses from the Canastra of Minas Gerais, Brazil. Journal of Food Science and Technology, 59(12), 4685-4694. https://doi.org/10.1007/s13197-022-05548-4

Arcari, S. G., Caliari, V., Sganzerla, M., & Godoy, H. T. (2017). Volatile composition of Merlot red wine and its contribution to the aroma: Optimization and validation of analytical method. Talanta, 174, 752-766. https://doi.org/10.1016/j.talanta.2017.06.074

Atanassova, M. R., Fernández-Otero, C., Rodríguez-Alonso, P., Fernández-No, I. C., Garabal, J. I., & Centeno, J. A. (2016). Characterization of yeasts isolated from artisanal short-ripened cows' cheeses produced in Galicia (NW Spain). Food microbiology, 53, 172-181. https://doi.org/10.1016/j.fm.2015.09.012

Bertuzzi, A. S., McSweeney, P. L., Rea, M. C., & 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), 371-390. https://doi.org/10.1111/1541-4337.12332

Bezerra, T. K. A., de Oliveira Arcanjo, N. M., de Araújo, A. R. R., de Queiroz, A. L. M., de Oliveira, M. E. G., Gomes, A. M. P., & Madruga, M. S. (2017). Volatile profile in goat coalho cheese supplemented with probiotic lactic acid bacteria. LWT-Food Science and Technology, 76, 209-215. https://doi.org/10.1016/j.lwt.2016.03.041

Boltar, I., Čanžek Majhenič, A., Jarni, K., Jug, T., & Bavcon Kralj, M. (2015). Volatile compounds in Nanos cheese: their formation during ripening and seasonal variation. Journal of Food Science and Technology, 52, 608-623. https://doi.org/10.1007/s13197-014-1565-6

Borelli, B. M., Ferreira, E. G., Lacerda, I. C., Franco, G. R., & Rosa, C. A. (2006). Yeast populations associated with the artisanal cheese produced in the region of Serra da Canastra, Brazil. World Journal of Microbiology and Biotechnology, 22, 1115-1119. https://doi.org/10.1007/S11274-006-9151-3

Boutrou, R., & Guéguen, M. (2005). Interests in Geotrichum candidum for cheese technology. International journal of food microbiology, 102(1), 1-20. https://doi.org/10.1016/j.ijfoodmicro.2004.12.028

BRASIL, 1996. Ministério da Agricultura, Pecuária e Abastecimento - MAPA. Portaria n° 146, de 07 de março de 1996. Aprova Regulamentos de Identidade de Produtos Lácteos.

BRASIL, 2006. Ministério da Agricultura, Pecuárias e Abastecimento – MAPA. Instrução Normativa nº 68, de 12 de dez de 2006. Oficializa os Métodos Analíticos Oficiais Físico-Químicos, para Controle de Leite e Produtos Lácteos.

Cardin, M., Cardazzo, B., Mounier, J., Novelli, E., Coton, M., & Coton, E. (2022). Authenticity and Typicity of Traditional Cheeses: A Review on Geographical Origin Authentication Methods. Foods, 11(21), 3379. https://doi.org/10.3390/foods11213379

Cardoso, V. M., Borelli, B. M., Lara, C. A., Soares, M. A., Pataro, C., Bodevan, E. C., & Rosa, C. A. (2015). The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese. Food Research International, 69, 331-340. https://doi.org/10.1016/j.foodres.2014.12.040

Castro, R. D., Oliveira, L. G., Sant'Anna, F. M., Luiz, L. M. P., Sandes, S. H. C., Silva, C. I. F. & Souza, M. R. (2016). Lactic acid microbiota identification in water, raw milk, endogenous starter culture, and fresh Minas artisanal cheese from the Campo das Vertentes region of Brazil during the dry and rainy seasons. Journal of Dairy Science, 99(8), 6086-6096. https://doi.org/10.3168/jds.2015-10579

Centeno, J. A., Lorenzo, J. M., & Carballo, J. (2022). Effects of autochthonous Kluyveromyces lactis and commercial Enterococcus faecium adjunct cultures on the volatile profile and the sensory characteristics of short-ripened acid-curd Cebreiro cheese. Food Microbiology, 108, 104101. https://doi.org/10.1016/j.fm.2022.104101

Chen, J., Li, Q., Wang, J., Chen, W., Zheng, Q., Zhong, Q., ... & Liao, Z. (2022). Comparative analysis of aroma components and quality of *Geotrichum candidum* after space mutation breeding. Frontiers in Microbiology, 13, 908329. https://doi.org/10.3389/fmicb.2022.908329

Cozzolino, R., Martignetti, A., De Giulio, B., Malorni, L., Addeo, F., & Picariello, G. (2021). SPME GC-MS monitoring of volatile organic compounds to assess typicity of Pecorino di Carmasciano ewe-milk cheese. International Journal of Dairy Technology, 74(2), 383-392. https://doi.org/10.1111/1471-0307.12756

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. International Dairy Journal, 12(12), 959-984. https://doi.org/10.1016/S0958-6946(02)00124-3
De Jesus Filho, M., Klein, B., Wagner, R., & Godoy, H. T. (2021). Key aroma compounds of Canastra cheese: HS-SPME optimization assisted by olfactometry and chemometrics. Food Research International, 150, 110788. https://doi.org/10.1016/j.foodres.2021.110788

De Souza, T. P., Evangelista, S. R., Passamani, F. R. F., Bertechini, R., de Abreu, L. R., & Batista, L. R. (2021). Mycobiota of Minas artisanal cheese: Safety and quality. International Dairy Journal, 120, 105085. https://doi.org/10.1016/j.idairyj.2021.105085

Delgado, G. C., & Bergamasco, S. M. P. P. (2017). Agricultura familiar brasileira: desafios e perspectivas de futuro. Brasília: Ministério do Desenvolvimento Agrário, 470.

Dieuleveux, V., Van Der Pyl, D., Chataud, J., & Gueguen, M. (1998). Purification and characterization of anti-Listeria compounds produced by Geotrichum candidum. Applied and environmental microbiology, 64(2), 800-803. https://doi.org/10.1128/aem.64.2.800-803.1998

Drake, M. A., & Delahunty, C. M. (2017). Sensory character of cheese and its evaluation. In Cheese (pp. 517-545). Academic Press. https://doi.org/10.1016/B978-0-12-417012-4.00020-X

Ferreira, D. F. (2014). Sisvar: um guia dos seus procedimentos de comparações múltiplas Bootstrap. Ciência e Agrotecnologia, 38, 109-112. https://doi.org/10.1590/S1413-70542014000200001

Gantzias, C., Lappa, I. K., Aerts, M., Georgalaki, M., Manolopoulou, E., Papadimitriou, K., ... & Vandamme, P. (2020). MALDI-TOF MS profiling of non-starter lactic acid bacteria from artisanal cheeses of the Greek island of Naxos. International journal of food microbiology, 323, 108586. https://doi.org/10.1016/j.ijfoodmicro.2020.108586

Gezginc, Y., Karabekmez-Erdem, T., Tatar, H. D., Dağgeçen, E. C., Ayman, S., & Akyol, İ. (2022). Metagenomics and volatile profile of Turkish artisanal Tulum cheese microbiota. Food Bioscience, 45, 101497. https://doi.org/10.1016/j.fbio.2021.101497

Gómez-Ruiz, J. Á., Ballesteros, C., Viñas, M. Á. G., Cabezas, L., & Martínez-Castro, I. (2002). Relationships between volatile compounds and odour in Manchego cheese: comparison between artisanal and industrial cheeses at different ripening times. Le Lait, 82(5), 613-628. https://doi.org/10.1051/lait:2002037

Graham, J. R., Ellis, J. D., Benda, N. D., Kurtzman, C. P., & Boucias, D. G. (2011). Kodamaea ohmeri (Ascomycota: Saccharomycotina) presence in commercial Bombus impatiens Cresson and feral Bombus pensylvanicus DeGeer (Hymenoptera: Apidae) colonies. Journal of Apicultural Research, 50(3), 218-226. https://doi.org/10.3896/IBRA.1.50.3.06
Guillén, M. D., & Abascal, B. (2012). Nature and distribution of the volatile components in the different regions of an artisanal ripened sheep cheese. Journal of dairy research, 79(1), 102-109. https://doi.org/10.1017/s0022029911000781

Hayaloglu, A. A., Yasar, K., Tolu, C., & Sahingil, D. (2013). Characterizing volatile compounds and proteolysis in Gokceada artisanal goat cheese. Small Ruminant Research, 113(1), 187-194. https://doi.org/10.1016/j.smallrumres.2013.01.001

Ianni, A., Bennato, F., Martino, C., Grotta, L., & Martino, G. (2020). Volatile flavor compounds in cheese as affected by ruminant diet. Molecules, 25(3), 461. https://doi.org/10.3390/molecules25030461

IPHAN. Instituto do Patrimônio Histórico e Artístico Nacional, 2008. Registro dos Queijos Artesanais de Minas, a ser inscrito no Livro dos Saberes.

Jollivet, N., Chataud, J., Vayssier, Y., Bensoussan, M., & Belin, J. M. (1994). Production of volatile compounds in model milk and cheese media by eight strains of Geotrichum candidum Link. Journal of Dairy Research, 61(2), 241-248. https://doi.org/10.1017/S0022029900028259

Júnior, L. C. G. C., Moreno, V. J., Magalhães, F. A. R., Costa, R. G. B., Resende, E. C., & Carvalho, K. B. A. (2014). Maturação do Queijo Minas Artesanal da Microrregião Campo das Vertentes e os efeitos dos períodos seco e chuvoso. Revista do Instituto de Laticínios Cândido Tostes, 69(2), 111-120. https://doi.org/10.14295/2238-6416.v69i2.326

Klich, M. A. (2002). Identification of common Aspergillus species. CBS.

Kothe, C. I., Mohellibi, N., & Renault, P. (2022). Revealing the microbial heritage of traditional Brazilian cheeses through metagenomics. Food Research International, 157, 111265. https://doi.org/10.1016/j.foodres.2022.111265

Leclercq-Perlat, M. N., Corrieu, G., & Spinnler, H. E. (2004). Comparison of volatile compounds produced in model cheese medium deacidified by Debaryomyces hansenii or Kluyveromyces marxianus. Journal of Dairy Science, 87(5), 1545-1550. https://doi.org/10.3168/jds.s0022-0302(04)73306-8

Mariani, C., Briandet, R., Chamba, J. F., Notz, E., Carnet-Pantiez, A., Eyoug, R. N., & Oulahal, N. (2007). Biofilm ecology of wooden shelves used in ripening the French raw milk smear cheese Reblochon de Savoie. Journal of Dairy Science, 90(4), 1653-1661. https://doi.org/10.3168/jds.2006-190

Matera, J., Luna, A. S., Batista, D. B., Pimentel, T. C., Moraes, J., Kamimura, B. A., ... & Cruz, A. G. (2018). Brazilian cheeses: a survey covering physicochemical characteristics, mineral content, fatty acid profile and volatile compounds. Food Research International, 108, 18-26. https://doi.org/10.1016/j.foodres.2018.03.014

McSweeney, P. L. H. (2017). Biochemistry of cheese ripening: introduction and overview. In: P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett, editors. Cheese: chemistry, physics, and microbiology. 4th ed. San Diego: Academic Press. Pp 379–88.

