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Effect of the use of carbon dioxide on Prato cheese making

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ABSTRACT

The effects of the addition of carbon dioxide (CO_2) under pressure (1.6×10^5 Pa at 8 °C) to pasteurised Prato cheese milk (pH 6.0) was investigated through 120 d of refrigerated storage. The addition of CO_2 decreased the curd formation time (30 min), the total manufacturing time (47 min), and the pH of Prato cheese, thus leading to reduced moisture content. The CO_2 treated cheese showed higher firmness and fracturability due to the greater whey loss. In contrast, the microorganism counts, cheese yield, protein loss, cohesiveness, springiness, and gumminess were not significantly affected by the treatment. For the lactose fermentation, no significant differences were observed. The addition of CO_2 did not change the proteolysis indexes, and no significant differences were observed in the sensory acceptance of the CO_2 treated cheese, which was well accepted by consumers.

1. Introduction

The addition of CO_2 to cheese milk as an acidifier to improve the manufacturing time and cheese yield, and to reduce the amount of coagulant has been of technological interest in Brazil. Milk acidification for cheese manufacture can be performed using starter cultures or the addition of lactic acid, acetic acid, or citric acid. However, the acid remaining in cheese and whey may compromise the quality of the product and/or its acceptance by consumers. Carbon dioxide (CO_2) may be an effective tool for milk pre-acidification, once it acts as an acidulant when dissolved in milk, and can be removed by syneresis during the cheese making (Nelson, Lynch, & Barbano, 2004).

Prato cheese is a Brazilian, semihard (moisture content 36–45.9%) and medium-scalded (≈42 °C) cheese produced by enzymatic coagulation, and ripened for at least 25 d (Alves, Merheb-Dini, Gomes, Silva, & Gigante, 2013). It is similar to Danbo and Gouda cheeses, with a soft texture and smooth taste (Mazal, Vianna, Santos, & Gigante, 2007), and widely consumed as a snack, or in sandwiches, pizzas, and ready-to-eat products.

Several authors have investigated the use of CO_2 in the cheese manufacturing process, and most studies have focused on the antimicrobial activity and the effects of the addition of CO_2 on milk coagulation and cheese ripening (Calvo, Montilla, & Olano, 1993; McCarney, Mullan, & Rowe, 1995; Ruas-Madiedo, Bada-Gancedo, Delgado, Gueimonde, & Reyes-Gavilán, 2003). The use of CO_2 in dairy foods, such as cottage cheese, can be used to extend the shelf life of the product, once the dissolved CO_2 slows the multiplication of microorganisms (Loss & Hotchkiss, 2003).

In addition to the microbiological effect, CO_2 may alter other milk properties; the acidification can lead to changes in milk composition and therefore in the micellar structure. Although the pH reduction can change the salt distribution of the medium, the typical salt distribution can be restored during the cheese manufacture using milk pre-acidified with CO_2 (Gastaldi, Lagaude, & de la Fuente, 1996).

The pH reduction is related to the amount of CO_2 dissolved, hydrated, and protonated in the aqueous phase, thus it depends on the intrinsic properties of the aqueous phase, such as the buffering capacity and initial pH (Ma & Barbano, 2003).

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Whereas the addition of CO_2 reduces the pH of milk and the coagulation time, it can be used to obtain the same clotting time using a lower amount of coagulant. CO_2 has been used in dairy industries, with a reduction of the amount of coagulant by a half without adverse effects, which can be a great advantage from an economic point of view. Reducing the pH of milk, especially by acidification, constitutes a fundamental step for cheese manufacture. Researchers have reported the effects of CO_2 on the production (McCarney et al., 1995; St-Gelais, Champagne, & Bélanger, 1997) and the proteolysis of Cheddar cheese during ripening (Nelson et al., 2004).

