

Effect of Adjunct Culture *Lactobacillus helveticus* (B02) on the Composition, Proteolysis, Free Amino Acids Release and Sensory Characteristics of Prato Cheese

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Abstract

At semi-industrial scale it was obtained two cheeses, one with aromatic culture (CHN) and the other with also *L. helveticus* (CHN + LH). Proteolysis during ripening was evaluated using proteolytic indices, electrophoretic profile Urea-PAGE and release of free amino acids. Sensory evaluation was performed by acceptability test. It was observed a 5-fold increase in the fraction corresponding to α_{s1-1} -casein in the CHN cheese and 6.5 times in the CHN + LH cheese and 7.2 and 8.2 increase respectively, for total amino acids. Prato cheese with the adjunct culture showed higher acceptability in terms of overall aroma and flavor.

Keywords

Amino Acids, Cheese, *L. helveticus*, Ripening, Sensory Analysis

1. Introduction

Prato is a typical Brazilian cheese that ranks among the most produced cheeses in the country. This type of cheese is of Danish origin, similar to Gouda and Danbo, despite its different flavor and texture [1]. Its industrial process involves enzymatic coagulation of pasteurized cow's milk by the action of rennet enzymes, with the addition of lactic ferment, calcium chloride and a small

amount of annatto dye (*Bixa orellana*) to give the characteristic yellow tint [2]. The distinctive characteristics of the manufacturing process are: semi-cooked and delactosed curd obtained at 40°C, by slowly replacing part of the whey (15% to 20% of the initial milk volume) with water at 74°C to 80°C directly on the curd to raise the temperature to no more than 1°C per minute. According to legal aspects, the ripening time of the Prato-type cheese must be at least 25 days [2].

According to Kabelová *et al.* [3], proteolysis contributes to the textural changes of the cheese matrix, due to the breakdown of the protein network, decrease of water activity through water binding by the liberated carboxyl and amino groups, and increase in pH which facilitates the release of sapid compounds during mastication.

Cheese taste and texture have strong influence depending on the types of cultures used. During cheese ripening, proteolysis of casein forms the basis for generating a wide range of peptides and amino acids, with the potential to influence cheese taste [4]. The acidifying mesophilic cultures *Lactococcus lactis* ssp. *cremoris* and *Lc. lactis* ssp. *lactis* have been the most widely used in Prato cheese for snacks, sandwiches and other culinary dishes. This culture provides closed textured characteristics, little proteolysis and mild flavor, the most suitable for this purpose.

The CHN culture is composed of *Lactococcus lactis* ssp. *cremoris* and *Lc. lactis* ssp. *lactis*, and two cultures that promote flavor: *Lc. lactis* ssp. *lactis* biovar. *diacetyllactis* and *Leuconostoc mesenteroides* ssp. *cremoris*. Besides lactic acid, *Lc. lactis* ssp. *lactis* biovar. *diacetyllactis* produces acetoin and diacetyl compounds that provide aroma. *Leuconostoc mesenteroides* ssp. *cremoris* is heterofermentative, producing from glucose many compounds: lactic and acetic acids, ethanol, carbon dioxide, acetoin and diacetyl [5]. The cheese produced by this CHN culture has consistency and buttery features, mild flavor (sweet, not acid and not spicy), oval-shape and shiny eyes [6]. Besides cheese, CHN culture can be used to obtain fermented buttermilk, promoting a buttery flavor, and a sense of spiciness and effervescence to this product [7] [8].

Lactobacillus helveticus (LH) is a thermophilic homofermentative species traditionally used as starter in the manufacture of long ripening Swiss-type cheese, Italian cheese, such as Emmental, Gruyere, Grana Padano and Parmigiano Reggiano and fermented milk beverages [9] [10]. LH strains have been used as adjunct cultures for other cheese types in order to accelerate ripening, for the degradation of hydrophobic peptides, to reduce the bitter taste and to enhance flavor notes [11] [12]. LH has also been considered a health promoting culture due to its potential for producing bioactive peptides and bacteriocins, and for exerting symbiotic effect when combined with probiotics in fermented milk products [10]. Some peptides formed from proteolysis have antihypertensive, antimicrobial and antioxidant biological properties, which add value to the product [13] [14]. The proteolytic system of LH is very efficient. It consists of cell-envelope proteases (CEP) for initial casein hydrolysis for oligopeptides and ami-

no acids; various intracellular peptidases for subsequent oligopeptides hydrolysis of small peptides and amino acids; and specific carriers for oligopeptides, tripeptides, dipeptides and free amino acids released from casein [9] [11].

