

Natural starter culture on artisanal cheese
Cultura *starter* natural em queijo artesanal
Cultivo iniciador natural en queso artesanal

Received: 09/19/2020 | Reviewed: 09/25/2020 | Accept: 09/25/2020 | Published: 09/27/2020

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Abstract

The use of natural starter culture in the manufacture of artisanal Minas cheeses is required by the government legislation in Brazil. However, there are no scientific support on role of the natural starter culture in these cheeses. The aim of this work was to compare artisanal Minas cheeses produced with and without natural starter culture to verify the influence on the physico-chemical and microbiological characteristics during the ripening process. The use of natural starter culture did not have a significant effect on the physico-chemical (dry matter, humidity, fat, pH, chlorides, water activity, ash, total protein, depth and extent of proteolysis) and microbiological (*Escherichia coli*, coliforms and *Staphylococcus aureus*) properties of the cheeses. The significant changes that occurred in the cheeses were mainly because of the ripening time. According to obtained results, natural starter has shown to be innocuous to traditional Minas Serro cheese. The present study is pioneering in the scientific literature and opens new horizons for the understanding of the requirement to use natural starter culture.

Keywords: Endogenous natural starter; Food safety; Raw milk.

Resumo

A utilização da cultura *starter* natural na fabricação de queijos Minas artesanais é exigida pela legislação governamental no Brasil. No entanto, não há suporte científico sobre o papel da cultura *starter* natural nesses queijos. O objetivo deste trabalho foi comparar os queijos Minas artesanais produzidos com e sem cultura *starter* natural para verificar a influência nas características físico-químicas e microbiológicas durante o processo de maturação. O uso da

cultura *starter* natural não teve efeito significativo sobre as propriedades físico-químicas (matéria seca, umidade, gordura, pH, cloretos, atividade de água, cinzas, proteína total, profundidade e extensão da proteólise) e microbiológicas (*Escherichia coli*, coliformes e *Staphylococcus aureus*) dos queijos. As mudanças significativas que ocorreram nos queijos foram principalmente devido ao tempo de maturação. De acordo com os resultados obtidos, a cultura *starter* natural tem se mostrado inócua ao queijo Minas Serro tradicional. O presente estudo é pioneiro na literatura do campo científico e abre novos horizontes para a compreensão da necessidade de usar a cultura natural *starter*.

Palavras-chave: Cultura starter endógena natural; Segurança de alimentos; Leite cru.

Resumen

El uso del cultivo iniciador natural en la fabricación de queso artesanal de Minas es requerido por la legislación del gobierno de Brasil. Sin embargo, no hay respaldo científico para el papel del cultivo iniciador natural en estos quesos. El objetivo de este trabajo fue comparar los quesos artesanales de Minas elaborados con y sin cultivo iniciador natural para verificar la influencia sobre las características físico-químicas y microbiológicas durante el proceso de maduración. El uso del cultivo iniciador natural no tuvo un efecto significativo sobre las propiedades fisicoquímicas (materia seca, humedad, grasa, pH, cloruros, actividad del agua, cenizas, proteína total, profundidad y extensión de la proteólisis) y microbiológicas (*Escherichia coli*, coliformes y *Staphylococcus aureus*) de quesos. Los cambios significativos que se produjeron en los quesos se debieron principalmente a la época de maduración. Según los resultados obtenidos, el cultivo iniciador natural se ha mostrado inofensivo para el queso tradicional Minas Serro. El presente estudio es pionero en la literatura científica y abre nuevos horizontes para comprender la necesidad de utilizar cultivos iniciadores naturales.

Palabras clave: Cultivo iniciador natural endógeno; Seguridad alimenticia; Leche cruda.

1. Introduction

The production of traditional Minas cheese (TMC) has a significant social, cultural, and economic role in Brazil. Cheeses are produced using raw milk, chymosin, and endogenous starter cultures obtained from whey or cheese surface of previous production (Mata, et al., 2016, Perin, et al., 2017, Prata, et al., 2020). The natural starter cultures are believed to play a fundamental role in the sensorial characteristics of each variety of these

cheeses and has been reported in several studies (Pinto, et al., 2009, Martins, et al., 2015, Perin, et al., 2017, Alexandre, et al., 2020).