McSweeney, P. L., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Le Lait, 80(3), 293-324. https://doi.org/10.1051/lait:2000127

Mdaini, N., Gargouri, M., Hammami, M., Monser, L., & Hamdi, M. (2006). Production of natural fruity aroma by *Geotrichum candidum*. Applied Biochemistry and Biotechnology, 128, 227-235. https://doi.org/10.1385/ABAB:128:3:227

MINAS GERAIS, 2009. Secretaria de Estado de Agricultura, Pecuária e Abastecimento. Instituto Mineiro de Agropecuária. Portaria nº 1022, de 03 de novembro de 2009. Identifica a Microrregião do Campo das Vertentes.

MINAS GERAIS, 2017. Instituto Mineiro de Agropecuária – IMA. Portaria n° 1736 de 27 de julho de 2017. Altera a Portaria nº 1305/2013, de 30 de abril de 2013, que dispõe sobre o período de maturação do Queijo Minas Artesanal.

MINAS GERAIS, 2022. Secretaria de estado de Agricultura, Pecuária e Abastecimento – SEAPA. Resolução N° 42, 27 de dezembro 2022. Reconhece e define sobre Queijo Minas Artesanal de Casca Florida (QMACF). Available in: <http://jornal.iof.mg.gov.br/xmlui/handle/123456789/276763 >.

Molimard, P., Issanchou, S., Brousse, M., & Spinnler, H. E. (1997). Effect of the association of surface flora on the sensory properties of mould-ripened cheese. Le lait, 77(1), 181-187. https://doi.org/10.1051/lait:1997112

Montel, M. C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N., & Berthier, F. (2014). Traditional cheeses: Rich and diverse microbiota with associated benefits. International journal of food microbiology, 177, 136-154. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019

Natrella, G., Gambacorta, G., De Palo, P., Maggiolino, A., & Faccia, M. (2020). Volatile organic compounds in milk and mozzarella: Comparison between two different farming systems. International Journal of Food Science & Technology, 55(11), 3403-3411. https://doi.org/10.1111/ijfs.14671

Ni, Q., Gasperi, F., Aprea, E., Betta, E., Bergamaschi, M., Tagliapietra, F., ... & Bittante, G. (2020). The volatile organic compound profile of ripened cheese is influenced by crude protein shortage and conjugated linoleic acid supplementation in the cow's diet. Journal of dairy science, 103(2), 1377-1390. https://doi.org10.3168/jds.2019-16495

Padilla B, Manzanares P, Belloch C (2014) Yeast species and genetic heterogeneity within Debaryomyces hansenii along the ripening process of traditional ewes' and goats' cheeses. Food Microbiology, 38: 160–166. https://doi.org/10.1016/j.fm.2013.09.002

Pasternak, J. (2012). New methods of microbiological identification using MALDI-TOF. Einstein (Sao Paulo), 10, 118-119. https://doi.org/10.1590/s1679-45082012000100026

Penland, M., Falentin, H., Parayre, S., Pawtowski, A., Maillard, M. B., Thierry, A., ... & Deutsch, S. M. (2021). Linking Pélardon artisanal goat cheese microbial communities to aroma compounds during cheese-making and ripening. International Journal of Food Microbiology, 345, 109130. https://doi.org/10.1016/j.ijfoodmicro.2021.109130

Pillonel, L., Ampuero, S., Tabacchi, R., & Bosset, J. (2003). Analytical methods for the determination of the geographic origin of Emmental cheese: volatile compounds by GC/MS-FID and electronic nose. European Food Research and Technology, 216, 179-183. https://doi.org/10.1007/s00217-002-0629-4

Pinheiro, A. C. M., Nunes, C. A., & Vietoris, V. (2013). SensoMaker: a tool for sensorial characterization of food products. Ciência e Agrotecnologia, 37, 199-201. https://doi.org/10.1590/s1413-70542013000300001

Pitt, J. I. A Laboratory guide to common Penicillium species (2000). North Ryde, NSW. Food Science Australia. https://doi.org/10.1007/978-1-4899-3579-3_24

Pyz-Łukasik, R., Gondek, M., Winiarczyk, D., Michalak, K., Paszkiewicz, W., Piróg-Komorowska, A., ... & Ziomek, M. (2021). Occurrence of Listeria monocytogenes in artisanal cheeses from Poland and its identification by MALDI-TOF MS. Pathogens, 10(6), 632. https://doi.org/10.3390/pathogens10060632

Reyes-Díaz, R., González-Córdova, A. F., del Carmen Estrada-Montoya, M., Méndez-Romero, J. I., Mazorra-Manzano, M. A., Soto-Valdez, H., & Vallejo-Cordoba, B. (2020).

Volatile and sensory evaluation of Mexican Fresco cheese as affected by specific wild Lactococcus lactis strains. Journal of Dairy Science, 103(1), 242-253. https://doi.org/10.3168/jds.2019-17188

Sánchez-Juanes, F., Teixeira-Martín, V., González-Buitrago, J. M., Velázquez, E., & Flores-Félix, J. D. (2020). Identification of species and subspecies of lactic acid bacteria present in spanish cheeses type “Torta” by MALDI-TOF MS and pheS gene analyses. Microorganisms, 8(2), 301. https://doi.org/10.3390/microorganisms8020301

Santamarina-García, G., Amores, G., Hernández, I., Morán, L., Barrón, L. J. R., & Virto, M. (2023). Relationship between the dynamics of volatile aroma compounds and microbial succession during the ripening of raw ewe milk-derived Idiazabal cheese. Current Research in Food Science, 6, 100425. https://doi.org/10.1016/j.crfs.2022.100425

Say, D. (2022). Physicochemical composition, nitrogen fraction and volatile profiles of goat cheese made with artisanal liquid coagulant. Journal of Food Science and Technology, 59(6), 2469-2478. https://doi.org/10.1007/s13197-021-05266-3

Schmidt, R., Cordovez, V., De Boer, W., Raaijmakers, J., & Garbeva, P. (2015). Volatile affairs in microbial interactions. The ISME journal, 9(11), 2329-2335. https://doi.org/10.1038/ismej.2015.42

Serhan, M., Linder, M., Hosri, C., & Fanni, J. (2010). Changes in proteolysis and volatile fraction during ripening of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. Small Ruminant Research, 90(1-3), 75-82. https://doi.org/10.1016/j.smallrumres.2010.01.008

Šipošová, P., Koňuchová, M., Valík, Ľ., & Medveďová, A. (2021). Growth dynamics of lactic acid bacteria and dairy microscopic fungus Geotrichum candidum during their co-cultivation in milk. Food Science and Technology International, 27(6), 572-582. https://doi.org/10.1177/1082013220976485

Sobral, D., Costa, R. G. B., de Paula, J. C. J., Teodoro, V. A. M., Moreira, G. D. M. M., & Pinto, M. S. (2017). Principais defeitos em queijo Minas artesanal: uma revisão. Revista do Instituto de Laticínios Cândido Tostes, 72(2), 108-120. https://doi.org/10.14295/2238-6416.v72i2.600

Starowicz, M. (2021). Analysis of volatiles in food products. Separations, 8 (9), 157. https://doi.org/10.3390/separations8090157

Taylor, K., Wick, C., Castada, H., Kent, K., & Harper, W. J. (2013). Discrimination of Swiss cheese from 5 different factories by high impact volatile organic compound profiles determined by odor activity value using selected ion flow tube mass spectrometry and odor threshold. Journal of food science, 78(10), C1509-C1515. https://doi.org/10.1111/1750-3841.12249

Tavaria, F. K., Ferreira, A. S., & Malcata, F. X. (2004). Volatile free fatty acids as ripening indicators for Serra da Estrela cheese. Journal of Dairy Science, 87(12), 4064-4072. https://doi.org/10.3168/jds.s0022-0302(04)73548-1

Teter, A., Barłowska, J., Król, J., Brodziak, A., Rutkowska, J., & Litwińczuk, Z. (2020). Volatile compounds and amino acid profile of short-ripened farmhouse cheese manufactured from the milk of the White-Backed native cow breed. LWT, 129, 109602. https://doi.org/10.1016/j.lwt.2020.109602

Wang, B., Wang, J., Xu, L. Y., Zhang, J. H., Ai, N. S., & Cao, Y. P. (2020). Characterization of the key odorants in kurut with aroma recombination and omission studies. Journal of dairy science, 103(5), 4164-4173.

Wang, J., Yang, Z. J., Wang, Y. D., Cao, Y. P., Wang, B., & Liu, Y. (2021). The key aroma compounds and sensory characteristics of commercial Cheddar cheeses. Journal of Dairy Science, 104(7), 7555-7571. https://doi.org/10.3168/jds.2020-19992

Zheng, X., Li, K., Shi, X., Ni, Y., Li, B., & Zhuge, B. (2018). Potential characterization of yeasts isolated from Kazak artisanal cheese to produce flavoring compounds. MicrobiologyOpen, 7(1), e00533. https://doi.org/10.1002/mbo3.533

Zheng, X., Ge, Z., Lin, K., Zhang, D., Chen, Y., Xiao, J., ... & Shi, X. (2021). Dynamic changes in bacterial microbiota succession and flavour development during milk fermentation of Kazak artisanal cheese. International Dairy Journal, 113, 104878. https://doi.org/10.1016/j.idairyj.2020.104878

ARTICLE 2 – MICROBIOTA DESCRIPTION OF ARTISANAL MINAS CHEESE PRODUCED IN THE CAMPOS DAS VERTENTES/MG REGION BY METAGENOMIC ANALYSIS

Formatted according to the guidelines of the International Journal of Food Microbiology
ISSN: 0168-1605
(preliminary version)

Fernanda Costa Prates[1], Vitória Luisa Soares[2], Fabiana Reinis Franca Passamani[3], and Luis Roberto Batista[4]*