The objective of this study was to evaluate the potential of using *L. helveticus* B02 as an adjunct culture of mesophilic aromatic culture to improve Prato cheese ripening and sensory characteristics.

2. Material and Methods

2.1. Culture Preparation

Mesophilic aromatic culture CHN-22 DVS (direct vat set) presently referred as CHN, and thermophilic culture *Lactobacillus helveticus* B02 DVS (LH culture) from Chr. Hansen (Valinhos, SP, Brazil) were rehydrated in 1 L of sterilized (110°C for 10 min), reconstituted (10%, w/v) powder skimmed milk (Molico, Nestle). Prior to manufacturing the cheese, the CHN culture was activated (growth at 22°C for 16 h) and the LH culture activated at 45°C for 18 h.

2.2. Cheese Manufacturing

Two batches of cheese were produced in parallel using 140 L of milk for each batch, at semi-industrial scale, using a 200 L capacity Queijomatic tank (Biasinox, Brazil). In batch 1, the CHN culture (200 mL 100 L⁻¹) was added to the pasteurized milk. In the 2nd batch, besides the addition to the CHN culture, the LH culture was added (215 mL 100 L⁻¹) after coagulation, according to the flowchart of Prato cheese processing (Figure 1). The following technological coadjuvants were employed: annato dye (5 mL 100 L⁻¹, Chr. Hansen, Brazil), calcium chloride solution 40% (60 mL 100 L⁻¹, Synth, Brazil) and rennet liquid CHY-MAX (7 mL 100 L⁻¹, 35 min coagulation, Chr. Hansen, Brazil). Heat-shrinkable plastic packaging was used for packaging the cheeses using a Selovac vacuum sealer (Supervac, GK185, Brazil).

2.3. Physicochemical Composition of Milk and Prato Cheese

Heat-treated milk was characterized for pH (pH meter Micronal B-375), titratable acidity (%lactic acid), and fat using the Gerber method [16], total dry matter [17], lactose [18], salt [19], ash [20] and total nitrogen (TN), using the Kjeldahl method (Conversion factor 6.38) [16] [21] [22].

After three days manufacturing and 10, 17, 24 31, 38 and 45 days of ripening the cheeses were analyzed for pH, titratable acidity and fat moisture, lactose, salt, ashes, total nitrogen, soluble nitrogen at pH 4.6 [23] and soluble nitrogen in 12% TCA [16] [21] [24], using the Kjeldahl method (Conversion factor 6,38). Three pieces of cheese from each treatment (CHN + LH cheese and CHN cheese) were randomly chosen. The analyses were performed in triplicate and the results were analyzed statistically using the Statsoft *Statistic* 5.0® software.

2.4. Microbiological Quality of the Milk and Cheese

The pasteurized milk was analyzed for *Salmonella* sp. (in 25 g), *Listeria mono-*

