Influence of the addition of natural antioxidant from mate leaves (*Ilex paraguariensis* St. Hill) on the chemical, microbiological and sensory characteristics of different formulations of Prato cheese

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Abstract The objective of this work is to evaluate the effects of the addition of dried extract from mate leaves and mesophilic cultures (*Lactococcus lactis* ssp. *lactis* and *cremoris*) on the chemical, microbiological and sensory characteristics of Prato cheese. The Prato cheese presented high moisture contents (49 to 53 %) and mean pH values of 5.15 for all tested formulations. The addition of mate leaves extract in the product did not influence the growth of the microbial cultures. During the maturation time, all formulations with the addition of adjunct cultures and mate leaves extract presented lower levels of lipid and protein oxidation compared to the control, proving the antioxidant effect of mate extract. The formulation of Prato cheese added of 0.1 wt.% of extract presented acceptability of about 80 % after 30 days of maturation. The sensory evaluation showed that only the formulation added by adjunct culture and 0.2 wt.% of mate extract presented lower values for the attributes global acceptance, texture and flavor, compared to the control. The formulations added of mate leaves extract presented residual bitter flavor after 45 days of storage.

Keywords Prato cheese · Natural antioxidant · *Ilex paraguariensis* · Mesophilic culture

Introduction

A recent tendency in food industries is related to the development of functional products and the substitution of synthetic by natural ingredients (Mattila-Sandholm et al. 2002; Michida et al. 2006; Hernández and Guzmán 2003). The dairy industry is contributing by promoting the expansion of functional products, such as yoghurts, dairy and soybean derivatives ones (Saarela et al. 2000; Biström and Nordström 2002; Sharma et al. 2011). In this context, cheese still presents a potential of technological development (Sharma et al. 2011). Food containing high concentrations of pro-oxidant substances (such as transition metal, heme protein) and polyunsaturated fatty acids are easily attached by free radicals and undergo oxidation. This may cause rancidity, decreasing both acceptability and nutritional value of the food. Lipid peroxidation causes degradation of polyunsaturated fatty acids and generation of residual products, such as malondialdehyde and lipid-derived volatiles, which may lead to sensory and nutritional deterioration (Gray et al. 1996; Oboh et al. 2012). To prevent and retard lipid oxidation, addition of synthetic antioxidants, such as BHA (tert-butyl-4-hydroxyanisole) and BHT (butyl-hydroxytoluene), are often necessary to increase the shelf life of food products.

The replacement of these synthetic antioxidants by plant extracts, the so-called natural antioxidants, is a public health concern, since extensive use of synthetic antioxidants and prolonged ingestion may be associated with the development of cancer (Sasse et al. 2009; Polorny 2007).

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Antioxidant properties of different plants extracts have been analyzed for this purpose, including: sage (*Salvia officinalis*), rosemary (*Rosmarinus officianalis*), oregano (*Origanum onites*), and many others (Hernández-Hernandez et al. 2009; Estévez et al. 2007; Chen et al. 2012; Kaur et al. 2012). Dried leaves of mate (*Ilex paraguariensis*), a native South American plant, are used to prepare an aqueous infusion which is rich in phenolic acids (Bastos et al. 2007a; Bastos et al. 2007b) and has shown in vitro and in vivo antioxidant activity, besides other pharmacological properties.

Recently, the mate leaves extract has been investigated as potential antioxidant agent in food products. The addition of the ethanolic extract of mate leaves to pork and back fat from pig salamis (5 mL/Kg) lowered the TBARS (thiobarbituric acid reactive substances) values (1.75 mg of malondialdehyde/kg of sample) and volatile compounds derived from lipids (Campos et al. 2007). Racanicci et al. (2008) verified that the addition of an aqueous extract of mate leaves (0.05 and 0.10 %) to chicken meat balls decreased lipid peroxidation, measured by TBARS assay and depletion of vitamin E. Milani et al. (2002) evaluated the antioxidant effect of hydro-ethanolic and methanolic extracts of apple peel, leaves of artichoke and mate leaves in mechanical minced meat (MMM) of chicken kept under refrigeration and freezing. The authors observed that the methanolic extract of mate leaves presented higher antioxidant potential compared to the other extracts. Despite describing good antioxidant activity in meat and milk products, none of these previous cited studies evaluated if the addition of mate leaves extract had an impact on products acceptance by the consumer, a fundamental parameter for the feasibility of replacing a synthetic antioxidant by a natural one.