Department of Food Science/ Federal University of Lavras – Minas Gerais/ Brazil

[1]fernanda.prates@estudante.ufla.br +5532991583784

[2]vitoria.soares@estudante.ufla.br +5535998171796

[3]fabireinis@gmail.com +5535988694908

[4]*Corresponding author: luisrb@ufla.br +5535984769559

Abstract

The artisanal cheese produced in Minas Gerais has great economic and cultural importance for the region and has been recognized as a Brazilian intangible heritage. Made from raw cow's milk, rennet, endogenous yeast (pingo), and salt, it stands out for having a straw-yellow color, medium to semi-hard texture, with mechanical holes and a slightly acid taste. Despite its known importance, little is known about its intrinsic characteristics and its microbiological profile, which are fundamental to guarantee its typical sensory characteristics. This work was a pioneer in revealing the microbiota present in Artisanal Minas Cheese (AMC) produced in this region, through the use of metagenomic analysis. Samples from certified cheesemakers were collected and the microbiota was revealed through sequencing of conserved ribosomal RNA regions, 16S for bacteria and ITS for fungi. The predominance of bacteria from the families Corynebacteriaceae, Lactobacillaceae, Streptococcaceae and Leuconostocaceae was revealed, highlighting *Corynebacterium variabile*, present in all analyzed samples. Among the lactic acid bacteria (LAB), the species *Lactobacillus brevis*, *L. buchneri* and *L. parabuchneri* were revealed. The presence of bacteria of the genus *Staphylococcus* was revealed were *Geotrichum candidum* was not identified, indicating that this fungi acts as a bioprotector. Among the fungi, *G. candidum* was predominant in the samples, besides *Diutina catenulata*, *Kluyveromyces lactis* and *Debaryomyces prosopidis*, which were present in all samples analyzed. The lack of *Debaryomyces hansenii* and *Yarrowia lipolytica*, widely described in artisanal cheeses, including Brazilian ones, was observed. This knowledge is a first step towards obtaining a Geographical Indication certificate, which would add value to the cheeses, benefiting producers and preventing fraud. The continuity of this study is needded to elucidate the relationship between microorganisms, their action during cheese maturation for the formation of products of their metabolism and specific sensory characteristics, which guarantee the typicality of the cheese produced in the Campos das Vertentes region - MG.

Keywords: microbiota, metagenomics, artisanal cheese, *Geotrichum candidum*.

1. Introduction

Brazil presents a wide variety of artisanal cheeses, which are differentiated by social and cultural aspects, influencing the traditional manufacturing methods. The manufacture of Artisanal Minas Cheese (AMC) is performed using raw milk, enzymatic rennet, endogenous yeast (pingo), and salt. In raw milk, more than 400 different species of lactic acid bacteria, yeasts, and fungi have been identified. This diversity decreases inside the cheeses, where only a few species of lactic acid bacteria and yeast become dominant. However, on the surface of the cheeses a wide diversity of microorganism species is reported, mainly due to the significant variations in the dynamics of these same species in each specific cheese and to the terroir of artisanal cheeses, due to the transfer of microorganisms present in the environment, on the surfaces, in the equipment and in the ripening chambers of the cheesemakers to the cheeses (MONTEL et al., 2014). Artisanal cheeses have typical and unique characteristics, such as texture and flavor, consequence of their origin, composition of starter cultures, raw materials, and maturation conditions (KAMIMURA et al., 2019). AMC is an intangible heritage of Brazil (IPHAN, 2008) and it is a product of extreme economic and sociocultural importance for the region, since its manufacture occurs mostly in a family production context (DELGADO, BERGAMASCO, 2017).

Currently there are 10 regions recognized as AMC producers, being Serro, Canastra, Cerrado, Araxá, Campo das Vertentes, Serra do Salitre, Triângulo Mineiro, Diamantina, Serras do Ibitipoca and the region "Entre Serras da Piedade ao Caraça". Among the AMC producing regions, the Campos das Vertentes region stands out officially recognized as a producer of AMC in 2009 (MINAS GERAIS, 2009) and the artisanal cheeses produced in this region must be matured for a minimum of 22 days (MINAS GERAIS, 2020). During cheese maturation, microorganisms play a key role in the formation of compounds that will ensure the specific sensory characteristics, due to the presence of microbial enzymes, responsible for the generation of various compounds that occur during cheese production and maturation period, through glycolysis, proteolysis and lipolysis (PENLAND et al., 2021).

The known techniques capable of revealing the microbiota in foodscan be culture-dependent or culture-independent. Among the techniques that are independent of cultivation in the laboratory, the high throughput sequencing (HTS) methodologies stand out, such as metagenomic analysis, a technique capable of revealing the entire microbiota existing in the samples through the sequencing of the total DNA extracted directly from the sample through DNA (or RNA) extraction, amplification, and sequencing (PUIG et al., 2018).

Metagenomic analysis arose from the interest in knowing the complete Genome sequence of different organisms, besides humans, sequenced in 1977 through the Sanger enzymatic method (RUPPERT; KLINE; RAHMAN, 2019). Today, sophisticated techniques for DNA sequencing have been developed, automating the process and reducing the high costs, called New Generation Sequencing (NGS), among which the Illumina company's sequencers stand out. Some research has been conducted using molecular methods to determine the microbiota present in foods, including artisanal cheeses (KOTHE, MOHELLIBI, RENAULT, 2022; CÉSAR et al., 2022; REMOR et al., 2021; NERO et al., 2021; ARAGÃO et al., 2021; KAMIMURA et al., 2020; SANT'ANNA et al., 2019; KAMIMURA et al., 2018; DE FREITAS MARTINS et al., 2018; PERIN et al., 2017; LUIZ et al., 2016; CASTRO et al., 2016). However, most of the studies conducted, revealed only the bacterial community present through the sequencing of the conserved 16S region, highlighting lactic bacteria and undesirable, spoilage or pathogenic bacteria. In contrast, there are still few studies that reveal the mycobiota, that is, the fungal community through the sequencing of the ITS region, given the importance of these microorganisms for the maturation and the biological signature of artisanal cheeses.

From this knowledge it will be possible to propose improvements in all stages of product manufacturing, adding value to the cheese, without ignoring the importance of ensuring its microbiological safety and stability, to support new health legislation that authorizes and regulates its manufacture and to endorse a future request for certification of Geographical Indication (GI). This certification provides a differential of quality, authenticity, and added value for the producer and protects consumers by ensuring that they are purchasing genuine and authentic products, thus avoiding fraud and imitations (INPI, 2023). There are several effective food authentication methods, such as those based on DNA sequencing, to detect cheese fraud. However, these methods have advantages and disadvantages, and there is no definitive method considered the most efficient for this purpose (CARDIN et al., 2022). The use of HTS techniques has become more frequent in revealing the microbiota of artisanal cheeses, which contributes to their authentication. HTS methodologies have provided greater insight into the microbial diversity present in cheeses and have revealed the impacts of microbial metabolism on the formation of sensory characteristics. Identifying specific microorganisms during fermentation and maturation, as well as understanding the factors that affect the diversity and abundance of the microbiota, and the functional and metabolic processes, constitute a unique signature for each type of cheese and are key to determining the quality of artisanal cheeses (KAMILARI et al., 2019).

The present work is a pioneer in metagenomics use to reveal the microbiota in artisanal cheeses produced and commercialized in the Campos das Vertentes/MG region. The acquisition of this knowledge can contribute to its regulation and to the establishment of a kind of microbiological signature for the cheeses, besides strengthening the region to achieve the certification of geographical indication (GI), denomination of origin (DO) modality, adding value to the cheeses and benefiting the producers of the region.

2. Material and methods

2.1. Sample preparation and DNA extraction

Three samples of artisan cheese with 22 days of manufacture were collected directly from the maturation chamber of 4 different producing cheese dairies in the Campos das Vertentes – MG region in August 2020. The samples were transported in sterile bags to the laboratory of Mycology and Mycotoxins, Department of Food Science, Federal University of Lavras (UFLA- MG). and were coded as CV1, CV2, CV3 and CV4. From each cheese collected, samples were taken from equidistant points, comprising center and rind. The samples of each of the 3 cheeses from each dairy were ground and mixed, and 100g of each sample was portioned to be sent to the analysis laboratory of the Biotecnologia Pesquisa e Inovação - BPI company, located at Av. Deputado Dante Delmanto, 1.649, Botucatu - SP, where the genetic material of the cheeses from each cheese dairy had its total DNA extracted using the ZR Fungal/Bacterial DNA MiniPrepTM kit (Zymo Research) according to the manufacturer's protocol and the amount of DNA necessary to proceed with amplification was obtained.

2.2. Amplification of the 16S region (V3-V4) and the ITS region (3F + 4R)

PCR amplification reactions were performed and an extract with a final volume of 20 µL was obtained, consisting of 10 µL of GoTaq® Green PCR Master Mix (Promega), 1 uL of the foward oligonucleotide at 10 µM, 1uL of the reverse oligonucleotide at 10 µM, 2uL of previously extracted genomic DNA and enough ultrapure sterile water for 20uL. For the identification of bacteria the primers primer sequence 5'-3' V3 -(5'-TCGTCGGCAGCGTCAGATGTGTATAAGACAGAGCCTACGGGNGGCWGCAG-3') and

V4 (5'-GTCTCGTGGCTCGGAGATGTGTATAAGACAGGACTACHVGGTATCTAATCC-3') were used, with 600bp amplicon.

For the identification of the filamentous fungi and yeasts, primers sequence 3' ITS_3-F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCATCGATGAAGAACGCAGC-3') ITS_4R 5'- and(5'-GTCTCGTGGGCTCGGAGATGTGTATAAGACAGTCCTCCGCTTATTGATATGC-3') with amplicon of 400bp.

A different extract was used for each amplification, which consisted of initial denaturation of the DNA strand at 95 °C for 3 min, followed by 27 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 s; extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. The amplification reactions were conducted in a VeritiTM Thermal Cycler (Applied Biosystems). After the amplification reaction of each sample, amplification was confirmed by electrophoresis in a 2% agarose gel stained with UniSafe Dye 0.03% (v/v).

2.3. Library purification and quantification

The generated libraries were submitted to purification steps (to remove very small fragments from the total population of molecules and primer remnants) using Agencourt AMPure XP magnetic bead (Beckman Coulter), according to the manufacturer's instructions. Then, quantification was performed by Real-Time PCR methodology using Kit KAPA-KK4824 (Library Quantification Kit - Illumina/Universal) in the QuantStudio 3 equipment (Applied Biosystems), all according to the manufacturer's protocol. An equimolar pool of DNA was generated by normalizing all samples to 4nM for sequencing, which was performed using the Illumina MiSeq sequencing system (Illumina® Sequencing) and MiSeq Reagent V2 kit 500 cycles - 2 x 250 bp reads.