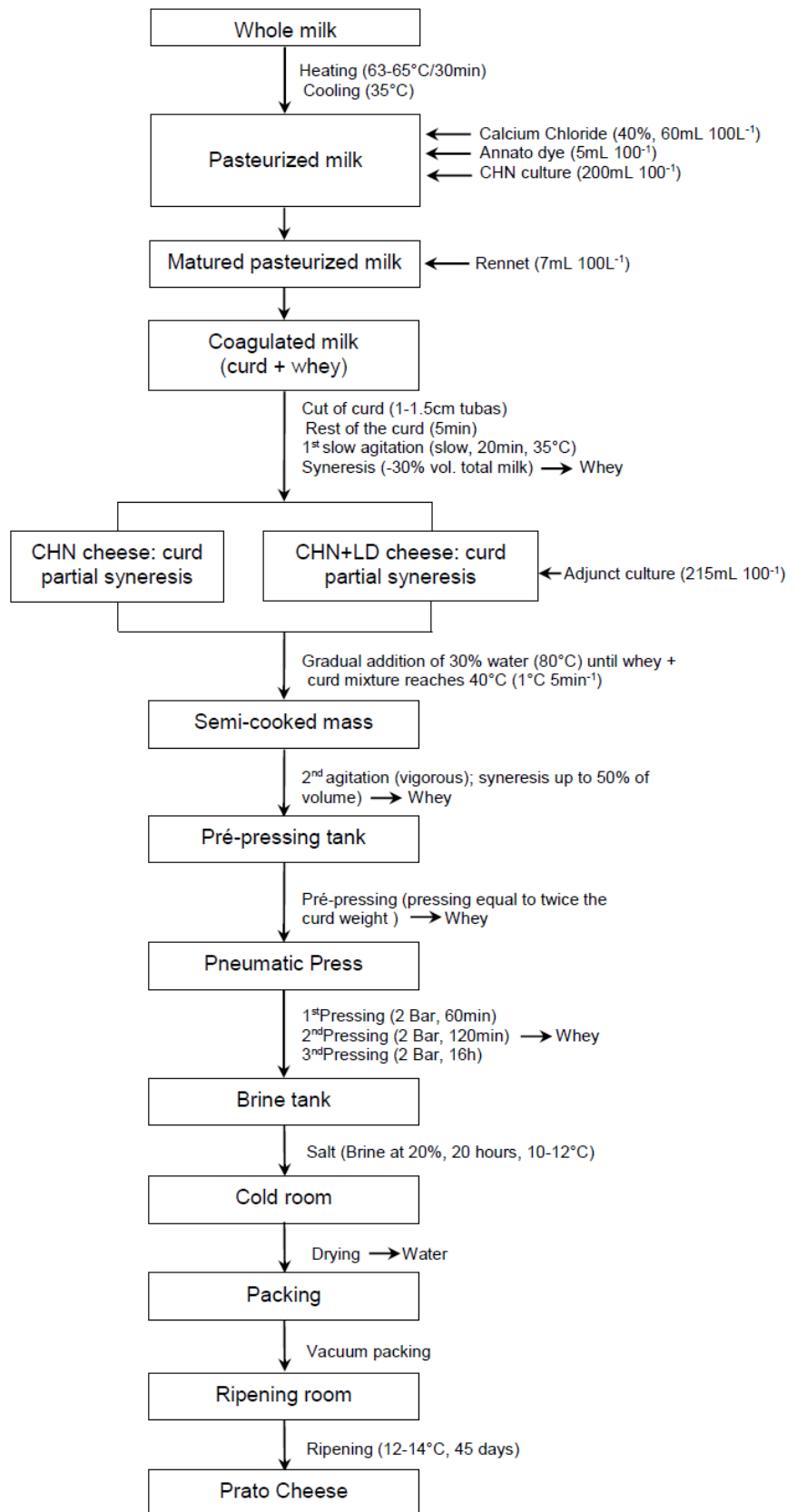


Figure 1. General flowchart of Prato cheese processing of according to Furtado and Lorenzo [15], with modifications.

cytogenes (in 25 g), coagulase-positive staphylococci (CFU g⁻¹), total mesophilic (CFU g⁻¹), mold and yeasts (CFU g⁻¹), coliforms at 35°C and 45°C (MPN g⁻¹) [21]. Cheeses were analyzed for the same microorganisms except for total mesophilic bacteria.

2.5. Characterization of Proteolysis during Ripening

Cheese at 3 days following manufacture and at 10, 17, 24, 31, 38 and 45 days of ripening were analyzed for proteolysis and electrophoretic profile of casein and at 3, 24 and 45 days for release of total and individual free amino acids.

Proteolysis was determined as soluble nitrogen at pH 4.6, expressed as a percentage of total nitrogen (Extension Index of Proteolysis—EIP), and as soluble nitrogen in trichloroacetic acid 12%, expressed as a percentage of total nitrogen (Depth of Proteolysis Index—DPI), determined by the macro-Kjeldahl method [23] [24] [25].

Polyacrylamide gel electrophoresis (urea-PAGE) was carried out using a vertical mini-PROTEAN® Casting System (Bio-Rad Laboratories Inc.), according to Andrews [26]. Briefly, the extracts for electrophoresis were prepared by dissolving 0.01 g of cheese sample (equivalent to 0.4% protein). 10 µL was applied to the gels. The bands separation was performed at 120 V. The bands were stained overnight by soaking the gels in Bio-Safe Coomassie G-250 Stain (Bio-Rad Laboratories Inc.) and destained with distilled water. Quantification of the casein fraction (relative % of peak area) was performed using ImageJ software.