Other studies have reported an increase in ionic calcium in CO_2-treated milk, thus improving the coagulation properties (De La Fuente, 1998). However, the decrease in pH due to the formation of carbonic acid can change the milk salt balance between soluble and colloidal phases of milk, thus changing the micelle structure and the curd formed, besides affecting the product composition (Gastaldi et al., 1996). In Cheddar cheese, it was found that the addition of CO_2 to milk resulted in a greater loss of fat and calcium in whey after cheese making, leading to a 4.4% reduction of cheese yield, despite no changes were observed in the protein recovery. In addition, there was a higher salt retention in cheese curd (Nelson et al., 2004).

Others authors also evaluated the use of CO_2 in the cheese manufacture, including Cheddar, Cottage, and Minas cheese (Chen & Hotchkiss, 1991; Dias, 2009; St-Gelais et al., 1997). However, to the best of our knowledge, there are no data about the effect of milk pre-acidification with CO_2 on the production of semihard cheese, such as Prato cheese. In addition, little is known about the effects of the addition of CO_2 on the milk components.

The study aim was to evaluate the effects of dissolved CO_2 on the pasteurised cheese milk for the manufacture and ripening of Prato Cheese. The technological aspects of cheese manufacture, acidification capacity of the starter culture, cheese yield, physicochemical properties, sensory acceptance, proteolysis, and texture profile of the cheese were also assessed.

2. Material and methods

2.1. Production of Prato cheese

Prato cheese was manufactured in 4 replicates on different days, according to Sobral et al. (2016), as described in Fig. 1. Two treatments were carried out as follows: A) CO_2 under pressure of 1.6×10^5 Pa at 8 °C, pH 6.0; and B) untreated milk (control). For each experiment, 100 L of cheese milk were used, which was divided into 50 L for each treatment (A and B).

Calcium chloride (50%, w/v, 0.40 mL L^{-1} milk); annatto dye (0.025 mL 100 L^{-1} milk; Chr. Hansen Brazil, Valinhos, Brazil), mesophilic starter culture (R 704, 0.5 g 100 L^{-1} milk; Chr. Hansen) and liquid fermentation produced chymosin (100% FPC, Chy Max M; Chr. Hansen) were used.

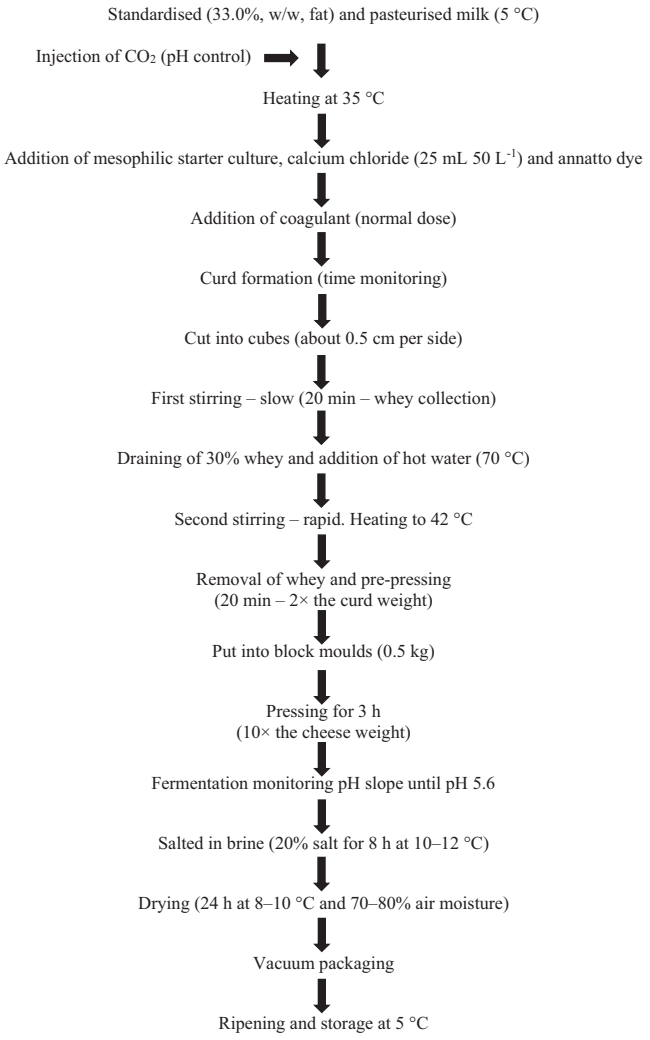

Fig. 1. Schematic flow chart of Prato cheese manufacture.

