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Evaluation of the melting capacity of Prato cheese obtained by modifications of the traditional manufacturing process

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Abstract – The objective of this research was to study the effect of the following modifications to the traditional manufacturing process of Prato cheese: use of milk concentrated by ultrafiltration (UFCM) up to a concentration factor (CF) of 4:1, and partial pre-fermentation of the UFCM and indirect curd cooking on the composition, pH, titratable acidity (TA) and melting capacity (MC) of the cheese as compared with the standard cheese. Three processings of each of the following five treatments were carried out: Treatment 1 (T1), without ultrafiltration; Treatment 2 (T2), ultrafiltration without pre-fermentation of the UFCM; Treatment 3 (T3), pre-fermentation of 100 g·kg^{-1} of the UFCM; Treatment 4 (T4), pre-fermentation of 200 g·kg^{-1} of the UFCM; and Treatment 5 (T5), pre-fermentation of 200 g·kg^{-1} of the UFCM, and the only one with indirect curd heating. There was a significant difference for total solids between cheeses T4 and T5 with respect to T2. T2 presented the lowest fat and fat in dry matter content, although the observed differences were not statistically significant. The UFCM cheeses presented lower values for fat recovery in cheeses with a significant difference between the treatments T2 and T3 with respect to T1, the fat losses in processing being the main factor affecting cheeses' composition. The pre-fermentation of 200 g·kg^{-1} of the UFCM increased the acidity and reduced the pH of the cheeses. The time did not exert an influence on the pH but it increased cheeses's TA and MC. There was no difference in MC between the treatments but there was a tendency for greater MC values for cheeses with a higher water to total protein ratio and higher pH value.

Cheese / ultrafiltration / pre-fermentation / cooking / melting

Résumé – **Évaluation de la capacité de fusion du fromage « Prato » obtenu par modifications du procédé de fabrication traditionnel.** L'objectif de cette recherche était d'étudier l'effet de modifications du procédé de fabrication traditionnel du fromage « Prato » sur la composition, le pH, l'acidité titrable (TA) et la capacité de fusion (MC) du fromage, comparé au standard traditionnel. Les modifications ont été les suivantes : utilisation de lait concentré par ultrafiltration (UFCM) jusqu'à un facteur de concentration (CF) de 4:1 ; pré-fermentation partielle de l'UFCM et

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cuisson indirecte du caillé. Trois répétitions ont été réalisées pour chacun des cinq traitements suivants : traitement 1 (T1), sans ultrafiltration ; traitement 2 (T2), ultrafiltration sans pré-fermentation de l'UFCM ; traitement 3 (T3), pré-fermentation de 100 g·kg^{-1} de l'UFCM, traitement (T4), pré-fermentation de 200 g·kg^{-1} de l'UFCM ; traitement 5 (T5), pré-fermentation de 200 g·kg^{-1} de l'UFCM. Une cuisson indirecte du caillé a été utilisée seulement dans le cas du traitement 5. L'analyse des données statistiques a montré que les fromages T4 et T5 ont présenté une différence significative par rapport au fromage T2. Ce fromage a présenté les plus faibles teneurs en matière grasse et en matière grasse dans la matière sèche, bien que les différences observées ne soient pas statistiquement significatives. Les fromages UFCM ont présenté une valeur plus faible pour la récupération de matière grasse dans les fromages avec une différence significative pour les traitements T2 et T3 par rapport à T1, la perte de matière grasse dans le processus étant le principal facteur affectant la composition des fromages. La pré-fermentation de 200 g·kg^{-1} du rétentat a augmenté l'acidité et réduit le pH ; le temps n'a pas eu d'influence sur le pH, mais la TA et la MC ont eu tendance à augmenter. Il n'y a pas eu de différence de MC entre les traitements mais les MC des fromages ayant un ratio eau/protéines totales et un pH plus élevés, ont eu tendance à augmenter.

Fromage / ultrafiltration / pré-fermentation / cuisson / fusion

1. INTRODUCTION

Prato cheese is one of the most produced cheeses in Brazil [34], being considered a cheese from semi-cooked and washed curd where the heating of the curd grains is obtained by the addition of hot water to the whey [1, 11, 38, 45, 47], this operation being indispensable to obtaining the organoleptic properties of the final product [45, 47]. However, the washing of the curd results in considerable losses of soluble constituents, which are removed together with the whey [12], tending to decrease the yield.

