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Assessment of proteolysis and sensory characteristics of Prato cheese with adjunct culture

Avaliação da proteólise e das características sensoriais de queijo Prato com cultura adjunta

Rafael Tamotsu Sato¹; Ariane Tayla Bisca Vieira²; Jaqueline Camisa³; Priscila Cristina Bizam Vianna⁴; Christiane Maciel Vasconcellos Barros De Rensis^{4*}

Abstract

Influence of adjunct cultures on the chemical and sensory characteristics, and proteolysis of Prato cheese was investigated. Cheeses were manufactured using a commercial starter culture and *Lactobacillus* strains (*Lactobacillus plantarum* or *Lactobacillus helveticus*) as adjunct cultures. Control cheeses lacked the adjunct culture. The chemical composition was analyzed at day 5 after manufacture and the proteolysis at days 5, 25, 45 and 65 of ripening. The sensory acceptance was assessed at 60 days. A split-plot design was used and the complete experiment was carried out in triplicate. The results were evaluated by ANOVA and Tukey's test at 5% significance level. There were no significant differences in chemical composition among the cheeses. A significant increase in proteolysis occurred during ripening period for the cheeses with adjunct culture when compared to cheeses without adjunct culture. Cheese with *Lactobacillus helveticus* showed higher scores for flavor, texture and purchase intent compared with the others treatments. Use of adjunct *Lactobacillus* suggests that the proteolysis of Prato cheese should be accelerated in order to reduce ripening period.

Key words: Adjunct culture, cheese, flavor, proteolysis, sensory analysis

Resumo

A influência de culturas adjuntas sobre as características químicas e sensoriais, e sobre a proteólise do queijo Prato foi avaliada. Os queijos foram fabricados com cultura *starter* comercial e cepas de *Lactobacillus* (*Lactobacillus plantarum* ou *Lactobacillus helveticus*) como culturas adjuntas. Os queijos controle não foram adicionados de cultura adjunta. A composição química foi analisada no dia 5 após a fabricação e a proteólise nos dias 5, 25, 45 e 65 de maturação. A aceitação sensorial foi avaliada após 60 dias. Um delineamento de parcelas subdivididas foi utilizado e o experimento completo foi realizado em triplicata. Os resultados foram avaliados pela análise de variância e teste de Tukey no nível de 5% de probabilidade. Os queijos não apresentaram diferenças significativas em relação à composição química. Um aumento significativo na proteólise ocorreu durante o período de maturação para os queijos com cultura adjunta, quando comparado aos queijos sem adição desse tipo de cultura. Os queijos com *Lactobacillus helveticus* apresentaram médias das notas mais altas para os atributos sabor, textura e intenção de compra em comparação aos demais tratamentos. A utilização de *Lactobacillus* como cultura adjunta indica que a proteólise do queijo prato pode ser acelerada a fim de reduzir o seu tempo de maturação.

Palavras-chave: Análise sensorial, cultura adjunta, queijo, proteólise, sabor

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Introduction

Prato cheese is one of the most produced and consumed cheese in Brazil. During the ripening period, it develops soft texture and mild flavor, as a result of biochemical reactions. It is very appreciated by consumers, and may be used as a table cheese or as a culinary ingredient (COSTA JUNIOR; PINHEIRO, 1998).

The ripening process is decisive for the development of suitable texture and sensory properties for many types of cheese (SOUZA; ARDÖ; MCSWEENEY, 2001), and the proteolysis is the main indicator of ripening (FOX; SINGH; MCSWEENEY, 1995). The understanding of the biochemistry of cheese ripening enables to control it during the process to obtain better quality cheeses (FOX, 1998).

Several approaches have been adopted for monitoring the biochemistry process and establishing the optimal ripening period to prevent excessive proteolysis, which contributes to the formation of low-molecular-mass hydrophobic bitter peptides, undesirable in cheese (MISTRY, 2001).

The optimal ripening period for Prato cheese is approximately 40 days. However, Brazilian legislation allows the product to be marketed after 25 days of storage (BRASIL, 1997), which is an insufficient time to develop the desirable sensory characteristics, therefore causing low quality and economic losses in the cheese industry (SILVA; VAN DENDER; BALDINI, 1999). Cheese ripening is a relatively expensive process for the cheese industry due to the slow-ripening process (SIUFE; ZORRILLA; RUBIOLO, 2010).

