



The effects of nisin on *Staphylococcus aureus* count and the physicochemical properties of Traditional Minas Serro cheese

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ABSTRACT

This study reports the effect of different concentrations of nisin (0, 100 and 500 IU mL⁻¹) against *Staphylococcus aureus* in Minas Traditional Serro cheese manufactured with raw milk. We also evaluated the influence of nisin on the physicochemical properties, mechanical characteristics and colour of the cheese over 60 days of ripening. Nisin was effective in reducing *S. aureus* count in Serro cheese; a reduction of 1.2 and 2.0 log cycles in *S. aureus* count was observed from the 7th day of ripening for cheese containing 100 IU mL⁻¹ and 500 IU mL⁻¹ of nisin, respectively, compared with the control sample. The major changes in physicochemical properties, mechanical characteristics and colour were associated with cheese ripening, except for the index of ripening, which was lower in the presence of nisin. The addition of nisin is a powerful tool to contribute to the safety of traditional cheese produced with raw milk.

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1. Introduction

In several countries, many varieties of cows', goats' and ewes' milk cheese are manufactured in farmhouses following traditional techniques, without the deliberate addition of commercial starter cultures. These cheeses are generally referred to as "artisanal" or "traditional". Technological parameters during different steps, such as renneting, acidification, heating, whey drainage, salting and ripening, have a great influence on the final characteristics of the cheese and play a major role in its microbial composition by enhancing biodiversity (Randazzo, Caggia, & Neviani, 2009).

Minas Traditional Serro cheese is widely consumed in Brazil, mainly as an accompaniment to meals and in a variety of sandwiches (Souza, Cruz, Moura, Vieira, & Sant'Ana, 2008). It is a semi-cured, semi-hard, enzymatically coagulated cheese that is manufactured in the Serro region, which is located in the state of Minas Gerais, Brazil. Like many other traditional cheeses, it has

great social and economic importance as a consequence of its historical and cultural context (Pinto, 2004).

Traditionally, cheeses have usually been made from raw milk, but due to hygiene and safety reasons most cheeses today are made from pasteurised milk in cheese dairies, using commercial starters (Manolopoulou et al., 2003). Nevertheless, Minas Traditional Serro cheese is still manufactured using raw milk (Pinto et al., 2009), which is a cause for concern because it can host pathogens such as *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella* sp. and *Listeria monocytogenes*, among others (De Buyser, Dufour, Maire, & Lafarge, 2001). Many studies have revealed the presence and/or survival of pathogenic bacteria in traditional cheese manufactured with raw milk in different countries (Alegria et al., 2009; Aygun, Aslantas, & Oner, 2005; Borelli et al., 2006; Carvalho, Viotto, & Kuaye, 2007; De Buyser et al., 2001; Hamana, El Hankouri, & El Ayadi, 2002; Pinto et al., 2009; Psoni, Tzanetakis, & Litopoulou-Tzanetaki, 2003; Rogga et al., 2005), including Minas cheeses (Carmo et al., 2002; Veras et al., 2008).

Staphylococcal food poisoning is a common ailment that is often transmitted by improperly handled or stored dairy products. In many countries, *S. aureus* is the second or third most common pathogen responsible for outbreaks of food poisoning (Veras et al., 2008). Some

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researchers have shown a reduction of *S. aureus* in Minas Traditional Serro cheese over the last few years (Martins, 2006; Ornelas, 2005; Pinto, 2004) thanks to better handling conditions and raw material quality. However, the observed *S. aureus* count reduction is not enough to ensure microbiological safety for consumers.

Nisin is produced by certain strains of *Lactococcus lactis* subsp. *lactis* (Cheigh & Pyun, 2005), and it exhibits antimicrobial activity against a wide range of spores and Gram-positive bacteria, including *Staphylococcus* (Arauz, Jozala, Mazzola, & Penna, 2009). It has been suggested as a natural antimicrobial to be used as a biopreservative in foods, including dairy products, and is generally regarded as safe (GRAS) (Adams, 2003). In addition, nisin is the only bacteriocin authorised for use in the food industry (Chollet, Swesi, Degraeve, & Sebti, 2009). Many recent studies have shown the effect of nisin against *S. aureus* and other Gram-positive bacteria (Cabo, Herrera, Crespo, & Pastoriza, 2009; Kim, Choi, Bajpai, & Kang, 2008; Malheiros, Daroit, Silveira, & Brandelli, 2010; Mitra, Chakrabarty, & Biswas, 2010; Pathanibul, Taylor, Davidson, & Harte, 2009).