This cheese is popularly used as an ingredient in various dishes in which the cheese is frequently melted [27]. The use of Mozzarella, Cheddar and Prato cheeses in pizzas, toasted sandwiches and frozen foods are examples of this. For these cheeses, good melting properties are one of the determinant factors in their quality evaluation [28].

Thus numerous research results have been reported about the influence of the production variables (starters, coagulant kind and concentration, whey draining pH, and heat treatment during curd cooking), composition (moisture, salt, fat and calcium content), proteolysis and microstructure of the cheeses on their MC [5, 18–20, 22, 27, 28, 33, 35, 43]. With respect to composition, cheeses with higher water and fat content present the tendency to larger MC [19, 20, 28, 33, 43] and cheeses with a higher salt and mineralization degree (calcium content) have the tendency to lower MC [19, 20].

In Brazil, Prato cheese, like the majority of the other cheeses produced there, is obtained from non-concentrated milk. However, in countries such as Australia, France, the USA and others, there are frequent reports of cheese manufacture from milk concentrated by the technique of ultrafiltration [8, 15–17, 26, 29, 31].

The use of this technique, known as MMV, was proposed by Maubois et al. [32], based on the concentration of milk by ultrafiltration (UF) until a liquid pre-cheese was obtained, with a total solids content similar to that found in a finished cheese. The process shows the advantages of eliminating the draining stage, allowing a greater retention of milk fat and whey proteins, principally α-lactalbumin and β-lactoglobulin, which are normally lost during this stage in the traditional cheese processing. Thus, in addition to producing a permeate with a smaller biological oxygen demand, the MMV method also results in an increase

in the yield of cheese, from 8 to 20% [17, 24, 26, 30, 32, 36].

Despite the advantages offered by the MMV method, it is difficult to concentrate up to the levels required for hard and semi-hard cheeses using UF [17], and when one does manage to reach these levels, the cheeses obtained tend to show inferior quality as compared with cheeses produced by the traditional process [13, 29]. According to Renner and Abd El-Salam [40], semi-hard cheeses obtained by UF either do not melt or melt to a lesser extent when compared with cheeses produced by the traditional method.

As a result of the above-mentioned motives, some researchers [16, 17], tested the production of semi-hard and hard cheeses from milks concentrated to inter-mediate CF (p/p) values (4:1 to 6:1), and subsequently following the traditional processing steps. Although it did not result in great gains in yield, the use of inter-mediate CF allowed an increase in yield as compared with that of the traditional proc-ess [16, 17]. In addition, it resulted in a reduction in milk transportation costs, decrease in water consumption, increase in plant productivity and the production of cheeses with functional and organoleptic characteristics closer to those of the tradi-tional cheeses [15, 40, 50].

In Australia, in the manufacture of Cheddar cheese from milk partially con-centrated by UF, pre-fermentation of part of the retentate has been used, normally about 100 g·kg^{-1}, in order to obtain curd of normal strength, since the fermentation results in a significant production of ion-ized calcium, which helps in the formation of a coagulum [16]. Such a technique is necessary since the production of cheeses by ultrafiltration is slightly quicker than the traditional process and the use of this technique provides an adequate number of lactic bacteria in the final product [16].

Ribeiro [41] produced Prato cheese from UFCM with various CF (1.5:1; 2.5:1; 3.5:1 and 5.0:1), and concluded that the use

of such retentates allowed one to obtain cheeses with typical organoleptic and tex-tural characteristics and a greater yield than the traditional process. In the case of the cheese produced from a retentate with a CF of 5:1, it was only possible to obtain an adequate texture by pre-fermenting part (100 g·kg^{-1}) of the retentate with a lactic culture. However, this author did not study the effect of the use of UFCM and partially-fermented UFCM on the melting capacity of Prato cheese.

