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RIPENED MINAS CHEESE WITH *ENTEROCOCCUS FAECIUM* EF1, *LACTOBACILLUS HELVETICUS* LH13 AND TURMERIC (*CURCUMA LONGA* L.)

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ABSTRACT

Background. Ripened cheeses undergo maturation periods during which biochemical changes occur, resulting in specific sensory characteristics in the final product. One crucial aspect of these changes is proteolytic reactions. The introduction of proteolytic microorganisms can significantly shorten the maturation time. Furthermore, probiotic microorganisms, due to their proteolytic activity, can contribute to both the technological process and the health effects of cheese production, providing consumers with added benefits. Turmeric, known for its phenolic pigments, which are responsible for its yellow color, can be added to foods to enhance their antioxidant activity. Proteolytic and probiotic microorganisms can reduce the maturation time of ripened cheeses. These microorganisms can also be used to assess how turmeric affects the sensory characteristics and health benefits of the cheeses. This study explored the enhancement of Minas cheese, cured for 60 days, by incorporating turmeric extract into the milk. The addition of *E. faecium* and *L. helveticus* was intended to improve the cheese's nutritional, sensory, and physicochemical properties.

Material and methods. Evaluation of the effects of adding *E. faecium*, *L. helveticus* and turmeric extract to ripened Minas cheese for up to 60 days. Three treatments were performed: cheese with the addition of starter culture only (Control), cheese with added *E. faecium* and *L. helveticus* (T1); and cheese with added *E. faecium*, *L. helveticus* and turmeric extract (T2). During maturation, analysis of centesimal composition, pH, titratable acidity, microbiological counts of viable cells, curcumin content, total phenolics, antioxidant activity (DPPH, ABTS+·and FRAP), extent and depth of proteolysis indexes (EPI and DPI), separation of polypeptides by molecular mass using SDS-PAGE and sensory analysis were carried out.

Results. There were differences in the centesimal composition parameters for all treatments during maturation. There was a reduction in pH values during maturation, reaching 4.7 to 4.8 after 14 days. The titratable acidity showed a progressive increase during maturation, reaching up to 1.3 g lactic acid·100 g^{-1} of cheese. The cheeses had *E. faecium* counts between 7 and 8 log CFU·g^{-1} during the entire ripening period. For total phenolics, T2 cheeses showed 174.29 ±0.46 mg GAE·100 g^{-1}, a significant increase ($p \leq 0.05$) when compared to the other treatments. Treatment T2 showed an increase in curcumin content during maturation, reaching 0.01 ±0.00 g·100 g^{-1} from 45 days with a significant increase ($p \leq 0.05$) in antioxidant activity. The proteolysis extension index of the three treatments was 12% at 60 days. The depth index showed values of 7.78% for the T1 treatment and 6.78% for the T1 and T2 treatments at 60 days. The electrophoretic profiles

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by SDS-PAGE of the three treatments, after 30 days, showed similarities. For sensory acceptance, the control and T1 treatments presented global acceptance scores between 7 and 8, and the T2 treatment between 6 and 7. **Conclusion.** The findings suggest that the ripened Minas cheese received satisfactory acceptance from potential consumers and could be a beneficial addition to the diet. When consumed regularly as part of a balanced diet and combined with exercise, it could enhance health. The addition of turmeric increased phenolic content and antioxidant activity, which remained at satisfactory levels after 60 days.

Keywords: antioxidant, curcumin, fermentation, probiotic, proteolysis

INTRODUCTION

The increasing demand for healthy foods is driving innovation and the development of new products in the global food industry. Food can be classified as functional if it is proven to have positive effects on specific physiological functions, in addition to providing necessary nutrients, thereby contributing to well-being, health promotion, and disease risk reduction (Cowan et al., 2023; Chaudhary et al., 2023).

Cheese is a protein-fat concentrate formed by milk coagulation. Its relatively high fat content protects probiotic bacteria as they pass through the gastrointestinal tract. Cheeses with high water content, low salt levels, and no preservatives create optimal conditions for probiotic strain survival and growth (Abdeen et al., 2024). The human gut microbiota plays an important role in maintaining health, and dietary supplementation with probiotics helps maintain a balanced intestinal tract, thereby preventing potentially harmful microorganisms from proliferating and causing negative effects (Ziemer and Gibson, 1998; Saad, 2006).

Probiotics are microorganisms that, when ingested regularly, offer various benefits to the consumer and are commonly found in fermented dairy products such as yogurt and cheese (Gao et al., 2021). The associated benefits, once consumed regularly, are in competition with pathogens for nutrients; adhesion and colonization of the surface of the intestinal mucosa; reduction of episodes of diarrhea; constipation relief; stimulation of the immune system; decreased risk of cardiovascular disease; lowering of serum cholesterol levels and antihypertensive effects (Roselino et al., 2012; Celiberto, 2014; Moraes Filho et al., 2019; Gao et al., 2021).

Enterococcus faecium is extensively employed both as a primary starter culture and adjunct culture in the crafting of traditional European cheeses. Its proteolytic activity accelerates maturation and the production of aromatic compounds, enhancing the sensory quality of mature cheeses (Moreno et al., 2006). Concerns arise regarding the safety of employing *E. faecium* in food production due to the presence of resistance genes. There is evidence indicating that these genes can potentially be transferred to other microorganisms when incorporated into food (Emaneini et al. 2008). *Lactobacillus helveticus* is a proteolytic microorganism used in the production of long-lasting matured cheeses such as parmesan (Furtado, 2011). Its proteolytic system consists of envelope proteinase cells that degrade proteins to oligopeptides, which are subsequently taken up by cells through a transport system specifically for further degradation into smaller peptides or amino acids. This hydrolysis of casein into peptides and free amino acids is important in the ripening process of cheeses, and *L. helveticus* has been shown to contribute to the acceleration of the maturation and reduction of the bitterness of aged cheeses (Sahingil et al., 2014).

Turmeric (*Curcuma longa* L.), widely used as a dye and condiment, contains phenolic pigments responsible for its yellow color (including curcumin), called curcuminoid pigments, which have several properties and beneficial effects, such as antioxidant, anti-inflammatory, antimicrobial, regulatory biliary and cholesterol-lowering functions (Odimegwu et al., 2019; Sahoo et al., 2021).

Considering the health benefits and sensory properties of turmeric, this study proposed the addition of turmeric extract to milk in the preparation of Minas cheese, cured for 60 days. The incorporation of *E. faecium* and *L. helveticus* was intended to enhance

the cheese's nutritional, sensory, and physicochemical properties.

MATERIAL AND METHODS

For the treatment of the cheeses, refrigerated pasteurized milk (HTST) was used and obtained in the region of Londrina/PR. The commercial fungal liquid coagulant used (*Aspergillus niger* var. *awamori*) was from the Estrella® brand, kindly supplied by company CHR-Hansen (Valinhos/SP). Turmeric powder from Kitano® was used. The commercial strains used were pure and in lyophilized form, composed of *E. faecium* EF 1, *L. helveticus* LH 13 and starter culture MW 031 R, mesophilic and homofermentative, composed of *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis* biovar. diacetylactis and *Leuconostoc* spp. All were supplied by Sacco® (Campinas/SP and Italy). The electrophoretic standard used was Precision Plus ProteinsTM (Bio-Rad®).

