

## Effect of the casein to fat ratio on the functional properties of Prato cheese

Efeito da relação caseína/gordura nas propriedades funcionais do queijo Prato

Efecto de la relación caseína/grasa sobre las propiedades funcionales del queso Prato

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### **Abstract**

This study aimed to evaluate the effect of casein to fat ratios (0.68, 0.72, and 0.76) on the functional properties of Prato cheese at 2, 15, 30, and 45 days of ripening. The different ratios did not interfere with the physicochemical characteristics, pH, Aw, proteolysis indices, color parameters, sliceability and sensory profile of the cheeses among the treatments. However, pH, proteolysis indices, texture profile, L\* and b\* color parameters of the cheeses changed through the ripening time. The cheeses made from milk standardized to casein/fat ratio of 0.68 presented lower chewiness values. The casein to fat ratio of 0.68 increased the protein and fat recoveries, and provided greater meltability and greater formation of free oil when compared to cheeses made from milk with casein to fat ratio of 0.76. Therefore, casein to fat ratio of 0.72 is recommended for the production of Prato cheese as the cheese presents good functional properties at this value, such as intermediate melting and chewiness, and less release of free oil.

**Keywords:** Brazilian cheese; Yield; Meltability; Free oil release.

### **Resumo**

O objetivo deste trabalho foi avaliar o efeito das relações caseína/gordura 0,68, 0,72 e 0,76 nas propriedades funcionais do queijo Prato com 2, 15, 30 e 45 dias de maturação. As diferentes relações não interferiram nas

características físico-químicas, pH, Aw, proteólise, cor, fatiamento e na avaliação sensorial entre os tratamentos. No entanto, houve diferença em relação ao tempo para o pH, índices de proteólise, perfil de textura, L\* e b\*. A mastigabilidade foi menor nos queijos fabricados com a relação caseína/gordura de 0,68. O uso da relação 0,68 aumentou as recuperações de proteína e gordura, além de proporcionar maior capacidade de derretimento e maior liberação de óleo livre, quando comparado aos queijos fabricados com a relação 0,76. Portanto, uma relação caseína/gordura de 0,72 é recomendada para a produção de queijo Prato, pois o queijo apresenta boas propriedades funcionais nesse valor, como derretimento e mastigabilidade intermediários, e menor liberação de óleo livre.

**Palavras-chave:** Queijo brasileiro; Rendimento; Derretimento; Liberação de óleo livre.

### Resumen

El objetivo de este trabajo fue evaluar el efecto de las relaciones caseína/grasa 0,68, 0,72 y 0,76 sobre las propiedades funcionales del queso Prato a los 2, 15, 30 y 45 días de maduración. Las diferentes relaciones no afectaron las características fisicoquímicas, pH, Aw, proteólisis, color, corte y evaluación sensorial entre tratamientos. Sin embargo, hubo diferencia en cuanto al tiempo para pH, índices de proteólisis, perfil de textura, L\* y b\*. La masticabilidad fue menor en los quesos elaborados con una relación caseína/grasa de 0,68. El uso de la relación 0,68 incrementó la recuperación de proteína y grasa, además de brindar mayor capacidad de fusión y mayor liberación de aceite libre, en comparación con los quesos elaborados con la relación 0,76. Por lo tanto, se recomienda una relación caseína/grasa de 0,72 para la producción de queso Prato, ya que el queso tiene buenas propiedades funcionales a este valor, como fusión y masticabilidad intermedias, y una menor liberación de aceite libre.

**Palabras clave:** Queso brasileño; Rendimiento; Fusión; Liberación de aceite libre.

## 1. Introduction

Prato cheese is a typical Brazilian cheese that stands out in the national dairy market to prepare quick snacks, and as an ingredient for sandwich making and other culinary preparations (Costa et al., 2018). It was originated from Danbo or Gouda cheese, with yellow color, smooth taste and a soft texture and represents one of the most consumed cheeses in Brazil (Silva et al., 2018).

The functional properties of cheeses are important attributes to maintain the desired characteristics for consumption and have been used as quality parameters (Martinez-Martínez & Velez-Ruiz, 2019). The functional characteristics of Prato cheese such as sliceability, meltability, and oil release are increasingly important criteria to meet the demand for its use as an ingredient (Ah & Tagalpallewar, 2017).

Sliceability is an important functional property of Prato cheese since Brazilians have the habit of consuming this cheese in sliced form (Furtado, 2019), while the meltability is important for cheeses used as ingredients in hot preparations. During heating, the cheese begins to melt uniformly, as water evaporates and milk fat begins to liquefy (Fox et al., 2017). Melting leads to the formation of free oil due to the weakening of the protein matrix, which allows the agglomeration of fat globules towards the surface of the cheese (Dai et al., 2019). The formation of a thin layer of free oil on the surface of the cheese is necessary to prevent excessive moisture loss during heating, in addition to visually providing a glossy appearance to the melted cheese (Fox et al., 2017).

In cheese manufacture, milk standardization is a necessary step, for example, due to the natural seasonal-related variations in milk composition. The standardization aims to obtain a cheese with consistent composition and texture, as well as maximum retention of milk components (Hill & Ferrer, 2021; Jurado & Ruiz-Navarro, 2018). Among the various protocols for milk standardization, the protein to fat ratio, or more specifically the casein to fat ratio (C/F ratio) stands out (Hill & Ferrer, 2021), and is specific to each cheese variety (Jurado & Ruiz-Navarro, 2018).