Based on these aspects, the objective of this work is to evaluate the effects of the addition of dried extract from mate leaves and mesophilic cultures (*Lactococcus lactis* ssp. *lactis* and *cremoris*) on the chemical and microbiological characteristics of Prato cheese. The acceptance of the products during the maturation time (60 days, 15 °C and 85 % of relative moisture) was also evaluated.

Material and methods

Extract of mate leaves

Mate tea leaves (*Ilex paraguariensis*) were collected of a homogeneous cultivation at sun and the enzymatic inactivation was carried out in experimental unit, at 180 °C, 5 min and 60 rpm. After, mate leaves were dried at 70 °C for 90 min in a mini fixed-bed drier. The leaves were then disintegrated in mixer (Walita, Brazil) and

classified by size. Samples of approximately 2.36 mm (Tyler mesh 16) were used in further steps.

The extraction of soluble compounds was carried out by percolation of solvent in batch mode (350 mL of water and 24 g of mate leaves at 96 °C). The extract was dried by atomization (LabPlant SD-05 Spray drying, USA) using air temperature of 190 °C, air rate of 47 m³/h and extract inlet rate of 600 mL/h. The dried extract was stored in amber flasks hermetically closed until the moment of use.

Antioxidant activity of the extract

The antioxidant activity in vitro of the essential oil was evaluated by the method DPPH• (free radical 2,2-diphenyl-1-picryl-hydrazil). The methodology was based on the measurement of extinction of absorption of DPPH•, related to solutions containing different concentrations of *I. paraguariensis* extract (2.5, 3.75, 5.0, 7.5, 10, 12.5, 15, 17.5 and 20 mg/mL) in ethanol. The scavenging percentage of radical DPPH• was calculated in terms of the percentage of antioxidant activity (AA%), using the following equation:

$$AA\% = 100 - \left\{ \left[(\text{Abs.}_{\text{sample}} - \text{Abs.}_{\text{blank}}) \times 100 \right] \div \text{Abs.}_{\text{control}} \right\}$$

A spectrophotometer was used (Agilent Technologies, model 8453E, USA) and the readings were carried out at 515 nm (Kulisic et al. 2004). The concentration of essential oil was calculated through regression analysis, in order to scavenge 50 % of radical DPPH•, which corresponded to the efficient concentration (EC) or inhibitory concentration (IC₅₀).

Formulation of Prato cheese

Six formulations of Prato cheese were evaluated in this work: Treatment 1 (Control - Prato cheese without addition of culture and extract of mate tea leaves), Treatment 2 (Prato cheese with addition of adjunct culture of *Lactococcus lactis* ssp. *lactis* and *cremoris*), Treatment 3 (Prato cheese with addition of adjunct culture of *Lactococcus lactis* ssp. *lactis* and *cremoris* and 0.1 wt.% of extract of mate tea leaves), Treatment 4 (Prato cheese with addition of adjunct culture of *Lactococcus lactis* ssp. *lactis* and *cremoris* and 0.2 wt.% of extract of mate tea leaves), Treatment 5 (Prato cheese without addition of adjunct culture and 0.1 wt.% of extract of mate tea leaves) and Treatment 6 (Prato cheese without addition of adjunct culture and 0.2 wt.% of extract of mate tea leaves).