Inoculum preparation

Stock cultures were prepared with the strains of *E. faecium* and *L. helveticus*, from the lyophilized culture, where 0.1% (w/v) were aseptically added to a medium composed of skimmed milk powder reconstituted at 10% (w/v) and supplemented with 15% (v/v) glycerol (previously sterilized and cooled to 37°C) before being stored at –20°C until the moment of use. Before use, the stock cultures were activated by transferring 1.0% (v/v) of stock cultures for skimmed milk powder, reconstituted at 10% (w/v), previously sterilized, and incubated at 37°C for 22 to 24 hours. The reactivation of the cultures occurred through two successive transfers. After incubation, aliquots were withdrawn to determine the colony count. The inoculum in milk used for cheese consisted of 1.0% (v/v) of *E. faecium* culture and 0.5% (v/v) of *L. helveticus*. For the starter culture, direct vat inoculation of 0.025 $g \cdot L^{-1}$ was used.

Turmeric extract

Turmeric extract was obtained according to the methodology described by Licón et al. (2012), where 0.05% (w/v) of ground turmeric powder was added to one liter of pasteurized milk and kept under agitation (50°C/10 minutes), followed by filtration.

Ripened Minas cheese production

The treated cheeses and their respective treatments are described in Table 1. The microorganisms used and curcumin extract varied with the treatments.

Table 1. Treatments for the production of ripened Minas cheese

Treatment	Starter culture*	*E. faecium*	*L. helveticus*	Turmeric extract
Control	+	–	–	–
T1	–	+	+	–
T2	–	+	+	+

**Lactococcus lactis* ssp. *lactis*, *Lac. lactis* ssp. *cremoris*, *Lac. lactis* ssp. biovar. *diacetylactis* and *Leuconostoc* spp.;
+ = addition; – = no addition.

The cheeses were produced following the procedures described by Furtado (2005). Pasteurized milk was heated to a temperature of 35°C and added to a solution of $CaCl_2$ 50% (w/v) in the proportion of 4 mL for every 10 liters of milk. Lactic cultures were added in the proportion 0.0025% (w/v) for the starter culture, 1.0% (v/v) for *E. faecium*, and 0.5% (v/v) for *L. helveticus*, and left for 40 minutes. Subsequently, 1.5% (w/v) of salt, 1.0 liter of extract of turmeric for the T2 treatment, and the liquid coagulant (in the proportion specified by the manufacturer) were added. The coagulation time was 50 minutes and the curd was cut with stainless steel lyres, guaranteeing grains of 0.8 cm on the edge. After cutting, the clot rested for 5 minutes, and stirring was then carried out until a time of 40 minutes. Soon thereafter, the draining process and the pre-pressed mass were carried out for 15 minutes.

Centesimal composition

The evaluation of the centesimal composition was performed according to methodologies described by Instituto Adolfo Lutz (2008) and AOAC (1995). To calculate the yield, the cheeses were weighed after the 24-hour draining period, calculated by the equation below:

$$\text{Yield} = \frac{V}{W} \quad (1)$$

where:

V = volume (liters of milk)

W = cheese weight (after 24 hours).

Microbiological analysis

For microbiological analysis, serial dilutions were prepared with 25 g samples. For the total lactic bacteria count, which included *L. helveticus* and non-starter lactic bacteria, MRS agar was used, with the addition of acetic acid (pH 5.4), and the samples were incubated under anaerobiosis, using an anaerobic generator (Probac®), according to the procedure described by Thierry et al. (1998), with modifications. The *E. faecium* count was carried out according to the International Dairy Federation – IDF (1991), using a KF Streptococcus agar selective medium, supplemented with triphenyl tetrazolium chloride at 1.0% (w/v) in the amount of 10 mL/L. For the count of total lactic acid bacteria in the control sample, with starter culture added, M17 agar and aerobic incubation were used, as described by Licón et al. (2012). The samples were incubated for 48 to 72 hours at 37°C.

Determination of curcumin content

The curcumin content in ground turmeric and cheeses was determined using a colorimetric method with a UV/VIS spectrophotometer (model Libra S22, Biochrom, Cambridge, UK), following the methodology of the Instituto Adolfo Lutz (2008). Approximately 0.1 g of the sample was extracted with 30 ml of absolute ethyl alcohol at 50°C for two and a half hours, filtered, and diluted in a 100 ml volumetric flask. Subsequently, 20 mL of this extract were added to another 250 mL volumetric flask, filled to volume, and read at 425 nm. The curcumin content is expressed in g/100 g of dry sample.

Total Phenolics and Antioxidant Capacity (DPPH, ABTS and FRAP)

Extraction followed the method described by Hung et al. (2009) using 80% ethanol. For the reaction of total phenolics, the methodology described by Swain and Hills (1959) with Folin-Ciocalteau reagent (Sigma-Aldrich) was used. Quantification was performed using a standard curve of gallic acid (0.1–0.5 mM) and the results were expressed in mg equivalents of gallic acid per 100 g of sample on a dry basis (mg EAG·100 g^{-1}).

The evaluation of the antioxidant capacity of the extracts in capturing the DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich) was evaluated following the method described by Brand-Williams et al. (1995). The methodology described by Sánchez-González et al. (2005) was used to evaluate the antioxidant capacity by scavenging the ABTS+• radical cation (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma-Aldrich). In both cases, quantification was performed using a Trolox standard curve (6-hydroxy-2v,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed in µmol Trolox per g of sample on a dry basis (100–1000 µmol Trolox. g^{-1}).

The FRAP method (Ferric Reducing Antioxidant Power), was evaluated according to the method of Rufino et al. (2006). A ferrous sulfate calibration curve (500–1500 µM) was used to quantify the antioxidant activity and the results were expressed in µmol of ferrous sulfate per g of sample on a dry basis (µmol ferrous sulfate·g^{-1}).

Extent and depth of proteolysis indexes (EPI and DPI)

The extension and depth indexes were evaluated by the precipitation method, using hydrochloric acid 1.41 mol/L (v/v) and trichloroacetic acid (TCA) 12% (w/v), respectively, and nitrogen was determined by the method of Kjeldahl, as described by the AOAC (1995). The extent of the proteolysis index was calculated, as shown in Equation 1 (Wolschoon-Pombo, 1983). The proteolysis depth index was calculated as shown in Equation 2 (Wolfschoon-Pombo, 1983).

$$\text{Extension of proteolysis} = \frac{\text{soluble nitrogen in pH 4.6}}{\text{\% Total Nitrogen}} \times 100 \quad (2)$$

$$\text{Depth of proteolysis} = \frac{\text{\% Soluble nitrogen in TCA 12\%}}{\text{\% Total Nitrogen}} \times 100 \quad (3)$$

Separation of polypeptides by molecular mass using SDS-PAGE

The SDS-PAGE was performed using sodium dodecyl sulfate polyacrylamide gel, containing 20%

acrylamide, described by Laemmli (1970), with modifications. Cheese samples were diluted in distilled water, submitted to the sonicator for 10 minutes and underwent constant agitation for 24 hours at 18°C. Then, they were diluted in Laemmli buffer containing 12.5% Tris-HCl buffer (Bio-Rad®) and 5% *β*-mercaptoethanol, pH 6.8, and boiled for 5 minutes before application. The Precision Plus ProteinsTM standard (Bio-Rad®) from 250 kDa to 10 kDa was used. The gel was run at 200 V, 10 mA, for 90 minutes and then stained with Coomassie® Brilliant Blue G-250 (Vetec®). After overnight staining, the gels were visualized using the Transiluminator L-PIX-HE equipment with an image capture system (Loccus® biotechnology) and L-PIX Image software.