The C/F ratio has a marked influence on cheese composition, functionality, and sensory attributes of cheeses (Sameen et al., 2016). However, although milk standardization using the casein to fat ratio is a very important tool, a standard protocol has not been established for all types of cheese (Costa et al., 2017). Moreover, this work was proposed using milk fat contents close to those commonly used for manufacturing Prato cheese, so that the results of the casein to fat ratios would be more

applicable. Therefore, this study aimed to evaluate the effect of the casein to fat ratio on the physicochemical characteristics, sensory parameters, texture profile, color measurements, functional properties, and yield of Prato cheese.

## 2. Material and Methods

### 2.1 Milk standardization for cheese making

The milk samples were standardized for three different casein to fat ratios within the range reported by Costa Júnior & Pinheiro, (1999): 0.68, 0.72, and 0.76. The calculations were performed using the formula 'fat = casein divided by C/F ratio', based on the casein (Silva et al., 1995) and fat contents of milk (Brasil, 2006). Cream from raw milk used in the cheese manufacture was used to standardize the milk fat content.

### 2.2 Prato cheese manufacture

The cheese manufacture was carried out using 100 liters of milk, on four different days, according to Costa, Sobral, Teodoro, Costa Júnior, et al. (2018)

The physicochemical composition of the cheeses was determined after 2 days of ripening. The determinations of pH, water activity ( $A_w$ ), proteolysis indices, texture profile, meltability, free oil formation, sliceability, and color measurements were carried out at 2, 15, 30, and 45 days of ripening. The sensory evaluation was performed after 30 days of ripening.

### 2.3 Physicochemical composition

Standardized milk and whey from cheese making (collected 20 minutes after the first mixing time) were analyzed for protein, moisture, fat and pH (Brasil, 2006).

The cheeses were characterized for moisture, fat, fat-in-dry-matter (FDM), protein, ash content and chloride (Brasil., 2006).

Water activity ( $A_w$ ) was measured by direct readings on the Aqualab® apparatus (Decagon, Washington, USA), and pH was measured in a pH meter (Brasil., 2006). Total nitrogen (TN) was determined by the Kjeldahl method (AOAC, 1995), and the pH 4.6-soluble nitrogen ( $SN_{pH4.6}$ ), and 12% trichloroacetic acid-soluble nitrogen ( $SN_{TCA12\%}$ ) was determined according to Nepomuceno, Costa Junior, & Costa (2016). Proteolysis was calculated by the extension and depth indices, using the following equations: Extension index =  $(SN_{pH4.6}/TN) \times 100$ , and Depth index =  $(SN_{TCA12\%}/TN) \times 100$ .

### 2.4 Recovery of milk constituents and cheese yield

Actual cheese yield (kg/100kg) and dry matter cheese yield (YDM) were calculated according to Alves et al. (2013), as shown in Equation 1:

$$YDM = R \times \frac{[100 - (\% \text{ actual moisture} + \% \text{ actual salt})]}{[100 - (\% \text{ desired moisture} + \% \text{ desired salt})]} \times 100 \quad (\text{Equation 1})$$

Where  $R$  is the actual cheese yield (kg cheese per 100kg milk), 1.2% desired salt, and 42% desired moisture.

The fat (FR) or protein (PR) recoveries to whey and cheese were calculated using Equation 2 (Alves et al., 2013):

$$FR \text{ or } PR = \frac{\text{weight of sample} \times \% \text{ component in the sample}}{\text{weight of milk} \times \% \text{ component in milk}} \times 100 \quad (\text{Equation 2})$$

\*protein or fat content of whey or cheese

## 2.5 Texture profile analysis

The texture profile analysis (TPA) was performed in a CT3 texture analyzer (Brookfield, Middleboro, USA). For the analysis, cheeses were cut into cubes with 20 mm edge and stored at 12 °C for 1 hour and 30 minutes before the tests. The test conditions were 30% compression; probe 20 mm height and 50.8 mm diameter, test speed 1 mm/s; load cell 4500g. For the cheese cut test, 65% compression, and probe: cutting wire 0.33 mm diameter and 40 mm wide was used. Hardness, chewiness, adhesiveness, elasticity, cohesiveness, and cut test were measured in at least five replicates for each treatment.

## 2.6 Color measurements

The color values (L\*, a\*, b\*) were measured by the CIE L\*a\*b\* system, using a CM-5 spectrophotometer, with a lower aperture (Konika Minolta, Sensing Americas, Inc.), with reflectance in the wavelength range from 360 nm to 740 nm, aperture size LAV 30 mm, using specular component included (SCI), CM-A158 target mask for mini Petri dish measurements, and conventional Petri dish CM-A128.

## 2.7 Sliceability

Sliceability was evaluated using a stainless steel manual slicer, according to Nepomuceno (2012). The samples were previously stored at 11-13 °C and then cut into slices proximate 2 mm thick. During the operation, the adherence of the cheese to the blade, the appearance, and the integrity of the slices were visually evaluated.

## 2.8 Melting capacity

The melting capacity (MC) was determined as reported by Kosikowski, (1982) according to Equation 3.

$$MC(\%) = \frac{R \times (Df^2 - Di^2) \times 100}{Di^2} \quad (\text{Equation 3})$$

Where Df and Di are the final and the initial diameter of the sample, respectively.