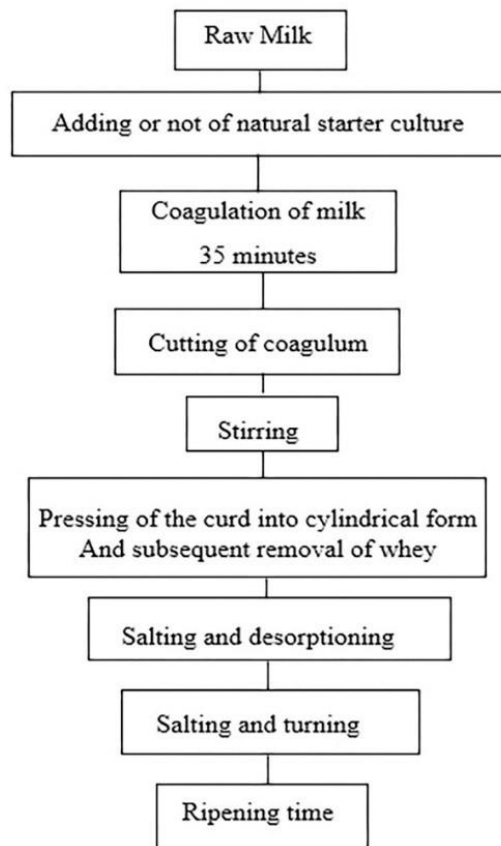
Santos et al. (2017) found that TMC from Serro, Canastra and Araxá can be easily discriminated by neural networks and linear discriminant analysis through their physicochemical characteristics. Therefore, to maintain the identity of these cheeses, it is important to evaluate the influence of different factors on their properties, like ripening temperature (Martins, et al., 2015).

To the best of the current knowledge, there are no studies evaluating the effect of the presence or absence of natural starter on the physicochemical and rheological properties of TMC during the ripening time. Although Brazilian legislation requires the use of natural starter in TMC, there is no definition regarding the amount of natural starter to be used. In addition, some studies found that the natural starter may be a source of contamination for TMC (Borelli, et al., 2006). If the natural starter culture may cause harm and does not improve cheese quality, why its use must remain obligatory? Therefore, the aim of this study was to compare TMC produced with or without natural starter to verify the influence on the physicochemical and microbiological properties of cheeses during the ripening time.

2. Methodology

Three producers from Serro were directed to produce their cheeses with milk from the same day, where half of this milk would be used to produce cheeses with 200 mL of natural starter culture per 100L of milk, and the other half of the milk would be used for cheese production without the natural starter. Each producer has used about 64 liters of raw milk for each production, obtained from his farmer, being eight cheeses per production, totaling twenty-four cheeses with natural starter and twenty-four cheeses without natural starter. The cheeses were made according to flowchart presented in Figure 1.

Figure 1. Process flowchart for the manufacturing of artisanal cheese from Serro.



Source: Authors.

Initially, the volume of milk was divided into two equal parts, where one received the starter culture and the other did not. Milk coagulation, clot cutting, shaking, and whey removal proceeded. Then salting and desorption, turning and ripening were carried out.

The cheeses were collected after eight days of production in the producing units, packed, transported and ripened without packaging, at room temperature for 60 days in adequate room for cheeses and were analyzed at times 10, 20, 40 and 60 days.

Physico-chemical analyses were performed for total dry matter (method A): moisture, ashes (Brazil, 2006), fat (ISO/IDF, 2008), and chlorides (ISO/IDF, 2006). The extent of ripening and depth of proteolysis were performed according ISO/IDF (2014) and Pombo & Lima (1989). The protein content was determined by the conversion of total nitrogen, using the factor 6.38. The a_w was determined using an Aqualab digital meter, model series 3TE and pH was measured using a pH meter model Qualxtron 8010 by inserting the electrode inside the cheeses.

For analysis of *S. aureus*, total coliforms and *E. coli*, were used, respectively, the Petrifilm™ STX and the Petrifilm Coliforms/*E.coli*, according to the procedures determined by the distributor, both indicated for analysis in milk and cheese (Schoeller & Ingham, 2001).

This project was approved by the research ethics committee of the Federal University of Minas Gerais under the number 44063315.1.0000.5149.

2.1. Statistical design

The experiments were carried out three times in a block randomized design with a factorial scheme (time × use or non-use of natural starter). The physicochemical and microbiological results were subjected to regression analysis and the angular coefficients of the curves for each attribute were compared by analysis of variance (ANOVA). The level of significance was set at $p < 0.05$.