Sensory analysis

The acceptance test was conducted using the procedure described by Moraes Filho et al. (2019). The cheeses were evaluated after 20 days of maturation. The judges (98 judges) evaluated the product's color, taste, aroma, texture, appearance and general acceptance using a hedonic scale of 9 points. All samples were presented individually, coded and randomized. The project was approved by the Research Ethics Committee (No. 1.036.296). During the analysis, the terms of free consent were collected for participation.

Statistical analysis

The experiment was conducted in triplicate and all results were submitted to analysis of variance (One-Way ANOVA) and the Tukey mean comparison test ($p<0.05$), using the Statistica 10.0 software (Statsoft Inc., 2011).

RESULTS AND DISCUSSION

Centesimal composition

The control and the treatments T1 and T2 presented, respectively, yields of 6.40, 6.03 and 5.99 liters of milk / kg of cheese. According to Furtado (2005), the average yield of Minas cheese is 7.5 to 8.5 liters of milk per kg of cheese matured for at least 20 days and with humidity between 46% and 49% and may vary with the milk composition and cheese moisture content, among other things.

The average results of the centesimal composition of the control, T1 and T2 on days 0, 7, 14, 30, 45 and 60 of the maturation period are shown in Table 2. There was no difference in protein content ($p > 0.05$) between treatments on the 14th day of maturation. Martins (2014) determined the protein levels in cheese cured for 45 days and found 22.50 ±1.43%, close to the values found in this study. According to Perry (2004), cheese with 48% fat in dry matter (FDM) contains between 23% and 25% protein. There were no significant differences in the moisture parameter up to 7 maturation days for the three treatments studied. It decreased with maturation time, stabilizing around 44 to 47% moisture. This is because moisture loss is a common and intrinsic phenomenon during cheese maturation, influenced by various process parameters (Ströher et al., 2020).

The salt levels were lower at time 0, but after 7 days of drying, there was no statistical difference in salt levels among all the cheeses. According to Furtado (2011), the salt content naturally increases during maturation due to the dehydration of the cheeses. In this study, the salt content for the control, T1 and T2, with 30 days of maturation, was 1.13 ±0.05, 1.28 ±0.26 and 1.14 ±0.09 g·100 g^{-1}, respectively.

Treatment T1 exhibited significantly lower FDM content ($p < 0.05$) than the other treatments throughout the maturation period. In the parameter fat, it was observed that the control did not present significant differences from other treatments within 14 days. This occurred due to the composition of the milk used to produce the control cheeses, which presented higher total nitrogen and lower fat content than the milk used to produce the T1 and T2 cheeses. The ash content showed a constant increase during the maturation period, with values close to those found by Martins (2014), who obtained 2.91 ±0.02 % ash (w/w). However, after 7 days of maturation, the T2 treatment showed significantly lower values ($p < 0.05$) than the other treatments. This difference might be due to specific microbial activity or enzymatic processes that occurred during maturation under T2 conditions. Certain microbial strains or enzymes in the T2 treatment could have caused a more efficient breakdown of organic matter, leading to a lower ash content compared to the other treatments.

Table 2. Physico-chemical parameters of samples of ripened Minas cheese during the maturation period, at 0, 7, 14, 30, 45 and 60 days

Parameters ($g \cdot 100\ g^{-1}$)	Treatments	Maturation period (days)					
		0	7	14	30	45	60
Protein	C	25.15 ±1.26^{Aa}	25.28 ±1.13^{Aa}	24.27 ±2.04^{Aa}	24.36 ±0.23^{Aa}	23.84 ±0.68^{Aa}	23.71 ±0.81^{Aa}
	T1	22.73 ±1.37^{ABa}	21.95 ±0.67^{Ba}	22.18 ±2.13^{Aa}	21.49 ±0.18^{Ba}	21.49 ±0.17^{Ba}	21.49 ±0.18^{Ba}
	T2	21.88 ±1.19^{Ba}	21.78 ±1.14^{Ba}	21.98 ±1.85^{Aa}	21.50 ±1.14^{Ba}	21.28 ±1.45^{Ba}	21.51 ±1.82^{Ba}
Moisture	C	49.22 ±0.99^{Aab}	49.80 ±0.12^{Aa}	48.42 ±0.11^{Babc}	47.64 ±0.16^{Abc}	47.25 ±1.11^{Abc}	46.72 ±0.83^{Ac}
	T1	51.25 ±0.39^{Aa}	49.07 ±0.65^{Ab}	48.74 ±0.11^{Bb}	46.04 ±1.40^{ABc}	44.70 ±0.84^{Ac}	43.86 ±0.98^{Bc}
	T2	50.41 ±0.93^{Aa}	49.87 ±0.07^{Aa}	50.00 ±0.70^{Aa}	45.08 ±0.60^{Bb}	44.55 ±1.72^{Ab}	45.39 ±1.03^{ABb}
Salt	C	0.75 ±0.03^{Bb}	0.99 ±0.06^{Aa}	1.10 ±0.08^{Aa}	1.13 ±0.05^{Aa}	1.16 ±0.08^{ABa}	1.13 ±1.00^{Aa}
	T1	1.05 ±0.02^{Aa}	1.05 ±0.04^{Aa}	1.26 ±0.10^{Aa}	1.28 ±0.26^{Aa}	1.36 ±0.13^{Aa}	1.31 ±0.16^{Aa}
	T2	1.12 ±0.05^{Ab}	1.05 ±0.07^{Ab}	1.28 ±0.02^{Aa}	1.14 ±0.09^{Aab}	1.06 ±0.02^{Bb}	1.13 ±0.08^{Aab}
Fat	C	21.17 ±0.29^{Bc}	21.83 ±0.29^{Bc}	24.00 ±1.00^{Ab}	24.33 ±0.58^{Bb}	25.17 ±0.29^{Bab}	26.67 ±0.58^{Ba}
	T1	24.50 ±0.00^{Ac}	24.53 ±0.06^{Ac}	25.33 ±0.58^{Ac}	29.00 ±0.00^{Ab}	30.00 ±1.00^{Aab}	31.00 ±1.00^{Aa}
	T2	24.50 ±0.00^{Ab}	24.50 ±0.00^{Ab}	24.10 ±0.17^{Ab}	30.00 ±1.73^{Aa}	31.50 ±0.87^{Aa}	30.33 ±1.15^{Aa}
FDM	C	41.68 ±0.57^{Cd}	43.49 ±0.58^{Bcd}	46.53 ±1.94^{Bb}	46.47 ±1.10^{Bbc}	47.71 ±0.55^{Bab}	50.05 ±1.08^{Ba}
	T1	50.26 ±0.00^{Ab}	48.17 ±0.11^{Ab}	49.05 ±1.12^{ABb}	53.74 ±0.00^{Aa}	54.25 ±1.81^{Aa}	55.22 ±1.78^{Aa}
	T2	49.40 ±0.00^{Bc}	48.87 ±0.00^{Ac}	50.20 ±0.35^{Abc}	54.63 ±3.15^{Aab}	56.81 ±1.56^{Aa}	55.54 ±2.11^{Aa}
Ash	C	2.60 ±0.02^{Ae}	2.79 ±0.02^{Ad}	2.91 ±0.02^{Abc}	3.03 ±0.06^{Aab}	3.08 ±0.08^{Aa}	2.81 ±0.03^{ABcd}
	T1	2.49 ±0.17^{Ab}	2.57 ±0.03^{Bb}	2.89 ±0.05^{Aa}	2.90 ±0.05^{Aa}	2.90 ±0.04^{Aa}	2.87 ±0.05^{Aa}
	T2	2.44 ±0.03^{Ab}	2.50 ±0.02^{Cab}	2.70 ±0.06^{Ba}	2.62 ±0.01^{Bab}	2.60 ±0.05^{Bab}	2.67 ±0.09^{Ba}