The product was elaborated by the traditional method, with some modifications. The adjunct culture (Chr Hansen, Brazil), at an initial concentration in the product of ~10⁸ CFU/g (2.5 % (wt/v)), was added to the standard

pasteurized milk (3.2 % of fat). Then, the sodium chloride (Quimex, Brazil), the dye *urucum* (Chr Hansen, Brazil) and the chymosin (Chymax, Chr Hansen, Brazil) were also added. The salt addition was performed in the mass, using sodium chloride at 2 wt.%, after removing of approximately 80 % of whey. The dried mate tea leaves extract at the different tested concentrations were added after the salt process. Then, the products were tinned and submitted to two steps of pressing: 0.141 kgf/cm² for 90 min and 0.211 kgf/cm² for the same time. After this, the products were packed under vacuum (−690 mmHg, Selovac) in thermo-shrinkable packages (Supravac VC2—55 µm, Brazil) and stored at 15 °C and 85 % of relative moisture for 60 days.

Chemical analysis

Samples of Prato cheese were randomly chosen and triturated in multiprocessor, homogenized and sliced manual for analytical determination after 1, 7, 15, 30, 45 and 60 days of storage.

The chemical parameters of cheese were analyzed using the IDF methods: moisture by IDF standard 4A (International Dairy Federation IDF 1982) and protein by IDF standard 20B (International Dairy Federation IDF 1993). The pH and titratable acidity were measured using AOAC standards (Association of Official Analytical Chemists 2000). Lipid oxidation was measured by spectrophotometer (Perkin Elmer model Lambda EZ150) at 531 nm using a standard curve with TEP (1.10^{-8} to 1.10^{-7} mol/mL) with tiobarbituric acid reactive substances (TBARS) following the methodology proposed by Raharjo et al. (1992) and modified. This analysis constitutes an estimation of lipid oxidation, since it determines the reactive substances to tiobarbituric acid. Results were expressed in mg of malondialdehyde (MA)/kg of product.

Protein oxidation was measured by assessment of carbonyl groups formed during the experiment using the methodology proposed by Levine et al. (1990) with slight modifications. The concentration of protein was measured at 280 nm in the chloride acid (HCl) control using bovine serum albumin (BSA) in 6 M guanidine as standard. Carbonyl concentration in the treated sample was measured with 2,4-dinitrophenylhydrazine (DNPH) incorporated on the basis of molar absorption coefficient of $21.0\text{ nM}^{-1}\cdot\text{cm}^{-1}$ at 370 nm of protein hydrazones. Results were expressed as nmol of carbonyl/mg of protein.

Microbiological analysis

The microbiological analysis (Counting of lactic acid bacteria) of cheese was carried out using the methodology proposed by Vanderzant and Splittstoesser (1992). Twenty five grams of cheese were placed in 225 mL peptone water

0.1 %, and homogenized in Stomacher (Sward—Laboratory Blender—Stomacher 400, USA), for 60 s, with a dilution of 10^1 . From this dilution the subsequent dilutions were generated (10^2 to 10^6). The inoculation for counting of lactic acid bacteria was performed in medium agar De Man, Rogosa & Sharpe—MRS (Franco and Landgraf 2005) and for adjunct culture in agar De Man, Rogosa & Sharpe -M-17 (González et al. 2007) by the method of plating in profundity and in surface, respectively. Samples were incubated for 48 h at 30 °C.

Sensory evaluation

Sensory evaluation was carried out after 30, 45 and 60 days of maturation, by a semi-trained panel of 20 tasters, evaluating 3 samples in 2 sessions. Samples of cheese (approximately 2 cm²) were distributed in plastic dishes coded with random numbers of 3 digits. The experiment was conducted in a sensory design balanced incomplete block of type I with the reference sample in each block (Pimentel-Gomes 1987). A group of 20 panelists evaluated the product in 2 sessions with 3 samples each one. The test of hedonic scale of 9 points (1 as the lowest and 9 as the highest values) was employed (Faria and Yotsuyanagi 2002). The attributes flavor, texture and global acceptance were evaluated separately.