In this context, the goal of our work was to evaluate the survival of *S. aureus* in Minas Traditional Serro cheese manufactured with raw milk containing different concentrations of nisin, during 60 days of ripening. The influence of nisin on the physicochemical characteristics, texture and colour of the cheese was also evaluated.

2. Material and methods

2.1. Culture and growth conditions

S. aureus ATCC 6538 was used in this study. The stock culture was maintained at -80°C in Brain Heart Infusion (BHI) broth (DIFCO[®], Becton, Dickinson and Company, Franklin Lakes, NJ, USA) containing 10% (v/v) glycerol. Stock cultures were revived in BHI broth and subsequently subcultured three times in BHI at 37°C for 24 h.

2.2. Pre-test

An overnight culture of *S. aureus* ATCC 6538 culture in BHI broth was added to reconstituted skim milk at a level of 12% (v/v) with five different nisin concentrations (0; 100; 200; 400 and 500 IU mL⁻¹). The culture was incubated at 37°C for 120 h and was inoculated into the milk in concentrations of 10^4 cfu mL⁻¹. Counts were determined after 0, 6, 12, 24, 48, 72, 96 and 120 h of incubation.

2.3. Cheese manufacturing

Minas Traditional cheese (500 g) was made with raw milk as described by Pinto et al. (2009), obtained from three different farms with inadequate conditions of milking, hygiene and handling, consequently giving milk with high counts of *S. aureus*. Commercial preparations of nisin (Chrisin[®], Christian Hansen (Chr. Hansen A/S, Hørsholm, Denmark); 2.5%, w/w, concentration) was added during the cheese preparation (Fig. 1) to final concentrations in the milk of 100 and 500 IU mL⁻¹. These concentrations were chosen based on previous tests (data not shown).

2.4. *S. aureus* analysis of cheese

S. aureus counts were determined in the milk used for cheese production, in the curd immediately after coagulation and in the cheese during the ripening period (at 7, 14, 22, 30, 45 and 60 d) using Petrifilm 3 M Rapid *S. aureus* (RSA) Count Plates (AOAC 981.15) according to the manufacturer's recommendations.

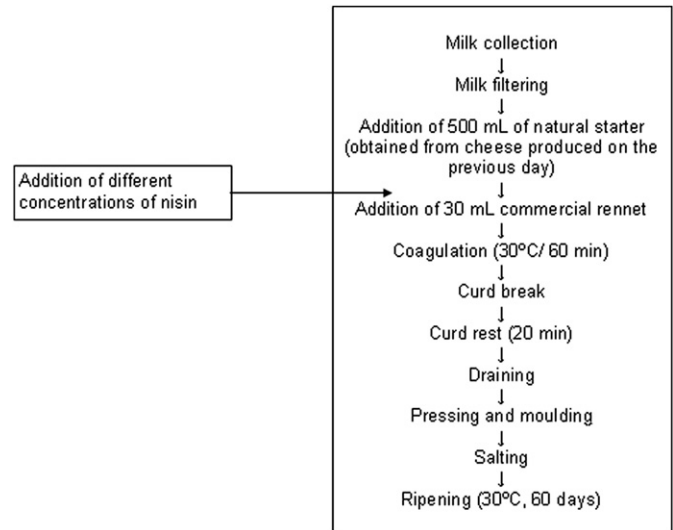


Fig. 1. Schematic flowchart of Minas Traditional Serro cheese production.

2.5. Physico-chemical analysis of cheese

Samples were analyzed to determine the moisture, fat, chloride and total nitrogen (TN) contents (Brasil, 1996). The pH (APHA, 1985) was also measured using a Tec-2 Tecnal pH meter (Tecnal, Piracicaba, SP, Brazil) at $20\text{--}30^{\circ}\text{C}$. The water activity was determined with an Aqualab water activity meter (model series CX2 T, Decagon Devices, Pullman, Washington, USA). Non-protein-nitrogen (NPN) content was determined using the trichloroacetic acid technique (Pereira, Silva, De Oliveira, & Costa Junior, 2001). True protein content was calculated by subtracting NPN from TN and multiplying by 6.38.

Index of ripening is measured by the degradation of casein or nitrogen originated from organic material and was quantified by the ratio (%) of soluble nitrogen at pH 4.6 and TN. This index should increase with advancing maturation. Ripening depth involves all of low molecular weight nitrogenous substances formed during processing and was quantified by the ratio of NPN to TN. Characteristics components are: amino acids, oligopeptides and amines (Wolfschoon-Pombo & Lima, 1989).

All analyses were carried out in triplicate, at 7, 14, 22, 30, 45 and 60 days of ripening.