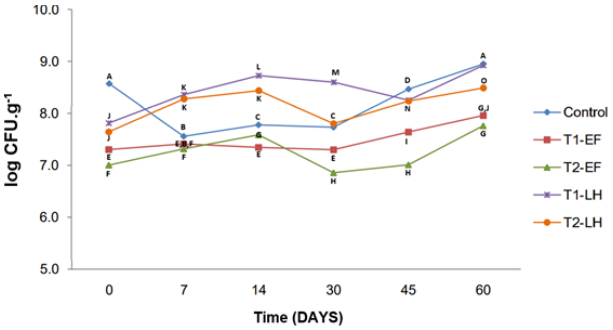
Mean ±standard deviation (n = 3).
Different capital letters in the same column indicate a significant difference (Tukey test $p \leq 0.05$); different lowercase letters on the same line indicate a significant difference (Tukey's test $p \leq 0.05$).
*FDM – fat in dry matter; C: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*; T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

Viable cell count, pH and titratable acidity

Viable *E. faecium* cells and total lactic acid bacteria in samples at 0 (the day after production), 7, 14, 30, 45 and 60 days of maturation are presented in Figure 1.

The initial counts of the control cheeses were 8.57 ±0.01 log $CFU \cdot g^{-1}$, and during maturation there was a significant decrease in the cell count to around 7.5 log $CFU.g^{-1}$. However, at the end of the maturation period, there was growth, reaching counts of 8.95 ±0.04 log $CFU \cdot g^{-1}$. The inoculum, consisting of 1.0% (v/v) of *E. faecium* culture, presented counts of around 8 log $CFU \cdot mL^{-1}$ of milk. When it was added to milk for cheese production, there was a dilution effect, and the initial treatment counts decreased by approximately 1 log cycle at time 0 (the day after production) to 7.30 ±0.23 log $CFU \cdot g^{-1}$ in treatment T1 and 7.00 ±0.07 log $CFU \cdot g^{-1}$ in treatment T2.

During maturation, the *E. faecium* counts of the T1 treatment remained constant until 30 days of maturation, presenting values between 7.0 and 7.5 log $CFU \cdot g^{-1}$. After 30 days, there was an increase in the counts, reaching 7.96 ±0.05 log $CFU \cdot g^{-1}$ with 60 days



Mean ±standard deviation (n = 3); Control: cheese with starter culture MW 031 R; T1: cheese with cultures from *L. helveticus* and *E. faecium*; T2: cheese with cultures of *L. helveticus* and *E. faecium* and extract of turmeric; Control: Count of total lactic acid bacteria capable of development in M17 agar; EF: Viable cell count of *E. faecium* in KF Streptococcus agar; LH: Total lactic acid bacteria count on acidified MRS agar, which includes *L. helveticus* and non-starter lactic acid bacteria. Different letters indicate a significant difference (Tukey's test, $p < 0.05$).

Fig. 1. Counts of viable *E. faecium* and total lactic acid bacteria in cheese samples at 0, 7, 14, 30, 45 and 60 days of maturation

of maturation. The T2 treatment showed a constant increase in counts up to 14 days, reaching values between 7.2 and 7.5 log UFC·g^{-1} and thereafter, counts decreased significantly to 6.86 ±0.05 log CFU·g^{-1}. However, at 60 days of maturation, there was a further increase in the count, which reached 7.76 ±0.07 log CFU·g^{-1}. The differences in *E. faecium* counts observed between the treatments can be attributed to the specific conditions and parameters associated with each treatment. In the case of the T1 treatment, the constant count of *E. faecium* up to 30 days of maturation, followed by a subsequent increase, suggests a gradual adaptation and proliferation of this bacterial strain over time. This could be due to factors such as the availability of nutrients, pH levels, and moisture content within the cheese matrix, which might have provided favorable conditions for the growth of *E. faecium*. On average, the decrease in moisture and increase in salt in cheeses may have favored the increase in *E. faecium* counts. According to Tzanetakis et al. (1995), during the maturation of Feta cheese, the increasing salt concentration gradually replaces the naturally predominant lactic acid bacteria (starter cultures) with more salt-resistant bacteria, such as *Lactobacillus (Lactiplantbacillus plantarum*) and *Enterococcus* (*E. faecium* and *E. durans*).

In this study, *E. faecium* viable counts remained between 10^7 and 10^9 CFU·g^{-1} throughout the maturation period per portion of cheese (30 grams), which provides high counts of the microorganism, with respectively 2.7 × 10^9 and 1.7 × 10^9 CFU per portion after 60 days of maturation. These high counts are beneficial for the cheese's technological properties.

The inoculum, consisting of 0.5% (v/v) of *L. helveticus*, presented counts above 9 log CFU·mL^{-1} in the milk. When added from milk production to cheese production, the same dilution effect was observed in counts of *E. faecium*, with a decrease of more than 1 logarithmic cycle. The initial count of the T1 treatment was 7.81 ±0.15 log CFU·g^{-1} and the initial count of the T2 treatment was 7.65 ±0.05 log CFU·g^{-1}, without significant differences ($p > 0.05$).

At 14 days of maturation, the treatments (T1-LH and T2-LH) increased by approximately 1 log cycle each. With 30 days of maturation, a decrease in the T2 treatment count of about 0.7 log cycles was observed; however, it soon began to multiply and, at 60 days of maturation, presented final counts of 8.49 ±0.02 log CFU·g^{-1} of cheese. A decline in the T1 treatment count of 0.5 log cycles was also observed, followed by an increase to 8.92 ±0.06 log CFU·g^{-1} at 60 days of maturation. The changes in microbial counts during cheese maturation are due to factors such as environmental changes (pH, moisture, nutrients), microbial competition, microbial adaptation to new conditions, and the metabolic activity of the microorganisms. Initially, the conditions favor microbial growth, but subsequent changes can cause temporary declines. Over time, microorganisms adapt and recover their growth, resulting in the final observed counts.