## 2.9 Free oil release

The free oil release was evaluated in duplicate, by the modified Gerber method (Kindsted t& Fox, 1991), and calculated according to Equation 4.

$$\% \text{ free oil released} = \frac{\% \text{ free oil of the cheese} \times 100}{\% \text{ fat of the cheese}} \quad (\text{Equation 4})$$

Where % free oil from the cheese = fat measured on the butyrometer scale

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## 2.10 Sensory evaluation

The sensory acceptance test was performed using a nine-point hedonic scale as described by Meilgaard, Civille, & Carr, (2006) with adaptations. For that, 120 untrained assessors (62 male and 58 female) aged 18 to 44 years, randomly selected, participated in the test after approval of the UFJF Research Ethics Committee (15517219.4.0000.5147). The cheese samples (cut into cubes of 3 cm of edge, served in plastic plates) were coded with three random digits in a monadic and randomized manner and presented to the assessors.

## **2.11 Experimental design**

The experiment was carried out in randomized blocks with split plots over time, in four replications. A block design was used to evaluate yield and the sensory profile, which were performed once. Data were subjected to analysis of variance, followed by Tukey's test, with a significance level of 5% ( $P < 0.05$ ), using R Core Team, version 2015.

## **3. Results and Discussion**

### **3.1 Physicochemical composition of milk**

The milk used for the cheese manufacturing processes presented similar proximate composition as it was collected from the same bulk tank, except for the fat content, which showed a significant difference ( $P < 0.05$ ) among the treatments due to the milk standardization as a function of casein to fat ratio (C/F ratio) (Table 1).

The standardization of milk fat (% w/v) as a function of the casein content (% w/v) aims to prevent drastic changes in fat-in-dry-matter (FDM) of the cheese, which is important to maintain the typical characteristics of the product and its functional properties and to prevent loss of these constituents in whey.

### **3.2 Physicochemical composition of Prato cheese**

The different C/F ratios in milk did not significantly affect the proximate composition of the cheeses (Table 1), thus all cheeses were in compliance with the Brazilian legislation for the moisture content and FDM, and were classified as a medium-moisture cheese (36.0%-45.9%) and full-fat cheese (45.0% - 59.9% of FDM) (Brasil, 1997). Several factors including the standardization of the manufacturing procedures, including coagulation time, curd particle, mixing time, curd washing, pressing time, and storage time in the drying chamber can explain the similarity among the treatments studied.

In addition, the values used for standardizing milk fat to achieve the C/F ratios were very close, ranging from 3.2% to 3.6%. These C/F ratios were defined to include the fat content of Prato cheese made in Brazil. Other works that evaluated C/F ratio use extreme values in determining these relationships, such as Sameen et al. (2016), who used C/F ratios ranging from 0.4 to 1.6, that impact in the cheese composition. Other studies (Kumar et al., 2011; Sheibani et al., 2019) worked with a difference between the evaluated C/F ratios of 0.10, for example, 0.6-0.7, which also gave different results in the composition of cheeses, while the differences between the C/F ratios in the present work were 0.04.

No significant differences were observed for the fat contents of the cheeses ( $P > 0.05$ ), probably due to the close values of the casein to fat ratio in milk. Also, the salt concentration was similar for all cheeses, due to the moisture contents that presented no significant differences (Fox et al., 2017; Fox & McSweeney, 1998).

**Table 1.** Composition and physicochemical characteristics of milk and Prato cheese at 2 day of ripening (mean  $\pm$  SD).

Milk	Treatments (C/F ratio)		
	0.68	0.72	0.76
Moisture (% w/w)	87.50 $\pm$ 0.22 <sup>a</sup>	87.53 $\pm$ 0.24 <sup>a</sup>	87.94 $\pm$ 0.34 <sup>a</sup>
Protein (% w/w)	3.42 $\pm$ 0.10 <sup>a</sup>	3.40 $\pm$ 0.35 <sup>a</sup>	3.44 $\pm$ 0.33 <sup>a</sup>
Casein (% w/w)	2.43 $\pm$ 0.01 <sup>a</sup>	2.43 $\pm$ 0.01 <sup>a</sup>	2.43 $\pm$ 0.01 <sup>a</sup>
Fat (% w/w)	3.60 $\pm$ 0.02 <sup>a</sup>	3.40 $\pm$ 0.02 <sup>b</sup>	3.20 $\pm$ 0.02 <sup>c</sup>
pH	6.41 $\pm$ 0.43 <sup>a</sup>	6.48 $\pm$ 0.30 <sup>a</sup>	6.46 $\pm$ 0.19 <sup>a</sup>
<b>Cheese</b>			
Moisture (% w/w)	44.63 $\pm$ 0.27 <sup>a</sup>	45.70 $\pm$ 2.25 <sup>a</sup>	44.42 $\pm$ 1.93 <sup>a</sup>
Fat (% w/w)	27.37 $\pm$ 0.75 <sup>a</sup>	26.12 $\pm$ 1.03 <sup>a</sup>	26.00 $\pm$ 0.82 <sup>a</sup>
Protein (% w/w)	21.94 $\pm$ 0.58 <sup>a</sup>	22.45 $\pm$ 0.84 <sup>a</sup>	22.47 $\pm$ 1.30 <sup>a</sup>
Fat in dry matter (% w/w)	49.44 $\pm$ 1.45 <sup>a</sup>	48.19 $\pm$ 2.91 <sup>a</sup>	46.79 $\pm$ 0.99 <sup>a</sup>
Ash content (% w/w)	3.03 $\pm$ 0.24 <sup>a</sup>	3.10 $\pm$ 0.08 <sup>a</sup>	3.09 $\pm$ 0.27 <sup>a</sup>
Chloride (% w/w)	0.73 $\pm$ 0.24 <sup>a</sup>	0.79 $\pm$ 0.28 <sup>a</sup>	0.80 $\pm$ 0.27 <sup>a</sup>

<sup>a,b</sup>Means within a row with different superscript letters differ ( $P < 0.05$ ). n=4. Source: Authors.