Free amino acids were determined by reverse phase-high performance liquid chromatography (RP-HPLC) using a Shimadzu HPLC system (Shimadzu Corporation, Japan), equipped with a Luna/Phenomenex C18 column (4.6 - 250 mm, 5 L). Identification and quantification was done by external standard (Pierce/PN 20088) with UV detection at 254 nm [27] [28]. Free amino acids were extracted from 1 g of the sample with 0.1 M HCl, 99.9% methanol (30:60) and the addition of internal standard alpha aminobutyric acid (10 mL), stirred for 1/2 hour using an orbital shaker at 1500 rpm.

2.6. Sensory Analysis

Acceptability tests were conducted at 7 and 30 days of manufacture with 100 cheese consumers, without restrictions to age, gender or social class. Three samples were presented: Prato cheese (made only with CHN starter culture), Prato cheese with adjunct culture (CHN + LH) and commercial Prato cheese (a market leader). The samples were served in a sequential monadic manner according to a complete balanced block design. The samples were evaluated according to Meilgaard *et al.* [29], for overall acceptability and in particular appearance, aroma, texture and flavor using a nine-point hedonic scale (9 = liked it very much, 5 = neither liked or disliked, and 1 = disliked very much). The test was conducted in individual cabins with fluorescent lamps and equipped with computerized Compusense Five, version 5.4, for data collection and analysis. The results of the sensory analysis were compared using analysis of variance and the Tukey test at

a significance level of 95% ($P \leq 0.05$) using the Fizz Sensory Software program version 2.47B, Fizz Calculation module. The effect of ripening time on color intensity, aroma, smoothness, characteristic Prato cheese taste, salty taste, acidic taste and sour taste was compared using the statistical test using the Excel program [29]. The research was approved by the Research Ethics Committee from FCM/UNICAMP (112.204/Campinas/Brazil).

3. Results and Discussion

3.1. Physicochemical and Microbiological Characterization of Pasteurized Milk and Prato Cheese

The microbiological and physicochemical results of the milk after pasteurization was in accordance with the Brazilian legislation parameters [30], according to **Table 1**.

The composition of CHN and CHN + LH cheeses at 3 days following manufacture also complies with the legal parameters established by Brazilian regulations for moisture (36.0% - 45.9%), fat on a dry basis (45.0% - 59.9%) and pH (5.2 - 5.4) [2], and also with previously published data [31]. The CHN and CHN + LH cheeses presented similar quantities of proteins, fat, acidity and pH. The addition of the adjunct LH culture after the partial syneresis step of Prato cheese production had no negative effect on the composition of CHN + LH cheese regarding the parameters presented above.

Microbiological characterization of CHN and CHN + LH cheeses three days following production (**Table 1**) complies with the Brazilian legal regulations [32] that establishes absence of *Salmonella* and *Listeria monocytogenes* in 25 g and maximum counts of 5×10^3 MPN g^{-1} for coliforms at 30°C; 5×10^2 MPN g^{-1} for coliforms at 45°C; 1×10^3 CFU g^{-1} for *Staphylococcus* coagulase positive. Therefore, the hygienic-sanitary measures applied during processing were sufficient to achieve quality and safety.

3.2. Characterization of Proteolysis during Ripening

Proteolysis during cheese ripening was determined by the concentration (%) of the soluble nitrogen compounds at pH 4.6-SN at pH 4.6 (extension of proteolysis) and SN 12% TCA (depth of proteolysis) related to the percentage of total nitrogen (TN), as shown in **Figure 2**, letters A and B, respectively. We observed for both CHN and CHN + LH cheeses a gradual increase of the Extension Index of Proteolysis (EIP) and Depth of Proteolysis Index (DPI), with 45 days of ripening time. The EIP represents the high molecular weight peptides which are released during casein hydrolysis. In the first weeks of ripening, proteolysis is due to milk proteases (especially plasmin), residual activity of the rennet, native proteinases and, to a lesser extent, to proteinases of lactic cultures and non-starter lactic acid bacteria [33]. A secondary proteolysis is represented by small peptides and free amino acids formed during ripening, mainly due to the action of proteases and peptidases in the nitrogenous compounds released from the casein hydrolysis. Small peptides and free amino acids contribute to the sensory

Table 1. Physical-chemical and microbiological characterization and viability of CHN and CHN + LH cultures in Prato cheese three days after production.