However, there are alternative technologies to accelerate the ripening of cheeses. A possible approach is the use of adjunct cultures, that contribute to a short lead time of ripening and, consequently, economic losses are reduced (DRAKE; SWANSON, 1995). The use of adjunct cultures can accelerate flavor development of the cheese through the increase of proteolysis, especially due to amino

peptidase activity, which reduces the bitter taste and increases the concentration of desirable flavored peptides and volatile flavor precursors (SILVA et al., 2004).

Lactobacilli are the most common microorganisms used as adjunct culture in cheese production. *Lactobacillus helveticus* and *Lactobacillus plantarum*, added during cheese production, contribute to the increase of proteolysis, development of desirable flavors and decrease of bitter flavors (BARROS et al., 2006; DRAKE; BOYLSTON; SWANSON, 1996).

The objective of this study was to evaluate the effect of the use of *Lactobacillus helveticus* and *Lactobacillus plantarum* as adjunct culture on the sensory characteristics and proteolysis of Prato cheese.

Material and Methods

Cheese manufacture

Raw milk was standardized with skim milk to obtain a final fat content of 3.0% and heat treated at 65°C for 30 min in a double wall tank (TMS 100 Incomar, Chavantes/SP, Brazil). The pasteurized standardized milk was cooled to 35°C and divided into three equal parts to be used for Prato cheese manufacture. The following treatments were carried out: 1) the control cheese (QC) using traditional mesophilic culture (1% v/v) MA11 – *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*; 2) cheese produced with traditional mesophilic culture and *Lactobacillus plantarum* (CHOOZIT FLAV 14 LYO 5D, 1% v/v; Q14); 3) cheese produced with traditional mesophilic culture and *Lactobacillus helveticus* (CHOOZIT FLAV 54 LYO 5D, 1% v/v; Q54). The cultures were supplied by Danisco® (Copenhagen, DK). The three processing procedures, one for each treatment, were carried out the same day and repeated three times, resulting in a total of nine tests previously randomized.

For each cheese, 50% calcium chloride solution (250 ppm) was added to the milk at 35°C, followed by the addition of mesophilic and adjunct culture (when used), annatto dye (80 ppm) and rennet (Bela Vista®, São Paulo/SP, Brazil) sufficient to coagulate the milk within 35 min.

After coagulation, the curd was cut into 0.5 cm cubes and submitted to slow continuous mixing for 15 min, followed by a removal of part of the whey (30%) and further heating of the curd to 40°C with the addition of hot water (80°C), to obtain the correct consistency for Prato cheese. After heating, the whey was removed and the curd was placed in rectangular plastic molds (0.5 kg) and pressed at room temperature in a vertical press with stainless steel weights. The cheeses were turned after 30 minutes (1st pressing stage) and after 1 h and 30 min (2nd pressing stage), with weights gradually increased 20 times the weight of the curd. The cheeses remained in the press for 12 h after the second pressing stage. After pressing was completed, cheeses were placed in a 20% NaCl solution for a period of 10 hours at 5°C. The cheeses were dried at 5°C for 24h and finally sealed under vacuum in plastic bags and stored at 12°C for 65 days.

Physicochemical composition

After 5 days of manufacture, the cheeses were evaluated for pH by the potentiometric method, titratable acidity (AOAC, 2003), fat content by Gerber method (BRITISH STANDARDS INSTITUTION, 1989), moisture content by gravimetric method at 105°C/16 h (AOAC, 2003), salt content by Volhard method (RICHARDSON, 1985) and ash content by incineration at 550°C/12 h (AOAC, 2003). Total nitrogen (TN) (IDF, 1962), soluble nitrogen in 12% trichloroacetic acid (NS-TCA12%) (AOAC, 2003) and soluble nitrogen at pH 4.6 (NS-pH4.6) (BARBANO; LYNCH; FLEMING, 1991) were determined by the Kjeldahl method. The nitrogen values were multiplied by the factor 6.38 to obtain the equivalent amount

of protein. All determinations were carried out in triplicate.

Evaluation of proteolysis

The cheeses were monitored for proteolysis after 5, 25, 45 and 65 days of refrigerated storage (12°C). Proteolysis was evaluated by the extent (EPI) and depth (DPI) of proteolysis indexes, according to the following equations: $EPI = (NS-pH4.6/NT) \times 100$ and $DPI = (NS-TCA12\%/NT) \times 100$.