2.6. Texture and colour determination

An Instron Universal Testing Machine model 3367 (Instron Ltd., Norwood, Massachusetts, USA) was used for the Texture Profile Analysis (TPA). Pre-test, test and post-test speeds of 1 mm s^{-1} were used, with a compression distance of 40% from the top of the sample. A cylindrical probe of 55 mm in diameter was used and a charge cell of 1 kN, which was moved perpendicularly through cylindrical cheese samples (25 mm diameter and 35 mm height) that were randomly collected from the whole cheese. The resistance exerted by the samples was automatically registered and the firmness (N), fracturability (N), gumminess (N), chewiness (N), springiness (mm) and cohesiveness were calculated by Blue Hill 2.0 software (Instron, Norwood, Massachusetts, USA), using the strength (N) \times time (s) data obtained during the test.

The colour of the cheese was evaluated using a Hunterlab Model Colour Quest II colorimeter (Hunter Associates Laboratory, Inc., Reston, VA, USA). HunterLab *L*, *a* and *b* values were read from the samples. The "*L*" value is a measure of lightness and ranges between

0 and 100. Positive or negative changes in the “a” value correspond to increases in the red or green colour proportions, respectively, while the “b” parameter varies from blue (–) to yellow (+).

Samples were taken from different points on the pieces of cheese, measured on eight consecutive occasions.

2.7. Data analysis

The experiments were carried out three times in a block randomised design with a factorial scheme (time × nisin concentrations). The decimal logarithms of *S. aureus* counts were subjected to regression analysis. The physicochemical and TPA results at each point in time were evaluated by Analysis of Variance (ANOVA), using the Tukey test to compare means. Level of significance was set at $P < 0.05$. The analyses were performed using the Statistical Analysis System (SAS) version 9.1 software.

3. Results and discussion

3.1. Effect of nisin on *S. aureus* growth in 12% reconstituted skin milk

There were no significant differences ($P \geq 0.05$) on growth of *S. aureus* between the concentrations of 100 and 200 IU mL⁻¹. Growth of *S. aureus* in skin milk samples containing 400 and 500 IU mL⁻¹ of nisin did not differ ($P \geq 0.05$) between themselves and was lower ($P < 0.05$) than that observed at concentrations of 100 and 200 IU mL⁻¹. The growth of *S. aureus* in absence of nisin was higher ($P \geq 0.05$) than in all others treatments. Because of such results, we selected for use in the experiment with traditional cheeses, the minimal and the maximum concentrations that were able to inhibit *S. aureus* growth (100 and 500 IU mL⁻¹).

3.2. Effect of nisin on *S. aureus* growth in Minas Traditional Serro cheese

Time and nisin concentration significantly influenced ($P < 0.05$) the behaviour of *S. aureus* in Serro cheese. Fig. 2 shows the *S. aureus* counts determined in cheeses ripened in the absence and in the presence of different levels of nisin. An increase in *S. aureus* population levels over the first 14 days was observed in the control curd compared with the milk; in contrast, the *S. aureus* count was

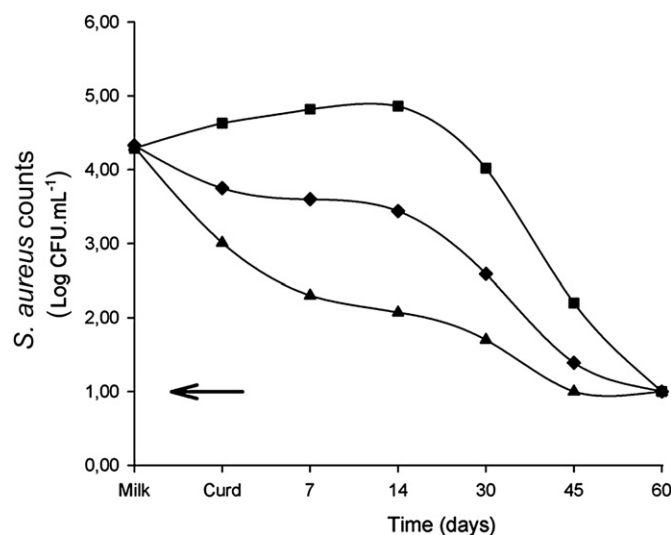


Fig. 2. Survival of *S. aureus* in Minas Traditional Serro Cheese manufactured using different nisin concentration: (■) no nisin; (◆) 100 IU mL⁻¹; (▲) 500 IU mL⁻¹.

reduced in the curd originating from nisin-treated milk, probably due to the bactericidal activity of nisin (Chalier, Cretenet, Even, & Le Loir, 2009; Cotter, Hill, & Ross, 2005). Nisin was considered to be the only factor responsible for reducing the *S. aureus* count of the milk before the curd was obtained, but other factors probably contributed to the death rate of *S. aureus* at later steps. For example, physicochemical changes in the cheese as a function of the process of ripening, as well as the production of organic acids and other compounds, might act synergistically to enhance the anti-bacterial properties of the nisin (Boussouel, Mathieu, Revol-Junelles, & Milliere, 2000; Chung & Hancock, 2000; Morgan, Bonnin, Mallereau, & Perrin, 2001; Pinto et al., 2009). On the other hand, other factors could decrease the efficacy of nisin during the ripening process, such as pH and the presence of nisin-resistant microbiota (Kramer et al., 2004; Martínez, Obeso, Rodríguez, & García, 2008).