Parameter	LD Prato Cheese	LD+LH Prato Cheese
Physico-chemical		
pH	5.36 ^a	5.40 ^a
Acidity (%)	0.6 ^a	0.61 ^a
Fat (%)	29.39 ^a	29.05 ^a
Fat on a dry basis (%)	53.01 ^a	49.12 ^a
Moisture (%)	44.56 ^a	40.86 ^b
Salt (%)	2.39 ^a	2.27 ^b
Salt humidity (%)	5.67 ^a	5.04 ^a
Total protein (%)	26.33 ^a	26.51 ^a
Microbiological		
<i>Salmonella</i> sp. (in 25 g)	Abs.	Abs.
<i>Listeria monocytogenes</i> (in 25 g)	Abs.	Abs.
Coagulase-positive staphylococci (CFU g ⁻¹)	<10	<10
Coliforms at 30°C - 35°C (MPN g ⁻¹)	<3	<3
Coliforms at 45°C (MPN g ⁻¹)	<3	<3
Molds and yeasts (CFU g ⁻¹)	<10	<10

Abbreviations are: MPN, most probable number per milliliter; CFU g⁻¹, colony-forming unit per milliliter; Abs., Absent.

characteristics of Prato cheese [33]. Published data show that the use of *L. helveticus* as adjunct culture in Prato cheese significantly increased the levels of DPI [34] [35] [36]. There were no significant differences in DPI nor EIP between the CHN and CHN + LH cheeses in this research. However, according to **Figure 2(a)** (EIP) and **Figure 2(b)** (DPI), the columns for each treatment started to split after 31 days of ripening and this tendency increased after 45 days of ripening for DPI.

The electrophoretic profile (**Figure 3**) of the cheeses samples shows an increase in the hydrolysis of the α_{s1} -casein fraction due to the action of rennet, clearly showing a higher intensity band corresponding to α_{s1-1} -casein, a polypeptide of high molecular weight, acidic and with slightly greater electrophoretic mobility than the α_{s1} -casein.

At 45 days of ripening, the % of the relative peak area from the electrophoretograms (not shown) we observed degradation of 56% and 67% of α_{s1} -casein fraction in the CHN and CHN + LH cheeses, respectively. The values found were very close to those found by Gorostiza *et al.* [37], which observed 65% of hydrolysis of α_{s1} -casein after 60 days of ripening. It was also observed that the corresponding fraction of α_{s1-1} -casein increased 5 times in CHN cheese and 6.5 times in CHN and CHN + LH cheese, and that the degradation process of the α_{s1} -casein fraction intensified at 31 days of ripening due to the presence of the adjunct culture. The degradation of β -casein was small, but it was mainly due to the presence of the adjunct culture, since *L. helveticus* has the potential to hydrolyze

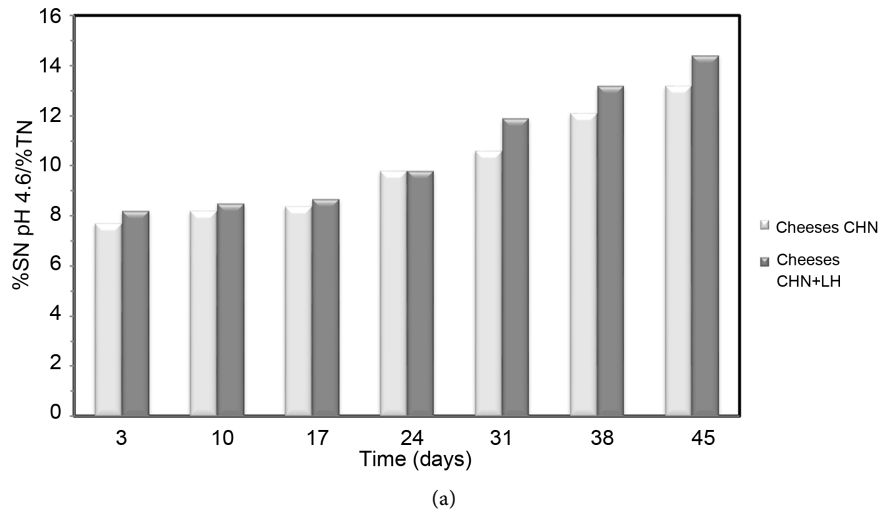


Figure 2. Extension index of proteolysis–EIP (a) and Depth of Proteolysis Index–DPI (b) in the CHN and CHN + LH cheeses during the 45 days of ripening period at 12°C ± 2°C (CHN–Aromatic mesophilic culture CHN-22; LH—*L. helveticus* B02; SN—Soluble Nitrogen; TN—Total Nitrogen; TCA—trichloroacetic acid).