Statistical analysis

The results obtained in each carried out analyses described previously were carried out in triplicate of samples and analyses and treated by analysis of variance followed by Tukey's post-hoc test, using the software Statistica 6.1 (Statsoft, Tulsa, OK, USA). All analyses were performed considering a 95 % confidence level ($p < 0.05$).

Results and discussion

In vitro antioxidant activity of mate leaves

The antioxidant activity of the mate leaves increased with the extract concentration, reaching 99.2 % of activity for concentration of 750 µg/mL. Bastos et al. (2007b) evaluated the aqueous extract of mate leaves (*Ilex paraguariensis*) and green tea (*Camellia sinensis*) by DPPH method and obtained antioxidant activities higher than 89 %. Miliuskas et al. (2004) affirm that extracts with DPPH values higher than 90 % are related to almost the totality of capture of this free radical due to the limitations of the method.

The correlation between the antioxidant activity (AA) (%) and the concentration of the extract led to an IC₅₀ of 234.48 µg/mL. The high value of IC₅₀ can be attributed to the extraction technique (percolation) and to the drying

method (spray-drier-190 °C). Moure et al. (2001) showed that the stability of polyphenol compounds during the extraction and dehydration is affected by chemical and enzymatic degradations, by the volatilization of compounds and mainly by the thermal decomposition.

Rivelli et al. (2007) determined the IC₅₀ values of different extracts of *I. paraguariensis*, reaching values from 10 to 15 µg/mL. Schinella et al. (2000) obtained IC₅₀ values from 18 to 28 µg/mL for aqueous extracts of *I. paraguariensis*, values lower than those obtained in the present work.

Physical-chemical analysis of formulations

The contents of fat (24.43 to 25.04 wt.%, wet basis) and protein (21.23 to 21.96 wt.%, wet basis) did not differ statistically ($p < 0.05$) among the formulations. Based on the moisture contents that varied from 49 to 53 wt.% the different formulations tested in this work were characterized as “cheese of high moisture” (Normative n 146, 07th March 1996, Ministério da Agricultura, Pecuária e Abastecimento, Brasil 1996).

Figure 1a presents the pH values of the different formulations tested in this work. A decrease in pH was observed after 7 and 45 days of storage, varying from 4.7 to

5.2. This fact can be attributed to the degradation of the lactose by lactic bacteria and the adjunct cultures, and the final products, as CO₂ and lactic acid (Narimatsu et al. 2003). The degradation of the lactose caused an increase in acidity content (Fig. 1b) and a consequent reduction in pH at 45 days of storage. However, an increase in pH values was verified at 60 days of maturation (5.38, 5.01, 5.15, 5.0, 5.20 and 5.28 for formulation 1, 2, 3, 4, 5 and 6, respectively). This behavior is common during the storage, normally after 30 days of maturation, due to the reduction in the lactose content and the proteolysis by the action of enzymes from the rennet and that produced by the bacteria. Costa et al. (2005) affirm that the consumption of proteins causes a high intense proteolytic process, which promotes a formation of alkaline nitrogen compounds (NH₃) by reactions of amino acids and lactic acid decomposition, leading to an increase in pH values. The pH values of cheeses results of a proportion of lactose and buffering substances of coagulant protein, with liberation of amine compounds, which tends to neutralize the natural acidity of the mass (Gutiérrez et al. 2004). This behavior was observed in the formulations, associated to the proteolysis promoted by the adjunct culture.