Serro cheese can be allowed to ripen for as long as 60 days; nevertheless, it is also often consumed after only 7 d of ripening. Initial values of *S. aureus* count were 4.30, 4.29 and 4.33 for the control group, 100 and 500 IU of nisin, respectively. Based on Fig. 2, a reduction of 0.7 and 2.0 log cycles in *S. aureus* count can be verified after 7 d of ripening for cheese containing 100 IU mL⁻¹ and 500 IU mL⁻¹ of nisin, respectively.

The addition of nisin to raw milk Camembert cheese was shown to decrease the *L. monocytogenes* count by 5–6 log cycles (Maisnier-Patin, Deschamps, Tatini, & Richar, 1992). Similarly, ricotta containing nisin shows an inhibition of *Listeria* growth for more than 8 weeks (Davies, Bevis, & Delves-Broughton, 1997). *L. monocytogenes* count was reduced by 7 log cycles in cottage cheese containing nisin after 3 days of storage at 20 °C, whereas in the absence of nisin there was only a 1 log reduction after 7 d under the same conditions (Ferreira & Lund, 1996). Hamana et al. (2002) inoculated Jben cheese with 10³ cfu mL⁻¹ of *S. aureus*. When the cheeses were manufactured with starter containing the nisin-producing *Lactococcus*, *S. aureus* was not detected after 3 d of storage at 4 °C. However, when the starter was a non-nisin-producer, the presence of the pathogenic bacterium was confirmed in the cheese samples.

3.3. Effect of nisin on the physicochemical characteristics of Minas Traditional Serro cheese

We verified significant differences ($P < 0.05$) in the pH values of the cheese only as a function of the whole ripening period, analyzed

Table 1
Changes in the physicochemical characteristics of Serro cheese during the ripening period as a function of nisin concentration^a.

Days	Nisin (IU mL ⁻¹)	Moisture (%)	Fat (%)	pH	Chloride (%)	<i>a_w</i>
7	0	44.63 ^b	24.17 ^a	4.85 ^a	1.78 ^b	0.94 ^a
	100	44.35 ^b	23.67 ^a	4.78 ^a	2.03 ^a	0.93 ^a
	500	46.73 ^a	23.17 ^a	4.84 ^a	2.21 ^a	0.94 ^a
14	0	38.47 ^a	27.83 ^a	5.01 ^a	1.96 ^b	0.92 ^a
	100	37.97 ^a	27.67 ^a	5.08 ^a	2.18 ^b	0.92 ^a
	500	37.07 ^a	26.38 ^a	5.12 ^a	2.44 ^a	0.92 ^a
30	0	29.29 ^b	32.37 ^a	5.13 ^a	2.24 ^b	0.88 ^a
	100	31.25 ^a	30.40 ^b	5.17 ^a	2.21 ^b	0.89 ^a
	500	32.39 ^a	32.10 ^a	5.21 ^a	2.55 ^a	0.89 ^a
45	0	24.78 ^b	34.47 ^a	5.20 ^a	2.37 ^b	0.86 ^a
	100	28.34 ^a	34.33 ^a	5.16 ^a	2.43 ^b	0.86 ^a
	500	27.16 ^a	33.03 ^a	5.24 ^a	2.87 ^a	0.87 ^a
60	0	23.12 ^a	37.67 ^a	5.22 ^a	2.41 ^b	0.83 ^a
	100	22.50 ^a	36.23 ^a	5.28 ^a	2.34 ^b	0.83 ^a
	500	23.26 ^a	36.17 ^a	5.28 ^a	2.90 ^a	0.85 ^a

^a For each time point, in the columns, the averages followed by the same superscript letters did not differ significantly ($P \geq 0.05$) when compared using the Tukey test.

by regression, varying from 4.84 to 5.26. These values are in agreement with those usually found for Serro cheese (Martins, 2006). However, the pH changes occurred in all cheeses over the course of the 60 days of ripening and were probably not responsible for the difference in *S. aureus* count between nisin-treated and control cheese because there was no significant pH variation ($P \geq 0.05$) between samples at each period of time, during the ripening, as seen in Table 1, where data were analyzed by the Tukey test.