2.2. Physicochemical characterisation of milk, Prato cheese, and whey

The pH, fat content (%, w/w), total solids (%, w/w), titratable acidity (%, w/w), protein content (%, w/w), density at 15 °C, and free calcium were determined in milk and whey at the day of the production, according to the methods described in Brasil (2006). The free calcium concentration was determined by the electrochemical method, using a Orion® model 4-Star calcium ion selective electrode at 25 °C (Thermo Fisher Scientific Inc, Minneapolis MN, USA). The equipment was calibrated as described by Pereira (2014). All analyses were performed in duplicate.

The pH, moisture, fat content (%, w/w), salt content (%, w/w), fixed mineral residue (%, w/w) and protein content (%, w/w) of Prato cheese were determined at the 4th d of refrigerated storage according to methods described in Brasil (2006).

The pH and proteolysis were assessed at 4, 30, 60, 90 and 120 d of refrigerated storage. The percentages (%, w/w) of total nitrogen (TN), pH 4.6-soluble nitrogen (SN) and 12% TCA-soluble nitrogen (SN) of cheeses were determined by the Kjeldahl method, according to the methodology described by Nepomuceno, Costa Junior, and Costa (2016). The conversion factor 6.38 was used to calculate the total protein content (%, w/w) and the total nitrogen was used to calculate the extent of proteolysis index (EPI, pH 4.6-SN:TN), and the depth of proteolysis index (DPI, 12%TCA-SN:TN). The pH of the cheeses was determined by direct reading using a pH Meter Tec-2 (Tecnal, Piracicaba, São Paulo, Brasil), equipped with spear tip pH electrode (Brasil, 2006).

The total inorganic calcium was determined in cheese by the atomic absorption spectrometric method (ISO/IDF, 2007; Silva & Queiroz, 2002).

The results of the physicochemical characterisation of milk, whey, and cheeses, and the final weight after 1 d of manufacture were used to calculate the production yield, according to the methodology described by Furtado (2005) and Sales et al. (2016),

using the following equations (the adjusted moisture value was 44%; equation (2)):

$$\text{Loss}(\%) = \frac{[(\text{kg milk} - \text{kg production}) \times \text{protein in whey} \times 100]}{[(\text{kg milk}/\text{milk density})\text{fat in milk} \times \text{whey density}]} \quad (1)$$

$$\text{GL coefficient}\left(\text{g TS L}^{-1}\right) = \frac{(\text{cheese TS} \times \text{kg production} \times 10)}{\text{volume of milk}} \quad (2)$$

$$\text{Adjusted cheese yield} = \frac{[(\text{volume of milk}) \times (100 - \text{adjusted volume})]}{\text{kg cheese} \times \text{TS cheese}} \quad (3)$$

where TS is total solids.

2.3. Texture profile analysis

Texture profile analysis was performed after 4, 30, 90, and 120 d of refrigerated storage using a CT3 Texture Analyzer (Brookfield, Middleboro, USA). The working conditions were: speed of 1 mm s^{-1}, and 40% compression distance from the sample. A cylindrical probe was used with a load cell of 1 kN, perpendicularly passed through the samples (cubes 25 mm × 25 mm × 25 mm), which were randomly collected from the whole cheese and later packed in plastic bags and stored at 10 °C for 1 h (Nepomuceno et al., 2016). The texture measurements were performed in sextuplicate for the parameters firmness, fracturability, cohesiveness, springiness, and gumminess (Golin et al., 2018; Soares et al., 2011).

2.4. Microbiological characterisation

Counts of *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* sp., coliforms at 30 °C and 45 °C (AOAC, 2016) and filamentous fungi and yeasts (Brasil, 2003) were performed at 4 d of refrigerated storage.