Sensory evaluation

The sensory evaluation of the cheeses was performed after 60 days of storage at 12°C. Cheeses were evaluated by 50 untrained panelists in one session made in isolated sensory evaluation booths under white light. The samples at 12°C were served (15g) in disposable white plates coded with three-digit numbers. Consumers were provided with water and unsalted crackers for palate cleansing. The attributes appearance, flavor, texture and overall impression were evaluated using a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely. The purchase intention was measured on a five point scale where 1 = definitely would not buy and 5 = definitely would buy (MEILGAARD; CIVILLE; CARR, 2006). The acceptance test results were evaluated by Analysis of Variance (ANOVA) and Tukey's test at 5% significance level. The results of the purchasing intention were changed as percentage and evaluated using a frequency histogram.

Experimental design and statistical analysis

The statistical design adopted for this experiment was a split-plot block design, treatments being the plots and the cold storage the sub-plot. The effect of treatment, ripening period and the interaction of these factors on proteolysis were assessed by Analysis of Variance and Tukey's test at 5% significance level.

Results and Discussion

Chemical composition of the cheeses

Table 1 presents the chemical composition of the cheeses manufactured with or without adjunct cultures. Comparing the results found in this study (Table 1) to the regulation, the moisture and

fat content on dry basis are in accordance with Brazilian law for all the samples. The addition of adjunct cultures of *Lactobacillus plantarum* and *Lactobacillus helveticus* did not significantly ($p > 0.05$) affect the chemical composition of the cheeses.

Table 1. Average composition (n=3) of the 5 day-old

cheeses produced with and without adjunct culture.

Component	QC	Q14	Q54
Moisture (%)	41.80 ^a	42.48 ^a	40.73 ^a
Fat (%)	29.75 ^a	27.75 ^a	28.50 ^a
FDM (%)	51.11 ^a	48.24 ^a	47.70 ^a
Protein (%)	23.00 ^a	22.76 ^a	20.19 ^a
Salt (%)	2.81 ^a	2.60 ^a	2.01 ^a
Ash (%)	3.82 ^a	3.17 ^a	3.55 ^a
Tritable acidity (% lactic acid)	0.76 ^a	0.95 ^a	1.23 ^a
pH	5.62 ^a	5.33 ^a	5.13 ^a

*Means with the same letter, in the same line, do not differ ($p > 0.05$). FDM = fat in dry matter. QC = control cheese. Q14 = cheese containing *Lactobacillus plantarum*. Q54 = cheese containing *Lactobacillus helveticus*.

Source: Elaboration of the authors.

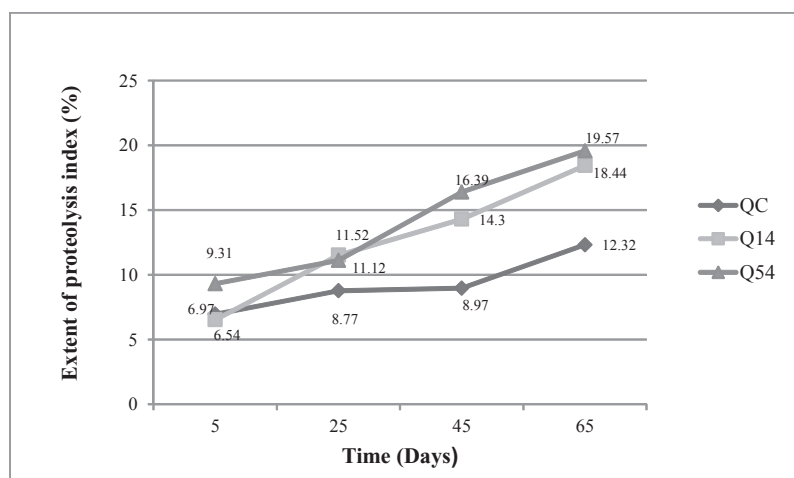
Barros (2005) studied light Prato cheese added by *Lactobacillus helveticus* and by *Lactobacillus casei*, and observed that the adjunct culture did not influence the composition of the cheese. Awad, Ahmed and El Soda (2010) obtained similar results for Domiati cheese using mixtures of adjunct cultures containing *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus paracasei* subsp. *paracasei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus casei* and three strains of *Enterococcus faecium*.