Table 3 shows the values of pH and titratable acidity of the control and treatments T1 and T2, at 0, 14, 30, 45 and 60 maturation days. There was a pH decrease in the three treatments throughout the maturation period, with average values of 4.73 to 4.82 after 14 days. Furtado (2005) mentions that Minas Padrão cheese matured for at least 20 days must present a pH between 5.0 and 5.1 and a humidity between 46% to 49%. Candioti et al. (2002) found pH values between 5.15 and 5.17 in cheeses without packaging with added *L. helveticus*, with a humidity of 35% to 36% at 180 days of maturation. In this work, the initial titratable acidity was between 0.27 to 0.39 g lactic acid·100 g^{-1}, values

Table 3. Values of pH and titratable acidity at 0, 14, 30, 45 and 60 days of maturation

Parameter	Treatments	Maturation time (days)					
		0	7	14	30	45	60
pH	C	4.77 ±0.08^{Ba}	4.86 ±0.04^{Ba}	4.73 ±0.04^{Aa}	4.73 ±0.04^{Aa}	4.64 ±0.20^{Aa}	4.65 ±0.22^{Aa}
	T1	5.05 ±0.08^{Aa}	5.10 ±0.01^{Aa}	4.82 ±0.06^{Aab}	4.82 ±0.06^{Aab}	4.63 ±0.19^{Ab}	4.67 ±0.22^{Ab}
	T2	4.72 ±0.15^{Ba}	5.00 ±0.08^{Aa}	4.79 ±0.06^{Aa}	4.79 ±0.06^{Aa}	4.64 ±0.21^{Aa}	4.72 ±0.19^{Aa}
Titratable acidity	C	0.39 ±0.00^{Ac}	0.85 ±0.25^{Ab}	0.90 ±0.07^{Ab}	1.29 ±0.09^{Aa}	1.06 ±0.12^{Aab}	0.94 ±0.10^{Aab}
	T1	0.27 ±0.01^{Ac}	0.83 ±0.01^{Ab}	0.98 ±0.06^{Ab}	1.28 ±0.14^{Aa}	0.99 ±0.18^{Ab}	0.96 ±0.01^{Ab}
	T2	0.31 ±0.01^{Ad}	0.81 ±0.08^{Ac}	1.17 ±0.11^{Aab}	1.27 ±0.05^{Aa}	1.10 ±0.13^{Aab}	0.95 ±0.07^{Abc}

Mean ±standard deviation (n = 3).
Different capital letters in the same column indicate a significant difference (Tukey test, $p \leq 0.05$); different lowercase letters on the same line indicate a significant difference (Tukey test, $p \leq 0.05$).
C: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*; T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

close to those found by Machado et al. (2004) of 0.28 ±0.08 g lactic acid·100 g^{-1}. During the maturation period, the acidity of the three treatments showed a significant increase in relation to the initial values, reaching around 1.3 g lactic acid·100 g^{-1} within 30 days and presenting a decrease after 45 days, stabilizing around 1.0 g lactic acid·100 g^{-1}. These values are higher than those found by Martins (2014), which were from 0.45 to 0.50 g lactic acid·100 g^{-1}, and may explain the drops in counts within 30 days of maturation. These changes are primarily due to the cheese maturation process and the formulation of treatments.

Curcumin content

Curcumin concentrations in turmeric and T2 treatment during the maturation period are shown in Table 4.

The average concentration of curcumin in commercial ground turmeric (2.99 ±0.03 g·100 g^{-1}) was lower than that found by Souza and Glória (1998), who evaluated rhizomes cultivated in several regions of Minas Gerais and found a mean value of 4.41 ±1.17 g·100 g^{-1} of sample. According to FAO (2004), indian turmeric cultivars present curcumin concentrations between 4.0 and 7.9 g·100 g^{-1}. The difference in curcumin concentration between commercial turmeric and cultivated rhizomes can be explained by variability in sources, cultivation conditions, varieties, processing methods, age and harvest time and storage conditions.

Table 4. Curcumin concentration in commercial ground turmeric and in the T2 treatment at 0, 14, 30 and 60 days of maturation

Treatments	Days	Curcumin* (g·100 g^{-1} sample on a dry basis)
Ground turmeric	–	2.999 ±0.033^{a}
T2	0	0.005 ±0.000^{c}
T2	14	0.005 ±0.000^{c}
T2	30	0.010 ±0.000^{b}

Mean ±standard deviation (n = 3) followed by different letters in the same column indicate a significant difference (Tukey test, $p \leq 0.05$).*Curcuminoid pigments expressed as curcumin, on a dry basis; T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

The cheese samples had a curcumin content of 0.005 ±0.000 g·100 g^{-1}, on a dry basis, after one day of manufacture, and with 60 days of maturation showed slightly higher content ($p < 0.05$), with 0.01 ±0.00 g·100 g^{-1}. This result was expected because a decrease in the product's humidity leads to a concentration of its components. The increased curcumin content is also evident in the enhanced yellow color of the product, as shown in Table 5.

Most clinical studies have found no significant toxicity associated with curcumin or curcumin-based

Table 5. Components *L**, *a** and *b** for the control, T1 and T2 at 0, 14, 30 and 60 days

Treatments	Days	*L**	*a**	*b**
C	0	89.10 ±0.45^{ab}	–2.03 ±0.05^{e}	15.41 ±0.54^{hi}
C	14	85.54 ±0.53^{cd}	–2.09 ±0.08^{e}	19.59 ±0.26^{ef}
C	30	84.93 ±1.53^{d}	–1.63 ±0.20^{bcde}	16.84 ±0.63^{fgh}
C	45	85.75 ±1.40^{bcd}	–1.75 ±0.19^{cde}	17.26 ±0.86^{efgh}
C	60	88.37 ±0.20^{abcd}	–1.89 ±0.07^{de}	16.83 ±1.07^{fgh}
T1	0	86.14 ±0.11^{bcd}	–1.09 ±0.17^{ab}	12.86 ±0.17^{i}
T1	14	86.23 ±0.91^{bcd}	–1.29 ±0.17^{abc}	16.53 ±0.04^{gh}
T1	30	89.85 ±0.25^{a}	–0.98 ±0.05^{a}	19.19 ±0.45^{efg}
T1	45	88.35 ±1.75^{abcd}	–1.44 ±0.08^{abcd}	20.04 ±1.38^{e}
T1	60	88.33 ±2.27^{abcd}	–1.43 ±0.06^{abcd}	19.97 ±1.07^{e}
T2	0	85.88 ±0.22^{bcd}	–6.64 ±0.12^{f}	26.33 ±0.46^{d}
T2	14	86.52 ±0.56^{abcd}	–7.69 ±0.23^{g}	32.20 ±0.14^{c}
T2	30	89.16 ±0.22^{ab}	–7.74 ±0.40^{g}	44.10 ±0.68^{a}
T2	45	85.26 ±0.30^{cd}	–7.72 ±0.13^{g}	40.38 ±1.15^{b}
T2	60	88.66 ±2.29^{abc}	–7.87 ±0.34^{g}	42.98 ±2.24^{ab}

Mean ±standard deviation (n = 3) followed by different letters in the same column indicate a significant difference (Tukey test, $p \leq 0.05$).
*L** (0 = black and 100 = white); *a** (red (+) and (–) green); b* (yellow (+) and (–) blue).
C: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*; T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

products. Trifan et al. (2021) mention a maximum acceptable daily intake for curcumin of 0.003 g·kg^{-1} of body weight. However, they also note that studies using higher doses have shown positive effects, indicating that consuming up to this level does not lead to toxicity in individuals. Gutierres et al. (2011) added curcumin to yogurt and tested it on diabetic rats, for 31 days, at concentrations between 0.015 and 0.09 g·kg^{-1} body weight/day, and concluded that the higher the concentration of curcumin, the better the anti-diabetic action. Thus, maturation is beneficial for increasing curcumin concentration, as observed in our results.