In a similar work, Kykkidou, Pournis, Kostoula, and Savvaidis (2007) found that the pH values of non-treated and nisin-treated Galotyri cheese were practically unchanged during 42 d of storage at 4 °C. However, in our cheese, the dose and activity of the natural starter could cause variations in the pH values. In the present study, natural starter doses were standardised to minimise interference with the cheese acidification of different treatments.

Minas Traditional Serro cheese is normally consumed after 7 d of ripening. An increase in the pH of all cheese samples was observed after 14 d of storage, which is related to proteolysis and the break-down of lactic acid by bacteria and yeasts. The pressing step also plays an important role in the final pH of the cheese. At this stage of processing the curd still contains a considerable amount of lactose, which should be appropriately reduced to avoid excessive fermentation and lactic acid production (Fox, McSweeney, Cogan, & Guinee, 2000).

An increase in chloride content was observed ($P < 0.05$) in cheeses of all treatments during the ripening due to moisture loss. Although the salt content was increased, the observed antimicrobial effect could be attributed to nisin and not to the salt, due to the fact that the water activity in the three treatments were similar ($P \geq 0.05$) after each period of time as shown in Table 1. Furthermore, after 14 days of ripening, there was no difference ($P \geq 0.05$) between the level of salt in cheese containing 0 and 100 IU mL⁻¹ of nisin and anyway the last treatment showed higher inhibition ($P < 0.05$) of *S. aureus* when compared with the control. *S. aureus* has shown a noteworthy resistance to salt (Jay, Loessner, & Golden, 2005). In addition, we believe that salt and nisin can act synergistically; Thomas and Wimpenny (1996) verified that salt content higher than 2% improves the bactericidal effect of nisin and that this effect is stronger at low temperatures. Many other studies report the use of nisin as a hurdle in cheese preservation (Pintado, Ferreira, & Sousa, 2010; Pires et al., 2008).

In Serro cheese, as well as in other traditional Brazilian cheeses, the salting step is carried out on the cheese surface and the salt diffuses rapidly, accelerating whey release from cheese.

Cheese moisture diminished ($P < 0.05$) during ripening. A slight variation ($P < 0.05$) in the moisture content was observed between differently treated cheeses on the 7th day and between the 30th and 45th day of ripening. After 7 days of ripening, the moisture content of the nisin-treated (500 IU mL⁻¹) sample was higher than that of the control and 100 IU mL⁻¹ nisin cheese. After 30 and 45 days, both nisin-treated cheeses had higher moisture contents than did the control. This result can be explained by the action of nisin on some bacteria responsible for cheese proteolysis. Because the growth of these bacteria was inhibited by nisin, more polypeptide chains are present in the cheese and more solvation water molecules are present around these large macromolecules.

The short time required for the manufacture of Serro cheese plays an important role in the high moisture content values at the beginning of ripening. The moisture content of this cheese is not significantly affected by pH during draining; however, the syneresis that occurs in this process is an important parameter because longer manufacture times lead to higher syneresis and lower moisture content in the final product (Kindstedt & Guo, 1997).

The fat content increased in all cheese samples ($P < 0.05$) during the ripening, due to moisture loss that causes an increase in the solids content in the cheese. The presence of nisin did not have an important effect on fat content. According to the Brazilian Technical Regulation of Cheese Identity, Serro cheese can be considered as semi-fat because its fat content varies between 25 and 44.9%.

The water activity (a_w) was reduced during ripening ($P < 0.05$) in all samples and a_w values were not affected ($P \geq 0.05$) by nisin treatment. The index of ripening and salt content, as well as moisture content, influence the a_w of cheese. The effect of the index of ripening is related to the presence of amino acids (due to proteolysis) with side chains containing polar groups that interact easily with water molecules and therefore reduce a_w . On the other hand, the effect of salt content is based on its low molecular weight and high solubility, which implies that there are strong interactions between NaCl and H₂O. Therefore, the chemical potential (μ) of water molecules is lower when the water molecules are interacting with NaCl than when they are interacting between themselves. In other words, a_w is reduced in the presence of NaCl (Equation (1)).

$$\mu_w = \mu_w^0 + RT \ln a_w \quad (1)$$

where μ_w is the chemical potential of water, μ_w^0 is the chemical potential of pure water at 25 °C and 1 atm, R is gas constant, T is temperature and a_w is water activity.

Even though the a_w values found in Serro cheese were under the minimum a_w required for pathogenic growth, a_w by itself is not enough to ensure cheese safety because its value can vary across the different micro-regions of the cheese (Beresford, Fitzsimons, Brennan, & Cogan, 2001).