### 3.3 Aw, pH and proteolysis indices

Concerning the Aw, there were no significant differences ( $P > 0.05$ ) among the treatments and during the ripening period. The aw mean values of cheeses made with milk standardized to C/F ratios of 0.68, 0.72, and 0.76 were  $0.9547 \pm 0.0356$ ,  $0.9660 \pm 0.0024$ , and  $0.9648 \pm 0.0074$ , respectively. There was no reduction of the Aw values during ripening, as the cheeses were packaged, which prevented changes in the moisture content.

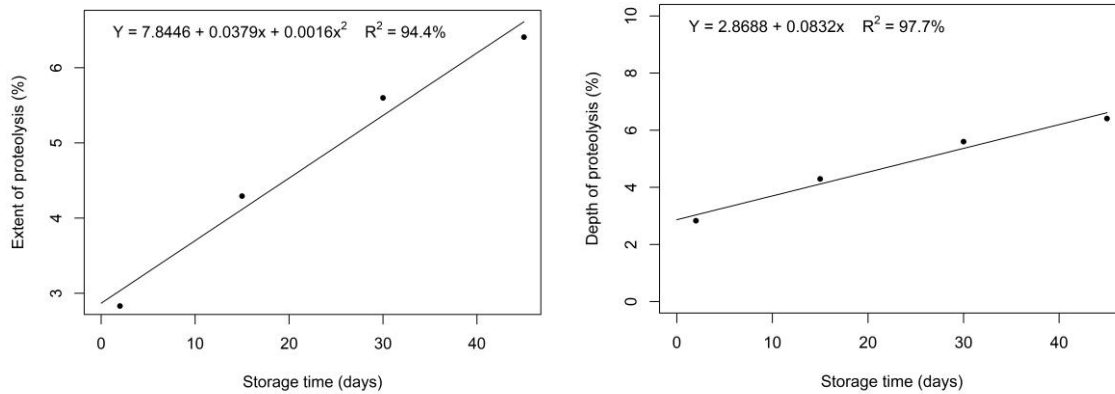
The mean pH values of the cheeses made from milk standardized to C/F ratios of 0.68, 0.72, and 0.76 were  $5.14 \pm 0.10$ ,  $5.17 \pm 0.10$ , and  $5.13 \pm 0.06$ , respectively, with no significant differences ( $P > 0.05$ ) among the treatments and during the ripening period. This parameter is influenced by the moisture contents, which did not present significant differences ( $P > 0.05$ ). The moisture content is directly related to the lactose content in cheese whey, which is fermented by starter culture, with pH reduction due to the release of lactic acid (Walstra et al., 1999). The same starter culture was used in all treatments, which can also explain the pH results. Changes in pH are also dependent on the cheese's buffering capacity, due to the concentration of proteins and mineral salts (Narimatsu et al., 2003).

Regarding the extent and depth of proteolysis, there was no significant difference ( $P > 0.05$ ) among the treatments. The mean values of extent of proteolysis of cheeses made from milk standardized to C/F ratios of 0.68, 0.72, and 0.76 were  $10.04 \pm 2.22$ ,  $9.92 \pm 2.26$ , and  $9.89 \pm 2.42$ , respectively. Concerning the depth of proteolysis, cheeses made from milk with C/F ratios of 0.68, 0.72, and 0.76 presented mean values of  $4.46 \pm 1.62$ ,  $4.96 \pm 2.20$ , and  $4.92 \pm 1.75$ , respectively. Several factors can directly affect the proteolytic activity of cheeses, including moisture, salt content, water activity, and pH (Rulikowska et al., 2013), which presented no significant differences in this study ( $P > 0.05$ ).

During cheese ripening, an increase in both proteolysis indices was observed, thus the results were plotted in a single curve since there were no significant differences among the treatments, and the models adjusted accordingly (Figure 1). The increase in proteolysis indices was due to the protein degradation by the residual action of the coagulant, which affects the extent of proteolysis. In turn, the proteolytic enzymes produced by lactic acid bacteria act on the nitrogen compounds

generated in the extent of proteolysis, which increases the depth of the proteolysis index (Alves et al., 2013). It is noteworthy that the amount of coagulant and the starter culture used in the processes was standardized for all treatments.

**Figure 1.** Extent and depth of proteolysis of Prato cheeses during the ripening period. Results expressed in average.



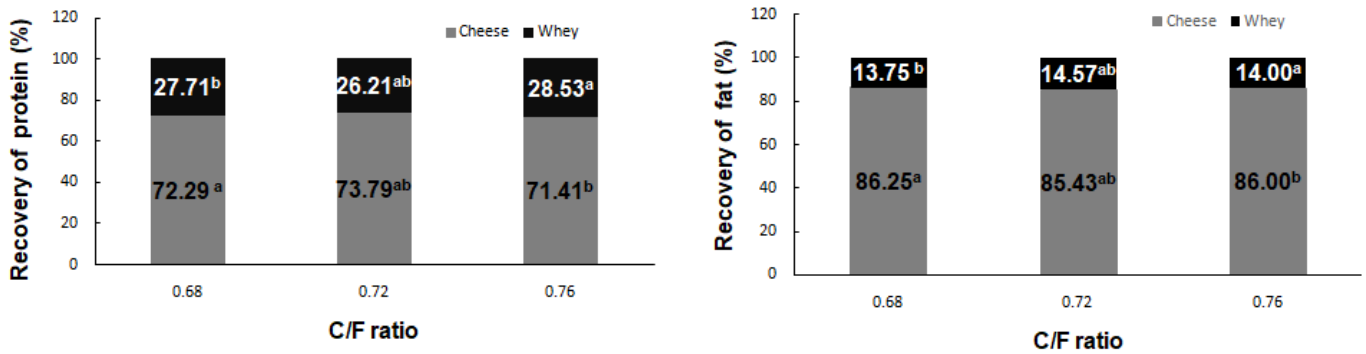
Source: Authors.