Tables 1 and 2 present the values of lipid and protein oxidation, respectively, for the formulations during the

Fig. 1 Chemical and microbial changes in Prato cheese formulations (T1–T6) during storage at 15 °C and 85 % of relative moisture ($n=9$). Each parameter is mentioned in Y axis

Table 1 Lipid oxidation (TBARS values as mg of MDA/kg of sample) for Prato cheese during storage at 15 °C

Treatment	Days of storage					
	1	7	15	30	45	60
T1	0.086 ^{aC} ±0.002*	0.212 ^{aA} ±0.014	0.187 ^{aB} ±0.006	0.205 ^{aAB} ±0.006	0.189 ^{aB} ±0.003	0.185 ^{aB} ±0.003
T2	0.085 ^{aB} ±0.002	0.103 ^{dA} ±0.003	0.065 ^{dC} ±0.004	0.075 ^{cB} ±0.001	0.046 ^{cD} ±0.002	0.042 ^{cD} ±0.002
T3	0.084 ^{aA} ±0.004	0.082 ^{eA} ±0.002	0.054 ^{eC} ±0.002	0.066 ^{cB} ±0.004	0.036 ^{cD} ±0.002	0.031 ^{eD} ±0.001
T4	0.084 ^{aA} ±0.003	0.091 ^{deA} ±0.002	0.059 ^{deB} ±0.004	0.078 ^{eA} ±0.004	0.045 ^{cC} ±0.001	0.035 ^{deD} ±0.002
T5	0.082 ^{aD} ±0.002	0.176 ^{bA} ±0.013	0.118 ^{bB} ±0.003	0.099 ^{bC} ±0.008	0.075 ^{bD} ±0.005	0.067 ^{bE} ±0.001
T6	0.080 ^{aC} ±0.003	0.136 ^{cA} ±0.002	0.105 ^{cB} ±0.002	0.078 ^{cC} ±0.004	0.071 ^{bC} ±0.003	0.042 ^{cdD} ±0.003

T1: Prato cheese without addition of mesophilic cultures and mate leaves extract, T2: Prato cheese with the addition of mesophilic cultures, T3: Prato cheese with the addition of mesophilic cultures and 0.1 wt.% of mate leaves extract, T4: Prato cheese with the addition of mesophilic cultures and 0.2 wt.% of mate leaves extract, T5: Prato cheese with the addition of 0.1 wt.% of mate leaves extract and T6: Prato cheese with the addition of 0.2 wt.% of mate leaves extract

MDA is the abbreviation of malondialdehyde

TBARS is the abbreviation of thiobarbituric acid reactive substances

*Mean ± standard deviation ($n=9$) followed by equal letters lower/uppercases on columns/lines do not differ at a significance level of 5 % (Tukey's test)

60 days of storage at 15 °C. The protein reactions of oxidation occur simultaneously to lipid oxidation in several kinds of food. The formation of carbonyl compounds (aldehydes and ketones) are one of the most common changes in oxidized proteins, giving an indicative of protein oxidation (Levine et al. 1990). The formulations presented significant differences ($p<0.05$) for lipid oxidation compared to treatment 1, during the storage period. The lowest values were found in treatment 3 (adjunct culture and 0.1 % of extract). This behavior was observed until the final period of maturation. Treatment 1 (without the addition of adjunct culture and mate leaves extract) showed the highest values of TBARS (0.086 to 0.212 mg of MDA/Kg) during the maturation period.

In a general way, a significant increase in TBARS values was observed until the 30th day of storage, except for

treatments 5 and 6. After this period, small changes were verified, with decrease from 45 days of storage. Several authors suggest that the reduction of TBARS values observed as a function of storage period is probably associated to the enhancement of polar product concentrations, resulting from the polymerization of secondary products of oxidation (Gatellier et al. 2007).

The rancidity odor can be detected by trained and non-trained tasters in the range from 0.5 to 1.0 and 0.6 to 2.0 mg MDA/kg (Trindade et al. 2008), respectively. Here, the TBARS values were lower than those cited by the previously cited authors and consequently the odor and flavor of rancidity was not detected in the sensory evaluation of formulations. The results showed that the treatment 3 (Table 1) from 7 days of storage presented the lowest values of TBARS. The formulations with addition of mate leaves extract (5 and 6) showed TBARS values higher than treatments 2, 3 and 4, but lower than the control.