The objective of this research was to study the effect of the following modifica-tions to the traditional manufacturing proc-ess of Prato cheese: use of milk concen-trated by ultrafiltration up to a CF value = 4:1, and partial (100 and 200 g·kg^{-1}) pre-fermentation of the UFCM and indirect curd cooking on the composition, pH, TA and MC of the resulting cheeses as com-pared with the standard cheese.

2. MATERIALS AND METHODS

2.1. Ultrafiltration of the milk

Type B raw milk (250 kg) from the Milk Producers' Cooperative in the Campinas Region was heat treated at 68 °C/2 min. One part of this milk (50 kg) was cooled to 35 °C and used to produce a batch of standard Prato cheese (T1) and the other part (200 kg) was cooled to 55 °C and submitted to concentra-tion by ultrafiltration. The milk was concen-trated with Carbosep mineral membranes (Tech-Sep – Groupe Rhône Poulenc, Saint-Maurice de Beynost, France) with a molec-ular mass cut-off of 20 000 g·mol^{-1} and inlet and outlet pressures of 2.45×10^5 and 1.47×10^5 Pa, respectively, up to a CF of 4:1. The ultrafiltration concentration process was performed in approximately 10 h, after which the ultrafiltered milk was pasteurized at 62 °C for 30 min, cooled to 7 °C and divided into four equal parts (T2, T3, T4 and T5).

2.2. Cheese manufacture

Three processing procedures were carried out in this experiment, on different dates. In each processing, five batches of Prato cheese were manufactured (T1, T2, T3, T4 and T5). Batch T1 was processed by the conventional method from non-concentrated milk on the same day the milk was delivered to the pilot plant. The other four batches were manufactured the next day from UFCM. In the case of the cheeses manufactured from UFCM (T2, T3, T4 and T5), after cooling each of the four parts, a certain portion was removed from each (0 $g \cdot kg^{-1}$ from T2; 100 $g \cdot kg^{-1}$ from T3 and 200 $g \cdot kg^{-1}$ from T4 and T5) and submitted to a pre-fermentation with a mesophilic starter (G3 MIX 6-VISBYVA®-B50, composed of multiple mixed strains of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) at 32 °C. The rest of the UFCM was stored at 5 ± 1 °C until the next day. After the separated portions had reached pH 5.0, they were mixed with their respective UFCM batches, then the retentate was heated to 35 °C to start the processing of the cheese. Both the standard cheese and those prepared from UFCM were manufactured according to the basic steps of the traditional processing of Prato cheese. For the standard cheese, 50% calcium chloride (0.4 $mL \cdot kg^{-1}$ milk) was added to the milk already heated to 35 °C, followed by the addition of freeze-dried ready-to-use starter (one-tenth of the quantity recommended by the manufacturer), annatto dye (*Bixa orellana*) (Chr. Hansen Ind. E Com. Ltda, Valinhos, SP, Brazil) and rennet (calf rennet – 90% chymosin) (BV Ind. E Com. Ltda, Valinhos, SP, Brazil) in a quantity sufficient to coagulate the milk at 35 °C. A similar procedure was used for the cheeses manufactured from UFCM, using starter, dye and coagulating agent in quantities equivalent to what would be used if one converted the UFCM into milk, but without the addition of calcium chloride. After coagulation (40 min for all treatments), the curd was cut into 0.4 cm × 0.5 cm cubes, and allowed to rest for 5 min. The curds were then submitted to slow continuous mixing for 20 min (1st mixing), followed by removal of part of the whey and further heating of the curd (2nd mixing) to 41 °C with the addition of hot water, in order to obtain the correct consistency for Prato cheese. For the cheeses obtained from UFCM, no 1st mixing or removal of the whey was carried out, the curd being heated soon after the resting period, resulting in a reduction of the processing time of about 30 min. For the cheeses T2, T3 and T4, the curd was heated directly by the addition of hot water. For T5, the curd was heated indirectly. After heating, the curds were pre-pressed for 20 min in a draining press with twice the weight of the curd, and then placed in plastic molds and pressed for 6 h in a vertical press, with stainless steel weights. The cheeses were turned after 20 min during pressing and then every hour until removed from the press. The weights were gradually increased from 10 to 20 times the weight of the curd. Having completed the pressing time, the cheeses were stored at 7 ± 1 °C and the next day placed in a 200 $g \cdot kg^{-1}$ (NaCl) brine solution for a period of 8 h at 4 ± 1 °C. Finally, they were dried at 7 ± 1 °C, weighed, sealed under vacuum in heat-shrinkable plastic bags and stored at 7 ± 1 °C for 45 d.