Color

The values of *L**, *a** and *b**, up to 60 days of maturation, are presented in Table 5. For the parameter *L** (0 = black and 100 = white), the samples remained between 85.0 and 89.0 during 60 days of maturation. Usually, in matured cheeses without packaging, there is a reduction in the values of *L**, caused by the constant drying (Tolentino, 2013). In this research, the cheeses were vacuum-packed after 12 days of drying, maintaining high luminosity.

The control and treatment T1 showed no variation in values of the *a** component (red (+) and (–) green) in 60 days of maturation. In the T2 treatment, turmeric promoted an increase in the green color, with significantly lower values ($p < 0.05$). For the *b** component (yellow (+) and (–) blue), after 14 days, the control sample showed no variation ($p > 0.05$), contrary to what was observed for T1. However, for the sample with turmeric (T2), there was an intensification in the yellow color during maturation, which was significantly different compared to time 0. This effect

was also observed by Licón et al. (2012), who noted that the yellow color tends to be more intense in acidified samples. The color changes in the cheese during maturation are mainly caused by the curcumin from turmeric, which intensifies the yellow color (b^*) and possibly increases the green color (a^*). Vacuum packaging maintains luminosity (L^*) by preventing moisture loss and oxidative degradation of the pigments. Enzymatic processes also contribute to these changes. Andrés-Bello et al. (2013) confirm that acidification affects pigment structures, enhancing yellow or orange hues and leading to higher color values in colorimeter readings. This is because acidic environments stabilize pigment forms that reflect more intensely in the yellow range of the visible spectrum.

Phenolic compounds and antioxidant capacity

Ground turmeric showed a content of phenolic compounds of 1,861.36 mg gallic acid equivalents (GAE)·100 g^{-1} (dry basis) (Table 6). For T2 cheeses, the phenolic content was higher than for other cheeses, but lower than for ground turmeric. This is due to the amount of turmeric added to the milk and the loss that occurs in whey during cheese production.

The antioxidant activity of turmeric was 42.48 µmol Trolox·g^{-1} as measured by DPPH• radical scavenging, 38.69 µmol Trolox·g^{-1} as measured by ABTS•+ radical scavenging and 44.78 µM ferrous sulfate·g^{-1} as measured by the FRAP method.

For the cheeses from the T2 treatment, the values obtained for antioxidant activity became significantly

Table 6. Phenolic compounds and antioxidant capacity by DPPH•, ABTS•+ and FRAP at 0, 14, 30, 45 and 60 days of maturation

Treatments	Days	Phenolic mg GAE·100 g^{-1}	DPPH• µmol Trolox·g^{-1}	ABTS•+ µmol Trolox·g^{-1}	FRAP µM ferrous sulfate·g^{-1}
Turmeric	–	1861.36 ±107.96^{a}	42.48 ±0.21^{a}	38.69 ±0.43^{a}	44.78 ±1.09^{a}
C	0	54.16 ±1.29^{de}	0.09 ±0.00^{d}	1.23 ±0.14^{h}	0.58 ±0.05^{b}
C	14	38.54 ±1.07^{de}	0.08 ±0.01^{d}	1.70 ±0.07^{dfg}	0.51 ±0.02^{b}
C	30	35.21 ±1.50^{de}	0.08 ±0.01^{d}	1.92 ±0.04^{df}	0.52 ±0.03^{b}
C	45	34.68 ±1.50^{de}	0.09 ±0.03^{d}	1.92 ±0.03^{f}	0.53 ±0.04^{b}
C	60	45.59 ±1.50^{de}	0.10 ±0.03^{d}	2.05 ±0.00^{d}	0.61 ±0.04^{b}
T1	0	39.07 ±1.82^{de}	0.09 ±0.01^{d}	1.22 ±0.04^{h}	0.51 ±0.02^{b}
T1	14	28.34 ±1.13^{de}	0.14 ±0.02^{d}	1.41 ±0.02^{gh}	0.60 ±0.07^{b}
T1	30	26.26 ±1.39^{e}	0.12 ±0.02^{d}	1.59 ±0.02^{fgh}	0.64 ±0.06^{b}
T1	45	31.53 ±1.02^{de}	0.13 ±0.01^{d}	1.66 ±0.05^{dfg}	0.77 ±0.13^{b}
T1	60	41.27 ±1.29^{de}	0.12 ±0.03^{d}	1.90 ±0.09^{df}	0.61 ±0.02^{b}
T2	0	77.29 ±0.96^{cde}	0.31 ±0.03^{cd}	3.03 ±0.11^{d}	0.93 ±0.02^{b}
T2	14	108.93 ±1.39^{bcd}	0.38 ±0.01^{cd}	3.14 ±0.12^{d}	1.05 ±0.03^{b}
T2	30	127.88 ±0.63^{bc}	0.36 ±0.06^{cd}	3.71 ±0.26^{c}	1.18 ±0.05^{b}
T2	45	168.52 ±0.76^{b}	0.60 ±0.33^{c}	4.05 ±0.01^{bc}	1.20 ±0.06^{b}
T2	60	174.29 ±0.46^{b}	0.94 ±0.01^{b}	4.18 ±0.04^{b}	1.11 ±0.03^{b}

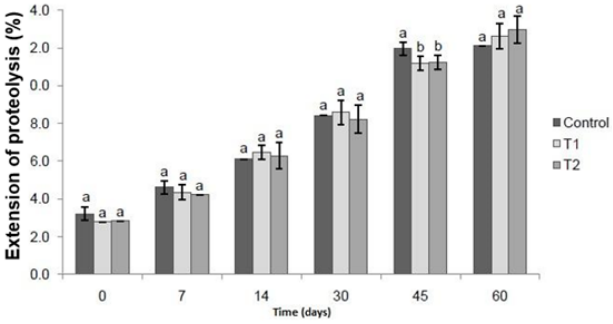
Different letters in the same column indicate a significant difference (Tukey's test, $p < 0.05$); GAE: Gallic acid equivalent.
C: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*; T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

different only at 60 maturation days (0.94 ±0.01 μmol Trolox·g^{-1}). For the ABTS•+ method, a difference was observed after 30 days, with a maximum value of 4.18 ±0.04 μmol Trolox·g^{-1}. As for the FRAP method, there were no significant differences between samples with and without turmeric throughout the period. These differences between the methods, according to Zhang et al. (2012), are due to their different chemical mechanisms, which do not correlate with each other. The differences in antioxidant activity observed in T2 cheese with turmeric using DPPH, ABTS, and FRAP assays are due to the distinct chemical mechanisms each method employs. The DPPH assay measures hydrogen donation, the ABTS assay measures electron donation, and the FRAP assay measures reducing power. Turmeric contains phenolic compounds like curcuminoids, which vary in their antioxidant properties and mechanisms. Curcumin, effective in DPPH and ABTS assays and FRAP, directly reduces ferric ions. These differences underscore the importance of using multiple assays to comprehensively assess antioxidant capacity (Ak and Gülçin, 2008; Xiao et al., 2020).