2.5. Sensory evaluation

The sensory evaluation of the samples was performed at 4, 30, 60, 90, and 120 d of storage. One hundred and fifty untrained assessors (30 consumers for each sampling point) consisting of students, professors, and staff from Candido Tostes Dairy Institute, of varied ages and both gender, were selected based on regular consumption of cheese. The acceptance test was evaluated by a 9-point hedonic scale (Nepomuceno et al., 2016) for the attributes appearance, flavour, texture, and overall acceptance. Cheese samples were cut into 2.5 × 2.5 × 2.5 cm pieces, and codified with random 3-digit numbers. The sensory evaluations were performed at room temperature (25 °C).

2.6. Statistical analysis

Four replications of each experiment were performed, in a split-plot design with treatment as the main factor and time as the subfactor. Data were analysed by analysis of variance (ANOVA) and Tukey's test, at a 5% probability. Regression analysis was used in case of significant effect of the variable time. The analyses were performed with Minitab 14 Statistical Software (Minitab Inc., State College, USA).

3. Results and discussion

3.1. Physicochemical characterisation of milk

The milk used in the manufacture of Prato cheese contained 3.21 ± 0.26% (w/v) protein, 3.12 ± 0.00% (w/v) fat, 11.83 ± 0.30% (w/v) total solids, and 0.17 ± 0.00% acidity, expressed in lactic acid. The milk density at 15 °C and pH were 1028.68 ± 0.36 kg m^{-3} and 6.61 ± 0.03, respectively.

3.2. Physicochemical characterisation of Prato cheese

The addition of CO_2 to milk changed pH and moisture values ($P \leq 0.05$) of Prato cheese when compared with the control treatment (Table 1), at 1 and 4 d of storage. The pH of the control cheese was higher than CO_2 treated cheese, probably due to the reduction of pH of milk (6.0), leading to a predominance of carbonic acid in cheese.

Regarding to the moisture content, a higher ($P \leq 0.05$) value was observed for the control cheese (48.7%, w/w) when compared to the CO_2 treated cheese (47.1%, w/w). This result is due to the greater syneresis in the CO_2 treated curd during the stirring, pre-pressing, and pressing steps. Dias (2009) evaluated the effect of the addition of CO_2 to pasteurised milk (pH 6.21) for the manufacture of Minas Frescal cheese, and also found a reduction of moisture 63.0% (w/w) to 57.4% (w/w), for the control and CO_2 treated cheese, respectively.

Table 1 shows the results (mean ± standard deviation) of the physicochemical composition of Prato cheese and the cheese yield for all treatments, on the 4th d after processing.

Cheese yield was calculated at the beginning of storage (4th day). No significant difference ($P \leq 0.05$) was observed between both treatments in relation to protein losses in the whey, GL coefficient (g TS L^{-1}) and adjusted cheese yield. These findings are probably due to the lower processing time of the treated cheese, leading to a lower syneresis and decreasing the loss of constituents, thus preventing great changes in the CO_2 treated cheese.

Table 1
Mean values and standard deviation of the chemical composition of the Prato cheese end cheese yield parameters, sampling point at 4th day of storage time.[a]

Parameters (n = 4)	Control cheese (mean ± SD)	CO_2 (mean ± SD)
pH	5.18 ± 0.06	5.08 ± 0.06
FAT (%, w/v)	26.50 ± 0.58	28.50 ± 1.29
Moisture (%, w/v)	48.66 ± 0.57	47.05 ± 0.86
Total solids (%, w/v)	51.30 ± 0.56	53.16 ± 0.98
Protein (%, w/v)	21.54 ± 2.70	22.11 ± 2.18
FMR (%, w/v)	3.90 ± 0.65	3.82 ± 0.21
FDM	51.66 ± 3.68	53.61 ± 1.24
Protein loss in the whey	25.22 ± 2.71	26.79 ± 2.60
GL coefficient (g TS L^{-1})	63.49 ± 1.54	63.92 ± 0.87
Adjusted cheese yield (L kg^{-1})	8.83 ± 0.21	8.76 ± 0.12
Litres of milk per kg yield (L kg^{-1})	8.517 ± 0.35	8.655 ± 0.27

[a] FMR, fixed mineral residue; FDE, fat in dry matter; n, number of replicate; SD, standard deviation. All differences between control and CO_2 treated cheese makes were not significant ($P > 0.05$), except pH and moisture that were significantly different at $P \leq 0.05$.