2.3. Analyses

2.3.1. Composition of the cheeses

Total nitrogen (TN) was determined using the Kjeldahl method according to AOAC method 991.20 [2], using a factor of 6.38 to determine total protein. Soluble nitrogen at pH 4.6 (non-casein nitrogen – NCN) was determined according to Barbano et al. [3]. Total solids (TS) content was determined using AOAC method 925.23 [2], and the moisture (M) content using the equation: M = 1000 – TS. The fat content was determined by the Gerber method [7] and the fat content in the dry matter (FDM)
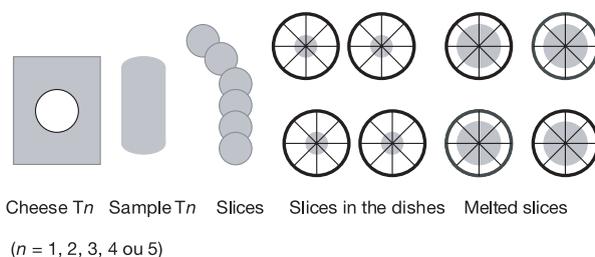
Cheese T*n*   Sample T*n*   Slices   Slices in the dishes   Melted slices

(*n* = 1, 2, 3, 4 ou 5)

Figure 1. Schematic representation of the test to determine the melting capacity of the cheeses.

using the equation: FDM = (Fat × 1000)/ TS. The moisture in defatted cheese (MDC) was calculated using the equation MDC = (M × 1000)/((TS – Fat) + M). The values for pH were determined by the potentiometric method and those for titratable acidity and the salt content using the AOAC method 920.124 [2] and the Volhard method [42], respectively. The salt content in the moisture (S/M) was calculated from the equation: S/M = (salt × 1000)/moisture. All the above determinations were carried out in triplicate on the cheeses after 10 d (D10) of refrigerated storage. In the same way, the pH, TA and NCN of the cheeses were monitored after 25 and 45 d of refrigerated storage (D25 and D45).

Total fat and protein cheese recovery from the milk (FCR and PCR) was evaluated with the following equation:

FCR or PCR = (cheese mass × % cheese component × 100)/(milk or UFCM mass × % milk or UFCM component),

where component means fat or protein.

2.3.2. Evaluation of the melting capacity of the cheeses

The melting capacity (MC) of Prato cheese was determined by the modified method of Schreiber, as described by Kosikowski [25]. The test was carried out in quadruplicate on the cheeses after 10, 25 and 45 d of storage (D10, D25 and D45).

For this test, one cheese from each treatment weighing about 0.5 kg was cut in half and a cylindrical sample removed from the center of one of the halves using a 0.036 m-diameter bore. This cylinder was cut into 0.007 m-thick slices using a modified egg slicer. Four slices of each sample (one from the surface, one from the center and 2 from the intermediary region) were placed individually in the center of a Petri dish marked with four lines at angles of 45° (Fig. 1). The dishes were covered and left at room temperature for 30 min. After this interval, the average initial diameter of each of the four slices was calculated as the average of the diameters in the four directions. Heating to evaluate melting was carried out for 7 min in an oven at 107 °C, after which the average final diameter was obtained in the same way as the average initial diameter. From these average diameters, the initial (Ai) and final (Af) areas of each slice were calculated and the melting capacity determined from these according to the equation: MC = (Af - Ai) × 100/Ai.

The arithmetic mean of the values for MC of the four slices of each sample provided the average melting capacity of each cheese.

2.3.3. Experimental design and statistical analysis

The statistical design adopted for this experiment was a split-plot block design, the treatments being applied to the plots and the time to the sub-plots. The batches of milk to be processed constituted the blocks. Three batches were carried out, one for each complete replicate, forming 3 blocks. The composition of the cheeses

Table I. Average composition (n = 3) of the cheeses: standard (T1), ultrafiltered without pre-fermentation (T2), ultrafiltered with 10% of pre-fermentation (T3), ultrafiltered with 20% of pre-fermentation (T4), and ultrafiltered with 20% of pre-fermentation, and with indirect heating (T5).