3.4. Effect of nisin on the index and depth of ripening of Minas Traditional Serro cheese

The index of ripening increased over the 60 d ripening period for all of the cheeses tested ($P < 0.05$). Nevertheless, nisin-treated samples showed lower ($P < 0.05$) index and depth of ripening than the control cheese. Figs. 3 and 4 show the behaviour of index and depth, respectively, of ripening for the three treatments used in this study (0, 100 and 500 IU mL⁻¹).

The lower index of ripening in the nisin-treated cheese compared with control samples can be explained by the action of

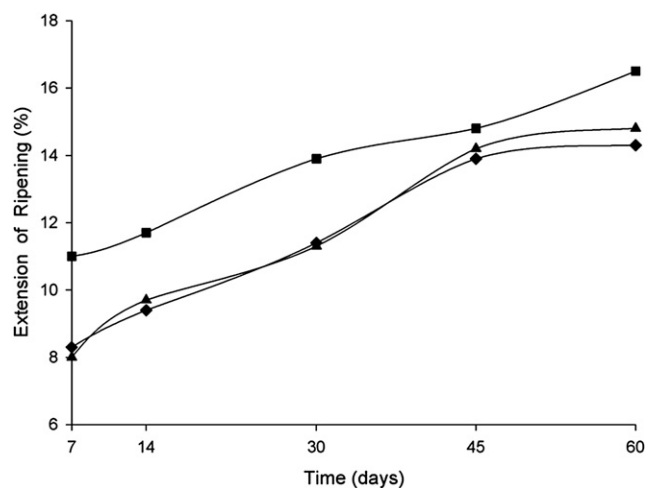


Fig. 3. Extension of ripening of Minas Traditional Serro cheese manufactured with different concentrations of nisin: (■) no nisin; (◆) 100 IU mL⁻¹; (▲) 500 IU mL⁻¹.

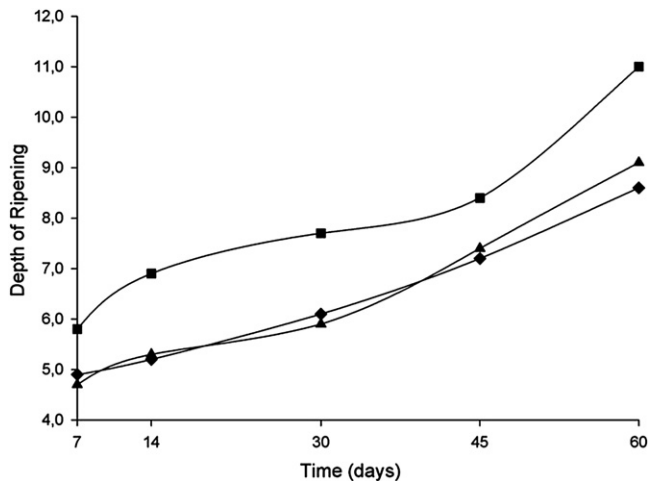


Fig. 4. Depth of ripening of Minas Traditional Serro cheese manufactured with different concentrations of nisin: (■) 0; (◆) 100 IU mL⁻¹; (▲) 500 IU mL⁻¹.

the bacteriocin. This damages the membranes of Gram-positive bacteria, thereby reducing the number of bacteria which are responsible for the proteolysis during the ripening (Montville & Chen, 1998).

3.5. Effect of nisin on the texture profile analysis and colour of Minas Traditional Serro cheese

Texture profile analysis (TPA) is an important tool for cheese characterisation. Serro cheese is a traditional product and, to the best of our knowledge, ours is the first published data providing the TPA parameters of this cheese. We believe that these results will contribute to a "Protected Designation of Origin" claim for Serro cheese. TPA is therefore a useful technique in this context (Foegeding & Drake, 2007).

Table 2 shows the TPA results at different times of ripening for each treatment. The correlation between time and treatment was significant ($P < 0.05$) for the parameters of firmness, fracturability, springiness, gumminess, chewiness and cohesiveness. On the other hand, adhesiveness did not vary over the course of ripening and was not affected by nisin treatment. The average adhesiveness values were -0.003 , -0.002 and -0.003 N cm⁻¹ for the control, 100 and 500 IU mL⁻¹ nisin-treated samples, respectively.