Milani et al. (2002) studied the antioxidant effects of alcoholic extracts of green, black and mate teas in mechanically minced meat and observed that all extracts demonstrated antioxidant action compared to the control samples (without antioxidants). Tang et al. (2001) observed equivalency of action between natural (tea catechins extracted from green tea) and synthetic (α -tocopheryl acetate) antioxidants, when used for supplementation of broiler feed.

Kumar et al. (1986) cites that the TBARS can be influenced by the pH values. As higher is the pH higher the lipid oxidation. The results obtained in this work corroborate this affirmation, in treatments 2, 3 and 4 the lowest values of TBARS and pH were observed. One can also observe that during the maturation period, the product

Table 2 Protein oxidation (nmol carbonyl/mg of protein) for Prato cheese during the storage at 15 °C

Treatment	Days of storage		
	1	30	60
T1	2.6 ^{cB} ±0.31	4.5 ^{dA} ±0.35	2.9 ^{cB} ±0.10
T2	4.7 ^{aB} ±0.50	8.6 ^{aA} ±0.23	5.5 ^{aB} ±0.49
T3	3.3 ^{bB} ±0.28	6.5 ^{bA} ±0.39	4.2 ^{bB} ±0.26
T4	3.1 ^{bCB} ±0.02	5.6 ^{cA} ±0.43	4.2 ^{bB} ±0.11
T5	2.6 ^{cB} ±0.41	3.4 ^{eA} ±0.19	2.5 ^{cB} ±0.10
T6	3.5 ^{bC} ±0.20	3.1 ^{eA} ±0.12	1.4 ^{dB} ±0.11

T1 to T6 as described in Table 1

*Mean ± standard deviation ($n=9$) followed by equal letters lower/uppercases on columns/lines do not differ at a significance level of 5 % (Tukey's test)

obtained from treatment 2 presented higher protein oxidation. However, treatments 5 (0.1 % of extract) and 6 (0.2 % of extract) led to lower carbonyl index after 30 days of maturation. This behavior was kept until 60 days of storage.

The mate tea extracts presented significant effect on protein oxidation after 30 days of storage in the products from formulations 5 and 6. After 60 days of storage, the formulation with 0.1 % of extract was not significantly different from the control. The addition of 0.2 % of extract seems to inhibit the protein oxidation. Several authors relate that the interactions between lipids and proteins have a significant effect on oxidative reactions in food (Sarker et al. 1995; Zamora et al. 2000; Howell et al. 2001). These oxidative phenomena can cause degradation, fragmentation or aggregation of the protein and enhancement of protein susceptibility (Decker 1998).

No information about protein oxidation in cheeses was found in the open literature. Most researches on oxidative processes and their implication in food quality are related to the lipid oxidation in meat products.

Microbiological analysis of formulations

Figure 2 presents the counting of lactic and mesophilic bacteria (log CFU/g) in each formulation of Prato cheese after 1, 7, 15, 30, 45 and 60 day of storage at 15 °C and relative moisture of 85 %. The counting of lactic bacteria, after elaboration (1 day), varied from 7.26 (formulation added by adjunct culture) to 6.04 log CFU/g (control), showing significant difference ($p<0.05$) among the treatments. The addition of mate leaves extract in the product did not influence the growth of the lactic bacteria.

The initial counting of adjunct lactic cultures (*Lactococcus lactis* ssp. *lactis* and *cremoris*) added to Prato cheese was about 10^8 CFU/g (Fig. 2). During the storage period, the treatments 2, 3 and 4 (7 to 9 log CFU/g) presented the highest values of log CFU/g of adjunct cultures until 15 days of storage. The treatments 3 (adjunct culture plus 0.1 % extract) and 4 (adjunct culture plus 0.2 % extract) did not present significant difference on the counting of adjunct cultures compared to treatment 2, demonstrating that the mate leaves extract did not present antimicrobial activity against the mesophilic cultures, as already presented in the literature (Heck and Mejia 2007; Efig et al. 2009), making possible to conclude that the concentrations tested here can be used without prejudice the final product.