Variable	T1	T2	T3	T4	T5	LSD[1]
pH	5.37[a]	5.37[a]	5.28[ab]	5.00[b]	5.09[ab]	0.23
Titratable acidity (% lactic acid)	0.64[b]	0.71[b]	0.86[ab]	1.28[a]	1.12[ab]	0.35
Total solids (g·kg^{-1})	517.70[ab]	487.70[b]	528.90[ab]	549.50[a]	554.70[a]	38.62
Moisture (g·kg^{-1})	482.30[ab]	512.30[a]	471.10[ab]	450.50[b]	445.30[b]	38.62
Fat (g·kg^{-1})	252.10[a]	208.40[a]	230.60[a]	246.60[a]	246.10[a]	36.59
FDM (g·kg^{-1})	487.80[a]	425.80[a]	433.80[a]	449.20[a]	443.00[a]	55.53
MDC (g·kg^{-1})	644.82[ab]	647.00[a]	610.68[ab]	597.77[ab]	590.41[b]	37.15
Fat recovery (%)	87.31[a]	64.39[b]	67.08[b]	72.37[ab]	70.74[ab]	11.98
Total protein (g·kg^{-1})	184.20[a]	213.80[a]	225.10[a]	218.60[a]	222.40[a]	30.99
Protein recovery (%)	70.58[b]	80.17[a]	80.54[a]	80.68[a]	82.46[a]	4.46
Salt (g·kg^{-1})	16.00[a]	16.70[a]	14.80[a]	13.60[a]	14.40[a]	2.16
Salt/Moisture (g·kg^{-1})	33.17[a]	32.60[a]	31.42[a]	30.90[a]	32.34[a]	4.66

* means with the same letter, in the same line, do not differ significantly from each other ($P > 0.05$) (Tuckey's multiple comparison method). [1] least significant difference (LSD).

from the 5 treatments was evaluated statistically by the analysis of variance, using the procedure of multiple comparisons between pairs of means (Tukey's method) on the tenth day after manufacture of the cheeses. The data for the analyses of pH, titratable acidity, non-casein nitrogen and melting capacity of the cheeses were evaluated in the same way as those for composition, on the tenth, twenty-fifth and forty-fifth days of storage. All the calculations were carried out with the aid of the SAS statistical program (SAS System for Windows, Release 8.02 – SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Chemical composition

3.1.1. Average composition of the cheeses

The cheeses made from non-ultrafiltered milk and from concentrated milk without pre-fermentation did not differ in terms of pH and TA (Tab. I). However, increasing the percentage of pre-fermented concentrate increased the TA of the cheeses and decreased the pH value, due to a greater incorporation of lactic acid from the pre-fermented UFCM and its considerable active starter incorporation, and as consequence, the pH and TA of cheeses T4 and T5 were different from those normally found in Prato cheese, which are: pH of 5.2 to 5.5 [11, 38] and about 0.86% lactic acid [44], respectively.

There was a significant difference for total solids between cheeses T4 and T5 with respect to T2, which presented the lowest total solids value, and although there was not a significant difference between treatments T1, T3, T4 and T5, from the practical point of view the treatments T3, T4 and T5 presented higher values than T1. The trend in the difference between treatments T3, T4 and T5 with treatments T1 and T2 can also be observed in terms of moisture in deffated cheese
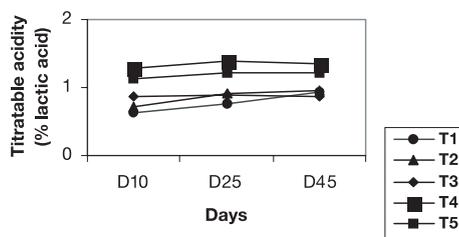
Figure 2. Evolution of TA during the 45 d of storage of the cheeses: standard (T1), ultrafiltered without pre-fermentation (T2), ultrafiltered with 10% of pre-fermentation (T3), ultrafiltered with 20% of pre-fermentation (T4), and ultrafiltered with 20% of pre-fermentation, and with indirect heating (T5).
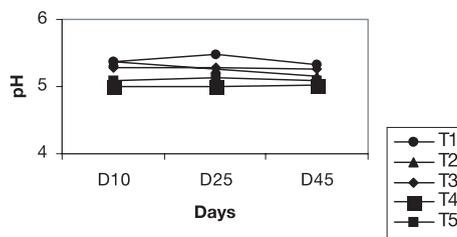