2.4. Bioinformatics Analysis

Gene sequences were processed using Quantitative Insights Into Microbial Ecology (QIIME 2) software version 2021.11 (BOLYEN et al. 2018). Raw sequence data were filtered using the q2-demux plugin followed by final cleaning with DADA2 (CALLAHAN et al., 2016). All amplicon sequence variants (ASVs) were aligned with mafft (KATOH et al., 2002) and used to construct the phylogenetic tree with fasttree2. Alpha and beta diversity metrics were estimated using q2 diversity after samples were rarefied (subsampled without

replacement) using 10000 sequences for bacteria and 5000 for fungi per sample. Taxonomy was assigned to the unique biological sequences (ASVs) using the q2 feature classifier (BOLYEN et al., 2018), naïve Bayes classify-sklearn taxonomy classifier for the bacterial sequences (QUAST et al., 2013; YILMAZ et al., 2014) and Unite-ver8-99 for fungi (NILSSON et al., 2018).

3. Results and discussion

3.1. Sequencing of the 16S (V3-V4) rRNA region

A predominance of bacteria belonging to the Corynebacteriaceae families was observed, except in sample CV4, where there was a predominance of Lactobacillaceae. Streptococcaceae, Leuconostococaceae and Lactobacillaceae were present in all samples analyzed (Figure 1). Among the genera of bacteria identified, Corynebacterium dominated, with the species *Corynebacterium variabile* standing out in samples CV1, CV2, and CV3. The presence of Corynebacterium variabile in artisanal cheese rinds was previously reported by de Freitas Martins et al. (2018) and Kothe, Mohellibi, Renault (2022) in cheeses produced in Pará and Serra da Canastra, respectively.Lactic acid bacteria of the genus *Lactococcus* and *Lactobacillus* were identified in all samples, with the latter predominating in sample CV4, highlighting the species *L. parabuchneri, L. bunchneri* and *L. brevis* (Figure 2). The occurrence of LAB in different producing regions in Minas Gerais are described by several authors (KOTHE, MOHELLIBI, RENAULT,2022; NERO et al., 2021; KAMIMURA et al., 2020; KAMIMURA et al., 2018; PERIN et al., 2017; LUIZ et al., 2016). The presence of LAB is related to the increase in the safety and quality of artisanal minas cheese, because in addition to participating in the process of fermentation and transformation of milk into cheese, they act as bioconservatives, producing antimicrobial substances that have antagonistic action on pathogens (FREIRE et al., 2021).

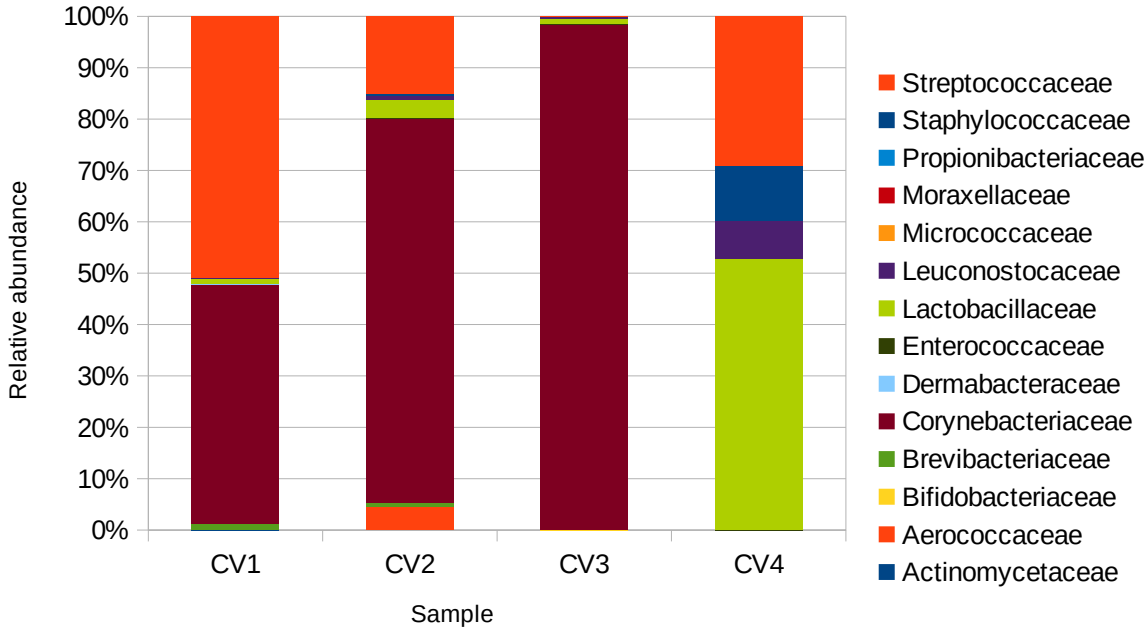


Figure 1. Identification and relative abundance of bacteria in Artisanal Minas Cheese by family.

The presence of *L. brevis*, *L. bunchneri* and *L. parabunchneri* in artisanal cheeses from Minas Gerais was also evidenced by Perin et al. (2017), in the Serro region. *L. brevis* are heterofermentative mesophilic bacteria that obligatorily use hexoses and pentoses as carbon source and can produce undesirable flavors and gas during cheese maturation (BRUNO; CARVALHO, 2009). *L. buchneri* and *L. parabuchneri* occur naturally in milk and can be undesirable in artisanal cheeses because some strains are producers of biogenic amines, especially histamine (ASCONE et al., 2017) and are related to the formation of lugs in cheeses (FRÖHLICH- WYDER et al., 2013).

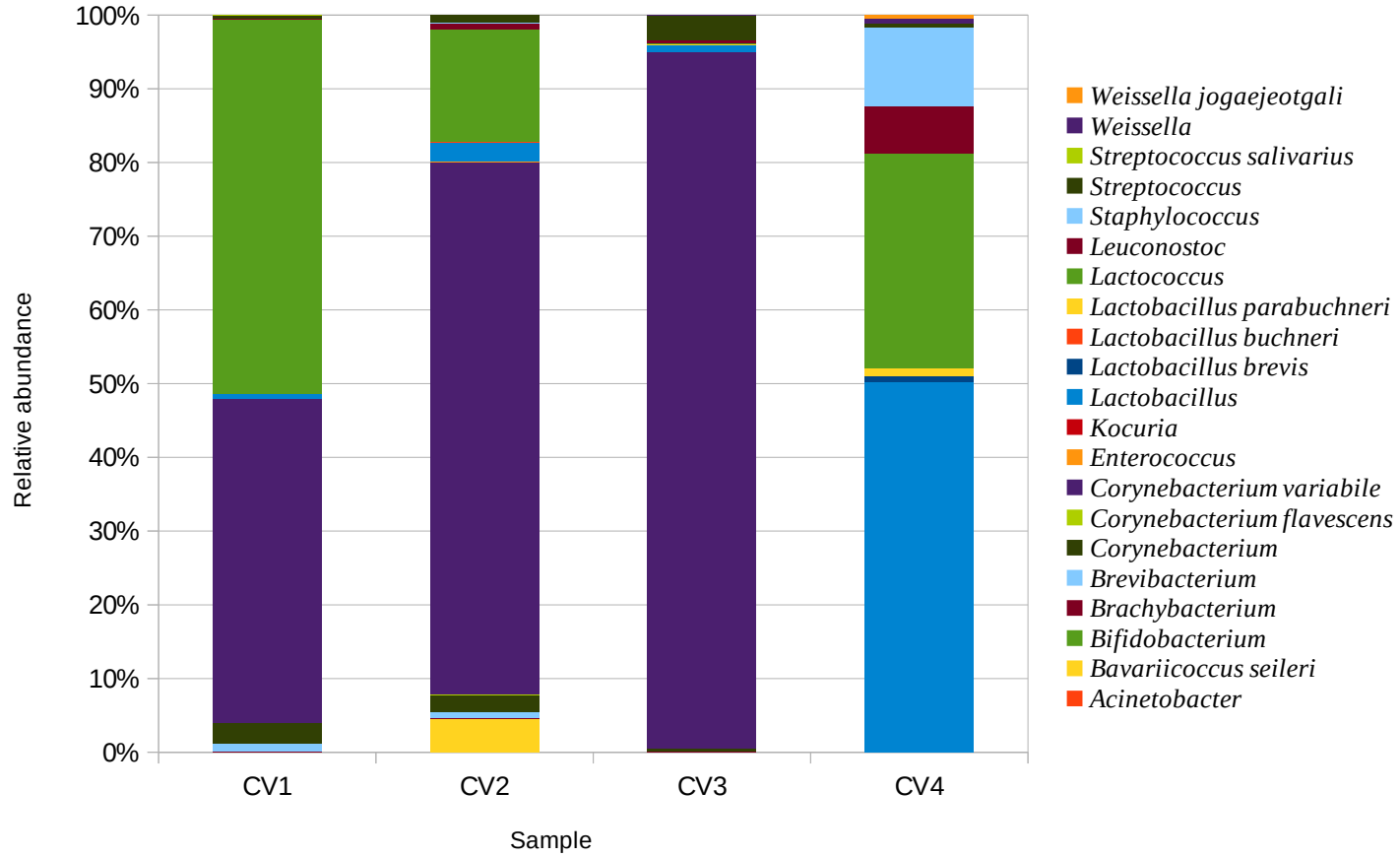


Figure 2. Identification and relative abundance of bacteria in Artisanal Minas Cheese by genus or species.

Bacteria of the genus *Weissella* were found in sample CV4, highlighting the presence of *Weissella jogaejeotgali*. *Weissella* is a LAB, reported in several fermented foods, with probiotic potential and has functional and technological properties, such as production of exopolysaccharides and bacteriocins that act respectively as thickeners and bioconservatives. Some strains of *Weissella* are able to decarboxylate phenolic compounds improving their bioavailability (FESSARD, REMIZE, 2017). However, some strains of *Weissella* are classified as opportunistic pathogens involved in human infections (FUSCO et al., 2015). The occurrence of *Weissella* in artisanal cheeses produced in Pará was reported by de Freitas Martins et al., 2018 and in cheeses produced in the Serro region by Perin et al. (2017). This is the first *Weissella jogaejeotgali* finding in artisanal cheeses. In addition to the reported bacteria, *acinetobacter* was revealed to be present in sample CV3 and *enterococcus* in samples CV2, CV3 and CV4 and (Figure 2). *Acinetobacter spp*. is a pathogen of great public health concern due to its resistance to known antibiotics and its association with various infections (JUNG and PARK, 2015). Multidrug resistance to antibiotics of *Acinetobacter spp* isolated from food has been reported, highlighting the importance of food as potential sources of dissemination of *Acinetobacter spp*. between community and clinical settings and reinforces the need for further investigations into the potential health risks of *Acinetobacter spp*. as foodborne pathogens (CARVALHEIRA, SILVA, TEIXEIRA, 2021). *Acinetobacter* has been reported to be present in various foods, such as fruits and vegetables (CARVALHEIRA; SILVA; TEIXEIRA, 2017), meat (RAFEI et al., 2015), and artisanal cheeses (REMOR et al., 2021; RIQUELME et al., 2015).