### 3.4 Recovery of milk constituents and cheese yield

The actual cheese yield and dry matter cheese yield (YDM) were similar among the treatments, with no significant differences ( $P > 0.05$ ) for the moisture and salt levels of the cheeses, which are parameters used to calculate YDM. The cheeses made from milk standardized to C/F ratios of 0.68, 0.72, and 0.76 presented percent yields of  $11.17 \pm 0.37$ ,  $10.99 \pm 0.29$ , and  $10.79 \pm 0.38$ , respectively, with no significant differences ( $P > 0.05$ ) among the treatments. Some processing factors can interfere with yield, including milk composition, coagulant used, coagulation time, and curd cutting step (Sales et al., 2016). In this study, the milk fat was the only variable, and all manufacturing procedures were standardized for the three treatments, which can explain no significant differences in cheese yields.

A significant difference ( $P < 0.05$ ) was observed for the protein and fat recoveries in cheese (Figure 2). Cheeses made from milk standardized to C/F ratio of 0.68 showed better protein and fat recovery, and lower loss of these components in whey when compared to cheeses made from milk with C/F ratio of 0.76. In contrast, no significant differences were observed for the protein and fat recoveries between the cheeses made from milk standardized to C/F ratios of 0.72 and 0.76 ( $P > 0.05$ ). Despite the differences in protein and fat recoveries, no significant differences ( $P > 0.05$ ) were observed for the protein and fat levels of the cheeses and whey. The difference among the results is due to the evaluation method used. The determination of the protein and fat content in the cheese was carried out through the analysis of the proximate composition, while the recovery of the constituents was determined through comparison among the content of the constituents in cheese and milk. This result highlights the importance of the method used to determine yield by the cheese industries, which generally assess only the fat or protein contents of whey, without determining the protein and fat recoveries in cheese. For the dairy industry, better protein and fat recoveries lead to an increase in process yield and consequently an increase in profitability.

**Figure 2.** Recovery of fat and protein in cheese and whey (%).



<sup>a,b</sup> Means with different superscript letters differ ( $P < 0.05$ ).  $n=4$ . Source: Authors.

### 3.5 Texture profile analysis e color measurements

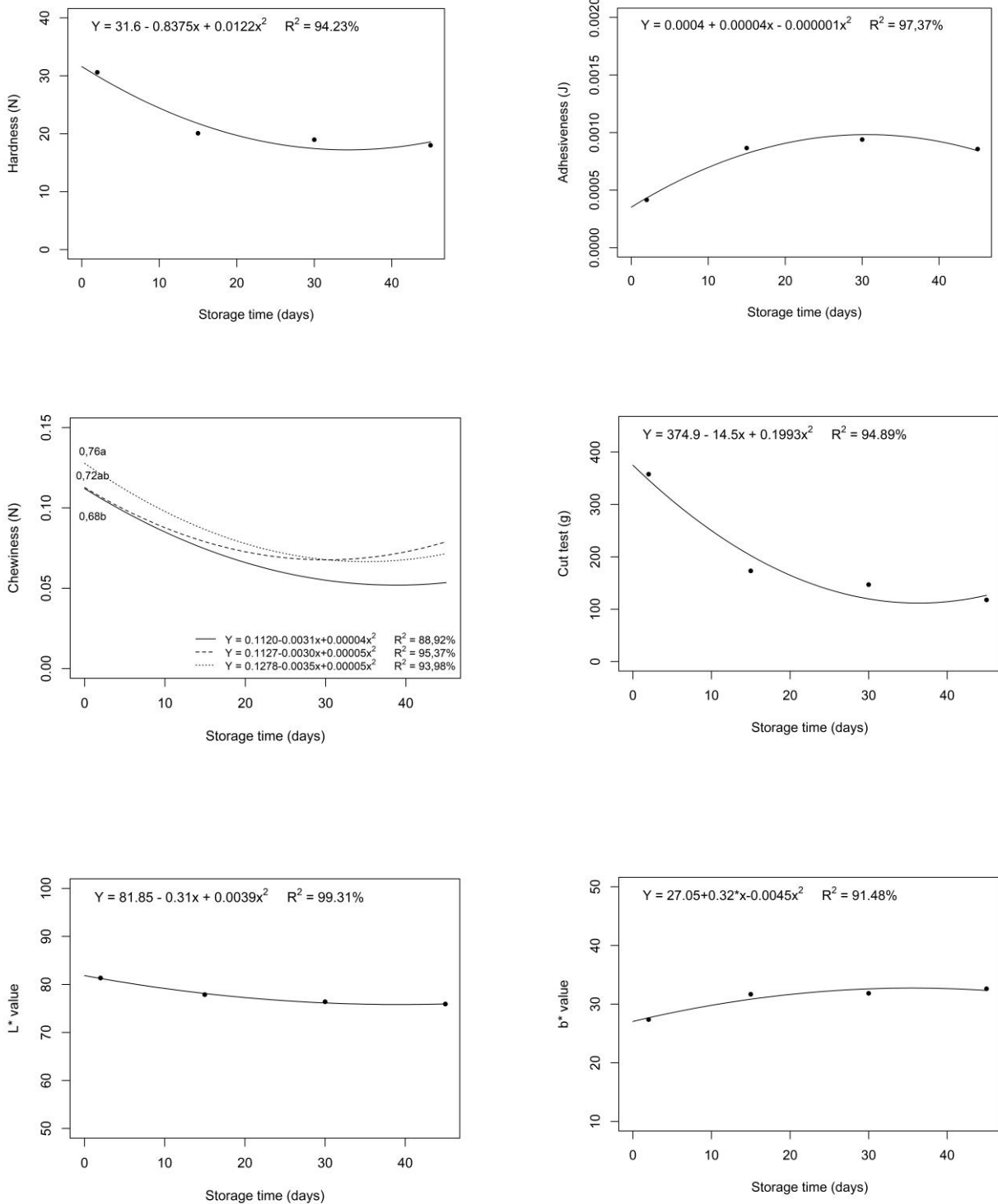
The different casein to fat ratios in milk of this study did not affect the parameters hardness, adhesiveness, cohesiveness, elasticity, and cut test of the cheeses ( $P > 0.05$ ), while significant differences ( $P < 0.05$ ) were observed for the parameter chewiness among the treatments. The cheeses made from milk standardized to C/F ratio of 0.68 had lower chewiness when compared to those made from milk with C/F ratio of 0.76 (Figure 3). Therefore, the cheeses made from milk with higher fat content required less force from chewing to swallowing (Sameen et al., 2016) even with no difference in their fat contents. No significant differences were observed for the texture parameters of the treatments made from milk standardized to C/F ratios of 0.72 and 0.76 ( $P > 0.05$ ). The increase in proteolysis during ripening led to a decrease in hardness, chewiness, and cut test, and an increase in adhesiveness. The cohesiveness mean values of the cheeses made from milk standardized to C/F ratios of 0.68, 0.72, and 0.76 were  $0.73 \pm 0.02$ ,  $0.73 \pm 0.02$ , and  $0.74 \pm 0.01$ , respectively, with no significant differences throughout the storage ( $P > 0.05$ ). A similar result was observed for elasticity, with mean values of  $4.93 \pm 0.13$ ,  $4.95 \pm 0.12$ , and  $4.96 \pm 0.09$  mm for the cheeses made from milk with C/F ratios of 0.68, 0.72, and 0.76, respectively. As reported by Fox & McSweeney (1998), pH can affect cohesiveness while moisture content affects elasticity. In the present study, no differences ( $P > 0.05$ ) were observed for these texture parameters, with no changes for the packaged ripened cheeses under refrigerated storage.