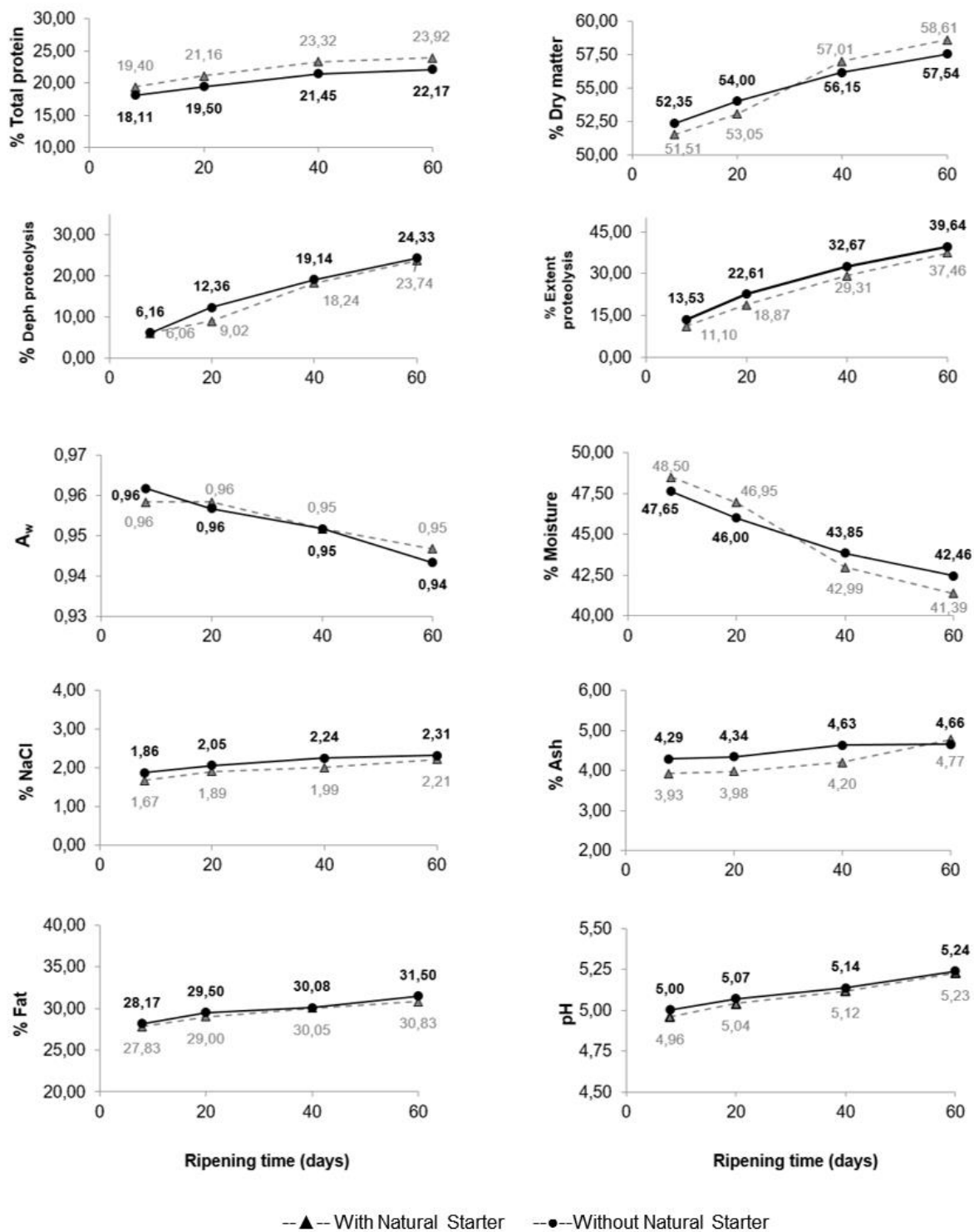
The analyses were performed using the Statistical Analysis System (SAS) version 9.1 software. For regression analysis were accepted models that presented a level of significance equal to or less than 5% for model and for linear and quadratic coefficients when this was the case.

3. Results and Discussion

3.1. Physicochemical evaluation of cheeses from the Serro - MG region produced with and without natural starter culture.

The behavior of the physicochemical properties of the Minas cheeses produced with and without natural starter culture is presented in Figure 2.

Figure 2. Physicochemical averages of cheeses produced with and without natural starter during ripening.



Source: Authors.

The addition or absence of natural starter culture did not cause any changes in the dry matter, moisture, fat, protein, salt, ash, a_w , and pH of the TMC ($p \geq 0.05$). Physicochemical

properties are important for characterizing cheeses from different regions. Among other factors, they are a result of the biological activity of the natural starter culture present in milk and the environment of different producer regions (Santos, et al., 2017).