Opposite results were observed by Nelson et al. (2004), who found lower adjusted cheese yield for Cheddar cheese made from milk pre-acidified with CO_2, which was 8.26 kg cheese 100 kg^{-1} pre-acidified milk, when compared with 9.29 kg cheese 100 kg^{-1} control milk.

3.3. Free calcium content of whey and total inorganic calcium of Prato cheese

Ca^{2+} ions play an important role in the casein stability and behaviour during milk processing, especially in the enzymatic coagulation of milk. The concentration of these ions is also associated with the solubility of colloidal calcium phosphate. Consequently, the determination of Ca^{2+} ions has a technological importance for cheese manufacturing (Fox & McSweeney, 1998).

A significant difference ($P \leq 0.05$) was observed in the free calcium content of whey, once higher values were observed for the treatment with addition of CO_2 (126.5 ± 13.99 mg L^{-1}) when compared with the control cheese (67.60 ± 12.59 mg L^{-1}), probably due to the lowering of milk pH during the CO_2 treatment. When it is soluble, calcium is found in the form of salts or free in solution in the form of a bivalent cation (Ca^{2+}). The reduction of pH can favour the demineralisation of colloidal calcium phosphate and lead to an increased activity of calcium ion (Ca^{2+}), which can reduce the repulsion between the negatively charged caseins, increasing the aggregation rate during milk coagulation (Fox & McSweeney, 1998). Thus, much of calcium is solubilised in whey during the early stages of syneresis.

Regarding the total inorganic calcium of Prato cheeses, the CO_2 treated cheese showed lower ($P \leq 0.05$) content when compared with the control sample, with values of 63.25 ± 0.01 and 66.75 ± 0.01 mg L^{-1}, respectively. According to Paula, Carvalho, Almeida, Costa, and Sobral (2012), CO_2 has a reversible effect on pH and an irreversible effect on inorganic colloidal calcium phosphate, which is transformed into other salts.

Although changes in milk pH, especially by acidification, is a fundamental step during the manufacturing process, changes may occur in milk composition, and therefore in the micelle structure (Gastaldi et al., 1996). Lucey et al. (1996) reported that the milk acidification followed by neutralisation improved the coagulation properties of rennet, due to a high Ca^{2+} activity.

3.4. Effect of carbon dioxide on the cheese manufacturing time

One of the technological interests of the addition of CO_2 to cheese milk as an acidifier is to reduce the manufacturing time. Prato cheese is produced on a large scale in Brazil, thus time saving during cheese making is very important for the productivity of the cheese industry.

When pre-acidified milk was used, the time required in curd setting dropped from 48 ± 2 to 18 ± 2 min. It is expected that the time for curd formation is affected by the low pH, which was close to the optimum pH of the coagulant enzyme, improving its performance. The enzymatic coagulation of cheese milk depends on several factors, including pH and ionic calcium in cheese milk. Some authors (Chen & Hotchkiss, 1991; De La Fuente, 1998; Dias, 2009; St-Gelais et al., 1997) have reported that an increase in ionic calcium in CO_2-treated milk can favour coagulation of milk, thus improving its technological performance for cheese manufacturing.

The milk pre-acidified with CO_2 led to a reduction of 64% in the processing time of Prato cheese, due to the lower time to curd setting and stirring. A similar result was found by Ruas-Madiedo, Alonso, Delgado, Bada-Gancedo, and De los Reyes-Gavilán (2002), who obtained 60% reduction of time to curd setting in the manufacture of a hard Spanish cheese, after a reduction in pH by 0.5 pH units with the addition of CO_2 to cheese milk.

St-Gelais et al. (1997), analysed the manufacturing time of Cheddar cheese made with milk preacidified with CO_2, and found less time for the pre-acidified milk (pH 6.56) when compared with the control. Shorter coagulation and manufacturing times were also reported by Nelson et al. (2004) in Cheddar cheese made with milk pre-acidified with CO_2 to pH 5.93.