When different treatments were studied at each time point, no difference ($P \geq 0.05$) was observed between them, except in

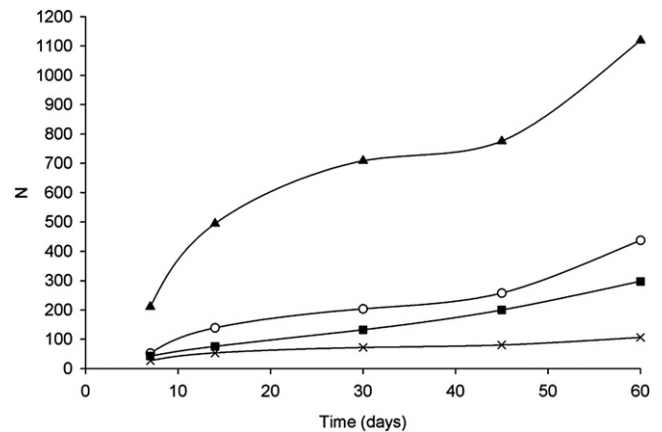


Fig. 5. Texture profile analysis parameters measured during the ripening of Minas Traditional Serro cheese: (○) Firmness, (■) Fracturability, (×) Gumminess, (▲) Chewiness.

relation to springiness. This parameter was higher inasmuch as ripening was raised, probably due to the residual action of the coagulant that is responsible for primary proteolysis (i.e., relaxes the protein chains and enhances cheese elasticity). Beginning at day 14 of ripening, the cheese containing 500 IU mL⁻¹ nisin showed a higher springiness compared with the other cheeses. The higher values of springiness observed in nisin cheese (500 IU mL⁻¹) can be explained by a synergistic result of coagulant action and antimicrobial effect of nisin against lactic acid bacteria (LAB). If the proteolytic LAB had grown strongly, the proteolytic action of these organisms would have resulted in smaller peptide fractions being formed and the protein network would have been weakened. Such a weak protein network would result in a reduction of springiness, differing from the primary proteolysis caused by residual action of rennet coagulant.

An increase of firmness, fracturability, gumminess and chewiness was also observed for all treatments as the ripening period progressed ($P < 0.05$). Fig. 5 demonstrates this behaviour for the average of three treated cheeses (Table 2). These parameters probably increased due to the decrease in cheese moisture during the ripening process (Dimitreli & Thomareis, 2007). Stampanoni and Noble (1991) also found similar results attributed to reduced moisture content.

According to Creamer and Olson (1986), Dimitreli and Thomareis (2007), and Marshall (1990), increasing the protein

Table 2
Texture Profile Analysis of Minas Traditional Serro cheese manufactured with different doses of nisin, during ripening.^a

Ripening (days)	Nisin dose	Firmness	Fracturability	Gumminess	Cohesiveness	Springiness	Chewiness
7	0	47.59 ^a	31.93 ^a	26.76 ^a	0.67 ^a	6.39 ^a	181.01 ^a
	100	53.83 ^a	59.44 ^a	31.07 ^a	0.60 ^a	6.51 ^a	210.50 ^a
	500	57.44 ^a	36.78 ^a	24.24 ^a	0.64 ^a	6.44 ^a	242.48 ^a
14	0	135.04 ^a	44.91 ^a	53.29 ^a	0.68 ^a	6.07 ^b	432.97 ^a
	100	132.29 ^a	87.55 ^a	48.71 ^a	0.66 ^a	6.17 ^b	487.16 ^a
	500	139.02 ^a	95.47 ^a	59.52 ^a	0.66 ^a	7.37 ^a	563.29 ^a
30	0	192.28 ^a	115.61 ^a	68.35 ^a	0.67 ^a	5.86 ^b	644.28 ^a
	100	217.94 ^a	146.97 ^a	71.47 ^a	0.66 ^a	6.49 ^b	714.86 ^a
	500	201.21 ^a	134.99 ^a	76.70 ^a	0.67 ^a	7.48 ^a	767.12 ^a
45	0	243.76 ^a	186.36 ^a	72.82 ^a	0.71 ^a	6.57 ^b	708.47 ^a
	100	256.95 ^a	180.97 ^a	84.42 ^a	0.71 ^a	7.13 ^a	780.79 ^a
	500	272.33 ^a	232.62 ^a	84.71 ^a	0.70 ^a	7.54 ^a	836.76 ^a
60	0	453.78 ^a	276.85 ^a	98.15 ^a	0.70 ^a	6.69 ^a	1116.45 ^a
	100	423.76 ^a	300.52 ^a	104.57 ^a	0.70 ^a	7.17 ^a	1073.68 ^a
	500	434.96 ^a	315.76 ^a	116.61 ^a	0.72 ^a	7.52 ^a	1166.33 ^a

^a For each time point, in the columns, averages followed by the same letters did not differ significantly ($P \geq 0.05$) when compared using the Tukey test.