The  $L^*$  values indicate luminosity, while positive  $a^*$  represents the intensity of the red color, and the positive  $b^*$  indicates a trend towards the yellow color. In the present study, there were no significant differences in these color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) among the treatments ( $P > 0.05$ ). Furthermore, no differences were observed for the positive  $a^*$  values ( $P > 0.05$ ) during ripening, with mean values of  $9.67 \pm 1.79$ ,  $9.12 \pm 2.17$ , and  $10.46 \pm 1.29$  for the cheeses made from milk standardized to C/F ratios of 0.68, 0.72, and 0.76, respectively.

Concerning the parameter  $L^*$ , lower values were observed, indicating a tendency to darkening ( $P < 0.05$ ), while an increase in  $b^*$  value was observed, indicating a slight tendency to yellow color during the ripening period ( $P < 0.05$ ) (Figure 3).. The coloring agent (annatto) used in the manufacture of this type of cheese is the main factor that affects the color parameters (Ramos, 2013). In the present study, the amount of dye, as well as the concentration of the original solution, and the product's manufacturer, was standardized for all treatments.



**Figure 3.** Texture profile and L\* and b\* color values of Prato cheeses manufacturing with different C/Fratios during the ripening period. Results expressed in average.

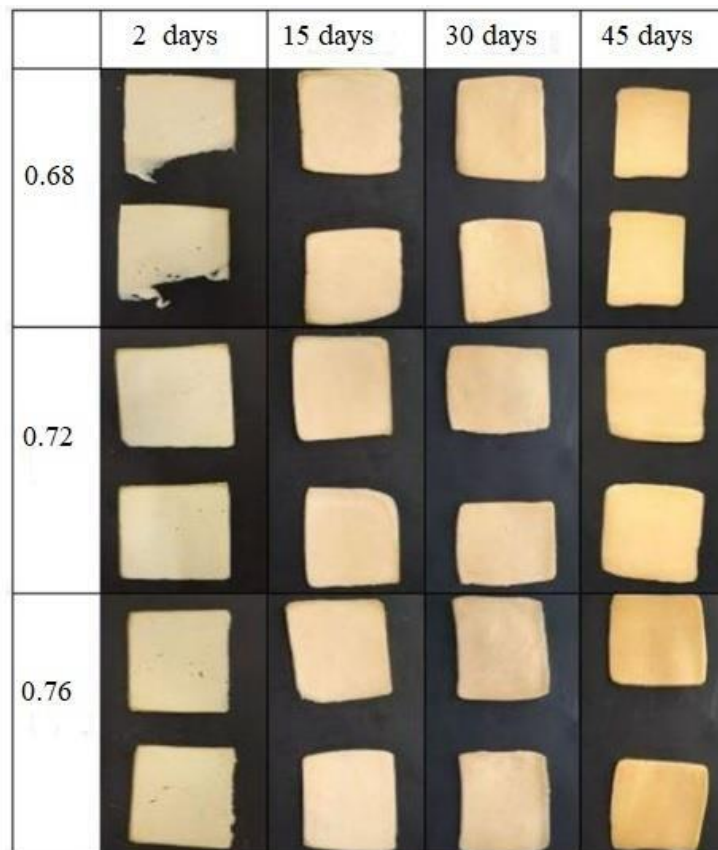


Source: Authors.