3.5. Lactose fermentation in Prato cheese

The lactose fermentation in Prato cheese should provide a pH reduction to 5.6 before the salting step, to reach the desirable sensory characteristics. There was no significant difference ($P > 0.05$) in the fermentation time between the treatments. The CO_2 treated cheese took 4 h 35 min to reach pH 5.6, while the control cheese took 4 h 10 min. This behaviour indicates that the lower processing time reduced the more intense draining, thus not affecting the amount of residual lactose in the treated cheese.

Although the CO_2 treated cheese showed lower pH in relation to the control treatment ($P \leq 0.05$), no significant effect of time was observed for pH of cheese during the storage ($P > 0.05$) (Table 2).

3.6. Texture profile analysis

The texture profile analysis of cheese is an important tool for cheese characterisation, once it is directly related to the sensory acceptance by consumers. Several factors including the physicochemical composition (protein, fat, salt, minerals and pH) are known to influence the texture of cheese (Visser, 1991).

Table 3 shows the TPA results at different days of storage (4, 30, 90, and 120 d) for each treatment. A significant difference ($P \leq 0.05$) was observed for the firmness and fracturability of Prato cheese between the treatments, with higher values for the treatment with CO_2. However, no significant effect ($P > 0.05$) was observed for both the storage time and the interaction between the treatment and time. The higher firmness and fracturability of the CO_2 treated cheese was due to the lower pH of the curd allowing higher syneresis, which provided a lower moisture in the treated cheese (Dimitreli & Thomareis, 2007).

No differences ($P > 0.05$) were observed for the parameters cohesiveness, springiness and gumminess of Prato cheese. However, a significant difference was observed for the storage time. Regarding the interaction between treatment × time, there was no significant difference ($P > 0.05$). The behaviour of the variables as a function of the storage time can be explained by the following equations: Cohesiveness = 0.747−0.00650 T + 0.000038 T^2; Elasticity = 12−0.0452 × T; and Gumminess = 3341−32.1 × T + 0.176 T^2.

3.7. Microbiological counts

The cheese from the two treatments showed an absence of *Salmonella* sp. (in 25 g) and *L. monocytogenes*. In addition, no coliforms at 45 °C and *S. aureus* counts were observed in the treatments.

Table 2
Mean values and standard deviation of the pH values of the Prato cheeses of control treatment and treatment with addition of CO_2 at all times (4, 30, 60, 90 and 120 days).

Treatment	pH (days)				
	4	30	60	90	120
Control	5.18 ± 0,06	5.10 ± 0,06	5.26 ± 0,04	5.20 ± 0,08	5.21 ± 0,07
CO_2	5.08 ± 0,06	5.06 ± 0,04	5.11 ± 0,16	5.02 ± 0,08	5.06 ± 0,08

Table 3
Texture profile analysis of Prato cheese from control and with CO_2 treatments.[a]

Storage Time (days)	Treatments (mean, n = 4)	Firmness (N)	Fracturability (N)	Cohesiveness	Springiness (mm)	Gumminess (N)
4	CO_2	44,34[a]	44,34[a]	0,73[a]	17,8[a]	34,44[a]
	Control	41,46[b]	41,46[b]	0,72[a]	11,20[a]	29,78[a]
30	CO_2	47,54[a]	47,54[a]	0,59[b]	8,44[b]	24,21[b]
	Control	38,26[b]	38,26[b]	0,59[b]	8,19[b]	22,91[b]
60	CO_2	48,40[a]	48,40[a]	0,40[c]	7,33[c]	21,00[c]
	Control	40,02[b]	40,02[b]	0,57[c]	7,94[c]	20,34[c]
90	CO_2	46,48[a]	46,48[a]	0,43[d]	7,49[d]	18,27[d]
	Control	37,25[b]	37,25[b]	0,54[d]	7,94[d]	19,08[d]
120	CO_2	40,81[a]	40,81[a]	0,43[e]	7,86[e]	17,72[e]
	Control	39,03[b]	39,03[b]	0,58[e]	8,20[e]	21,16[e]

[a] In each columns, averages followed by the same letters did not differ significantly ($P > 0.05$) when compared using the Tukey test. Abbreviations are: N, Newton; n, number of replicate.