Figure 3. Evolution of pH during the 45 d of storage of the cheeses: standard (T1), ultrafiltered without pre-fermentation (T2), ultrafiltered with 10% of pre-fermentation (T3), ultrafiltered with 20% of pre-fermentation (T4), and ultrafiltered with 20% of pre-fermentation, and with indirect heating (T5).

(Tab. I) which shows that the treatments with retentate pre-fermentation presented similar lower values for MDC than treatments without it.

According to the Brazilian technical regulation for cheese identity and quality [6], Prato cheese is classified as a medium moisture content cheese (between 360 and 459 g·kg^{-1}) and as a high fat cheese (FDM content between 450 and 599 g·kg^{-1}), and thus only the cheeses from treatments T4 and T5 attained the legal norms with respect to moisture content, and only the standard cheese for FDM content (Tab. I). The cheeses made from ultrafiltered milk showed lower fat contents than those normally encountered in Prato cheese, which is from 260 to 290 g·kg^{-1} [11], and consequently did not present adequate FDM values.

The data presented in Table I show that, although statistically there was no significant difference between the fat contents or between the protein contents of the cheeses from the different treatments, in practical terms there were differences. Treatment T2 presented the lowest fat and fat in dry matter content and the use of pre-fermentation presented a tendency to increase the fat content of the ultrafiltered cheeses, such that T4 and T5 cheeses presented FDM values near to the minimum required by

the current legislation. The UFCM cheeses presented lower values for fat recovery in cheeses, with a significant difference between the treatments T2 and T3 with respect to T1, with T2 presenting the lowest value. Fat losses in processing were the main factor affecting the cheeses' composition. Such losses have already been reported in the literature [29] and have been attributed to a coarser UFCM cheese structure. Data from these authors being prepared for publication have shown differences in microstructure between UFCM cheeses with and without pre-fermentation, which helps to explain such differences in fat losses.

There was a significant difference for cheese protein recovery between cheeses T2, T3, T4 and T5 with respect to T1, which presented the lowest value, and as consequence T1 presented the smallest total protein content.

3.1.2. Evolution of pH and titratable acidity of the cheeses

Figures 2 and 3 show, respectively, the evolution of TA and of the pH of the cheeses from the five treatments during the 45 d of refrigerated storage.

A statistical analysis of the data for TA provided the following information: there

was no interaction for treatment × time ($P = 0.24$) and the values for TA of the cheeses showed statistically significant variations with time ($P < 0.01$) and with the type of treatment adopted ($P = 0.02$). The TA of the treatments increased significantly between the dates D10 and D25, although there were no significant differences between days D25 and D45. With respect to the treatments, according to Tukey's test, T1 did not differ significantly from treatments T2, T3 and T5, although it did differ from T4. T4 also differed from T1, but not from the other treatments.

An analysis of the data for pH showed there was no interaction of treatment × time ($P = 0.75$) and the factor time did not significantly influence ($P = 0.45$) the pH values of the different cheeses. However, these values were significantly influenced ($P = 0.03$) by the type of treatment adopted for the cheeses. Based on Tukey's test, it was shown that the pH data for T1 did not differ statistically from those obtained for treatments T2, T3 and T5 but did differ from those of T4. The values for T4 also differed from those observed for T1, but not from those observed for the other treatments.

The use of both UFCM and UFCM with 100 g·kg^{-1} pre-fermentation of the retentate did not significantly alter the acidity or pH of the cheeses. However, 200 g·kg^{-1} pre-fermentation of the UFCM increased the TA of the cheeses (Fig. 2) and decreased the pH value (Fig. 3). The type of heating adopted did not interfere in the TA and pH of these cheeses (Figs. 2 and 3), this result disagreeing with information found in the literature [45, 47] which attributed the lower acidity of cheeses elaborated with direct heating to a leaching effect of the curd by the hot water.