The decrease in the pH of fresh TMC when compared to milk pH was mainly due to the conversion of lactose to lactic acid by lactic acid bacteria (Macedo & Malcata, 1997). Wyder & Puhán (1999) and Martins, et al. (2015) report that the increase in pH during ripening resulted from the alkaline products released during proteolysis like alkaline nitrogen compounds resulting from the degradation of protein by native milk proteases in MTC.

Fox et al. (2004), indicate that degradation of lactate by yeast results in deacidification on cheese's surface with consequent increase in pH, favoring the growth of molds, leading to an increase in pH, proteolysis and consequently softening of the cheese.

Machado et al. (2004) report that the amount of natural starter culture added to milk can vary, resulting in different concentrations of glucose fermenting bacteria in the cheese curd, thus causing variations in pH.

The empirical form of salting applied on cheese's surface by the producers from Serro becomes a factor that makes salt content standardization difficult in these cheeses, which leads to considerable variations in this parameter in the cheeses of a single producer (Pinto, et al., 2011).

Cheese ashes are composed of salt, usually added to the curd, and the mineral components derived from different salts which constitute the milk salts and interfere in their organic and inorganic composition (Sanjuán, et al., 2002). The mineral composition of milk varies with the species and the feed to which the animals are subjected, for example, type of feed, soil composition, and period of the year.

The a_w has decreased during ripening ($p < 0.05$) (Figure 2) due to moisture reduction and consequent salt increase. In addition, according to Pinto, et al. (2011), this reduction could be due to proteolysis, which releases amino acids with side chains containing polar groups that interact easily with water molecules and thus reduce the a_w .

Proteolysis is indicated by the increasing extension and depth indices over time. Extension is mainly related to the action of the coagulating agent that transforms the proteins into peptides. Depth is related to the activity of endoenzymes and exoenzymes in the lactic culture employed in the degradation of the peptides (Pombo & Lima, 1989, Narimatsu, et al., 2003).

The results of the depth and extent of ripening obtained in this study are consistent evidence that the natural starter, under the conditions in which it was used, is innocuous for

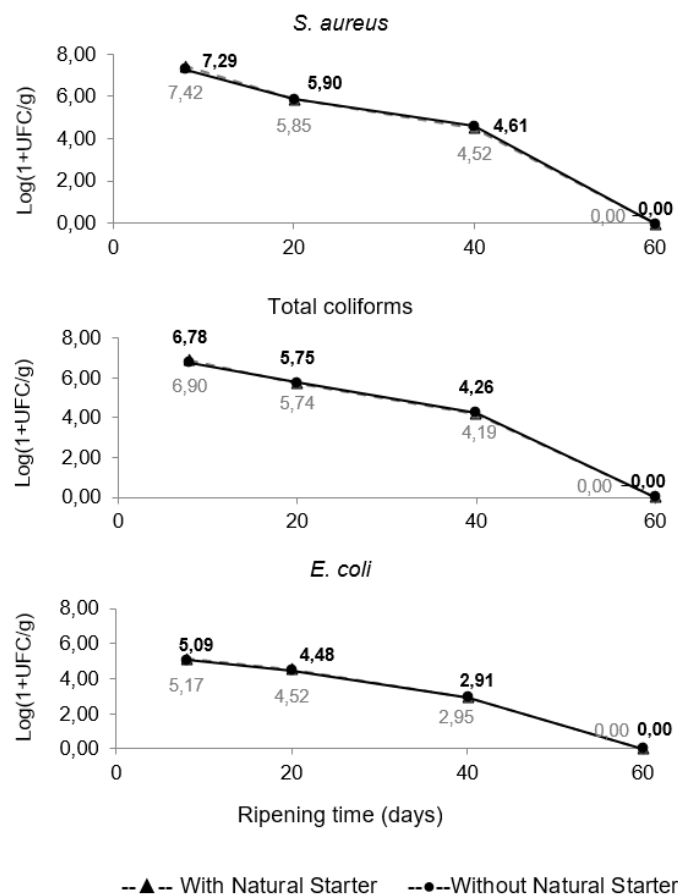
the cheese at least regarding the physicochemical characteristics. If there was a significant effect of the microbiota contained in this starter, the cheeses would acidify more rapidly causing increased syneresis and consequently higher levels of salt, ash, and fat, and low moisture and pH (Walstra, et al., 2006).

Microbiota from the natural starter would inevitably result in greater cell death during ripening, availability of proteolytic enzymes and consequently, greater extension and depth of ripening.