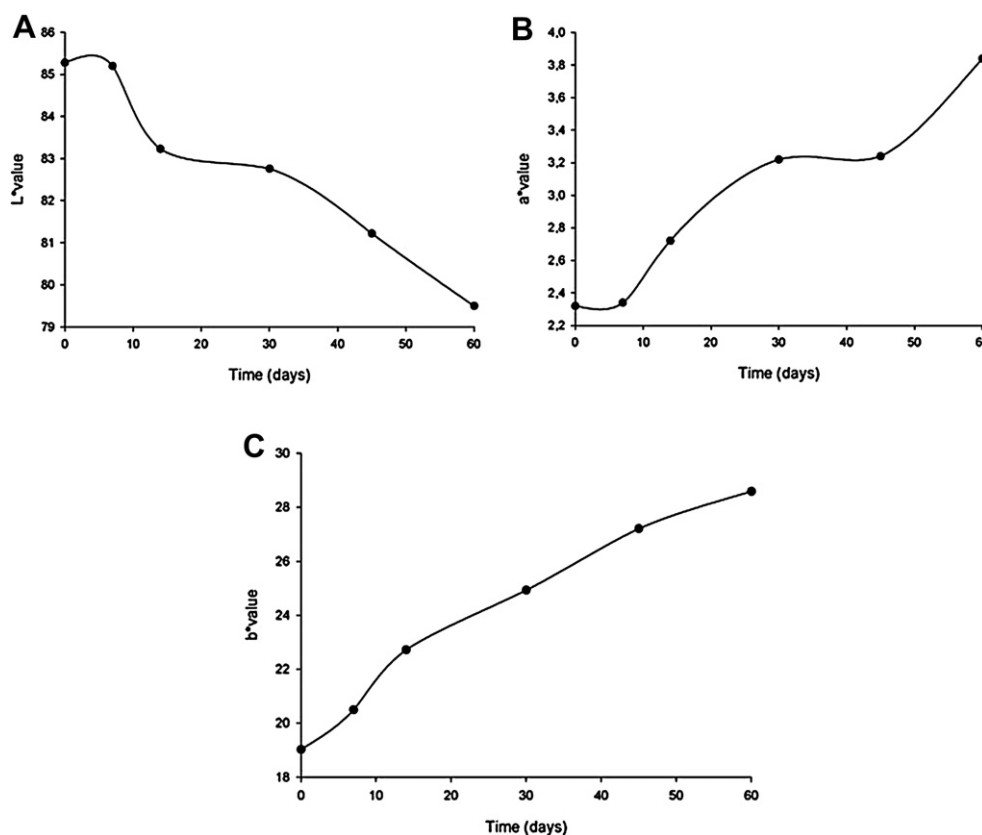


Fig. 6. Colour changes (L^* , a^* and b^* parameters) in Minas Traditional Serro cheese over 60 days of ripening.

content of cheese allows better salt penetration into the protein matrix of the cheese. The resulting increase in firmness, which is related to a_w , pH, moisture and salt content, caused an increase in fracturability (Buffa, Trujillo, Pavia, & Guamis, 2001; Fernandez Del Pozo et al., 1985; Lawrence, Creamer, & Gilles, 1987).

Colour analysis is another simple but important tool to characterise cheeses, especially those that are manufactured artisanally. No influence of nisin treatment on the colour of the cheese ($P \geq 0.05$) could be detected; however, this parameter was strongly affected by the length of the ripening period (Fig. 6). A decrease in the L^* value is clearly observed over the course of ripening, indicating that the lightness of the sample decreased during ripening, and an increase in a^* and b^* parameters demonstrates the development of red and yellow colour, respectively, as the cheese aged. Our results were similar to previous reports of cheese colour as a function of the ripening period (Buffa et al., 2001). Age-related changes in cheese colour have previously been attributed to changes in protein hydration during ripening, which alters the amount of free moisture and thus the light-scattering properties of the cheese matrix (Paulson, McMahon, & Oberg, 1998).

4. Conclusions

Nisin proved to be an efficient antimicrobial agent against *S. aureus* in Minas Traditional Serro cheese prior to the step of milk coagulation, resulting in the inhibition of growth of *S. aureus* in the cheese.

The use of nisin does not imply that acceptable levels of *S. aureus* in raw cheese milk have been attained, because bacteriocin efficacy depends on the initial contamination of the milk as well as cheese handling practices. Nevertheless, the addition of nisin to raw milk containing low counts of *S. aureus* allows the manufacture of safer Serro cheese.

The addition of nisin during the Serro cheese manufacturing process resulted in a lower index of ripening. However, Brazilian consumers generally demand fresh cheese, which implies that a lower index of ripening probably will not diminish the acceptability of Serro cheese. In addition, nisin did not cause significant changes in the physicochemical and mechanical characteristics of the cheese.

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