The T2 sample, enriched with turmeric, exhibits higher phenolic content and antioxidant activity than the control and T1 samples. Phenolic compounds, particularly curcuminoids found in turmeric, are well known for their potent antioxidant properties. These compounds can neutralize free radicals, thereby reducing oxidative stress and potentially lowering the risk of chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. The significant antioxidant activity observed in the T2 cheese was especially notable after 30 to 60 days of maturation. This enhanced antioxidant capacity can offer additional health benefits to consumers, making the T2 cheese a functional food with added health-promoting properties.

Extent and depth of proteolysis indexes (EPI and DPI)

The variation in the extent of proteolysis of cheeses at 0, 7, 14, 30, 45 and 60 days of maturation is represented in Figure 2. The extension index measures soluble nitrogen at pH 4.6, which includes high, medium, and low molecular mass peptides and free amino acids, obtained by primary proteolysis conducted by chymosin and plasmin residues (Macedo and Malcata, 1997; Furtado, 2011).



Mean ±standard deviation (n = 3) followed by different letters between treatments, for each time,
indicate a significant difference (Tukey's test, $p < 0.05$);
Control: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*;
T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

Fig. 2. Variation of the extent of proteolysis index of ripened Minas cheese at up to 60 days of maturation of the control and treatments T1 and T2

The soluble nitrogen content at pH 4.6 of the treatments T1 and T2 did not differ ($p > 0.05$) from the control during the first 30 days of maturation. In this initial phase, primary proteolysis driven by chymosin and plasmin progressed similarly across all treatments. This uniformity could be due to the similar enzymatic activity and initial conditions in all cheese samples, resulting in comparable levels of high, medium, and low molecular mass peptides and free amino acids. At 45 days of maturation, the control showed a significant difference from the T1 and T2 cheeses. This phase indicates a deviation in proteolytic activity, possibly due to differences in the microbial populations, enzyme concentrations, or environmental conditions such as pH and moisture, specific to each treatment. The control might have experienced either accelerated or decelerated proteolysis relative to T1 and T2, leading to significant variation. However, at 60 days of maturation, the three treatments did not show statistical differences. As maturation progresses, the proteolytic processes might reach a point of equilibrium where the accumulated effects of proteolysis even out the differences observed at the mid-maturation stage. The convergence of proteolytic activity suggests that the enzymes and microbial communities across all treatments eventually stabilize, leading to similar levels of peptide and amino acid production. Martins (2014),

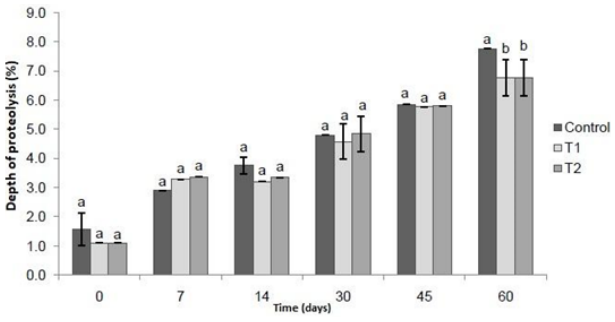
using a microbial liquid coagulant (*Aspergillus niger* var. *awamori*), obtained 14% proteolysis extension in Minas Padrão cheese matured for 45 days, a value higher than those found in this study. The lower extent of proteolysis observed in this study compared to Martins (2014) can be attributed to differences in the coagulants used, namely the use of a microbial liquid coagulant (*Aspergillus niger* var. awamori) with higher proteolytic activity. Additionally, variations in microbial populations, environmental conditions, cheese composition, and enzyme concentrations likely contributed to the reduced proteolysis. These factors collectively influence the efficiency of protein breakdown during cheese maturation, resulting in lower proteolysis levels in the current study.

Figure 3 shows the variation of proteolysis during the maturation period. The depth of proteolysis, evaluated by the ratio of soluble nitrogen in TCA 12% / NT, is influenced by the activity of endoenzymes and exoenzymes of lactic cultures used in cheese making and possible contaminants. Peptides with a chain length between 2 and 20 amino acid residues and free amino acids are obtained from secondary proteolysis (Furtado, 2008; 2011).

There was no significant difference between cheeses aged up to 45 days of maturation; however, at 60 days, the control differed from the samples ($p < 0.05$). In cheese matured for 90 days, Milesi et al. (2011) observed a depth of proteolysis of around 6.5% for two strains of *L. helveticus*.

Although several strains of *L. helveticus* have been demonstrated to provide a highly efficient proteolytic system when compared to other lactic acid bacteria (Valence et al., 2000; Griffiths and Tellez, 2013; Sahingil et al., 2014), this distinction was not observed in this study. The undifferentiated behavior at the end of maturation may have been caused by the reduction in the total lactic bacteria count in MRS after 14 days, as seen in Figure 1, a higher percentage of salt in these cheeses and/or low pH values, causing milk proteases and microbial to present low activity (Olarte et al., 2000). Furthermore, this distinction varies greatly from one lineage to another (Milesi et al., 2011) and its activity can be influenced by the type of cheese produced.

L. helveticus differs not only in the intensity of its caseinolytic activity but also in the specificity of its endopeptidases (Jensen et al., 2009), as observed by Matar et al. (1997), who found that milk fermented with *L. helveticus* R389 produced bioactive peptides, while another strain (L89) did not show such proteolytic capacity.



Mean ±standard deviation (n = 3) followed by different letters between treatments, for each time,
indicate a significant difference (Tukey's test, $p < 0.05$);
Control: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*;
T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

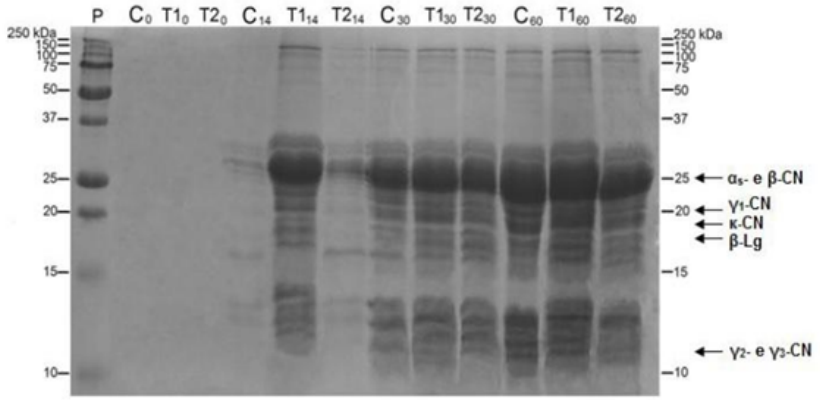
Fig. 3. Variation of the proteolysis depth index of ripened Minas cheese at up to 60 days of maturation of the control, T1 and T2 treatments

Polypeptides by molecular mass using SDS-PAGE

The electrophoretic profile of the control and the T1 and T2 treatments during maturation is shown in Figure 4. The high-intensity band around 25 kDa represents the α_s- and β-caseins. Bands between 20 and 18 kDa may indicate the presence of e γ_1-casein, κ-casein and β-Lactoglobulin, and bands between 11 and 12 kDa may indicate the presence of γ_2- and γ_3 caseins. As there was no presence of 14 kDa bands, there seems to have been an absence of α-lactalbumin (Fox et al., 2000; Damodaran et al., 2010; Paul et al., 2014; Miyamoto et al., 2015).