Bacteria of the genus *Enterococcus* are important for the dairy industry, acting as non-starter lactic acid bacteria (NSLAB) in artisan cheeses and related to the development of desirable sensory characteristics during cheese maturation due to their lipolytic activity and production of aromatic volatile compounds. In addition, there are reports of the production of bacteriocins that inhibit food spoilage and pathogenic bacteria (GIRAFFA, 2003). The presence of enterococcus was evaluated in 582 Brazilian artisan cheeses produced in different regions, 262 of which in Minas Gerais (Araxá, Cerrado, Serra da Canastra, Serro, and Campo das Vertentes) and its presence was detected in more than 50% of the sequences obtained, highlighting this bacterium as a component of the native microbiota of Brazilian artisan cheeses. In isolates obtained from AMC produced in the Campos das Vertentes region, 39.5% were identified as enterococcus (MARGALHO et al., 2020), corroborating the findings of the present study.

It is worth noting that, although enterococcus is recognized as part of the natural microbiota of artisanal cheeses, these microorganisms originate from the gastrointestinal tract of warm-blooded animals and their presence may be related to poor sanitary conditions and the lack of adoption of good agricultural practices and good manufacturing practices (MARGALHO et al., 2020). It was also observed the identification of the genus *Staphylococcus* in all analyzed samples, and more frequently in sample CV4. The occurrence of this genus is usually associated with external contamination, during the process of obtaining milk and making cheese, without the adoption of measures to ensure Good Management and Manufacturing Practices, due to the artisanal nature and also the lack of knowledge and resources of producers regarding control measures to prevent the occurrence of microorganisms of this genus, which includes the pathogenic bacteria *S. aureus*. De Sá et al (2021) analyzed artisanal cheeses produced in the Campos das Vertentes region and detected the presence of coagulase positive *staphylococcus* in quantities higher than those allowed for marketing in cheeses with 31 days of aging. Kothe, Mohellibi, Renault (2022) also reported that 45% of samples of AMC produced in Minas Gerais accused the presence of the genus *Staphylococcus* in different Brazilian artisanal cheeses was reviewed and reported by Camargo et al. (2021).

3.2. ITS region sequencing

From the sequencing of the ITS region, the fungal community present in artisanal cheeses was revealed, with the families Dipodascaceae and Debaryomycetaceae predominating (Figure 3). The fungal species predominantly found in the sampled artisan cheeses was *Dipodascus geotrichum* or *Geotrichum candidum* in samples CV1 and CV3. Although also present in sample CV2, *Diutina catenulata* was predominant, as well as in sample CV4 (Figure 4). The presence of *G. candidum* in artisan cheeses produced in Brazil has been reported by other authors (ARAGÃO et al., 2021; DE SOUZA et al., 2021), and this fungus has been shown to be important in the maturation of cheeses, contributing to the development of the characteristic flavor (ARAGÃO et al, 2021) and acting as a bioprotective agent (BOUTROU, GUÉGUEN, 2005), inhibiting the growth of deteriorating microorganisms such as *Mucor spp*., *Aspergillus ochraceus*, and pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus cereus* (MARIANI et al., 2007; DIEULEVEUX, V. et al., 1998).

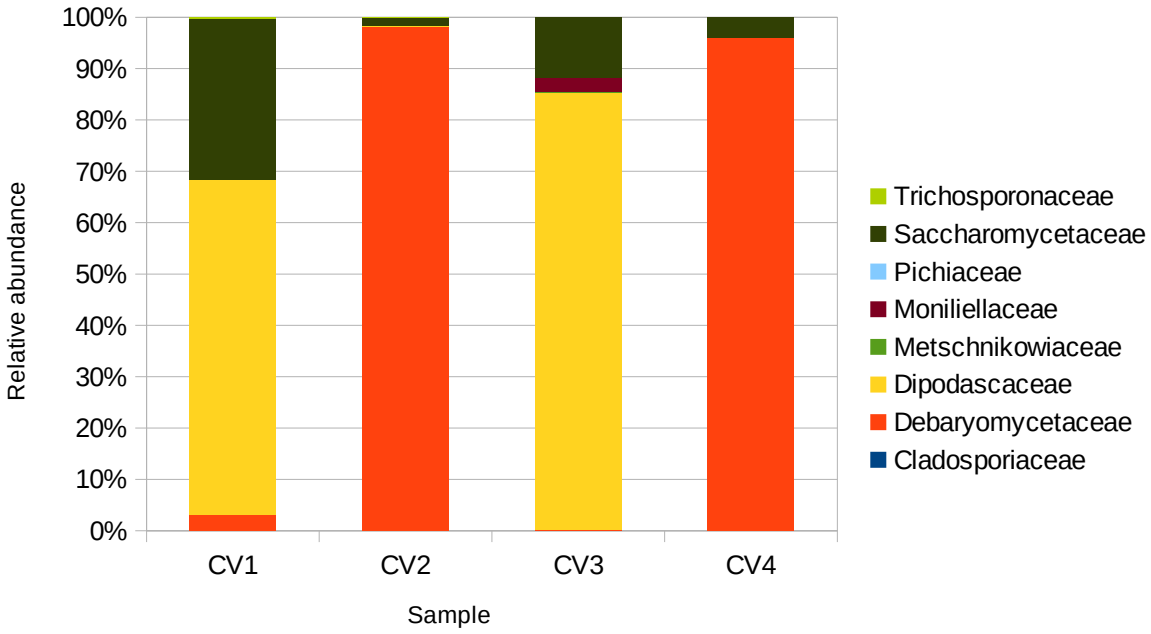


Figure 3. Identification and relative abundance of filamentous fungi and yeasts in Artisanal Minas Cheese by family.

G. candidum is able to alkalize the medium through lactic acid metabolism, which allows its survival and growth during cheese maturation, coexisting with the predominant bacteria in the cheese, and is responsible for the production of several organic compoundsvolatiles such as aldehydes and carboxylic acids through deamination reactions, influencing the sensory perception of cheese (ŠIPOŠOVÁ et al., 2021).

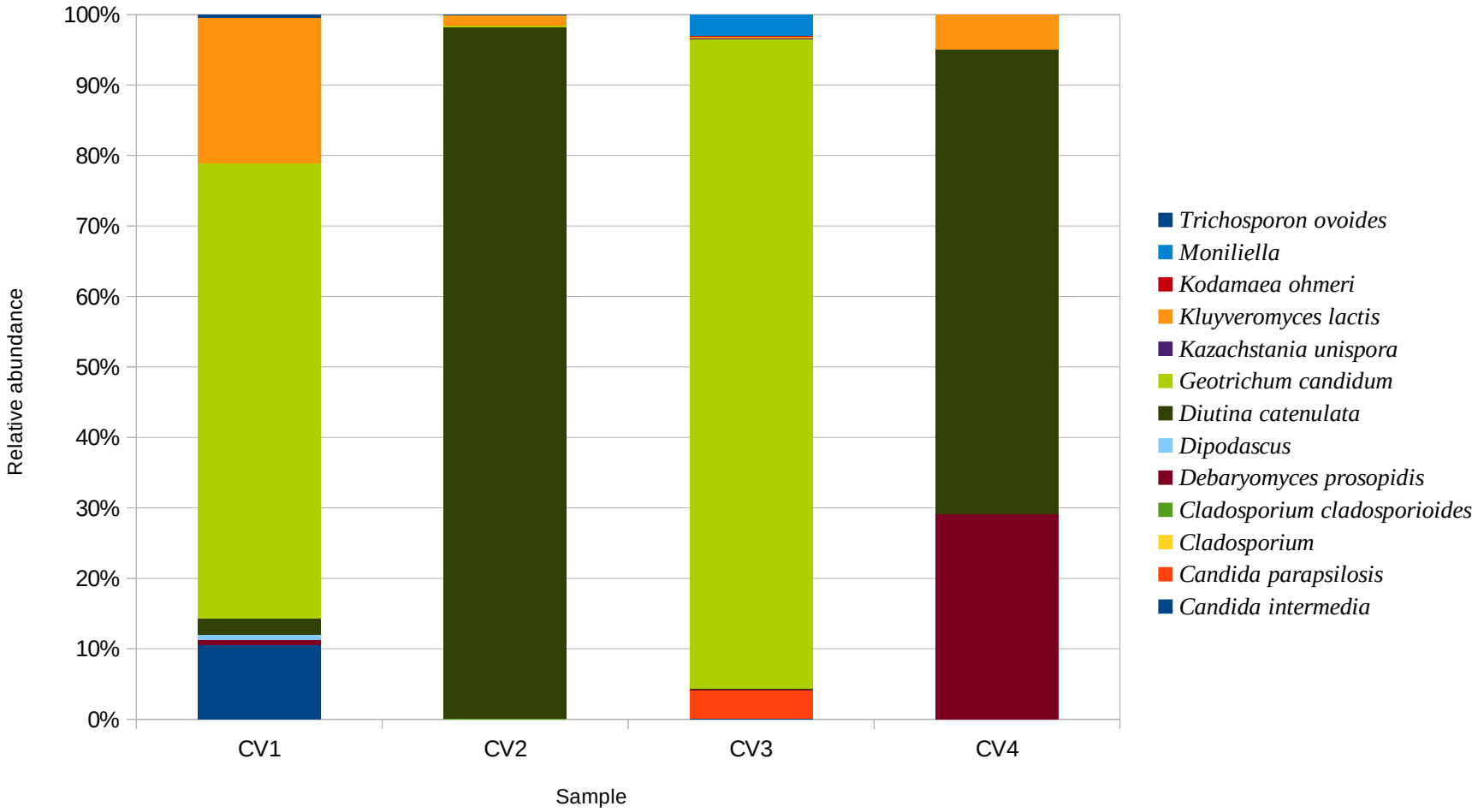


Figure 4. Identification and relative abundance of filamentous fungi and yeasts in Artisanal Minas Cheese by genus or species.