There was no significant difference ($P > 0.05$) in coliforms at 30 °C, and filamentous fungi and yeasts counts between the treatments, with mean counts of 2.68 log cfu g^{-1} and 4.63 log cfu g^{-1}, respectively.

The microbial counts were in accordance with those recommended by the legislation. Despite the CO_2 treatment can be used for inhibition of microbial contaminants in cheese milk, such effect was not observed in this study, probably due to a great part of CO_2 can be removed during the cheese manufacture in the whey draining step (Nelson et al., 2004).

3.8. Sensory evaluation

The analysis of variance did not indicate significant differences ($P > 0.05$) between the treatments, storage time ($P > 0.05$) and the interaction treatment × time ($P > 0.05$) in relation to the sensory acceptance of Prato cheese. There was no significant difference at a 5% probability level among the samples in the preference test. Both CO_2 treated and control cheeses scored 6.65 and 6.85, respectively, in the nine-point hedonic scale at 120 d, thus the CO_2 treated cheese was well accepted by consumers.

3.9. Evolution of proteolysis in Prato cheese ripening

Proteolysis is a complex biochemical event that occurs during cheese ripening, caused by several agents including the residual coagulant, starter culture, natural enzymes of milk, and microorganisms such as non-starter bacteria (NSLAB) and exogenous enzymes (Sousa, Ardo, & McSweeney, 2001). The extent of proteolysis is related to the hydrolysis of protein matrix, mainly by the action of the residual coagulant, while the depth of proteolysis is related to the enzymatic activity of the starter culture used and possible contaminants (Wolfschoon-Pombo & Lima, 1989).

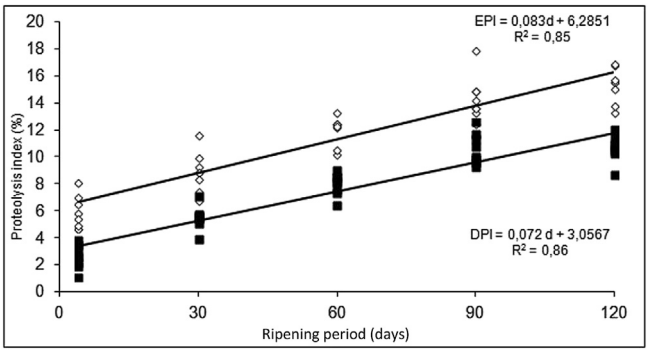

Fig. 2. Extent of proteolysis index (◇; EPI) and depth of proteolysis index (■, DPI) of Prato cheese from treatments.

Although no significant differences were observed for the extent and depth of proteolysis among the treatments ($P > 0.05$), changes were observed throughout the 120 d of storage ($P \leq 0.05$). In addition, no significant interaction was observed between treatment and time ($P > 0.05$). In general, a linear increase was observed for the proteolysis indexes of cheeses from both treatments throughout 120 d storage (Fig. 2).

In the present study, the proteolysis of the treated cheese was not affected by the CO_2 treatment. However, the extent and depth of proteolysis indexes increased during the 120 d of refrigerated storage of Prato cheese for both treatments. This result may be due to cheeses were made using the same raw material and manufactured in a single batch, using similar amounts of coagulant and lactic acid culture.

4. Conclusion

The pre-acidification of the pasteurised cheese milk with CO_2 up to pH 6.0 affected the physicochemical properties, pH, moisture, and texture profile of Prato cheese. The CO_2 treated cheese showed a greater reduction of time to curd setting and processing time. In relation to the cheese yield, the addition of CO_2 did not affect the Prato cheese production yield. The lactose fermentation and the evolution of the proteolysis indexes of Prato cheeses were not affected by the addition of CO_2 during 120 d of storage. The CO_2 treated cheese was well accepted by consumers, demonstrating that it is possible to use carbonation of cheese milk for the manufacture of Prato cheese.

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