The TA of the five treatments increased between the tenth and twenty-fifth days of storage (Fig. 2), probably, according to Fox et al. [10], due to fermentation of the residual lactose by the starter. Although the TA of the cheeses increased during this
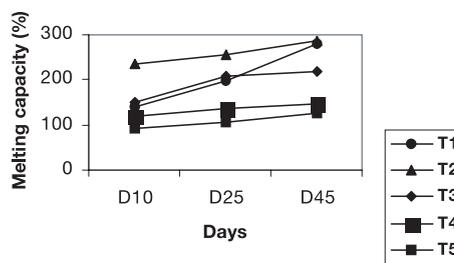

Figure 4. Evolution of melting capacity during the 45 d of storage of the cheeses: standard (T1), ultrafiltered without pre-fermentation (T2), ultrafiltered with 10% of pre-fermentation (T3), ultrafiltered with 20% of pre-fermentation (T4), and ultrafiltered with 20% of pre-fermentation, and with indirect heating (T5).

period, the pH values did not decrease, due to the buffering capacity presented by the cheeses after 10 d of storage. According to Fox et al. [10], the buffering capacity of cheeses greatly increases when the pH presents values below 5.5, and all the cheeses manufactured in this experiment presented pH values below 5.5 on D10 (Fig. 3).

The cheeses made from partially pre-fermented (100 and 200 g·kg^{-1}) UFCMs, showed similar behavior for pH to that found by Metzger et al. [35]. These researchers studied the behavior of Mozzarella cheese made from milk chemically pre-acidified to different pH values, during 90 d of storage, and showed that the pH values either remained stable or decreased with time.

3.1.3. Evolution of the melting capacity of the cheeses

The melting capacity of the cheeses can be visualized in Figure 4.

The statistical analysis of the MC data provided the following information: there was no interaction between treatment × time ($P = 0.49$), and the type of treatment did not significantly influence ($P = 0.25$)

Table II. Average non-casein nitrogen ($n = 3$) of the cheeses: standard (T1), ultrafiltered without pre-fermentation (T2), ultrafiltered with 10% of pre-fermentation (T3), ultrafiltered with 20% of pre-fermentation (T4), and ultrafiltered with 20% of pre-fermentation, and with indirect heating (T5).

Storage	Non-casein nitrogen ($g \cdot kg^{-1}$)				
	T1	T2	T3	T4	T5
D10	2.80	4.30	4.70	6.20	5.50
D25	4.00	5.70	6.30	7.40	6.90
D45	4.80	6.60	7.60	9.50	8.50

this functional property, although the latter did suffer significant alterations ($P < 0.01$) with time. According to Tukey's test, $MC_{D10} < MC_{D25} = MC_{D45}$.

The MC of the cheeses from the five treatments increased during storage, probably due to cheese proteolysis and degradation of the protein matrix, which probably resulted in the loss of the cheese's ability to maintain its structure during heating [4, 20, 33, 43, 46]. The loss of matrix integrity is reported as one of the main factors determining the increase in MC of cheeses [28, 33]. The cheeses manufactured in this experiment suffered primary proteolysis (PP) during storage (Tab. II) (PP-D10 < PP-D25 < PP-D45), which in part justifies the increase in MC observed during refrigerated storage.

Although the increase in MC of cheeses observed during storage is normally associated with cheese proteolysis, it has also been reported that changes in water partitioning from expressible water to entrapped water can be responsible for changes in the cheeses' melting capacity [14, 28, 33].

During storage, a decrease in expressible water of the cheese usually occurs (water whose mobility is not impeded by the macro-structure of the protein matrix and which can be expressed by centrifugation), such water being found together with the fat and the starter organisms in vacuoles and elongated pockets [33, 39]. According to McMahon et al. [33], after salting of the cheese, reorganization of the protein matrix possibly occurs, with subsequent absorption of water by these vacuoles/pockets during refrigerated storage. Thus the expressible water decreases since it becomes entrapped water (water physically retained in the spaces between the sub-aggregates of protein that form the basic structure of the protein matrix), and as this phenomenon progresses, so the protein network becomes more hydrated and some interactions between proteins are substituted by interactions between protein and entrapped water, factors which allow the cheese to melt more easily [33].