These specific characteristics of *G. candidum* and the wide discussion on the benefits of its presence in artisanal cheeses from Minas Gerais, ensuring their microbiological safety, were fundamental to regulate its production and authorize its commercialization through Resolution 42 of the Secretary of State of Agriculture, Livestock and Supply / Minas Gerais, which defines the bloomy rind variety of artisanal cheese from Minas Gerais as having "visual dominance of filamentous fungi, predominantly *Galactomyces Geotrichum* (*Geotrichum candidum* or *Geotrichum silvicola*)" (MINAS GERAIS, 2022). It was observed that in sample CV4, where the presence of *G. candidum* was not detected, there was greater growth of *Staphylococcus,* endorsing the action of *G. candidum* as a bioprotector, inhibiting the growth of pathogenic microorganisms. Due to this characteristic, the production was recognized and the producers are waiting for the regulation of the rules applied to the production and commercialization of this Minas Artesanal Cheese from “Casca Florida” by the government of the state of Minas Gerais.

Diutina catenulata is an ascomycota yeast found in humans, animals and environmental sources and is known to be a contaminant in dairy products such as cheese. This species, formerly called *Candida catenulata* has been associated with superficial and invasive infections in both humans and animals. Previous phylogenetic analyses initially classified this species in the family Saccharomycetaceae, however, more recent studies have reclassified *Diutina catenulata* as belonging to the family Debaryomycetaceae. This reclassification occurred due to the discovery that a codon, previously translated as leucine, is actually translated as serine. To confirm this taxonomic change, phylogenetic analyses were performed using 204 family alignments which confirmed the correct placement of the species in the family Debaryomycetaceae (O'BRIEN et al., 2018). Kothe, Mohellibi, Renault (2022) using similar methodology to that used in the present study, identified the presence of *Diutina catenulata*, *Debaryomyces hansenii* and *Kodamaea ohmeri* in cheeses produced in the South (colonial and highland) and Southeast (Araxá, Serra da Canastra and Serro) regions of Brazil. The results obtained in the mentioned work suggest the dominance of *D. catenulata* in the cheese samples and the occurrence of *Kodamaea ohmer*i, *G. candidum*, *Trichosporon sp*. and *Moniliella sp*., all of which were also found in at least one of the cheese samples evaluated in the present study. *K. ohmeri* was also reported in AMC produced in the Serro region, where it exhibited low lipolytic activity and β-galactosidase production (CARDOSO et al., 2015). This yeast was also identified in endogenous yeast (pingo and rala) used for artisanal cheese production in the Serro-MG region (DE MIRANDA et al., 2023).

D. catenulata, Kluyveromyces lactis and *Debaryomyces prosopidis* were found in all samples analyzed in this study. Silva (2020) when evaluating AMC produced in 16 properties located in Serra da Canastra, also found *D. catenulata* and *Debaryomyces* in the samples, besides *Candida* and *Trichosporon*, stating that this is the core mycobiota of AMC produced in the region. However, in another study conducted in the same region, after sequencing, the dominance of *G. candidum, D.* c*atenulata* and *Kluyveromyces* and the presence of *Torulaspora, Debaryomyces, Fusarium sp, Paecilomyces sp., Trichosporon (T. coremiforme and T. japonicum), Aspergillus sp. (A. oryzae* and *A. ochraceus), Penicillium sp* and *Cladosporium cladosporioides* (Aragão et al., 2021). The presence of *G. candidum* and *K. lactis* in artisanal cheese is related to the formation of unique sensory characteristics of the cheese and was also revealed in AMC produced in the Serro region (DE SOUZA et al., 2021).

K. lactis is capable of producing acetaldehyde, ethanol, aldehydes, branched-chain alcohols, and acetic acid esters that contribute fruity and acetic notes in artisanal cheeses (ATANASSOVA et al., 2016). Zheng et al. (2018) also isolated this yeast in artisanal cheese produced in China and described its ability to produce volatile compounds important for flavor formation in artisanal cheese. The presence of species belonging to the genus *candida* in artisanal cheese has been widely described, as compiled by Bintsis (2021). There are reports of the presence of *Candida intermedia*, in artisanal cheese produced in Serra da Canastra (ANDRADE et al., 2017) and in artisanal ewe cheese produced in Portugal (PEREIRA-DIAS, 2000), and *Candida parapsilosis* in Italian Pecorino di Farindola cheese (TOFALO et al., 2014) and in traditional goat and ewe cheeses (PADILLA et al., 2014).

Among the filamentous fungi found, the presence of the *Cladosporium cladosporioides* complex stands out in the CV2 cheese dairy. This fungus was also reported in cheeses produced in Serra da Canastra (CÉSAR ET AL., 2022; ARAGÃO et al. 2021) and its presence is undesirable, as they contribute to the formation of black stains on the surface of the cheeses (MESSINI et al., 2017) affecting the sensory perception of the consumer and impacting the commercialization, although there are no reports of mycotoxin production and that its consumption causes harm to health.

For a unique characterization of the entire Campos das Vertentes region, the data obtained in all analyzed cheesemaking facilities were combined, allowing a better observation of all the microbiota present in the artisanal cheeses (Figure 5), where it was possible to observe the dominance of *G. candidum* over other species of microorganisms (bacteria and fungi). This result is very important to encourage the production of Artisanal Minas Cheese

Bloomy Rind among producers, because this knowledge guarantees the producer the needded security to maintain the manufacture of this type of cheese and the incentive for consumption through the dissemination of information, since *G. candidum* is recognized as a bioprotector and the commercialization is being regulated.

The presence of at least one of the yeasts *Yarrowia lipolytica* or *Debaryomyces hansenii* was revealed in cheeses produced in different types of cheeses (BANJARA, SUHR and HALLEN- ADAMS, 2015), including artisanal cheeses produced in different regions of Brazil, such as cheese colonial produced in the South of the country (LANDELL, HARTFELDER, and VALENTE, 2006), Canastra (KOTHE, MOHELLIBI, RENAULT, 2022; ARAGÃO et al., 2021; BORELLI et al., 2006), Serro (DE MIRANDA et al., 2023; DE SOUZA et al., 2021; CARDOSO et al., 2015) and Salitre (LIMA et al., 2009). The presence of these yeasts in cheeses produced in the Campos das Vertentes region was not revealed in this study, suggesting that the simultaneous absence of these species can be an authenticity marker of cheeses produced in this region, alone or combined with other characteristics validated by different authentication methods. However, it is recognized the need for the continuity of this study, aiming at expanding the knowledge of the microbiota of artisan cheeses produced in all regions of Minas Gerais, in order to confirm this marker and to develop the microbiological signature of cheeses produced in different regions of Minas Gerais.

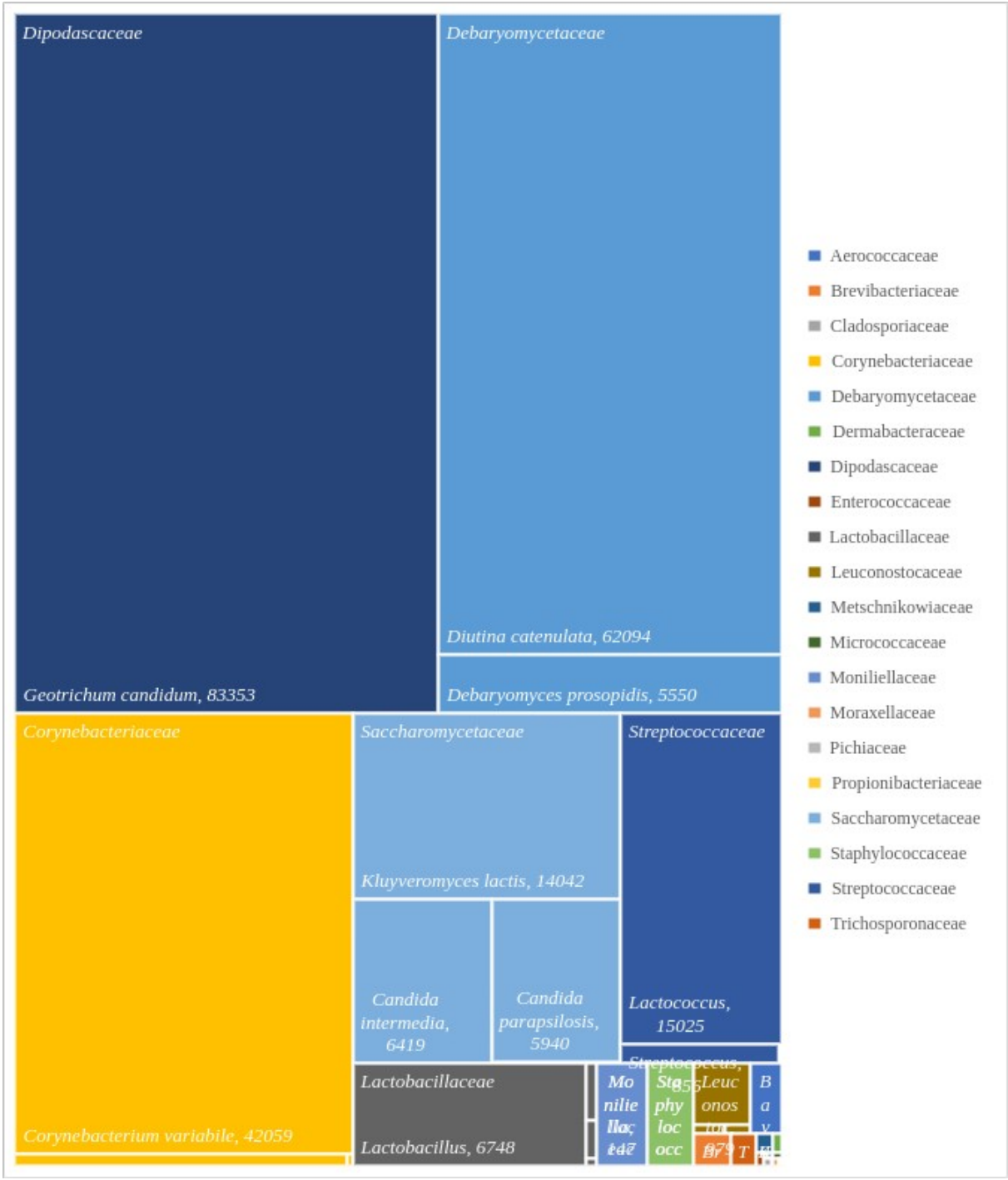


Figure 5: Microbiota of Artisanal Minas Cheese produced in the Campos das Vertentes/ MG region by family and species or genus.