Sensory analysis of the formulations

Table 3 presents the results of the sensory attributes global acceptance, flavor and texture of the different formulations

Fig. 2 Counting (mean of 9 replicate experiments) of lactic (a) and mesophilic bacteria (b) changes in Prato cheese formulations during storage at 15 °C and 85 % of relative moisture

of Prato cheese after 30, 45 and 60 days of storage at 15 °C and relative moisture of 85 %. Related to the global acceptance, only the formulation 4 (adjunct culture plus 0.2 % of extract) differed significantly ($p<0.05$) from the treatment 1 (control), showing the lowest mean acceptance (6.23 to 6.43) after 30, 45 and 60 days of maturation, corresponding to “like slightly” on hedonic scale. The formulation 5, after 30 and 45 days of storage, presented a global acceptance from 81.44 to 80.77 %, equivalent to “like much” in hedonic scale. After 60 days, treatments 1 (control) and 3 (adjunct culture plus 0.1 % of extract) presented the highest global acceptance, of 85.89 and 80.33 %, respectively. Related to the acceptability, after 30 and 45 days of maturation, the treatments 5 and 6 presented similar behavior compared to the control, with acceptance levels of 79 and 81 %, respectively. After 60 days, the acceptance of these two formulations suffered a decrease, as can be seen in Table 3.

Table 3 Sensory parameters of global acceptance, flavor and texture for Prato during the storage at 15 °C and 85 % of relative moisture

Treatment*	Acceptance			Flavor			Texture		
	Days of storage								
	30	45	60	30	45	60	30	45	60
T1**	7.3 ^{abA} ±1.44	7.2 ^{aB} ±0.87	7.7 ^{aA} ±0.78	7.0 ^{abA} ±1.58	7.3 ^{aA} ±0.84	7.2 ^{abA} ±1.15	7.2 ^{abA} ±2.07	7.0 ^{aA} ±1.16	7.2 ^{aA} ±1.25
T2	6.6 ^{bcB} ±1.33	7.1 ^{aA} ±1.04	7.1 ^{bcdA} ±0.98	6.5 ^{bcB} ±1.41	6.7 ^{abA} ±1.51	6.5 ^{bcB} ±1.48	6.3 ^{bcA} ±1.68	6.2 ^{abA} ±1.83	6.5 ^{aA} ±1.38
T3	6.7 ^{bcB} ±1.19	6.8 ^{abAB} ±1.09	7.2 ^{abCA} ±0.90	6.7 ^{bcB} ±1.45	6.1 ^{bcC} ±1.34	7.1 ^{abA} ±1.12	6.1 ^{cB} ±1.65	6.1 ^{abB} ±1.50	6.8 ^{aA} ±1.47
T4	6.4 ^{ca} ±1.41	6.2 ^{ba} ±1.04	6.4 ^{ca} ±1.28	5.9 ^{ca} ±1.94	5.4 ^{ca} ±1.48	5.8 ^{ca} ±1.71	5.8 ^{ca} ±1.89	5.7 ^{ba} ±1.60	5.6 ^{ba} ±1.54
T5	7.3 ^{abA} ±0.99	7.3 ^{abA} ±1.08	6.5 ^{deB} ±1.07	7.2 ^{abA} ±1.13	6.9 ^{abAB} ±1.52	6.6 ^{deB} ±1.52	7.2 ^{abA} ±1.14	6.8 ^{ab} ±1.35	6.5 ^{ab} ±1.25
T6	7.1 ^{abcA} ±1.63	7.3 ^{aA} ±1.03	6.6 ^{ceB} ±1.77	7.7 ^{aA} ±1.34	6.8 ^{abB} ±1.35	7.7 ^{aA} ±1.36	7.5 ^{aA} ±1.04	6.8 ^{ab} ±1.34	6.6 ^{ab} ±1.73

T1 to T6 as described in Table 1

*Mean ± standard deviation (20 panelists semi-trained) followed by equal letters lower/upercases on columns/lines do not differ at a significance level of 5 % (Tukey's test)