3.2. Evaluation of the microbiological counts in the artisanal Minas cheese from Serro – MG, produced with and without natural starter culture.

We evaluated the effect of the presence or absence of natural starter culture in the microbiological aspects of the Minas cheeses (Figure 3).

Figure 3. Average of total counts of *S. aureus*, total coliforms and *E. coli* in cheeses produced with and without natural starter during ripening.



Source: Authors.

The angular coefficients of the curves referring to the population counts of *S. aureus*, total coliforms and *E. coli* for the cheeses produced with and without natural starter did not differ among them ($p \geq 0.05$) (Figure 3). This means that the use of natural starter is innocuous to the bacteria studied. The assumption that the natural starter selects resistant bacteria capable of overcoming the growth of pathogenic groups was not sustainable in this study.

Paiva, et al. (2015) investigated the effect of the addition of natural starter on the lactic bacteria count of the traditional Minas cheese from Serro region and verified that there was not influence on the lactic bacteria count when compared to the same cheese manufactured without natural starter during the 60 days of ripening.

The presence of bacteria from total coliforms group, *E. coli* and *staphylococci* coagulase positive above the recommended amounts by the legislation indicates poor microbiological quality (Apolinário, et al., 2014).

In the present study, even with 40 days of ripening, the microbial counts for the three groups studied are high compared to the study conducted by Martins, et al. (2015), in which was demonstrated the food unsafety of cheeses with less than 25 days of ripening. That study has served as basis for current legislation to recommend the marketing of cheeses with less than 60 days of ripening.

According to Borges, et al. (2008), coagulase positive *staphylococci* counts above 5 Log UFC/mL⁻¹ may induce the production of enterotoxins if they are under adequate environmental conditions and that this occurrence of producing staphylococcal enterotoxins in dairy products appears to be more related to the ability of strains to produce enterotoxins than to the degree of *S. aureus* contamination.

In fact, even though the legislation allows the commercialization of cheeses with less than 30 days of ripening, the contamination of this food cause concern.

Studies show that the counts of lactic bacteria contained in the natural starter are close to those found in raw milk (Borelli, et al., 2006, Nóbrega, et al., 2008). Providing that natural starter has a count of 10⁷ UFC. mL⁻¹ of lactic bacteria according to a study conducted by Nóbrega, et al. (2008) and providing that 200 mL of natural starter are used per 100 liters of milk, it can be inferred that 2x10⁹ UFC is being added to the milk. As there are 100 liters of milk in the vat, the initial concentration of the lactic bacteria from the natural starter in the vat milk would be 2x10⁴ UFC/mL, which is well below the count of lactic bacteria found in the milk itself.

Although the above-mentioned studies are only quantitative, the results disqualify a natural starter that in theory should have high concentrations of lactic bacteria that will

outweigh the growth of contaminants and dominate the environment. The hypothesis that although the natural starter contains the same concentrations of milk lactic bacteria but which would be more resistant due to the selectivity of the natural starter, is practically discarded with the results of depth of ripening found in the present study.

There are not scientific studies that give to the use of natural starter and there are many evidences in the literature showing that it could be innocuous in relation to the microbiological safety of cheeses, since all these studies were made with cheeses elaborated with natural starter.

Castro, et al. (2016) also related the use of natural starter at high counts with the contamination of *S. aureus*, besides the inadequate handling of the cheeses. It is also mentioned the water supply of the dairies, which are used both in the cleaning and hygiene of the utensils, as well as in the washing of the cheese, as another possible source of contamination of this bacterium. Pinto, et al. (2011) verified that cheeses produced with natural starters containing higher counts of *S. aureus* resulted in cheeses with higher contamination of this microorganism. However, this statement cannot be considered, since there was no control group in this study, neither the same milk was used.

The presence of total coliforms and *E. coli* in cheeses is attributed by several authors to insufficient sanitary conditions of the herds, failure to obtain hygienic milk, lack of hygiene during cheeses manufacturing and inadequate conditions for the commercialization of the product (Souza, et al., 2003, Brant, et al., 2007, Baranceli, et al., 2014).

4. Conclusions

No significant differences were observed in the physical-chemical and microbiological parameters of traditional Minas Serro cheeses made with and without the natural starter. Taking these findings into account, qualitative studies can be carried out involving the amino acid profile of cheeses made with and without starter, to validate its use. The results of the present work are pioneering in scientific literature and open new horizons for understanding the need to use natural starter culture.

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