Bands around 16 kDa are related to para-κ-casein (Paul et al., 2014; Miyamoto et al., 2015). According to Damodaran et al. (2010), para-κ-casein results from the hydrolysis of κ- casein in the Fen105-Met106 bond by the action of chymosin, not being hydrolyzed during maturation (Sousa et al., 2001).

Any bands in the 30 to 60 kDa range are assumed to be microorganisms since the electrophoretic profile



P: standard from 250 kDa to 10 kDa.
C: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*.
T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.
CN: casein; Lg: lactoglobulin.

Fig. 4. Electrophoretic profile (SDS-PAGE) of control (C) and treatments T1 and T2, at 0, 14, 30 and 60 days of maturation

of milk does not contain proteins in this range (Valence et al., 2000). The rest of the bands are assumed to be degradation products.

At time 0, it was observed that there was no apparent band for the three treatments. As there had not been sufficient time for proteolysis to occur, the proteins were not yet soluble in water. After 14 days, it was observed that there was protein hydrolysis, allowing their solubilization and appearance in the gel. There was a differentiation in the intensity of the bands between treatments at 14 days, with treatment T1 showing a higher amount and intensity of bands, indicating more proteolysis.

After 30 days of maturation, the three treatments showed accentuation and progression in proteolysis, with bands of high intensity between 23 to 31 kDa, as observed by Kırmacı et al. (2014), and formation of bands of low molecular weight between 10 and 15 kDa, which correspond to products of degradation (Paul et al., 2014). At 60 days, the samples showed similar peptide profiles to the samples at 30 days, but with accentuated intensity and a greater number of bands for the T1 treatment, containing *L. helveticus*. Although peptide fragments smaller than 10 kDa may be present, the technique used in this study was not able to detect them.

Bands between 75 and 150 kDa from 14 days of maturation showed no hydrolysis after 60 days, indicating that there was no enzymatic action on these proteins. Moatsou et al. (2002) observed in Feta cheese, by an electrophoretic method in the presence of urea (UREA-PAGE), that the β-casein band remained constant during the maturation period and, in some cases, increased, probably due to the accumulation of hydrolysis products in this area. They also observed that the area of γ-caseins was not notably changed, apparently due to the pH of the cheese being unfavorable to plasmin activity.

According to the authors, most peptides soluble in water originate from αS-casein, due to rennet action that progresses rapidly during the maturation period (Moatsou et al., 2002; Kırmacı et al., 2014). According to Sihufe et al. (2010), this behavior is common in most cheeses, where the activity of the residual coagulant is more important than the plasmin activity.

Sensory analysis

Samples at 20 days of maturation were used for the sensory analysis since the objective was to verify if there were significant differences in the sensory attributes of probiotic cheeses supplemented with extract of turmeric.

Table 7. Sensory evaluation scores of control and treatments T1 and T2 at 20 days of maturation

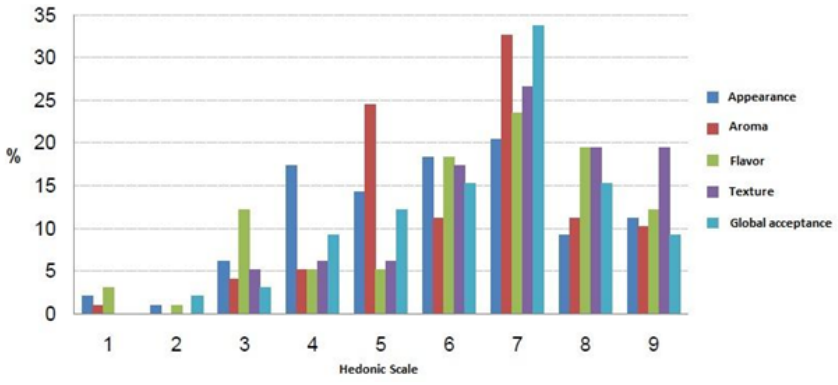
Treatments	Appearance	Aroma	Flavor	Texture	Global
Control	7,57 ±1,15[a]	7,16 ±1,31[a]	7,55 ±1,32[a]	7,23 ±1,53[a]	7,51 ±1,13[a]
T1	7,63 ±1,19[a]	6,98 ±1,41[a]	7,58 ±1,29[a]	7,27 ±1,49[a]	7,48 ±1,12[a]
T2	5,91 ±1,92[b]	6,34 ±1,64[b]	6,28 ±2,09[b]	6,90 ±1,67[a]	6,44 ±1,64[b]

9-point hedonic scale (9 = like very much; 1 = dislike very much).
Different letters in the same column indicate a significant difference (Tukey's test, $p \leq 0.05$).
Control: cheese with starter culture MW 031 R; T1: cheese with cultures of *L. helveticus* and *E. faecium*; T2: cheese with cultures of *L. helveticus* and *E. faecium* and turmeric extract.

The sensory evaluation scores of the three treatments (Control, T1 and T2) at 20 days of maturation, are shown in Table 7. Attributes were around 6 (like slightly) and 7 (like moderately), indicating that the three treatments were accepted. There were no differences in acceptance ($p > 0.05$) between the control and T1 regarding the evaluated attributes. The cheese with turmeric extract (T2) received the lowest ratings for appearance, aroma, and flavor, which had an impact on overall acceptance. Nonetheless, it still achieved a relatively positive level of acceptance based on the hedonic scale used.

Evaluating the percentage of acceptance of treatment T2 (Fig. 5), the texture attribute obtained the highest average, 6.90 ±1.67, where 82.66% of the judges assigned grades between 6 (like slightly) and 9 (like extremely). For the appearance attribute (5.91 ±1.92), only 40.81% of judges attributed grades between 7 (like moderately) and 9 (like extremely) and 26.53% grades from 1 (dislike extremely) to 4 (dislike slightly). Despite this, most judges (73.47%) assigned scores between 6 and 9 for the global acceptance attribute. The most prominent aspect in the category 'neither liked nor disliked' was the aroma, with 24.49%.

Gardiner et al. (1999) evaluated the addition of *E. faecium* in Cheddar cheese and concluded that the strain did not affect the appearance, aroma or texture of the product. This cheese did not present significant differences when compared to the control cheese that



9-point hedonic scale (9 = like very much; 1 = dislike very much).

Fig. 5. Percentage of judges giving different acceptance scores for cheese with added *E. faecium*, *L. helveticus* and turmeric extract (T2), based on a sensory acceptance test with a 9-point hedonic scale

had only been supplemented with starter culture. In the cheese treatments analyzed in our study, the addition of the probiotic culture did not influence acceptance. However, we suggest that the attributes of the T2 treatment received lower acceptance scores due to the addition of turmeric extract.

CONCLUSION

The ripened Minas cheese showed good acceptance among consumers and could be beneficial to health when consumed regularly. Proteolytic microorganisms like *E. faecium* and *L. helveticus*, along with turmeric extract, contributed to enhanced phenolic content and antioxidant activity in the cheese. Despite slightly lower sensory scores for the cheese with added turmeric, its enriched health benefits make it a potentially valuable dietary component.

DECLARATIONS

Data statement
All data supporting this study has been included in this manuscript.

Ethical Approval
Not applicable.

Competing Interests
The authors declare that they have no conflicts of interest.

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