4. Conclusions

The microbiota of the AMC produced in the Campos das Vertentes region has great diversity, highlighting the dominance of *G. candidum, D. catenulata, C. variabile, K. lactis*, and other species of *candida*, which are related to the formation of specific sensory characteristics. The results suggest that the presence of *G. candidum* has a protective effect against the growth of undesirable pathogens, because in the cheeses in which its presence was not reported, the presence of undesirable microorganisms such as bacteria of the genus *Staphylococcus* and contaminating fungi of the *Cladosporium cladosporioides* complex were detected. *G. candidum* proved to be the dominant fungus among all species found, ensuring the necessary safety for the effective production of bloomy rind cheese among producers in the region. It is suggested that the absence of the yeasts *D. hansenii* and *Y. Lipolytica*, widely described in the literature in artisan cheeses can be a marker of authenticity, alone or combined with other characteristics of artisan cheeses produced in the Campos das Vertentes region. The results obtained in this study increase the knowledge about the microbiota naturally present in the handmade cheeses produced in the Campos das Vertentes region and are an important step to ensure the production of the bloomy rind cheeses, to support new legislation that aims to regulate the production of these cheeses and are also a document that can be used to endorse the request for the certification of Denomination of Origin, to the INPI, ensuring a higher added value to the cheeses, benefiting both the producer and the consumer, by encouraging the consumption of this product with unique and typical characteristics. However, new studies must be conducted in order to find ways to ensure the development of authentication methods for artisan cheeses produced in this region.

Disclosure of Conflict of interest

The authors declare that they have no financial or personal interests that could exert any influence on the work reported in this article. Thanks to this study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES). The authors also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq) and the Fundação de Amparo à Pesquisa de MG (FAPEMIG) for financial support.

Acknowledgments

This study was partly financied by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES). The authors also thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil (CNPq) and Fundação de Amparo a Pesquisa de MG (FAPEMIG) for their financial support.

References

Andrade, R.P., Melo, C.N., Genisheva, Z., Schwan, R.F., Duarte, W.F., 2017. Yeasts from Canastra cheese production process: Isolation and evaluation of their potential for cheese whey fermentation. Food Res. Int. 91, 72–79. https://doi.org/10.1016/j.foodres.2016.11.032

Aragão, M. O., Evangelista, S. R., Passamani, F. R. F., Guimarães, J. P. M., Abreu, L. R., & Batista, L. R., 2022. Fungal community and physicochemical profiles of ripened cheeses from the Canastra of Minas Gerais, Brazil. Journal of Food Science and Technology, 59(12), 4685-4694. https://doi.org/10.1007/s13197-022-05548-4

Ascone, P., Maurer, J., Haldemann, J., Irmler, S., Berthoud, H., Portmann, R., ... & Wechsler, D., 2017. Prevalence and diversity of histamine-forming Lactobacillus parabuchneri strains in raw milk and cheese–A case study. International dairy journal, 70, 26-33. https://doi.org/10.1016/j.idairyj.2016.11.012

Atanassova, M.R., Fernández-Otero, C., Rodríguez-Alonso, P., Fernández-No, I.C., Garabal, J.I., Centeno, J.A., 2016. Characterization of yeasts isolated from artisanal short-ripened cows' cheeses produced in Galicia (NW Spain). Food Microbiol. 53, 172–181. https://doi.org/10.1016/j.fm.2015.09.012

Banjara, N., Suhr, M. J., & Hallen-Adams, H. E., 2015. Diversity of yeast and mold species from a variety of cheese types. Current microbiology, 70, 792-800. https://doi.org/10.1007/s00284-015-0790-1

Bintsis, T., 2021. Yeasts in different types of cheese. AIMS microbiology, 7(4), 447. https://doi.org/ 10.3934/microbiol.2021027

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... & Caporaso, J. G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature biotechnology, 37(8), 852-857. https://doi.org/10.1038/s41587-019-0209-9

Borelli, B. M., Ferreira, E. G., Lacerda, I. C., Franco, G. R., & Rosa, C. A., 2006. Yeast populations associated with the artisanal cheese produced in the region of Serra da Canastra, Brazil. World Journal of Microbiology and Biotechnology, 22, 1115-1119. https://doi.org/10.1007/S11274-006-9151-3

Boutrou, R., & Guéguen, M., 2005. Interests in Geotrichum candidum for cheese technology. International journal of food microbiology, 102(1), 1-20. https://doi.org/10.1016/j.ijfoodmicro.2004.12.028

BRUNO, L. M., & CARVALHO, J. D. G., 2009. Microbiota lática de queijos artesanais. URL. https://www.infoteca.cnptia.embrapa.br/bitstream/doc/748514/1/Doc124.pdf

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods, 13(7), 581-583. https://doi.org/10.1038/nmeth.3869

Camargo, A. C., de Araújo, J. P. A., Fusieger, A., de Carvalho, A. F., & Nero, L. A., 2021. Microbiological quality and safety of Brazilian artisanal cheeses. Brazilian Journal of Microbiology, 52(1), 393-409. https://doi.org/10.1007/s42770-020-00416-9

Cardin, M., Cardazzo, B., Mounier, J., Novelli, E., Coton, M., & Coton, E., 2022. Authenticity and Typicity of Traditional Cheeses: A Review on Geographical Origin Authentication Methods. Foods, 11(21), 3379. https://doi.org/10.3390/foods11213379

Cardoso, V. M., Borelli, B. M., Lara, C. A., Soares, M. A., Pataro, C., Bodevan, E. C., & Rosa, C. A., 2015. The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese. Food Research International, 69, 331-340. https://doi.org/10.1016/j.foodres.2014.12.040

Carvalheira, A., Silva, J., & Teixeira, P., 2017. Lettuce and fruits as a source of multidrug resistant Acinetobacter spp. Food microbiology, 64, 119-125. https://doi.org/10.1016/j.fm.2016.12.005

Carvalheira, A., Silva, J., & Teixeira, P., 2021. Acinetobacter spp. in food and drinking water–A review. Food Microbiology, 95, 103675. https://doi.org/10.1016/j.fm.2020.103675

Castro, R. D., Oliveira, L. G., Sant'Anna, F. M., Luiz, L. M. P., Sandes, S. H. C., Silva, C. I. F. & Souza, M. R., 2016. Lactic acid microbiota identification in water, raw milk, endogenous starter culture, and fresh Minas artisanal cheese from the Campo das Vertentes region of Brazil during the dry and rainy seasons. Journal of Dairy Science, 99(8), 6086-6096.https://doi.org/10.3168/jds.2015-10579

César, Isabel Cristina da Rocha et al. Filamentous Fungi from Artisanal Cheeses Produced in Canastra Region, Brazil. SSRN. URL. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4142335

De Freitas Martins, M. C., de Freitas, R., Deuvaux, J. C., Eller, M. R., Nero, L. A., & de Carvalho, A. F., 2018. Bacterial diversity of artisanal cheese from the Amazonian region of Brazil during the dry and rainy seasons. Food Research International, 108, 295-300. https://doi.org/10.1016/j.foodres.2018.03.060

De Miranda, N. M. Z., de Souza, A. C., de Souza Costa Sobrinho, P., Dias, D. R., Schwan, R. F., & Ramos, C. L. (2023). Novel yeasts with potential probiotic characteristics isolated from the endogenous ferment of artisanal Minas cheese. Brazilian Journal of Microbiology, 1-13.

De Sá, L. F. C., Martins, J. M., Martins, M. L., Ellena, E. A., de Cássia Moreira, B., & Pereira, L. L. C., 2021. Qualidade microbiológica do queijo Minas artesanal do Campo das Vertentes Microbiological quality of Campo das Vertentes artisanal Minas cheese. Brazilian Journal of Development, 7(11), 110013-110028. https://doi.org/10.34117/bjdv7n11-567

De Souza, T. P., Evangelista, S. R., Passamani, F. R. F., Bertechini, R., de Abreu, L. R., & Batista, L. R., 2021. Mycobiota of Minas artisanal cheese: Safety and quality. International Dairy Journal, 120, 105085. https://doi.org/10.1016/j.idairyj.2021.105085

Delgado, G. C., & Bergamasco, S. M. P. P., 2017. Agricultura familiar brasileira: desafios e perspectivas de futuro. Brasília: Ministério do Desenvolvimento Agrário, 470. URL. https://www.fao.org/family-farming/detail/ar/c/522540/

Dieuleveux, V., Van Der Pyl, D., Chataud, J., & Gueguen, M., 1998. Purification and characterization of anti-Listeria compounds produced by Geotrichum candidum. Applied and environmental microbiology, 64(2), 800-803. https://doi.org/10.1128/aem.64.2.800-803.1998

Fessard, A., & Remize, F., 2017. Why are Weissella spp. not used as commercial starter cultures for food fermentation?. Fermentation, 3(3), 38. https://doi.org/10.3390/fermentation3030038

Freire, T. T., Tolentino, A. L., Ferreira, B. K. O., & dos Santos, T. M., 2021. Bactérias ácido lácticas suas características e importância: revisão. Research, Society and Development, 10(11), e513101119964-e513101119964. https://doi.org/10.33448/rsd-v10i11.19964

Fröhlich-Wyder, M. T., Guggisberg, D., Badertscher, R., Wechsler, D., Wittwer, A., & Irmler, S., 2013. The effect of Lactobacillus buchneri and Lactobacillus parabuchneri on the eye formation of semi-hard cheese. International Dairy Journal, 33(2), 120-128. https://doi.org/10.1016/j.idairyj.2013.03.004

Fusco, V., Quero, G. M., Cho, G. S., Kabisch, J., Meske, D., Neve, H., ... & Franz, C. M., 2015. The genus Weissella: taxonomy, ecology and biotechnological potential. Frontiers in microbiology, 6, 155. https://doi.org/10.3389/fmicb.2015.00155

Giraffa, G., 2003. Functionality of enterococci in dairy products. International journal of food microbiology, 88(2-3), 215-222. https://doi.org/10.1016/S0168-1605(03)00183-1

INPI, 2023. Indicações geográficas. URL. https://www.gov.br/inpi/pt-br/servicos/indicacoes-geograficas.