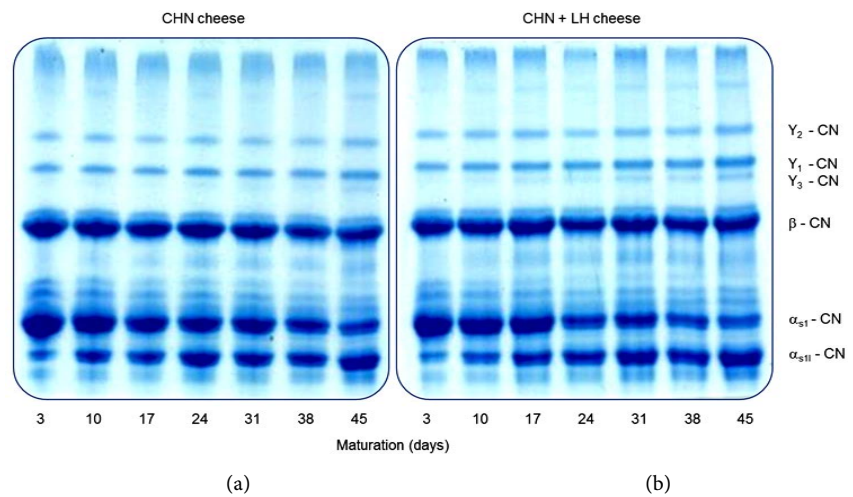


Figure 3. Electrophoresis profile of CHN (a) and CHN + LH (b) cheeses during ripening (CHN: Aromatic mesophilic culture CHN-22 and LH: *L. helveticus* B02).

β -casein. We observed γ_3 -casein band fraction after 17 days of ripening, while in the cheese without the addition of an adjunct culture this fraction was evident only at 45 days of ripening. El-Soda *et al.* [38] found that the ability to degrade casein depended on the species: *L. lactis* and *L. bulgaricus* hydrolyzed both, α_{s1} - and β -casein; *L. helveticus* only hydrolyzed β -casein and *L. acidophilus* only α_{s1} -casein.

Table 2 shows the composition and evolution of the total and individual free amino acids of CHN and CHN +LH cheeses after 3, 24 and 45 days of ripening.

The individual and total free amino acid levels progressively increased in CHN and CHN +LH cheeses ($P < 0.05$). However, there was no significant difference in the amount of any of the free amino acids due to the use of the adjunct culture. Glutamic acid and leucine were the free amino acids found in greater quantities in the two cheeses produced. The former is known to be a main contributor to umami taste. Glutamic acid and mineral salts were the key drivers for umami taste in Cheddar cheese according to research conducted by Andersen *et al.* [39]. In that work glutamic acid was equivalent to 31% - 34% of the total amount of amino acids in Cheddar. Presently, glutamic acid was equivalent to 18% of the total amount of amino acids in Prato cheese.

Table 2. Profile of individual and total free amino acids (mg 100 g⁻¹) of the CHN and CHN + LH cheeses at 3, 24 and 45 days of ripening.