Evaluation of proteolysis

The index pH 4.6-SN:TN increased significantly ($p < 0.05$) (Table 2) for all the cheeses during the 65 days of ripening (Figure 1). The index pH 4.6-SN:TN evaluates the high molecular weight

peptides formed in primary proteolysis due to the degradation of caseins mainly by the action of residual coagulant. The evolution of this index is an important factor for the final composition and the sensory characteristics of the cheese (CHOISY et al., 1987; FOX et al., 2000).

For all the treatments, there was a significant increase of the index 12%TCA-SN:TN during ripening period (Table 2). The index 12%TCA-SN:TN% evaluates the small peptides and amino acids accumulated during ripening, mainly due to proteolytic action of bacterial proteases and peptidases on the nitrogen compounds released in the degradation of caseins. Small peptides and free amino acids contribute to the sensory characteristics of cheeses (FOX et al., 2000; MORENO et al., 2002).

Figure 1. Extent of proteolysis index (EPI) of Prato cheese during the 65 days of ripening period.

Source: Elaboration of the authors.

Table 2. Effect of treatment, ripening period, and their interactions on pH 4.6-soluble N (SN):totalN (TN), and 12%TCA-SN:TN of cheese with or without adjunct cultures (n = 3).

Item	P-value	
	pH 4.6-SN:TN	12%TCA-SN:TN
Treatment ⁽¹⁾	0.2202	0.0290
Ripening period ⁽²⁾	0.0000	0.0000
Treatment × ripening period	0.9599	0.3960

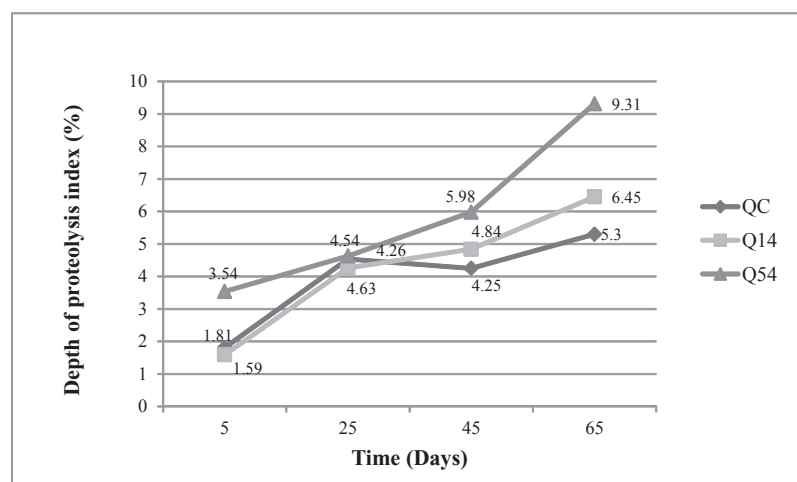
⁽¹⁾Treatment: chesse with and without adjunct culture; ⁽²⁾65 days.

NS = P > 0.05

Source: Elaboration of the authors.

The degree of secondary proteolysis in terms of the 12%TCA-SN was higher in cheeses produced using *L. helveticus* than in cheeses produced using *L. plantarum* throughout the ripening period (Figure

2). These results showed that the higher proteolytic activity of *L. helveticus* could contribute to the 12%TCA-SN formation and consequently increased proteolysis for cheeses.

Figure 2. Depth of proteolysis index (DPI) of Prato cheese during the 65 days of ripening period.

Source: Elaboration of the authors.

Kiernan et al. (2000) found that Cheddar cheeses containing *L. helveticus* as adjunct culture showed an increase in proteolysis and an improvement in flavor when compared to the control cheese. Barros (2005) studied Prato cheese containing *L. casei* or *L. helveticus* and observed an increase in the index 12%TCA-SN:TN of proteolysis for the cheese containing *L. helveticus* at 65 days of ripening.

Sensory analysis

The average scores obtained for the sensory attributes of the cheeses are shown in Table 3. In general, the cheeses were well accepted by consumers, with scores between 6 and 7 (slightly to moderately liked) for all the sensory attributes studied.

Table 3. Average scores for sensory attributes of the cheeses with and without adjunct culture.