### 3.6 Sliceability

Sliceability analysis is a subjective perception rather than an instrumental determination. After two days of manufacture, the samples of all treatments showed adherence to the blade of the equipment, damaging the slices, which was more evident for the cheese made from milk standardized to C/F ratio of 0.68 (Figure 4). Despite the similar cheese composition, a higher fat recovery was observed for the cheese made from milk with C/F ratio of 0.68, which explains the sliceability behavior of this treatment. In addition, for brine-salted cheeses, salt penetrates inside the cheeses gradually, thus affecting the moisture content. Throughout the ripening period, when the salt-in-moisture is equal throughout the cheese, a more effective sliceability is achieved, with intact, flexible, and foldable slices (Furtado, 2019). Therefore, the C/F ratios of milk in this study did not interfere with the sliceability of Prato cheeses.

**Figure 4.** Slicing of Prato cheeses with different C/F ratios during the ripening period.



Source: Authors.

### 3.7 Melting capacity and free oil release

The melting capacity differed significantly ( $P < 0.05$ ) among the treatments, and the cheeses made from milk standardized to C/F ratio of 0.68 presented greater meltability when compared to cheeses made from milk with C/F ratio of 0.76 (Figure 5). No significant differences were observed among the treatments made from milk standardized to C/F ratios of 0.68 and 0.72 ( $P > 0.05$ ). The melting capacity tended to increase over time for all treatments.

Melting is influenced by the fat content, moisture, and proteolysis indices (Wadhvani et al., 2011), and none of these factors differed among the treatments of the present study. Although the milk fat content varied as a function of the C/F ratios in milk, there were no changes in the fat content of cheeses ( $P > 0.05$ ). A higher fat recovery was observed in cheeses made from milk standardized to C/F ratio of 0.68, which led to a higher melting capacity. It is known that fat plays a lubricating role

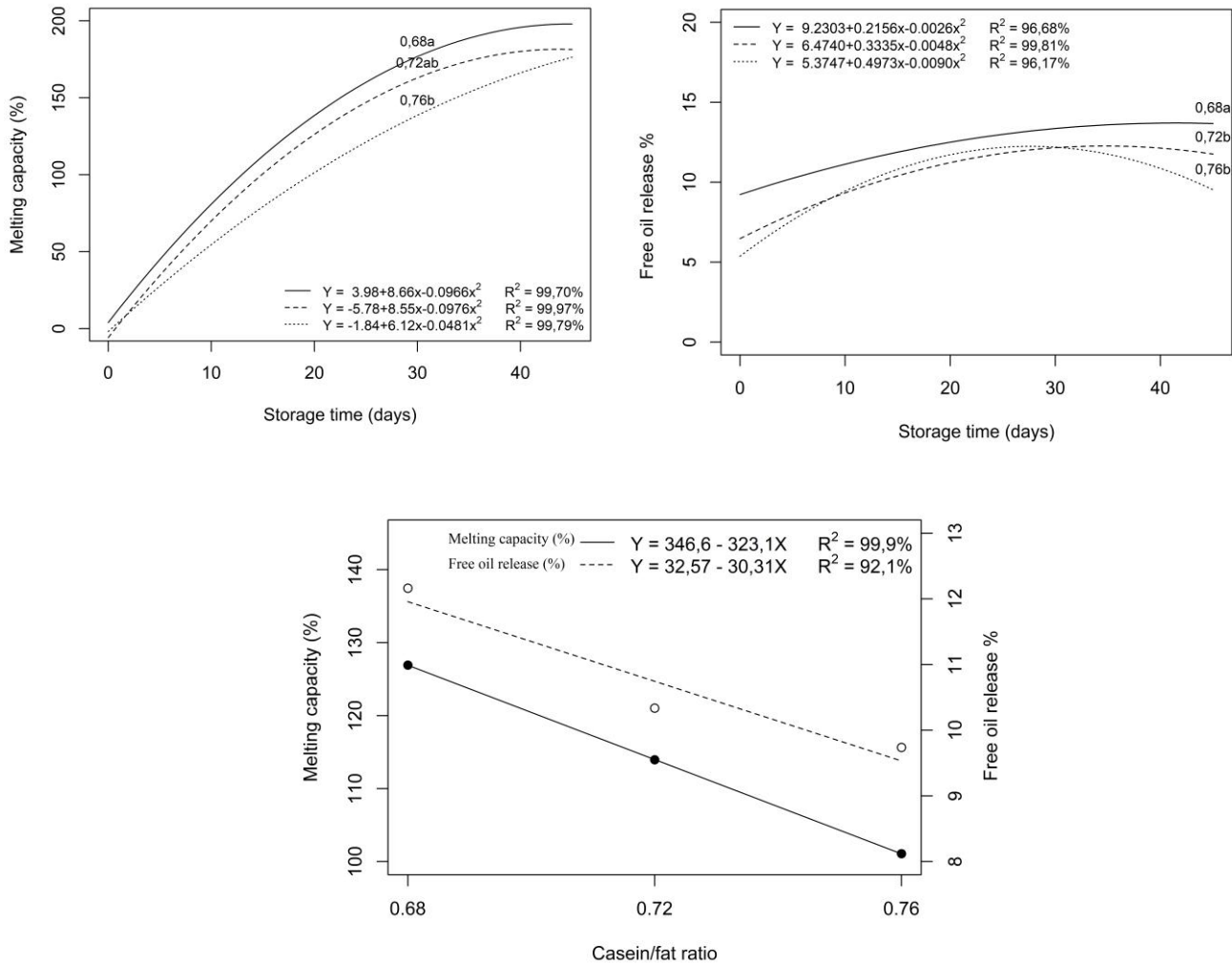
between the cheese protein matrices, thus an increase in the fat recovery in cheese is associated with a greater meltability (Dai et al., 2019).