The mean values of the attribute flavor, also presented in Table 3, showed that the formulations 1, 5 and 6, after 30 days of storage, could be characterized as “like moderately”. Only the treatment 4 was significantly different from the control at 45 days of storage. After this time, a decrease on mean values was observed for all formulations, except for treatments 1 and 2. The tasters detected a residual bitter flavor after 45 days, probably due to the addition of mate leaves extracts in these formulations. It is well related the presence of polyphenols and flavonoids in mate leaves, that probably conferred this characteristic to the product. The bitter flavor can also be related to the formation of low chain peptides, produced by the action of proteases of rennet and some lactic bacteria (Souza et al. 2001).

Spadoti et al. (2005) evaluated the sensory characteristics of Prato cheese obtained by modification of traditional process using milk concentrated by ultrafiltration and pre-fermentation of 10 % of total volume. The authors observed the presence of a slight bitter flavor on the formulations after 45 days of storage at 7 °C.

Law et al. (1993) observed an increase in the proteolysis during the storage of cheddar cheese, due to the presence of starter culture of *L. lactis*. The authors considered that the proteases of the starter plus the rennet could be responsible for the increase of the content of peptides in the product. The substrate for the action of the peptidases from the starter becomes abundant, making easy the production of low chain peptides and amino acids that contribute to the characteristic flavor of the product. Menéndez et al. (2000) also obtained improvement on the sensory characteristics of Arzúa-Ulloa cheese by reduction of bitter flavor compared to the control sample. The authors tested, individually, five different strains of *Lactobacillus*: *Lactobacillus casei* ssp. *casei*, *Lactobacillus plantarum*, *Lactobacillus casei* ssp. *pseudopantarum* (two strains) and *Lactobacillus casei* (commercial strain)—in conjunct to the starter culture of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *lactis* var. *diacetylactis*.

The attribute texture (Table 3) showed higher punctuation (7.17 to 7.51) after 30 days of maturation for formulations 1, 5 and 6, characterized as “like moderately” in hedonic scale. Treatments 3 and 4 presented the lowest values for this attribute, differing statistically from the control ($p<0.05$), and classified in the hedonic scale as “like slightly” and “not like”, respectively. After 45 and 60 days of maturation, only formulation 4 (adjunct culture plus 0.2 % of extract) differed significantly from treatment 1, showing the lowest punctuation for this attribute.

Lawrence et al. (1987) relates that the texture of cheeses is directly related to the pH values and the relation between the α -caseins and the moisture content. The authors showed a good correlation between the firmness of the product and

the α -casein present. The hydrolysis of casein by the residual rennet retained by the mass and by the plasmin promotes the change of texture on the protein matrix, provoking the softening of the product. The products of the hydrolysis are short and long chain peptides and amino acids, precursors of compounds that contribute to the final flavor of the product. The main problems of texture include increase firmness, hardness and elasticity. This important attribute is commonly used to differ and classify the varieties of cheeses and is considered by the consumers as a reference of quality and global preference (Banks 2004; Kealy 2006).

Conclusions

The Prato cheese presented high moisture contents (49 to 53 %) and mean pH values of 5.15 for all tested formulations. The addition of mate leaves extract in the product did not influence the growth of the microbial cultures. During the maturation time, all formulations with the addition of adjunct cultures and mate leaves extract presented lower lipid oxidation, proving the antioxidant activity of mate extract. The mate tea extracts presented significant effect on protein oxidation after 30 days of storage in the products from formulations with 0.1 % and 0.2 % of extract. After 60 days of storage, the formulation with 0.2 % of extract seems to inhibit the protein oxidation, indicating that in this concentration the extract may have had the function of antioxidant toward proteins oxidation. The formulation of Prato cheese added of 0.1 % of extract presented acceptability of about 80 % after 30 days of maturation. The flavor of the product added by mate leaves extract presented residual bitter flavor after 45 days of storage.

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