Amino acid	CHN Cheese			CHN + LH Cheese		
	3	24	45	3	24	45
Aspartic Acid.	1.37 ^b	2.46 ^b	14.08 ^a	1.10 ^b	3.58 ^b	13.77 ^a
Glutamic acid	7.40 ^c	42.53 ^b	197.67 ^a	8.43 ^c	55.93 ^b	219.76 ^a
Serine	2.38 ^c	7.28 ^b	23.55 ^a	2.81 ^c	15.54 ^b	21.26 ^a
Glycine	0.83 ^c	13.68 ^b	19.90 ^a	1.03 ^c	7.53 ^b	16.74 ^a
Histidine	8.16 ^b	12.99 ^b	50.53 ^a	8.00 ^c	16.32 ^b	45.11 ^a
Arginine	3.10 ^c	12.48 ^b	26.86 ^a	1.19 ^c	11.75 ^b	21.74 ^a
Threonine	2.31 ^c	13.81 ^b	28.22 ^a	2.99 ^c	16.68 ^b	30.20 ^a
Alanine	6.84 ^c	16.51 ^b	25.02 ^a	7.07 ^c	16.87 ^b	25.47 ^a
Proline	20.90 ^c	29.90 ^b	41.34 ^a	19.22 ^c	27.93 ^b	44.88 ^a
Tyrosine	10.77 ^c	23.63 ^b	40.04 ^a	9.58 ^c	22.46 ^b	38.94 ^a
Valine	9.44 ^c	43.62 ^b	79.77 ^a	8.43 ^c	42.61 ^b	90.10 ^a
Methionine	4.15 ^c	11.86 ^b	15.90 ^a	4.34 ^c	13.20 ^b	18.46 ^a
Cystine	9.92 ^{ab}	9.05 ^b	15.20 ^a	10.04 ^a	11.02 ^a	21.26 ^a
Isoleucine	3.89 ^c	15.76 ^b	35.67 ^a	7.42 ^b	17.55 ^b	39.82 ^a
Leucine	15.54 ^c	99.92 ^b	205.01 ^a	25.22 ^c	103.52 ^b	232.72 ^a
Phenylalanine	10.48 ^c	48.46 ^b	85.65 ^a	8.37 ^c	51.03 ^b	100.06 ^a
Lysine	18.17 ^c	49.19 ^b	87.37 ^a	18.18 ^c	51.93 ^b	98.01 ^a
Tryptophan	12.12 ^c	50.66 ^b	93.03 ^a	11.78 ^c	68.67 ^b	114.66 ^a
Total	149.86 ^c	503.55 ^b	1084.87 ^a	147.29 ^c	554.42 ^b	1215.04 ^a

Different letters in the same row are significantly different (Tukey's test, $P < 0.05$). Abbreviations: Ac. Acid; LD: Aromatic mesophilic culture CHN-22; LH: *L. helveticus* B02.

According to Kabelová *et al.* [3] there are distinctive taste groups of amino acids: bitter tasting amino acids (leucine, lysine, and phenylalanine), bitter sweet amino acids (proline and valine), and salty-umami amino acids (glutamic acid and aspartic acid). In the present study, glutamic acid, arginine, glycine, aspartic acid, phenylalanine, valine, and threonine were the most evident free amino acids during the ripening period-in descending order. Their concentration increased 10 to 25 times in the 45 day period and the main ones observed corresponded to approximately 40% of the total free amino acids. However, considering the sum observed in the 45 days of ripening, the increase of free amino acids was 7.2 times for CHN and 8.2 times for CHN +LH (1084 and 1215 mg 100⁻¹ of Prato cheese, respectively).

Considering the sum of Glu and Asp for CHN or CHN + LH cheeses, both of them presented 19% of these umami amino acids released by ripening. However, in absolute numbers, in a 30 g portion of cheese, 63 mg and 70 mg of these umami amino acids were found, respectively in CHN or CHN + LH samples, and that higher quantity found in the cheese made with adjunct culture could be the reason for the taste improvement observed in the sensory test.

There are few published works on the release of free amino acids in Prato cheese. In one of the published works (in which adjunct culture was not used) an 8-fold increase of free amino acids was achieved during ripening (60 days) with a final average value of 2.454 mg 100g⁻¹ of total solids [37]. In the mentioned work, the main amino acids were taurine / GABA, glutamic acid, leucine, lysine, tryptophan and phenylalanine, corresponding to 62% of the total free amino acids found [37].

Moreno [36] showed an increase in the levels of total free amino acids in four commercial samples of Prato cheese (named A, B, C and D). The progressive evolution of total amino acid levels at the beginning and end of the 45 days ripening was higher for Cheese D (734.6 - 2037.7 mg 100 g⁻¹), followed by cheese C (141.7 - 1240.9 mg 100 g⁻¹), B (127.7 - 557.9 mg 100 g⁻¹) and A (47.3 - 225.1 mg 100 g⁻¹). The faster accumulation of free amino acids in the cheeses C and D compared to cheeses A and B was due to the autolysis of *L. helveticus*.