The free oil release differed significantly ( $P < 0.05$ ) among the treatments (Figure 5). Cheeses of treatment 0.68 C/F showed greater oil release when compared to cheeses made from milk with C/F ratios of 0.72 and 0.76. No differences were observed for the oil release between the treatments made with C/F ratios of 0.72 and 0.76 ( $P > 0.05$ ), with an increase during ripening ( $P < 0.05$ ) (Figure 5).

These results were similar to those found in the meltability of the cheeses. The treatment 0.68 C/F provided greater meltability and free oil release, probably due to the higher fat content of milk, with a linear correlation of 96% (Figure 5). The plot allows establishing a desired C/F ratio in milk (among 0.68-0.76) and predicting the meltability and oil release of Prato cheese. As the functional properties of Prato cheese can be used as cheese quality parameters, using C/F ratios is possible to standardize the manufacture of Prato cheese according to the functional properties to meet different demands, including cheese with a greater meltability and formation of free oil, or a cheese with a lower meltability with less free oil.

Although no differences were observed for the proximate composition of all treatments, it was evident that the C/F ratio affected the functional properties of Prato cheese. The proteolysis reactions can favor the meltability and formation of free oil, due to the breakage of the protein network, which acts as a physical barrier between the fat globules. When cheese is heated and melted, the fat globules form excessive free oil (Kindstedt, 1993), which was evident with the increase in meltability and free oil release in the treatments during ripening.

**Figure 5.** Melting capacity (%), free oil release and relation of melting capacity (%) and free oil release of Prato cheeses with different C/F ratios during the ripening period. Results expressed in average.



Source: Authors.

### 3.8 Sensory evaluation

There were no significant differences in the consumer acceptance test among the treatments ( $P > 0.05$ ) (Table 2), which demonstrates that the different C/F ratios in milk did not affect the appearance, aroma, flavor, texture, and overall acceptance of cheeses after 30 days of refrigerated storage.

Among the remarkable features in the appearance of Prato cheese, color is an extremely important attribute (Kubo et al., 2013). During cheese making, the use of dye was standardized in terms of quantity, concentration, and manufacturer, which did not impact the color parameters of the cheeses ( $P > 0.05$ ).

Concerning flavor and taste, according to Fox et al. (2017), these characteristics are strongly influenced by the composition of cheeses and proteolysis during cheese ripening. In this study, no significant differences ( $P > 0.05$ ) were detected in the proximate composition or in the proteolysis indices of Prato cheeses among treatments, which did not cause any difference in the characteristics of the cheeses evaluated by the tasters.

**Table 2.** Sensory evaluation of Prato cheeses with 30 days of ripening (mean  $\pm$  SD).

Attributes	Treatments (C/F ratio)		
	0.68	0.72	0.76
Appearance	8.13 $\pm$ 0.93 <sup>a</sup>	7.97 $\pm$ 1.02 <sup>a</sup>	8.16 $\pm$ 0.97 <sup>a</sup>
Aroma	7.81 $\pm$ 1.06 <sup>a</sup>	7.81 $\pm$ 1.14 <sup>a</sup>	7.84 $\pm$ 1.16 <sup>a</sup>
Flavour	7.71 $\pm$ 1.20 <sup>a</sup>	7.47 $\pm$ 1.39 <sup>a</sup>	7.56 $\pm$ 1.37 <sup>a</sup>
Texture	7.93 $\pm$ 1.02 <sup>a</sup>	7.91 $\pm$ 1.16 <sup>a</sup>	7.82 $\pm$ 1.17 <sup>a</sup>
Overall acceptance	7.94 $\pm$ 1.10 <sup>a</sup>	7.73 $\pm$ 1.24 <sup>a</sup>	7.81 $\pm$ 1.25 <sup>a</sup>

<sup>a,b</sup>Means within a row with different superscript letters differ ( $P < 0.05$ ). n=4. Source: Authors.

For texture, in the evaluation of the texture profile using the texturometer, there was a significant difference ( $P < 0.05$ ) in chewiness, which was lower in cheeses made with a casein/fat ratio of 0.68. This difference was not perceived by consumers in the texture attribute, probably because the use of equipment makes the analysis more accurate, excluding the possibility of sensory fatigue by the tasters (Foegeding & Drake, 2007; Fox et al., 2017). Therefore, as there was no difference in appearance, flavor, taste, and texture, the overall impression also did not differ ( $P > 0.05$ ) among treatments.

#### 4. Conclusions

The casein to milk fat ratios in milk did not affect the physicochemical characteristics, pH, Aw, proteolysis indices, color parameters, sliceability, and sensory profile of the cheeses. However, pH, proteolysis indices, texture profile, L\* and b\* color parameters of the cheeses changed through the ripening time. Concerning the texture profile, lower chewiness values were observed for the cheeses made from milk standardized to casein/milk fat ratio of 0.68. The actual and dry matter cheese yields were similar among the treatments, while the C/F ratio of 0.68 in milk led to higher protein and fat recoveries and greater meltability and formation of free oil when compared to the casein/milk fat ratio of 0.76.

Therefore, a C/F ratio of 0.72 is recommended for the production of Prato cheese as the cheese presents good functional properties at this value, such as intermediate melting and chewiness, and less release of free oil.

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