Attribute	QC	Q14	Q54
Appearance	7,82 ^a	7,38 ^a	7,66 ^a
Aroma	7,44 ^a	7,08 ^b	7,62 ^a
Flavor	6,74 ^b	6,26 ^b	7,42 ^a
Texture	7,40 ^b	6,86 ^c	7,78 ^a
Overall Impression	7,50 ^a	7,12 ^a	7,24 ^a

*Means with the same letter, in the same line, do not differ ($p > 0,05$). QC = control cheese. Q14 = cheese containing *Lactobacillus plantarum*. Q54 = cheese containing *Lactobacillus helveticus*.

Source: Elaboration of the authors.

The cheese containing *L. helveticus* (Q54) showed higher acceptability for flavor and texture attributes than the other samples. However, there was no difference between the samples for the attributes appearance and overall impression.

Proteolysis plays a critical role in determining the typical sensory characteristics and represents a

significant indicator of quality (flavor and texture) (KARIMI; MORTAZAVIAN; KARAMI, 2012). Bacterial cell wall associated proteinases and intracellular peptidases released after cell lysis in the curd are considered to play an important role in casein hydrolysis during cheese preparation (WILKINSON et al., 1994). The differences of

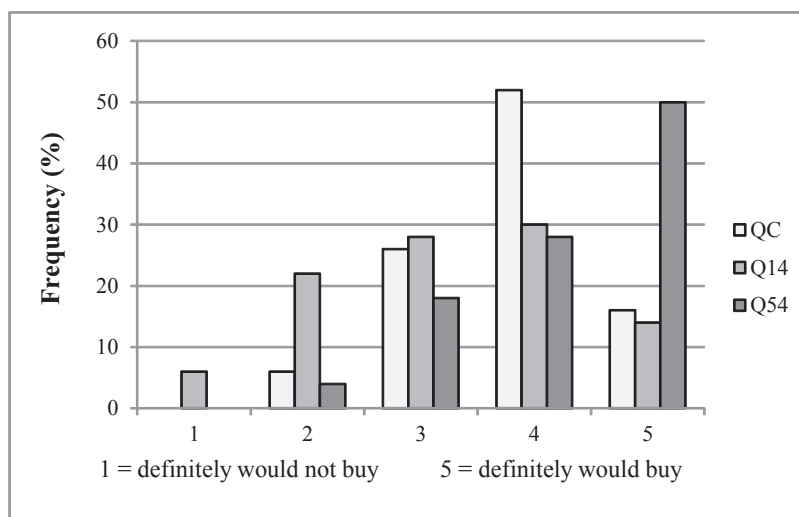
flavor and texture attributes can be related to the greater proteolysis of the cheese Q54 when compared to control cheese and Q14 (see Figure 2).

Improvement in cheese flavor can be linked to the high levels of free amino groups in cheese made with the adjunct culture that possess considerable levels of aminopeptidolytic activity (PIROUZIAN et al., 2012). According to Slattery et al. (2010), the *L. helveticus* is often used as adjunct culture in order to enhance the flavor of cheese. The results found in our study are in agreement with previously reported results. Barros et al. (2006) found that Prato cheese containing *L. helveticus* as adjunct culture showed a texture improvement probably due to the action of the proteolytic system of this microorganism on the casein matrix. In another study, Fenelon, Beresford

and Guinee (2002) used various types of cultures containing combinations of *Lactococcus* and adjunct cultures (including various strains of *L. helveticus*) and found that the adjunct culture improved flavor and increased the acceptability of low-fat Cheddar cheese when compared to control cheese containing only *Lactococcus*.

Figure 3 shows the frequency histogram for purchase intention. A better flavor perception for the cheese Q54 reflected positively on the buying intention of consumers. About 80% of the panelists certainly or probably buy the Prato cheese containing *L. helveticus* as adjunct culture (Q54), indicating a better acceptability of this cheese when compared to control cheese (QC) and cheese containing *L. plantarum* (Q14).

Figure 3. Frequency histogram analysis for buying intention for Prato cheese with or without adjunct culture.



Source: Elaboration of the authors.

Conclusion

The most pronounced impact of the addition of adjunct culture on Prato cheese was observed on the proteolysis index and on the organoleptic properties of the ripened cheese. The addition of *L. helveticus* showed technological potential to accelerate ripening and, consequently, to improve the sensory characteristics of the Prato cheese.

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Notes

This study was approved by the “Comitê de ética e biossegurança da Universidade Norte do Paraná (CAAE – 0087.0.078.000-10)” and was carried out according with biosecurity and bioethics rules.

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