3.3. Sensory Analysis

Table 3 and **Table 4** presents the average results obtained in the acceptability tests of CHN and CHN + LH Prato cheeses at the beginning (7 days) and the end (45 days) of the ripening period and a commercial sample.

At the beginning of ripening (**Table 3**) the results showed no significant differences ($P > 0.05$) for the attribute texture among the three cheeses: CHN and CHN + LH and commercial product, with the averages close to "I like it".

Cheese flavor is one of the most important criteria for product quality and consumer acceptance [39]. The three cheeses showed overall aroma and flavor differences ($P \leq 0.05$), which showed higher means and close to "I like it" for CHN + LH cheese. As for the appearance of CHN + LH cheese, it did not differ significantly from the CHN cheese.

Table 3. Acceptability of the Prato cheese samples 7 days after production.

Attribute	CHN Cheese	CHN + LH Cheese	Commercial cheese	L.S.D.
Appearance	6.9 (1.4) ^{ab}	7.2 (1.1) ^a	6.5 (1.7) ^b	0.45
Aroma	6.5 (1.5) ^b	7.1 (1.1) ^a	6.5 (1.5) ^b	0.42
Texture	6.4 (1.5) ^a	6.8 (1.4) ^a	6.8 (1.6) ^a	0.45
Flavor	6.3 (1.6) ^b	7.0 (1.4) ^a	6.0 (1.8) ^b	0.50
Overall Quality	6.4 (1.6) ^b	7.1 (1.3) ^a	6.1 (1.8) ^b	0.49

Results expressed as mean (standard deviation) from 100 evaluations. L.S.D. least significant difference at 5% significance level (Tukey test). For each attribute (line), values followed by the same letters are not statistically different from each other ($P < 0.05$). Abbreviations: CHN: Aromatic mesophilic culture CHN-22; LH: *L. helveticus* B02.

Table 4. Acceptability of the Prato cheese samples 30 days after production.

Attribute	CHN Cheese	CHN + LH Cheese	Commercial cheese	L.S.D.
Appearance	7.0 (1.2) ^b	7.4 (0.9) ^a	7.2 (1.2) ^{ab}	0.33
Aroma	6.7 (1.3) ^b	7.1 (1.1) ^a	6.6 (1.4) ^b	0.41
Texture	7.0 (1.1) ^a	6.9 (1.3) ^a	7.1 (1.2) ^a	0.36
Flavor	6.7 (1.4) ^{ab}	7.1 (1.2) ^a	6.4 (1.8) ^b	0.49
Overall Quality	6.7 (1.3) ^b	7.3 (1.2) ^a	6.5 (1.6) ^b	0.43

Results expressed as mean (standard deviation) from 100 evaluations. L.S.D. Least significant difference at 5% significance level (Tukey test). For each attribute (line), values followed by the same letters are not statistically different from each other ($P < 0.05$). Abbreviations: CHN: Aromatic mesophilic culture CHN-22; LH: *L. helveticus* B02.

At 30 days (**Table 4**) of ripening the samples differed ($P \leq 0.05$) in terms of overall quality, aroma and flavor, and the sample containing the adjunct culture had higher averages and ranked between “I liked it” and “I really liked it”. As for appearance, there was no significant difference between the CHN +LH cheeses and the CHN cheese.

Therefore, the adjunct culture *L. helveticus* helped to improve the sensory and overall characteristics of Prato Cheese, in terms of aroma and flavor, giving it a higher quality standard when compared to cheese prepared without this culture and a commercial brand obtained at the market.

Improvements in flavor, texture and overall impression of Prato cheese with the addition of adjunct culture (*L. helveticus*) were observed in the work of Barros *et al.* [35]. Another study on Prato cheese, also with the addition of *L. helveticus* and *L. plantarum*, also reported acceptance improvements, with higher averages for the attributes flavor, texture and purchase intent in samples with the addition of *L. helveticus* [34].

4. Conclusion

The addition of *Lactobacillus helveticus* B02 as an adjunct culture of mesophilic aromatic culture CHN-22 contributed to the improvement of sensory characteristics of Prato cheese, in terms of overall aroma and flavor. The electrophoretic

profile of cheeses and total free amino acids highlighted the role of *L. helveticus* in the degradation process of caseins, particularly β -casein.

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