According to Guo and Kindstedt [14] and McMahon et al. [33], one can obtain expressible water from cheeses for a maximum of 20 d. In this experiment, the cheeses presented an increase in MC during storage (Fig. 4), although this increase was only significant between the tenth and twenty-fifth days of storage, the increase in MC occurring during this period being a function of proteolysis occurring in the cheeses and of changes in the protein-protein and protein-moisture interactions. After this period the MC continued to increase, although not significantly, since it only suffered an additional effect of proteolysis, which continued to occur.

Since the property of melting is influenced by numerous physical and chemical factors, even cheeses belonging to the same category may respond differently to the same time × temperature binomial of heating [21]. Thus it becomes difficult to compare the results in Figure 4 with those obtained by other authors using different processing conditions. It was only possible to verify that in this experiment, as in those carried out by other authors [35, 37, 43, 48, 49], the MC of the cheeses increased during storage, mainly during the first 25 d.

Metzger et al. [35], working with Mozzarella cheese from milk chemically pre-acidified to different pH values and comparing them with Mozzarella made with normal milk, also showed that the MC of the cheeses was

not affected by pre-acidification ($P > 0.05$), but was affected by time ($P < 0.05$).

Although no significant differences in MC were observed between the treatments in this experiment, in data of Figure 4 a tendency for the following behavior can be observed: MCT4 = MCT5 < MCT1 = MCT3 < MCT2, mainly in the period between D10 and D25.

Treatments T1, T2 and T3 presented a greater tendency to melt due to their greater ratio of water to protein (2.62, 2.4 and 2.10, respectively), resulting probably in a more hydrated protein matrix, which, according to McMahon et al. [33], allows the proteins to flow more easily when heated, increasing the melting capacity of the cheese. However, considering that these cheeses were exposed to the same time × temperature heating binomial, the less moist cheeses with greater fat contents (T1 and T3) tended to transfer heat more rapidly to the protein matrix, which thus received a greater amount of energy [9].

Lefevere et al. [28] observed that, during the heating of cheese, part of the heat is used to rearrange the protein-protein interactions. Thus when the cheese is heated, the protein matrix absorbs part of the energy, which influences the interactions that maintain the protein structure, and in the range from 60 to 80 °C, protein unfolding starts to occur, exposing hydrophobic sites and leading to a greater aggregation between the protein molecules [27] and, according to Lefevere et al. [28], greater protein-protein interaction results in lower MC. Thus the T2 cheese may have presented a higher MC as a function of less protein-protein aggregation.

Treatments T4 and T5, in addition to having smaller ratios of water to protein in the cheese, also presented lower pH values, justifying their lower MC values (Fig. 4). Ramkumar et al. [39] observed that the amount of whey centrifuged from a model Cheddar cheese decreased with increase in pH of the curd, suggesting that the capacity of the curd to physically retain water mol-

ecules, like the casein-moisture interactions in the cheese, were pH-sensitive. Kindstedt et al. [23], working with Mozzarella cheese, also reported that the pH strongly affected the protein-moisture interactions and consequently the melting of the cheese, the cheeses losing their melting capacity at pH values below 5.0.

4. CONCLUSIONS

It is possible to manufacture Prato-type cheese from milk concentrated by ultrafiltration (CF = 4:1) with a proximate composition similar to that of cheese produced from non-concentrated milk.

The pre-fermentation of 200 $g \cdot kg^{-1}$ of the retentate increases the acidity and decreases the pH of the cheeses. The type of cooking does not interfere with these parameters.

The time factor does not influence the pH values but does increase the titratable acidity and melting capacity of the cheeses.

There was statistically no difference in melting capacity between the cheeses from the different treatments, although there was a tendency for the cheeses with a greater amount of entrapped water, due to a greater water, to protein ratio and higher pH values, to melt more.

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