IPHAN, 2008. Registro dos Queijos Artesanais de Minas, a ser inscrito no Livro dos Saberes. URL. http://portal.iphan.gov.br/

Jung, J., & Park, W., 2015. Acinetobacter species as model microorganisms in environmental microbiology: current state and perspectives. Applied microbiology and biotechnology, 99, 2533-2548. https://doi.org/10.1007/s00253-015-6439-y

Kamilari, E., Tomazou, M., Antoniades, A., & Tsaltas, D., 2019. High throughput sequencing technologies as a new toolbox for deep analysis, characterization and potentially

authentication of protection designation of origin cheeses?. International Journal of Food Science. https://doi.org/10.1155/2019/5837301

Kamimura, B. A., Cabral, L., Noronha, M. F., Baptista, R. C., Nascimento, H. M., & Sant'Ana, A. S., 2020. Amplicon sequencing reveals the bacterial diversity in milk, dairy premises and Serra da Canastra artisanal cheeses produced by three different farms. Food microbiology, 89, 103453. https://doi.org/10.1016/j.fm.2020.103453

Kamimura, B. A., De Filippis, F., Sant'Ana, A. S., & Ercolini, D., 2018. Large-scale mapping of microbial diversity in artisanal Brazilian cheeses. Food microbiology, 80, 40-49. https://doi.org/10.1016/j.fm.2018.12.014

Kamimura, B. A., Magnani, M., Luciano, W. A., Campagnollo, F. B., Pimentel, T. C., Alvarenga, V. O., & Sant'Ana, A. S., 2019. Brazilian artisanal cheeses: an overview of their characteristics, main types and regulatory aspects. Comprehensive Reviews in Food Science and Food Safety, 18(5), 1636-1657. https://doi.org/10.1111/1541-4337.12486

Katoh, K., Misawa, K., Kuma, K. I., & Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic acids research, 30(14), 3059-3066. https://doi.org/10.1093/nar/gkf436

Kothe, C. I., Mohellibi, N., & Renault, P., 2022. Revealing the microbial heritage of traditional Brazilian cheeses through metagenomics. Food Research International, 157, 111265. https://doi.org/10.1016/j.foodres.2022.111265

Landell, M. F., Hartfelder, C., & Valente, P., 2006. Identification and enzymatic profile of yeasts isolated from artisanal cheese in Southern Brazil. Acta Scientiae Veterinariae, 34(1), 49-55. URL. https://www.redalyc.org/pdf/2890/289021847007.pdf

Lima, C. D. L. C., Lima, L. A., Cerqueira, M. M. O. P., Ferreira, E. G., & Rosa, C. A., 2009. Bactérias do ácido láctico e leveduras associadas com o queijo-de-minas artesanal produzido na região da Serra do Salitre, Minas Gerais. Arquivo brasileiro de medicina veterinária e zootecnia, 61, 266-272. https://doi.org/10.1590/S0102-09352009000100037

Luiz, L. M. P., Castro, R. D., Sandes, S. H. C., Silva, J. G., Oliveira, L. G., Sales, G. A., ... & Souza, M. R., 2017. Isolation and identification of lactic acid bacteria from Brazilian Minas artisanal cheese. CyTA-Journal of Food, 15(1), 125-128. https://doi.org/10.1080/19476337.2016.1219392

Margalho, L. P., van Schalkwijk, S., Bachmann, H., & Sant'Ana, A. S., 2020. Enterococcus spp. in Brazilian artisanal cheeses: Occurrence and assessment of phenotypic and safety properties of a large set of strains through the use of high throughput tools combined with multivariate statistics. Food Control, 118, 107425. https://doi.org/10.1016/j.foodcont.2020.107425

Mariani, C., Briandet, R., Chamba, J. F., Notz, E., Carnet-Pantiez, A., Eyoug, R. N., & Oulahal, N., 2007. Biofilm ecology of wooden shelves used in ripening the French raw milk smear cheese Reblochon de Savoie. Journal of Dairy Science, 90(4), 1653-1661. https://doi.org/10.3168/jds.2006-190

Messini, A., Buccioni, A., Minieri, S., Mannelli, F., Mugnai, L., Comparini, C. & Rapaccini, S., 2017. Effect of chestnut tannin extract (Castanea sativa Miller) on the proliferation of Cladosporium cladosporioides on sheep cheese rind during the ripening. International Dairy Journal, 66, 6-12. https://doi.org/10.1016/j.idairyj.2016.10.012

MINAS GERAIS, 2009. Portaria nº 1.022, de 3 de novembro de 2009. Identifica a microrregião do Campo das Vertentes como produtora de queijo Minas artesanal. URL. http://ima.mg.gov.br/index.php?preview=1&option=com_dropfiles&format=&task=frontfile.download&catid=1576&id=16558&Itemid=1000000000000

MINAS GERAIS, 2020. Portaria nº 1.969, de 26 de março de 2020. Dispõe sobre a produção de Queijo Minas Artesanal em queijarias e entrepostos localizados dentro de microrregiões definidas e para as demais regiões do Estado, caracterizadas ou não como produtora de Queijo Minas Artesanal. URL. https://www.legisweb.com.br/legislacao/?id=391762

Montel, M. C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N., & Berthier, F., 2014. Traditional cheeses: Rich and diverse microbiota with associated benefits. International journal of food microbiology, 177, 136-154. https://doi.org/10.1016/j.ijfoodmicro.2014.02.019

Nero, L. A., Andretta, M., Almeida, T. T., Ferreira, L. R., Camargo, A. C., Yamatogi, R. S., ... & Call, D. R., 2021. Lactic microbiota of the minas artisanal cheese produced in the serro region, Minas Gerais, Brazil. LWT, 148, 111698. https://doi.org/10.1016/j.lwt.2021.111698

Nilsson, R. H., Taylor, A. F., Adams, R. I., Baschien, C., Bengtsson-Palme, J., Cangren, P., ... & Abarenkov, K., 2018. Taxonomic annotation of public fungal ITS sequences from the built environment–a report from an April 10–11, 2017 workshop (Aberdeen, UK). MycoKeys, (28), 65. https://doi.org/10.3897/mycokeys.28.20887

O'Brien, C. E., McCarthy, C. G., Walshe, A. E., Shaw, D. R., Sumski, D. A., Krassowski, T. & Butler, G., 2018. Genome analysis of the yeast Diutina catenulata, a member of the Debaryomycetaceae/Metschnikowiaceae (CTG-Ser) clade. PLoS One, 13(6), e0198957. https://doi.org/10.1371/journal.pone.0198957

Padilla B, Manzanares P, Belloch C, 2014. Yeast species and genetic heterogeneity within Debaryomyces hansenii along the ripening process of traditional ewes' and goats' cheeses. Food Microbiology, 38: 160–166. https://doi.org/10.1016/j.fm.2013.09.002

Penland, M., Falentin, H., Parayre, S., Pawtowski, A., Maillard, M. B., Thierry, A. & Deutsch, S. M., 2021. Linking Pélardon artisanal goat cheese microbial communities to aroma compounds during cheese-making and ripening. International Journal of Food Microbiology, 345, 109130. https://doi.org/10.1016/j.ijfoodmicro.2021.109130

Pereira-Dias S, Potes ME, Marinho A, et al., 2000. Characterisation of yeast flora isolated fron artisanal Portuguese ewes' cheese. Int J Food Microbiol 60: 55–63. https://doi.org/10.1016/S0168-1605(00)00323-8

Perin, L. M., Sardaro, M. L. S., Nero, L. A., Neviani, E., & Gatti, M., 2017. Bacterial ecology of artisanal Minas cheeses assessed by culture-dependent and-independent methods. Food microbiology, 65, 160-169. https://doi.org/10.1016/j.fm.2017.02.005

Puig, M. S., Lorenzo, F., Bertó, R., & Orihuel, E., 2018. Metagenómica aplicada a la industria alimentaria. Tecnoalimen: tecnología alimentaria y packaging, (23), 24-26.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., ... & Glöckner, F. O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research, 41(D1), D590-D596. https://doi.org/10.1093/nar/gks1219

Rafei, R., Hamze, M., Pailhoriès, H., Eveillard, M., Marsollier, L., Joly-Guillou, M. L. & Kempf, M., 2015. Extrahuman epidemiology of *Acinetobacter baumannii* in Lebanon. Applied and environmental microbiology, 81(7), 2359-2367. https://doi.org/10.1128/AEM.03824-14

Remor, A., de Vasconcelos, T. C. B., Belo, V. S., Zanella, R., dos Santos, E. D., & Dickel, E. L., 2021. Investigação metagenômica em queijos tipo coloniais, produzidos com leite pasteurizado e comercializados no estado do Rio Grande do Sul, Brasil. Veterinária e Zootecnia, 28, 1-9. https://doi.org/10.35172/rvz.2021.v28.475

Riquelme, C., Câmara, S., Maria de Lurdes, N., Vinuesa, P., da Silva, C. C. G., Malcata, F. X., & Rego, O. A., 2015. Characterization of the bacterial biodiversity in Pico cheese (an artisanal Azorean food). International Journal of Food Microbiology, 192, 86-94. https://doi.org/10.1016/j.ijfoodmicro.2014.09.031

Ruppert, K. M., Kline, R. J., & Rahman, M. S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation, 17, e00547. https://doi.org/10.1016/j.gecco.2019.e00547

Sant'Anna, F. M., Wetzels, S. U., Cicco, S. H. S., Figueiredo, R. C., Sales, G. A., Figueiredo, N. C. & Souza, M. R., 2019. Microbial shifts in Minas artisanal cheeses from the Serra do Salitre region of Minas Gerais, Brazil throughout ripening time. Food microbiology, 82, 349-362. https://doi.org/10.1016/j.fm.2019.02.016

Silva, J. M. M., 2020. Micobiota core de queijos de leite cru produzidos na região da Serra da Canastra. URL. https://www.locus.ufv.br/handle/123456789/27934

Šipošová, P., Koňuchová, M., Valík, Ľ., & Medveďová, A., 2021. Growth dynamics of lactic acid bacteria and dairy microscopic fungus *Geotrichum candidum* during their co-cultivation in milk. Food Science and Technology International, 27(6), 572-582. https://doi.org/10.1177/1082013220976485

Tofalo, R., Fasoli, G., Schirone, M., Perpetuini, G., Pepe, A., Corsetti, A., & Suzzi, G., 2014. The predominance, biodiversity and biotechnological properties of Kluyveromyces marxianus in the production of Pecorino di Farindola cheese. International journal of food microbiology, 187, 41-49. https://doi.org/10.1016/j.ijfoodmicro.2014.06.029

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., & Glöckner, F. O., 2014. The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. Nucleic acids research, 42(D1), D643-D648. https://doi.org/10.1093/nar/gkt1209

Zheng, X., Li, K., Shi, X., Ni, Y., Li, B., & Zhuge, B., 2018. Potential characterization of yeasts isolated from Kazak artisanal cheese to produce flavoring compounds. MicrobiologyOpen, 7(1), e00533. https://doi.org/10.1002/mbo3.533

APPENDIX A – Species identified in artisan minas cheese produced in the Campos das Vertentes/MG region

Fungi
Candida intermedia
Candida parapsilosis
Cladosporium cladosporioides
Debaryomyces prosopidis
Diutina catenulata
Geotrichum candidum
Kazachstania unispora
Kluyveromyces lactis
Kodamaea ohmeri
Trichosporon ovoides
Bacteria
Acidipropionibacterium acidipropionici
Bavariicoccus seileri
Corynebacterium flavescens
Corynebacterium variabile
Lactobacillus brevis
Lactobacillus buchneri
Lactobacillus parabuchneri
Streptococcus salivarius
Weissella jogaejeotgali

APPENDIX B – Information booklet for artisan cheese producers in the Campos das Vertentes/MG region